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Subclassification of α_1-adrenoceptor recognition sites by urapidil derivatives and other selective antagonists

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1 The affinities of urapidil derivatives and other antagonists for α_1-adrenoceptors labelled by [^{3}H]-prazosin were determined on membranes of six different rat tissues.

2 Urapidil and its 5-acetyl-, 5-formyl- and 5-methyl-derivative displaced [^{3}H]-prazosin from α_1-adrenoceptor binding sites in a concentration-dependent manner which varied with tissue. IC_{50} values were lower in vas deferens, hippocampus and cerebral cortex than in heart, liver and spleen. For 5-methyl-urapidil, binding to two distinct sites could be demonstrated with mean K_I values of about 0.6 and 45 nM. Saturation binding studies with [^{3}H]-prazosin in the presence of 5-methyl-urapidil indicated a competitive type of interaction between 5-methyl-urapidil and [^{3}H]-prazosin.

3 The proportion of [^{3}H]-prazosin binding sites with high affinity for 5-methyl-urapidil was 58% in vas deferens, 69% in hippocampus, 41% in cerebral cortex and 23% in myocardium. In liver and spleen virtually no high affinity sites were found. These values were in good agreement with the percentages of binding sites with high affinities for WB-4101 and phentolamine, indicating that all these antagonists bind to the same subtype of α_1-recognition sites, whereas other α-antagonists like BE 2254, yohimbine and unlabelled prazosin did not discriminate between two binding sites.

4 Preincubating membranes of the cerebral cortex with chloroethylclonidine preferentially inactivated [^{3}H]-prazosin binding sites with low affinity for 5-methyl-urapidil.

5 The antagonist potencies of 5-methyl-urapidil and WB-4101 against α_1-adrenoceptor-mediated contractile responses were higher in vas deferens than in myocardium. The α_1-mediated effects in vas deferens but not in the heart were highly susceptible to nitrendipine.

6 Using 5-methyl-urapidil, the existence of two distinct α_1-adrenoceptor recognition sites could be demonstrated which correspond to the proposed α_{1A}- and α_{1B}-subtypes. Since 5-methyl-urapidil is one of the ligands with most selectivity between these subtypes in binding studies it may serve as a valuable tool for such investigations.

Introduction

Urapidil and some of its derivatives which are substituted at the 5-position of the uracil moiety (5-acetyl-, 5-formyl-, 5-methyl-urapidil, see Figure 1) lower blood pressure by a central nervous mechanism as well as by a peripheral action (Sanders & Jurna, 1985; Kolassa *et al.*, 1986; Ramage, 1986; 1988; Fozard & Mir, 1987; Gillis *et al.*, 1987; Doods *et al.*, 1988). The high affinity for 5-hydroxytryptamine (5-HT) receptors of the 5-HT_{1A} subtype and the intrinsic activity at these receptors seems to be responsible for the central component of the antihypertensive action whereas α_1-adrenolytic properties contribute to the vasodilator effect in the periphery (van Zwieten *et al.*, 1985; Ramage, 1986; 1988; Gillis *et al.*, 1987; Gross *et al.*, 1987).

In a preliminary paper, we were able to demonstrate that 5-methyl-urapidil inhibited [^{3}H]-prazosin binding to α_1-adrenoceptors of various rat tissues with two distinct affinity constants (Gross *et al.*, 1988a). Although there is increasing evidence from functional as well as from radioligand binding studies that α_1-adrenoceptors may be divided into subtypes this issue remains highly controversial (for reviews see McGrath, 1982; Hieble *et al.*, 1986; 1987; Morrow & Creese, 1986; McGrath & Wilson, 1988). Since the decision whether α_1-adrenoceptors are a homogeneous group of receptors or not is critically dependent on the availability of subtype-selective

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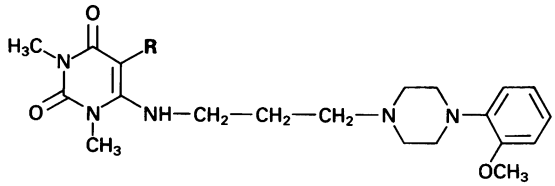

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—H	Urapidil
—CH_3	5-Methyl-urapidil
—C(=O)—H	5-Formyl-urapidil
—C(=O)—CH_3	5-Acetyl-urapidil

Figure 1 Chemical structures of urapidil and its derivatives substituted at the 5-position of the uracil moiety.

antagonists, we investigated whether 5-methyl-urapidil and other urapidil derivatives (Figure 1) discriminate between distinct α_1-adrenoceptor recognition sites and/or receptors and whether these sites can be reconciled with subdivisions of α_1-adrenoceptors proposed previously.

Methods

Male adult Wistar rats weighing 250 to 400 g were used for all experiments. Organs were removed immediately after the rats had been killed and used for radioligand binding and functional studies.

