Regulation of Adrenergic Receptors in the Rat Kidney

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Abstract—Chronic daily application of ($\pm$)-isoprenaline induced a selective-down regulation of β-adrenoceptors in the kidney: the concentration of [^{3}H]dihydroalprenolol binding sites was significantly lowered by isoprenaline treatment while [^{3}H]prazosin and [^{3}H]rauwolscine binding, representing α_1- and α_2-adrenoceptors, respectively, was not markedly altered. Since the proportion of high- and low-affinity sites for the non-selective α- but relatively β_1-selective agonist ($-$)-noradrenaline remained constant and since in [^{3}H]dihydroalprenolol competition experiments the high- and low-affinity site ratio fitted well to the β_1/β_2 relation, determined independently by employing ICI 118551 as a β_2-selective ligand, a parallel decrease of both β_1- and β_2-adrenoceptor density can be concluded.

The kidneys are densely innervated by sympathetic nerve fibres (Di Bona 1982). Locally-released as well as circulating catecholamines can exert a variety of effects on renal function by interaction with adrenoceptors and their subtypes present in the kidney. α_1-, α_2-, β_1- and β_2-Adrenoceptors have all convincingly been demonstrated in the kidney (for review see Insel & Snavely 1981). Agonist interaction with α_1-adrenoceptors can cause arteriolar vasoconstriction, stimulation of gluconeogenesis and sodium chloride reabsorption in proximal tubular cells associated with increased phosphoinositide hydrolysis (Smyth et al 1985; Baines & Ho 1987; Neylon & Summers 1987; Wolff et al 1987). α_2-Adrenoceptor activation itself has probably a negligible influence on renal function, parathyroid hormone, arachidonic acid and vasopressin. Vasopressin stimulation of adenylate cyclase activity, however, can be antagonized via α_2-adrenoceptors (Pettinger et al 1987). Thus a reduction of phosphate excretion, an increase as well as an attenuation of sodium and chloride reabsorption can be mediated via α_2-adrenoceptors (Smyth et al 1984; Pettinger et al 1987; Rouse et al 1990). Conversely, β_1- and β_2-adrenoceptor-induced stimulation of adenylate cyclase increases renin secretion as well as fractional sodium re-absorption in the distal part of the nephron (Insel & Snavely 1981; Desaulles et al 1978).

The adrenoceptors as important effector molecules of sympathetic neurotransmission undergo agonist-induced regulation of cell-surface receptor density, which has been termed up- and down-regulation and extensively studied in the heart (for review see Coupe & Clarke 1988).

Adrenoceptors as initial sites of catecholamine action are selectively distributed in the cortex and medulla of the kidney as well as in various sections of the nephron. β-Adrenoceptors have been demonstrated in glomeruli, ascending limb, distal tubules, and collecting ducts of cortex and outer medulla in the rat kidney by autoradiography (Münzel et al 1984). In contrast to the guinea-pig, the predominant subtype in this species is the β_1-adrenergic receptor (Engel et al 1985; Summers et al 1985). The autoradiographic distribution correlates well with the localization of isoprenaline-sensitive adenylate cyclase in the nephron (Morel 1981). In

contrast to β-adrenoceptors, α_1- and α_2-adrenergic receptors could be identified in kidney arterioles and mainly in the proximal convoluted tubules (Calianos & Muntz 1990). Since all adrenoceptors appear to be accessible to circulating catecholamines (Baines & Ho 1987) down-regulation phenomena can be induced in this organ by systemic agonist application (Snavely et al 1985).

