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REDUCED VASCULAR BETA-ADRENERGIC RECEPTORS IN DEOXYCORTICOSTERONE–SALT HYPERTENSIVE RATS

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Abstract—A method has been established for measuring beta-adrenergic receptors in membranes prepared from rat mesenteric arteries. The beta-adrenergic antagonist ^{125}I-iodohydroxybenzylpindolol was used as ligand in direct binding experiments. In the presence of 10^{-4}M phentolamine, ^{125}I-iodohydroxybenzylpindolol binding was saturable, stereospecific, rapid and reversible. The relative potencies of beta-adrenergic agonists in displacing ^{125}I-iodohydroxybenzylpindolol correlated with their relative potencies in stimulating adenylate cyclase activity in artery homogenates. This method was used to measure beta-receptors in mesenteric arteries of rats made hypertensive by deoxycorticosterone–salt treatment. Arteries from rats with established hypertension contained a reduced concentration of beta-adrenergic receptors. This decrease was from 10.1 ± 1.5 (S.E.M.) to 7.0 ± 1.2 fmoles/mg protein. There was no change in affinity for ^{125}I-iodohydroxybenzylpindolol (0.10 ± 0.02 n*M*).

In established hypertension there is an increase in the total peripheral resistance [1–3]. Some of this increase in peripheral resistance is due to structural changes in the vessels [4] but a substantial portion is mediated by the sympathetic nervous system [5]. In the peripheral circulation α-adrenergic receptors are vasoconstrictor whereas β-adrenergic receptors are vasodilator. Hence hypertension and the increased total peripheral resistance may be brought about by an imbalance between α- and β-adrenergic activity. Altered sensitivity to adrenergic stimulation has been demonstrated to be due to changes in the concentration of α- and β-receptors in different physiological and pathological conditions. As an example, experimental thyrotoxicosis in rats is associated with increases in cardiac β-receptors and decreases in cardiac α-receptors [6, 7]. In experimental hypertension, reduction in the numbers of both α- and β-receptors have been observed in myocardial membranes [8–10]. Since changes in adrenergic control in the smaller vessels may be important in regulating total peripheral resistance and thus in the development of hypertension, we have established methods for measuring β-adrenergic receptors in membrane preparations from rat mesenteric arteries. Using this method, β-adrenergic receptors have been measured in artery membranes from doca–salt hypertensive and control rats.

METHODS

Doca–salt hypertension. Male Sprague–Dawley rats (70–90 g) were unilaterally nephrectomized under ether anaesthesia. They were then injected weekly with 30 mg/kg of deoxycorticosterone pivalate (Percorten M, Ciba Geigy) and given 1% saline drinking water. Control animals were unilaterally nephrectomized and given saline drinking water. Blood pressures of conscious animals were measured by a tail plethysmographic method [11].

Beta-receptor characterization. Rats (200–300 g) were killed by cervical dislocation. Mesenteric arteries were removed by gently dissecting the vessels from the gut beginning at the duodenum, working progressively around the intestine and finally cutting the artery from the aorta. The tissue was placed in saline at 0°C. Fatty tissue and veins were removed by repeatedly combing the arteries with forceps. Microscopic examination showed that the material so prepared was largely arterial with little contamination from veins and adipose tissue. Arteries from 4–5 rats were pooled, finely minced with scissors and homogenized in 5 vol. of buffer (50 mM sodium phosphate, pH 7.4, 4 mM $MgCl_2$) containing 0.25 M sucrose using a 'Polytron' homogenizer. Three 5 sec passes were used separated by 30 sec chilling on ice. The homogenate was filtered through gauze and then centrifuged at 30,000 *g* for 15 min and the membrane pellet was washed twice with the above buffer. The membranes were finally resuspended in buffer at a final protein concentration of 1–2 mg/ml. Protein concentration was determined by the method of Lowry *et al.* [12] using bovine serum albumin as standard.

