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Mediation of noradrenaline-induced contractions of rat aorta by the α_{1B}-adrenoceptor subtype

[1]R. Testa, L. Guarneri, E. Poggesi, I. Simonazzi, C. Taddei & A. Leonardi

Research and Development Division, Recordati S.p.A., 20148 Milan, Italy

1 The subtypes of α_1-adrenoceptor mediating contractions to exogenous noradrenaline (NA) in rat aorta have been examined in both biochemical and functional studies.

2 Incubation of rat aortic membranes with the irreversible α_{1B}-adrenoceptor antagonist, chloroethylclonidine (CEC: 10 μM) did not change the K_D of [^{3}H]-prazosin binding in comparison to untreated membranes, but reduced by 88% the total number of binding sites (B_{max}).

3 Contractions of rat aortic strips to NA after CEC (50 μM for 30 min) incubation followed by repetitive washing, showed a marked shift in the potency of NA and a partial reduction in the maximum response. The residual contractions to NA after CEC incubation were not affected by prazosin (10 nM).

4 The competitive antagonists prazosin, terazosin, (R)-YM-12617, phentolamine, 5-methylurapidil and spiperone inhibited contractions to NA with estimated pA_2 values of 9.85, 8.54, 9.34, 7.71, 7.64 and 8.41, respectively.

5 The affinity of the same antagonists for the α_{1A}- and α_{1B}- adrenoceptors was evaluated by utilizing membranes from rat hippocampus pretreated with CEC, and rat liver, respectively. 5-Methylurapidil and phentolamine were confirmed as selective for the α_{1A}-adrenoceptors, whereas spiperone was α_{1B}-selective.

6 A significant correlation was found between the pA_2 values of the α_1-adrenoceptor antagonists tested and their affinity for the α_{1B}-adrenoceptor subtype, but not for the α_{1A}-subtype.

7 In conclusion, these findings indicate that in rat aorta most of the contraction is mediated by α_{1B}-adrenoceptors, and that the potency (pA_2) of an antagonist in this tissue should be related to its antagonistic effect on this subtype of the α_1-adrenoceptor population.

Keywords: Rat aorta; α_{1A}-adrenoceptors; α_{1B}-adrenoceptors; prazosin; terazosin; (R)-YM-12617; phentolamine; 5-methylurapidil; spiperone

Introduction

Since the subdivision of α_1-adrenoceptors into α_{1A}- and α_{1B}-subtypes (Morrow & Creese, 1986; Han *et al.*, 1987a,b), the subtype(s) of α_1-adrenoceptor present in rat aorta have been variously classified as α_{1B} (Han *et al.*, 1990; Eltze & Boer, 1992) both α_{1A} and α_{1B} (Tian *et al.*, 1990) or atypical (Muramatsu *et al.*, 1991; Oriowo *et al.*, 1992).

Recently, Aboud *et al.* (1993) stated that contractions of rat aorta induced by noradrenaline (NA) are mediated by non-α_{1A}, non α_{1B}-adrenoceptors, due both to the high potency of the α_{1A}-selective antagonists and the sensitivity to the selective α_{1B}-adrenoceptor alkylating agent, chloroethylclonidine (CEC) (Han *et al.*, 1987a).

This paper describes our attempts to characterize the α-adrenoceptor(s) in rat aorta by use of CEC both in biochemical and functional studies. In addition, since no absolutely subtype-selective antagonists are available, the potency of several α_1-adrenoceptor antagonists on rat aorta was also evaluated and compared to their affinity for the α_{1A}- and α_{1B}-adrenoceptor subtypes determined in rat hippocampus pretreated with CEC and rat liver, respectively. Some of the affinities of the antagonists for the α_1-adrenoceptor subtypes were previously published by our group (Testa *et al.*, 1993; Taddei *et al.*, 1993).

Methods

Male Sprague Dawley rats (300–450 g) were obtained from Charles River Italia.

[1] Author for correspondence.

Radioligand binding in rat aortic membranes

Preparation of membranes Rats were killed by cervical dislocation and the thoracic aorta dissected, washed in saline and homogenized in 50 vols of 50 mM Tris-HCl buffer (pH 7.4) in a Polytron (speed 7, 2 × 20 s). The homogenate obtained was filtered through four layers of cheesecloth, divided in two parts and centrifuged at 49000 *g* for 10 min. The pellets were resuspended in 50 vol of the ice-cold buffer, incubated for 30 min at 37°C and recentrifuged at 49000 *g* for 10 min. When the effects of irreversible receptor inactivation were studied, the buffer contained CEC (10 μM). The pellets were washed once more in drug-free ice-cold buffer and finally suspended in 160 vol of Tris-HCl.