Radioligand binding

Hearts were perfused through the aorta with ice-cold saline and atria were cut off. After connective tissue had been removed the organs were blotted, weighed and homogenized twice for 15 s with an Ultra Turrax in 10 ml of ice-cold buffer (Tris HCl 50 mM, NaCl 100 mM, EDTA 2 mM, pH 7.4). The homogenates were filtered through 4 layers of gauze and centrifuged at 80,000 *g* for 20 min at 4°C. The pellets were resuspended in 8 ml of fresh buffer (Tris HCl 50 mM, EDTA 1 mM, pH 7.4), incubated for 10 min at 37°C, centrifuged again as described above and washed once more with 8 ml buffer.

[^{3}H]-prazosin binding was performed as described previously (Gross & Lues, 1985) with minor modifications. Membranes corresponding to 1.7 to 10 mg of initial wet weight, depending on the density of [^{3}H]-prazosin binding sites in the respective tissue, were incubated with 0.05 to 0.75 nM (saturation experiments) or 0.2 nM (competition experiments) [^{3}H]-prazosin in a final volume of 2 and 1 ml, respectively. The incubation buffer consisted of Tris HCl 50 mM, EDTA 1 mM, pH 7.4 as final concentrations. Incubations were carried out in duplicate or triplicate at 30°C and terminated after 45 min or 2 h (saturation experiments with 5-methyl-urapidil) by rapid filtration through Whatman GF/C filters using a Brandel M24R cell harvester. The filters were washed with 15 ml ice-cold buffer (Tris HCl 50 mM, pH 7.4) and subsequently dried at 100°C. Membrane-bound radioactivity retained on the filters was measured by liquid scintillation counting in a toluene/Triton x 100 mixture with an efficiency of 49%. Phentolamine (10 μM) was used to define non-specific binding which was usually 15% at 0.2 nM [^{3}H]-prazosin. The protein content of membrane suspensions was determined by the method of Lowry *et al.* (1951).

α_1-Adrenoceptor-mediated functional responses

The α_1-adrenoceptor-mediated inotropic response of the myocardium was studied as described previously (Gross *et al.*, 1988b). Strips of the right ventricle were tied to a tissue holder and mounted in an organ bath containing 20 ml of a modified Krebs-Henseleit solution saturated with 95%O_2:5%CO_2 (30°C); the solution had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 10. Propranolol (10 μM) was used to block completely any β-adrenoceptor-mediated response, oxaprotiline (0.3 μM) and corticosterone (40 μM) were added to inhibit neuronal and extraneuronal uptake, respectively. The preparations were stimulated electrically via platinum electrodes (1 Hz, 3 ms, amplitude 20% above threshold voltage). Contractions were registered with an isometric transducer (preload 9.8 mN) and recorded with a Hellige amplifier and recorder.

Contractions of vas deferens were measured under the same conditions as described above for the positive inotropic response of heart ventricles except that the contractions were recorded without electrical stimulation; the resting tension was 4.9 mN.

Cumulative concentration-response curves in the heart and single concentration-response curves in vas deferens were constructed after 40 min of equilibration. 5-Methyl-urapidil and WB-4101 were added 30 min before the agonist.

Calculations

All values are expressed as mean ± s.e.mean of *n* independent experiments. IC_{50} values and pseudo Hill coefficients (n_H) were calculated as described by

McPherson (1983). Two-site analysis of the data was performed as described by Barlow (1983), F-test analysis was used to decide whether a one- or a two-site model was more appropriate ($P < 0.05$). IC_{50} values were transformed into K_I values by the method of Cheng & Prusoff (1973). The significance of difference between two means was assessed by use of a two-tailed unpaired Student's t test. ANOVA and Duncan's multiple range test were used for the comparison of more than two means. pA_2-values were calculated by linear regression analysis according to the method of Arunlakshana & Schild (1959).

Drugs

The following drugs were used: [^{3}H]-prazosin (specific activity 82 Ci mmol^{-1}, NEN, Boston, U.S.A.), 5-methyl-, 5-acetyl- and 5-formyl-urapidil (synthesized by Dr W. Prüsse, Konstanz, F.R.G.), (−)-adrenaline base (Hoechst, Frankfurt, F.R.G.), BE 2254 (2[β-(4-hydroxyphenyl)-ethyl-aminomethyl]tetralone hydrochloride, Beiersdorf, Hamburg, F.R.G.), chloroethylclonidine (Research Biochemicals Inc., Natick, U.S.A.), corticosterone (Sigma, Munich, F.R.G.), nitrendipine (Bayer, Leverkusen, F.R.G.), (+)-oxaprotiline hydrochloride (Ciba-Geigy, Basle, Switzerland), phentolamine hydrochloride (Ciba-Geigy, Basle, Switzerland), prazosin hydrochloride (Pfizer, Karlsruhe, F.R.G.), (±)-propranolol hydrochloride (Sigma, Munich, F.R.G.), urapidil (Byk Gulden Pharmazeutika, Konstanz, F.R.G.), WB-4101 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4 benzodioxane hydrochloride. (Research Biochemicals Inc., Natick, U.S.A.), yohimbine hydrochloride (Sigma, Munich, F.R.G.).