Membranes (0.05 ml, 50–100 μg protein) were incubated with ^{125}I-iodohydroxybenzylpindolol (^{125}IHP) (Searle Nucleonics) (50–500 pM) in a final volume of 0.15 ml containing 50 mM sodium phosphate pH 7.4, 4 mM $MgCl_2$, and 10^{-4} M phentolamine. Incubation was carried out at 37° for 45 min and was terminated by filtration through Whatman glass fiber filters GF/C. The filters were washed with 25 ml of 20 mM sodium phosphate, 4 mM $MgCl_2$ pH 7.4, at 37°, dried and counted in a Packard autogamma scintillation counter. Specific binding was defined as binding displaceable by 10^{-6} M propranolol. Under

the conditions used specific binding ranged from 40 to 70 per cent of the total counts bound per filter.

Adenylate cyclase measurement. Since the coupling of β-receptors to adenylate cyclase is very labile in membranes prepared from mesenteric arteries, homogenization was reduced to two 3 sec passes with a 'Polytron' homogenizer when adenylate cyclase activity was measured. The homogenate was filtered through gauze and used immediately. Adenylate cyclase was assayed by the method of Harden *et al.* using [^{3}H] ATP [13]. All assay tubes contained 10^{-4} M GTP. Separation of cAMP was achieved by the method of Krishna *et al.* [14]. Recovery of cAMP was determined using absorbance measurements.

RESULTS AND DISCUSSION

Characterization of ^{125}I-HYP binding to membranes prepared from rat mesenteric arteries. Specific binding of ^{125}I-HYP to artery membranes had the characteristics expected of β-receptor binding as discussed below. Binding sites exhibited satisfactory β-adrenergic specificity only when binding was carried out in the presence of 10^{-4} M phentolamine (see below). Under these conditions ^{125}I-HYP binding was saturable, and Scatchard analysis [15] produced a straight line indicating a single class of non-cooperative binding sites (Fig. 1). The dissociation constant calculated from Scatchard analysis was 0.10 ± 0.02 nM and there were 10 ± 1.5 fmoles of receptors per mg of protein in membranes prepared from untreated rats.

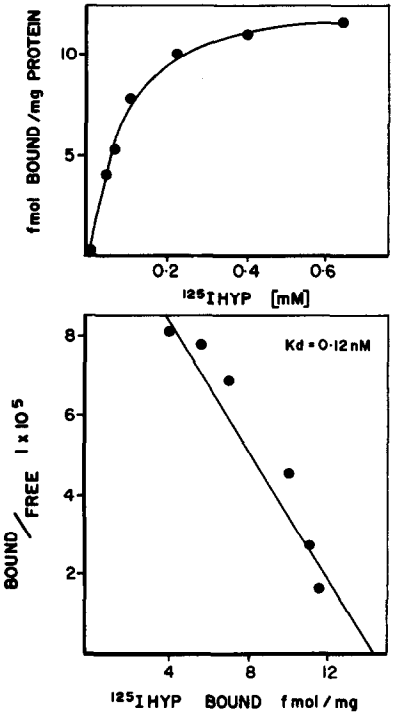

Fig. 1. (Upper panel) Binding of ^{125}I-iodohydroxybenzylpindolol to mesenteric artery membranes. (Lower panel) Scatchard analysis of the binding data expressed as ^{125}I-HYP bound (moles) over ^{125}I-HYP free (moles/litre) against ^{125}I-HYP bound (fmoles/mg protein).

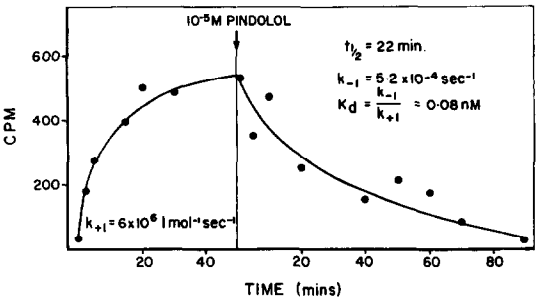

Fig. 2. Kinetics of ^{125}I-iodohydroxybenzylpindolol binding to mesenteric artery membranes. The binding reaction was initiated by adding membranes. Dissociation was initiated by adding pindolol as shown. Association and dissociation rate constants were calculated as described in the text.

