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Functional evidence equating the pharmacologically-defined α_{1A}- and cloned α_{1C}-adrenoceptor: studies in the isolated perfused kidney of rat

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1 The present study characterizes and classifies α_1-adrenoceptor-mediated vasoconstriction in the isolated perfused kidney of rat using quantitative receptor pharmacology and compares the results to radioligand binding studies (made in cloned α_1-adrenoceptor subtypes, native α_{1A}-adrenoceptors in submaxillary gland of rat, and α_{1A}-adrenoceptors in several other tissues of rat).

2 Concentration-effect curves to noradrenaline in the presence of 5-methyl-urapidil were biphasic, indicating α_1-adrenoceptor heterogeneity. The α_1-adrenoceptor subtype mediating the first phase (low affinity for 5-methyl-urapidil) could not be 'isolated' for detailed pharmacological characterization but was defined by a sensitivity to inhibition by chloroethylclonidine and an inability of methoxamine to activate the site. Additionally, vasoconstriction mediated by this α_1-adrenoceptor subtype or subtypes was abolished by nitrendipine (1 μM), thereby allowing characterization of the second, high affinity site for 5-methyl-urapidil.

3 The following antagonists interacted competitively with noradrenaline at the α_1-adrenoceptor for which 5-methyl-urapidil exhibits high affinity (pK_B value): WB 4101 (10.3)$>$prazosin (9.5)$\approx$HV 723 (9.3)$\approx$5-methyl-urapidil (9.2)$>$phentolamine (8.6)$>$spiperone ($pA_2 = 8.1$)$\approx$oxymetazoline (7.9). In contrast, insurmountable antagonism was seen with **S(+)**- and **R(−)**-niguldipine, the **S(+)**-isomer being approximately 30 fold more potent than the **R(−)**-isomer. Receptor protection experiments indicated that **S(+)**-niguldipine interacted directly with α_1-adrenoceptors. Dehydroniguldipine acted as a competitive antagonist ($pK_B = 9.0$). Thus, the results with antagonists define the α_1-adrenoceptor as an α_{1A}-adrenoceptor.

4 An agonist 'fingerprint' was constructed in the presence of nitrendipine to define further the α_{1A}-adrenoceptor. The following order and relativity of agonist potency was obtained: cirazoline (1)$\approx$adrenaline (2)$>$noradrenaline (5)$>$phenylephrine (23)$\approx$amidephrine (31)$>$methoxamine (71)$\gg$isoprenaline (1456)$\approx$dopamine (2210).

5 A high correlative association was shown between the affinity of antagonists obtained functionally in the isolated perfused kidney of rat and pK_i values obtained from binding experiments with the cloned bovine α_{1C}-adrenoceptor ($R^2 = 0.85$), native α_{1A}-adrenoceptors in submaxillary gland of rat ($R^2 = 0.79$), and α_{1A}-adrenoceptors from several other tissues of rat (values taken from the literature, $R^2 = 0.89$).

6 The present study demonstrates that the α_{1A}-adrenoceptor is the predominant α_1-adrenoceptor subtype mediating vasoconstrictor responses to exogenously administered noradrenaline in the isolated perfused kidney of rat. More importantly, α_{1A}-adrenoceptors mediating vasoconstrictor responses to noradrenaline exhibited a pharmacological equivalency to the cloned bovine α_{1C}-adrenoceptor. Thus, definitive functional pharmacological data are provided for equating the two receptors and support results derived recently from molecular and radioligand binding studies.

Keywords: Native α_1-adrenoceptor subtypes; cloned α_1-adrenoceptor subtypes; α_{1A}-adrenoceptors; 5-methyl-urapidil; **S(+)**-niguldipine; chloroethylclonidine; submaxillary gland; kidney

Introduction

Considerable evidence exists for the division of α_1-adrenoceptors into subtypes. In 1986, Flavahan & Vanhoutte proposed the existence of two distinct α_1-adrenoceptor subtypes (α_{1H}- and α_{1L}-adrenoceptors) based on the work of Holck *et al.* (1983) and observations made by Drew (1985). α_{1H}-Adrenoceptors were reported to possess a high affinity for prazosin ($pA_2 > 9$) and yohimbine ($pA_2 > 6.4$) whereas α_{1L}-adrenoceptors were reported to possess a low affinity (pA_2 values <9 and <6.4 respectively). This proposal received little attention due to possible alternative explanations, such as species variation (McGrath *et al.*, 1989), experimental rigour (Docherty *et al.*, 1987; McGrath *et al.*, 1989), and the potential for the involvement of α_2-adrenoceptor subtypes (Bylund, 1985). It should be noted, however, that since the article by Flavahan & Vanhoutte (1986), α_1-adrenoceptors exhibiting both high and low affinity for prazosin have been identified in gerbil (Kimura *et al.*, 1993), rat (Oshita *et al.*, 1991; Ohmura *et al.*, 1992), rabbit (Muramatsu *et al.*, 1990a; Oshita *et al.*, 1993) and dog (Flavahan *et al.*, 1987; Kohno *et al.*, 1994). Thus, a classification scheme proposing α_1-adrenoceptor subtypes based upon divergent affinity estimates for prazosin remains in line with a body of current thinking (Muramatsu *et al.*, 1990b; Oshita *et al.*, 1991). However, the most widely accepted classification scheme for α_1-adrenoceptors operative today considers only those α_1-adrenoceptor subtypes with high affinity for prazosin, and stemmed directly from work by Morrow & Creese (1986).

Morrow & Creese (1986) observed that WB 4101 and phentolamine displaced high affinity [^{3}H]-prazosin ($pK_D \approx$

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9–10) binding in cortex of rat in a manner consistent with the presence of two distinct binding sites. Binding sites for [^{3}H]-prazosin with high affinity for WB 4101 and phentolamine (Morrow & Creese, 1986), as well as oxymetazoline (Hanft & Gross, 1989a), benoxathian (Han *et al.*, 1987), YM 12617 (Hanft *et al.*, 1989), 5-methyl-urapidil (Gross *et al.*, 1988) and S(+)-niguldipine (Boer *et al.*, 1989) were designated α_{1A}-adrenoceptors, whereas binding sites with low affinity for these ligands were designated α_{1B}-adrenoceptors. Subsequently, the α_{1B}-adrenoceptor subtype has been shown to bind selectively spiperone (Michel *et al.*, 1989) and risperidone (Sleight *et al.*, 1993) and to undergo preferential alkylation by chloroethylclonidine (CEC; Johnson & Minneman, 1987). The identification of α_{1A}- and α_{1B}-adrenoceptor binding sites in human brain (Gross *et al.*, 1989) and the demonstration of functional correlates to these binding sites (Han *et al.*, 1987) served to substantiate the α_{1A}- and α_{1B}-adrenoceptor classification scheme.

In contrast to pharmacological approaches, molecular biological techniques have demonstrated the existence of three α_1-adrenoceptors, all of which exhibit high affinity for prazosin: the cloned α_{1B}-, α_{1C}- and α_{1D}-adrenoceptors (for references, see Ford *et al.*, 1994). Difficulty has arisen, however, in equating cloned and pharmacologically-defined α_1-adrenoceptors. Until recently only the cloned and pharmacologically-defined α_{1B}-adrenoceptors were considered to represent the same molecular entity (Pimoule *et al.*, 1992). Currently, however, the cloned α_{1C}-adrenoceptor, which exhibits high affinity for 5-methyl-urapidil, WB 4101, oxymetazoline and S(+)-niguldipine, is also considered to be the same receptor as the α_{1A}-adrenoceptor described pharmacologically above (Ford *et al.*, 1994; Faure *et al.*, 1994; Clarke *et al.*, 1994; Price *et al.*, 1994; Michel & Insel, 1994). The cloned α_{1D}-adrenoceptor, characterized by its low affinity for 5-methyl-urapidil, oxymetazoline and (+)-niguldipine and its relative resistance to alkylation by CEC, is without a clear-cut functional correlate (Ford *et al.*, 1994), although the α_1-adrenoceptor mediating contraction of the rat aorta *in vitro* is claimed as an α_{1D}-adrenoceptor (Ko *et al.*, 1994; Saussy *et al.*, 1994).

It has been known for more than a decade that α_1-adrenoceptors mediate vasoconstrictor responses to noradrenaline (NA) in the isolated perfused kidney of rat, despite a preponderance of α_2-adrenoceptors in this organ (Schmitz *et al.*, 1981). Radioligand binding and biochemical studies have demonstrated the presence of both α_{1A}- and α_{1B}-adrenoceptor subtypes in rat kidney (Han *et al.*, 1990; Wilson & Minneman, 1990; Michel *et al.*, 1993a). Furthermore, α_{1A}-adrenoceptors have been reported to mediate vasoconstrictor responses to α_1-adrenoceptor agonists both *in vitro* (Eltze *et al.*, 1991; Eltze & Boer, 1992; Blue *et al.*, 1992) and *in vivo* (Elhawary *et al.*, 1992; Sattar & Johns, 1994a,b).

The present study was undertaken to identify and characterize more fully α_1-adrenoceptor-mediated vasoconstriction in the isolated perfused kidney of rat and to compare the results with ligand binding data derived from the cloned hamster α_{1B}-, bovine α_{1C}- and rat α_{1D}-adrenoceptors. Thus, we now present details of functional, pharmacologically-derived data that substantiate the claim for equating the native α_{1A}-adrenoceptor with the cloned, bovine α_{1C}-adrenoceptor (as proposed originally by Ford *et al.*, 1994). Preliminary accounts of some of this work have been presented (Blue & Clarke, 1990; Clarke *et al.*, 1991; Sharif *et al.*, 1991; Blue *et al.*, 1991; Clarke *et al.*, 1994).

Methods

Isolated perfused kidney of rat

Male Sprague-Dawley (Charles River) rats (350–500 g) were anaesthetized with sodium pentobarbitone (55 mg kg^{-1}, i.p.) and the right renal artery and kidney were isolated as described previously (Blue *et al.*, 1992). A cannula was inserted into the mesenteric artery and advanced across the abdominal aorta into the renal artery. The kidney was removed and perfused immediately at a constant rate (6 ml min^{-1}; Masterflex pump, pump head size 7014-21, Cole-Parmer, Chicago, IL, U.S.A.) with Krebs bicarbonate solution (pH 7.4, 37°C) of the following composition (mM): NaCl 118.5, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and dextrose 5. The Krebs solution was bubbled continuously with 95% O_2 and 5% CO_2. Perfusion pressure was measured by a Spectramed physiological pressure transducer (model number: P23XL) positioned near the kidney and displayed on a Beckman physiograph (model R611). Following removal of the renal capsule, kidneys were allowed to equilibrate for 30 min before exposure to a bolus dose of NA (0.1 μg in 0.1 ml of 0.9% saline). Following this priming dose, kidneys were allowed to equilibrate for an additional 15 min before experiments were begun.