Results

Radioligand binding studies

[^{3}H]-prazosin in concentrations ranging from 0.01 to 0.75 nM was used to label α_1-adrenoceptors in crude particulate membrane preparations of six rat tissues. Binding of [^{3}H]-prazosin to 5-hydroxytryptamine receptors could be excluded, since high concentrations of 5-hydroxytryptamine and 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetraline, up to 0.3 μM) did not displace [^{3}H]-prazosin (data not shown). Scatchard transformation of saturation binding data resulted in linear plots and pseudo Hill coefficients (n_H) not significantly different from unity ($P > 0.05$) suggesting a single population of binding sites in all tissues investigated (Table 1, see also Figure 4). However, equilibrium dissociation constants (expressed as pK_D, −log M) differed slightly between the tissues. In liver, spleen and heart

Table 1 Binding of [^{3}H]-prazosin to α_1-adrenoceptors of various rat tissues

	n	pK_D	n_H	B_{max}
Cerebral cortex	3	10.4 ± 0.03	1.09	11.4 ± 0.3
Heart	3	10.6 ± 0.03	1.05	5.9 ± 0.3
Hippocampus	4	10.2 ± 0.05	1.08	5.6 ± 0.1
Liver	5	10.7 ± 0.04	1.13	11.5 ± 0.6
Spleen	3	10.6 ± 0.12	0.96	2.7 ± 0.1
Vas deferens	3	10.2 ± 0.03	1.07	8.4 ± 0.9

B_{max} (maximal number of binding sites in fmol per mg initial wet weight) and pK_D values (−log equilibrium dissociation constants, −log M) were calculated by linear regression analysis after transformation of the data according to Scatchard (1949). n_H = Hill coefficient. Values are means ± s.e.mean of n experiments.

mean pK_D values of 10.6 and 10.7 were measured, whereas pK_D values of 10.2 were found in vas deferens and hippocampus.

As shown in Figure 2, significantly lower concentrations ($P < 0.01$, $n = 6$) of 5-methyl-urapidil are required for half-maximum inhibition of [^{3}H]-prazosin binding in vas deferens and hippocampus as compared to liver and spleen membranes. Similar results were obtained when inhibition experiments with 5-methyl-urapidil were carried out in a buffer containing NaCl 160 mM, KCl 4.6 mM, $MgCl_2$ 1 mM, $CaCl_2$ 2.5 mM, Tris HCl 5 mM, glucose 10 mM as final

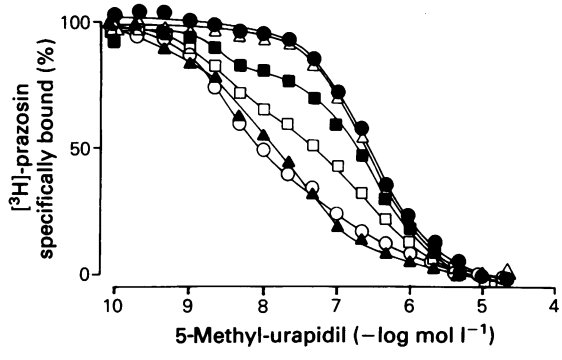

Figure 2 Inhibition of specific [^{3}H]-prazosin binding to α_1-adrenoceptors in various tissues of the rat by 5-methyl-urapidil. Crude membrane preparations of vas deferens (▲), hippocampus (○), cerebral cortex (□), heart ventricles (■), spleen (●) and liver (△) were incubated with 0.2 nM [^{3}H]-prazosin and increasing concentrations of 5-methyl-urapidil at 30°C for 45 min. Non-specific binding was determined in the presence of 10 μM phentolamine and subtracted from total binding. Values given represent means of at least 6 experiments, s.e.means were less than 4%.