[^{3}H]-prazosin binding studies Aliquots (2 ml) of aortic membranes were incubated for 30 min at 25°C with different concentrations of [^{3}H]-prazosin ranging from 0.05 to 8 nM. Non-specific binding was determined in the presence of 10 μM phentolamine. Incubations were terminated by vacuum filtration over 0.2% polyethyleneimine pretreated Whatman GF/B fibre filters using a Brandel cell harvester. The filters were then washed with 3 × 3 ml of ice-cold buffer and the radioactivity retained on the filters was counted in 10 ml of Filter Count (Packard) in a liquid scintillation spectrometer (counting efficiency of 40%).

Functional studies using rat aortic strips

Spiral strips were prepared from each artery (1 strip per aorta, fragments about 3 cm long were used) and suspended in 20 ml organ baths containing Krebs bicarbonate buffer of

the following composition (mM): NaCl 112.0, KCl 5.0, $CaCl_2$ 2.5, KH_2PO_4 1.0, $MgSO_4$ 1.2, $NaHCO_3$ 12.0 and glucose 11.1, equilibrated at 37°C with 95% O_2: 5% CO_2 and containing desmethylimipramine (0.1 μM) and corticosterone (1 μM) to block neuronal and extraneuronal uptake of NA, (±)-propranolol (1 μM) to block β-adrenoceptors and yohimbine (0.1 μM) to block α_2-adrenoceptors.

Tissues were subject to a passive load of 1.5 g and change in length measured with isotonic transducers (Basile 7006).

Tissues were allowed to equilibrate for 60 min and primed twice with 10 μM NA. After washing, the preparations were equilibrated for a further 60 min and a cumulative concentration-response curve to NA generated (generally from 1 nM to 100 μM). Following washout of NA and re-equilibration of the tissue (45 min), the drug to be tested was added and after 30 min, a second NA cumulative concentration-response curve constructed.

When CEC was the test-drug, following the 30 min incubation with the alkylating agent, the tissues were washed extensively three times (in 0.5 h) before constructing the second NA concentration-response curve. In order to evaluate the effects of prazosin in tissues previously treated with CEC, vascular preparations, subjected to the same protocol as above and after developing the second NA concentration-response curve, were washed with drug-free Krebs solution. After re-equilibration (45 min) the tissues were further incubated for 30 min with the antagonist before generating a third NA concentration-response curve. Proper control preparations treated with CEC and following vehicle incubation were prepared to verify any time-dependent change of NA response.

Vascular endothelium was not specifically removed; however, no particular care was taken to avoid stretching or damaging the luminal surface of the vessels, or to preserve its integrity.

Affinity for the α_{1A}- and α_{1B}-adrenoceptor subtypes

The affinity of different antagonists for the α_{1A}- and α_{1B}-adrenoceptor subtypes was evaluated by studying their ability to displace specific [^{3}H]-prazosin binding from membranes of rat hippocampus pretreated with CEC (α_{1A}) and rat liver (α_{1B}) according to the method of Morrow & Creese (1986) and Taddei *et al.* (1993), respectively.

Data analysis

Values are arithmetic means ± s.e.mean. Saturation binding data were obtained from pools of rat thoracic aortae, and the experiments were conducted on four different pools of tissue.

The saturation curves (Scatchard analysis) were analyzed according to the method of Munson & Rodbard (1980), using the non-linear curve-fitting programme LIGAND (from N.I.H.) to determine the apparent dissociation constant (K_D) and maximum number of binding sites (B_{max}) for [^{3}H]-prazosin.

The displacement curves of the antagonists on the α_{1A}- and α_{1B}- adrenoceptor subtypes were analysed by non-linear curve fitting of the logistic equation according to the method reported by De Lean *et al.* (1978), utilizing the ALLFIT programme (from N.I.H.). The IC_{50} values and pseudo-Hill slope coefficients were estimated by the programme. The value for the inhibition constant, K_i, was calculated by use of the Cheng & Prusoff (1973) equation:

$$K_i = IC_{50}/(1 + L/K_D),$$

where L is the concentration of [^{3}H]-prazosin used.

In the isolated vascular preparations, 5–10 preparations were used when the effects of CEC were studied, and at least 2 preparations were taken from different animals for each concentration of the other antagonists tested. Only one antagonist concentration was used for each preparation.