Unless indicated otherwise, experiments were performed in the presence of cocaine (30 μM), corticosterone (30 μM), propranolol (1 μM), indomethacin (10 μM) and EDTA (100 μM) to inhibit neuronal and extraneuronal uptake of catecholamines, vascular β-adrenoceptors, the involvement of prostanoids and auto-oxidation of catecholamines respectively. High concentrations of cocaine and corticosterone are necessary to prevent NA from overcoming uptake blockade when used in high concentrations (e.g. in competition studies with antagonists). Propranolol was omitted from the Krebs solution in experiments where 5-hydroxytryptamine (5-HT) was used as agonist as propranolol is also a 5-HT_2 receptor antagonist (Bond *et al.*, 1989). SCH 23390 (30 nM; a D_1 receptor antagonist) was added to the Krebs solution when dopamine was used as agonist. Nitrendipine (1 μM) was added to the Krebs solution in experiments where blockade of L-type Ca^{2+} channels was desired.

Non-cumulative concentration-effect curves to agonists were obtained by perfusing kidneys until a steady-state response was obtained. Perfusion pressure was allowed to return to baseline levels between each concentration of agonist (studied in 0.5 log increments). In experiments with phentolamine and angiotensin II (see Methods), dose-effect curves were constructed by measuring vasoconstrictor responses to bolus injection of agonist (0.1 ml in 0.9% saline) into the perfusion fluid close to the kidney. Perfusion pressure was allowed to return to baseline before injection of subsequent doses. All results are expressed as a rise in perfusion pressure (mmHg) over baseline, rather than absolute pressure, to normalize for variations in baseline perfusion pressure which averaged 55 mmHg ($n = 200$).

With the exception of CEC, all experiments with antagonists were performed as follows. After construction of a control agonist concentration-effect or dose-effect curve, kidneys were perfused for 60 min with Krebs solution containing antagonist or vehicle. A second curve to the agonist was then constructed in the presence of antagonist or vehicle (time control). For experiments with S(+)-niguldipine, a third concentration-effect curve was constructed after an additional 60 min washout with Krebs solution devoid of antagonist. The entire perfusion apparatus was dismantled and cleaned thoroughly following each experiment with either S(+)- or R(−)-niguldipine due to lasting contamination with these compounds. In time control experiments, both in the absence and presence of nitrendipine (1 μM), concentration-effect curves to infusion of NA were shifted 1.24 fold and 1.5 fold to the right respectively with an insignificant depression of the maximal response ($n = 8$; data not shown). This time-dependent shift in the concentration-effect curve to NA was used to correct dextral shifts produced by antagonists in experiments using NA as agonist. In contrast, time controls for dose-effect curves to bolus injection of NA did not differ in $EC_{100\ mmHg}$ or maximal response ($n = 4$; data not shown).

Experiments with CEC were performed in one of two

ways. First, after construction of a control concentration-effect curve to NA, kidneys were perfused with CEC (10 or 100 μM) for 20 min and then perfused with Krebs solution free of CEC for 40 min before construction of a second concentration-effect curve to NA. Secondly, kidneys were perfused initially with CEC (100 μM) for 20 min and then perfused with Krebs solution free of CEC for 40 min before exposure to the priming dose of NA. Concentration-effect curves to NA, after administration of a priming dose, were then constructed as detailed above.

Preliminary experiments were conducted to rule out a potential role for α_2-adrenoceptors. First, in the presence of prazosin (30 nM), concentration-effect curves to NA were not shifted significantly from corresponding time controls by the selective α_2-adrenoceptor antagonist idazoxan (300 nM; $n = 4$; data not shown). Secondly, Dunn and coworkers (1989) have shown that exogenously administered angiotensin II (50 nM) can unmask α_2-adrenoceptors in the isolated distal saphenous artery of rabbits. Thus, dose-effect curves to NA and the selective α_2-adrenoceptor agonist, UK 14,304, were constructed in the absence and presence of angiotensin II (50 nM). UK 14,304 failed to elicit a vasoconstrictor response (0.01–1000 μg) either in the absence or presence of angiotensin II ($n = 4$, data not shown). Likewise, dose-effect curves to NA were not potentiated by angiotensin II ($n = 4$, data not shown).

Calculation of pK_B and pA_2 values

The pK_B values for competitive antagonists were determined by Schild regression analysis. Regression lines with slopes not significantly different from 1 were constrained to 1 for the estimation of pK_B values. Concentration-ratios were calculated using equiactive responses to agonist and were determined only when control concentration-effect and test curves were parallel. pA_2 estimates for single concentrations of antagonist were calculated as follows:

$$pA_2 \text{ value} = -\log [\text{Antagonist concentration}/(\text{Concentration ratio} - 1)]$$

This method assumes a linear relationship, with a slope of 1, between the $-\log$ molar concentration of the antagonist and the log (concentration-ratio − 1).

Autoradiography

Kidneys were isolated as described above and perfused with CEC (100 μM) or vehicle for 20 min and then perfused with Krebs solution free of CEC for 40 min. Control and CEC-treated kidneys were removed and immediately frozen in dry-ice on microtome chucks. Longitudinal sections (20 μM) were cut from control and CEC-treated kidneys and collected on gelatinised microscope slides. The slides were stored at −20°C for 2 weeks prior to binding assays.

For α_1-adrenoceptor autoradiography, sections were thawed at room temperature and pre-incubated in 170 mM Tris HCl (pH 7.4, 23°C) for 60 min in order to remove possible interfering agents (e.g. neurotransmitters) and residual CEC. Slides were then covered with 0.8 ml of solution containing 0.3 nM [^{3}H]-prazosin (specific activity: 82 Ci mmol^{-1}) in 170 mM Tris HCl plus 50 mM NaCl for 60 min at 23°C to attain equilibrium. Adjacent sections received [^{3}H]-prazosin solution containing 100 μM phentolamine to define non-specific binding. Slides were rinsed in ice-cold buffer for 25 min followed by a rinse in ice-cold water for 15 s. Slides were then dried rapidly in a stream of cold air. Following desiccation overnight, the slides, together with radiation standards, were apposed to ^{3}H-sensitive film in X-ray cassettes for 3 months.

For angiotensin II receptor autoradiography, kidney sections were thawed at 23°C and allowed to dry before being pre-incubated in 500 ml of $NaPO_4$ buffer (20 mM $NaPO_4$, 150 mM NaCl, 10 mM $MgCl_2$ and 10 mM EDTA, pH 7.4) at 23°C for 30 min. Sections were removed from the pre-incubation buffer, air dried and laid flat over glass rods. Sections were then covered with 0.5–0.7 ml of sodium phosphate buffer containing 0.1 nM [^{125}I]-Sar^1-Ile^8-angiotensin II (specific activity: 2200 Ci mmol^{-1}) in the presence or absence of unlabelled competing compound. Nonspecific binding was defined with 10 μM angiotensin II. The assay buffer contained a mixture of the following peptidase inhibitors: captopril (1 μM), bacitracin (60 μg ml^{-1}), phosphoramidon, pepstatin A, bestatin, chymostatin and antipain (each at 1.3 μg ml^{-1}) and amastatin (0.13 μg ml^{-1}). Incubations were terminated after 90 min by rinsing the slides in ice-cold 50 mM Tris HCl (pH 7.4) for 10 min followed by rapid drying in a stream of cold air. The labelled sections were desiccated overnight and then apposed, together with radiation standards, to a radiation-sensitive film in an X-ray cassette. The autoradiograms were generated after 2 weeks exposure.

Autoradiograms for [^{3}H]-prazosin and [^{125}I]-Sar^1-Ile^8-angiotensin II binding were analysed by digital subtraction image analysis.

Radioligand binding

Competition binding assays were performed with [^{3}H]-prazosin (specific activity: 82 Ci mmol^{-1}; 0.1 nM final assay concentration) with approximately 0.3 mg protein in 400 μl of Tris (50 mM)-EDTA (0.5 mM) buffer (pH 7.4). Incubations were at 25°C for 60 min. Reactions were terminated and membranes were harvested by rapid filtration through GF-B filters pretreated with a 0.1% solution of polyethylenimine. Filters were washed three times with 3 ml of ice-cold 0.1 M NaCl solution and dried. The bound radioactivity was determined by liquid scintillation counting. Specific binding was defined as the difference between the binding of [^{3}H]-prazosin in the absence and presence of 10 μM phentolamine. Competition binding assays were performed using 10 different concentrations of displacing antagonist.

Data were first analysed by iterative curve fitting to a four parameter logistic equation to yield Hill coefficients and IC_{50} values. K_i values were calculated according to the Cheng-Prusoff equation.

Statistics

Confidence limits (CL: at 95% probability) were calculated for the slopes of Schild regressions using StatView 512+ (Brain Power Inc., Calabasas, CA, U.S.A.). Differences between mean values were tested for statistical significance ($P < 0.05$) using Student's unpaired t test (two tailed).

For correlations, each pair of concordant tissues was fitted to a linear regression model which included both the slope and intercept. Confirmatory analyses normalizing the intercept to zero were also performed. Tests for correlation and inference on slopes were performed using standard statistical software (SAS PROC REG and PROG CORR, SAS Inc., Cary, NC, U.S.A.). Significance levels were set at the $P < 0.05$ level.

Materials

Drugs were obtained from the following sources: [^{125}I]-Sar^1-Ile^8-angiotensin II and [^{3}H]-prazosin from Dupont-New England Nuclear, Boston, MA, U.S.A.; (−)-noradrenaline HCl, (−)-isoprenaline HCl, (−)-phenylephrine HCl, methoxamine HCl, oxymetazoline HCl, dopamine HCl, 5-hydroxytryptamine HCl, (±)-propranolol HCl, cocaine HCl, corticosterone, indomethacin and EDTA from Sigma Chemical Co., St. Louis, MO, U.S.A.; chloroethylclonidine 2HCl, WB 4101 ((2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane HCl), 5-methyl-urapidil HCl, (−)-adrenaline bitartrate, idazoxan HCl, (+)-SCH 23390 HCl (**R**-(+)-7-chloro-8-hydroxy-3methyl-1-phenyl-2,3,4,5-tetrahydro-1h-3-benzazepine HCl), **S**(+)-niguldipine, **R**(−)-niguldipine from Re-

search Biochemicals Inc., Natick, MA, U.S.A.; nitrendipine, dehydroniguldipine, amidephrine, HV 723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxyethyl)-amino)-propyl) benzene-aceto-nitrile fumarate from the Institute of Organic Chemistry, Syntex Research, Palo Alto, CA, U.S.A.; UK 14,304 (5-bromo-6-[2-imidazolin-2ylamino]-quinoxaline) and prazosin HCl from Pfizer Central Research, U.K. and Pfizer Inc., Groton, CT, U.S.A. respectively; phentolamine mesylate from Ciba Geigy, Summit, NJ, U.S.A.; and cirazoline from Syntelabo, Paris, France. Peptides were obtained from Peninsula Labs (Belmont, CA, U.S.A.).

Stock solutions of drugs were prepared in deionised water with following exceptions: corticosterone (dimethylsulphoxide), indomethacin (0.5% sodium carbonate), 5-methyl-urapidil (predissolved in a drop of 1 M HCl), nitrendipine, S(+)- and R(−)-niguldipine and dehydroniguldipine (ethanol).

The cloned hamster smooth muscle α_{1B}-adrenoceptor (Cotecchia *et al.*, 1988), the cloned rat cerebral cortex α_{1D}-adrenoceptor (Lomasney *et al.*, 1991) and the cloned bovine α_{1C}-adrenoceptor (Schwinn *et al.*, 1990) stably expressed in rat-1 fibroblast cell lines, were purchased from Dr Lee Allen, Duke University, Durham, NC, U.S.A.