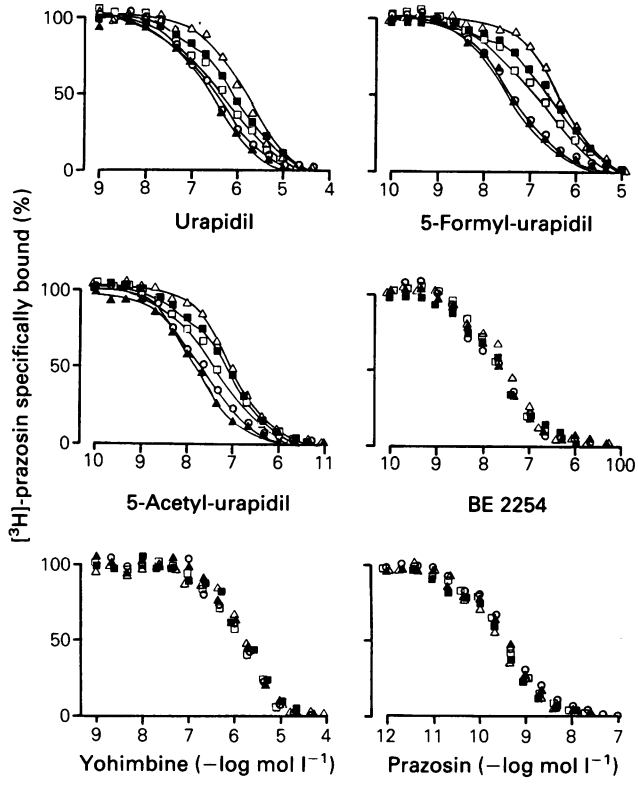

Figure 3 Inhibition of specific [^{3}H]-prazosin binding to α_1-adrenoceptors by various antagonists. Crude membrane preparations of vas deferens (▲), hippocampus (○), cerebral cortex (□), heart ventricles (■) and liver (△) were incubated with 0.2 nM [^{3}H]-prazosin and increasing concentrations of antagonists at 30°C for 45 min. Non-specific binding was determined in the presence of 10 μM phentolamine and subtracted from total binding. Values given represent means of at least 3 experiments, s.e.means were less than 6%.

concentrations, pH 7.4 (data not shown) instead of the routinely used Tris/EDTA buffer. The displacement of [^{3}H]-prazosin by 5-methyl-urapidil in cortical and myocardial membranes was not influenced by high concentrations of guanosine triphosphate (GTP, 1 mM, data not shown), whereas agonist binding (adrenaline) was clearly affected by GTP.

A tissue-specific displacement of the radioligand was also found for urapidil and its 5-acetyl- and 5-formyl-derivative (Figure 3) as well as for WB-4101 and phentolamine (Table 2). The order in which [^{3}H]-prazosin binding was inhibited in the different tissues was similar for all these compounds. The lowest IC_{50} values were always found in vas deferens and hippocampus, intermediate values in cerebral cortex and heart and the highest IC_{50} values in liver. All compounds tested inhibited [^{3}H]-prazosin binding to liver membranes with pseudo Hill coefficients (n_H) not different from unity whereas n_H values smaller than 1 were measured in the other tissues for those α_1-antagonists which discriminated most clearly between two [^{3}H]-prazosin binding sites (5-methyl-urapidil, WB-4101 and phentolamine, see Table 2).

In contrast to these compounds, unlabelled prazosin, BE 2254 and yohimbine inhibited [^{3}H]-prazosin binding to membranes of different tissues in a monophasic manner (n_H not significantly different from 1) with similar K_I values (Figure 3). Computerized analysis of the binding data revealed that 5-methyl-urapidil, WB-4101 and phentolamine bound to two distinct sites in vas deferens, hippocampus, cerebral cortex and heart but to only one site in liver and spleen (Table 2). pK_I values for the low affinity sites of tissues containing two populations of [^{3}H]-prazosin binding sites were in the same range as pK_I

Table 2 Inhibition of [^{3}H]-prazosin binding to α_1-adrenoceptors of various tissues by subtype-selective α_1-antagonists

	Tissue	n	n_H	$pK_{I\ high}$	$pK_{I\ low}$	*% high*
5-Methyl-urapidil	Vas deferens	6	0.67 ± 0.09	9.21 ± 0.21	7.55 ± 0.19	57.54 ± 7.19
	Hippocampus	6	0.53 ± 0.02	9.00 ± 0.08	7.08 ± 0.07	68.54 ± 2.44
	Cortex	20	0.57 ± 0.01	9.27 ± 0.04	7.33 ± 0.02	41.20 ± 1.52
	Heart	18	0.77 ± 0.03	9.24 ± 0.08	7.41 ± 0.02	22.82 ± 1.32
	Spleen	6	0.97 ± 0.03	—	7.53 ± 0.07	2.8 ± 2.8
	Liver	16	1.01 ± 0.02	—	7.63 ± 0.03	—
WB-4101	Vas deferens	5	0.93 ± 0.31	9.82 ± 0.05	8.89 ± 0.13	89.31 ± 9.86
	Hippocampus	6	0.65 ± 0.03	9.85 ± 0.04	8.95 ± 0.08	75.85 ± 4.10
	Cortex	6	0.66 ± 0.01	9.99 ± 0.11	8.51 ± 0.05	44.27 ± 1.30
	Heart	6	0.85 ± 0.03	10.31 ± 0.24	8.89 ± 0.05	22.28 ± 1.93
	Liver	7	1.02 ± 0.02	—	8.85 ± 0.04	—
Phentolamine	Vas deferens	3	0.75 ± 0.02	8.92 ± 0.01	7.69 ± 0.03	59.79 ± 2.51
	Hippocampus	3	0.64 ± 0.03	8.86 ± 0.49	7.19 ± 0.31	62.65 ± 8.92
	Cortex	3	0.70 ± 0.02	9.05 ± 0.02	7.65 ± 0.02	41.88 ± 1.51
	Heart	3	0.87 ± 0.02	9.26 ± 0.28	7.76 ± 0.00	17.57 ± 2.75
	Liver	3	0.93 ± 0.01	—	7.81 ± 0.02	—

$pK_I = -\log K_I$ calculated from IC_{50} values according to Cheng & Prusoff (1973). n_H = Hill coefficient. Values are means ± s.e.mean of *n* experiments.