The effects of adrenoceptor down-regulation have been extensively studied in the heart. In experimental models of prolonged catecholamine administration, the non-selective β-agonist isoprenaline, as the most cardiotoxic catecholamine (Rona 1985), produced mainly a loss of β_2-adrenoceptors in the heart (Lu & Barnett 1985; Molenaar et al 1990). This is in contrast to failing human hearts, in which a selective down-regulation of β_1-adrenoceptors, possibly as a consequence of elevated catecholamine levels, has been observed (Bristow et al 1989). In an experimental standard model of catecholamine cytotoxicity (Csapo et al 1972) using daily intraperitoneal injections of the β-receptor agonist ($\pm$)-isoprenaline, we observed—beside the expected disappearance of β-adrenoceptors—a concomitant decrease in α_1-adrenoceptor density (Lübbecke et al 1988). This observation, which can probably be explained by an increase in noradrenaline release via isoprenaline-activated presynaptic β_2-adrenoceptors (Gillespie 1980) gave rise to extend our investigation to the kidney, which—similar to the heart—contains sympathetic nerve fibres as well as all known adrenoceptor subtypes.

Materials and Methods

Male Wistar rats, ca 220 g, were randomly assigned to isoprenaline-treated and control groups. Experimental rats received 40 μg g^{-1} ($\pm$)-isoprenaline in 0·9% NaCl containing 1·7 mg mL^{-1} ascorbic acid by daily intraperitoneal injections. Injections were performed between 0700 and 0800 h. After 8 days of treatment, rats were killed 24 h after the last injection by cervical dislocation. The kidneys were removed, trimmed of fat, connective tissue, renal pelvis and capsula. They were weighed and stored in liquid nitrogen.

Preparation of a kidney membrane fraction
Immediately before receptor assay, kidney membranes were prepared according to the method of Neylon & Summers

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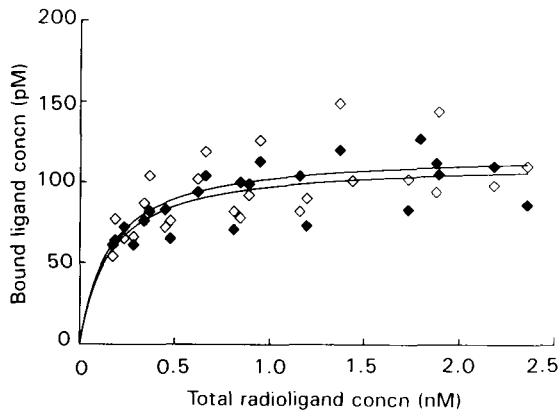
Fig. 1. Saturation isotherm of [³H]prazosin binding to α_1-adrenoceptors in kidney membranes from isoprenaline-treated (◆) and control (◇) rats. Pooled data of four separate experiments. The small difference in receptor density was not statistically significant.
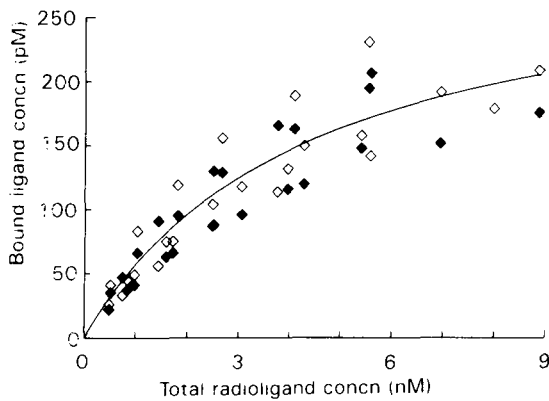

Fig. 2. Saturation isotherm of [³H]rauwolscine binding to α_2-adrenoceptors in rat kidney from isoprenaline-treated (◆) and control (◇) rats. Curves of control and treated experiments are superimposed by computerized fitting procedures. Pooled data of four separate assays.

(1985). Briefly, kidneys were thawed and minced in ice-cold 50 mM Tris-HCl (pH 7·7), 10 mM $MgSO_4$, 250 mM saccharose solution first with scissors and then with an Ultra-Turrax tissue homogenizer. Homogenization with a Potter-Elvhej-hem homogenizer, 10 strokes at 1500 rev min⁻¹, was followed by centrifugation at 5000 g for 5 min. The supernatant was again spun at 5000 g (5 min) and 30 000 g (15 min). The resulting pellet was resuspended in the same buffer and washed twice with intervening centrifugation at 30 000 g for 15 min. The final pellet was resuspended in 50 mM Tris-HCl (pH 7·7), 10 mM $MgSO_4$, 1 mM mercaptoethanol buffer.

