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α_{1A}-Adrenoceptor-mediated contractile responses of the human vas deferens

K. Furukawa, *D.J. Rosario, *D.J. Smith, *C.R. Chapple, †T. Uchiyama & [1]R. Chess-Williams

Department of Biomedical Science, University of Sheffield, Sheffield, *Department of Urology, Royal Hallamshire Hospital, Sheffield and †Department of Pharmacology, Toho University School of Medicine, Tokyo, Japan

1 The predominant α_1-adrenoceptor mediating contractions of the human vas deferens has been characterised *in vitro* by use of subtype selective antagonists.

2 Responses of human epididymal vas deferens were obtained to phenylephrine in the presence of amine uptake inhibitors and propranolol. The effects of the α_1-adrenoceptor antagonists, 5-methylurapidil, oxymetazoline, WB4101, prazosin and chloroethylclonidine were examined and also the L-type calcium channel blocker, nifedipine.

3 5-Methylurapidil, WB4101, oxymetazoline and prazosin acted as competitive antagonists of the responses to phenylephrine, yielding pA_2 values of 8.8, 9.2, 7.7 and 8.8 respectively. All four antagonists produced Schild plots with slopes similar to unity and maximum responses to phenylephrine were not altered in the presence of any of the antagonists.

4 Tamsulosin (1 nM) caused rightward shifts of phenylephrine concentration-response curves yielding an apparent pK_B value of 10.0. However, maximum responses were also reduced by 51% with this concentration of antagonist.

5 Incubation of tissues with chloroethylclonidine (100 μM for 40 min) failed to alter responses significantly but the presence of nifedipine (1 μM) reduced maximum responses to phenylephrine by 32%.

6 The high affinity of 5-methylurapidil, oxymetazoline and WB4101, together with the failure of chloroethylclonidine to antagonize responses, indicate that the predominant α_1-adrenoceptor mediating contraction of the human vas deferens has the characteristics previously described for the pharmacologically-defined α_{1A}-adrenoceptor. The data are also consistent with those described for the cloned α_{1c}-adrenoceptor subtype thereby supporting the hypothesis that the two receptors are identical. The human vas deferens therefore represents a readily accessible preparation for functional studies of the human α_{1A}-adrenoceptor.

Keywords: Human vas deferens; α_1-adrenoceptor; α_1-adrenoceptor subtype; WB4101; chloroethylclonidine; oxymetazoline; 5-methylurapidil

Introduction

α_1-Adrenoceptor subtypes have been identified by use of a variety of techniques; functional studies, radioligand binding and molecular biology. Although differences in protocol and technique have resulted in several classification schemes, the most widely accepted at present is based on the properties of the three cloned receptors: the α_{1b}-adrenoceptor (Cotecchia *et al.*, 1988), the α_{1c}-adrenoceptor (Schwinn *et al.*, 1990) and the α_{1d}-adrenoceptor (Perez *et al.*, 1991). Pharmacological experiments have supported the subdivision of α_1-adrenoceptors, functional α_1-adrenoceptors initially being classified as α_{1A}- or α_{1B} based on different affinities for the competitive antagonist WB4101. Subsequently, chloroethylclonidine was found to inactivate the α_{1B}-adrenoceptor subtype which is the receptor with a low affinity for WB4101 (Morrow & Creese, 1986; Minneman, 1988).

The precise relationship between cloned and native receptors continues to be a matter of debate. It is generally accepted that tissues such as the rat spleen possess functional α_{1B}-adrenoceptors, the properties of which agree with those described for the cloned α_{1b}-adrenoceptor, but functional correlates for the α_{1d}- and α_{1c}-adrenoceptors appear to be less readily identifiable. It has been suggested however, that the widely distributed pharmacologically-defined α_{1A}-adrenoceptor may represent the cloned α_{1c}-adrenoceptor (Fauve *et al.*, 1994; Ford *et al.*, 1994; Rokosh *et al.*, 1994), and the term α_{1A}-adrenoceptor is now often used to describe this receptor. Recently, it has been proposed that the contractile responses of the rat aorta represent a functional correlate for the α_{1d}-adrenoceptor (Kenny *et al.*, 1995).