The concentration-response curves (before and after antagonist incubation) were analysed by non-linear curve fitting of the logistic equation as reported for the binding studies. The statistical significance of the differences between the parameters of the control concentration-response curve and the curve obtained after drug incubation (EC_{50}, slope, E_{max}), was examined by simultaneous fitting with the considered parameter shared as reported by De Lean *et al.* (1978).

The dissociation constant of the agonist and the fraction of receptors inactivated by incubation with CEC were evaluated according to Furchgott & Bursztyn (1967).

Schild-plot parameters (pA_2 and slope) were evaluated by linear regression analysis according to Tallarida & Murray (1987). The dose-ratios (DRs) (EC_{50} ratios of NA) observed after incubation with the different concentrations of the antagonists were always corrected for time-dependent changes in first and second curve locations, determined in vehicle-treated time-matched control curves. Identity of the experimentally derived DRs with a Schild plot slope of unity was assessed by the *F* test for goodness of fit, according to Munson & Rodbard (1980).

Correlation analysis between the functional activity of the antagonists in rat aortic strips and their binding affinities at α_1-adrenoceptor subtypes was assessed by least-squares linear regression.

Drugs

The following drugs were used: [^{3}H]-prazosin (7-methoxy-^{3}H: specific activity 76.2 Ci mmol^{-1}, batch 2825-078, NEN Research Product, Dupont de Nemours Italiana); chloroethylclonidine 2HCl, spiperone HCl, desmethylimipramine HCl, 5-methylurapidil, (Research Biochemical Incorporated, Natick, U.S.A.); pargyline HCl, (−)-noradrenaline $C_4H_6O_6$, prazosin HCl, phentolamine-HCl, corticosterone, (±)-propranolol HCl, yohimbine HCl, (Sigma-Aldrich, Italy); nicardipine HCl, and (R)-YM-12617 HCl (tamsulosin) were synthesized in Recordati laboratories.

In binding studies, the compounds were dissolved in absolute ethanol. For the isolated vascular preparations, CEC, prazosin, terazosin, and phentolamine were dissolved in distilled water; nicardipine, 5-methylurapidil, and spiperone were dissolved in aqueous N,N-dimethylformamide and Tween 80 (both 1% v/v final concentration) as 1 mM stock solutions and further diluted as necessary with distilled water; (R)-YM-12617 was dissolved in dimethylsulphoxide and water (1:1) at 1 mM, and further diluted as necessary with distilled water.

Results

Effects of CEC on [^{3}H]-prazosin binding in rat aortic membranes

The incubation of rat aortic membranes with 10 μM CEC did not change the K_D of [^{3}H]-prazosin binding in comparison to untreated membranes (K_D values were 1.68 ± 0.53 and 1.28 ± 0.33 nM, respectively). In contrast, the total number of binding sites labelled by [^{3}H]-prazosin were significantly reduced (by about 88%) from 1.80 ± 0.22 pmol g^{-1} fresh tissue (untreated), to 0.22 ± 0.08 pmol g^{-1} (after CEC). A representative Scatchard plot of saturation assay in rat aorta membranes is shown in Figure 1.

The nonlinear regression analysis revealed that the data were best described by a model for one binding site, both before and after CEC incubation.

Antagonism of noradrenaline-induced contractions in rat aortic strips

The cumulative addition of NA to spiral strips of rat thoracic aorta produced concentration-dependent contractions with a

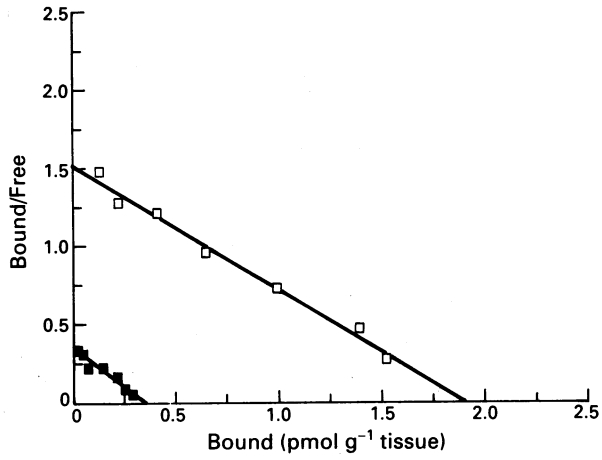

Figure 1 Scatchard plot representative from a single experiment of [^{3}H]-prazosin saturation binding in rat aortic membranes pretreated (■) or not pretreated (□) with 10 μM chloroethylclonidine. In both cases, the data were consistent with binding to a single site with B_{max} = 0.22 pmol g^{-1} (K_D = 1.68 nM) and B_{max} = 1.80 pmol g^{-1} (K_D = 1.28 nM), respectively.