Protein content was determined by the method of Lowry et al (1951) using bovine serum albumin as standard.

Adrenoceptor determinations

Receptors were assayed by radioligand binding. Bound and unbound radioligand were separated by rapid vacuum filtration using glass fibre filters (Schleicher & Schüll, Germany, No. 9). Filters were dried, scintillation fluid (Quickscint 1, Zinsser, Frankfurt, Germany) was added; counting for radioactivity was performed in a Searle Isocap 300 scintillation counter (counting efficiency 40%).
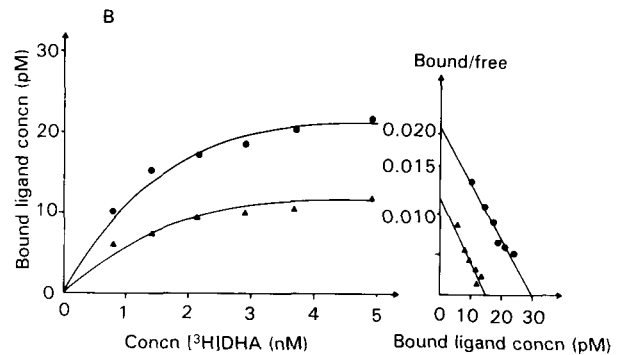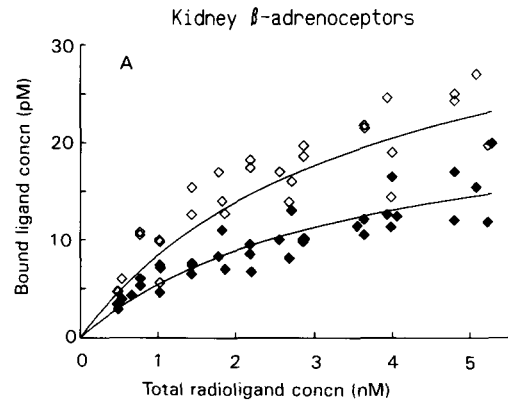
Kidney β-adrenoceptors

Fig. 3. A. Saturation isotherms of six pooled experiments showing a significant decrease in [³H]dihydroalprenolol binding in isoprenaline-treated (◆) compared with control (◇) rats. B. A single experiment with added Scatchard plots. ● Control (K_D 1·5 nM, B_{max} 30·7 fmol mg⁻¹), ▲ isoprenaline (K_D 1·2 nM, B_{max} 14·3 fmol mg⁻¹).

α_1-*Adrenoceptors*

[³H]Prazosin (sp. act. 26 Ci mmol⁻¹, NEN, Dreieich, Germany) was employed as a selective ligand for α_1-adrenoceptors. In saturation experiments, kidney membranes (250 μL, protein content 0·1–1·2 mg mL⁻¹) were incubated with varying concentrations (0·2–2·5 nM) of [³H]prazosin for 45 min at 30°C. In time-course experiments, this incubation period was sufficient to reach equilibrium. Non-specific binding was determined by inclusion of 0·1 μM hydroxyphenyl-ethyl-aminoethyl-tetralone (HEAT).

In competition experiments, a fixed radioligand concentration (1·2–16 nM) was incubated with varying concentrations of yohimbine, isoprenaline (0·1 μM–1 mM), prazosin, and HEAT (0·1 nM–10 μM) for adrenoceptor characterization as well as with noradrenaline (0·1 nM–1 mM) for determination of agonist affinity. The binding reaction in all experiments was stopped after 45 min by addition of 4 mL ice-cold 10 mM Tris-HCl buffer, pH 7·4, containing polyethyleneglycol (PEG 6000, 10% w/v). The incubation buffer was 10 mM Tris-HCl, 1 mM EDTA, pH 7·4.

