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The α_{1A}-adrenoceptor subtype mediates contraction in rat femoral resistance arteries

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Abstract

In this study, α_1-adrenoceptor subtypes were characterised in rat femoral resistance arteries mounted on a small vessel myograph. A-61603 was found to be more potent than noradrenaline and phenylephrine in these arteries. Brimonidine (UK 14304) could not evoke any contractile responses and the sensitivity to noradrenaline and phenylephrine was not affected by (8*aR*,12*aS*,13*aS*)-5,8,8*a*,9,10,11,12,12*a*,13*a*-decahydro-3-methoxy-12-(ethylsulphonyl)-6*H*-isoquino[2,1-*g*][1,6]-naphthyridine (RS 79948), ruling out the presence of α_2-adrenoceptors. Prazosin, 5-methyl-urapidil and 2-([2,6-dimethoxyphenoxyethyl]aminomethyl)-1,4-benzodioxane (WB 4101) produced rightward shifts in the sensitivity to noradrenaline, giving pA_2 values of 9.6, 9.4 and 10.4, respectively, in agreement with the presence of α_{1A}-adrenoceptors. (8-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY 7378; 1 μM) produced a small shift in the sensitivity of noradrenaline giving a pK_B of 7.2. In the presence of 300 nM 5-methyl-urapidil, sensitivity to noradrenaline was not further shifted by 1 μM BMY 7378. Responses to noradrenaline were unaffected by the α_{1B}-adrenoceptor alkylating agent chloroethylclonidine (1 μM). These results suggest α_{1A}-adrenoceptors mediate contractile responses to noradrenaline in rat femoral resistance arteries.

Keywords: α_1-Adrenoceptor subtype; (Rat); Femoral resistance artery

1. Introduction

α_1-Adrenoceptors are a heterogeneous family of receptors belonging to the superfamily of G-protein coupled receptors. It is now accepted that there are three functional α_1-adrenoceptor subtypes— α_{1A}, α_{1B} and α_{1D}—corresponding to the cloned subtypes—α_{1a}, α_{1b} and α_{1d} (Hieble et al., 1995; Bylund et al., 1998). These three receptor phenotypes show high affinity to prazosin ($pA_2 > 9$) in functional and radioligand binding experiments. In blood vessels, post-junctional α_1-adrenoceptors control vascular smooth muscle tone and thus modulate peripheral arterial resistance. α_1-Adrenoceptors with low affinity for prazosin ($pA_2 < 9$) have also been observed in functional studies in blood vessels and classified as α_{1L} and α_{1N} subtypes (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990). These receptor subtypes with low affinity for prazosin have not been cloned. Recently it has been proposed that the α_{1L} may not be derived from a different gene, instead it may be derived from the same gene as the α_{1A}-adrenoceptor and may represent an energetically more favourable conformational state of the α_{1A} subtype (Ford et al., 1997).

The three cloned adrenoceptor subtypes are present in vascular smooth muscle at the mRNA level (Piascik et al., 1997; Hrometz et al., 1999; Piao et al., 2000) but the functional dominance in adrenergic responses varies with the tissue and species. For example, in rat, in vivo studies showed evidence for the significant role of α_{1D} in the regulation of systemic arterial pressure (Zhou and Vargas, 1996; Villalobos-Molina et al., 1999). Moreover, from studies with the α_{1B}-adrenoceptor selective antagonist, L-765,314, it appears that the α_{1B}-adrenoceptor does not contribute to phenylephrine-induced pressor response, nor may it be important for the maintenance of basal mean arterial pressure in SHR (Patane et al., 1998). In contrast, in vitro studies showed evidence for the involvement of other subtypes in the contractile responses to the adrenergic agonists in various vascular beds. The α_{1A}-adrenoceptor was identified as a predominant subtype in rat mesen-

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teric arteries (Kong et al., 1994; Williams and Clarke, 1995). However, Piascik et al. (1997) and, recently, Stam et al. (1999) showed evidence for α_{1B} and α_{1L}, respectively, as the predominant subtypes in the same vasculature. The α_{1A} and/ α_{1D} subtypes are predominant in the renal vascular bed (Eltze et al., 1991; Blue et al., 1995; Eltze et al., 1999; Salomonsson et al., 2000) and the α_{1D} subtype in rat cremaster muscle arterioles (Leech and Faber, 1996). Thus, the α_1-adrenoceptor subtype involved in the regulation of peripheral arterial resistance in rat is controversial.