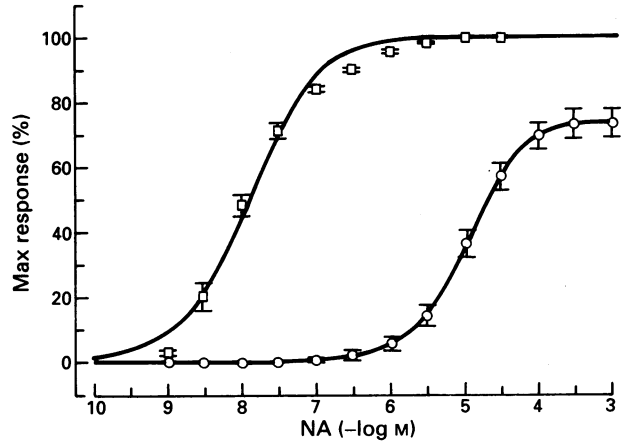

Figure 2 Effects of chloroethylclonidine (50 μM, 30 min incubation followed by washout) on contractions to noradrenaline (NA) in rat aorta. Responses, expressed as a percentage of the maximum contraction obtained in the control (□) cumulative concentration-response curve, are presented as mean ± s.e. from at least 5 experiments.

mean pEC_{50} value ($-\log EC_{50}$ = concentration that produced 50% of the maximal tension attainable) of 7.35 (range 6.82–7.96), in agreement with that (7.77) reported by Ruffolo & Waddel (1982).

The second of the paired control concentration-response curves to NA was, in general, slightly shifted to the right of the first curve, yielding a DR in the range 1.3–2.5.

The slopes and maxima of these curves were not statistically different.

The NA concentration-response curve was partially but significantly depressed (by about 25%) and markedly shifted to the right after 30 min incubation (followed by repetitive washout) with 50 μM CEC (Figure 2).

The plot of reciprocals of equieffective NA concentrations before and after CEC incubation (not shown), according to the method of Furchgott & Bursztyn (1967), enabled calculation of K_A and the fraction (q) of the initial receptor pool remaining after receptor inactivation. The pK_A value (4.15; $-\log K_A$, M) estimated for the agonist was clearly different from that previously reported (6.58) by other authors utilizing dibenamine as an irreversible antagonist (Ruffolo & Waddel, 1982). The number of remaining receptors (q = 0.0008; <0.1%) suggests that almost all the adrenoceptors were inactivated by CEC.

To verify this hypothesis, the effect of prazosin (10 nM, 30 min incubation) on NA-induced contraction of aorta strips was evaluated in tissues pretreated, or not, with CEC. As shown in Figure 3, prazosin induced a marked shift to the right (by about 85 fold) of the NA concentration-response curve (corrected DR = 34; apparent pK_B = 9.5) in untreated strips, but was practically inactive on strips previously treated for 30 min (followed by repetitive washout) with 50 μM CEC.

The addition of the calcium entry blocker, nicardipine, (up to 10 μM) caused only a non-significant (in comparison to the matched control preparations), non-concentration-dependent rightward shift and a 8–15% depression of the maximum of the concentration-response curve for the NA-induced contractions (Figure 4). This suggests that dihydropyridine-sensitive Ca^{2+} channels are unlikely to be involved in receptor-effector coupling in this tissue.

In order to confirm the results described above, the potency of different α_1-adrenoceptor antagonists in inhibiting the noradrenaline-induced contractions of rat aorta was assessed.

The antagonists caused parallel, concentration-related shifts to the right of the NA concentration-response curves,

