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Functional characterization of α_1-adrenoceptor subtypes in longitudinal and circular muscle of human vas deferens

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Abstract

The α_1-adrenoceptor subtype(s) mediating contraction to noradrenaline in longitudinal and circular muscle of human epididymal vas deferens was studied using competitive antagonists. The effects of the alkylating agents, phenoxybenzamine and chloroethylclonidine were also investigated. Noradrenaline evoked concentration-dependent contractions of longitudinal and circular muscle with comparable potencies (pD_2; 5.6 and 5.5 respectively). The contractions in longitudinal and circular muscle respectively were inhibited by prazosin (pA_2, 8.6 and pK_B, 9.2), 5-methylurapidil (pK_B, 8.7 and 9.1) and less potently by spiperone (pA_2, 7.1) or BMY 7378 (pK_B, 6.3 and 6.6). Contractions of the circular but not longitudinal muscle was comparatively insensitive to pretreatment with phenoxybenzamine. In contrast pretreatment with chloroethylclonidine reduced the contractions in both muscle types and also enhanced phenoxybenzamine-sensitivity in longitudinal but not circular muscle. The results suggest that contractions evoked by noradrenaline in both muscle types of human vas deferens is mediated via activation of α_1-adrenoceptors with pharmacological profile of the α_{1A}-subtype. However the involvement of α_{1A}-adrenoceptor variants, such as the hypothesised α_{1L}-subtype may underlie the differential effects of phenoxybenzamine in longitudinal and circular muscle. Factors contributing to chloroethylclonidine-sensitivity are discussed.

Keywords: Vas deferens, human; Noradrenaline; α_1-Adrenoceptor subtype

1. Introduction

Evidence from molecular cloning and pharmacological studies have shown that α_1-adrenoceptors constitute a heterogenous group. This has led to a classification scheme that recognises three α_1-adrenoceptor subtypes (α_{1A}-, α_{1B}- and α_{1D}-; Hieble et al., 1995). A fourth α_1-adrenoceptor subtype (α_{1L}-) has also been proposed based on the detection of low-affinity binding of [^{3}H]prazosin in a variety of tissues (Muramatsu et al., 1991; Ohmura et al., 1992; Oshita et al., 1993; Ford et al., 1994; Hieble and Bond, 1994 but see Esbenshade et al., 1993). However recent studies indicate that the α_{1L}-adrenoceptor may represent a different conformation of the α_{1A}-adrenoceptor subtype (Ford et al., 1997).

The contraction of human vas deferens is mediated mainly by the action of noradrenaline on postjunctional α_1-adrenoceptors (Birmingham, 1968; Anton and McGrath, 1977; Hedlund et al., 1985; Holmquist et al., 1990). Furukawa et al. (1995) have reported that phenylephrine-induced contraction of whole tissue specimens of human vas deferens is mediated by the stimulation of α_{1A}-adrenoceptors. However, there is some evidence (Amobi and Smith, 1992, 1995a,b) that (i) α_1-adrenoceptor agonists more readily evoke contraction of longitudinal than circular muscle; in comparison both muscle types respond reliably to noradrenaline and (ii) the muscle types are differentially sensitive to inhibition by phenoxybenzamine, an irreversible α_1-adrenoceptor antagonist or by thioridazine, a phenothiazine antipsychotic (non α_1-subtype selective, Sleight et al., 1993; α_{1A}-subtype selective, Chess-Williams et al., 1995). It is unclear whether these effects reflect the involvement of different α_1-adrenoceptor sub-

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types in longitudinal and circular muscle of human vas deferens. This is of particular interest as its physiological function depends on the coordinated contraction of both muscle types (Batra, 1974; Amobi and Smith, 1995a). In the present study, contractions evoked by the primary neurotransmitter, noradrenaline in longitudinal muscle strips and rings of circular muscle were investigated in the presence of a variety of α_1-adrenoceptor antagonists that have been shown in other tissues to exhibit relative selectivity for the different α_1-adrenoceptor subtypes. The effects of phenoxybenzamine and the alkylating agent, chloroethylclonidine on these preparations were also investigated.