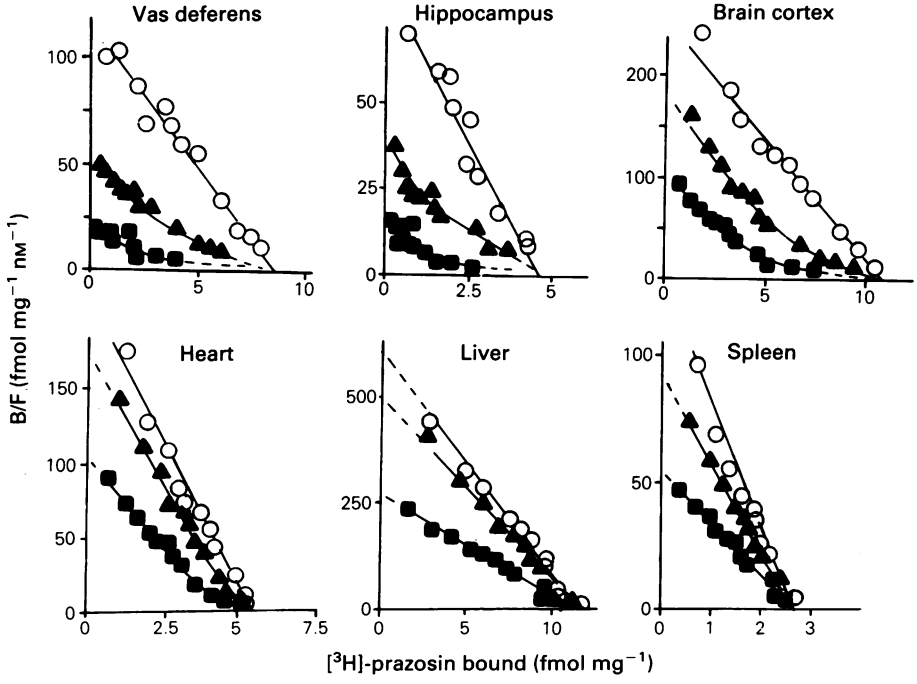

Figure 4 Binding of [^{3}H]-prazosin to α_1-adrenoceptors of various rat tissues in the absence (○) and in the presence of 5-methyl-urapidil, 3 (▲) and 30 nM (■). Membranes were incubated with increasing concentrations of [^{3}H]-prazosin ranging from 0.01 to 0.75 nM for 2 h at 30°C. Non-specific binding was determined in the presence of 10 μM phentolamine and subtracted from total binding. Results are presented as Scatchard plots. Values represent means of at least 3 experiments, s.e.means were less than 3%.

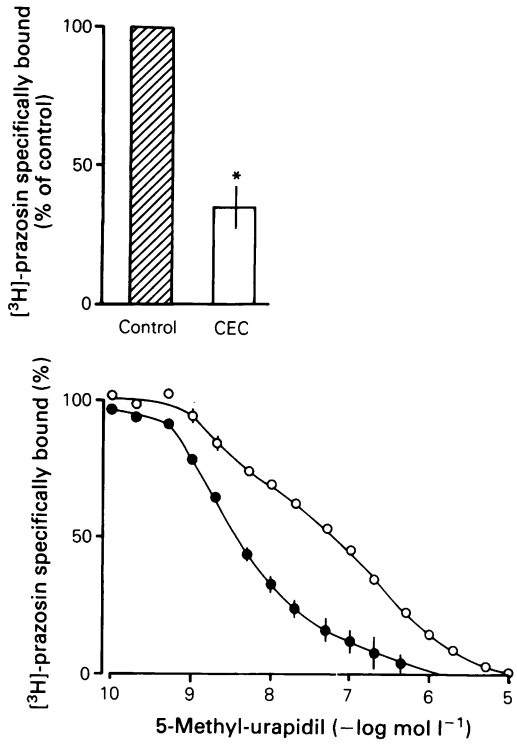

Figure 5 Effect of chloroethylclonidine (CEC) on the inhibition of [^{3}H]-prazosin binding by 5-methyl-urapidil. Membrane suspensions of the cerebral cortex were incubated without (○) and with 10 μM CEC (●) at 37°C. After 30 min the membrane suspensions were centrifuged and washed twice. CEC pretreatment caused a 63% loss of [^{3}H]-prazosin binding. Hill coefficients for the displacement of the radioligand by 5-methyl-urapidil were increased from 0.55 ± 0.01 (controls) to 0.76 ± 0.10 (CEC treatment, $P < 0.01$). Values represent means of 4 experiments with s.e.mean shown by vertical lines. * $P < 0.01$ compared to controls.

values for the respective single sites in liver and spleen. Of all compounds investigated 5-methyl-urapidil discriminated most clearly between these two populations of [^{3}H]-prazosin binding sites.

In saturation binding studies with [^{3}H]-prazosin, addition of 5-methyl-urapidil (3 and 30 nM) resulted in an apparent increase in K_D values in liver and spleen, the Scatchard plots remained linear. In those tissues, however, in which a major proportion of binding sites with high affinity for 5-methyl-urapidil had been found, the Scatchard plots became curvilinear (Figure 4).

