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# Contractile actions of imidazoline α-adrenoceptor agonists and effects of noncompetitive $\alpha_1$-adrenoceptor antagonists in human vas deferens

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**Abstract**

The contractile actions of imidazoline α-adrenoceptor agonists were investigated in human vas deferens longitudinal and circular muscle. The effects of phenoxybenzamine were studied in comparison to dibenamine and SZL-49 (4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-bicyclo[2,2,2]octa-2,5-dienylcarbonyl-2-piperazine), an alkylating prazosin analogue that discriminates between $\alpha_{1H}$- and $\alpha_{1L}$-adrenoceptor subtypes. The imidazoline α-adrenoceptor agonist, A-61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulfonamide hydrobromide), was a potent agonist ($pD_2$; longitudinal muscle 6.9, circular muscle 6.4) and cirazoline a partial agonist ($pD_2$; longitudinal muscle 6.1, circular muscle 5.1). Oxymetazoline was less effective, indanidine and clonidine were ineffective. SZL-49 produced a differential inhibition of contractions evoked by A-61603 in circular ($\alpha_{1H}$) compared to longitudinal ($\alpha_{1L}$) muscle and phenoxybenzamine had the opposite effect. Dibenamine inhibited the contractions comparably in both muscle types and analyses of its partial alkylation of receptors yielded identical estimates of equilibrium dissociation constant ($pK_d$) for A-61603 in longitudinal (5.82) and circular (5.84) muscle. Receptor occupancy–response relationships revealed that whilst the muscle types are not different in receptor reserves for A-61603, contraction to the potent imidazoline is more efficiently coupled in longitudinal than in circular muscle. This underlies the markedly different responsiveness of the muscle types to cirazoline or oxymetazoline (α-adrenoceptor agonists with lower efficacies relative to A-61603).The differential inhibitory actions of phenoxybenzamine and SZL-49 are discussed.


*Keywords:* Vas deferens, human; A-61603; Cirazoline; Oxymetazoline; Phenoxybenzamine; SZL-49; Dibenamine

## 1. Introduction

Imidazoline α-adrenoceptor agonists stimulate contraction in a variety of smooth muscles via $\alpha_1$-adrenoceptors (Horie et al., 1995; Knepper et al., 1995) and have been reported to interact differently with the receptor compared to phenethylamines such as noradrenaline (Demarinis et al., 1987; Ruffolo et al., 1980, 1983). Furthermore, it has been reported that contractions evoked by some imidazoline α-adrenoceptor agonists but not by noradrenaline in rat or mouse anococcygeus are preferentially inhibited by phenoxybenzamine (Coates et al., 1982; Coates and Weetman, 1983; Kenakin, 1984). This finding was the basis for an initial attempt to subclassify $\alpha_1$-adrenoceptors. Currently, $\alpha_1$-adrenoceptors are classified on the basis of gene cloning and pharmacological criteria into subtypes that display high affinity ($\alpha_{1H}$) for prazosin ($pK_B \geq 9$; $\alpha_{1A}$, $\alpha_{1B}$ or $\alpha_{1D}$). A second group is characterised solely in terms of its low affinity ($\alpha_{1L}$) for prazosin and some $\alpha_1$-adrenoceptors antagonists ($pK_B \leq 8.5$; Ford et al., 1994; Hieble et al., 1995; Ohmura et al., 1992). However, it has been suggested that the $\alpha_{1A}$- and $\alpha_{1L}$-subtypes may be functional variants of the $\alpha_{1a}$ gene product (Daniels et al., 1999; Ford et al., 1997).

The contraction of human vas deferens is mediated mainly by neurally released noradrenaline and stimulation

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of postjunctional $\alpha_1$- but not $\alpha_2$-adrenoceptors (Hedlund et al., 1985). In situ hybridization studies have reported a predominance of $\alpha_{1a}$ mRNA in human vas deferens smooth muscles (Moriyama et al., 1997), and in agreement, functional studies of vasectomy specimens have characterised the $\alpha_1$-subtype mediating contraction as the $\alpha_{1A}$- or $\alpha_{1L}$-subtype (Davis et al.,1999; Furukawa et al., 1995; Moriyama et al., 1997). Subsequent studies with a variety of competitive $\alpha_1$-adrenoceptor antagonists (Amobi et al., 1999, 2002) showed that contraction of human vas deferens muscle types involves the stimulation of $\alpha_1$-adrenoceptors that exhibit the pharmacological characteristics of the $\alpha_{1L}$-subtype in longitudinal muscle ($pA_2/pK_B$; prazosin, 8.6; WB 4101, 8.6; 5-methylurapidil, 8.7; and RS-17053, 7.07) and $\alpha_{1H}$- (mainly $\alpha_{1A}$-) subtype in circular muscle ($pA_2/pK_B$; prazosin, 9.2; WB 4101, 9.5; 5-methylurapidil, 9.1; and RS-17053, 9.01). Other antagonists had similar inhibitory potencies in longitudinal and circular muscle (Rec 15/2739, 9.2 and 9.8; HV 723, 8.3 and 8.4; spiperone, 7.1 both muscle types; and BMY 7378, 6.3 and 6.6). In these studies, it was also observed that contractions evoked in the muscle types by noradrenaline were differentially blocked by noncompetitive $\alpha_1$-adrenoceptor antagonists such as SZL-49 (4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-bicyclo[2,2,2]octa-2,5-dienylcarbonyl-2-piperazine) or phenoxybenzamine but not by chloroethylclonidine or benextramine or dibenamine. Noradrenaline-induced contraction of longitudinal muscle was more sensitive to phenoxybenzamine than circular muscle contraction whilst SZL-49 produced the opposite effect.

