British Journal of Pharmacology (2002) 136, 127–135

www.nature.com/bjp

Discrimination by SZL49 between contractions evoked by noradrenaline in longitudinal and circular muscle of human vas deferens

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1 The effects of irreversible α_1-adrenoceptor antagonists, SZL-49 (an alkylating analogue of prazosin), dibenamine and benextramine on contractions to noradrenaline (NA) in longitudinal and circular muscle of human epididymal vas deferens were investigated. Competitive α_1-adrenoceptor antagonists were also used to further characterize the α_1-adrenoceptor subtype stimulated by NA in longitudinal and circular muscle.

2 NA evoked concentration-dependent contractions of both muscle types (pD_2; 5.4 and 5.2 respectively). The contraction of circular muscle was comparatively more sensitive than that of longitudinal muscle to pretreatment with SZL-49. In contrast, dibenamine or benextramine produced comparable effects in both muscle types.

3 The relationship between receptor occupancy and contraction in either longitudinal or circular muscle was nonlinear, with half-maximal response requiring similar receptor occupancy (longitudinal muscle 14%, circular muscle 16%). Maximal response in both muscle types occurred with little or no receptor reserve ($<10\%$).

4 The competitive α1-adrenoceptor antagonists produced dextral shifts of the dose-response curves to NA in longitudinal and circular muscle. The inhibitory potencies, estimated from the apparent pK_B values were significantly different in longitudinal and circular muscle respectively for either WB 4101 (pK_B, 8.6 and 9.5) or RS-17053 (pK_B, 7.1 and 9.0) but not for Rec 15/2739 (pK_B, 9.2 and 9.8) or HV 723 (pK_B, 8.3 and 8.4).

5 In conclusion, the potency profile of the competitive α_1-adrenoceptor antagonists and the lack of different receptor reserves for NA in the muscle types suggest that the discriminatory effects of SZL-49 is primarily due to a predominance of the α_{1L}-adrenoceptor subtype in longitudinal muscle and α_{1A}-subtype in circular muscle.

British Journal of Pharmacology (2002) **136,** 127–135

Keywords: Human vas deferens; SZL-49; dibenamine; benextramine; α_{1A}-/α_{1L}- subtype antagonists

Abbreviations: A61603, N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthaleen-1-yl] methanesulphonamide hydrobromide; BMY7378, 8-[2-(4-(2-methoxyphenyl) piperazin-1-yl)ethyl]-8-azaspiro[4,5]decane-7,9-dione; DMSO, dimethyl sulphoxide; HV 723, α-ethyl-3,45-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)-amino)-propyl)-benzene acetonitrile fumarate; NA, noradrenaline; PBZ, phenoxybenzamine; Rec 15/2739, 8-3-[4-(2-methoxyphenyl)–1-piperazinyl]-propylcarbamoyl-3-methyl-4-oxo-2-phenyl-4H-1-benzopyran dihydroch;oride; RS-17053, N-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride; SZL-49, 4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-bicyclo[2,2,2]octa-2,5-dienylcarbonyl-2-piperazine; WB 4101, (2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride

Introduction

Three native α_1-adrenoceptors currently classified as α_{1A}-, α_{1B}- and α_{1D}-subtypes are well characterized by the isolation of distinct cDNAs , detection of corresponding mRNAs (α_{1a}-, α_{1b}- and α_{1d}-) and pharmacological discrimination by a variety of antagonists (Hieble *et al*., 1995; Ford *et al*., 1994). However α_1-adrenoceptors at which prazosin displays different affinities formed the basis for an earlier proposal to subclassify α_1-adrenoceptors into subtypes with high (α_{1H}, $pK_B \geqslant 9$) and low (α_{1L}, $pK_B \leqslant 8.5$) affinities for the antagonist (Hieble & Bond, 1994; McGrath & Wilson, 1988; Flavahan & Vanhoutte, 1986). A subclassification incorporating both current and earlier schemes but using only pharmacological criteria posits that the α_{1H}- group includes α_{1A}-, α_{1B}- and α_{1D}-subtypes whilst the low affinity group consists of two subtypes, α_{1L} and α_{1N} (Oshita *et al*., 1993; Muramatsu *et al*.,1991; 1990). The α_{1L} and α_{1N} subtypes can be distinguished by their low ($pK_B < 8.5$) and high affinity ($pK_B \geqslant 9$) respectively for the α_1-adrenoceptor antagonist, HV723 (Kohno *et al*., 1994; Ohmura *et al*., 1992; Oshita *et al*., 1993). Distinct cDNAs for the α_{1L}- and α_{1N}-subtypes have not been isolated. However, recent studies have found that

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native α_1-adrenoceptors characterised in ligand binding experiments as the α_{1A}-subtype or cloned α_{1A}-adrenoceptor isoforms exhibit pharmacological properties of the α_{1L}-subtype in functional studies (Daniels *et al*., 1999; Hiraoka *et al*., 1999; Ford *et al*., 1997). This has led to the current view that the α_{1L}-subtype represents a functional phenotype of the α_{1A}-adrenoceptor (Daniels *et al*., 1999; Ford *et al*., 1997).