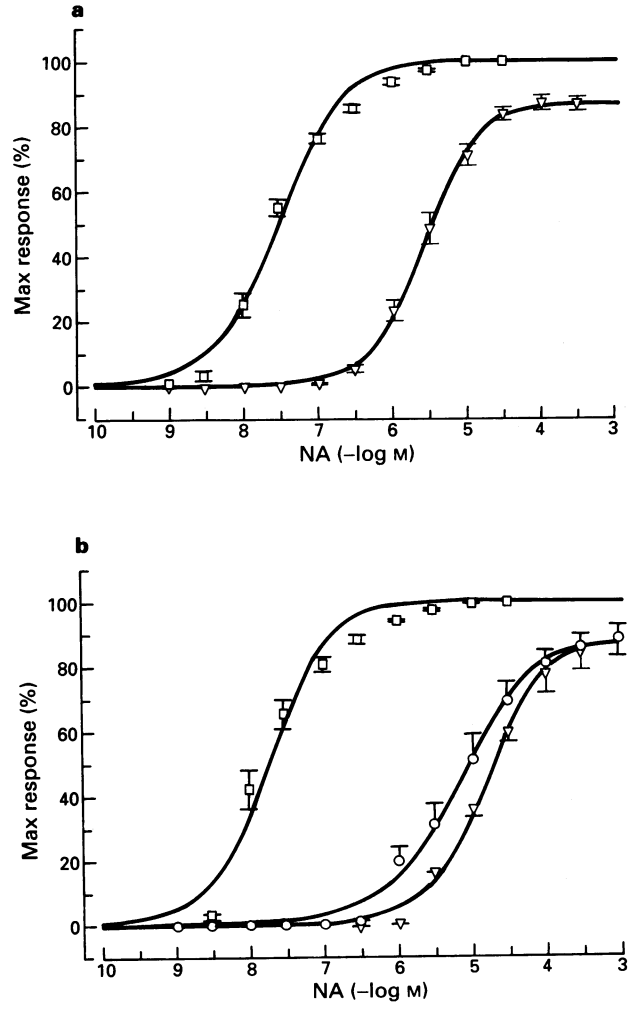

Figure 3 Effects of prazosin (10 nM, 30 min incubation) on contractions to noradrenaline (NA) in rat aorta untreated and previously treated with chloroethylclonidine (CEC). In both cases the responses are expressed as a percentage of the maximum contraction obtained in the control (□) cumulative concentration-response curve. (a) NA-response curve before (□) and after prazosin (▽) in untreated tissues. (b) Responses to NA in tissues before (□), following 30 min CEC incubation and washout (○), and after further addition of prazosin (▽). Data are presented as mean ± s.e. from at least 3 experiments in both cases.

without affecting significantly the maximum contraction caused by the agonist in comparison to that observed in the matched control preparations. The slopes of the Schild plots obtained with these compounds were found to be close to, and not significantly different from, unity, suggesting that simple competitive antagonism occurred.

The calculated pA_2 values (slope constrained to unity) given in Table 1 show that prazosin and (R)-YM-12617 were the most potent antagonists. It is noticeable that, in our experimental conditions, spiperone proved more potent than 5-methylurapidil as an antagonist of NA-induced contractions.

Affinity for the α_{1A}- and α_{1B}-adrenoceptor subtypes

The potency of the compounds in displacing the [^{3}H]-prazosin specific binding in the different tissues tested is summarized in Table 2. Prazosin and terazosin showed the same affinity for the two α_1-adrenoceptor subtypes, whereas 5-methylurapidil (Figure 5a) and (R)-YM-12617 were selective for the α_{1A}-subtype, and spiperone (Figure 5b) was selective for the α_{1B}-subtype.

The pseudo-Hill coefficients for the inhibition of [^{3}H]-prazosin binding were found to be close to and not significantly different from unity (not shown), indicating a simple competitive antagonism.

The investigated antagonists generated a significant linear correlation between their functional potency in terms of pA_2 values evaluated on rat aorta and their binding affinity for the α_{1B}-adrenoceptor subtype, but not with the affinity for α_{1A}-subtype (Figure 6). The obtained equations are the following (Y = X for identity; in parentheses the s.e. mean of the slope; r^2 = correlation coefficient):

(α_{1A}-adrenoceptor) $Y = 0.978\ (\pm 0.031)X$; $r^2 = 0.38$, NS
(α_{1B}-adrenoceptor) $Y = 0.946\ (\pm 0.024)X$; $r^2 = 0.77$, $P < 0.01$

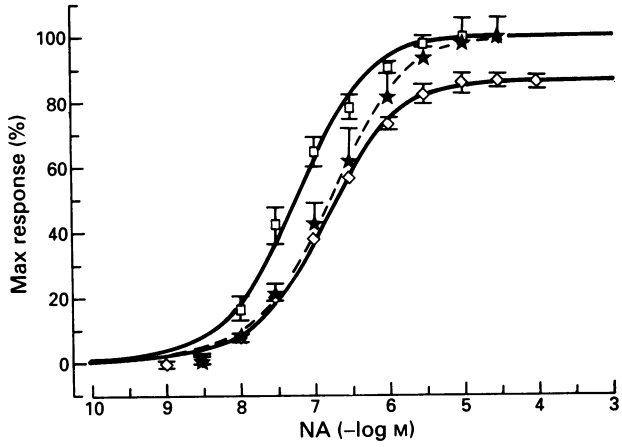

Figure 4 Effects of vehicle (★) and 10 μM nicardipine (◇) on contractions to noradrenaline (NA) in rat aorta. In both cases the responses are expressed as percentage of the maximum contraction obtained in the control (□) cumulative concentration-response curve. The points are the mean ± s.e. from at least 3 experiments.