In the present study, the functional adrenoceptors mediating contractile responses in femoral resistance arteries were characterised. These arteries control the blood flow to the limbs and significantly contribute to the systemic arterial pressure. Potencies of the agonists noradrenaline (non-selective), phenylephrine (α_1-selective), brimonidine (UK 14304) (α_2-selective) (Cambridge, 1981) and A-61603 (α_{1A}-selective) (Knepper et al., 1995) were examined. Affinities of the competitive reversible antagonists prazosin (α_1-selective) (Cambridge et al., 1977), RS 79948 (α_2-selective) (Brown et al., 1993; Uhlen et al., 1998), 5-methyl-urapidil (α_{1A}-selective) (Gross et al., 1988), WB 4101 (selective for α_{1A} and α_{1D}) (Morrow and Creese, 1986; Kenny et al., 1995) and BMY 7378 (α_{1D}-selective) (Goetz et al., 1995) were determined. The effect of chloroethylclonidine which is known to preferentially alkylate α_{1B}-adrenoceptor subtype (Han et al., 1987), was also evaluated.

2. Materials and methods

2.1. Preparation of rat femoral resistance arteries

Male Wistar rats, of age 12–14 weeks and weighing 200–250 g, were killed by stunning and exsanguination. Hind limbs were removed and transported to the lab in physiological saline solution (PSS) under ice cold conditions. Third order femoral small arteries (normalised diameter of 271 ± 6, $n = 110/23$, no. of arterial segments/no. of rats) were dissected out under a microscope (Zeiss) within an hour. Four to eight arterial segments from each rat were used for experimentation.

2.2. Small vessel wire myography

Arterial segments of 2-mm length were mounted in a four channel 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 (1.2), $MgSO_47H_2O$ (1.2), (+)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 and Halpern, 1977). Then, the resting tension was set to a normalised 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 an effective resting transmural pressure (ERTP) 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 K^+ 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 ERTP of more than 100 mm Hg (13.3 kPa) when stimulated with 123 mM KCl. ERTP was calculated from the Laplace equation:

$$\text{ERTP} = \text{wall tension}/(\text{internal circumference}/2\pi)$$

which corrects for differences in length and diameter of arterial segments (Mulvany and 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 pre-contracting with 1 μM noradrenaline. All the vessels in the study produced more than 60% relaxation. In some vessels, the endothelium was mechanically removed by gently rubbing the luminal side of the vessel wall with hair that had been stored in ethanol and rinsed in PSS before use. Endothelial removal was confirmed by the lack of relaxation to carbachol when tested as above.

After an equilibration period, two to four concentration response curves to the agonists were obtained per each arterial segment. Preliminary experiments showed that repeated concentration response curves were reproducible and no corrections for time-dependent changes were required. The first concentration response curve was taken as control and the subsequent curves were obtained after

