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Functional characterization of α_1-adrenoceptor subtypes in human skeletal muscle resistance arteries

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1 α_1-adrenoceptor subtypes in human skeletal muscle resistance arteries were characterized using agonists noradrenaline (non-selective) and A61603 (α_{1A}-selective), the antagonists prazosin (non-selective), 5-methyl-urapidil (α_{1A}-selective) and BMY7378 (α_{1D}-selective) and the alkylating agent chloroethylclonidine (preferential for α_{1B}).

2 Small arteries were obtained from the non-ischaemic skeletal muscle of limbs amputated for critical limb ischaemia and isometric tension recorded using wire myography.

3 Prazosin antagonized responses to noradrenaline with a pA_2 value of 9.18, consistent with the presence of α_1-adrenoceptors, although the Schild slope (1.32) was significantly different from unity.

4 5-Methyl-urapidil competitively antagonized responses to noradrenaline with a pK_B value of 8.48 and a Schild slope of 0.99, consistent with the presence of α_{1A}-adrenoceptors. In the presence of 300 nM 5-methyl-urapidil, noradrenaline exhibited biphasic concentration response curves, indicating the presence of a minor population of a 5-methyl-urapidil-resistant subtype.

5 Contractile responses to noradrenaline were not affected by 1 μM chloroethylclonidine suggesting the absence of α_{1B}-adrenoceptors. Maximum responses to noradrenaline and A61603 were reduced to a similar extent by 10 μM chloroethylclonidine, suggesting an effect of chloroethylclonidine at α_{1A}-adrenoceptors at the higher concentration.

6 BMY7378 (10 and 100 nM) had no effect on responses to noradrenaline. BMY7378 (1 μM) poorly shifted the potency of noradrenaline giving a pA_2 of 6.52. These results rule out the presence of the α_{1D}-subtype.

7 These results show that contractile responses to noradrenaline in human skeletal muscle resistance arteries are predominantly mediated by the α_{1A}-adrenoceptor subtype with a minor population of an unknown α_1-adrenoceptor subtype.

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Abbreviations: A61603, N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methane sulphonamide; BMY7378, (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione; CEC, chloroethylclonidine; CRC, concentration response curve; RS 79948, (8aR,12aS,13aS)-5,8,8a,9,10,11,12,12a,13a-Decahydro-3-methoxy-12-(ethylsulphonyl)-6H-isoquino[2,1-g][1,6]-naphthyridine

Introduction

Vascular post-junctional α_1-adrenoceptors mediate sympathetic vasoconstriction and thus modulate systemic vascular resistance. Functional, radioligand binding and molecular biological studies, over the last two decades, have demonstrated heterogeneity among the post-junctional α_1-adrenoceptors (Hieble *et al*., 1995). According to the current IUPHAR accepted classification, α_1-adrenoceptors mediating functional responses are classified into three subtypes denoted by α_{1A}, α_{1B} and α_{1D} corresponding to the cloned subtypes α_{1a}, α_{1b} and α_{1d} (Bylund *et al*., 1998). The existence of a fourth subtype with low affinity for prazosin, the α_{1L}-adrenoceptor, has been proposed on the basis of functional studies (Flavahan & Vanhoutte, 1986; Muramatsu *et al*., 1990), though a molecular correlate is lacking. There is now evidence to support the idea that the α_{1L}-adrenoceptor may represent a low affinity state of the α_{1A}-adrenoceptor (Ford *et al*., 1997).

Currently there is limited information on α_1-adrenoceptor subtypes in human blood vessels. Human conduit vessels show the presence of mRNAs for all three subtypes (Shibata *et al*., 1998; Rudner *et al*., 1999; Moriyama *et al*., 2000) but not all of them are functionally coupled since contractile responses to agonists were found to be mediated only by $\alpha_{1A/L}$- in renal artery (Moriyama *et al*., 2000), α_{1A}- in mesenteric artery (Shibata *et al*., 1998), α_{1A}- and α_{1D}- in mammary artery (Rudner *et al*., 1999) and α_{1B}- in internal iliac artery (Hatano *et al*., 1994). Characterization of α_1- subtypes is not yet reported in human resistance arteries, vessels which play a major role in controlling the systemic vascular resistance and maintaining the mean arterial pressure sufficient for tissue perfusion.