Table 1 Estimated potency of α_1-antagonists, expressed as pA_2 values from constrained Schild plots (slope = 1.00), for competitive antagonism against noradrenaline-induced contractions of rat aortic strips

Drugs	*pA_2*
Prazosin	9.85 ± 0.10
Terazosin	8.54 ± 0.05
(R)-YM-12617	9.34 ± 0.07
Phentolamine	7.71 ± 0.13
5-Methylurapidil	7.64 ± 0.04
Spiperone	8.41 ± 0.11

Results are presented as mean ± s.e. of $n = 6-10$ preparations for each pA_2 determination.

Table 2 Affinities of antagonists (expressed as K_i, nM) for the α_{1A}- and α_{1B}-adrenoceptor subtypes

Drugs	*α_{1A}*	*α_{1B}*
Prazosin	0.93 ± 0.19	0.36 ± 0.11
Terazosin	5.81 ± 1.57	3.55 ± 0.46
(R)-YM-12617	0.20 ± 0.03	4.06 ± 0.95
Phentolamine	16.37 ± 4.75	89.33 ± 2.51
5-Methylurapidil	4.66 ± 1.82	220.24 ± 30.29
Spiperone	37.81 ± 5.82	2.03 ± 0.09

Rat hippocampus membranes pretreated with CEC (α_{1A}) and rat liver membranes (α_{1B}) were utilized as the source of receptors. The slope of all the displacement curves were not statistically different from unity. Data are the mean ± s.e.mean of 2–4 experiments, each in triplicate.

Discussion

To characterize the population of α_1-adrenoceptors present in rat aorta, we studied the effects of CEC incubation on [^{3}H]-prazosin binding in membrane preparations from this tissue, and the effects of this selective α_{1B} irreversible antagonist on

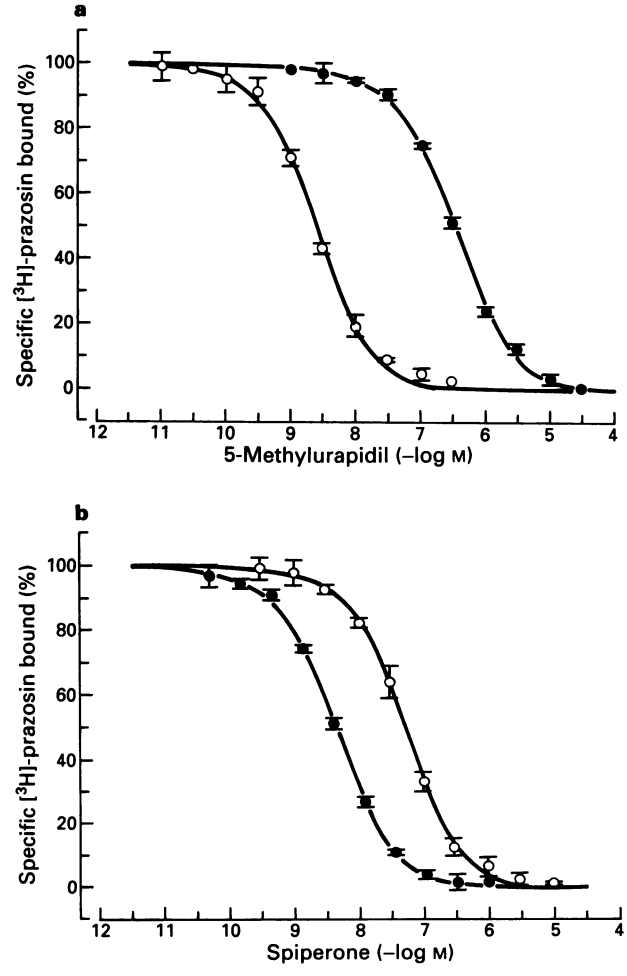

Figure 5 (a) 5-Methylurapidil and (b) spiperone/[^{3}H]-prazosin competition in rat hippocampal (α_{1A}, ○) and liver (α_{1B}, ●) membranes. The points represent the mean ± s.e. percentage reduction of the specific binding from 3 experiments each in triplicate. Lines through the data were fitted by computer, assuming displacement from a single recognition site.