Results

Antagonist characterization

Increasing concentrations of noradrenaline (NA; 0.01–3 μM) produced concentration-dependent increases in perfusion pressure spanning approximately two orders of magnitude and attaining maximal vasoconstrictor responses of 200–250 mmHg. Prazosin (1–30 nM), phentolamine (30–300 nM) and WB 4101 (0.3–30 nM) produced parallel dextral shifts of concentration-effect curves to NA and caused a slight but insignificant reduction in the maximal response (Figure 1). Schild regression analysis yielded lines with slopes not significantly different from 1 and pK_B values of 9.5, 8.6 and 10.3, respectively (Table 1).

CEC is a commonly used tool to identify α_{1B}-adrenoceptors. Although CEC binds to all α_1-adrenoceptor subtypes with high affinity for prazosin, as well as α_2-adrenoceptors (Michel *et al.*, 1993b), CEC preferentially alkylates α_{1B}-adrenoceptors. Figure 2 shows concentration-effect curves to NA after pretreatment of kidneys with CEC (10 and 100 μM) for 20 min. Only the highest concentration of CEC (100 μM) produced a statistically significant ($P<0.05$) dextral shift (2.6 ± 0.1) in the concentration-effect curve to NA. Neither concentration of CEC produced a significant depression of the maximal response compared with time controls. The results suggest a limited role for CEC-sensitive α_1-adrenoceptors (α_{1B}- and α_{1D}-adrenoceptors) in NA-mediated vasoconstriction.

Figure 3 shows the effect of perfusion for 20 min with CEC (100 μM) on [^{3}H]-prazosin binding in kidney. CEC reduced [^{3}H]-prazosin binding by approximately 70% without affecting [^{125}I]-Sar^1-Ile^8-angiotensin II binding, suggesting a specific action of CEC.

The most selective ligand available for the α_{1A}-adrenoceptor is the S(+)-isomer of the dihydropyridine Ca^{2+}

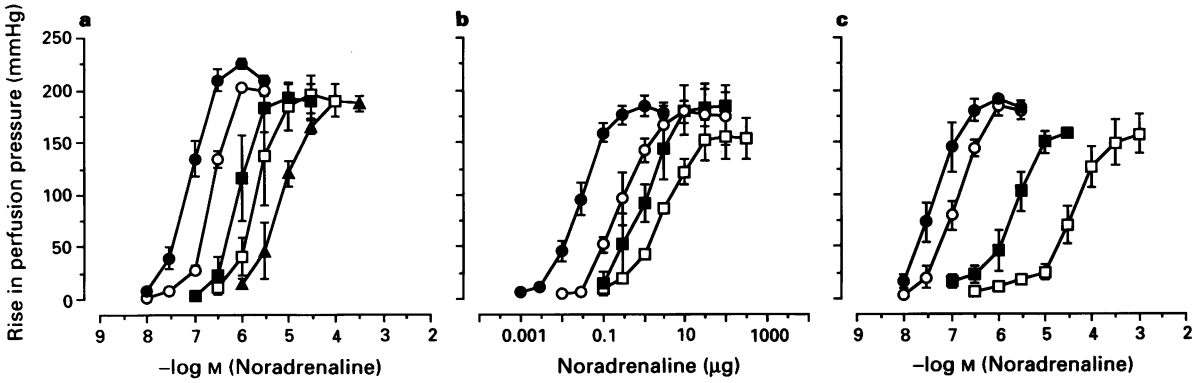

Figure 1 Interaction of noradrenaline (NA) with α_1-adrenoceptor antagonists. (a) Concentration-effect curves to NA in the absence (●, $n=16$) and presence of prazosin: 1 nM (○, $n=4$), 3 nM (■, $n=4$), 10 nM (□, $n=4$) and 30 nM (▲, $n=4$). (b) Dose-effect curves to NA in the absence (●, $n=12$) and presence of phentolamine: 30 nM (○, $n=4$), 100 nM (■, $n=4$) and 300 nM (□, $n=4$). (c) Concentration-effect curves to NA in the absence (●, $n=12$) and presence of WB 4101: 0.3 nM (○, $n=4$), 3 nM (■, $n=4$), 30 nM (□, $n=4$). Kidneys were perfused with Krebs solution containing antagonist for 60 min prior to construction of the second concentration- or dose-effect curve to NA. Each point represents the mean ± s.e.mean where larger than the symbol.

Table 1 Affinity estimates for α_1-antagonists versus noradrenaline in isolated perfused kidney of rat

Antagonist	*Concentrations* (nM)	pK_B	*Slope*	*95% confidence limits*
Prazosin	1, 3, 10, 30	9.5[a]	1.07	0.93–1.21
WB 4101	0.3, 3, 30	10.3[a]	1.01	0.89–1.13
Phentolamine[b]	30, 100, 300	8.6[a]	1.06	0.63–1.49
S(+)-Niguldipine	0.03, 0.1, 0.3	10.5[c]		
R(−)-Niguldipine	3, 30, 300	9.1[c]		
Dehydroniguldipine	10, 30, 100	9.0[a]	1.07	0.80–1.35
5-Methyl-urapidil[d]	3, 10, 30, 100	9.2[a]	1.07	0.94–1.21
	3, 30	9.1[ae]	1.05	0.89–1.25
Oxymetazoline[d]	100, 1000	7.9[a]	1.11	0.83–1.39
HV 723[d]	1, 10	9.3[a]	0.95	0.79–1.11
Spiperone[d]	100	8.1[f]		

[a]Slope constrained to 1. [b]Versus bolus doses of NA. [c]Insurmountable antagonist, affinity estimate based on lowest concentration. [d]Experiment conducted in the presence of nitrendipine (1 μM). [e]Methoxamine as agonist. [f]pA_2 (slope of 1 assumed).

channel antagonist, niguldipine (Boer *et al.*, 1989). The **R**(−)-isomer has lower affinity and is less selective than the **S**(+)-isomer (Boer *et al.*, 1989). Figure 4a and b show composite data for the interaction of NA with **S**(+)- and **R**(−)-niguldipine respectively. **S**(+)-niguldipine (0.03–0.3 nM; Figure 4a) potently, but insurmountably antagonized vasoconstrictor responses to NA. Antagonism by **S**(+)-niguldipine (0.1 nM) was not reversible even after perfusion for 3 h with **S**(+)-niguldipine-free Krebs solution ($n = 4$; data not shown). **R**(−)-niguldipine (3–300 nM; Figure 4b) had approximately 30-times less affinity than **S**(+)-niguldipine and also appeared to deviate from simple competitive antagonism. Antagonism by **S**(+)-niguldipine was not attributable to antagonism of L-type Ca^{2+} channels as other experiments revealed that the dihydropyridine Ca^{2+} channel antagonist, nitrendipine, produced only a limited (5.8 fold) parallel dextral shift of concentration-effect curves to NA (1 μM; $n = 4$; data not shown). Limited dextral shifts (<6 fold) have also been observed using high concentrations of other L-type Ca^{2+} channel antagonists (Blue *et al.*, 1991). Likewise, antagonism by **S**(+)-niguldipine appeared specific for α_1-adrenoceptors as vasoconstrictor responses to 5-hydroxytryptamine (0.01–3 μM) were not affected by **S**(+)-niguldipine (3 nM; $n = 4$; data not shown). In contrast, dehydroniguldipine (10–100 nM; Figure 4c) produced parallel dextral shifts in the concentration-effect curves to NA with no significant reduction in the maximal response, yielding a pK_B estimate of 9.0 (Table 1).

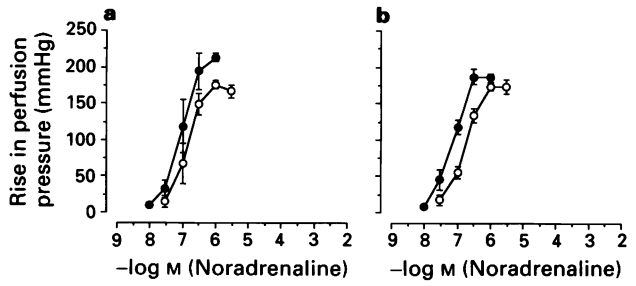

Figure 2 Concentration-effect curves to noradrenaline (NA) before (●) and after (○) treatment with chloroethylclonidine (CEC): (a) 10 μM or (b) 100 μM. Kidneys were perfused with Krebs solution containing CEC for 20 min and then perfused with CEC-free Krebs solution for 40 min before construction of a second concentration-effect curve to NA. Each point represents the mean ± s.e.mean obtained from 4 kidneys.

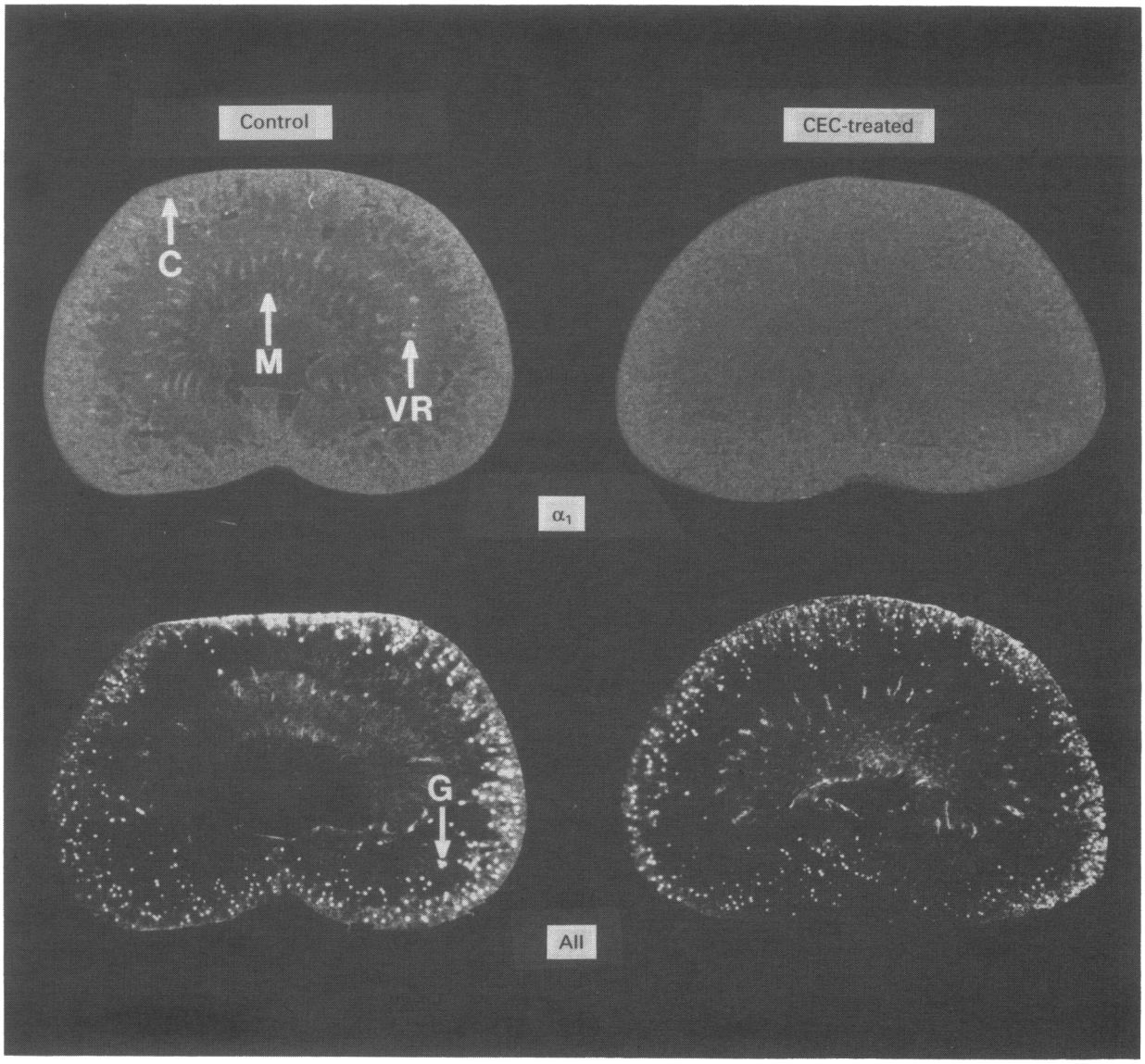

Figure 3 Effect of chloroethylclonidine (CEC; 100 μM) on [^{3}H]-prazosin and [^{125}I]-Sar^1-Ile^8-angiotensin II binding in rat kidney. Kidneys were perfused with Krebs solution containing CEC (100 μM) or vehicle for 20 min and then perfused with CEC-free Krebs solution for 40 min before preparation for autoradiography. C = cortex, M = medulla, VR = vasa recta, G = glomeruli.