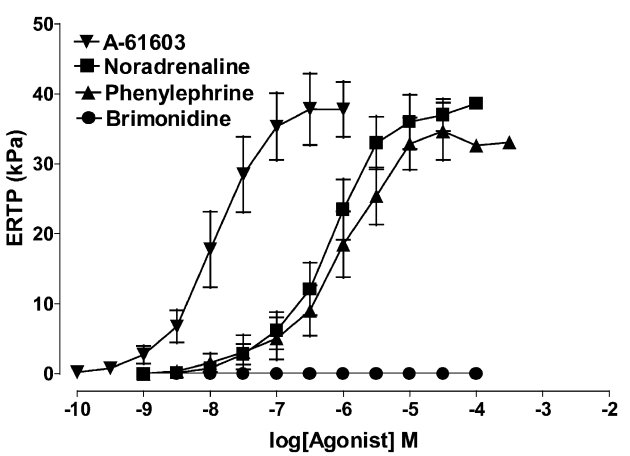

Fig 1. Contractile responses, expressed as effective resting transmural pressure to (ERTP) noradrenaline ($n = 10$), phenylephrine ($n = 10$), brimonidine ($n = 6$) and A-61603 ($n = 7$) in rat femoral resistance arteries. Given are means ± S.E.M.

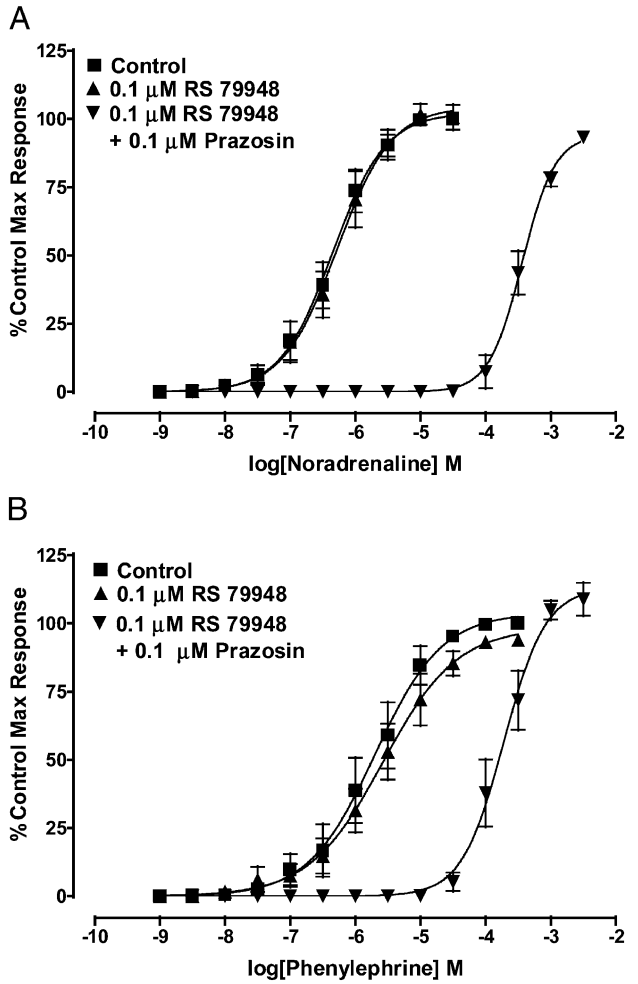

Fig 2. Effects of prazosin ($n = 5$) and RS 79948 ($n = 7$) on the contractile responses to (A) noradrenaline and (B) phenylephrine in rat femoral resistance arteries. Given are means ± S.E.M.

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 concentration response curves to noradrenaline were obtained (to block β-adrenoceptors, neuronal and non-neuronal uptake of noradrenaline, respectively). EDTA (0.023 mM) and ascorbic acid (0.3 mM) were included in the PSS to prevent oxidation of noradrenaline. RS 79948 (0.1 μM), a selective α_2-adrenoceptor antagonist (Brown et al., 1993; Uhlen et al., 1998) was present in PSS during the experimental protocols for α_1-adrenoceptor subtype characterisation.

Results are expressed as mean ± S.E.M., n being expressed as the no. of rats or no. of arterial segments/no. of rats. Agonist potency is expressed as pEC_{50} (the negative logarithm of the concentration required to produce 50% of the maximum response). EC_{50} values and maximum responses were calculated using the software program GraphPad Prism which fits CRCs to a four parameter logistic equation given below:

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

where X is the logarithm of the molar concentration of the agonist, Y is the response and P is the Hill slope. Antagonist affinities are expressed either as pK_B or pA_2 values. pK_B was used when one concentration of the antagonist was used to obtain the affinity and calculated using the following equation (Schild, 1949):

$$pK_B = -\log\{[B]/(r-1)\}$$

where K_B is the dissociation constant, $[B]$ 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. pA_2 values were obtained when three different concentrations of the antagonist were used. These values were obtained from the x-intercept of the plot of $\log(r-1)$ and $\log[B]$ (Arunlakshana and Schild, 1959) after linear regression, using GraphPad prism.