The skeletal muscle vascular bed is a major vascular bed with a large contribution to the peripheral arterial resistance. Our previous studies in human skeletal muscle resistance

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arteries showed that contractile responses to noradrenaline are predominantly mediated by α_1-adrenoceptors with a small contribution of α_2-adrenoceptors (Jarajapu *et al*., 2001a). In the present study, the functional α_1-adrenoceptor subtypes in these arteries were characterized using the α_1-adrenoceptor agonists noradrenaline (non-selective) and A-61603 (α_{1A}-selective) (Knepper *et al*., 1995), the antagonists prazosin (selective for α_1- over α_2-), 5-methyl-urapidil (α_{1A}-selective) (Gross *et al*., 1988) and BMY 7378 (α_{1D}-selective) (Goetz *et al*., 1995) and the alkylating agent chloroethylclonidine, which preferentially alkylates α_{1B}-adrenoceptors (Han *et al*., 1987). A preliminary account of these results has been presented to the British Pharmacological Society (Jarajapu *et al*., 2001b).

Methods

The study was approved by the appropriate Ethics Committee and each patient gave informed consent. Skeletal muscle biopsies (~ 1 cm^3) were obtained from non-ischaemic areas of skeletal muscle of limbs amputated for critical limb ischaemia ($n=21$, which includes three females and 18 males with a mean age of 66 ± 3 years). The level of amputation was always selected to be in a non-ischaemic area where the haemodynamics were physiologically normal. This is assessed by Doppler ultrasound and is essential to ensure adequate wound healing after surgery. Skeletal muscle specimens were obtained either from medialis or gastrocnemius muscle. Biopsies were transported to the laboratory in physiological saline solution (PSS) under ice cold conditions. Small arteries (normalized diameter ($L_{0.9}$) of 307 ± 10 μm, $n=68$) were isolated from the biopsies under a stereomicroscope (Zeiss) within 2–3 h of the time of amputation.

Small vessel myography

Arterial segments of 2 mm length were mounted in a small vessel wire myograph (Danish Myotech, Aarhus, Denmark) for isometric tension measurements. The vessel segments were incubated in PSS of composition (mM): NaCl (119), KCl (4.5), $NaHCO_3$ (25), KH_2PO_4 (1), $MgSO_47H_2O$ (1), (+)glucose (11) and $CaCl_2$ (2.5), at 37°C and gassed with carbogen. One hour after mounting, the resting tension–internal circumference relation was determined for each vessel segment (Mulvany & Halpern, 1977). Then, the resting tension was set to a normalized internal circumference of $L_{0.9}$ where $L_{0.9}=0.9L_{100}$ and L_{100} is the internal circumference that the vessel would have under a transmural pressure of 100 mm Hg (13.3 kPa). The software program Myodaq-Myodata was used for data-acquisition. Subsequently, vessel viability was checked by exposure to high potassium solution (123 mM) twice and then to 10 μM noradrenaline in the presence of high potassium solution. Arterial segments were considered viable if they produced an effective pressure of more than 100 mm Hg (13.3 kPa) when stimulated with 123 mM KCl. Effective pressure was calculated from the Laplace equation:

$$\text{Effective pressure} = \text{wall tension}/(\text{internal circumference}/2\pi) \quad (1)$$

which corrects for differences in length and diameter of arterial segments (Mulvany & Halpern, 1977). All the vessels were found to be viable according to this criterion. The presence of functional endothelium was checked with 1 μM carbachol after precontracting with 1 μM noradrenaline. All the vessels in the study produced more than 60% relaxation.

After an equilibration period, three to five concentration response curves (CRCs) were obtained per each arterial segment. In preliminary experiments ($n=8$) no significant changes in maximum responses (% of CRC1 maximum: CRC2, 95 ± 2; CRC3, 90 ± 8; CRC4, 93 ± 10; CRC5, 91 ± 8) or pEC_{50} values (CRC1, 6.3 ± 0.04; CRC2, 6.1 ± 0.03; CRC3, 6.2 ± 0.04; CRC4, 6.0 ± 0.04; CRC5, 6.1 ± 0.06) of noradrenaline were observed showing that repeated CRCs were reproducible and that no corrections for time-dependent changes were required. The second CRC was taken as control and the subsequent CRCs were obtained after incubating the arterial segments for 30 min with antagonists at different concentrations. In the experiments with chloroethylclonidine the arterial segments were exposed to chloroethylclonidine (1 or 10 μM) for 30 min and then washed for 60 min (three times every 15 min) (Hancock, 1996). Propranolol (1 μM), cocaine (3 μM) and corticosterone (3 μM) were added to the PSS when CRCs to noradrenaline were obtained (to block β-adrenoceptors, neuronal and non-neuronal uptake of noradrenaline respectively). The selective α_2-adrenoceptor antagonist RS79948 (0.1 μM), which was shown previously to almost abolish the responses to an α_2-adrenoceptor agonist, brimonidine (UK14304), without affecting the CRCs to phenylephrine (Jarajapu *et al*., 2001a), was included in the PSS throughout the experimental protocol. EDTA (0.023 mM) and ascorbic acid (0.3 mM) were included in the PSS to prevent oxidation of noradrenaline.