One problem that has previously given rise to difficulties in fully characterizing new receptors is the small differences that may exist between homologues of the receptor in different species. Thus it was originally concluded that the cloned bovine α_{1c}-adrenoceptor was not expressed in any rat tissue and therefore could not represent the native α_{1A}-adrenoceptor (Schwinn *et al.*, 1990); However, a recent study discovered a wide distribution for this receptor in the rat when a primer for the rat homologue of the α_{1c}-adrenoceptor was used to measure mRNA levels (Rokosh *et al.*, 1994). This demonstrates the importance of small differences between species and the need to study the human receptor whenever possible if the ultimate aim is to develop selective drugs for use in man.

Investigations of functional receptor subtypes in man however, are difficult because of the problem of obtaining healthy viable tissues. The human vas deferens is a tissue which is readily available from a healthy and relatively homogeneous group of patients undergoing routine vasectomy. This tissue is innervated by adrenergic postganglionic nerve fibres (Birmingham, 1968) from which noradrenaline appears to be the sole transmitter, mediating contractile responses via α_1-adrenoceptors (Holmquist *et al.*, 1990). The present study was therefore undertaken to characterize the predominant α_1-adrenoceptor subtype mediating responses of this tissue. The effects of 5-methylurapidil and oxymetazoline (high affinity for the cloned α_{1c}-adrenoceptor and the native α_{1A}-adrenoceptor), WB4101 (low affinity for the cloned α_{1b}- and native α_{1b}-

[1] Author for correspondence.

adrenoceptor) and chloroethylclonidine (proposed as an irreversible antagonist of all the α_1-adrenoceptor subtypes except the native α_{1A}) have been examined on the responses of the human vas deferens to phenylephrine. The dependency of these responses on the influx of extracellular calcium through L-type voltage-regulated channels has also been investigated by use of the dihydropyridine, nifedipine.

Methods

Segments (1 cm) of epididymal vas deferens obtained from routine vasectomy (healthy males, 30–40 years of age) were set up in a Krebs-bicarbonate solution (composition in mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 1.9, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.7) gassed with 5% CO_2 in O_2 and maintained at 37°C. Tissues were set up under 1 g resting tension and the tension developed following the addition of phenylephrine was measured by means of isometric force transducers (Lectromed UF1, 57 g sensitivity) connected to a Tandon PCA-sl computer via an analogue to digital converter (Cambridge Electronic Design). Developed tension was recorded using 'CHART' and analysed using 'SPIKE 2' software.

Drug administration

Tissues were equilibrated for 120 min with several changes of bathing medium. Non-cumulative concentration-response curves were constructed to phenylephrine by adding 3 fold incremental concentrations of phenylephrine, washing for 10–30 min between each addition. After obtaining an initial concentration-response curve, tissues were washed for 90 min before incubating with antagonist for 30 min and then obtaining a further non-cumulative concentration-response curve to phenylephrine in the presence of antagonist. Only two concentration-response curves were obtained on each tissue. All experiments were performed in the presence of cocaine (10 μM) and corticosterone (10 μM) to inhibit amine uptake, and propranolol (1 μM) to antagonize β-adrenoceptors.

Determination of pA_2 and pK_B values

Phenylephrine concentration-response curves were obtained in the absence of antagonist and in the presence of 5-methylurapidil (3–30 nM), WB4101 (3–30 nM), prazosin (10–100 nM), oxymetazoline (0.1–1.0 nM) and tamsulosin (1 nM). Tissues were incubated with the antagonists for 30 min (60 min for tamsulosin) before obtaining responses to phenylephrine. Control experiments were performed with an identical protocol but without the addition of antagonist, and these were used to correct for time-dependent changes in tissue sensitivity which may occur during the course of the experiment.

Effect of incubation with chloroethylclonidine

Tissues were equilibrated for 120 min and a non-cumulative concentration-response curve obtained to phenylephrine. After washing, tissues were incubated with chloroethylclonidine (25 or 100 μM) for 40 min followed by washout every 10 min for 60 min. Phenylephrine administrations were then repeated. Control tissues were incubated without the addition of chloroethylclonidine.