A number of studies have shown that SZL-49 more potently blocks contraction involving $\alpha_{1H}$- than $\alpha_{1L}$-adrenoceptor subtypes (Flavahan et al., 1998; Piascik et al., 1990). In contrast, phenoxybenzamine has not been identified in functional studies as a selective antagonist of the $\alpha_1$-subtypes as currently classified. In view of earlier reports that phenoxybenzamine inhibits contractions of rat or mouse anococcygeus induced by imidazoline α-adrenoceptor agonists but not contractions induced by noradrenaline (Coates et al., 1982; Coates and Weetman, 1983; Kenakin, 1984), the aims of the present study are to investigate the contractile actions of imidazoline α-adrenoceptor agonists in the human vas deferens and determine whether the differential inhibitory effect of phenoxybenzamine in human vas deferens muscle types is agonist-dependent. For comparison, dibenamine, a prototype β-haloalkylamine from which phenoxybenzamine is derived, and SZL-49, a noncompetitive antagonist that discriminates between $\alpha_{1H}$- and $\alpha_{1L}$-adrenoceptor subtypes were included. The effects of the noncompetitive $\alpha_1$-adrenoceptor antagonists against A-61603 and noradrenaline-induced contractions were studied simultaneously and were referred to briefly in a previous publication describing the discriminatory effects of SZL-49 against noradrenaline-induced contractions of human vas deferens (Amobi et al., 2002).

## 2. Materials and methods

### 2.1. 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; ≈ 3 mm in length). The tissues were suspended horizontally (resting tension 5–7 mN) in Perspex chamber superfused at 2 ml/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 and monoamine oxidase, respectively, and the β-adrenoceptor antagonist, propranolol (1 μM). Contractions were recorded via a force-displacement transducer coupled to a Gould WindoGraf recorder.

### 2.2. Imidazoline agonists and pretreatment with irreversible $\alpha_1$-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. After noradrenaline washout and re-equilibration with Krebs' medium for 30–45 min, the following protocols were used.

(i) Tissues were exposed to different concentrations of noncompetitive antagonists using procedures for progressive partial alkylation of $\alpha_1$-adrenoceptors (Ruffolo, 1982; Salles and Badia, 1991). Thus the tissues were exposed to either phenoxybenzamine (10 nM for 15 min or 0.1 μM for 30 min or 1 μM for 15 min) or SZL-49 (10 nM for 15 min or 0.1 μM for 30 min or 1 μM for 15 min) or dibenamine (0.1 μM for 30 min or 1 μM for 15 min). At the end of drug exposures, the tissues were washed repeatedly (over 10 min) with drug-free Krebs' medium and then superfused for a further 45 min with fresh Krebs' medium. Noncumulative dose–response curves to A-61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulfonamide hydrobromide) with exposure times of 5–7 min at intervals of 15–40 min were then determined in tissues pretreated with drugs or drug-free medium (time/protocol-matched controls). In all experiments, only one concentration–response curve was determined per longitudinal or circular muscle prepa-

ration. Tissues pretreated with drugs or drug-free medium 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 as the drug-treated group or as A-61603 controls. These were not invariably from the same patient.

(ii) The tissues were treated exactly as described above without exposure to the noncompetitive $\alpha_1$-adrenoceptor antagonists but superfused for 45 min with fresh Krebs' medium. Subsequently, noncumulative concentration–response curves to other imidazoline α-adrenoceptor agonists (cirazoline, oxymetazoline, indanidine or clonidine) were determined. The contractile actions of the different agonists were usually determined using preparations (either ring segments or longitudinal strips) from a single vas deferens. In other cases, longitudinal (strip) and circular (ring) muscle preparations (not always from the same vas deferens) were run in parallel and exposed the same imidazoline α-adrenoceptor agonist.

### 2.3. Data analysis

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 agonist concentration is expressed as a percentage of the initial response to noradrenaline(100 μM). $EC_{50}$ 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, Durham, NC, USA).

The apparent equilibrium dissociation constant for A-61603, expressed as $pK_d$ ($-\log K_d$), was determined by the methods of Furchgott(1966) and Furchgott and Bursztyn (1967). Briefly, equieffective concentrations of A-61603 were determined by interpolation from the concentration–response curves (controls and dibenamine-pretreated tissues, [A] and [A′], respectively). The reciprocals of these were plotted and values for $K_d$ 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:

$$1/[A] = 1/(q[A']) + (1-q)/(qK_d)$$

from which

$$K_d = (\text{slope} - 1)/\text{intercept and } q = 1/\text{slope}$$

Fractional $\alpha_1$-adrenoceptor occupancy in longitudinal and circular muscle at each concentration of A-61603 was calculated using the equation:

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

A plot of the relative response to A-61603 in longitudinal and circular muscle against the calculated $\alpha_1$-adrenoceptor occupancy was used to verify the presence of receptor reserve for A-61603 in the muscle types.