Binding was rapid, equilibrium being reached within 20–30 min at 37°. Binding was rapidly reversible, the half life of the dissociation being 22 min (Fig. 2). Association and dissociation rate constants were calculated from this data using the formulae

$$\frac{\ln 2}{t_{\frac{1}{2}}\text{ (association)}} = k_{+1}[L] + k_{-1} \qquad (1)$$

and

$$k_{-1} = \frac{\ln 2}{t_{\frac{1}{2}}\text{ (dissociation)}}, \qquad (2)$$

where k_{+1} and k_{-1} are, respectively, the association and dissociation rate constants, $[L]$ is the concentration of free ^{125}I-HYP, $t_{\frac{1}{2}}$ in equation (1) is the time required to reach ½ equilibrium bound concentration, and $t_{\frac{1}{2}}$ in equation (2) is the half life of the dissociation.

Calculation of the K_d from k_{-1}/k_{+1} thus measured, gave a value of 0.08 nM in good agreement with the value obtained by Scatchard analysis (Fig. 2).

The affinities of β-adrenergic agonists for ^{125}I-HYP binding sites were compared with their potencies in stimulating adenylate cyclase in mesenteric artery homogenates. Basal adenylate cyclase activity was 4.6 ± 0.5 pmoles/mg protein. Maximum stimulation produced by adrenergic agonists was 50 per cent above basal. The relative affinities of agonists measured in competition binding studies were—isoprenaline > adrenaline > noradrenaline. Similar affinities for these compounds were calculated from their potencies in stimulating adenylate cyclase (Table 1). Because the maximum stimulation of adenylate cyclase was only 50 per cent, it was not possible to obtain reliable data for affinities of antagonist compounds by competition for adenylate cyclase activation. The antagonist compounds pindolol and propranolol competed for ^{125}I-HYP binding with high affinity. The pharmacologically active stereoisomer *L*-propranolol was 50–100 times more potent in competing for binding than the inactive isomer D-propranolol, demonstrating stereospecificity of binding.

Requirement for phentolamine for detection of β-receptors on artery membranes. Large amounts of

Table 1. Affinity of adrenergic agonists and antagonists for mesenteric artery beta-receptors

Compound	K_{act} (adenylate cyclase)* (nM)	K_I (^{125}I-HYP binding) (nM)
Isoprenaline	1.2×10^{-7}	6×10^{-8}
Adrenaline	2×10^{-6}	6×10^{-7}
Noradrenaline	10^{-5}	2×10^{-5}
Pindolol		5×10^{-9}
L-propranolol		2×10^{-9}
D-propranolol		$>10^{-7}$

* K_{act} was calculated as the concentration of drug producing 50% maximum stimulation of adenylate cyclase.

† K_I was calculated using the formula

$$K_I = \frac{I_{50}}{(1 + [L][K_L])},$$

where I_{50} is the concentration of drug causing 50 per cent displacement of ^{125}I-HYP binding, $[L]$ is the concentration of ^{125}I-HYP and K_L is the dissociation constant of ^{125}I-HYP binding to mesenteric artery membranes.