α_2-*Adrenoceptors*

The incubation buffer was 50 mM Tris-HCl, 1 mM ascorbic acid, 10 mM $MgCl_2$ in 250 μL assay volume. In saturation experiments the concentration of the α_2-selective radioligand [³H]rauwolscine (sp. act. 73·7 Ci mmol⁻¹, NEN, Dreieich, Germany) ranged between 0·5 and 9 nM; non-specific binding

Table 1. α_1-, α_2- and β-Adrenoceptor density and radioligand affinity determined in saturation experiments with [³H]prazosin, [³H]rauwolscine and [³H]dihydroalprenolol.

	Control			Isoprenaline		
	B_{max} (fmol mg^{-1})	K_D (nM)	n	B_{max} (fmol mg^{-1})	K_D (nM)	n
α_1-Adrenoceptors	128·8 ± 12·4	0·19 ± 0·03	4	120·5 ± 13·9	0·18 ± 0·02	4
α_2-Adrenoceptors	317·5 ± 14·5	5·7 ± 0·6	4	301 ± 8·3	6·1 ± 0·5	4
β-Adrenoceptors	39·4 ± 4·1	4·1 ± 0·4	6	26·7 ± 1·9*	3·8 ± 1·0	6

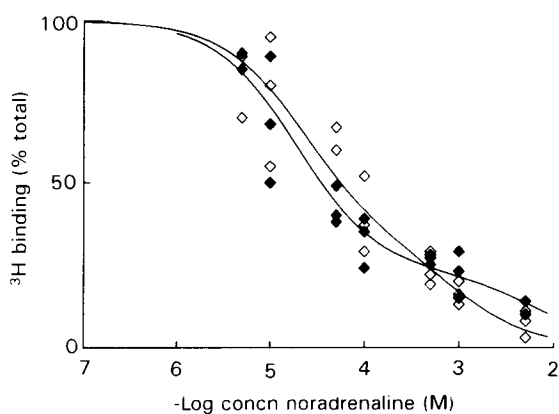n = number of experiments, mean values ± s.e.m., *$P < 0.02$.

FIG. 4. Competition curves of (−)-noradrenaline against [³H]dihydroalprenolol. The shallow shape of the curves fitted a two-affinity state of the receptors. No significant differences between control (◇) and treated (◆) animals could be observed (data of four experiments each).

was defined by addition of 0·1 μM yohimbine. In competition experiments (radioligand concentration 2·5–4 nM) yohimbine (0·1 nM–0·1 μM), prazosin, phentolamine or isoprenaline (0·1 μM–1 mM) was added for binding site characterization, noradrenaline (1 nM–1 mM) for agonist affinity determination. After 45 min at 35°C, assays were terminated by addition of 4 mL ice-cold 10 mM Tris-HCl washing buffer, pH 7·4, and vacuum filtration. It was experimentally established that the assay conditions were sufficient to reach equilibrium. The protein concentration varied between 1·0–1·4 mg mL^{-1}.

β-Adrenoceptors

[³H]Dihydroalprenolol (sp. act. 95 Ci mmol^{-1}, NEN, Dreieich, Germany) was employed as radioligand, oxprenolol (10 μM) for definition of non-specific binding. Incubation volume and time was the same as for [³H]rauwolscine, the temperature was 37°C. The radioligand concentration was 0·4–5·3 nM in saturation experiments 1·7–2·2 nM in competition experiments. For initial receptor characterization, oxprenolol (10 nM–100 μM), phentolamine or isoprenaline (100 nM–1 mM) was added to the assay for determination of agonist affinity (−)-noradrenaline 1 nM–5 mM, and for differentiation of β_1- and β_2-adrenoceptors the relative β_2-selective antagonist ICI 118551 in concentrations between 1 nM and 1 mM were added. The protein content in the assay was between 0·9 and 1·4 mg mL^{-1}.

Evaluation of data and statistics

Non-specific binding was subtracted from total binding.

Data from the saturation and competition experiments were analysed by employing two fitting procedures (Munson & Rodbard 1980; Motulsky 1990). Normal distribution was tested (Pearsons & Stephens 1964) and comparison between experimental and control groups was by Student's t-test for unpaired data. Significance was attached to $P < 0.05$.