2. Materials and methods

2.1. Preparation of tissues

Epididymal specimens of human vas deferens (5–8 mm long, 2–3 mm diameter) were obtained after elective vasectomies of healthy fertile men (30–45 years old). Connective tissue and blood vessels were removed using a light microscope. The specimens were cut either longitudinally into strips (longitudinal muscle preparation; ≈ 5–6 mm long and 1 mm wide) or transversely into rings (circular muscle preparations; 2–3 mm in length). The tissues were suspended horizontally (resting tension 5–7 mN) in a jacketed Perspex chamber superfused at 2 ml/min with Krebs' bicarbonate saline (35–36°C), composition (mM): NaCl, 118.8; $NaHCO_3$, 25; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 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 and monoamine oxidase respectively and the β-adrenoceptor blocker, propranolol (1 μM). Mechanical responses were recorded via a force-displacement transducer (compliance 0.4 mm/g) coupled to a Gould WindoGraf recorder.

2.2. Competitive α_1-adrenoceptor antagonists

Tissues were equilibrated in Krebs' medium by superfusion for 180 min and were then stimulated two to three times with noradrenaline (100 μM, 45–60 min interval) to obtain a reproducible initial response. Following a further 45–60 min reequilibration, the preparations were exposed to α_1-adrenoceptor antagonists or drug-vehicle (time-matched controls) for 60 min. Subsequently noncumulative concentration–response curves to noradrenaline, with exposure times of 5–8 min at intervals of 15–40 min were determined in the continued presence of the antagonists. Circular or longitudinal muscle (rings or strips respectively) preparations from the same patient were always exposed to different concentrations of antagonists. In this and other experiments, only one concentration–response curve to noradrenaline was determined per preparation and separate time/protocol-matched controls were used to correct for any change in tissue sensitivity. Contractions were analysed by using computer software to measure the total response (i.e., rhythmic activity plus sustained tonic response). The response at each concentration is expressed as a percentage of the initial response to noradrenaline (100 μM).

2.3. Pretreatment with phenoxybenzamine or chloroethylclonidine

Tissues were equilibrated in Krebs' superfusate and stimulated with noradrenaline (100 μM) as described above and then exposed to phenoxybenzamine, an irreversible nonsubtype selective α_1-adrenoceptor antagonist (1 μM for 15 or 30 min). At the end of phenoxybenzamine exposure, tissues were repeatedly washed (over a period of 10 min) with drug-free Krebs' medium. In other experiments, tissues were exposed to chloroethylclonidine (100 μM) for a total of 90 min. Repeated pretreatment has been shown to overcome its inaccessibility to α_1-adrenoceptor binding sites and to increase irreversible inactivation of the receptors (Minneman et al., 1988; Suzuki et al., 1990; Hatano et al., 1994). Thus the preparations were superfused with chloroethylclonidine, initially for 20 min then washed for 1–2 min. This was followed by a second 30-min treatment with 1–2 min washout and reexposure for a further 40 min. At the end of chloroethylclonidine exposure, tissues were repeatedly washed (over a period of 10 min). In some experiments, the tissues were then superfused for 20–25 min with chloroethylclonidine-free Krebs' medium and pretreated as described above with phenoxybenzamine (1 μM for 30 min). Following drug pretreatments and washout, all preparations were superfused for a further 45 min with fresh drug-free Krebs' medium. Subsequently, noncumulative concentration–response curves to noradrenaline was determined in preparations pretreated with either phenoxybenzamine or chloroethylclonidine or chloroethylclonidine followed by phenoxybenzamine or drug-free medium (time/protocol-matched controls).

2.4. 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, USA). 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 at the EC_{50} level for different concentrations of antagonist.