Studies of human vas deferens using gene cloning techniques have reported a predominance of α_{1a} mRNA in the tissue (Moriyama *et al*., 1997). Functional studies have reported that its contraction involves the stimulation of α_{1A}-adrenoceptors (Moriyama *et al*., 1997; Furukawa *et al*., 1995) or α_{1L}-adrenoceptor (Davis *et al*., 1999). A previous study (Amobi *et al*., 1999), found that 5-methylurapidil, an α_{1A}-subtype selective antagonist and prazosin were moderately more potent at inhibiting contractions evoked by NA in circular muscle (pA_2/pK_B, 9.2 and 9.1 respectively) than in longitudinal muscle (pA_2/pK_B, 8.6 and 8.7 respectively). A differential inhibition was not found with other α_1-adrenoceptor antagonists such as spiperone (α_{1B}-subtype selective, pA_2, 7.1 both muscle types) or BMY 7378 (α_{1D}-subtype selective, (pK_B, 6.3 and 6.6 respectively). On the basis of these findings, it was proposed tentatively that the functional response of human vas deferens involves a predominance of α_1-adrenoceptors with the pharmacological characteristics of α_{1L}-subype in longitudinal muscle but α_{1A}-subtype in circular muscle.

The present study was undertaken in order to clarify this further by using (i) SZL-49, an alkylating analogue of prazosin reported to be more potent at inhibiting responses involving stimulation of α_{1H}- than α_{1L}-adrenoceptor subtypes (Flavahan *et al*., 1998; Piascik *et al*., 1990), (ii) other irreversible α_1-adrenoceptor antagonists such as dibenamine and benextramine that have not been reported to discriminate between subtypes; it was hoped that the inclusion of dibenamine (a prototype β-haloalkylamine) will clarify and extend an earlier finding that its analogue, phenoxybenzamine more reliably inhibited longitudinal than circular muscle contractions (Amobi *et al*., 1999) and (iii) reversible competitive antagonists that exhibit different affinities for the α_{1A}-, α_{1L}- and α_{1N}-adrenoceptor subtypes.

Methods

Preparation of tissues

Specimens of human vas deferens (epididymal portion) were obtained after elective vasectomies of healthy fertile men. College ethical approval and patient consent were obtained. Connective tissue and blood vessels were removed and the specimens cut longitudinally into strips (longitudinal muscle preparation; 3–5 mm long and 1 mm wide) or transversely into rings (circular muscle preparations; $\approx$3 mm in length). The tissues were suspended horizontally (resting tension 5–7 mN) in Perspex chamber superfused at 2 ml per min with Krebs' medium (36°C), composition (mM): NaCl 118.8, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.1, ascorbic acid 0.1 and continuously gassed with 95% O_2 and 5% CO_2. In all experiments, the perfusate contained oestradiol (1 μM) and desipramine (0.1 μM) as inhibitors of extraneuronal and neuronal uptake respectively, tropolone (10 μM) and iproniazid (10 μM) inhibitors of catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) respectively and the β-adrenoceptor blocker, propranolol (1 μM). An α_2-adrenoceptor antagonist was not added as earlier studies have found no evidence for the involvement of α_2-adrenoceptors in the contractile response of human vas deferens (Hedlund *et al*., 1985; Amobi & Smith, 1995). Contractions were recorded *via* a force-displacement transducer coupled to a Gould WindoGraf recorder.

Experiments with irreversible α_1-adrenoceptor antagonists or competive α_1-adrenoceptor antagonists

Tissues were equilibrated in Krebs' medium by superfusion for 180 min and then stimulated two to three times with NA (100 μM, 60 min interval) to obtain a reproducible initial response. After re-equilibration with Krebs' medium for 30–45 min, the tissues were exposed to either SZL-49 (10 nM or 0.1 μM for 15 min or 0.1 μM for 30 min) or dibenamine (1 μM for 15 or 30 min) or benextramine (1–100 μM for 15–30 min under reduced light illumination). At the end of drug exposures, tissues were repeatedly washed (over a 10 min period) with drug-free Krebs' medium and then superfused for a further 45 min with fresh Krebs' medium. Subsequently, non cumulative concentration-response curves to NA with exposure times of 5–7 min at intervals of 15–40 min were determined in tissues pretreated with drugs or drug-free medium (time/protocol-matched controls).

In other experiments, tissues were treated exactly as described above without exposure to the irreversible α_1-adrenoceptor antagonists but superfused for 45 min with Krebs' medium containing competitive α_1-adrenoceptor antagonists. Subsequently non cumulative concentration-response curves to NA were determined in the continued presence of the antagonists. In all experiments, only one concentration-response curve to NA was determined per longitudinal or circular muscle preparation and separate time/protocol-matched controls were used to correct for any change in tissue sensitivity. Tissues pretreated with drugs or drug-free medium (time/protocol-matched controls) were generally prepared from a single vas deferens specimen. However in other experiments, longitudinal (strip) and circular (ring) muscle preparations from different vasa deferentia were run in parallel either as the drug-treated group or controls. These were not invariably from the same patient. Contractions were analysed by using computer software developed in-house to measure the total response (i.e. rhythmic activity plus peak tonic response). The response at each NA concentration is expressed as a percentage of the initial control response (NA 100 μM).

Data analysis

EC_{50} values, (expressed as pD_2; the negative log of agonist concentration giving 50% of maximum response) were determined using a logistic curve-fitting programme (FP 60 ver 6.0a, FIG.P Software Corporation, Durham, NC, U.S.A.). For the competitive antagonists, dose-ratios (DR, i.e. the ratio of NA concentration producing 50% of maximum response in the presence and in the absence of

antagonist) were determined for different concentrations of antagonist. Antagonist potency was estimated from the apparent pK_B values (− log antagonist dissociation constant) determined from the Gaddum equation:

$$pK_B = \log (DR\text{-}1) - \log [B] \quad (1)$$

where DR is the dose ratio determined for each concentration of antagonist [B] that did not produce a significant reduction of the maximum response. Apparent pK_B values were calculated from at least two different concentrations of each antagonist. Schild analysis was not performed because with higher concentrations of the antagonists, the maximum contraction was not attained with the highest concentration of NA or was depressed.