Results

Influence of isoprenaline treatment on body and kidney weight
Before treatment, body weight was nearly identical in control (221 ± 14 g) and treated (219 ± 11 g) animals. After 8 days of treatment, weight gain was significantly lower in the treated group: 234 ± 19 g (n = 40) vs 250 ± 16 g (n = 39, mean ± s.d.). The kidney weights also were significantly lower after isoprenaline treatment: 1·9 ± 0·3 g or, when normalized to body weight 0·77 ± 0·1% in control (n = 75) and 1·7 ± 0·2 g and 0·72 ± 0·09%, respectively in treated rats (n = 86).

Radioligand-binding experiments
Initial experiments with the three radioligand antagonists showed a saturable, rapidly reversible binding to the kidney membrane preparation. In competition experiments with [³H]prazosin, the rank order of affinities of unlabelled ligands was prazosin > HEAT ≫ yohimbine, while for [³H]rauwolscine the sequence was yohimbine > phentolamine > prazosin > isoprenaline. These results are typical for a selective α_1-adrenoceptor and in the latter case, for α_2-adrenoceptor interaction. For [³H]dihydroalprenolol the affinity rank order was oxprenolol > isoprenaline = noradrenaline > phentolamine, suggesting a specific binding to β-adrenoceptors.

Saturation experiments show no evidence for multiple binding sites for the radioligands (Figs 1, 2, 3). The data of all experiments were fitted best to a one-site model. As shown in Table 1, α_2-adrenoceptors appear to be the predominant adrenoceptor subtype in the kidney, while β-adrenoceptor density is low in this organ. However, only this receptor showed a significant diminution after isoprenaline treatment, while receptor densities of α_1- and α_2-adrenoceptors as well as of the affinities represented by their K_D values showed no differences between the treated and control groups (Table 1).

Competition experiments with (−)-noradrenaline with the three radioligands resulted in shallow displacement curves fitting best to an interaction model with two receptors or two affinity states of one receptor (Fig. 4). The slopes of the displacement curves were not markedly altered by addition of 100 μM 5′guanylylimidodiphosphate (GppNHp). As can be seen in Table 2, neither K_i values nor the

Table 2. High- and low-affinity binding constants of noradrenaline in kidney membranes of rats.

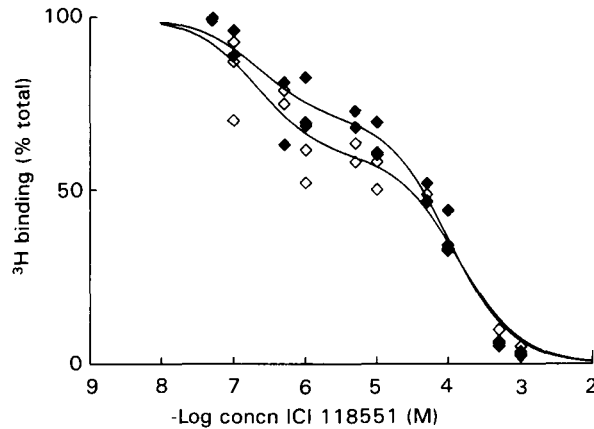|	Control	% of sites	n	Isoprenaline	% of sites	n
[³H]Prazosin						
K_i high	$3\cdot3\pm2\cdot2$ µM	54 ± 8	4	$0\cdot53\pm0\cdot21$ µM	56 ± 1	4
K_i low	176 ± 63 µM	46 ± 8	4	104 ± 38 µM	44 ± 1	4
[³H]Rauwolscine						
K_i high	$3\cdot9\pm1\cdot3$ µM	50 ± 4	4	$3\cdot3\pm1\cdot3$ µM	51 ± 9	4
K_i low	85 ± 21 µM	50 ± 4	4	79 ± 24 µM	49 ± 9	4
[³H]Dihydroalprenolol						
K_i high	$29\cdot2\pm12\cdot9$ µM	79 ± 3	4	$12\cdot9\pm3\cdot3$ µM	76 ± 1	4
K_i low	$1\cdot7\pm0\cdot9$ mM	21 ± 3	4	$3\cdot7\pm1\cdot8$ mM	23 ± 1	4

Mean values ± s.e.m.