Results are expressed as mean ± s.e.m. Agonist potency is expressed as pEC_{50} (the negative logarithm of the concentration required to produce 50% of the maximum response). pEC_{50} values and maximum responses were calculated using the software program GraphPad Prism which fits CRCs to a four parameter logistic equation given below:

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(\log EC_{50} - X)p}} \quad (2)$$

where X is the logarithm of the molar concentration of the agonist, Y is the response and P is the Hill slope. In one case the four parameter logistic equation for a two-site fit was used as given below:

$$Y = \text{Bottom1} + \frac{(\text{Top1} - \text{Bottom1})}{1 + 10^{(\log EC_{50}1 - X)P1}} + \frac{(\text{Top2} - \text{Bottom2})}{1 + 10^{(\log EC_{50}2 - X)P2}} \quad (3)$$

where 1 and 2 refer to the first and second sites.

Antagonist affinities are expressed either as pA_2 or pK_B values. When three different concentrations of the antagonist were used, pA_2 values were obtained from the x-intercept of the plot of log(r-1) and log[B] without constraining the slope of the line (Arunlakshana & Schild, 1959). If the antagonism met the criteria of competition (Schild slope close to unity) then the affinity was expressed as the pK_B. When only one concentration of the antagonist was used the pA_2 was obtained from the following equation (Schild, 1949):

$$pA_2 = -\log([B]/(r-1)) \quad (4)$$

where [B] is the molar concentration of the antagonist and 'r' is the ratio of EC_{50} of the agonist in the presence of the antagonist to that in the absence.

Drugs

(−)-Noradrenaline (arterenol) bitartrate, propranolol hydrochloride, corticosterone acetate and prazosin HCl were obtained from Sigma (Poole, Dorset, U.K.); Cocaine HCl was obtained from Thornton and Ross Ltd. (U.K.); RS 79948 ((8aR,12aS,13aS)-5,8,8a,9,10,11,12,12a,13a-Decahydro-3-methoxy-12-(ethylsulphonyl)-6H-isoquino[2,1-g][1,6]-naph-thyridine) and A61603 (N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulphonamide) were obtained from Tocris (Avonmouth, Bristol, U.K.); 5 methyl urapidil, chloroethylclonidine 2HCl and BMY 7378 ((8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione) were obtained from RBI (Natick, U.S.A.). The stock solution of 5-methyl-urapidil was prepared in 5% dimethyl sulphoxide and that of corticosterone acetate was prepared in 25% absolute ethanol. Stock solutions of all the other drugs were prepared in distilled water. PSS containing 123 mM KCl was prepared by replacing NaCl with an equimolar quantity of KCl.

Statistics

pEC_{50} values and maximum responses were compared by using paired '*t*'-test or two-way ANOVA followed by the Newman-Keuls range test for multiple comparisons. An F-test was used to compare the curve fits to one-site and two-site models. Confidence limits were obtained from GraphPad prism.

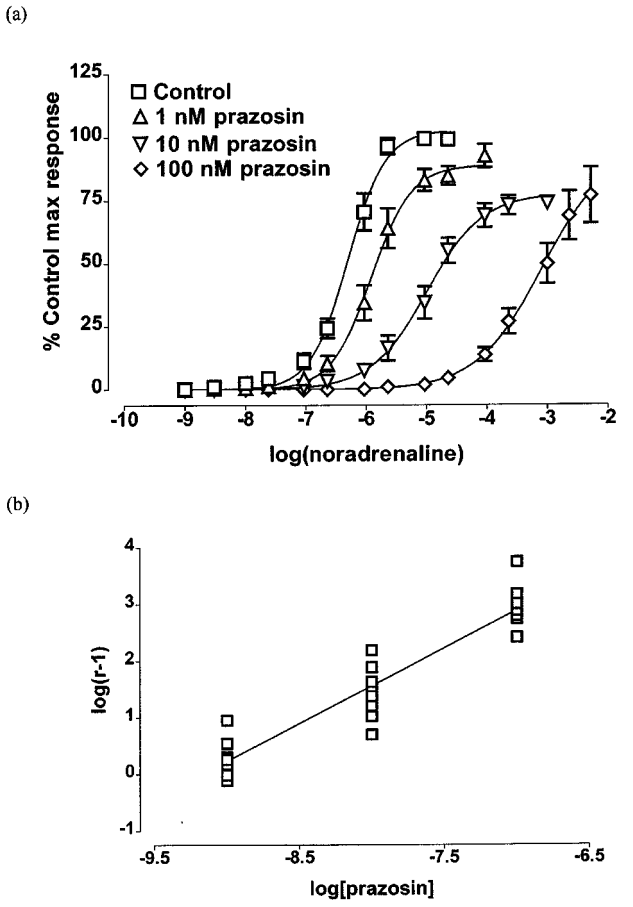

Figure 1 (a) Prazosin antagonism of responses to noradrenaline in human skeletal muscle resistance arteries ($n=11$). (b) Schild plot for the prazosin antagonism of noradrenaline responses in human skeletal muscle resistance arteries ($n=28$).