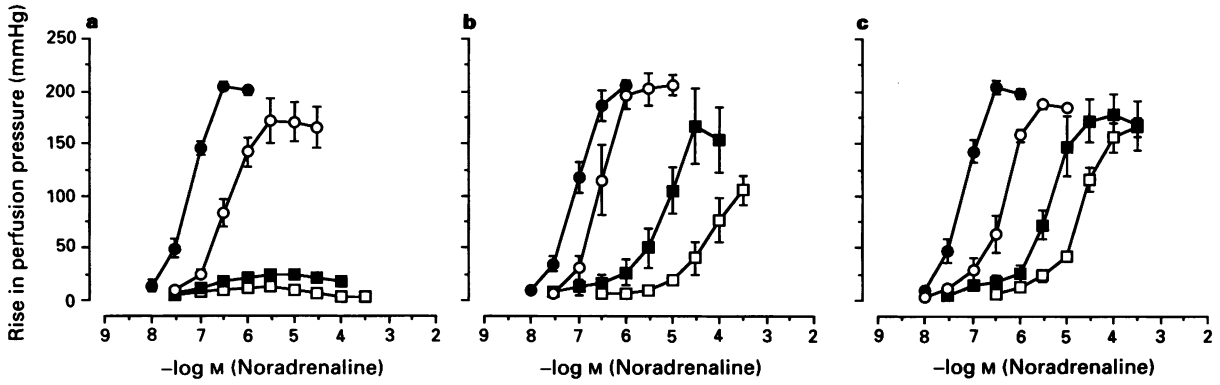

Figure 4 Interaction of noradrenaline (NA) with niguldipine. (a) Concentration-effect curves to NA in the absence (●, $n = 12$) and presence of S(+)-niguldipine: 0.03 nM (○, $n = 4$), 0.1 nM (■, $n = 4$) and 0.3 nM (□, $n = 4$). (b) Concentration-effect curves to NA in the absence (●, $n = 12$) and presence of R(−)-niguldipine: 3 nM (○, $n = 4$), 30 nM (■, $n = 4$) and 300 nM (□, $n = 4$). (c) Concentration-effect curves to NA in the absence (●, $n = 12$) and presence of dehydroniguldipine 10 nM (○, $n = 4$), 30 nM (■, $n = 4$), 100 nM (□, $n = 4$). Kidneys were perfused with Krebs solution containing antagonist for 60 min prior to construction of the second concentration-effect curve to NA. Each point represents the mean ± s.e.mean where larger than the symbol.

The mechanism of insurmountable antagonism was investigated by determining whether prazosin could protect against insurmountable antagonism produced by S(+)-niguldipine. Prazosin (50 nM) competitively antagonized concentration-effect curves to NA (169 fold dextral shift; $pA_2 = 9.5$) and was reversible, in part, after a 60 min washout (Figure 5a). When kidneys were co-perfused with both prazosin (50 nM) and S(+)-niguldipine (0.1 nM), prazosin afforded protection against S(+)-niguldipine (Figure 5b).

5-Methyl-urapidil is another ligand selective for the α_{1A}-adrenoceptor subtype (Gross *et al.*, 1988). In the presence of 5-methyl-urapidil (0.03–3 μM; Figure 6a), concentration-effect curves to NA were biphasic, the first phase being antagonized in an apparently insurmountable fashion by the highest concentration of 5-methyl-urapidil.

The interaction of 5-methyl-urapidil (1 and 10 nM) with methoxamine as agonist was investigated because functional studies in rabbit aorta (mixed population of α_{1L}- and α_{1B}-adrenoceptors) have shown that α_{1B}-adrenoceptors are not activated by methoxamine (Oshita *et al.*, 1993). In contrast to NA, concentration-effect curves to methoxamine were shifted in a parallel, monophasic fashion by 5-methyl-urapidil, yielding a pK_B estimate of 9.1 (Figure 6b). Thus, methoxamine failed to stimulate the low affinity site for 5-methyl-urapidil.

Figure 6c shows concentration-effect curves to NA constructed in the presence of 5-methyl-urapidil (1 μM) both before and after pretreatment of kidneys with CEC (100 μM). The first phase of the concentration-effect curve to NA was significantly reduced, but not abolished, by pretreatment with CEC (Figure 6c; compare closed triangles with open diamonds). This results suggests that the initial phase of the concentration-effect curve to NA in the presence of 5-methyl-urapidil is mediated, in part, by a CEC-sensitive α_1-adrenoceptor.

As nitrendipine produced a small dextral shift similar to that seen with CEC (see above), the ability of nitrendipine (1 μM) to inhibit the first phase of the concentration-effect curve to NA in the presence of 5-methyl-urapidil was investigated. Nitrendipine exerted complete inhibition of the first phase of the concentration-effect curve to NA constructed in the presence of 5-methyl-urapidil (Figure 6c; compare closed triangles with inverted triangles). In light of this result, the interaction of NA and 5-methyl-urapidil (3–100 nM) was re-evaluated with nitrendipine (1 μM) present in the Krebs solution throughout. 5-Methyl-urapidil produced parallel dextral shifts of concentration-effect curves to NA with no significant reduction in the maximal response compared to that seen normally in time controls (Figure 6d). Schild regression analysis yielded a line with a slope not significantly different from 1 and a pK_B value of 9.2 for 5-methyl-urapidil (Table 1).

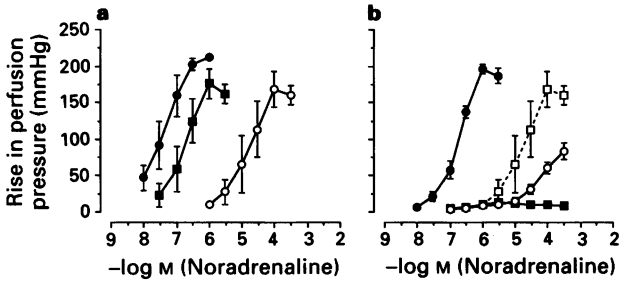

Figure 5 Receptor protection with prazosin against insurmountable antagonism by S(+)-niguldipine. (a) Concentration-effect curves to noradrenaline (NA) in the absence (●) and presence (○) of prazosin (50 nM for 60 min) and following a 60 min washout with prazosin-free Krebs solution (■). (b) Concentration-effect curves to NA before (●) and after coperfusion (○) for 60 min with S(+)-niguldipine (0.1 nM) and prazosin (50 nM) and following removal of prazosin from the Krebs solution (■). The dashed line (□) represents the position of the concentration-effect curve to prazosin (50 nM) alone and is taken from (a). Each point represents the mean ± s.e.mean obtained from 4 kidneys; s.e. are shown where larger than the symbol.

Receptor characterization in the presence of nitrendipine

To characterize further the α_1-adrenoceptor for which 5-methyl-urapidil exhibits high affinity, an agonist profile was obtained with 9 α_1-adrenoceptor agonists in the absence and presence of nitrendipine (1 μM; Figure 7a and b). With the exception of oxymetazoline, all agonists were full agonists relative to NA. The relative order of agonist potency was nearly identical irrespective of whether nitrendipine was present or absent from the Krebs solution (Table 2). Agonist independent pA_2 values for prazosin (obtained in the presence of nitrendipine) suggest that all the agonists produced vasoconstriction via activation of α_1-adrenoceptors with high affinity ($pA_2 > 9$) for prazosin (i.e. α_{1H}-adrenoceptors).

As oxymetazoline behaved as a partial agonist, the ability of oxymetazoline to antagonize vasoconstrictor responses to NA was evaluated. Oxymetazoline (0.1 and 1 μM) produced parallel dextral shifts in the concentration-effect curves to

NA without affecting the maximal response (Figure 7c), yielding a pK_B estimate of 7.9 (Table 1).

Finally, antagonism by HV 723 (an antagonist reported to be selective for α_{1N}- over α_{1L}-adrenoceptors; Muramatsu *et al.*, 1990b) and spiperone (α_{1B}-adrenoceptor-selective: Michel *et al.*, 1989) was evaluated in the presence of nitrendipine (1 μM). Both HV 723 (1 and 10 nM) and spiperone (0.1 μM) produced parallel dextral shifts in the concentration-effect curves to NA with no change in the maximal response. Schild regression analysis yielded a pK_B estimate of 9.3 for HV 723. A pA_2 value of 8.1 was estimated for spiperone (Table 1).