Results are given as means ± S.E.M. and $n$ refers to the number of experiments. Statistical analysis was by Student's $t$-test when two groups were analysed and by one-way analysis of variance (ANOVA) when more than two groups were analysed. A significant $F$ value from ANOVA tests ($P<0.05$) was followed by a priori comparison with Student's $t$-test (directional where appropriate) using the within-group variance (mean square) from ANOVA. Differences were considered significant at $P<0.05$.

### 2.4. Drugs

Drugs used were as follows: propranolol hydrochloride (ICI, Macclesfield, Cheshire), noradrenaline tartrate (Winthrop Laboratories, Surrey), dibenamine (TCI, Tokyo, Japan), indanidine (Sgd 101/75, Siegfried, Switzerland). Cirazoline hydrochloride, oxymetazoline hydrochloride and A-61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulfonamide hydrobromide) from Tocris (UK). Phenoxybenzamine hydrochloride, SZL-49 (4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-bicyclo[2,2,2]octa-2,5-dienylcarbonyl-2-piperazine) and clonidine hydrochloride from Research Biochem International (Natick, MA, USA). Desipramine hydrochloride,17β-oestradiol, tropolone, iproniazid hydrochloride and ascorbic acid from Sigma (Poole, Dorset). Stock solutions of dibenamine, SZL-49 or phenoxybenzamine were prepared in dimethyl sulfoxide, 17β-oestradiol in ethanol and other drugs in distilled water. Aliquots were added to the perfusate with a final dimethyl sulfoxide or ethanol concentration of less than 0.01%.

## 3. Results

### 3.1. Contractions to imidazoline α-adrenoceptor agonists

The concentration-dependent contractions evoked by imidazoline α-adrenoceptor agonists in the longitudinal and circular muscle of human vas deferens are shown in Fig. 1. A-61603 activated both muscle types but was more potent in longitudinal than in circular muscle ($pD_2$; 6.91 ± 0.11 and 6.36 ± 0.07, $n=13$ and 17, respectively). Cirazoline also activated both muscle types but was less effective in circular than in longitudinal muscle ($pD_2$; 5.06 ± 0.08 and 6.05 ± 0.06, $n=12$ and 8, respectively). Oxymetazoline (up to 300 μM) produced a weak contractile action in longitudinal muscle and was ineffective in circular muscle. Clonidine and indanidine (up to 1 mM, not shown) were comparatively ineffective in both muscle types.

The potencies and intrinsic activities of the effective imidazoline α-adrenoceptor agonists relative to noradrenaline are shown in Table 1. In both muscle types, A-61603 behaved as a full agonist (maximal response comparable to

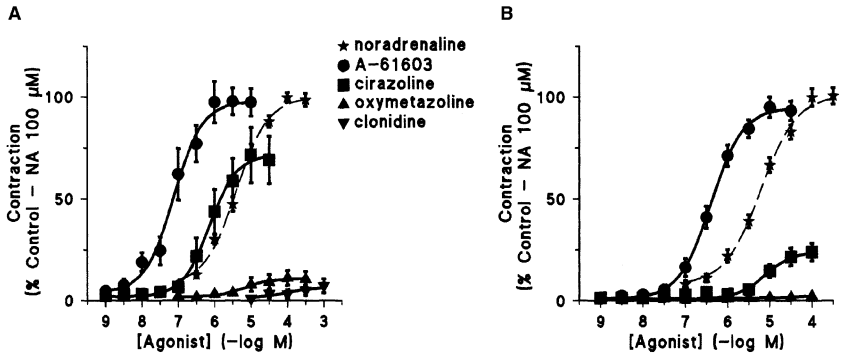


Fig. 1. Activation profiles of imidazoline α-adrenoceptor agonists in (A) longitudinal and (B) circular muscle of human vas deferens: A-61603 ($n$ = 13–17), cirazoline ($n$ = 8–12), oxymetazoline ($n$ = 5–6) and clonidine (longitudinal muscle, $n$ = 5; circular muscle, $n$ = 4; not shown). The dotted line shows concentration–response curves to noradrenaline (Amobi et al., 2002; see Introduction). Each data point represents the mean ± S.E.M. and $n$ is the number of experiments.

that of noradrenaline) but was more potent than noradrenaline. Cirazoline behaved as a partial agonist in both muscle types and was more potent than noradrenaline in longitudinal but not in circular muscle.

### 3.2. Effects of noncompetitive $\alpha_1$-adrenoceptor antagonists on A-61603-induced contractions

The effects of noncompetitive $\alpha_1$-adrenoceptor antagonists were studied against contractions evoked by A-61603, a full agonist in both longitudinal and circular muscle. The effects of phenoxybenzamine and SZL-49 on the concentration–response curves to A-61603 are shown in Fig. 2 and summarised in Table 2. Partial alkylation of $\alpha_1$-adrenoceptors with phenoxybenzamine produced a progressive inhibition of longitudinal compared to circular muscle contractions evoked by the agonist. In longitudinal muscle, phenoxybenzamine (0.01 μM for 15 min or 0.1 μM for 30 min) effectively reduced both the potency of A-61603 and the maximum contraction (Fig. 2A). In comparison, circular muscle contraction was less sensitive: the same concentration of (0.01 μM for 15 min or 0.1 μM for 30 min) produced little to moderate change in the potency of A-61603 and the maximum contraction (Fig. 2B; Table 2). A higher concentration of phenoxybenzamine (1 μM for 15 min) reduced the maximum contraction by 35% in circular muscle compared to 79% reduction in longitudinal muscle (Table 2).