Results

Prazosin produced concentration-dependent rightward shifts in the sensitivity of noradrenaline (Figure 1a). Maximum responses were unaffected by 1 nM and significantly decreased to 77% of the control by 10 nM prazosin ($P<0.05$, ANOVA), while a true maximum could not be established by noradrenaline $\leqslant 3$ mM in the presence of 100 nM prazosin. The Schild plot (Figure 1b) gave a pA_2 value of 9.18 with a slope of 1.32 (95% CL: 1.14–1.51), significantly different from unity ($P<0.01$, $n=11$). If the curves were constructed as a per cent of their own maximum, the shifts by 1 and 10 nM prazosin were parallel and pA_2s were 9.33 ± 0.08 and 9.27 ± 0.16 respectively.

5-Methyl-urapidil produced concentration-dependent rightward shifts in the sensitivity of noradrenaline without significantly affecting the maximum responses (Figure 2a). Schild regression analysis (Figure 2b) yielded a pK_B of 8.48 with a slope of 0.99 (95% CL: 0.74–1.25). With the two lower concentrations the shift was parallel but in the presence of 300 nM 5-methyl-urapidil the slope of the CRC was significantly lowered if the relationship was modelled to the one-site fit (Hill slopes: control, 1.16 ± 0.12; 300 nM 5-methyl-urapidil, 0.70 ± 0.12, $P<0.05$). This low Hill slope could be accounted for by a biphasic concentration-response curve since the data could be modelled to a two-site fit (Figure 3) with the first, minor, phase consisting of approximately 20% of the maximum response. The upper part of the curve remained parallel to the control curve, satisfying the criteria for Schild analysis. An F-test showed that the two-site model fits significantly better than the one-site model (F-ratio = 1.8, $P<0.05$). The log EC_{50} of the upper part of the curve (3.98) was almost identical to the log EC_{50} obtained from the one-site fit (4.04), thus the outcome of the Schild analysis was not affected by the two-site fit. This biphasic CRC in the presence of 300 nM 5-methyl-urapidil was not seen when employing A61603 as the agonist (see below).

Incubation of arterial segments with 3, 30 and 300 nM 5-methyl-urapidil for 30 min did not increase the basal tension ruling out the possible complicating effect of partial agonism at $5HT_{1A}$ receptors (Schoeffter & Hoyer, 1988).

The potency of noradrenaline was not shifted by 0.1 and 1 nM BMY7378 but was shifted 6 fold rightwards by 1 μM BMY7378 without a significant change in maximum, giving a pA_2 of 6.52 ± 0.30 (Figure 4).

Neither sensitivity nor the maximum contractile responses to noradrenaline were affected by 1 μM chloroethylclonidine (Figure 5). With 10 μM chloroethylclonidine the maximum response was decreased to 69 ± 8 per cent of the control ($P<0.05$, Figure 5) with a 3 fold decrease in the potency. Chloroethylclonidine (1 and 10 μM) had no effect on basal tension, ruling out an agonist action at α_1-adrenoceptors (Docherty & O'Rourke, 1997; Ibarra *et al*., 2000).