Noradrenaline (NA) dissociation constant (K_A) was determined by the method of Furchgott (1966) and Furchgott & Bursztyn (1967). Equieffective concentrations of NA before and after pretreatment with dibenamine ([A] and [A′] respectively) were determined by interpolation from the concentration-response curves. The reciprocals of these were plotted and the value for K_A and fraction of receptors remaining active (q) were calculated from the slope and intercept of the straight line (linear regression) fitting the points according to the equation (Furchgott, 1966):

$$1/[A] = 1/(q[A'] + (1\text{-}q)/(qK_A) \quad (2)$$

from which

$$K_A = (\text{slope-}1)/\text{intercept and } q = 1/\text{slope} \quad (3)$$

Fractional α_1-adrenoceptor occupancy in longitudinal and circular muscle at each concentration of NA was calculated using the equation (Furchgott & Bursztyn, 1967):

$$\%\ \text{receptor occupancy} = ([A]/(K_A + [A])) \times 100 \quad (4)$$

A plot of the relative response to NA in longitudinal and circular muscle against the calculated α_1-adrenoceptor occupancy was used to verify the presence of receptor reserve for NA in the muscle types.

Results are given as means ± s.e.mean (n = number of experiments). Statistical analysis was by one way analysis of variance (ANOVA). A significant F value from ANOVA tests ($P<0.05$) was followed by *a priori* comparison with Student's t-test (directional or non directional where appropriate) using the within groups variance (mean square) from ANOVA. Differences were considered significant at $P<0.01$.

Drugs

Drugs used were propranolol hydrochloride (ICI, Macclesfield, Cheshire, U.K.), noradrenaline acid tartrate (NA; Winthrop Laboratories, Guildford, Surrey, U.K.), dibenamine (TCI, Tokyo, Japan), HV 723 (α-ethyl-3,45-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)-amino)-propyl)-benzene acetonitrile fumarate, gift from Professor I. Muramatsu) RS-17053 (N-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride, Tocris, UK), Rec 15/2739 (8-3-[4-(2-methoxyphenyl)−1-piperazinyl]-propylcarbamoyl-3-methyl-4-oxo-2-phenyl-4H-1-benzopyran dihydroch;oride, a gift from Professor R. Testa), from RBI (Natick, U.S.A.) WB 4101((2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride), SZL-49 (4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-bicyclo[2,2,2]octa-2,5-dienylcarbonyl-2-piperazine) and Sigma (Poole, Dorset, U.K.) dimethyl sulphoxide (DMSO), desipramine, tropolone, iproniazid, 17β-oestradiol, benextramine and ascorbic acid. Stock solutions of dibenamine, SZL-49, Rec 15/2739 or RS-17053 were prepared in DMSO, 17β-oestradiol in ethanol and other drugs in distilled water or dilute aqueous acid. Aliquots added to controls or drug perfusates had a final concentration <0.01% for DMSO or ethanol.

Results

Contractions to NA and effects of pretreatment with irreversible α_1-adrenoceptor antagonists

Noradrenaline (NA) evoked concentration-dependent contractions of longitudinal and circular muscle (pD_2; 5.4 ± 0.1, n = 28 and 5.21 ± 0.1, n = 24 respectively; maximum contraction; 2.5 ± 0.8 mN, and 3.9 ± 1.3 mN respectively). The effects of the irreversible α_1-adrenoceptor antagonists, SZL-49, dibenamine or benextramine on the concentration-response curves are shown in Figure 1.

SZL-49 inhibited contractions of both muscle types but had a greater effect in circular than longitudinal muscle. SZL-49 (10 nM for 15 min) reduced NA potency by about 2 fold in longitudinal muscle and approximately 4 fold in circular muscle (Table 1). A higher concentration of SZL-49 (100 nM for 15 or 30 min) reduced the potency of NA in longitudinal muscle 10–14 fold and its maximum contraction by 27–30%. In circular muscle, the same concentration of SZL-49 (100 nM for 15 or 30 min) reduced NA potency 7 fold but this was associated with 70–80% reduction of the maximum (Table 1). This contrasting effect of SZL-49 could originate from different receptor reserves for NA. This was investigated by studying the effects of other irreversible α_1-adrenoceptor antagonists.

Dibenamine produced comparable effects in longitudinal and circular muscle (Figure 1b,e). Pretreatment with 1 μM for 15 min, caused a modest reduction of NA potency (1.3 fold) and depressed the maximum contraction in both muscle types by 17–21%. Increasing the duration of pretreatment (1 μM for 30 min) produced little additional change in NA potency but reduced the maximum by 35–40% (Table 1). Benextramine (1 μM for 15 min or 10 μM for 15–30 min; not shown) did not produce a consistent effect in longitudinal (n = 3–7) or circular muscle (n = 5–11). Higher concentrations (30 μM) or (100 μM) for 30 min reduced NA potency by between 1–2 fold in longitudinal muscle and about 3 fold in circular muscle. With the increase in benextramine concentration, maximum contractions were progressively depressed from 16–47% in longitudinal muscle and from 10–35% in circular muscle with little further reduction in NA potency (Table 1).