Preincubation of cortical membranes with 10 μM chloroethylclonidine (Minneman *et al.*, 1988) for 30 min resulted in a 63% loss of specific [^{3}H]-prazosin binding ($P < 0.01$, $n = 4$). In membranes not treated with chloroethylclonidine, 5-methyl-urapidil displaced the radioligand with a shallow inhibition curve (n_H 0.55 ± 0.01, $51 \pm 2\%$ low affinity sites). After chloroethylclonidine treatment the inhibition curve became significantly steeper (n_H 0.76 ± 0.10, $P < 0.01$) and the proportion of sites with low affinity decreased ($12 \pm 1\%$, $P < 0.01$, see Figure 5).

Functional studies

Electrical stimulation of right heart ventricles produced a basal force of contraction of 10.6 ± 0.8 mN ($n = 18$). Adrenaline (0.01 to 100 μM) in the presence of 10 μM propranolol caused a positive inotropic effect of 7.3 ± 0.7 mN by stimulating α_1-adrenoceptors. The $-\log EC_{50}$ value was 6.46 ± 0.06. Preliminary experiments showed that this high concentration of propranolol was sufficient to suppress a β-adrenoceptor-mediated response even at the highest adrenaline concentration used (Gross *et al.*, 1988b). 5-Methyl-urapidil (30 to 300 nM) and WB-4101 (10 to 100 nM) caused a parallel shift to the right of the concentration-response curve. The maximum increase in tension due to α_1-adrenoceptor activation was not significantly affected by 5-methyl-urapidil in concentrations up to 0.3 μM ($n = 4$) or WB-4101 0.1 μM ($n = 6$). pA_2 values calculated from Arunlakshana & Schild plots were 7.65 (slope -1.04, $r = 1.00$) and 8.21 (slope -0.97, $r = 0.99$) for 5-methyl-urapidil and WB-4101, respectively (Figure 6).

The dihydropyridine Ca^{2+}-channel blocker, nitrendipine, at a concentration of 0.1 μM had no influence on the α_1-adrenoceptor mediated positive inotropic effect (Figure 7).

In vas deferens, α_1-adrenoceptor activation by adrenaline (0.03 to 100 μM) resulted in a maximum increase by 19.4 ± 1.7 mN ($n = 8$) with a $-\log EC_{50}$ value of 6.67 ± 0.08. The shift of concentration-response curves to the right produced by 5-methyl-urapidil (10 to 100 nM) and WB-4101 (1 to 30 nM) was significantly greater in vas deferens as compared to heart ventricles. pA_2 values of 8.47 (slope -1.04, $r = 0.98$) and 9.01 (slope -1.33, $r = 1.00$) were calculated for 5-methyl-urapidil and WB-4101, respectively (Figure 6). Both antagonists at the highest concentrations used had no significant effect ($P > 0.05$) on the maximum increase in tension.

In contrast to the α_1-mediated positive inotropic effect in myocardium, the α_1-adrenoceptor response in vas deferens was clearly affected by nitrendipine (Figure 7).

In both tissues investigated, 5-methyl-urapidil in concentrations of up to 1 μM had no intrinsic activity and can thus be considered as a pure antagonist.