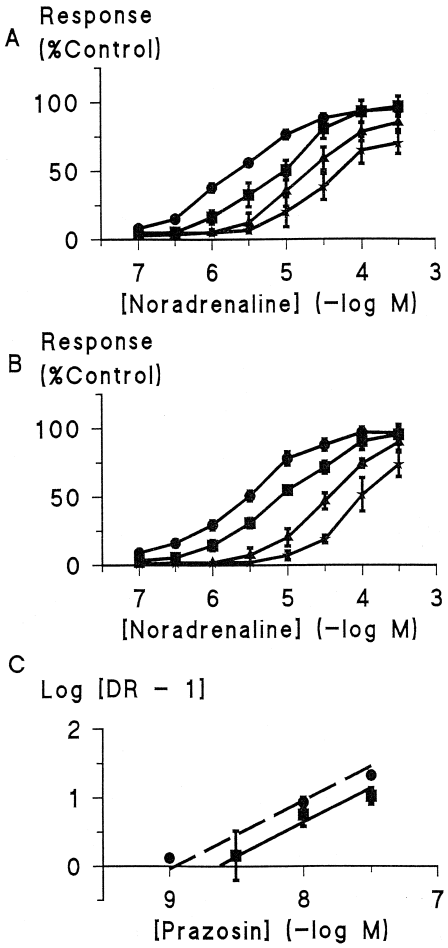

Fig. 1. Inhibitory effects of prazosin on concentration–response curves to noradrenaline in (A) longitudinal and (B) circular muscle of human vas deferens. Controls (● in A and B, $n = 22$ and 19 respectively) and in the presence of prazosin (A) 3 nM (■), 10 nM (▲) and 30 nM (★) and (B) 1 nM (■), 10 nM (▲) and 30 nM (★). The controls in this and in Fig. 2, Fig. 3, and Fig. 4 are the same data. In these figures, responses are expressed as a percentage of the contraction to noradrenaline (100 μM) before exposure to the antagonist or drug vehicle for 60 min and each point represents the mean ± S.E.M. of four to six experiments. (C) Schild plots for the inhibitory effects of prazosin (■) in longitudinal muscle and (●) constrained linear regression (slope = 1) for the antagonism by prazosin in circular muscle (dashed line).

Schild plots (log (DR-1) against log antagonist concentration [B]) were constructed. Antagonist potency (pA_2 value) was obtained from the intercept on the abscissa by linear regression (FIG.P software see above, Arunlakshana and Schild, 1959). In cases where the Schild slope was significantly different from unity, antagonist potency, expressed as apparent pK_B values (−log antagonist dissociation constant) was determined from the Gaddum equation:

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

where DR is the dose ratio produced by the lowest concentration of antagonist [B] to reliably displace the concentration–response curve.

Results are given as mean ± S.E.M. and n refers to the number of experiments. Statistical comparison was carried out using Student's t- test. Differences between the mean of control and experimental groups were considered significant at $P < 0.05$.

2.5. Drugs

Drugs used were propranolol hydrochloride (ICI, Macclesfield, Cheshire), noradrenaline acid tartrate (Winthrop Laboratories, Guildford, Surrey), prazosin hydrochloride (Pfizer, Sandwich) and from Semat (St. Albans, Herts, UK) 5-methylurapidil, spiperone hydrochloride, chloroethylclonidine dihydrochloride, BMY 7378 dihydrochloride, phenoxybenzamine hydrochloride and from Sigma (Poole, Dorset) 17β-oestradiol, desipramine hydrochloride, tropolone, iproniazid hydrochloride and ascorbic acid. Stock solutions of prazosin and oestradiol were prepared in