Radioligand binding

The binding of [^{3}H]-prazosin in the rat submaxillary gland and at cloned hamster α_{1B}-, bovine α_{1C}- and rat α_{1D}-adrenoceptors expressed in rat-1 fibroblasts was saturable, of high affinity and consistent with the presence of a single population of binding sites. K_D and B_{max} values respectively were as follows: α_{1B}-clone (49.3 ± 0.70 pM; 2.44 ± 0.17 pmol mg^{-1} protein), α_{1C}-clone (84.7 ± 3.58 pM; 5.2 ± 0.57 pmol mg^{-1} protein), α_{1D}-clone (78.3 ± 0.59 pM; 1.88 ± 0.08 pmol mg^{-1} protein) and submaxillary gland of rat (39 ± 1.7 pM; 193 ± 9.5 fmol mg^{-1} protein). Displacement curves in all

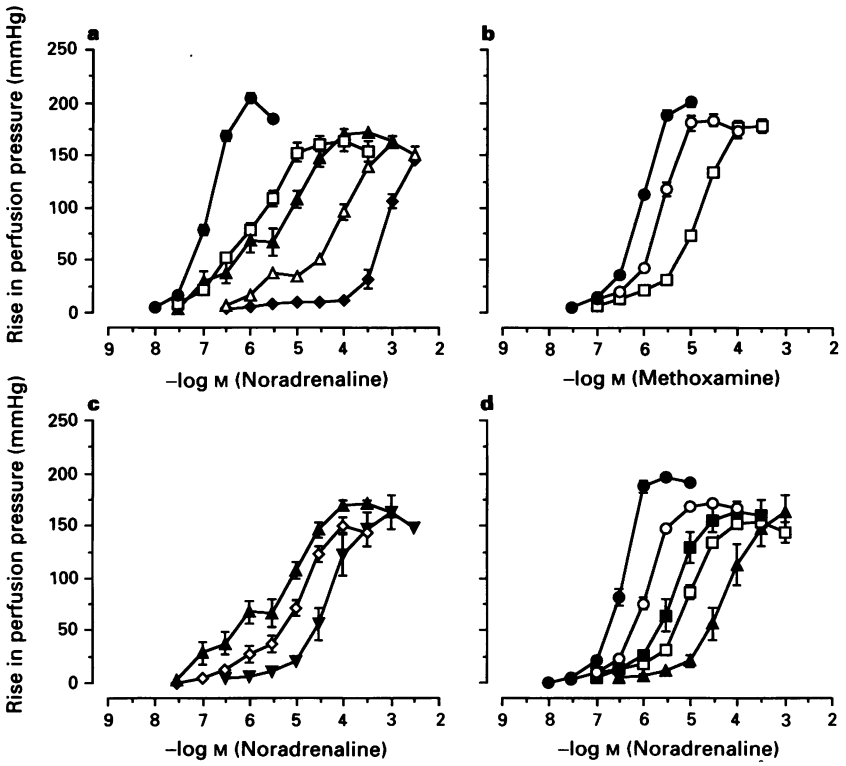

Figure 6 Interaction of 5-methyl-urapidil with α_1-adrenoceptors. (a) Concentration-effect curves to NA in the absence (●, $n = 16$) and presence of 5-methyl-urapidil: 0.03 nM (□, $n = 4$), 0.1 μM (▲, $n = 4$), 1 μM (△, $n = 4$) and 3 μM (◆, $n = 4$). (b) Concentration-effect curves to methoxamine in the absence (●, $n = 8$) and presence of 5-methyl-urapidil: 0.003 μM (○, $n = 4$) and 0.03 μM (□, $n = 4$). (c) Concentration-effect curves to NA obtained in the presence of 0.1 μM 5-methyl-urapidil (▲, $n = 4$) and after pretreatment (100 μM for 20 min) with chloroethylclonidine (◇, $n = 4$) or in the presence of 1 μM nitrendipine (▼, $n = 4$). (d) Concentration-effect curves to NA (constructed in the presence of 1 μM nitrendipine) and measured in the absence (●, $n = 16$) and presence of 5-methyl-urapidil: 0.003 μM (○, $n = 4$), 0.01 μM (■, $n = 4$), 0.03 μM (□, $n = 4$) and 0.1 μM (▲, $n = 4$). Each point represents the mean ± s.e.mean where larger than the symbol.

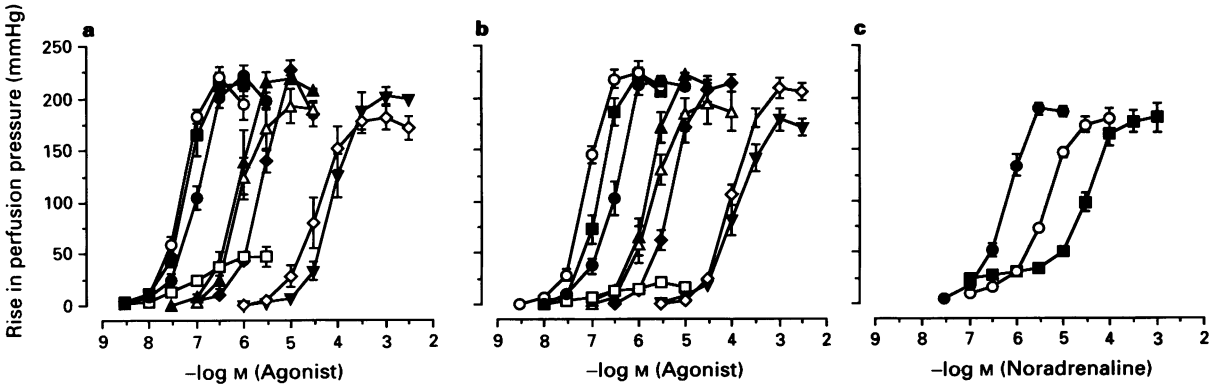

Figure 7 Effect of α_1-adrenoceptor agonists. Agonist profile in (a) the absence and (b) the presence of nitrendipine (1 μM): (○) cirazoline, $n = 4$; (■) adrenaline, $n = 4$; (●) noradrenaline, $n = 4$; (□) oxymetazoline, $n = 4$; (▲) phenylephrine, $n = 4$; (△) amidephrine, $n = 4$; (◆) methoxamine, $n = 4$; (◇) dopamine, $n = 4$; (▼) isoprenaline, $n = 4$. (c) Concentration-effect curves to noradrenaline in the presence of nitrendipine (1 μM) obtained in the absence (●, $n = 8$) and presence of oxymetazoline: 0.1 μM (○), $n = 4$) and 1 μM (●, $n = 4$). Each point represents the mean ± s.e.mean where larger than the symbol.

four preparations were best described by a single site model. Estimated pK_i values are shown in Table 3. Of the cloned α_1-adrenoceptors, only pK_i values from the bovine α_{1C}-adrenoceptor showed a high correlative association with pA_2 values obtained in rat kidney ($R^2 = 0.85$; Figure 8). Likewise, pK_i values from rat submaxillary gland and pK_i values from other α_{1A}-adrenoceptors of rat (abstracted from the literature) correlated well with pA_2 values obtained in rat kidney ($R^2 = 0.79$ and 0.89 respectively; Table 4). As might be expected, strong correlations were observed between pK_i values from rat submaxillary gland, cloned bovine α_{1C}-adrenoceptors, and rat α_{1A}-adrenoceptors, the latter values being drawn from the literature (Table 4).

Discussion

The antagonist affinity profile obtained here demonstrates unequivocally that the α_{1A}-adrenoceptor, as originally defined pharmacologically (see Introduction), is the predominant subtype mediating vasoconstriction in the isolated perfused kidney of rat. Furthermore, we now show that this subtype can be 'isolated' for quantitative evaluation by adding nitrendipine to the perfusion fluid. Thus, the present results extend previous studies on α_1-adrenoceptor classification in kidney of rat (Eltze *et al.*, 1991; Eltze & Boer, 1992; Blue *et al.*, 1992; Elhawary *et al.*, 1992; Sattar & Johns, 1994a,b).

Clear evidence that more than one subtype of α_1-adrenoceptor mediates vasoconstriction to exogenous NA in the kidney was obtained with 5-methyl-urapidil. Concentration-effect curves to NA were found to be biphasic in the presence of 5-methyl-urapidil, the initial phase being antagonized in an apparently insurmountable manner by increasing concentrations of 5-methyl-urapidil. A comprehensive characterization of the α_1-adrenoceptor(s) mediating the initial phase to NA could not be undertaken, however, as the site or sites could not be 'isolated' pharmacologically for study. Partial sensitivity to CEC, and an apparent inability of methoxamine to activate the site(s), provide some descriptive characteristics. Certainly, CEC-sensitive α_1-adrenoceptors were demonstrated independently in functional (Figure 2) and autoradiographic (Figure 3) experiments.

Another characteristic of the α_1-adrenoceptor(s) for which 5-methyl-urapidil exhibited low affinity is that responses mediated by this receptor(s) are sensitive to inhibition by the L-type Ca^{2+} channel antagonist, nitrendipine. Although sen-

Table 2 Relative potency of α_1-adrenoceptor agonists estimated in the absence and presence of nitrendipine in isolated perfused kidney of rat

	No nitrendipine		*Nitrendipine* (1 μM)		
Agonist	$EC_{100\,mmHg}$ (μM)	*Relative potency*	$EC_{100\,mmHg}$ (μM)	*Relative potency*	*Prazosin* pA_2
Cirazoline	0.04	1	0.06	1	9.5[a]
Adrenaline	0.05	1	0.13	2	9.3[a]
Oxymetazoline	0.09[b]	2	NR	–	9.6[cd]
Noradrenaline	0.09	2	0.29	5	9.5[a]
Phenylephrine	0.62	14	1.43	23	9.3[a]
Amidephrine	0.75	17	1.97	31	9.2[a]
Methoxamine	1.90	43	4.55	71	9.3[d]
Dopamine	70.0	1590	138.0	2210	9.6[d]
Isoprenaline	71.0	1610	91.0	1456	9.1[d]

[a]Versus 30 nM prazosin. [b]Calculated at $EC_{50\%}$. [c]pA_2 value obtained in the absence of nitrendipine as oxymetazoline produced no vasoconstriction in the presence of nitrendipine. [d]Versus 3 nM prazosin. NR = no response.

Table 3 Summary of affinity estimates for α_1-adrenoceptor ligands in isolated perfused kidney of rat (pK_B or pA_2 values), submaxillary gland of rat, cloned hamster α_{1B}-, bovine α_{1C}- and rat α_{1D}-adrenoceptors and α_{1A}-adrenoceptors in several different tissues of rat taken from the literature (pK_i values ± s.e.mean)[a]

		Cloned						
Ligand	*Kidney*[b]	α_{1B}	α_{1C}	α_{1D}	*Submaxillary Gland* (α_{1A})	*Literature*[c] (α_{1A})	(n)	*References*[d]
Prazosin	9.5	10.1 (0.02)	9.9 (0.02)	9.9 (0.07)	10.1 (0.01)	10.1 (0.05)	(38)	1–18, 20–27
WB 4101	10.3	8.6 (0.05)	10.0 (0.06)	9.6 (0.04)	10.1 (0.08)	9.9 (0.10)	(18)	1, 2, 5, 7, 9, 12–16, 18, 20–23, 26, 27
Phentolamine	8.6	7.9 (0.07)	8.9 (0.05)	8.1 (0.19)	8.8 (0.10)	8.7 (0.11)	(13)	1–3, 5, 7, 9, 11, 15, 16, 22, 24, 26, 27
S(+)-Niguldipine	10.5	7.0 (0.57)	9.8 (0.30)	7.1 (0.13)	9.7 (0.09)	10.3 (0.18)	(8)	5, 6, 11, 12, 14, 17, 19, 27
R(−)-Niguldipine	9.1	ND	ND	ND	ND	8.9	(2)	5, 11
5-Methyl-urapidil	9.2	7.5 (0.02)	9.4 (0.05)	8.0 (0.03)	9.2 (0.08)	9.0 (0.08)	(22)	4, 5, 7, 10–12, 14, 18–20, 22, 24–27
Oxymetazoline	7.9	6.4 (0.06)	7.8 (0.05)	6.5 (0.13)	8.5 (0.04)	8.3 (0.17)	(6)	7, 9, 14, 24
HV 723	9.3	8.4 (0.11)	9.6 (0.03)	9.4 (0.09)	ND	8.9 (0.11)	(4)	2, 15, 16, 20
Spiperone	8.1	8.9 (0.06)	8.5 (0.04)	8.5 (0.16)	8.1 (0.03)	8.2 (0.08)	(4)	9, 17, 25, 26

[a]All pK_i values were determined with [^{3}H]-prazosin as ligand. [b]pK_B or pA_2 values versus noradrenaline in isolated perfused kidney of rat were taken from Table 1. [c]pK_i values from high affinity binding sites in rat brain, heart, lung, kidney, vas deferens and submaxillary gland. [d]Literature source for α_{1A}-adrenoceptor affinity estimates. ND = not determined. References: (1) Morrow *et al.*, 1985; (2) Morrow & Creese, 1986; (3) Honda *et al.*, 1987; (4) Gross *et al.*, 1988; (5) Boer *et al.*, 1989; (6) Hanft & Gross, 1989a; (7) Hanft & Gross, 1989b; (8) Hanft *et al.*, 1989; (9) Michel *et al.*, 1989; (10) Hanft & Gross, 1990; (11) Michel *et al.*, 1990; (12) Eltze *et al.*, 1991; (13) Feng *et al.*, 1991; (14) Klijn *et al.*, 1991; (15) Oshita *et al.*, 1991; Tsuchihashi *et al.*, 1991; (17) Eltze & Boer, 1992; (18) Hiramatsu *et al.*, 1992; (19) Jackson *et al.*, 1992; (20) Muramatsu, 1992; (21) Ohmura *et al.*, 1992; (22) Yazawa *et al.*, 1992; (23) Ivorra *et al.*, 1993; (24) Michel *et al.*, 1993a; (25) Schwietert *et al.*, 1993; (26) Sleight *et al.*, 1993; (27) Salles & Badia, 1994.