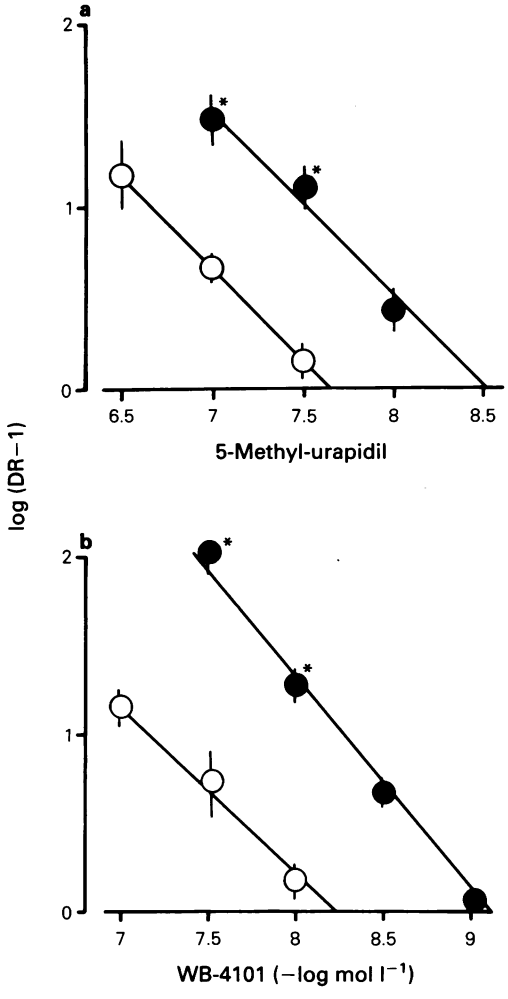

Figure 6 Concentration-dependent antagonism of the α_1-adrenoceptor-mediated positive inotropic response of right heart ventricles (○) and of the contractile response of rat vas deferens (●). Arunlakshana-Schild plots for 5-methyl-urapidil (a) and WB-4101 (b). Adrenaline (0.01 to 100 μM) in the presence of propranolol (10 μM) was used as α_1-adrenoceptor agonist. Neuronal and extraneuronal catecholamine uptake were blocked by oxaprotiline and corticosterone, respectively. Least squares fit to data reveal the following pA_2 values: pA_2 for 5-methyl-urapidil 7.65 in the heart and 8.47 in vas deferens, slopes −1.0. pA_2 values for WB-4101: 8.21 in the heart and 9.01 in vas deferens, slopes −1.0 and −1.3, respectively. Values given represent means (with s.e.mean shown by vertical lines) of at least 4 experiments for each antagonist concentration. * $P < 0.01$.

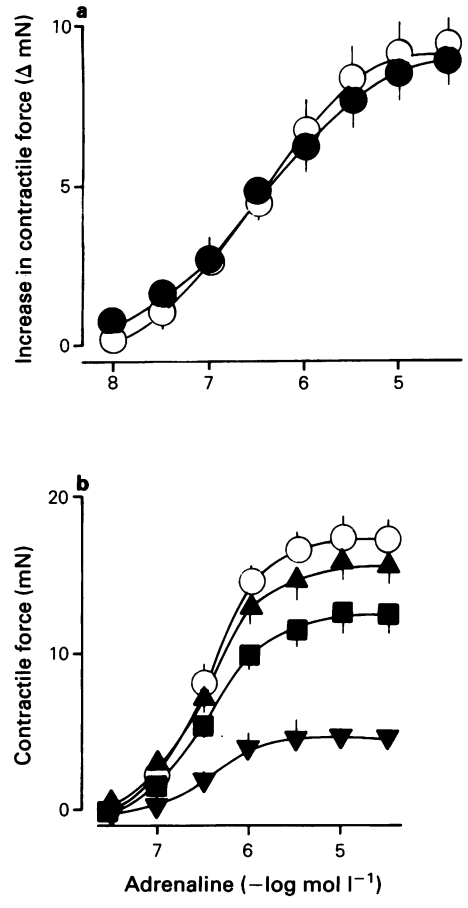

Figure 7 Effect of nitrendipine on α_1-adreno- ceptor-mediated functional responses. The positive inotropic effect in right heart ventricles (a) and the contractile response of vas deferens (b) caused by adrenaline in the presence of 10 μM propranolol was measured in the absence (○) and in the presence of nitrendipine 3 (▲), 10 (■), 30 (▼) and 100 (●) nM. Neuronal and extraneuronal catecholamine uptake were blocked by oxaprotiline and corticosterone, respectively. Values represent means of 6 experiments with s.e.means shown by vertical lines.

Discussion

In a preliminary paper (Gross *et al.*, 1988a) and in the present study we have demonstrated that 5-methyl-urapidil displaced the α_1-adrenoceptor ligand [^{3}H]-prazosin from its binding sites on membranes of six rat tissues with different affinity constants (K_I). Inhibition curves were shallow or clearly biphasic in vas deferens, hippocampus, cerebral cortex and heart

but monophasic in liver and spleen. Two-site analysis of the data strongly suggests the existence of two distinct binding sites with affinities of about 0.6 nM and 45 nM. Liver and spleen membranes seem to contain only the site with low affinity for 5-methyl-urapidil, whereas both high and low affinity sites were found in the other tissues investigated. These two binding sites in vas deferens, hippocampus, cortex and myocardium could also be detected in saturation experiments: the linear Scatchard plots of [^{3}H]-prazosin binding became curvilinear after addition of 5-methyl-urapidil, confirming the existence of two binding sites. These sites were labelled by [^{3}H]-prazosin with similar or equal affinities. In liver and spleen, addition of 5-methyl-urapidil resulted in an apparent decrease in K_D values but Scatchard plots remained linear. Unaltered B_{max} values indicated a competitive type of interaction between 5-methyl-urapidil and [^{3}H]-prazosin.

Among a series of urapidil derivatives that all inhibited [^{3}H]-prazosin binding in a tissue-dependent manner, 5-methyl-urapidil most clearly discriminated between two classes of α_1-recognition sites. The order of selectivity was: 5-methyl-urapidil > 5-formyl-urapidil ≥ 5-acetyl-urapidil > urapidil. On the other hand, several well-known α-adrenoceptor antagonists like BE 2254, yohimbine and unlabelled prazosin did not recognize distinct α_1-binding sites.