FIG. 5. Inhibition of [³H]dihydroalprenolol binding by unlabelled ICI 118551 to kidney β-adrenoceptors in isoprenaline (◆) and control (◇) rats. The unlabelled compound has a 100-fold selectivity for β_2-adrenoceptors, this is clearly demonstrated in this competition experiment with a non-selective radioligand.

proportion of high- and low-affinity sites were significantly altered by isoprenaline treatment. In the case of β-adreno-ceptors, in which receptor density was decreased about 42%, the percentage of high- and low-affinity sites remained nearly identical. Competition experiments of [³H]dihydroalpreno-lol with the β_2-selective antagonist ICI 118551 showed more than a hundredfold selectivity of this compound to β_2-adrenoceptors (Fig. 5): the K_i value for the high-affinity site was $1\cdot1\pm0\cdot35\times10^{-7}$ M in controls and $1\cdot3\pm0\cdot45\times10^{-7}$ M in the treated animals, the corresponding K_i values for the low-affinity sites were $7\cdot6\pm0\cdot9\times10^{-5}$ and $5\cdot1\pm0\cdot4\times10^{-5}$ M, respectively. The percentage of high-affinity sites was $42\pm4\%$ ($58\pm4\%$ low-affinity sites) and $30\pm3\%$ (low-affinity sites $70\pm6\%$) in the treated group. Because of limitation in available material only three ICI 118551 competition experiments could be performed in the control group and two in the treated group, and significant differences could not be demonstrated.

Discussion

Following daily intraperitoneal injection of isoprenaline we previously found a diminution of both β- and α_1-adrenocep-tors (Lübbecke et al 1988). However, we could not confirm this result in this study using kidney membranes. In this organ, only the β-adrenergic receptor density was signifi-

cantly diminished, while numbers of α_1- and α_2-adrenocep-tors were not markedly affected. To examine agonist interac-tion with catecholaminergic receptors, $(-)$-noradrenaline competition experiments over a wide concentration range of the agonist were performed with all three radioligands. Interestingly the shape of the concentration/effect curves was flat (Fig. 4) adequately fitting a two-affinity state of all three receptors for this naturally occurring agonist. The slope of the agonist competition curves was not markedly influenced by addition of the stable GTP analogue GppNHp. Thus, an interaction of $(-)$-noradrenaline with receptor subtypes, which has been demonstrated for renal β- and α_2-adrenocep-tors (Insel & Snavely 1981; Lorenz et al 1990), would be a plausible explanation. However, the kidney had to be frozen in liquid nitrogen before membrane preparation. An irrever-sible uncoupling of the cell surface receptors and the G-proteins could be a possible consequence of this treatment. Other authors (Woodcock & Johnston 1982) observed an interconversion of the two-affinity state only in the presence of high NaCl concentrations.

In the case of β-adrenergic receptors, the high- and low-affinity site ratio was consistent with the β_1/β_2 ratio reported in the literature and determined by us, employing the β_2-selective antagonist ICI 118551. Since in β-adrenoceptors, as in all other assayed receptors, the proportion of binding sites of differing affinities was not altered by isoprenaline treat-ment, a parallel decrease of β_1- and β_2-adrenoceptors can be assumed. This is in agreement with results obtained by intravenous isoprenaline infusion (Snavely et al 1985). In contrast to results in the heart (Lübbecke et al 1988), no obvious changes in α_1-adrenoceptor density and affinity could be found. In contrast to the heart (Lübbecke et al 1988), the weight of the kidney was not increased but was slightly lower after isoprenaline exposure. Since the weight of the musculi gastrocnemius was also unaffected (data not shown), this trophic catecholamine effect appears to be exclusive for the heart muscle.

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