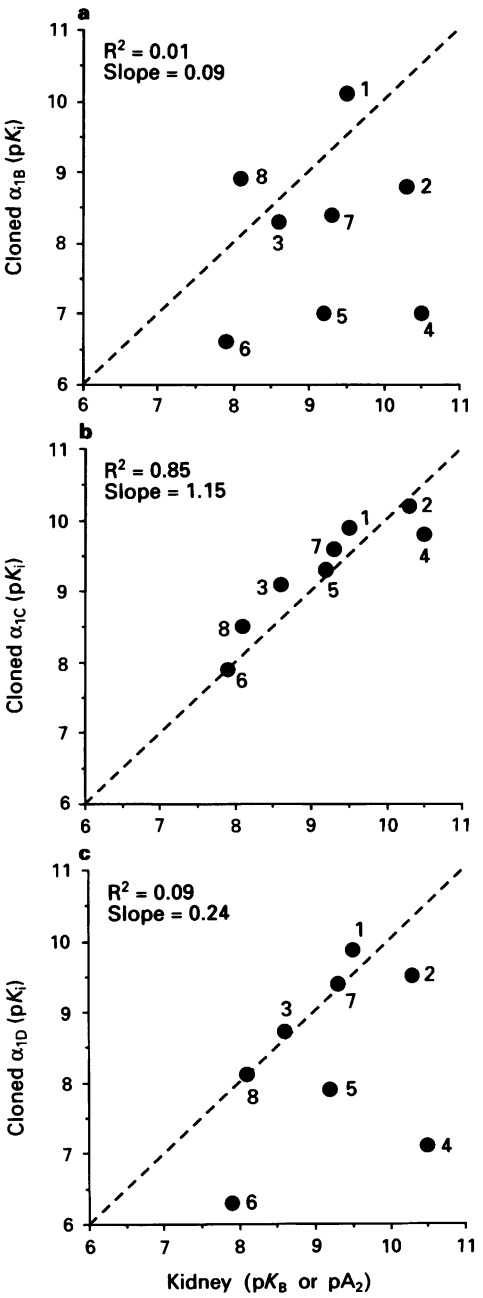

Figure 8 Correlations of antagonist affinity estimates (pA_2 values) at α_1-adrenoceptors in isolated perfused kidney of rat and antagonist affinity estimates (pK_i values) in cloned (a) α_{1B}-adrenoceptors, (b) α_{1C}-adrenoceptors and (c) α_{1D}-adrenoceptors: (1) prazosin; (2) WB 4101; (3) phentolamine; (4) S(+)-niguldipine; (5) 5-methyl-urapidil; (6) oxymetazoline; (7) HV 723; (8) spiperone. Dashed lines indicate the line of identity.

sitivity to nitrendipine does not provide insight into the identity of the receptor, it is nevertheless an important finding as it allows pharmacological 'isolation' of the α_1-adrenoceptor at which 5-methyl-urapidil exerts *high* affinity and, therefore, characterization and classification of the site. For this reason, agonists and antagonists (tested subsequently to using 5-methyl-urapidil) were studied in the presence of nitrendipine (see Table 1).

The α_1-adrenoceptor for which 5-methyl-urapidil exhibited high affinity was characterized using several α_1-adrenoceptor subtype-selective antagonists. Competitive antagonism was observed with prazosin and the α_{1A}-adrenoceptor selective antagonists, WB 4101, phentolamine, 5-methyl-urapidil and oxymetazoline, as well as spiperone (α_{1B}-selective; Michel *et al.*, 1989) and HV 723 (reported to be selective for α_{1N}- over α_{1L}-adrenoceptors; Muramatsu *et al.*, 1990b). The following relative order of antagonist affinity (pK_B value) was obtained: WB 4101 (10.3)> prazosin (9.5)≈HV 723 (9.3)≈5-methyl-urapidil (9.2)> phentolamine (8.6)> spiperone≈oxymetazoline (7.9). This order and relativity of antagonist affinity clearly defines an α_{1A}-adrenoceptor.

The S(+)-isomer of the dihydropyridine Ca^{2+} channel antagonist, niguldipine, is the most selective ligand available for the α_{1A}-adrenoceptor subtype (Boer *et al.*, 1989). In the present study, concentration-effect curves to NA were insurmountably and irreversibly antagonized by subnanomolar concentrations of S(+)-niguldipine. Insurmountable antagonism by S(+)-niguldipine is not attributable to blockade of L-type Ca^{2+} channels as vasoconstrictor responses to NA in rat kidney are largely resistant to nitrendipine (and other L-type Ca^{2+} channel antagonists: see Blue *et al.*, 1991). Indeed, the failure of S(+)-niguldipine to antagonize vasoconstrictor responses to 5-hydroxytryptamine, coupled with the ability of prazosin to protect partially against antagonism by S(+)-niguldipine, suggests strongly that direct binding of S(+)-niguldipine to α_1-adrenoceptors is involved. The insurmountable nature and irreversibility of S(+)-niguldipine may result because of high affinity for the α_{1A}-adrenoceptor (see Rang, 1966) as well as high lipophilicity (Boer *et al.*, 1989). The relative antagonistic potency observed with the S(+)- versus R(−)-isomer of niguldipine (approximately 30 fold) is fully consistent with reported characteristics of α_{1A}-adrenoceptors (Boer *et al.*, 1989; Michel *et al.*, 1990). It is also of interest to note that dehydroniguldipine, a non-chiral analogue of niguldipine, antagonized concentration-effect curves to NA competitively with a potency similar to that of R(−)-niguldipine. This confirms the importance of stereochemistry in the interaction of niguldipine with α_{1A}-adrenoceptors.

In addition to the antagonist affinity profile discussed above, the present study provides a comprehensive agonist potency profile at the α_{1A}-adrenoceptor subtype using 9 ligands. To date, the usefulness of agonists as tools for discrimination and classification of α_1-adrenoceptor subtypes

Table 4 Correlations of affinity estimates for α_1-adrenoceptor ligands in isolated perfused kidney of rat, submaxillary gland of rat, cloned hamster α_{1B}-, bovine α_{1C}- and rat α_{1D}-adrenoceptors and α_{1A}-adrenoceptors in several different tissues of rat taken from the literature

			R-squared (slope)			
	Kidney (α_{1A})	*Submaxillary gland* (α_{1A})	*Literature* (α_{1A})	*Cloned* (α_{1B})	*Cloned* (α_{1C})	*Cloned* (α_{1D})
Kidney (α_{1A})	–	0.79	0.89	0.02*	0.85	0.12*
	–	(1.15)	(1.09)	(0.12)*	(1.14)	(0.28)*
Submaxillary gland (α_{1A})	0.79	–	0.89	0.13*	0.84	0.29*
	(1.15)	–	(0.85)	(0.22)*	(0.95)	(0.35)*
Literature (α_{1A})	0.89	0.89	–	0.08*	0.81	0.16*
	(1.09)	(0.85)	–	(0.20)*	(0.84)	(0.29)*
Cloned α_{1C}	0.85	0.82	0.82	0.22*	–	0.39*
	(1.15)	(0.94)	(0.85)	(0.31)*	–	(0.42)*

*Significantly different from 1 ($P<0.05$). Other R-squared values and slopes of regression lines are not significantly different from 1 and slopes do not differ significantly from the line identity ($P<0.05$).

has been limited. Comparison of agonist potency ratios in rat kidney (Eltze & Boer, 1992; present study) with those obtained in both guinea-pig spleen (α_{1B}-adrenoceptor; Eltze, 1994) and rat thoracic aorta (putative α_{1D}-adrenoceptor; Saussy *et al.*, 1994; Eltze & Boer, 1992), however, suggest that certain agonists (e.g. methoxamine) may exhibit selectivity for the α_{1A}-adrenoceptor subtype (see also Figure 6b).

A most important finding from the present study is the high correlative association between antagonist affinity estimates obtained functionally in the perfused kidney and pK_i values obtained from radioligand binding experiments using the cloned bovine α_{1C}-adrenoceptor. It is this correlation which led us to conclude that the α_{1A}-adrenoceptor mediating vasoconstrictor responses to NA in rat kidney represents a species homologue of the cloned α_{1C}-adrenoceptor (see Ford *et al.*, 1994). This conclusion is now supported by high correlative associations between the affinity of ligands for the cloned bovine α_{1C}-adrenoceptor and their binding affinity for α_{1A}-adrenoceptors in submaxillary gland of rat as well as other rat tissues (values abstracted from the literature). Radioligand binding studies reported recently by others (Faure *et al.*, 1994; Forray *et al.*, 1994; Laz *et al.*, 1994) also support the equivalency of the α_{1A}-adrenoceptor with the α_{1C}-adrenoceptor, and considerable molecular biological evidence now exists for the presence of the α_{1C}-adrenoceptor gene in rat (Alonso-Llamazares *et al.*, 1993; Laz *et al.*, 1994; Rokosh *et al.*, 1994; Faure *et al.*, 1994; Price *et al.*, 1994) despite an initial negative report (Schwinn & Lomasney, 1992).

Apart from radioligand binding data, only one published functional study has attempted to equate the α_{1A}- and α_{1C}-adrenoceptors (Forray *et al.*, 1994). A high correlative association was found between the pA_2 values for 6 α_1-adrenoceptor antagonists measuring human prostatic contraction *in vitro* and their pK_i values at the cloned α_{1C}-adrenoceptor of man. However, only 3 of these antagonists (indoramin, 5-methyl-urapidil, and SNAP 1069) exhibited selectivity for the cloned α_{1C}-adrenoceptor over the cloned human α_{1B}- and α_{1D}-adrenoceptors. Thus, conclusions must be guarded. Indeed, we have reported recently that the α_1-adrenoceptor mediating contraction in prostate of man differs from the α_{1A}-adrenoceptor subtype (Ford *et al.*, 1995) and remains for further definition.