During recent years, several investigators suggested that α_1-adrenoceptors may be divided into subclasses. These attempts were based on functional data, e.g. different potencies of agonists and antagonists in various tissues and species, on differences in Ca^{2+} utilization (for reviews see Hieble *et al.*, 1986; 1987; McGrath & Wilson, 1988), on the possibility that α_1-adrenoceptors may be coupled to different second messenger pathways (García-Sainz & Hernandez-Sotomayor, 1985; Johnson & Minneman, 1986; Han *et al.*, 1987a,b) and on radioligand binding studies (Morrow *et al.*, 1985, Morrow & Creese, 1986, Han *et al.*, 1987a,b; Minneman *et al.*, 1988). However, as delineated above (see Introduction), these issues remained highly controversial and there is still no unequivocal classification. Our data presented above clearly support the findings of Morrow *et al.* (1985), Morrow & Creese (1986) and Minneman and coworkers (Han *et al.*, 1987a,b; Minneman *et al.*, 1988). Both groups found that WB-4101 and phentolamine bind to α_1-adrenoceptors in cerebral as well as in other tissues with two different affinities. The binding sites with high affinity for WB-4101 and phentolamine were designated α_{1A} (or α_{1a}), the sites with low affinity α_{1B} (or α_{1b}). Using [^{3}H]-prazosin instead of [^{125}I]BE 2254 as radioligand, we could confirm the results of these investigators. In our hands, WB-4101 as well as phentolamine proved to be selective for subtypes of α_1-recognition sites. Several lines of evidence indicate that 5-methyl-urapidil binds preferentially to α_1-sites with high affinity for WB-4101 and phentolamine and can thus be considered as a selective α_{1A}-ligand: (1) The order in which these chemically different antagonists displaced [^{3}H]-prazosin binding was similar. (2) The percentages of high- and low-affinity sites in various tissues as determined by the use of these different antagonists were in good agreement. (3) Chloroethylclonidine which has been reported to inhibit irreversibly [^{125}I]-BE 2254 binding sites with low affinity for WB-4101 (Minneman *et al.*, 1988) inactivated [^{3}H]-prazosin binding sites with low affinity for 5-methyl-urapidil as well, leaving most of the high affinity sites unaffected. Thus, our experiments with 5-methyl-urapidil support the assumption that two pharmacologically distinct α_1-binding sites exist. However, the approximately 70 fold selectivity of this new compound for α_{1A}-recognition sites clearly exceeds that of WB-4101 and phentolamine (20 to 30 fold). Therefore, 5-methyl-urapidil may serve as a valuable tool for further functional investigations of these subtypes.

Another attempt to subclassify α_1-adrenoceptors is based on the largely variable potencies of prazosin in functional experiments (Flavahan & Vanhoutte, 1986; Hieble *et al.*, 1986). In our experiments, however, K_D values for [^{3}H]-prazosin binding to various tissues differed by a ratio of about 3 or less. The fact that [^{3}H]-prazosin had the highest affinity for α_1-adrenoceptors in those organs with a high proportion of α_{1B}-sites may indicate that [^{3}H]-prazosin itself is slightly subtype-selective, but this selectivity is obviously too small to be detected by two-site analysis. However, the existence of a further α_1-recognition site with much lower affinity for [^{3}H]-prazosin cannot be excluded definitively since we used only radioligand concentrations up to 0.75 nM in our experiments.

The demonstrations of two distinct α_1-adrenoceptor recognition sites raises the question whether both sites are functionally relevant. In order to answer this question we chose two organs with a different content of α_{1A} and α_{1B}-sites in which contractile responses could be easily measured. In vas deferens at least 58% of [^{3}H]-prazosin binding sites belonged to the α_{1A}-type but in the heart only about 20%. The antagonist potencies of 5-methyl-urapidil and WB-4101 against the α_1-mediated contractile or inotropic response were about ten fold higher on vas deferens as compared to right heart ventricle, suggesting that both α_1-subtypes may contribute to functional responses. In the heart, pA_2 values of both antagonists were in good agreement with pK_I values for the α_{1B} site as determined by radioligand binding.

However, a contribution of the small amount of α_{1A}-binding sites may remain undetected by Schild analysis (Milnor, 1986). In vas deferens, on the other hand, pA_2 values intermediate between K_I values of the α_{1A} and α_{1B} sites were measured. These results may be due to a possible functional involvement of both subtypes on this tissue.

It has been suggested that α_{1A}-binding sites mediate contractions of smooth muscle which require the influx of extracellular Ca^{2+} through dihydropyridine-sensitive channels whereas activation of α_{1B}-sites stimulates inositol phosphate formation independent of extracellular Ca^{2+} (Han *et al.*, 1987a). Our experiments on heart muscle and vas deferens are in agreement with this assumption. The α_1-mediated contraction of the vas deferens, a tissue in which α_{1A}-binding sites prevail, was highly susceptible to the Ca^{2+}-channel blocker, nitrendipine. In contrast, the inotropic effect of the myocardium with 80% α_{1B}-sites was not at all inhibited by high concentrations of nitrendipine. In our opinion, however, these results are not sufficient to prove definitely that the α_{1A} and the α_{1B} subtype are linked to different second messenger pathways. Additional investigations on other tissues are clearly needed.

In conclusion, our results demonstrate that two distinct α_1-adrenoceptor binding sites can be distinguished in rat tissues by radioligand binding techniques. It remains to be established whether the same sites can be identified in other species and in man. For an unequivocal subclassification of α_1-adrenoceptors, however, further studies are needed to characterize better the biochemical and functional events mediated by these recognition sites.

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