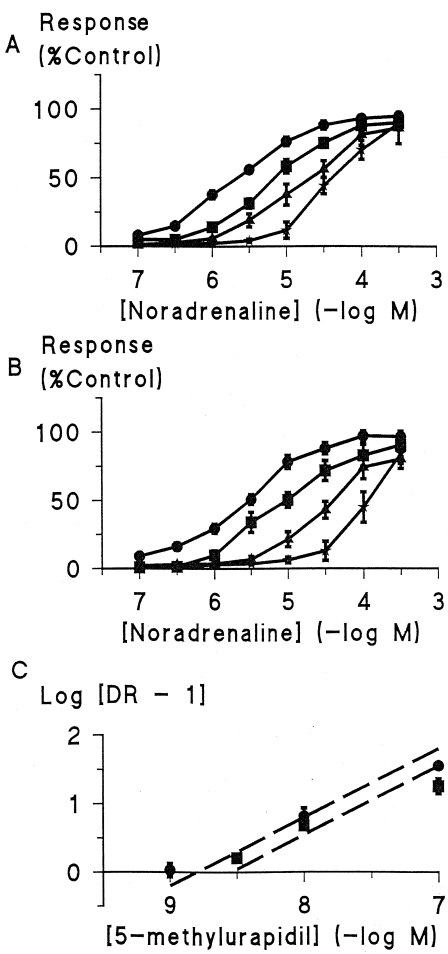

Fig. 2. Inhibitory effects of 5-methylurapidil on concentration–response curves to noradrenaline in (A) longitudinal and (B) circular muscle of human vas deferens. Controls (● in A and B, $n = 22$ and 19 respectively) and in the presence of 5-methylurapidil (A) 3 nM (■), 10 nM (▲) and 100 nM (★) and (B) 1 nM (■), 10 nM (▲) and 100 nM (★). (C) Constrained linear regression (slope = 1) for the antagonism by 5-methylurapidil in (■) longitudinal and (●) circular muscle.

ethanol, phenoxybenzamine in dimethyl sulfoxide and other drugs in distilled water or dilute aqueous acid.

3. Results

3.1. Contractions to noradrenaline and effects of competitive antagonists

Noradrenaline evoked concentration-dependent contractions of longitudinal and circular muscle (Fig. 1) with pD_2 values respectively of 5.6 ± 0.03 ($n = 22$) and 5.5 ± 0.22 ($n = 19$) and maximum contraction of 1.2 ± 0.23 and 1.7 ± 0.36 mN. Prazosin, 5-methylurapidil, spiperone and BMY 7378 produced dose-dependent shifts of the response curve in both muscle types (Figs. 1–4) but in some experiments, the maximum contraction was not attained in the presence of high concentrations of some antagonists.

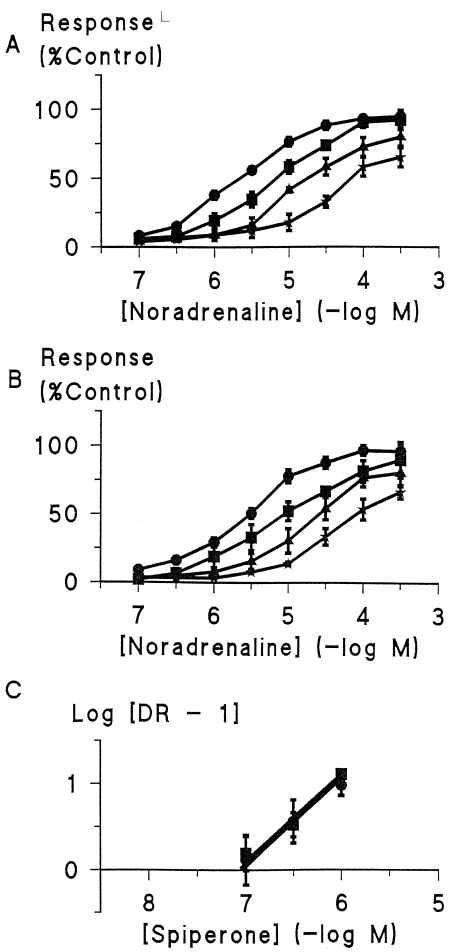

Fig. 3. Inhibitory effects of spiperone on concentration–response curves to noradrenaline in (A) longitudinal and (B) circular muscle of human vas deferens. Controls (● in A and B, $n = 22$ and 19 respectively) and in the presence of spiperone (A and B) 100 nM (■), 300 nM (▲) and 1 μM (★). (C) Schild plots for the inhibitory effects of spiperone (■) in longitudinal and (●) circular muscle.