To summarize, the α_{1A}-adrenoceptor is the predominant α_1-adrenoceptor subtype mediating vasoconstrictor responses to exogenously administered NA in the isolated perfused kidney of rat. These α_{1A}-adrenoceptors, characterized functionally, exhibit equivalency to other native α_{1A}-adrenoceptors from rat as well as to the cloned α_{1C}-adrenoceptor, making it unnecessary to postulate the existence of a fourth α_1-adrenoceptor with high affinity for prazosin (Schwinn & Lomasney, 1992). Thus, the following classification of α_1-adrenoceptor with high affinity ($pA_2 > 9$) for prazosin is recommended: α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (Hieble *et al.*, 1995). Finally, the isolated perfused kidney of rat, studied in the presence of nitrendipine, appears to offer an unequivocal functional preparation for the singular study of the α_{1A}-adrenoceptor. This preparation is important because a candidate functional preparation for the 'classical' α_{1A}-adrenoceptor (vas deferens of rat; Han *et al.*, 1987) has been shown to exhibit clear-cut α_1-adrenoceptor heterogeneity (Ohmura *et al.*, 1992). The presence of α_{1A}-adrenoceptors mediating vasoconstriction in mesenteric (Williams & Clarke, 1994) and renal vascular smooth muscle of rat suggests a role in the maintenance of blood pressure under physiological conditions. Innervation of mesenteric and renal vascular α_{1A}-adrenoceptors by postganglionic sympathetic nerves has been demonstrated (Blue & Clarke, 1992; Williams & Clarke, 1995).

References

ALONSO-LLAMAZARES, A., ZAMANILLO, D., FERNANDEZ, A., CHINCHETRU, M.A. & CALVO, P. (1993). Differential expression of the α_{1C} adrenergic receptor subtype in rat tissues. *NeuroReport*, **4**, 1266–1268.

BLUE, D.R. & CLARKE, D.E. (1990). Characterisation and modulation of vascular α-adrenoceptor responses in rat kidney. *Br. J. Pharmacol.*, **99**, 204P.

BLUE, D.R. Jr., VIMONT, R.L. & CLARKE, D.E. (1992). Evidence for a noradrenergic innervation to α_{1A}-adrenoceptors in rat kidney. *Br. J. Pharmacol.*, **107**, 414–417.

BLUE, D.R., WHITING, R.L. & CLARKE, D.E. (1991). Pharmacological profile of an alpha$_{1A}$-adrenoceptor in the renal vasculature: studies with (+)- and (−)-niguldipine. In *Adrenoceptors: Structure, Mechanisms, Function: Advances in Pharmacological Science*, ed. Abadi, S.Z. pp. 373–374. Basel: Birkhauser verlag.

BOER, R., GRASSEGGER, A., SCHUDT, C. & GLOSSMANN, H. (1989). (+)-Niguldipine binds with very high affinity to Ca^{2+} channels and to a subtype of α_1-adrenoceptors. *Eur. J. Pharmacol.-Mol. Pharmacol. Sec.*, **172**, 131–145.

BOND, R.A., ORNSTEIN, A.G. & CLARKE, D.E. (1989). Unsurmountable antagonism to 5-hydroxytryptamine in rat kidney results from pseudoirreversible inhibition rather than multiple receptors or allosteric receptor modulation. *J. Pharmacol. Exp. Ther.*, **249**, 401–410.

BYLUND, D.B. (1985). Heterogeneity of alpha-2-adrenergic receptors. *Pharmacol. Biochem. Behav.*, **22**, 835–843.

CLARKE, D.E., FORD, A.P.D.W., WILLIAMS, T.J., BONHAUS, D., VIMONT, R. & BLUE, D.R. Jr. (1994). Pharmacological evidence for the equivalency of the α_{1A} adrenoceptor of rat and the cloned bovine α_{1C}-adrenoceptor: a revised α_1-adrenoceptor classification scheme. Abstract from the *Pharmacology of Adrenoceptors Satellite*, King of Prussia, July 22–23.

CLARKE, D.E., WHITING, R.L., PFISTER, J. & BLUE, D.R. (1991). Antagonism of α_1-adrenoceptor subtypes by niguldipine and 5-methyl-urapidil in rat kidney. *Br. J. Pharmacol.*, **102**, 196P.

COTECCHIA, S., SCHWINN, D.A., RANDALL, R.R., LEFKOWITZ, R.J., CARON, M.G. & KOBILKA, B.K. (1988). Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 7159–7163.

DOCHERTY, J., FLAVAHAN, N.A. & VANHOUTTE, P.M. (1987). α_1-Adrenoceptor subclassification in vascular smooth muscle: a premature proposal? *Trends Pharmacol. Sci.*, **8**, 123–125.

DREW, G.M. (1985). What do antagonists tell us about α-adrenoceptors? *Clin. Sci.*, **68**, 15s–19s.

DUNN, W.R., MCGRATH, J.C. & WILSON, V.G. (1989). Expression of functional postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery – a permissive role for angiotensin II? *Br. J. Pharmacol.*, **96**, 259–261.

ELHAWARY, A.M., PETTINGER, W.A. & WOLFF, D.W. (1992). Subtype-selective alpha-1 adrenoceptor alkylation in the rat kidney and its effect on the vascular pressor response. *J. Pharmacol. Exp. Ther.*, **260**, 709–713.

ELTZE, M. (1994). Characterization of the α_1-adrenoceptor subtype mediating contraction of guinea-pig spleen. *Eur. J. Pharmacol.*, **260**, 211–220.

ELTZE, M. & BOER, R. (1992). The adrenoceptor agonist, SDZ NVI 085, discriminates between α_{1A}- and α_{1B}-adrenoceptors subtypes in vas deferens, kidney and aorta of the rat. *Eur. J. Pharmacol.*, **224**, 125–136.

ELTZE, M., BOER, R., SANDERS, K.H. & KOLASSA, N. (1991). Vasodilatation elicited by 5-HT_{1A} receptor agonists in constant-pressure-perfused rat kidney is mediated by blockade of α_{1A}-adrenoceptors. *Eur. J. Pharmacol.*, **202**, 33–44.

FAURE, C., PIMOULE, C., ARBILLA, S., LANGER, S.Z. & GRAHAM, D. (1994). Expression of α_1-adrenoceptor subtypes in rat tissues: implications for α_1-adrenoceptor classification. *Eur. J. Pharmacol. Mol. Pharmacol. Sec.* **268**, 141–149.

FENG, F., PETTINGER, W.A., ABEL, P.W & JEFFRIES, W.B. (1991). Regional distribution of α_1-adrenoceptor subtypes in rat kidney. *J. Pharmacol. Exp. Ther.*, **258**, 263–268.

FLAVAHAN, N.A. & VANHOUTTE, P.M. (1986). α_1-Adrenoceptor subclassification in vascular smooth muscle. *Trends Pharmacol. Sci.*, **7**, 347–349.

FLAVAHAN, N.A., VOS, A.A.A.M. & VANHOUTTE, P.M. (1987). Subclassification of α_1-adrenoceptors mediating contraction of the canine pulmonary artery. *Br. J. Pharmacol.*, **91**, 331P.

FORD, A.P.D.W., ARREDONDO, N.F., BLUE, D.R., BONHAUS, D.W., KAVA, M.S., WILIMANS, T.J., VIMONT, R.L., ZHU, Q.-M., PFISTER, J.R. & CLARKE, D.E. (1995). Do α_{1A}- (α_{1C}-) adrenoceptors (AR) mediate prostatic smooth muscle contraction in man? Studies wtih RS 17053. *Br. J. Pharmacol.*, (in press).

FORD, A.P.D.W., WILLIAMS, T.J., BLUE, D.R. & CLARKE, D.E. (1994). α_1-Adrenoceptor classification: sharpening Occam's razor. *Trends Pharmacol. Sci.*, **15**, 167–170.

FORRAY, C., BARD, J.A., WETZEL, J.M., CHIU, G., SHAPIRO, E., TANG, R., LEPOR, H., HARTIG, P.R., WEINSHANK, R.L., BRANCHEK, T.A. & GLUCHOWSKI, C. (1994). The α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1C} subtype. *Mol. Pharmacol.*, **45**, 703–708.

GROSS, G., HANFT, G. & MEHDORN, H.M. (1989). Demonstration of α_{1A}- and α_{1B}-adrenoceptor sites in human brain tissue. *Eur. J. Pharmacol.*, **169**, 325–328.

GROSS, G., HANFT, G. & RUGEVICS, C. (1988). 5-Methyl-urapidil discriminates between subtypes of the α_1-adrenoceptor. *Eur. J. Pharmacol.*, **151**, 333–335.

HAN, C., WILSON, K.M. & MINNEMAN, K.P. (1990). α_1-Adrenergic receptor subtypes and formation of inositiol phosphates in dispersed hepatocytes and renal cells. *Mol. Pharmacol.*, **37**, 903–910.

HAN, C., ABEL, P.W. & MINNEMAN, K.P. (1987). α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. *Nature*, **329**, 333–335.

HANFT, G. & GROSS, G. (1989a). Heterogeneity of α_1-adrenoceptor binding sites in the rat heart – subtype-selective agonists and antagonists. *Br. J. Pharmacol.*, **98**, 652P.

HANFT, G. & GROSS, G. (1989b). Subclassification of α_1-adrenoceptor recognition sites by urapidil derivatives and other selective antagonists. *Br. J. Pharmacol.*, **97**, 691–700.

HANFT, G. & GROSS, G. (1990). The effect of reserpine, desipramine and thyroid hormone on α_{1A}- and α_{1B}-adrenoceptor binding sites: evidence for a subtype-specific regulation. *Br. J. Clin. Pharmacol.*, **30**, 125S–127S.

HANFT, G., GROSS, G., BECKERINGH, J.J. & KORSTANJE, C. (1989). α_1-Adrenoceptors: the ability of various agonists and antagonists to discriminate between two distinct [^{3}H]prazosin binding sites. *J. Pharm. Pharmacol.*, **41**, 714–716.

HIEBLE, J.P., BYLUND, D.B., CLARKE, D.E., EIKENBURG, D.C., LANGER, S.Z., LEFOKOWITZ, R.T., MINNEMAN, K.P. & RUFFOLO, R.R. Jr. (1995). Recommendation for nomenclature of α_1-adrenoceptors: consensus update. *Pharmacol. Rev.*, (in press).

HIRAMATSU, Y., MURAOKA, R., KIGOSHI, S. & MURAMATSU, I. (1992). 5-Methylurapidil may discriminate between α_1-adrenoceptors with a high affinity for WB4101 in rat lung. *Br. J. Pharmacol.*, **105**, 6–7.

HOLCK, M.I., JONES, C.H.M. & HAEUSLER, G. (1983). Differential interactions of clonidine and methoxamine with the postsynaptic α-adrenoceptors of rabbit main pulmonary artery. *J. Cardiovasc. Pharmacol.*, **5**, 240–248.

HONDA, K., NAKAGAWA, C. & TERAI, M. (1987). Further studies on (±)-YM-12617, a potent and selective α_1-adrenoceptor antagonist and its individual optical enantiomers. *Naunyn-Schmied. Arch. Pharmacol.*, **336**, 295–302.

IVORRA, D., GASCON, S., VILA, E. & BADIA, A. (1993). α_1-Adrenoceptor subtypes and inositol phosphates production in heart ventricles of spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, **21**, 931–936.