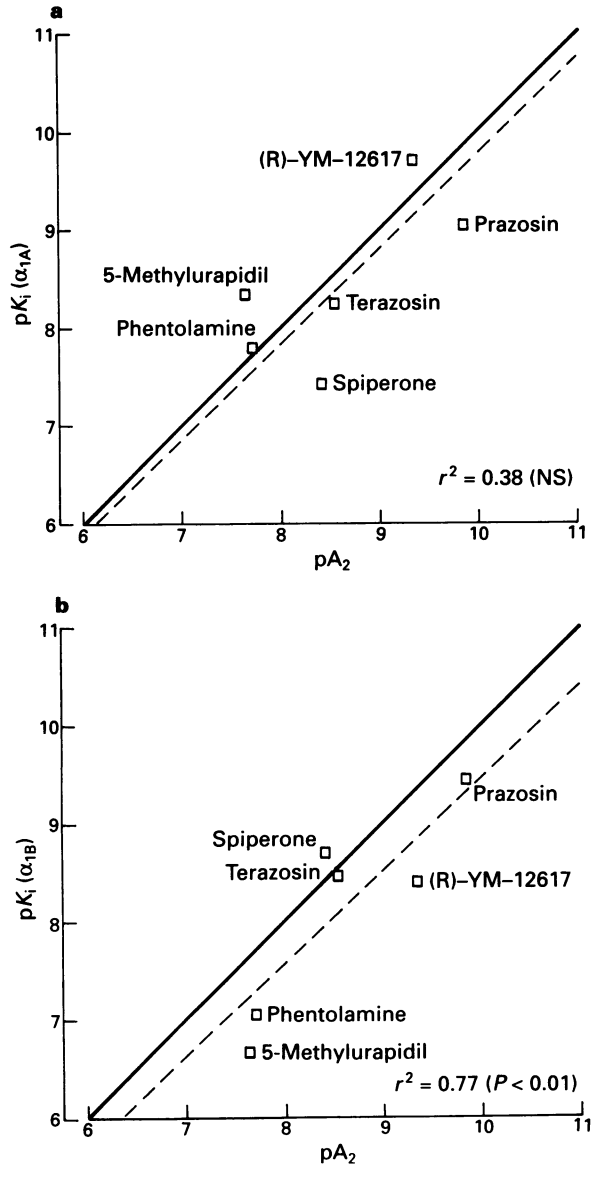

Figure 6 Correlation between the pA_2 values of the investigated α-antagonists on noradrenaline-induced contractions of rat aorta, and their affinity expressed as pK_i values for α_{1A} (a) and α_{1B}-adrenoceptor subtypes (b). Solid lines represent the 'equivalence' lines (slope = 1). Dashed lines are the evaluated regression lines, Y = mX (in parentheses the s.e. of the slope): α_{1A}-adrenoceptor Y = 0.978 (±0.031)X; α_{1B}-adrenoceptor Y = 0.946 (±0.024)X.

NA-induced contractions, as well as the potency of several competitive antagonists in inhibiting contractions to NA.

Incubation of rat aorta membranes with CEC induced a significant decrease (88%) in the total number of binding sites without affecting the K_D for [^{3}H]-prazosin.

Nonlinear regression analysis of the saturation experiments revealed that the data best fit to a model for one binding site, indicating that a single population of receptors is labelled by [^{3}H]-prazosin.

In the functional studies with rat aorta, 50 μM CEC induced a marked parallel shift to the right of the NA concentration-response curve and a depression in the maximum tension attainable. The EC_{50} of the agonist increased from 12 nM to 10 μM. The results are in agreement with previously published data reported by other authors (Han *et al.*, 1990; Aboud *et al.*, 1993). Moreover, we found that the receptors remaining after incubation of rat aortic strips with CEC were insensitive to prazosin. In these experiments, while prazosin (10 nM) in absence of CEC treatment showed a 34 fold shift to the right of the NA concentration-response curve, after CEC incubation a non-significant shift of the NA curve was observed with the drug. These findings are in agreement with those reported by Oriowo & Bevan (1990), who demonstrated that CEC unmasks a non-α-adrenoceptor site in the rat aorta, insensitive to phenoxybenzamine, prazosin, WB4101 and yohimbine. The K_A value of the agonist calculated after CEC incubation (4.15) is in agreement with these observations.

In fact, it has been reported that the K_A of NA in this tissue utilizing dibenamine as irreversible antagonist (Ruffolo & Waddel, 1982) is 6.58 and we confirmed this result utilizing phenoxybenzamine as irreversible antagonist (K_A = 6.46; data not shown). These findings indicate, on the whole, that the receptors remaining after CEC incubation cannot be considered α_1-adrenoceptors.