Determination of K_A and receptor reserve for NA and receptor occupancy-response relationship

The equilibrium dissociation constant (K_A) for NA and the relationship between receptor occupancy and response were determined by analysing the effects of partial alkylation of α_1-adrenoceptors with dibenamine (1 μM for 30 min). This concentration produced a comparable reduction of the maximum contraction in both muscle types. The K_A for NA and the fraction of receptors remaining active (q) was

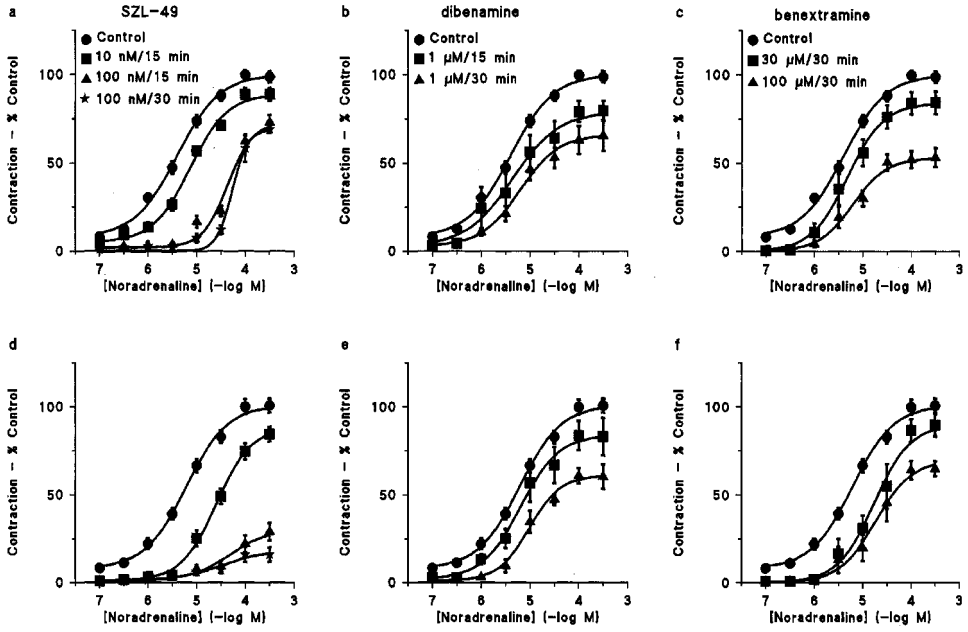

Figure 1 Effects of different irreversible α_1-adrenoceptor antagonists on concentration-response curves evoked by noradrenaline in longitudinal muscle (upper panels) and circular muscle (lower panels) of human vas deferens. Controls ($n=24-28$) and tissues pretreated with (a,d) SZL-49 (10 nM for 15 min, $n=5-6$; 100 nM for 15 min, $n=5-7$ and 100 nM for 30 min, $n=3$) or (b,e) dibenamine (1 μM for 15 min, $n=6$ and 1 μM for 30 min, $n=7$) or (c,f) benetramine (30 μM for 30 min, $n=6$ and 100 μM for 30 min, $n=6$). Data points represent means ± s.e.mean (n = number of experiments).

Table 1 Effects of irreversible α_1-adrenoceptor antagonists on contractions to noradrenaline in longitudinal and circular muscle of human vas deferens

	Longitudinal muscle				*Circular muscle*			
	pD_2	*Change in NA potency*	*% reduction of maximum*	(n)	*pD_2*	*Change in NA potency*	*% reduction of maximum*	(n)
Control	5.40 ± 0.1	–	–	(28)	5.21 ± 0.1	–	–	(24)
SZL-49								
10 nM, 15 min	5.12 ± 0.05	1.9 fold	11.2 ± 3.5	(6)	4.64 ± 0.03	3.7 fold	15.6 ± 4.1	(5)
100 nM, 15 min	4.38 ± 0.04	10.5 fold	27.0 ± 3.1*	(5)	4.37 ± 0.1	7.0 fold	71.0 ± 4.9*	(7)
100 nM, 30 min	4.27 ± 0.08	13.7 fold	31.0 ± 2.0*	(3)	(nd)	(nd)	84.0 ± 4.1*	(3)
Dibenamine								
1 μM, 15 min	5.29 ± 0.25	1.3 fold	21.0 ± 5.6†	(6)	5.11 ± 0.12	1.3 fold	17.5 ± 7.8	(6)
1 μM, 30 min	5.17 ± 0.08	1.7 fold	34.5 ± 8.6*	(7)	4.97 ± 0.06	1.7 fold	39.7 ± 6.9*	(7)
Benextramine								
30 μM, 30 min	5.29 ± 0.06	1.3 fold	15.8 ± 6.4	(6)	4.77 ± 0.16	2.8 fold	10.3 ± 6.5	(6)
100 μM, 30 min	5.24 ± 0.14	1.5 fold	46.9 ± 5.3*	(6)	4.72 ± 0.16	3.1 fold	35.1 ± 4.2*	(6)

Data are means ± s.e.mean (n = number of experiments). NA potency is expressed as pD_2 ($-\log EC_{50}$). nd, not determined because of marked depression of the maximum contraction. †$0.01 < P < 0.05$ and *$P < 0.01$.

determined as described in Methods, from double reciprocal plots of equieffective concentrations of NA in controls and tissues pretreated with dibenamine. Representative plots are shown in Figure 2. The calculated mean K_A and q values were respectively 21.5 ± 5.2 μM and 0.362 ± 0.07 ($n=7$) in longitudinal muscle. The corresponding values in circular muscle were 27.1 ± 6.2 μM and 0.35 ± 0.07 ($n=7$). The mean K_A value for NA in each muscle type was used in the calculation of theoretical receptor occupancy for that muscle. A plot of contractile response as a function of the theoretical receptor occupancy shows a similar nonlinear occupancy-response relationship in longitudinal and circular muscle (Figure 2). It was determined that NA evoked maximal contraction with about 93% and 91% receptor occupancy respectively in longitudinal and circular muscle with 7–9% as receptor reserve for the agonist. Half maximal contraction required about 14% and 16% receptor occupancy in longitudinal and circular muscle respectively. The ratio K_A/EC_{50}, a measure of coupling efficiency (Ruffolo, 1982) yielded values of 5.4 and 4.4 in longitudinal and circular muscle respectively. Analysis of the effects produced by benextramine (100 μM for 30 min) yielded K_A and q values (longitudinal muscle; 21.5 ± 7.4 μM and 0.29 ± 0.07 and $n=6$; circular muscle; 27.4 ± 8.2 μM and 0.48 ± 0.12; $n=6$) that are comparable to values obtained with dibenamine.