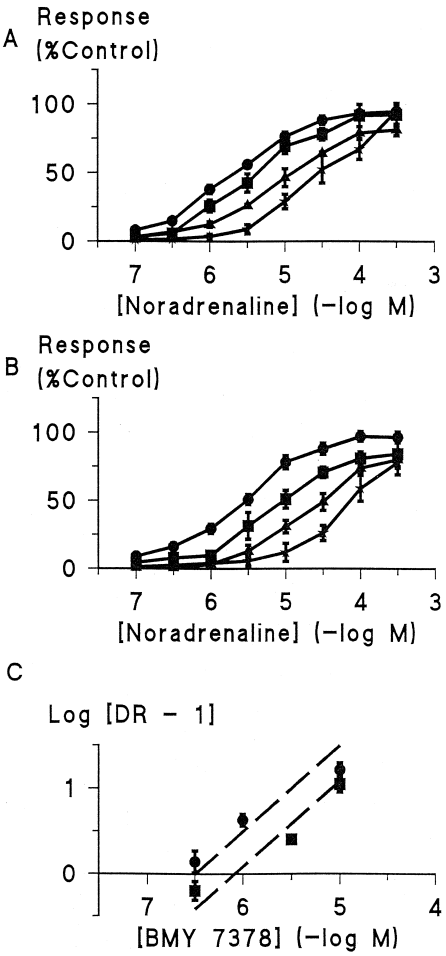

Fig. 4. Inhibitory effects of BMY 7378 on concentration–response curves to noradrenaline in (A) longitudinal and (B) circular muscle of human vas deferens. Controls (● in A and B, $n = 22$ and 19 respectively) and in the presence of BMY 7378 (A) 300 nM (■), 3 μM (▲) and 10 μM (★) and (B) 300 nM (■), 1 μM (▲) and 10 μM (★). (C) Constrained linear regression (slope = 1) for the antagonism by BMY 7378 in (■) longitudinal and (●) circular muscle.

In longitudinal muscle, Schild analysis of the inhibition by prazosin produced a slope not significantly different from unity (0.86 ± 0.18) and yielded an *x*-axis intercept (pA_2 value) of 8.64 ± 0.07 (Fig. 1A and C). In circular muscle, the Schild slope was significantly different from unity (0.8 ± 0.01). The pK_B determined from the lowest concentration of prazosin (1 nM) to displace the response curve was 9.2 ± 0.05 ($n = 4$). Linear regression with the slope constrained to unity yielded an *x*-axis intercept of 8.96 ± 0.09 (Fig. 1C). For both muscle types, inhibition by 5-methylurapidil produced Schild plots with slopes significantly different from unity (0.68 ± 0.09 in longitudinal and 0.76 ± 0.01 in circular muscle). Affinity estimates (pK_B) determined from lowest concentrations of 5-methylurapidil (longitudinal muscle, 3 nM and circular muscle, 1 nM) to reliably displace the response curves were respectively 8.71 ± 0.03 ($n = 4$) and 9.1 ± 0.1 ($n = 4$). Linear regres-

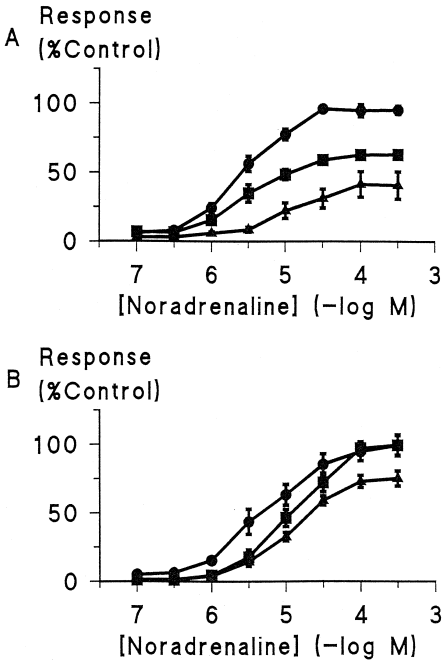

Fig. 5. Effects of pretreatment with phenoxybenzamine on concentration–response curves to noradrenaline in (A) longitudinal and (B) circular muscle of human epididymal vas deferens. Concentration–response curves in time/protocol matched controls (● in A and B, $n = 6$) and in tissues pretreated with phenoxybenzamine (1 μM) for either 15 min (■ in A and B, $n = 4$) or for 30 min (▲ in A and B, $n = 6$ and 5 respectively).