Partial alkylation of the receptors with SZL-49 also produced a differential inhibition of longitudinal and circular muscle contractions to A-61603 but in a manner opposite to that of phenoxybenzamine. In longitudinal muscle, SZL-49 (0.01 μM for 15 min) produced a dextral shift of the concentration–response curve to A-61603, thus reducing its potency but not the maximum contraction. In circular muscle, the same concentration of SZL-49 (0.01 μM for 15 min) reduced both the potency of A-61603 and the maximum contraction (Fig. 2C and D; Table 2). Higher concentrations of SZL-49 (0.1 μM for 30 min or 1 μM for 15 min) progressively reduced the potency of A-61603 in both muscle types but this was associated with 29–44% reduction of the maximum contraction in longitudinal muscle compared to 53–70% reduction of the maximum in circular muscle (Table 2).

The effects of inactivating the $\alpha_1$-adrenoceptors with dibenamine (0.1 μM for 30 min or 1 μM for 15 min) on the concentration–response curves to A-61603 are shown in

Table 1
Characteristics of the contractile action of imidazoline α-adrenoceptor agonists and noradrenaline in human vas deferens

| Agonist | Longitudinal muscle | | | | Circular muscle | | | |
|---|---|---|---|---|---|---|---|---|
| | p$D_2$ | Relative potency | Intrinsic activity | $n$ | p$D_2$ | Relative potency | Intrinsic activity | $n$ |
| Noradrenaline[a] | 5.40 ± 0.1 | 1.00 | 1.00 | 28 | 5.21 ± 0.1 | 1.00 | 1.00 | 24 |
| A-61603[b] | 6.91 ± 0.11 | 32-fold | 0.98 ± 0.07 | 13 | 6.36 ± 0.07 | 14-fold | 0.95 ± 0.05 | 17 |
| Cirazoline[b,c] | 6.05 ± 0.06 | 4.5-fold | 0.72 ± 0.12 | 8 | 5.06 ± 0.08 | 0.7-fold | 0.24 ± 0.04 | 12 |
| Oxymetazoline | nd | – | 0.11 ± 0.03 | 5 | no response | – | – | 6 |

Data are mean ± S.E.M. ($n$ = number of experiments); p$D_2$ = – log $EC_{50}$.
nd: not determined due to weak agonist action.
[a] Data from simultaneous experiments (noradrenaline maximum contraction in longitudinal and circular muscle: 2.5 ± 0.8 and 3.9 ± 1.3 mN, respectively; Amobi et al., 2002). Relative potency and intrinsic activity of imidazoline α-adrenoceptor agonists are expressed with reference to noradrenaline.
[b] $P$ < 0.01: comparison of agonist potencies and intrinsic activities.
[c] $P$ < 0.01: comparison of agonist potencies between longitudinal and circular muscle.