Role of extracellular calcium

Following the initial phenylephrine concentration-response curve, tissues were washed and the bathing solution changed to one containing nifedipine (1 μM) for 30 min. Phenylephrine was then reapplied to tissues in the presence of nifedipine. Experiments with nifedipine were performed in the dark.

Data analysis

Increases in developed tension to phenylephrine were plotted as a percentage of the maximum increase for each concentration-response curve. Individual EC_{50} values (concentration for a half maximal response) were determined and geometric mean EC_{50} values with 95% confidence limits calculated. Differences in mean EC_{50} values were analysed with Student's t test applied to individual logarithmic EC_{50} values. Dissociation constants (pK_B) for antagonists were determined from the equation:

$$pK_B = \log(CR-1) - \log[B]$$

where CR is the concentration-ratio (ratio of the EC_{50} values in the presence and absence of the antagonist) obtained with a concentration [B] of antagonist. Schild plots were also constructed and pA_2 values determined from the intercept on the abscissa scale (Arunlakshana & Schild, 1959). Data given in the text represent the mean with s.e.mean for pK_B values and maximal responses, whilst EC_{50} values are given as the geometric mean with 95% confidence limits.

Drugs

5-Methylurapidil, WB4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane) and chloroethylclonidine were obtained as the hydrochloride salts from Research Biochemicals Inc. (Natick, MA, U.S.A.). (-)-Tamsulosin (YM617) and prazosin base were kindly suppled as gifts by Yamanouchi Europe B.V. (Leiderdorp, Netherlands) and Pfizer Central Research (Sandwich, Kent, U.K.) respectively. (-)-Phenylephrine hydrochloride, cocaine hydrochloride, nifedipine, oxymetazoline hydrochloride, corticosterone-21-acetate and (±)-propranolol hydrochloride were obtained commercially from Sigma. All reagents were of Analar grade.

Results

Results with competitive antagonists

With a 2 h washing protocol between curves, two non-cumulative concentration-response curves could be constructed on each tissue with no loss of sensitivity to phenylephrine (Figure 1a). 5-Methylurapidil (3–30 nM, Figure 1), WB4101 (3–30 nM, Figure 2) and prazosin (10–100 nM) produced parallel rightward shifts of concentration-response curves to phenylephrine without affecting maximum responses. Oxymetazoline (0.1–1.0 nM) failed to elicit a response over the concentration-range examined but acted as a competitive antagonist of the contractile responses to phenylephrine, causing rightward shifts of concentration-response curves without affecting maximum responses. The shifts produced by these antagonists were used to calculate mean pK_B values (−log dissociation constant, ± s.e.mean) and to construct Schild plots. All four antagonists had a high affinity for the α_1-adrenoceptors of the human vas deferens and the slopes of Schild plots were not significantly different from unity (Table 1, Figure 3).

Antagonism by tamsulosin

Tamsulosin at a concentration of 1 nM caused rightward shifts of concentration-response curves to phenylephrine yielding an apparent pK_B value of 10.0 ± 0.1. However, maximum responses were also reduced (Figure 4), tamsulosin (1 nM) reducing responses by 51% from 1.58 ± 0.31 g to 0.77 ± 0.18 g ($P<0.05$, $n=6$).

Antagonism by chloroethylclonidine

Incubation of tissues with chloroethylclonidine (25 or 100 μM) did not significantly depress maximum responses to phenylephrine (Figure 5). EC_{50} values and maximum responses to