sion with the slopes constrained to unity yielded *x*-axis intercepts of 8.5 ± 0.15 and 8.8 ± 0.6 respectively in longitudinal and circular muscle (Fig. 2C).

Schild analysis of the inhibition by spiperone (Fig. 3C) produced slopes not significantly different from unity in longitudinal and circular muscle and yielded respectively, pA_2 values of 7.11 ± 0.05 (slope, 0.92 ± 0.15) and 7.05 ± 0.04 (slope, 0.87 ± 0.16).

The Schild plot for BMY 7378 produced slopes significantly different from unity (longitudinal muscle, 0.8 ± 0.18; circular muscle, 0.7 ± 0.1). The pK_B determined from the lowest concentration of BMY (0.3 μM) to reliably displace noradrenaline response curve was 6.3 ± 0.11 ($n = 4$) in longitudinal muscle and 6.64 ± 0.07 ($n = 4$) in circular muscle whilst linear regression with the slopes constrained to unity yielded *x*-axis intercepts of 6.1 ± 0.11 and 6.5 ± 0.14 respectively (Fig. 4C).

3.2. Effects of irreversible antagonists

Pretreatment with phenoxybenzamine (1 μM) markedly reduced the maximum contraction to noradrenaline in longitudinal muscle by 37 ± 2% (15 min pretreatment, $n = 4$) and by 59.1 ± 10% (30 min pretreatment, $n = 6$) and produced up to a fourfold reduction in the potency of noradrenaline (Fig. 5A). In contrast, the maximum contraction evoked by noradrenaline in circular muscle was not changed after 15 min pretreatment with phenoxybenzamine (1 μM; $n = 4$, Fig. 5B). Prolonged pretreatment for 30 min reduced the maximum contraction by 25.1 ± 5.5% ($n = 5$) and produced a modest (1.4-fold) reduction in the potency of noradrenaline (Fig. 5B).

The effect of pretreatment with the alkylating agent, chloroethylclonidine was investigated using procedures reported to overcome inaccessibility of the agent to α_1-adrenoceptor binding sites (see methods). Fig. 6A and B shows that noradrenaline-induced contractions of longitudinal and circular muscle were both sensitive to repeated pretreatment with chloroethylclonidine (100 μM, for a total of 90 min); maximum contraction was reduced by 59.6 ± 6.4% ($n = 5$) in longitudinal muscle and by 54.9 ± 10.4% ($n = 5$) in circular muscle. Fig. 6A and B also show that the sensitivity of longitudinal but not circular muscle contractions to phenoxybenzamine was significantly enhanced ($P < 0.01$) by prior treatment with chloroethylclonidine; the maximum contraction in longitudinal muscle was reduced by 83.8 ± 2.7% when the tissues were pretreated with chloroethylclonidine followed by phenoxybenzamine (1 μM for 30 min).