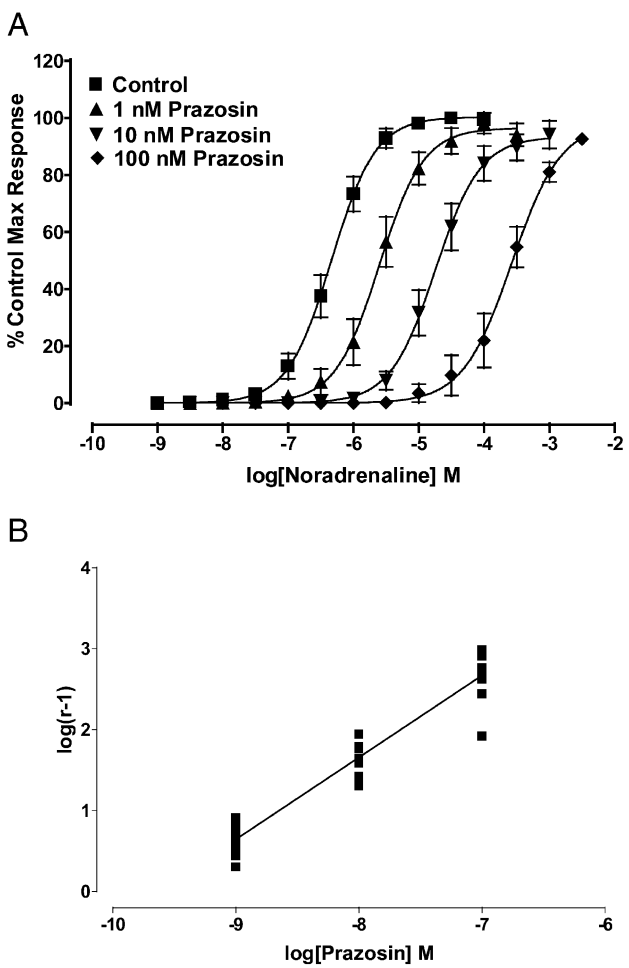

Fig 3. (A) Effect of prazosin on the contractile responses to noradrenaline in rat femoral resistance arteries ($n = 10$). Given are means ± S.E.M. (B) Schild plot for the antagonism of noradrenaline-mediated responses by prazosin (no. of points: 30).