In addition, it is well known that the smooth muscle contractions elicited by α_{1A}-subtype receptor activation are dependent on Ca^{2+} influx through the dihydropyridine-sensitive channels, while contractions elicited by the activation of α_{1B}-adrenoceptors are independent of extracellular Ca^{2+} influx (Minneman, 1988; Han *et al.*, 1990). In our experimental conditions, the calcium entry blocker, nicardipine caused only a non-significant, non-dose-dependent slight rightward shift and depression of the maximum of the concentration-response curve to NA, as reported by Han *et al.* (1990) after nifedipine incubation.

The competitive antagonists prazosin, terazosin, (R)-YM-12617, phentolamine, 5-methylurapidil and spiperone inhibited contractions to NA with pA_2 values of 9.85, 8.54, 9.34, 7.71, 7.64 and 8.41, respectively. Though the present functional evaluations did not provide the most precise estimates of the antagonists affinity, owing to the limited series of experiments and at least in some cases the narrow range of concentrations tested, the pA_2 value of prazosin (or the apparent pK_B = 9.5) derived is in agreement with previously reported data showing affinity values (pA_2) of 10.42 (Hamed *et al.*, 1983), 10.30 (Martinotti *et al.*, 1991), 9.89 (Muramatsu *et al.*, 1990), 9.45 (Aboud *et al.*, 1993) and 10.6 (Oriowo *et al.*, 1990). Also our pA_2 values for phentolamine and 5-methylurapidil are similar to those previously obtained by other authors (Muramatsu *et al.*, 1990; Hamed *et al.*, 1983; Aboud *et al.*, 1993).

Recently, Aboud *et al.* (1993) stated that contractions of rat aorta to NA are mediated by non-α_{1A}, non-α_{1B}-adrenoceptors, due to the simultaneous presence of sensitivity to CEC (α_{1B}) and to the high potency of selective α_{1A}-antagonists. Therefore, we evaluated the affinity of the tested antagonists for the α_{1A}-adrenoceptor subtype in rat hippocampal membranes after CEC inactivation (Morrow & Creese, 1986), and the affinity for the α_{1B}-adrenoceptor subtype in rat liver membranes since this tissue contains mainly this α_1-subtype (Garcia-Sainz *et al.*, 1992; Taddei *et al.*, 1993). Rat aortic membranes were not utilized to evaluate the affinity for the α_{1B}-adrenoceptors because of the low number of specific binding sites for [^{3}H]-prazosin. We confirmed that prazosin and terazosin do not discriminate between the α_1-adrenoceptor subtypes, whereas 5-methylurapidil and (R)-YM-12617 were confirmed as clearly the most active on α_{1A}-adrenoceptors, and spiperone on α_{1B}-adrenoceptor subtypes. The selectivity ratios (pK_i hippocampus/pK_i liver) obtained by us with phentolamine and spiperone (5.5 and 0.04, respectively) were fully in agreement with those previously reported by Michel *et al.* (1989), who used rat submaxillary gland and liver as sources of α_{1A}- and α_{1B}-adrenoceptor subtypes, respectively.

The pA_2 values obtained with the antagonists in the course of our experiments gave significant correlation with their affinity for the α_{1B}-adrenoceptor, and showed a lower degree of correlation with their affinity for the α_{1A}-adrenoceptor subtype.

When some of these antagonists were tested on rabbit urethra, a tissue containing mainly the α_{1A} subtype, their

functional potency correlated better with their binding affinity for the α_{1A}-adrenoceptor than for the α_{1B}-subtype (Testa *et al.*, 1993), supporting the soundness of this approach.

In our opinion, all the findings reported in the present work clearly indicate that in rat aorta most of the contraction to NA is mediated by α_{1B}-adrenoceptors, and that the potency (pA_2) of an antagonist in this tissue parallels its receptor binding affinity for this subtype of the α_1-adrenoceptor population.

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Note added during revision of the manuscript

In a recent paper, Eltze (1994) reported an excellent correlation between the affinities (pA_2 values) of several α_1-adrenoceptor antagonists (including those used in our study) evaluated on guinea-pig spleen, and their pK_i values for the rat liver α_{1B}-adrenoceptors (reported by Taddei *et al.*, 1993), concluding that the α_1-adrenoceptor mediating smooth muscle contraction of this tissue can be best characterized as being of the B subtype.

Furthermore, this author showed a significant correlation between the affinities (pA_2 values) of these antagonists on guinea-pig spleen, and the affinities (pA_2 values) of the same compounds evaluated on rat aorta, but not with the affinities (pA_2 values) evaluated on rat vas deferens (a tissue expressing the α_{1A}-adrenoceptors).

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