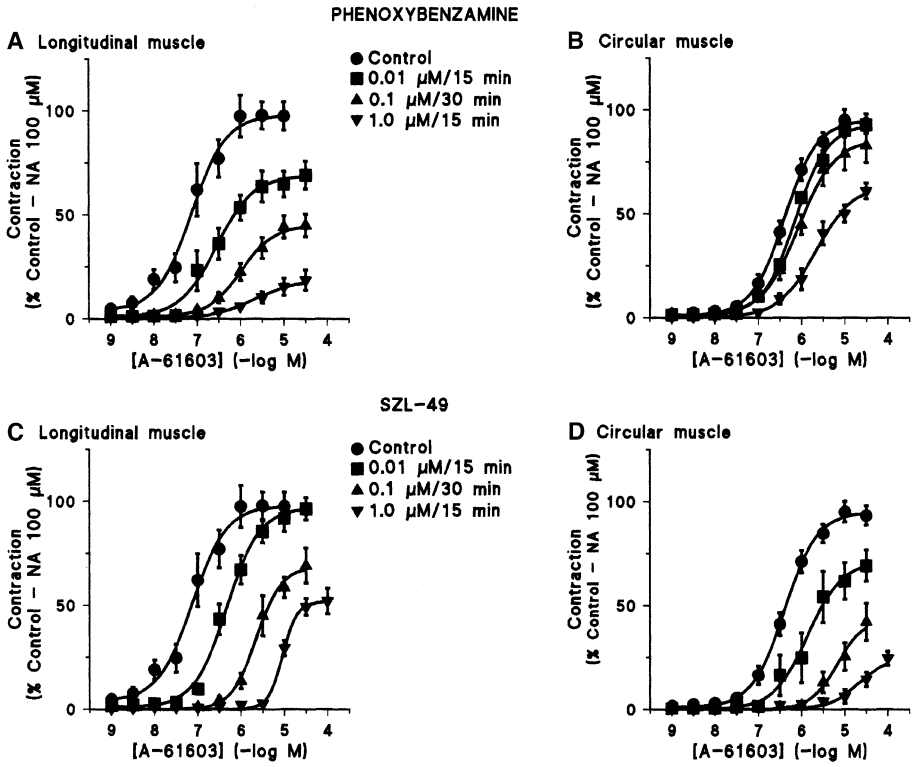


Fig. 2. Effects of partial alkylation of $\alpha_1$-adrenoceptors with phenoxybenzamine (upper panels) and SZL-49 (lower panels) on concentration–response curves evoked by A-61603 in longitudinal and circular muscle of human vas deferens. Controls ($n = 13–17$), phenoxybenzamine (A and B; 0.01 μM for 15 min, $n = 6$; 0.1 μM for 30 min, $n = 10$; 1 μM for 15 min, $n = 4–8$), SZL-49 (C and D; 0.01 μM for 15 min, $n = 6$; 0.1 μM for 30 min, $n = 8$; 1 μM for 15 min, $n = 5$). Data points represent mean ± S.E.M. and $n$ is the number of experiments.

Fig. 3. The potency of A-61603 was reduced by 3–6-fold in longitudinal muscle and by 2–3-fold in circular muscle. The two pretreatment procedures with dibenamine produced comparable reduction of the maximum contraction to A-61603 in longitudinal muscle (22–36%) and in circular muscle (25–35%, Table 2) and thus were used in all subsequent analysis.

### 3.3. Determination of $K_d$, receptor occupancy–response relationship and receptor reserve for A-61603

The equilibrium dissociation constant ($K_d$) for A-61603 was determined by analysing the effects of partial alkylation of $\alpha_1$-adrenoceptors with dibenamine as described in Materials and methods. Representative double-reciprocal plots of

Table 2
Effects of noncompetitive $\alpha_1$-adrenoceptor antagonists on contractions evoked by A-61603 in longitudinal and circular muscle of human vas deferens

| | Longitudinal muscle | | | | Circular muscle | | | |
|---|---|---|---|---|---|---|---|---|
| | $pD_2$ | Change in potency | $E_{max}$ (% decrease) | $n$ | $pD_2$ | Change in potency | $E_{max}$ (% decrease) | $n$ |
| Control | 6.91 ± 0.11 | – | – | 13 | 6.36 ± 0.07 | – | – | 17 |
| Phenoxybenzamine | | | | | | | | |
| 0.01 μM, 15 min | 6.50 ± 0.09 | 2.6-fold | 28.7 ± 6.7[a] | 6 | 6.17 ± 0.05 | 1.5-fold | 2.3 ± 3.1 | 6 |
| 0.1 μM, 30 min | 5.93 ± 0.07 | 9.6-fold | 51.6 ± 5.6[a] | 10 | 6.12 ± 0.07 | 1.7-fold | 11.5 ± 8.4 | 10 |
| 1.0 μM, 15 min | nd | nd | 79.2 ± 4.9[a] | 4 | 5.73 ± 0.1 | 4.3-fold | 34.5 ± 3.8[a] | 8 |
| SZL-49 | | | | | | | | |
| 0.01 μM, 15 min | 6.31 ± 0.06 | 4.0-fold | 0.85 ± 5.6 | 6 | 5.83 ± 0.19 | 3.4-fold | 25.7 ± 7.6[a] | 6 |
| 0.1 μM, 30 min | 5.62 ± 0.06 | 19.5-fold | 28.5 ± 8.4[a] | 8 | 5.13 ± 0.05 | 17.0-fold | 52.8 ± 9.0[a] | 8 |
| 1.0 μM, 15 min | 5.03 ± 0.01 | 76-fold | 44.4 ± 5.6[a] | 5 | nd | nd | 69.6 ± 3.3[a] | 5 |
| Dibenamine | | | | | | | | |
| 0.1 μM, 30 min | 6.43 ± 0.11 | 3.0-fold | 22.0 ± 3.6[b] | 6 | 6.07 ± 0.03 | 2.0-fold | 25.1 ± 3.3[a] | 6 |
| 1.0 μM, 15 min | 6.14 ± 0.08 | 5.9-fold | 36.1 ± 3.8[a] | 6 | 5.93 ± 0.12 | 2.7-fold | 35.4 ± 3.3[a] | 6 |

Data are mean ± S.E.M. ($n$ = number of experiments). A-61603 potency is expressed as $pD_2$ ($-\log EC_{50}$).
nd: not determined because of marked depression of the maximum contraction.
[a] $P < 0.01$.
[b] $0.01 < P < 0.05$.