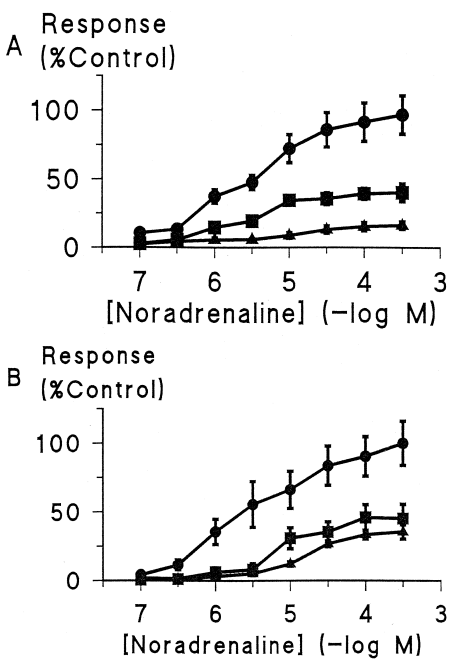

Fig. 6. Effects of repeated pretreatment with chloroethylclonidine on concentration–response curves to noradrenaline in (A) longitudinal and (B) circular muscle of human epididymal vas deferens. Concentration–response curves in time/protocol matched controls (● in A and B, $n = 5$) and effects of pretreatment with chloroethylclonidine (100 μM for a total of 90 min, ■ in A and B, $n = 5$) or combined pretreatment with chloroethylclonidine (100 μM) followed by phenoxybenzamine (1 μM for 30 min, ▲ in A and B, $n = 4$ and 5 respectively).

4. Discussion

The objective of the present study was to characterise the α_1-adrenoceptors in the longitudinal and circular muscle of human vas deferens. The present study differs in a number of ways from an earlier work on the whole tissue specimens by Furukawa et al. (1995). First, longitudinal muscle strips and rings of circular muscle were studied with noradrenaline as the agonist. Secondly, other α_1-adrenoceptor antagonists with relative subtype selectivity (spiperone; α_{1B}-subtype selective in some tissues, Ford et al. (1994) and BMY 7378; α_{1D}-subtype selective, Deng et al. (1996)) were included in the present work. Thirdly, the effects of chloroethylclonidine was reexamined but using procedures that have been reported to reduce its inaccessibility to α_1-adrenoceptor binding sites in intact tissues. Fourthly, the effect of phenoxybenzamine, an agent with putative contraceptive action in the male (Homonnai et al., 1984) was also examined. The main findings from these experiments are discussed below.

The longitudinal and circular muscle showed comparable sensitivity to activation with noradrenaline and were inhibited by prazosin (pA_2/pK_B; 8.6 and 9.2 respectively) consistent with activation of α_1-adrenoceptors. The α_1-adrenoceptor subtype selective antagonists inhibited contractions of both muscle types with 5-methylurapidil showing the highest potency (pK_B; 8.7 and 9.1) in comparison to spiperone (pA_2; 7.1 both muscle types) or BMY 7378 (pK_B; 6.3 and 6.6). The potency profile of these antagonists in both muscle types is comparable to their affinities (pA_2/pK_B) at either native or cloned α_{1A}-adrenoceptors (Aboud et al., 1993; Burt et al., 1995; Kenny et al., 1994, 1995; Testa et al., 1995; Deng et al., 1996). This suggests that noradrenaline contracts both muscle types by stimulating α_1-adrenoceptors with characteristics of the α_{1A}-subtype.

However, chloroethylclonidine and phenoxybenzamine, agents that irreversibly inactivate α_1-adrenoceptors produced different effects. Repeated pretreatment with chloroethylclonidine produced comparable inhibition of longitudinal and circular muscle contractions. This contrasts with the lack of effect of a single chloroethylclonidine pretreatment on phenylephrine- or noradrenaline-induced contractions of human vas deferens (Amobi and Smith, 1995b; Furukawa et al., 1995). Although chloroethylclonidine was introduced as an agent that selectively inactivates the α_{1B}-adrenoceptor subtype (Han et al., 1987; Mallard et al., 1992), subsequent studies have shown that other α_1-adrenoceptor subtypes including the α_{1A}-subtype are sensitive to the agent when procedures that enhance its access are used (Forray et al., 1994; Hatano et al., 1994; also see Oriowo and Ruffolo, 1992; Daniel et al., 1996). Overall, our finding confirms this and is consistent with the view that its inaccessibility and large receptor reserve for agonists contribute to previously reported chloroethylclonidine-insensitivity of α_1-adrenoceptor mediated responses (Tian et al., 1990; Ford et al., 1994; Burt et al., 1995).