JACKSON, C.A., MICHEL, M.C. & INSEL, P.A. (1992). Expression of renal α_1-adrenergic subtypes in established hypertension. *J. Cardiovasc. Pharmacol.*, **19**, 857–862.

JOHNSON, R.D. & MINNEMAN, K.P. (1987). Differentiation of α_1-adrenergic receptors linked to phosphatidylinositol turnover and cyclic AMP accumulation in rat brain. *Mol. Pharmacol.*, **31**, 239–246.

KIMURA, T., MURAMATSU, I., KIGOSHI, S. & MURAOKA, R. (1993). Characterization of α_1- and β-adrenoceptor subtypes in the mongolian gerbil cerebral cortex. *Mol. Neuropharmacol.*, **3**, 69–73.

KLIJN, K., SLIVKA, S.R., BELL, K. & INSEL, P.A. (1991). Renal α_1-adrenergic receptor subtypes: MDCK-D1 cells, but not rat cortical membranes possess a single population of receptors. *Mol. Pharmacol.*, **39**, 407–413.

KO, F.-N., GUH, J.-H., YU, S.-M., HOU, Y.-S., WU, Y.-C. & TENG, C.-M. (1994). (−)-Discretamine, a selective α_{1D}-adrenoceptor antagonist, isolated from *Fissistigma glaucescens*. *Br. J. Pharmacol.*, **112**, 1174–1180.

KOHNO, Y., SAITO, H., TAKITA, M., KIGOSHI, S. & MURAMATSU, I. (1994). Heterogeneity of α_1-adrenoceptor subtypes involved in adrenergic contractions of dog blood vessels. *Br. J. Pharmacol.*, **112**, 1167–1173.

LAZ, T.M., FORRAY, C., SMITH, K., BARD, J.A., VAYSSE, P.J.-J., BRANCHEK, T.A. & WEINSHANK, R.L. (1994). The rat homologue of the bovine α_{1C}-adrenergic receptor shows the pharmacological properties of the classical α_{1A}-subtype. *Mol. Pharmacol.*, **46**, 414–422.

LOMASNEY, J.W., COTECCHIA, S., LORENZ, W., LEUNG, W.-Y., SCHWINN, D.A., YANG-FENG, T.L., BROWNSTEIN, M., LEFKOWITZ, R.J. & CARON, M.G. (1991). Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor. *J. Biol. Chem.*, **266**, 6365–6369.

MCGRATH, J.C., BROWN, C.M. & WILSON, V.G. (1989). Alpha-adrenoceptors: a critical review. *Med. Res. Rev.*, **9**, 407–533.

MICHEL, A.D., LOURY, D.N. & WHITING, R.L. (1989). Identification of a single α_1-adrenoceptor corresponding to the α_{1A}-subtype in rat submaxillary gland. *Br. J. Pharmacol.*, **98**, 883–889.

MICHEL, M.C., BUSCHER, R., KERKER, J., KRANEIS, H., ERDBRUGGER, W. & BRODDE, O.E. (1993a). α_1-Adrenoceptor subtype affinities of drugs for the treatment of prostatic hypertrophy: evidence for heterogeneity of chloroethylclonidine-resistant rat renal α_1-adrenoceptor. *Naunyn-Schmied. Arch. Pharmacol.*, **348**, 385–395.

MICHEL, M.C., KERKER, J., BRANCHEK, T.A. & FORRAY, C. (1993b). Selective irreversible binding of chloroethylclonidine at α_1- and α_2-adrenoceptor subtypes. *Mol. Pharmacol.*, **44**, 1165–1170.

MICHEL, M.C., HANFT, G. & GROSS, G. (1990). α_{1B}- but not α_{1A}-adrenoceptors mediate inositol phosphate generation. *Naunyn-Schmied. Arch. Pharmacol.*, **341**, 385–387.

MICHEL, M.C. & INSEL, P.A. (1994). Comparison of cloned and pharmacologically defined rat tissue α_1-adrenoceptor subtypes. *Naunyn-Schmied. Arch. Pharmacol.*, **350**, 136–142.

MORROW, A.L., BATTAGLIA, G., NORMAN, A.B. & CREESE, I. (1985). Identification of subtypes of [^{3}H]-prazosin-labelled α_1 receptor binding sites in rat brain. *Eur. J. Pharmacol.*, **109**, 285–287.

MORROW, A.L. & CREESE, I. (1986). Characterization of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB4104 and [^{3}H]prazosin binding. *Mol. Pharmacol.*, **29**, 321–330.

MURAMATSU, I. (1992). A pharmacological perspective of α_1-adrenoceptors: subclassification and functional aspects. In *α-Adrenoceptors*. pp. 193–202. Tokyo: Excerpta Medica, Ltd.

MURAMATSU, I., KIGOSHI, S. & OSHITA, M. (1990a). Two distinct α_1-adrenoceptor subtypes involved in noradrenaline contraction of the rabbit thoracic aorta. *Br. J. Pharmacol.*, **101**, 662–666.

MURAMATSU, I., OHMURA, T., KIGOSHI, S., HASHIMOTO, S. & OSHITA, M. (1990b). Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. *Br. J. Pharmacol.*, **99**, 197–201.

OHMURA, T., OSHITA, M., KIGOSHI, S. & MURAMATSU, I. (1992). Identification of α_1-adrenoceptor subtypes in the rat vas deferens: binding and functional studies. *Br. J. Pharmacol.*, **107**, 697–704.

OSHITA, M., KIGOSHI, S. & MURAMATSU, I. (1991). Three distinct binding sites for [^{3}H]-prazosin in the rat cerebral cortex. *Br. J. Pharmacol.*, **104**, 961–965.

OSHITA, M., KIGOSHI, S. & MURAMATSU, I. (1993). Pharmacological characterization of two distinct α_1-adrenoceptor subtypes in rabbit thoracic aorta. *Br. J. Pharmacol.*, **108**, 1071–1076.

PIMOULE, C., GRAHAM, D. & LANGER, S.Z. (1992). Characterization of the α_{1B}-adrenoceptor gene product expressed in a stably transfected HeLa cell-line. *Br. J. Pharmacol.*, **105**, 233P.

PRICE, D.T., CHARI, R.S., BERKOWITZ, D.E., MEYERS, W.C. & SCHWINN, D.A. (1994). Expression of α_1-adrenergic receptor subtype mRNA in rat tissues and human SK-N-MC neuronal cells: implications for α_1-adrenergic receptor subtype classification. *Mol. Pharmacol.*, **46**, 221–226.

RANG, H.P. (1966). The kinetics of action of acetylcholine antagonists in smooth muscle. *Proc. R. Soc. B.*, **164**, 488–510.

ROKOSH, D.G., BAILEY, B.A., STEWART, A.F.R., KARNS, L.R., LONG, C.S. & SIMPSON, P.C. (1994). Distribution of α1C-adrenergic receptor mRNA in adult rat tissues by RNase protection assay and comparison with α1B and α1D. *Biochem. Biophys. Res. Commun.*, **200**, 1177–1184.

SALLES, J. & BADIA, A. (1994). Selective enrichment with α_{1A}- and α_{1B}-adrenoceptor subtypes in rat brain cortical membranes. *Eur. J. Pharmacol.*, **266**, 301–308.

SATTAR, M.A. & JOHNS, E.J. (1994a). Evidence for an α_1-adrenoceptor subtype mediating adrenergic vasoconstriction in Wistar Normotensive and stroke-prone spontaneously hypertensive rat kidney. *J. Cardiovasc. Pharmacol.*, **23**, 232–239.

SATTAR, M.A. & JOHNS, E.J. (1994b). α_1-Adrenoceptor subtypes mediating adrenergic vasoconstriction in kidney of two kidney, one-clip Goldblatt and deoxycorticosterone acetate-salt hypertensive rats. *J. Cardiovasc. Pharmacol.*, **24**, 420–428.

SAUSSY, D.L. Jr., GOETZ, A.S., KING, H.K. & TRUE, T.A. (1994). BMY 7378 is a selective antagonist of α_{1D} adrenoceptors (AR): evidence that rat vascular α_1 AR are of the α_{1D} subtype. *Can. J. Physiol. Pharmacol.*, **72** (Suppl.), 323.

SCHMITZ, J.M., GRAHAM, R.M., SAGALOWSKY, A. & PETTINGER, W.A. (1981). Renal alpha-1 and alpha-2 adrenergic receptors: biochemical and pharmacological correlations. *J. Pharmacol. Exp. Ther.*, **219**, 400–406.

SCHWIETERT, H.R., BATINK, H., WILFFERT, B., PFAFFENDORF, M. & VAN ZWIETEN, P.A. (1993). Pharmacological analysis of α_1-adrenoceptor binding sites in rat left ventricular tissue with subtype selective antagonists. *Br. J. Pharmacol.*, **108**, 139P.

SCHWINN, D.A. & LOMASNEY, J.W. (1992). Pharmacological characterization of cloned α_1-adrenoceptor subtypes: selective antagonists suggest the existence of a fourth subtype. *Eur. J. Pharmacol.*, **227**, 433–436.

SCHWINN, D.A., LOMASNEY, J.W., LORENZ, W., SZKLUT, P.J., FREMEAU, R.T. Jr., YANG-FENG, T.L., CARON, M.G., LEFKOWITZ, R.J. & COTECCHIA, S. (1990). Molecular cloning and expression of the cDNA for a novel α_1-adrenergic receptor subtype. *J. Biol. Chem.*, **265**, 8183–8189.

SHARIF, N.A., SHIEH, I.A., BLUE, D.R. & CLARKE, D.E. (1991). Localization and function of alpha$_1$-adrenoceptor (α_1AR) subtypes in rat kidney. *Pharmacologist*, **33**, 214.

SLEIGHT, A.J., KOEK, W. & BIGG, D.C.H. (1993). Binding of antipsychotic drugs at α_{1A}- and α_{1B}-adrenoceptors: risperidone is selective for the α_{1B}-adrenoceptors. *Eur. J. Pharmacol.*, **238**, 407–410.

TSUCHIHASHI, H., MARUYAMA, K., BABA, S., MANO, F., KINAMI, J. & NAGATOMO, T. (1991). Comparison of α_1-adrenoceptors between rat brain and spleen. *Jpn. J. Pharmacol.*, **56**, 523–530.

WILLIAMS, T.J. & CLARKE, D.E. (1995). Pharmacological characterization of α_1-adrenoceptors mediating vasoconstriction to noradrenaline and nerve stimulation in isolated perfused mesentery of rat. *Br. J. Pharmacol.*, **114**, 531–536.

WILSON, K.M. & MINNEMAN, K.P. (1990). Different pathways of [^{3}H]inositol phosphate formation mediated by α_{1a}- and α_{1b}-adrenergic receptors. *J. Biol. Chem.*, **265**, 17601–17606.

YAZAWA, H., TAKANASHI, M., SUDOH, K., INAGAKI, O. & HONDA, K. (1992). Characterization of [^{3}H]YM617, R-(−)-5-[2[[2[ethoxy-ring(n)-^{3}H] (*o*-ethoxyphenoxy)ethyl]amino]-propyl-2- methoxybenzenesulfonamide HCl, a potent and selective alpha-1 adrenoceptor radioligand. *J. Pharmacol. Exp. Ther.*, **263**, 201–206.

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