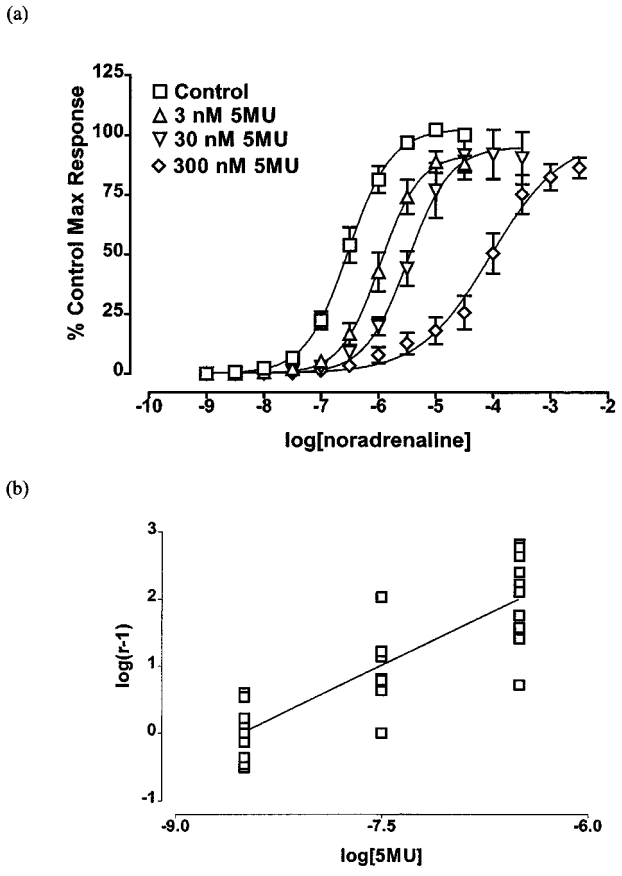

Figure 2 (a) Antagonism by 5-methyl-urapidil (5MU) of responses to noradrenaline in human skeletal muscle resistance arteries ($n=10$). (b) Schild plot for the 5-methyl-urapidil (5MU) antagonism of noradrenaline responses in human skeletal muscle resistance arteries ($n=29$).

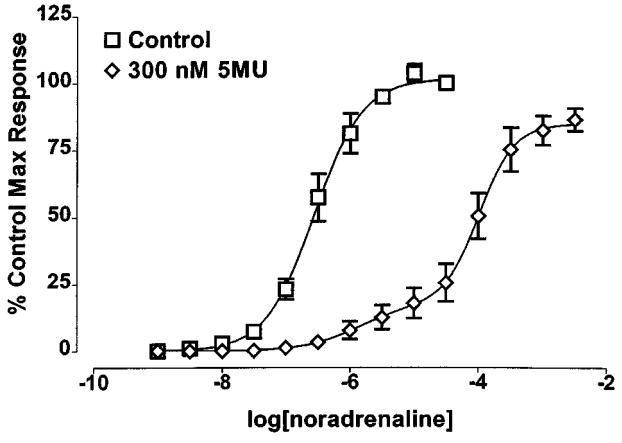

Figure 3 Two-site fit for the data shown in 2(a) for 300 nM 5-methyl-urapidil.

The α_{1A}-selective agonist A61603 produced concentration-dependent contractions in the arterial segments (Figure 6). The maximum contractile responses produced by A61603 (per cent of KCl responses 72 ± 7, $n=5$) were significantly higher than those produced by noradrenaline (per cent of

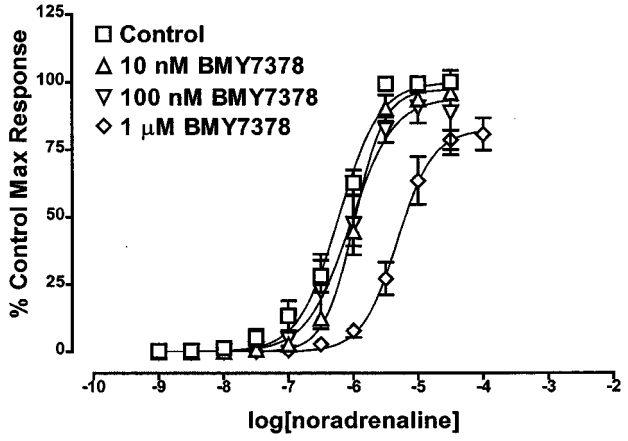

Figure 4 Effect of BMY7378 on the contractile responses to noradrenaline in human skeletal muscle resistance arteries ($n=6$).

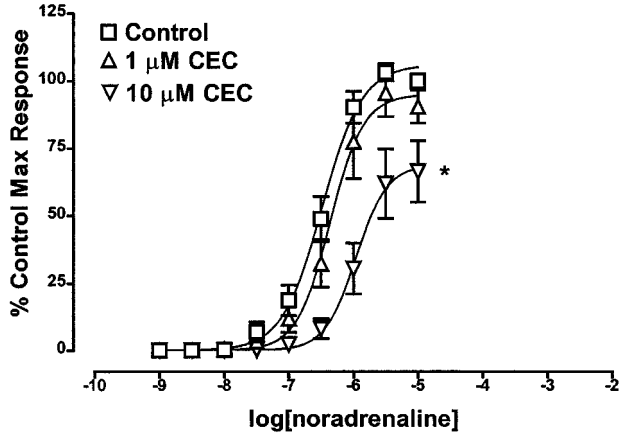

Figure 5 Effect of chloroethylclonidine (CEC), at concentrations of 1 μM ($n=5$) and 10 μM ($n=8$), on the contractile responses to noradrenaline in human skeletal muscle resistance arteries. *Maximum responses significantly smaller than control ($P<0.05$).