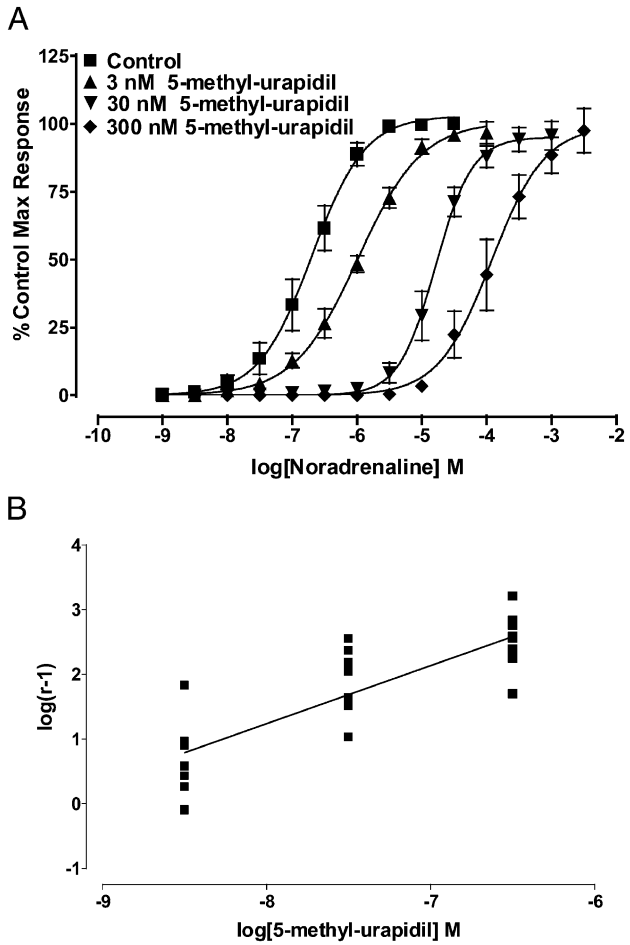

Fig 4. (A) Effect of 5-methyl-urapidil on the contractile responses to noradrenaline in rat femoral resistance arteries ($n = 8$). Given are means ± S.E.M. (B) Schild plot for the antagonism of noradrenaline-mediated responses by 5-methyl-urapidil (no. of points: 24).

2.3. Drugs

(−)-Noradrenaline (arterenol) bitartrate, brimonidine (UK14304), propranolol hydrochloride, corticosterone acetate, 2-([2,6-dimethoxyphenoxyethyl]aminomethyl)-1,4-benzodioxane (WB 4101) hydrochloride and prazosin HCl were obtained from Sigma (Poole, Dorset, UK); Cocaine HCl was obtained from Thornton and Ross (UK); (8*aR*,12*aS*,13*aS*)-5,8,8*a*,9,10,11,12,12*a*,13*a*-Decahydro-3-methoxy-12-(ethylsulphonyl)-6*H*-isoquino[2,1 − g][1,6]-naphthyridine (RS 79948) and *N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1 yl]methanesulphonamide (A-61603) were obtained from Tocris (Avonmouth, Bristol, UK); 5-methyl-urapidil, chloroethylclonidine 2 HCl and (8-[2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY 7378) were obtained from RBI (Natick, USA). The stock solution of 5-methyl-urapidil was prepared in 5% dimethyl sulfoxide 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.

2.4. Statistics

pEC_{50} values and maximum responses were compared by using the paired *t*-test or two-way analysis of variance (ANOVA) followed by the Newman–Keuls range test for multiple comparisons. Confidence limits were obtained from GraphPad prism.

3. Results

3.1. Contractile responses to different adrenoceptor agonists in rat femoral resistance arteries

Noradrenaline, phenylephrine and A-61603 produced concentration-dependent contractile responses in the rat femoral resistance arteries (Fig. 1). Maximum responses, expressed as ERTP, produced by all these three agonists were not significantly different. Significantly higher pEC_{50} values were observed with A-61603 (7.9 ± 0.1) compared to noradrenaline (6.2 ± 0.1, $P < 0.001$) and phenylephrine (6.0 ± 0.1, $P < 0.001$). A-61603 was found to be 55 and 85 times more potent than noradrenaline and phenylephrine, respectively. Brimonidine did not evoke any contractions in these arteries over a concentration range of 1 nM to 0.1 mM.

3.1.1. Contractile responses to brimonidine in endothelium-denuded arterial segments

In order to check the influence of endothelium on the contractile responses to brimonidine, experiments were carried out in arterial segments denuded of endothelium. Arterial segments which were denuded of endothelium showed less than 10% relaxation to carbachol whereas