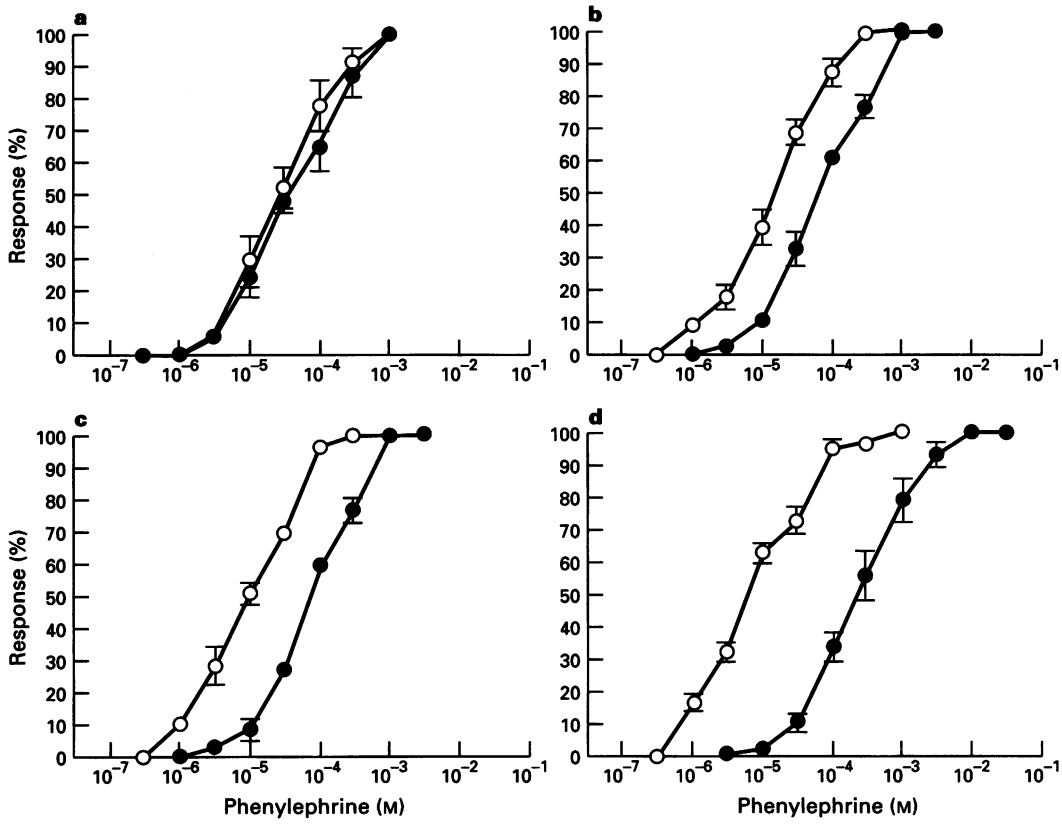

Figure 1 First (○) and second (●) concentration-response curves of human vas deferens to phenylephrine. In (a), no antagonist was added. In separate tissues, the second curve was obtained in the presence of 5-methylurapidil at concentrations of (b) 3 nM, (c) 10 nM or (d) 30 nM. Responses are plotted as a percentage of the first maximum response.

phenylephrine following incubation with the higher concentration of chloroethylclonidine [49.1(27.1–88.8) μM; 0.89 ± 0.26 g, $n = 6$] were not significantly different from values for the control curves [28.5(11.8–68.7) μM, 1.19 ± 0.36 g].

Role of extracellular calcium

The presence of nifedipine (1 μM) abolished spontaneous activity and also significantly depressed responses to phenylephrine. Maximum responses were reduced by 32% from 0.90 ± 0.24 g to 0.61 ± 0.16 g ($P < 0.05$, $n = 6$). Phenylephrine EC_{50} values were similar in the absence [9.5(6.4–13.2) μM] and in the presence of nifedipine [14.6(11.9–17.9) μM].

Table 1 pK_B and pA_2 values for antagonists obtained at the α_1-adrenoceptors of the human vas deferens with phenylephrine used as agonist

Antagonist	pK_B	n	pA_2	*Slope*
5-Methylurapidil	8.9 ± 0.1	18	8.8	1.08 ± 0.28
Oxymetazoline	7.7 ± 0.1	20	7.7	0.94 ± 0.14
WB4101	9.2 ± 0.1	18	9.2	0.99 ± 0.07
Prazosin	8.6 ± 0.1	12	8.8	1.07 ± 0.34
Tamsulosin*	10.0 ± 0.1	5	–	–

pK_B values are the mean (± s.e.mean) values of n experiments. pA_2 values and slopes are the values obtained from Schild plots. *The pK_B value for tamsulosin is an apparent value since additional non-competitive effects were also observed.