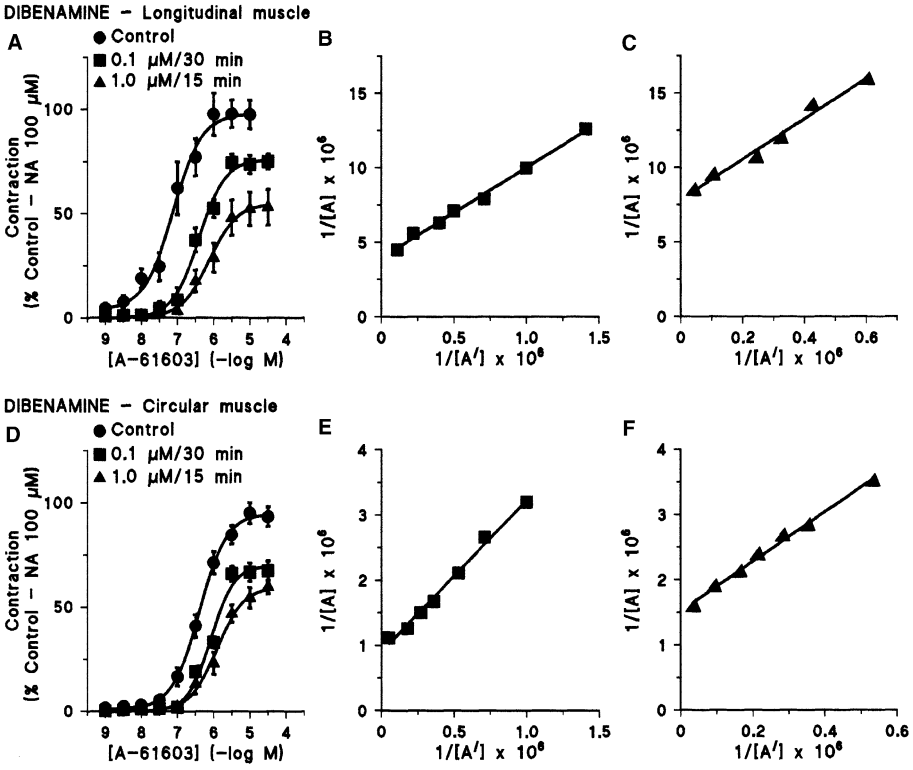


Fig. 3. Effects of partial alkylation of $\alpha_1$-adrenoceptors with dibenamine on concentration–response curves evoked by A-61603 in (A) longitudinal and (D) circular muscle of human vas deferens (controls, $n=13–17$; dibenamine, 0.1 μM for 30 min, $n=6$; 1 μM for 15 min, $n=6$). Data points represent mean ± S.E.M. and $n$ is the number of experiments. Typical double-reciprocal plots of equieffective concentrations of A-61603 (data derived from A and D). In these examples, $K_d$ (expressed as p$K_d$) was calculated as described in Materials and methods from the linear functions: in longitudinal muscle (B; 0.1 μM dibenamine for 30 min) $y=6.05x+(3.957\times10^6)$, p$K_d=5.89$; (C; 1 μM dibenamine for 15 min) $y=13.53x+(7.845\times10^6)$, p$K_d=5.80$; and in circular muscle (E; 0.1 μM dibenamine for 30 min) $y=2.32x+(0.905\times10^6)$, p$K_d=5.83$; (F; 1 μM dibenamine for 15 min) $y=3.80x+(1.521\times10^6)$, p$K_d=5.73$. Mean $K_d$ values for A-61603, determined from individual experiments in longitudinal and circular muscle, were used in subsequent receptor analysis.

equieffective concentrations of A-61603 from controls and tissues pretreated with dibenamine are shown in Fig. 3. Estimates of $K_d$ for A-61603 (expressed as p$K_d$) and q values are summarised in Table 3. In both muscle types, the p$K_d$ values for A-61603, obtained after pretreatment with dibenamine (0.1 μM for 30 min), are comparable to values obtained with a higher concentration of the antagonist (1 μM for 15 min). However, the two pretreatment procedures progressively inactivated a greater proportion of the $\mu_1$-adrenoceptors in longitudinal than in circular muscle as reflected by the $q$ values (Table 3). Subsequent analysis examined the relationship between receptor occupancy and response evoked in the muscle types by A-61603.

The mean $K_d$ value for A-61603 in longitudinal and circular muscle was used in the calculation of theoretical receptor occupancy for that muscle. A plot of contractile

Table 3
Apparent equilibrium dissociation constant (p$K_d$) for A-61603 and the fraction of $\alpha_1$-adrenoceptors remaining active ($q$) in longitudinal and circular muscle of human epididymal vas deferens after pretreatment with different concentrations of dibenamine

| | Longitudinal muscle | | | Circular muscle | | |
|---|---|---|---|---|---|---|
| | p$K_d$ | $q$ (%) | $n$ | p$K_d$ | $q$ (%) | $n$ |
| Dibenamine | | | | | | |
| 0.1 μM, 30 min | 5.90 ± 0.18 | 21.9 ± 6.9 | 6 | 5.82 ± 0.06 | 45.0 ± 4.3 | 6 |
| 1.0 μM, 15 min | 5.73 ± 0.10 | 8.42 ± 1.9 | 6 | 5.86 ± 0.19 | 35.1 ± 9.1 | 6 |

Data are mean ± S.E.M. and $n$ is number of experiments. p$K_d$ ($-\log K_d$) values not significantly different (ANOVA, P>0.05) and mean p$K_d$ from pooled data: 5.82 ± 0.11 ($n=12$) in longitudinal muscle and 5.84 ± 0.10 in circular muscle ($n=12$).