Effects of competitive α_1-adrenoceptor antagonists

In longitudinal muscle (Figure 3a–d), WB 4101 (3, 10 and 30 nM), RS-17053 (30, 300 and 1000 nM), Rec 15/2739 (0.3, 1

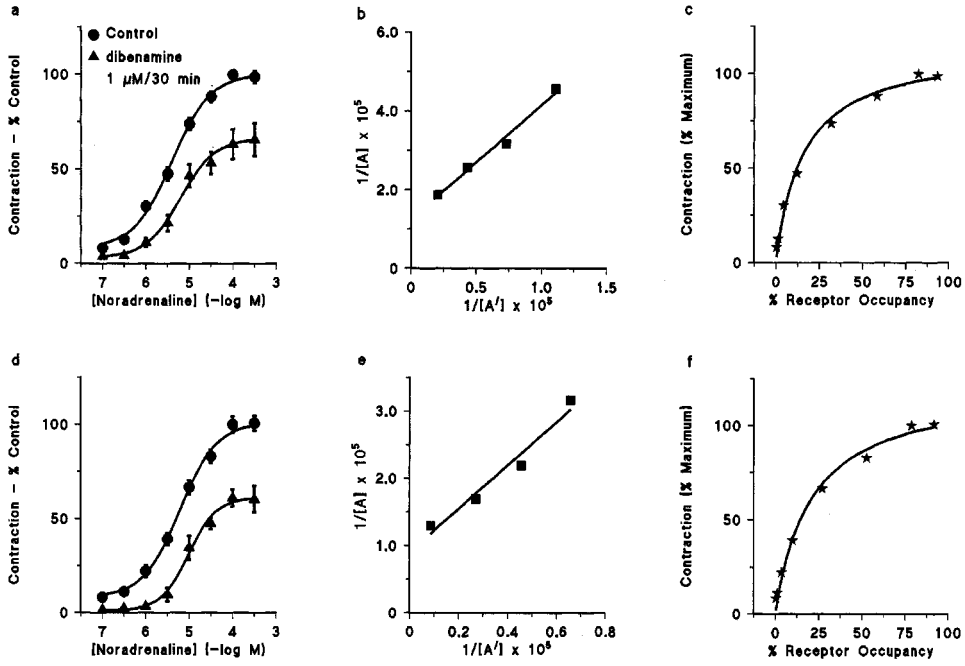

Figure 2 Determination of equilibrium dissociation constant (K_A) for noradrenaline (NA), receptor reserve for the agonist and receptor occupancy-response relationship in longitudinal muscle (upper panels) and circular muscle (lower panels) of human vas deferens. (a,d) NA concentration-response curves in controls and tissues pretreated with dibenamine (1 μM for 30 min as in Figure 1). (b,e) Double-reciprocal plots of equieffective concentrations of NA in controls and tissues pretreated with the antagonist. In these examples, K_A was calculated as described in Methods from the linear function (b, longitudinal muscle) $y=7.669x+(2.879\times10^5)$; $K_A=23.2$ μM and (e, circular muscle) $y=3.57x+(1.458\times10^5)$; $K_A=17.6$ μM. (c,f) Plots of contraction as a function of receptor occupancy respectively in longitudinal and circular muscle used mean K_A values from individual experiments (longitudinal muscle mean K_A, 21.5±5.2 μM; $n=7$ and circular muscle mean K_D, 27.1±6.2 μM; $n=7$).

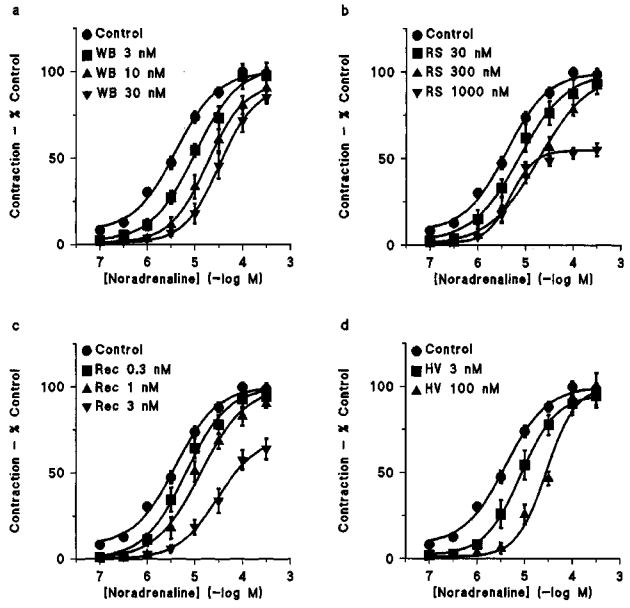

Figure 3 Concentration-response curves evoked by noradrenaline in longitudinal muscle of human vas deferens in the absence (controls) and presence of (a) WB 4101, (WB); (b) RS-17503, (RS); (c) Rec 15/2739, (Rec) and (d) HV723, (HV). Data points represent the means±s.e.mean ($n=28$ in controls and 4–8 in the presence of each antagonist concentration).

and 3 nM) and HV 723 (3 and 100 nM) produced dextral shifts of the concentration-response curves to NA. However with WB 4101 (30 nM, Figure 3a) and Rec (3 nM, Figure 3c) maximum contraction was not attained with the highest NA concentration used and RS-17053 (1000 nM, Figure 3b) significantly depressed maximum contraction without producing a further shift of the concentration response curve. No reliable shift was produced by lower concentrations of WB 4101 (1 nM, $n=4$; not shown) or RS-17053 (10 nM, $n=4$; not shown).