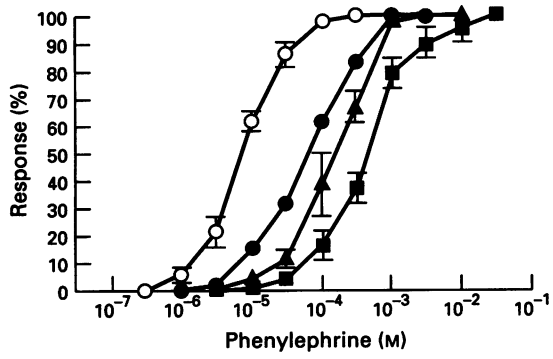

Figure 2 Concentration-response curves of human vas deferens to phenylephrine in the absence (○) and presence of WB4101 at concentrations of 3 nM (●), 10 nM (▲) and 30 nM (■). The control curve for 3 nM WB4101 is presented but was similar to controls for experiments with 10 nM and 30 nM.

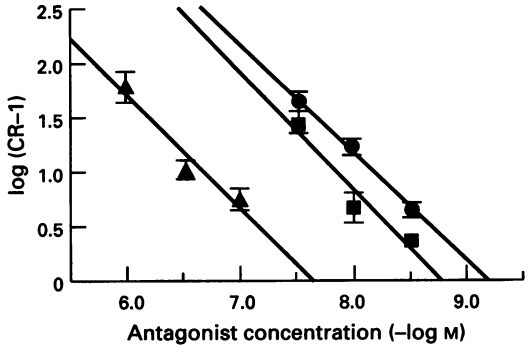

Figure 3 Schild plots for the antagonism of contractile responses to phenylephrine by WB4101 (●), 5-methylurapidil (■) and oxymetazoline (▲). The slopes of the lines are similar to unity.

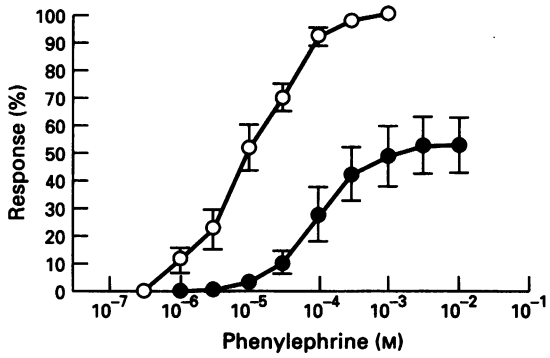

Figure 4 Concentration-response curves of human vas deferens to phenylephrine in the absence of antagonist (○) and in the presence of 1 nM tamsulosin (●). Responses are plotted as a percentage of the initial control curve maximum.

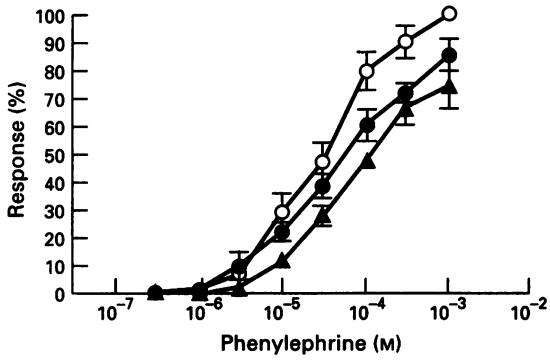

Figure 5 Concentration-response curves to phenylephrine of human vas deferens before incubation (○) and following a 40 min incubation with chloroethylclonidine at 25 μM (●) or 100 μM (▲). Responses are plotted as a percentage of the first concentration-response curve. The control curve for the 100 μM experiment is presented and is similar to that for the 25 μM experiment.

Discussion

The existence of three α_1-adrenoceptor subtypes has been clearly demonstrated in recent years by use of molecular biological techniques. Clone α_{1d}-adrenoceptors with a high affinity for the competitive antagonist WB4101 (Perez *et al.*, 1991): cloned α_{1b}-adrenoceptors with a lower affinity for this competitive antagonist (Cotecchia *et al.*, 1988); and an α_{1c}-adrenoceptor with a high affinity for oxymetazoline and 5-methylurapidil have been sequenced and cloned (Schwinn *et al.*, 1990). The precise relationship between these cloned receptors and those receptors (native receptors) mediating functional responses to α_1-adrenoceptor agonists is a matter of debate but it has been proposed that the cloned α_{1c}-adrenoceptor represents the classical functional α_{1A}-adrenoceptor found in tissues such as the rat vas deferens (Faure *et al.*, 1994; Ford *et al.*, 1994; Rokosh *et al.*, 1994). This native α_{1A}-adrenoceptor has a higher affinity for WB4101 and 5-methylurapidil than the native α_{1B}-adrenoceptor found in tissues such as the rat spleen. It has also been suggested that the α_{1d}-adrenoceptor, which has a high affinity for WB4101 but a low affinity for 5-methylurapdil, mediates contraction of the rat aorta (Kenny *et al.*, 1995).