Given that sensitivity to chloroethylclonidine is not incompatible with the involvement of α_{1A}-adrenoceptors in longitudinal and circular muscle, the basis for the different action of phenoxybenzamine remains enigmatic. Phenoxybenzamine is generally considered to be a potent irreversible, non subtype selective α_1-adrenoceptor antagonist. Our finding with chloroethylclonidine imply that the effect of phenoxybenzamine is unlikely to be associated with different α_{1A}-adrenoceptor reserves for noradrenaline in the muscle types, especially as the differential inhibition of longitudinal but not circular muscle contraction by phenoxybenzamine remained even after chloroethylclonidine pretreatment.

A second but more provocative possibility for the dissimilar actions of phenoxybenzamine is that the α_1-adrenoceptors in longitudinal and circular muscle of human vas deferens may be variants of the α_{1A}-adrenoceptor subtype. Splice variants of human α_{1A}-adrenoceptor subtype have been described (Hirasawa et al., 1995). Indeed, noradrenaline-induced contraction of human prostatic tissue is mediated via the stimulation of α_1-adrenoceptors considered to be 'atypical' α_{1A}-adrenoceptors (Chess-Williams et al., 1996; Kenny et al., 1996; Noble et al., 1997) and designated as the α_{1L}-subtype by Muramatsu et al. (1994, low affinity for prazosin, pK_B, 8.3). Recently, Ford et al. (1997) have suggested that the conventionally defined (by gene cloning and pharmacological criteria) α_{1A}-adrenoceptor and the α_{1L}-subtype may be different conformational states of the same receptor. Apparently, prazosin and 5-methylurapidil exhibit marginally different affinities for the α_{1L}- and conventional α_{1A}-adrenoceptors (Ford et al., 1997) but no data exists for phenoxybenzamine.

In the present study, the inhibitory potency of prazosin or 5-methylurapidil in both muscle types fall within the wide range of affinity values reported for these antagonists (prazosin; 8.17–11.88 and 5-methylurapidil; 7.7–9.3) at the conventional α_{1A}-adrenoceptor (Salles and Badia, 1991; Aboud et al., 1993; Kenny et al., 1994; Burt et al., 1995; Furukawa et al., 1995). However, it is intriguing that the inhibitory potency of either prazosin or 5-methylurapidil (pA_2/pK_B) in longitudinal muscle (8.6 and 8.7 respectively) and in circular muscle (9.2 and 9.1) matches closely the affinity estimates (pA_2) of these antagonists at the α_{1L}-adrenoceptors of human lower urinary tract (prazosin, 8.7 and 5-methylurapidil, 8.2) and the conventional α_{1A}-adrenoceptor in rat kidney (prazosin, 9.5 and 5-methylurapidil, 9.2; Ford et al., 1997). It is noteworthy that a difference in inhibitory potency was not shown by spiperone (pA_2, 7.1 both muscle types) or BMY 7378 (pK_B, 6.3 in longitudinal and 6.6 in circular muscle). Our results indicate that the α_1-adrenoceptors, presumably α_{1L}- and α_{1A}-subtypes at which prazosin and 5-methylurapidil exhibit different affinities may be present in both muscle types albeit to different extents. The results suggest a

predominance of α_{1L}-subtype in longitudinal muscle and α_{1A}-subtype in circular muscle. Further confirmation of this will depend on the development of better α_{1L}/α_{1A}-subtype selective antagonists.

In conclusion, the results of this study show that contractions evoked by noradrenaline in longitudinal and circular muscle of human epididymal vas deferens is mediated by the stimulation of α_1-adrenoceptors with pharmacological characteristics of the α_{1A}-subtype, perhaps with the α_{1L}-subtype dominating in longitudinal muscle. The results show that chloroethylclonidine inhibited NA-induced contraction of both muscle types and have identified inaccessibility as a contributory factor in the previously reported lack of effect of the agent. Phenoxybenzamine produced a differential inhibition of the longitudinal but not circular muscle, without and with chloroethylclonidine pretreatment.

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