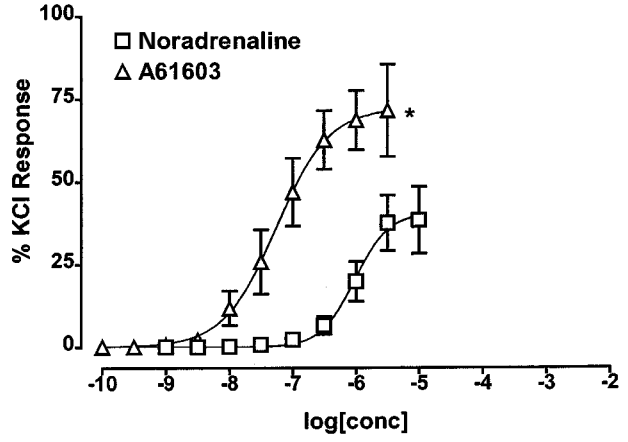

Figure 6 Contractile responses to noradrenaline and A61603 in human skeletal muscle resistance arteries expressed as per cent of KCl response ($n=5$). *Maximum responses and pEC_{50} values (see text) significantly higher than those of noradrenaline ($P<0.01$).

KCl responses, 41 ± 5, $n=5$, $P<0.05$). The pEC_{50} value for A61603 (7.26 ± 0.15) was significantly higher than that for noradrenaline (6.00 ± 0.14, $P<0.05$) giving a potency ratio for A61603:noradrenaline of 17.

5-Methyl-urapidil (300 nM) shifted the potency of A61603 by 54 fold giving a pA_2 of 8.28 ± 0.16 (Figure 7). Chloroethylclonidine (10 μM) significantly decreased the maximum responses produced by A61603 to 69 ± 6 per cent of the control ($P<0.05$, Figure 8) with a 7 fold decrease in the potency.

Discussion

This study for the first time characterized functional α_1-adrenoceptor subtypes in human skeletal muscle resistance arteries showing that the contractile responses to noradrenaline in these arteries are predominantly mediated by the α_{1A}-adrenoceptor with a minor population of another subtype which is yet to be characterized.

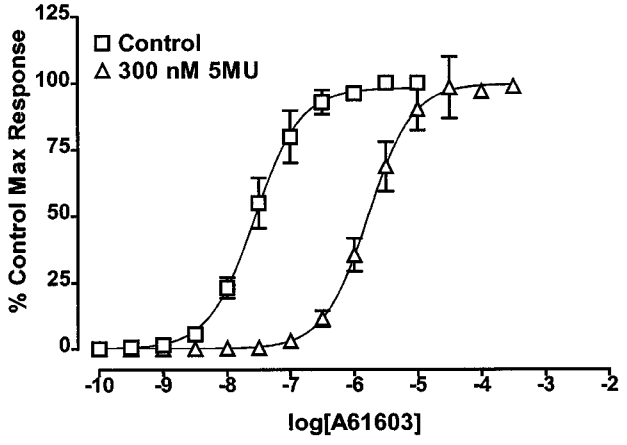

Figure 7 Antagonism of contractile responses to A61603 by 300 nM 5-methyl-urapidil (5MU) in human skeletal muscle resistance arteries ($n=7$).

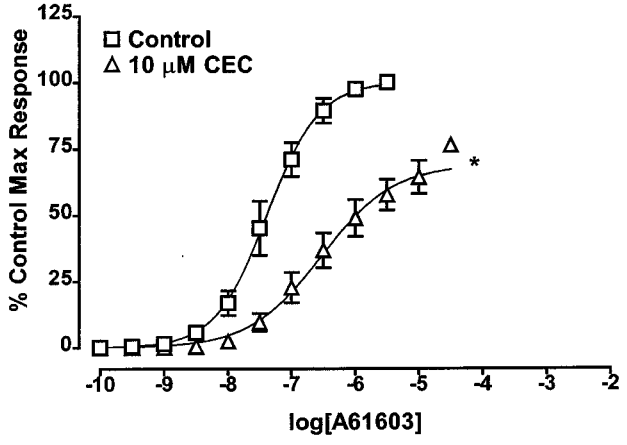

Figure 8 Effect of 10 μM chloroethylclonidine (CEC) on the contractile responses to A61603 in human skeletal muscle resistance arteries ($n=7$). *Maximum responses significantly smaller than the control ($P<0.05$).