Our knowledge of the distribution and functional characteristics of α_1-adrenoceptor subtypes in human tissues is limited because of the difficulties involved in obtaining fresh viable tissue specimens. Human prostate samples obtained from prostate resection procedures have been used successfully for functional studies although results are inconclusive. Several studies have concluded that the predominant functional receptor in this tissue has the pharmacological characteristics of the native α_{1A}-adrenoceptor which appears to be identical to the cloned α_{1c}-adrenoceptor subtype (Chapple *et al.*, 1992; Lepor *et al.*, 1993; Testa *et al.*, 1993) but the receptor has a low affinity for RS17053, a compound which has been proposed to be selective for α_{1A} and α_{1c}-adrenoceptors (Ford *et al.*, 1995). Receptor transcript studies support the concept that the cloned α_{1c}-adrenoceptor is the predominant receptor in the human prostate (Price *et al.*, 1993), although mRNA levels may not necessarily represent true receptor levels.

The aim of the present study was to characterize the α_1-adrenoceptors responsible for mediating contractile responses of the human vas deferens. In animal experiments the vas deferens and spleen of the rat have traditionally been used to investigate functional α_{1A} and α_{1B}-adrenoceptors respectively. At the α_{1A}-adrenoceptor of the rat vas deferens, WB4101 has a pK_B of 9.4 whilst at the α_{1B}-adrenoceptor of the rat spleen this antagonist has a lower affinity of 8.4 (Couldwell *et al.*, 1993). These values were obtained in our laboratory under identical conditions to those of the present study where a value of 9.2 was obtained for the human vas deferens. Similarly for 5-methylurapidil, the pK_B value of 8.9 is similar to the value obtained at the α_{1A}-adrenoceptors of the rat vas deferens ($pA_2 = 8.5$, Hanft & Gross, 1989) and these values are signficantly greater than the values reported for the splenic α_{1B}-adrenoceptors in radioligand binding studies to native receptors (7.6, Michel *et al.*, 1994). Both WB4101 and 5-methylurapidil therefore exhibit a high affinity for the functional α_1-adrenoceptors of the human vas deferens, indicating that the α_{1B}-adrenoceptor is not the predominant subtype mediating responses of this tissue.

The results with tamsulosin support this conclusion. In radiologand binding studies this compound has been shown to have a higher affinity for α_{1d}- (10.1) and α_{1c}-adrenoceptors (10.6) than the α_{1b}-adrenoceptor (9.1, Michel & Insel, 1994). In functional studies with rat spleen, tamsulosin acts as a competitive antagonist with a low affinity for these α_{1B}-adrenoceptors ($pK_B = 8.9$, Noble *et al.*, 1994) but at the α_{1A}-adrenoceptors of the rat vas deferens it acts as a non-surmountable antagonist reducing maximum responses to phenylephrine by 51% at a concentration of 1 nM (Furukawa *et al.*, 1995). Although it was not possible to calculate an accurate affinity value for tamsulosin, the apparent dissociation constant ($pK_B = 10.0$) and the qualitative effects (ie. non-surmountable effects) of this antagonist at the α_1-adrenoceptors of the human vas deferens are similar to those reported previously for α_{1A}-adrenoceptors and add further support to the conclusion that α_{1B}-adrenoceptors do not mediate contractions of this tissue.