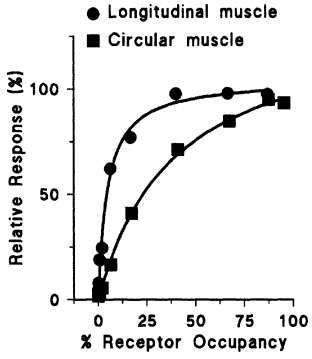


Fig. 4. Plots of contraction to A-61603 as a function of receptor occupancy in longitudinal and circular muscle. Receptor occupancy was calculated as described in Materials and methods using the mean $K_d$ values for A-61603 in longitudinal and circular muscle.

response to A-61603 as a function of the theoretical receptor occupancy yielded different nonlinear occupancy–response curves in longitudinal and circular muscle (Fig. 4). It was determined from these curves that half-maximal contraction required about 5% and 24% receptor occupancy in longitudinal and circular muscle, respectively, but virtually all the receptors were required for maximal contraction in both muscle types. The efficiency of coupling between receptor occupancy and response in the muscle types estimated from the ratio $K_d/EC_{50}$ were 12.3 and 3.3 in longitudinal and circular muscle, respectively.

## 4. Discussion

Two aspects of this study are, first, the differential activation of human vas deferens muscle types by imidazoline α-adrenoceptor agonists and, second, the effects of noncompetitive $\alpha_1$-adrenoceptor antagonists. The inhibitory profiles of phenoxybenzamine, dibenamine and SZL-49 against A-61603-induced contractions (present study) are similar to their action against contractions to noradrenaline (Amobi et al., 1999, 2002). However, the present results show a more efficient coupling between receptor occupancy and response to A-61603 in longitudinal than in circular muscle. This is remarkably different from that observed with noradrenaline.

Evidences from ligand binding and functional studies indicate that imidazoline α-adrenoceptor agonists exhibit a greater selectivity for $\alpha_{1A}$- over $\alpha_{1B}$- and $\alpha_{1D}$-subtypes (Ford et al., 1997; Horie et al., 1995; Knepper et al., 1995) and that A-61603 acts as a full agonist relative to noradrenaline in tissues that express $\alpha_1$-adrenoceptors characterised either as $\alpha_{1A}$- or $\alpha_{1L}$-subtypes (rat vas deferens and canine prostate—Knepper et al., 1995; rat caudal artery—Lachnit et al., 1997; rat small mesenteric artery—Stam et al., 1999). In contrast, cirazoline, oxymetazoline and other imidazoline α-adrenoceptor agonists display a spectrum of action as partial or full agonists in these and other tissues that express the $\alpha_{1A}$- or $\alpha_{1L}$-subtypes (rat perfused kidney—Blue et al., 1995; rat vas deferens—Buckner et al., 1996; Kenakin, 1984; rodent anococcygeus—Coates et al., 1982; Coates and Weetman, 1983; rat prostate—Hiraoka et al., 1999). In the present study of five imidazolines, only A-61603 and cirazoline reliably activated the human vas deferens. A-61603 was 14–32-fold more potent than noradrenaline. This is comparable to its relative potency in human resistance arteries ($\alpha_{1A}$, 17-fold, Jarajapu et al., 2001) and in studies of cloned $\alpha_{1a}$-subtype (21-fold, Knepper et al., 1995), but higher potencies have been reported (canine prostate and rat vas deferens—Knepper et al., 1995; rat small mesenteric artery—Stam et al., 1999). The relative potency of cirazoline (0.7- to about 5-fold) is similar to values (0.6–3.4-fold) reported in rat small mesenteric artery (Stam et al., 1999) and studies of cloned $\alpha_{1a}$-subtype (Horie et al., 1995; Minneman et al., 1994). Oxymetazoline was a weak agonist in longitudinal muscle and ineffective in circular muscle. Indanidine and clonidine were ineffective in both muscle types. The findings with oxymetazoline and clonidine match reports that human vasectomy specimens are quiescent to both drugs (Furukawa et al., 1995; Hedlund et al., 1985).

The variable action of imidazoline α-adrenoceptor agonists in human vas deferens is reminiscent of their action in tissues that express the $\alpha_{1A}$- or $\alpha_{1L}$-adrenoceptor subtype and clearly is not due to selective activation of these subtypes. What then is the basis for the differential activation of human vas deferens muscle types by imidazoline α-adrenoceptor agonists? A factor that can influence tissue responsiveness independent of receptor subtype is the intrinsic efficacy of the agonist. Although it cannot be measured directly, it is considered to be a drug-dependent property and therefore is constant. Yet, it is evident that the longitudinal and circular muscle differ markedly in responsiveness (Fig. 1) and in the maximum contraction evoked by the effective imidazoline α-adrenoceptor agonists (Table 1, intrinsic activities of cirazoline or oxymetazoline compared to A-61603). Kenakin (1984) and Salles and Badia (1991) showed that such selective activation by agonists is mainly due to tissue differences in efficiency of stimulus–response coupling. The present results show that receptor occupancy and response to A-61603 is more efficient in longitudinal than in circular muscle. The increased amplification in longitudinal muscle could involve additional effector mechanisms via the same or a different G-protein, its subunits or second messengers (Hoyer and Boddeke, 1993; Macrez-Lepretre et al., 1997; Taguchi et al., 1998; Zhong and Minneman, 1999). A possible contributory factor may involve $Ca^{2+}$ handling. Longitudinal muscle contractility relies more on intracellular $Ca^{2+}$ and a "$Ca^{2+}$-independent" mechanism (perhaps $Ca^{2+}$ sensitization) than does circular muscle (Amobi and Smith, 1993, 1998a,b). Evidences from studies in other tissues indicate that intracellular $Ca^{2+}$ release is more effective than its influx in activating smooth muscle (Bramich and Hirst, 1999; Chiu et al., 1986).