Prazosin competitively antagonized the responses of noradrenaline, allowing for a small reduction in the maximum responses from 10 nM prazosin, an effect previously reported in rat aorta (Godfraind & Alossachie, 1988) and which can account for the steep slope of the Schild regression analysis (1.32). The pA_2 values (>9) indicate the presence of α_1-adrenoceptors with high affinity for prazosin, and provide no evidence for the presence of α_{1L}-adrenoceptors (Bylund *et al*., 1998).

The effects of 5-methyl-urapidil were consistent with competitive antagonism of noradrenaline. Schild regression analysis gave a pK_B of 8.48, which is in agreement with the reported affinity value at human α_{1a}-adrenoceptors and higher than at α_{1b} and α_{1d} (Kenny *et al*., 1995). The Schild slope of 0.99 indicates competitive antagonism. This is all consistent with the presence of a functional α_{1A}-subtype in human skeletal muscle resistance arteries.

In the presence of 300 nM 5-methyl-urapidil the CRC to noradrenaline was biphasic, shown by a two-site fit. The curve had a component with a relatively small maximum (20%) and which appeared at low concentrations before the main phase started. This gave the illusion of a low Hill slope if the curve was modelled to a single site. This lower part of the curve is thus relatively resistant to 5-methyl-urapidil suggesting the presence of another population of α_1-adrenoceptors. Biphasic CRCs to noradrenaline in the presence of 5-methyl-urapidil have been observed previously in studies with perfused rat kidney (Blue *et al*., 1995), although detailed pharmacological characterization of the α_1-subtype with low affinity for 5-methyl-urapidil was not carried out. This confirms that this antagonist has the ability to separate the concentration response curves where two receptors for which it has different affinities had produced control responses within the same concentration range.

Chloroethylclonidine was first identified as a tool to subclassify α_1-adrenoceptor subtypes with preference for α_{1B} (Han *et al*., 1987). Later, it was found that it binds to all receptor sites though α_{1A}- is relatively insensitive to alkylation and the effect was concentration and time-dependent (Docherty & O'Rourke, 1997). There is no consensus for selective antagonists for the α_{1B}-subtype. In the present study, 1 μM chloroethylclonidine did not affect the contractile responses to noradrenaline as might have been expected had a significant population of α_{1B}-adrenoceptors been involved. Chloroethylclonidine (10 μM) did decrease the maximum responses to noradrenaline with a rightward shift. However, at this concentration chloroethylclonidine also reduced the responses to the selective α_{1A}-agonist, A61603, to a similar degree, suggesting that the effect may not be related to alkylation of the α_{1B}-subtype. For example, studies have shown significant inactivation of the α_{1A}-subtype by chloroethylclonidine in murine tissues (Yang *et al*., 1998). It can be difficult to draw conclusions using chloroethylclonidine to characterize α_1-subtypes since alkylation depends on the time of exposure and effectiveness of washout and also varies with the species and preparation.

BMY 7378 is believed to be a selective antagonist that can help to characterize α_{1D}-adrenoceptors. This compound has approximately 100 fold higher affinity for the α_{1d}-compared to α_{1a}- and α_{1b}- subtypes in human and non-human recombinant systems (Goetz *et al*., 1995). It has a pA_2 value of 8.9 in rat aorta and forms a part of the evidence that this

vessel contains a significant population of α_{1D}-adrenoceptors (Goetz *et al*., 1995; Saussy *et al*., 1996). In the present study 10 and 100 nM BMY 7378 did not produce any shift in the potency of noradrenaline but a 6 fold shift was observed with 1 μM BMY 7378 giving a pA_2 value of 6.52. This is far less than the reported affinity values at the human α_{1d}-subtype (8.2) but comparable to that for the α_{1a}- (6.2) or α_{1b}-subtypes (6.7) (Kenny *et al*., 1995). This rules out the α_{1D}- subtype as the mediator of the major component of noradrenaline-mediated contraction in human skeletal muscle resistance arteries.