The experiments with chloroethylclonidine also support this conclusion. In radioligand binding studies to native receptors this compound has been shown to irreversibly and selectively inactivate α_{1B}-adrenoceptors without affecting α_{1A}-adrenoceptors (Han *et al.*, 1987a; Minneman *et al.*, 1988). In functional studies, we have previously shown that the incubation of tissues with chloroethylclonidine at a concentration of 25 μM for 40 min will significantly depress splenic α_{1B}-adrenoceptor-mediated responses (Couldwell *et al.*, 1993). Incubation of the human vas deferens with a high concentration of chloroethylclonidine (100 μM) for 40 min did not significantly affect the responses of this tissue, supporting the conclusion that responses to phenylephrine in this tissue are not mediated via the α_{1B}-adrenoceptor subtype.

Until recently no tissue had been identified in which responses were mediated via the α_{1D}-adrenoceptor. However Kenny *et al.* (1995) have demonstrated that the affinities of a range of α_1-adrenoceptor antagonists on the rat aorta correlate with the values obtained at the cloned α_{1d}-adrenoceptor. The responses of the human vas deferens do not appear to be mediated via α_{1d}-adrenoceptors because both 5-methylurapidil and oxymetazoline had high affinities for the receptor. The values of 8.9 and 7.7 obtained for 5-methylurapidil and oxymetazoline respectively in the present study are almost identical to the values of 8.9 and 7.8 for the cloned α_{1c}-adrenoceptor whilst affinity values are more than 10 fold lower for 5-methylurapidil and more than 50 fold lower for oxyme-

tazoline at the α_{1d}-adrenoceptor (Faure *et al.*, 1994; Kenny *et al.*, 1995). It is therefore unlikely that α_{1d}-adrenoceptors are involved in the responses of the human vas deferens to phenylephrine.

The human vas deferens may therefore provide a useful model for the study of the human α_{1A}-adrenoceptor. In preliminary experiments we encountered two problems when using this tissue. When attempting to construct cumulative concentration-response cuves to phenylephrine, tissues developed significant spontaneous activity which made it impossible to measure drug-induced increases in tension. This problem was overcome by use of single concentrations of agonist, washing between each addition. The second problem encountered was tachyphylaxis to the agonist which was overcome by using long washout periods of up to 30 min between each addition of phenylephrine. The protocol finally employed in this study resulted in accurate, highly reproducible concentration-response curves to phenylephrine as illustrated in Figure 1.

The role of extracellular calcium influx in α_1-adrenoceptor-mediated responses of the human vas deferens was also studied. The α_{1A}-adrenoceptor-mediated responses of the vas deferens of the rat are totally dependent on the influx of extracellular calcium and removal of extracellular calcium or the presence of nifedipine completely abolishes responses to noradrenaline and phenylephrine (Han *et al.*, 1987b; Jackson *et al.*, 1992). Also in radioligand binding studies (+)-niguldipine, a calcium channel blocker, has been found to have a high affinity for the α_{1A}-adrenoceptor subtype (Boer *et al.*, 1989) and it has been suggested that α_{1A}-adrenoceptors are linked to a dihydropyridine-sensitive calcium channel (Han *et al.*, 1987b). Some, but not all pharmacological experiments support this hypothesis (see Minneman, 1988; Jackson *et al.*, 1992). Although it is not possible to use dependence on calcium influx as a means of receptor classification, it is of interest to examine this feature to characterize the response fully. With the human vas deferens approximately 60% of the response remained in the presence of nifedipine. Under identical conditions in the rat vas deferens, nifedipine abolished responses to phenylephrine (Jackson *et al.*, 1992). Thus responses of the human vas deferens, unlike the rat vas deferens, are only partially dependent on the influx of calcium through L-type calcium channels.

In conclusion, the high affinity of the competitive antagonists, 5-methylurapidil, WB4101 and oxymetazoline, together with the failure of chloroethylclonidine to antgaonize responses, indicate that the predominant functional α_1-adrenoceptor in the human vas deferens is the α_{1A}-adrenoceptor subtype. The high affinity of 5-methylurapidil and oxymetazoline rule out an involvement of the α_{1B} or α_{1D}-adrenoceptors in contractions of this tissue. These characteristics are also consistent with those reported for the cloned α_{1c}-adrenoceptor and the study lends support to the suggestion that the cloned α_{1c}-adrenoceptor represents the native α_{1A}-adrenoceptor. The human vas deferens therefore provides a suitable and readily accessible tissue for studies of the pharmacologically defined α_{1A}-adrenoceptor present in human tissue.

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