In circular muscle (Figure 4a–d), WB 4101 (0.1, 1 and 3 nM), RS-17053 (1, 3 and 10 nM), Rec 15/2739 (0.1, 1 and 10 nM) and HV 723 (3 and 30 nM) produced dextral shifts of the concentration-response curve to NA. However, the maximum contraction was significantly depressed in the presence of WB 4101 (3 nM, Figure 4a) or Rec 15/2739 (10 nM, Figure 4c). RS-17053 (10 nM, Figure 4b) significantly depressed maximum contraction without producing a further shift of the concentration response curve. The inhibitory potency of the various antagonists against NA-induced contractions of longitudinal and circular muscle was estimated by calculating apparent pK_B values from the effects of antagonist concentrations that did not significantly change the maximum contraction. The pK_B values so determined and mean values are presented in Table 2.

Discussion

Previous studies have established a predominance of α_{1a} mRNA in the smooth muscle of human vas deferens (Moriyama *et al*., 1997). Functional studies have reported

that NA activates the tissue by stimulating postjunctional α_1- but not α_2-adrenoceptors (Hedlund *et al*., 1985) and subsequent work have characterized the α_1-subtype as either α_{1A}- (Moriyama *et al*., 1997; Furukawa *et al*., 1995) or α_{1L}- (Davis *et al*., 1999). The present study shows that SZL-49 discriminates between contractions evoked by NA in the muscle types of human vas deferens: longitudinal muscle contraction was less sensitive to SZL-49 compared to circular muscle contraction. Evidently, this is not caused by the involvement of a greater receptor reserve for NA in longitudinal than in circular muscle. Other irreversible α_1-adrenoceptor antagonists (dibenamine or benextramine) or chloroethylclonidine (Amobi *et al*., 1999) produced a comparable inhibition of longitudinal and circular muscle contraction. Analysis of the effects of dibenamine revealed that the fraction of total receptor pool required for maximal contraction in longitudinal muscle was similar to that in circular muscle. This indicates that the muscle types are not different in terms of receptor reserve for NA and suggests that the discriminatory effects of SZL-49 is not due to a greater receptor reserve for NA in longitudinal than in circular muscle. Functional studies in other tissues have reported that SZL-49 was less potent at inhibiting contractions involving α_{1L}- than α_{1H}- adrenoceptors (Flavahan *et al*., 1998; Piascik *et al*., 1990). Thus a possible explanation for the discriminatory effect of SZL-49 in human vas deferens is that longitudinal muscle contraction is mediated predominantly by the stimulation of the α_{1L}-subtype whilst circular muscle contraction is mainly *via* the α_{1A}-adrenoceptors. This was investigated further using α_1-adrenoceptor antagonists such as Rec 15/2739 and HV 723 and the α_{1A}-/α_{1L}-adrenoceptor subtype discriminating (in functional studies) antagonists such as WB 4101and RS-17053 (Ford *et al*., 1996; 1997; Marshall *et al*., 1996; Blue *et al*., 1995).

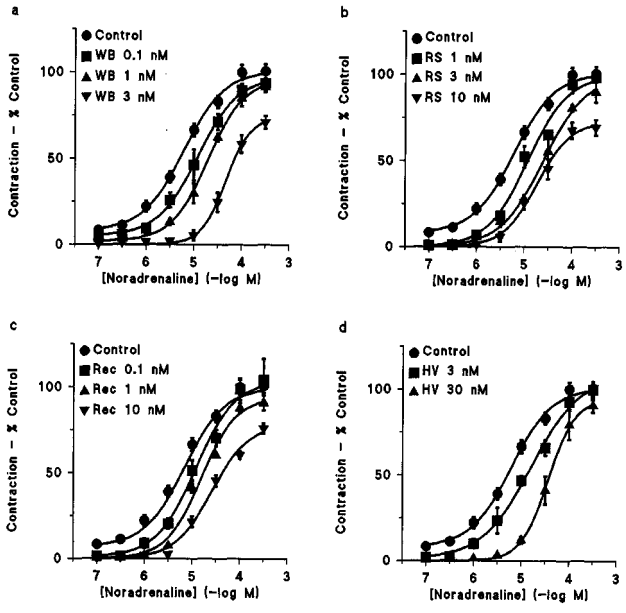

Figure 4 Concentration-response curves evoked by noradrenaline in circular muscle of human vas deferens in the absence (controls) and presence of (a) WB 4101, (WB); (b) RS-17503, (RS); (c) Rec 15/2739, (Rec) and (d) HV723, (HV). Data points represent the means ±s.e.mean ($n=24$ in controls and 4–8 in the presence of each antagonist concentration).

All four antagonists produced dextral shifts of the concentration-response curve to NA but showed evidence of noncompetitive interaction: the maximum contraction was either not attained or significantly depressed with the highest concentration of antagonist used (except HV 723). Reliable displacement of NA response curve in both muscle types occurred within a narrow range of antagonist concentrations similar to the range reported in intact human vasectomy specimens (Davis *et al*., 1999; Furukawa *et al*., 1995), human prostate and lower urinary tract (Ford *et al*., 1996; Marshall *et al*., 1995; 1996; Chess-Williams *et al*., 1996; Testa *et al*., 1996), rat epididymal and prostatic vas deferens (Chess-Williams *et al*., 1996; Marshall *et al*., 1996; Burt *et al*., 1995; 1998) and rat portal vein and perfused kidney (Ford *et al*., 1996; Marshall *et al*., 1996; Blue *et al*., 1995). With WB 4101 or RS-17053, there was a marked (30 fold) difference in the lowest concentration required to reliably displace the response curve in longitudinal and in circular muscle. These