The finding that A-61603 (an imidazoline) stimulates contraction of the muscle types with different efficiencies was unexpected and contrasts with the observation that noradrenaline (a phenethylamine) stimulates both muscle types with comparable efficiencies (Amobi et al., 2002). Imidazolines and phenethylamines interact differently with $\alpha_1$- or indeed $\alpha_2$-adrenoceptors and their different chemical structures have been reported to influence the degree of G-protein coupling (Demarinis et al., 1987; Eason et al., 1994; Ruffolo et al., 1983; Spedding and Dacquet, 1997). Thus the present finding in human vas deferens muscle types that the efficiency of stimulus–response coupling in a tissue depends on ligand structure is noteworthy and can best be explained in terms of agonist–receptor trafficking and receptor promiscuity (Kenakin, 1995, 1997; Perez et al., 1996).

The second aspect of this study relates to the effects of noncompetitive $\alpha_1$-adrenoceptor antagonists. The results indicate that the opposite effects of phenoxybenzamine and SZL-49 cannot be explained in terms of different

receptor reserves for A-61603 in the muscle types. Given this, how can their opposite actions be explained? SZL-49 is known to be more potent at inhibiting contractions involving $\alpha_{1H}$- than $\alpha_{1L}$-adrenoceptor subtypes (Flavahan et al., 1998; Piascik et al., 1990). Previous studies of human vas deferens using competitive $\alpha_1$-subtype antagonists established that the discriminatory effect of SZL-49 against noradrenaline-induced contractions of the muscle types is due to a predominance of $\alpha_{1H}$-subtype (mainly $\alpha_{1A}$) in circular muscle and $\alpha_{1L}$-subtype in longitudinal muscle (Amobi et al., 2002). The inhibitory profile of SZL-49 in the present study with A-61603 is consistent with this characterisation and confirms the agonist independence of its action in this tissue.

For phenoxybenzamine, the evidence for $\alpha_1$-subtype selectivity is more equivocal. Coates et al. (1982) and Coates and Weetman (1983) suggested that phenoxybenzamine could distinguish between two $\alpha_1$-adrenoceptor subtypes in rat or mouse anococcygeus ("$\alpha_{1S}$" and $\alpha_1$, both activated by noradrenaline, and "$\alpha_{1S}$", preferentially activated by the imidazoline, indanidine and sensitive to phenoxybenzamine). In contrast, Kenakin (1984) showed that the differential inhibitory action of phenoxybenzamine originates from the actions of two agonists that differ in intrinsic efficacies and is not due to activation of different $\alpha_1$-subtypes. However, this is unlikely to be the explanation for the finding that phenoxybenzamine caused a differential inhibition of longitudinal compared to circular muscle contractions evoked by the same agonist, A-61603 (present study) or noradrenaline (Amobi et al., 1999).

In more recent ligand binding studies, Maruyama et al. (1992) and Takeda et al. (1997) reported that phenoxybenzamine but not dibenamine exhibits a higher affinity for $\alpha_{1H}$- over $\alpha_{1L}$-subtype. This is the reverse of the effect observed in the present functional study; phenoxybenzamine was more effective against longitudinal ($\alpha_{1L}$) than circular muscle contraction ($\alpha_{1H}$, mainly $\alpha_{1A}$). The basis for the divergence in the functional inhibitory action of phenoxybenzamine and its affinity at $\alpha_{1H}$- and $\alpha_{1L}$-subtypes in ligand binding studies is unclear. However, it is well documented that functional inhibitory potencies of some antagonists differ from their ligand binding affinity estimates at $\alpha_{1H}$- (mainly $\alpha_{1A}$-) adrenoceptor (Ford et al., 1997; Daniels et al., 1999). A more compelling evidence suggesting that the action of phenoxybenzamine in human vas deferens is unlikely to be a greater selectivity for $\alpha_{1L}$- over $\alpha_{1H}$-subtype is that dibenamine inhibited the contractions comparably in both muscle types. Inactivation of $\alpha_1$-adrenoceptors or other transmitter receptors by phenoxybenzamine or by dibenamine is mediated by the same reactive ethyleneimmonium ion. It may be that the agonist-independent action of phenoxybenzamine in human vas deferens involves a specific drug and/or tissue-related factor that is yet to be identified.

In conclusion, the results shows that contractions evoked by the potent imidazoline α-adrenoceptor agonist, A-61603, is more efficiently coupled in longitudinal than in circular muscle of human vas deferens. This underlies the marked differences in the responsiveness of the muscle types to other imidazoline α-adrenoceptor agonists with lower relative efficacies. A caveat noted in this study is that the efficiency of stimulus–response coupling in a tissue may depend on the chemical structure of the ligand. The results also show that the differential inhibitory actions of non-competitive $\alpha_1$-adrenoceptor antagonists against A-61603-induced contraction is not due to different receptor reserves for the agonist in longitudinal and circular muscle. The differential action of SZL-49 but not phenoxybenzamine can be accounted for by the functional predominance of $\alpha_{1H}$-subtype (mainly $\alpha_{1A}$) in circular muscle and $\alpha_{1L}$-subtype in longitudinal muscle.

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