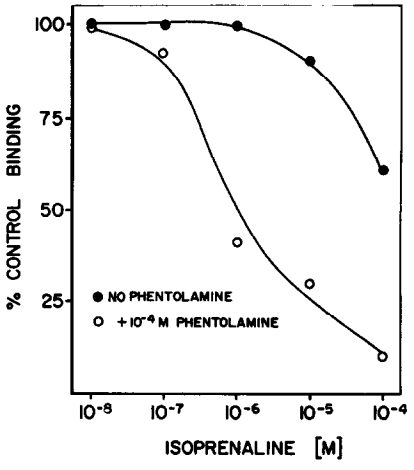

Fig. 3. Competition by isoprenaline for ^{125}I-iodohydroxybenzylpindolol binding sites in the presence and absence of phentolamine.

^{125}I-HYP bound at sites in artery membranes which were not β-receptors and much of this 'non-receptor' binding was displaced by low concentrations of propranolol (10^{-6} M). 'Specific' ^{125}I-HYP binding (defined as bound counts displaceable by 10^{-6} M propranolol) did not have the characteristics of β-receptor binding unless phentolamine was present in the binding tubes. In the absence of phentolamine, binding was not stereospecific and β-adrenergic agonists had very low affinity for the binding sites. This propranolol displaceable, non-receptor binding was eliminated by carrying out binding assays in the presence of 10^{-4} M phentolamine. The displacement of bound ^{125}I-HYP by isoprenaline in the presence and absence of 10^{-4} M phentolamine is shown in Fig. 3. In the absence of phentolamine, isoprenaline had low affinity for ^{125}I-HYP binding sites. But in the presence of phentolamine the affinity for isoprenaline was high, consistent with β-receptor binding. Similar requirement for phentolamine has been reported by Pochet and Schmitt [16] for β-receptor binding in muscle cell preparations and by Sporn and Molinoff [17] for β-receptor binding in brain membranes. Since high concentrations of phentolamine were required to block this 'non-receptor' binding (10^{-4}–10^{-5} M), it is unlikely that specific α-adrenergic blockade is involved.

Beta-adrenergic receptors in mesenteric arteries of doca–salt hypertensive rats. Systolic blood pressures and ventricular weights of doca–salt treated rats and control rats 4–6 weeks after surgery are shown in Table 2. Beta-receptor concentrations and affinities were measured in membranes prepared from mesenteric arteries from both groups of rats. For all determinations, concentration and affinity of receptors were measured by the Scatchard method [15].

Receptor concentration was lower in membranes prepared from doca–salt hypertensive rats but the affinities of the receptors were unchanged (Table 2).

Recently Limas and Limas [18] have shown that the aortae and the inferior vena cavae of spontaneously hypertensive rats contain reduced concentrations of β-adrenergic receptors. In agreement with these findings, we have shown that mesenteric arter-

Table 2. Blood pressure, heart weight and artery beta-receptor concentration in doca–salt hypertensive and control rats

	Doca–salt treated	Salt treated controls
Systolic blood pressure (mmHg)	173 ± 5.7*	134 ± 3.6
Heart/body weight (%)	0.42 ± 0.01*	0.32 ± 0.01
Mesenteric artery beta-receptor concentrations (fmoles/mg)	7.0 ± 1.2†	10.1 ± 1.5
Dissociation constant (nM)	0.10 ± 0.02‡	0.10 ± 0.02

* $P < 0.01$ compared to controls.

† $P < 0.0125$ compared to controls. Calculated using an unpaired Student's *t*-test.

‡ N.S.

ies of doca–salt hypertensive rats also contain fewer β-receptors than controls. Taken together, these results obtained with membranes from different blood vessels in spontaneously hypertensive rats and doca–salt hypertensive rats strongly suggest that reduced numbers of β-adrenergic receptors in the vasculature may be a common feature of experimental hypertension. Increased intensity of sympathetic stimulation can lead to a reduced responsiveness to catecholamines which is partly due to a reduced number of adrenergic receptors [19]. The reduction in arterial β-receptors in doca–salt hypertensive rats may be a reflection of increased sympathetic activity [20] in this model. The reduction in arterial β-receptors which mediate vasodilation may conceivably be partly responsible for the increased peripheral resistance characteristic of established hypertension.

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