Table 2 Inhibitory potencies of α_1-adrenoceptor antagonists against contractions evoked by noradrenaline in longitudinal and circular muscle of human vas deferens

Longitudinal muscle		*Circular muscle*	
WB4101			
3 nM	8.68±0.1 (8.9–8.5; $n=6$)	0.1 nM	9.68±0.3 (10.5–8.9; $n=6$)
10 nM	8.57±0.1 (8.8–8.4; $n=5$)	1 nM	9.34±0.1 (9.6–9.1; $n=8$)
Mean pK_B	8.63±0.1 ($n=11$)*	Mean pK_B	9.48±0.13 ($n=14$)*
RS-17053			
30 nM	7.12±0.2 (7.9–6.7; $n=5$)	1 nM	9.02±0.1 (9.4–8.7; $n=4$)
300 nM	7.04±0.2 (7.4–6.8; $n=8$)	3 nM	9.0±0.1 (9.4–8.8; $n=6$)
Mean pK_B	7.07±0.13 ($n=13$)†	Mean pK_B	9.01±0.1 ($n=10$)†
Rec 15/2739			
0.3 nM	9.23±0.2 (9.8–8.7; $n=6$)	0.1 nM	10.3±0.1 (10.7–10.1; $n=4$)
1 nM	9.19±0.2 (10–8.6; $n=8$)	1 nM	9.37±0.1 (9.4–9.1; $n=4$)
Mean pK_B	9.21±0.13 ($n=14$)	Mean pK_B	9.82±0.2 ($n=8$)
HV 723			
3 nM	8.6±0.2 (8.9–8.0; $n=5$)	3 nM	8.56±0.2 (9.0–7.9; $n=4$)
100 nM	7.98±0.1 (8.2–7.8; $n=4$)	30 nM	8.25±0.1 (8.3–8.0; $n=4$)
Mean pK_B	8.32±0.15 ($n=9$)	Mean pK_B	8.41±0.13 ($n=8$)

Data shown are pK_B values±s.e.mean (range of pK_B value and n=number of experiments) determined from the effects of individual concentrations of each antagonist. Mean pK_B values were calculated from pooled data and each symbol (* or †) indicates pairs that are significantly ($P<0.01$) different.

observations may indicate a heterogeneity of the α_1-adrenoceptor subtypes in longitudinal and circular muscle for which the antagonists exhibit slightly different affinities. The apparent pK_B estimates are well within the range reported for the α_{1A}-/α_{1L}-subtype but for some of the antagonists, the values tended to decrease with antagonist concentration (Table 2). Whether this originates from 'pharmacological pleiotropism' of the α_{1A}-adrenoceptor subtype (Ford *et al*., 1997) or is caused by the presence of different proportions of both α_{1A}- and α_{1L}- subtypes in longitudinal and in circular muscle preparations from different patients remain unclear (see below).

Studies of intact human vasectomy specimens with phenylephrine have reported pA_2/pK_B values of 9.2 for WB 4101 and 7.1–7.2 for RS-17053 (Davis *et al*., 1999; Furukawa *et al*., 1995). In the present study, the apparent pK_B values for these antagonists in circular muscle (WB 4101; 9.5 and RS-17053; 9.0) are comparable to their published affinity estimates ($pA_2/pK_B \geqslant 9$) at α_1-adrenoceptors conventionally defined as the α_{1A}-subtype (Ford *et al*., 1996; 1997; Lachnit *et al*., 1997; Burt *et al*., 1995; Aboud *et al*., 1993). In comparison, the lower inhibitory potency of these antagonists (pK_B, WB 4101; 8.6 and RS-17053; 7.1) in longitudinal muscle is distinctive of α_{1L}-adrenoceptor pharmacology (see Table 3) and agrees best with their functional affinities ($pA_2/pK_B < 9$) in human prostate and other tissues (Stam *et al*., 1999; Kava *et al*., 1998; Ford *et al*., 1996; Marshall *et al*., 1996). The inhibitory potency of HV 723, (purported α_{1N}-subtype selective antagonist, Hiraoka *et al*., 1995; Muramatsu *et al*., 1990) was similar in both muscle types with pK_B values (8.3–8.4) that correspond to its reported affinity at either α_{1A}- or α_{1L}-adrenoceptor subtypes (Honner & Docherty, 1999; Muramatsu *et al*., 1994).

Rec 15/2739 potently inhibited longitudinal and circular muscle contractions with similar pK_B values $\geqslant 9$. This matches the affinity estimate reported for the antagonist in functional studies of human recombinant α_{1A}-adrenoceptors (Ford *et al*., 1997), native α_{1A}-adrenoceptors (rat caudal artery, Lachnit *et al*., 1997) or tissues displaying the α_{1L} -adrenoceptor pharmacology (lower urinary tract tissues of man, Ford *et al*., 1997 and rabbit bladder neck, Kava *et al*., 1998) and at α_1-adrenoceptors in rat anococcygeus muscle and vas deferens (Chess-Williams *et al*., 1996). However lower affinity (3–10 fold) estimates for Rec 15/2739 have been reported in some studies (rabbit urethra and prostate, 8.64 and 8.52, Leonardi *et al*., 1997; human prostate, 8.1, Chess-Williams *et al*., 1996; 8.57, Testa *et al*., 1996). Nevertheless, the similar inhibitory potency of Rec 15/2739 in longitudinal and circular muscle is more compatible with the view that the drug displays a comparable high affinity at α_{1A}- and α_{1L}-adrenoceptor subtypes (Daniels *et al*., 1999; Ford *et al*., 1997; Leonardi *et al*., 1997; Kenny *et al*., 1996).