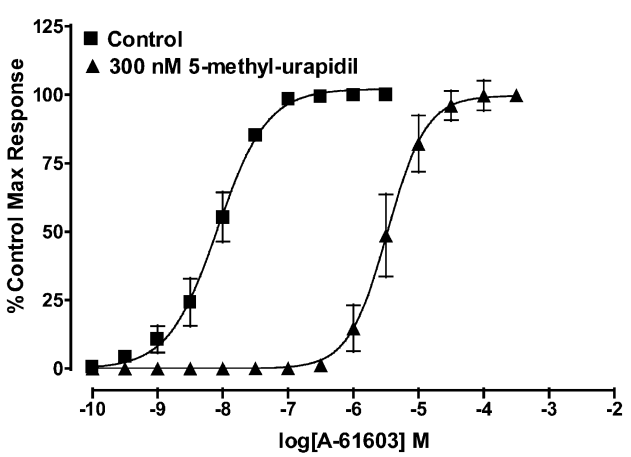

Fig 5. Effect of 300 nM 5-methyl-urapidil on the contractile responses to A-61603 in rat femoral resistance arteries ($n = 5$). Given are means ± S.E.M.

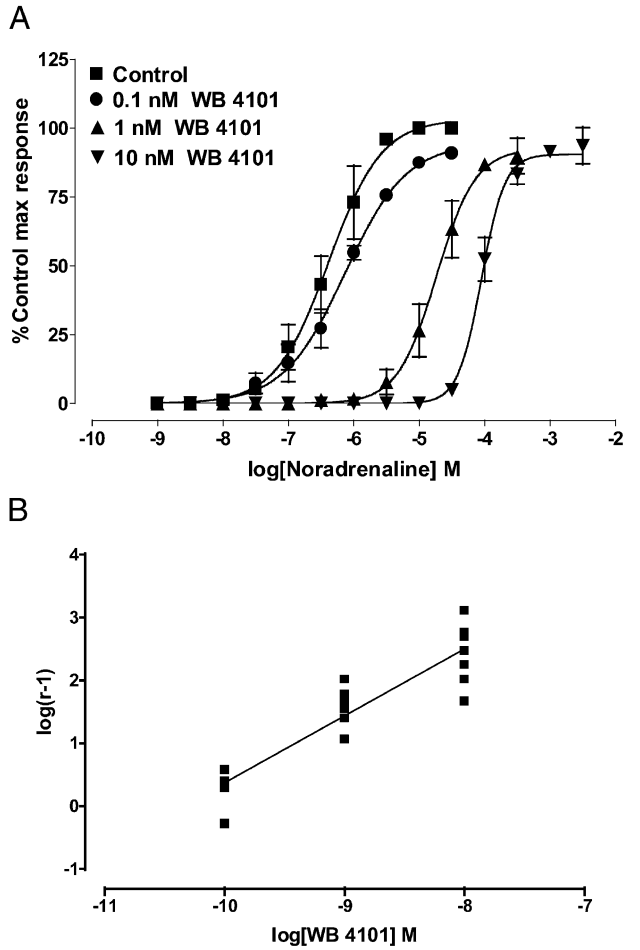

Fig 6. (A) Effect of WB 4101 on the contractile responses to noradrenaline in rat femoral resistance arteries ($n = 7/4$). Given are means ± S.E.M. (B) Schild plot for the antagonism of noradrenaline-mediated responses by WB 4101 (no. of points: 18).

matching arterial segments with intact endothelium showed 75–100% relaxation ($n = 6$). Brimonidine produced small concentration-dependent contractile responses in two of the six endothelium-denuded arterial segments (maximum ERTP = 6.4 and 4.8 kPa) with no contraction observed in the other four arteries (not shown). No contraction was observed in the corresponding arterial segments with intact endothelium ($n = 6$). Brimonidine-mediated contractile responses in the absence of endothelium were antagonized by 0.1 μM RS 79948 (not shown).

3.2. Effect of selective α_1- and α_2-adrenoceptor antagonists on the contractile responses to noradrenaline and phenylephrine

The selective α_2-adrenoceptor antagonist RS 79948 (0.1 μM) did not produce any shift in the potency of noradrenaline (Fig. 2A) or phenylephrine (Fig. 2B). The selective α_1-adrenoceptor antagonist prazosin (0.1 μM) shifted the potency of noradrenaline and phenylephrine rightwards giving pK_B values of 9.9 ± 0.1 and 9.1 ± 0.3, respectively (Fig. 2A and B).