These effects of antagonists suggest the predominant involvement of α_{1A}-adrenoceptors in the contractile responses to noradrenaline in these arteries. This is further supported by the results with agonists. In the present study A61603, an α_{1A}- selective agonist, was found to be 17 fold more potent than noradrenaline which is comparable to the reported 21 fold greater potency in stimulating IP_3 production in mouse fibroblasts expressing bovine α_{1a}-adrenoceptors (Knepper *et al*., 1995). In the same report A61603 was shown to be 200 times less potent than noradrenaline at the α_{1d}-subtype and not at all active at the α_{1b}-subtype in stimulating IP_3 production. The relatively higher potency and efficacy of A61603 in the present study reinforce the predominant contribution of α_{1A}- adrenoceptors to the contractile responses to the agonists, as opposed to the α_{1B}- or α_{1D} subtypes. The maximum responses produced by A61603 in the present study were significantly higher than that of noradrenaline, which has not been observed in previous studies (Knepper *et al*., 1995; Smith *et al*., 1997; Argyle & McGrath, 2000). We recently reported that in these arteries the maximum responses to noradrenaline were greater than that to phenylephrine (Jarajapu *et al*., 2001a). There is therefore a spectrum of maxima across these three agonists, A61603 > noradrenaline > phenylephrine, that is not characteristic of resistance arteries in other species. The basis for the variable maximum response in human skeletal muscle resistance arteries deserves further investigation. 5-methyl-urapidil (300 nM) shifted the potency of A61603 with a pA_2 value of 8.28, in agreement with the reported value for 5-methyl-urapidil (Kenny *et al*., 1995) and similar to the pK_B obtained in the present study with noradrenaline as the agonist. In contrast to the situation with noradrenaline as the agonist, 5-methyl-urapidil (300 nM) produced a parallel shift in the CRCs of A61603, in agreement with activation of a single population of α_1-adrenoceptors by A61603. The additional component in the biphasic response to noradrenaline under similar conditions does not, therefore, emerge for A61603. This shows that the receptor involved in the first phase of the biphasic response has, compared with α_{1A}, lower affinity for 5-methyl-urapidil and a potency ratio that favours noradrenaline over A61603. This description could fit either α_{1B} or α_{1D}.

Animal studies show both species and tissue variation in the putative post-junctional α_1-adrenoceptor subtypes in resistance arteries, with all known subtypes having been postulated in some case or other (Vargas & Gorman, 1995; Leech & Faber, 1996; Zhou & Vargas, 1996; Smith *et al*., 1997; Stam *et al*., 1999; Villalobos-Molina *et al*., 1999; Argyle & McGrath, 2000; Zhu *et al*., 1997; Salomonsson *et al*., 2000). It has been suggested that variations in the distribution of different α_1-adrenoceptor subtypes may be related to the density of adrenergic innervation (Stassen *et al*., 1998). The presence of post-junctional α_1-adrenoceptors has been shown in human resistance arteries from subcutaneous fat, skeletal muscle, pericardial fat and omentum (Jarajapu *et al*., 2001a; Nielson *et al*., 1990; 1991) although no subtype characterization has yet been reported. The significance of the present study is that it shows the predominant role of the α_{1A}-subtype in the responsiveness to noradrenaline of human skeletal muscle resistance arteries. At rest, sympathetic tone to skeletal muscle resistance vessels is a major determinant of peripheral resistance. Thus, this subtype of α_1-adrenoceptor should be considered of major physiological importance. Drugs that block it selectively should be expected to reduce resistance in the normally constricted skeletal muscle beds. All α-blockers that are employed clinically, either for benign prostatic hyperplasia (Martin, 1999; Kirby, 1999) or heart failure (Brodde, 1990; Strein & Sponer, 1990) have either equal affinity at all three subtypes or highest affinity for α_{1A}. The implication from the current study is that any α-blocker with α_{1A}-antagonism (non-selective or α_{1A}-selective) might cause vasodilatation in skeletal muscle. Theoretically, this may be of value in heart failure where muscle fatigue is an important symptom that should be relieved by increasing resting blood flow. On the other hand, it is not known what the consequence would be of selective α_{1A}-blockade on other organ systems. Indeed, vasodilatation might cause a baroreceptor reflex to mediate an activation of the sympathetic nervous system that impacts on organs not protected by α-blockade. There is already compensatory activation of the sympathetic nervous system in heart failure (Francis, 1985) and this might be exacerbated by selective α_{1A}-adrenoceptor blockade. This would provide a logical basis for not using α_{1A}-adrenoceptor selective antagonists in heart failure. It is interesting that tamsulosin, an α_1-blocker with selectivity for α_{1A}-, used in very low doses for benign prostatic hyperplasia, shows no evidence of blocking vascular α_1-adrenoceptors (Djavan & Marberger, 1999; Harada & Fujimura, 2000). This may be explained by the very high affinity of human prostate smooth muscle α_{1A}-adrenoceptors for antagonist ligands (Mackenzie *et al*., 2000).

In conclusion, the present study shows the predominant involvement of the α_{1A}-adrenoceptor subtype in noradrenaline-mediated contractile responses in human skeletal muscle resistance arteries. Further work is required to characterize the functional α_1-adrenoceptor subtypes in resistance arteries from other vascular beds, e.g. subcutaneous, mesenteric and renal, which make a significant contribution to peripheral arterial resistance.

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