On balance, the findings with the four competitive α_1-adrenoceptor antagonists seem consistent with a predominance of α_1-adrenoceptors that exhibit pharmacological characteristics of the α_{1L}-subtype in longitudinal muscle but α_{1A}-subtype in circular muscle of human vas deferens (see Table 3). Given the current view that the α_{1L} -and α_{1A}-subtypes may represent functional phenotypes of the α_{1a}-gene product and the suggestion that the pleiotropic behaviour of α_{1a}-gene product may originate from tissue-specific influences on its conformational state (Daniels *et al*., 1999; Ford *et al*., 1997), the question arises as to the significance of their coexistence in human vas deferens. It is possible that the predominant functional phenotype of the receptor in longitudinal and circular muscle reflects physiological adaptions induced by different microenvironments in the muscle layers.

An intriguing finding from the present study is that the discriminatory action of SZL-49 is the opposite of the effect previously reported for phenoxybenzamine (PBZ): circular muscle contraction to NA was less sensitive to PBZ compared to longitudinal muscle contraction (Amobi *et al*., 1995; 1999). The opposite effects of SZL-49 and PBZ can be reproduced on contractions evoked by a different agonist, A61603 (unpublished observation). Does this imply that PBZ has a greater selectivity for α_{1L}- over α_{1A}-adrenoceptor subtype? Perhaps not, as dibenamine, a prototype β-haloalkylamine, produced a comparable inhibition of contractions evoked by NA in longitudinal (α_{1L}-) and circular (α_{1A}-) muscle. Inactivation of α_1-adrenoceptors and indeed

Table 3 A comparison of published functional affinities (pA_2 or pK_B) of α_{1A}- and α_{1L}-adrenoceptor discriminating antagonists in rat and human tissues

	Isolated perfused kidney of rat (α_{1A})	*Rat epididymal vas deferens* (α_{1A}/α_{IL})	*Human prostate and lower urinary tract tissues* (α_{1L})	*Intact human epididymal vas deferens* (α_{1A}/α_{1L})	*Longitudinal muscle of human epididymal vas deferens* (α_{1L})	*Circular muscle of human epididymal vas deferens* (α_{1A})
Prazosin	9.5[1] 9.5[2]	9.2[3] 8.39±0.1[5]	8.25[6] 8.29±0.13[7] 8.7±0.1[2]	8.8[8] 8.6±0.1[9]	8.6±0.07[10]	9.2±0.05[10]
5-Methylurapidil	9.2[1] 9.2[2]	8.7[3]	7.93[6] 8.28±0.13[7] 8.2±0.1[2]	8.8[8]	8.7±0.03[10]	9.1±0.1[10]
WB 4101	10.3[1] 10.3[2]	9.6[3] 8.7±0.06[5]	8.39±0.09[7] 8.9±0.1[2]	9.2[8]	8.63±0.1[11]	9.48±0.13[11]
RS-17053	9.8[2]	9.5[4]	7.1[4] 7.3±0.1[2]	7.1, 7.2[9]	7.07±0.13[11]	9.01±0.1[11]

Data (values±s.e.mean) are from Blue *et al*., 1995[1]; Ford *et al*., 1996[2]; Burt *et al*., 1995[3]; Marshall *et al*., 1996[4]; Ohmura *et al*., 1992[5]; Testa *et al*., 1996[6]; Muramatsu *et al*., 1994[7]; Furukawa *et al*., 1995[8]; Davis *et al*., 1999[9]; Amobi *et al*., 1999[10] and current study[11]. Note that the functional response of intact human vas deferens[8,9] probably reflects longitudinal muscle contraction.

other transmitter receptors by dibenamine and PBZ is mediated by the formation of the same reactive aziridinium ion. The basis for the differential effect of PBZ in the human vas deferens is currently under study.

A point that deserves some comment relates to the K_A (21.5–27.1 μM) and pD_2 (5.4–5.21) values for NA determined in the current study of human vas deferens. The low potency of NA in both muscle types is close to published pD_2 for NA in human prostate (5.9–5.5, Testa *et al*., 1996; Marshall *et al*., 1995) or human mesenteric artery (5.2, Testa *et al*., 1996) but despite extensive search of the literature no published K_A for NA in functional studies of human tissue was found. The K_A values (present study) are relatively higher than published K_A for NA (6.3–12.3 μM) in epididymal or whole rat vas deferens (Salles & Badia, 1991; Diaz-Toledo & Marti, 1988; Minneman & Abel, 1984; Minneman *et al*., 1983). The pD_2 for NA in both muscle types of human vas deferens are also lower than the pD_2 (6.92–6.36) reported for the agonist in the rodent tissue. Judging from the ratio K_A/EC_{50}, (4.4–5.4 in human compared to 18–79 in rat vas deferens), it seems that stimulus response coupling may not be as efficient in human as in rodent vas deferens and perhaps underlies its weaker response to neural stimulation (Smith & Bray, 1990).

In conclusion, the results of the present study suggest that SZL-49 discriminates between the muscle types in human vas deferens because it inhibits contractions mediated through the stimulation of α_{1A}-adrenoceptors (circular muscle) more potently than contractions involving α_{1L}-adrenoceptors (longitudinal muscle).The results obtained using the non-discriminating irreversible α_1-adrenoceptor antagonists indicate a comparable receptor reserve for NA in both muscle types.

This work is supported by the Wellcome Trust. We thank Maggie Gormley, at Margaret Pyke Centre, Sister C. Voos and Raj Rammohun at the Royal Free Hospital, nurses/staff at Elliot Smith Clinic for their assistance and Professor I. Muramatsu and Professor R. Testa respectively for gifts of HV723 and Rec 15/2739.

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(Received September 24, 2001
Revised January 29, 2002
Accepted February 20, 2002)