3.3. Affinities of non-selective and subtype-selective α_1-adrenoceptor antagonists in rat femoral resistance arteries

Prazosin (1–100 nM) produced concentration-dependent parallel rightward shifts in the potency of noradrenaline (Fig. 3A) without affecting the maximum responses. The Schild regression analysis (Fig. 3B) gave a pA_2 value of 9.6 with a slope of 1.0 (95% CL: 0.9–1.1).

5-Methyl-urapidil (3–300 nM) antagonised noradrenaline-mediated contractile responses in a concentration-dependent fashion (Fig. 4A) without affecting the maximum responses. Schild regression analysis (Fig. 4B) gave a pA_2 value of 9.4 with a slope of 0.9 (95% CL: 0.6–1.2). The potency of A-61603 was also shifted by 5-methyl-urapidil (300 nM) (Fig. 5) giving a pK_B value of 9.3 ± 0.2. Incubation of arterial segments with 5-methyl-urapidil for 30 min did not increase the basal tension ruling out a

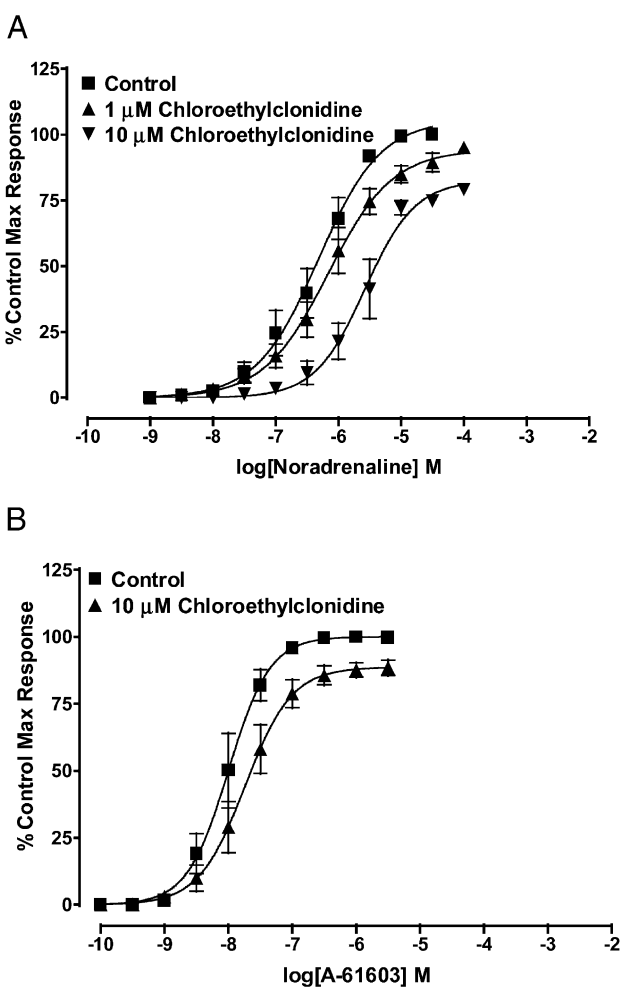

Fig 7. (A) Effect of chloroethylclonidine on the contractile responses to noradrenaline in rat femoral resistance arteries ($n = 9$). (B) Effect of 10 μM chloroethylclonidine on the contractile responses to A-61603 in rat femoral resistance arteries ($n = 7$). Given are means ± S.E.M.

possible agonist effect on $5HT_{1A}$ receptors (Schoeffter and Hoyer, 1988).

WB 4101 (0.1–10 nM) produced concentration-dependent rightward shifts in the sensitivity to noradrenaline (Fig. 6A) without affecting the maximum responses. Schild regression analysis (Fig. 6B) gave a pA_2 value of 10.4 with a slope of 1.1 (95% CL: 0.8–1.3).

Neither sensitivity nor the maximum contractile responses to noradrenaline were affected by 1 μM chloroethylclonidine (Fig. 7A). With 10 μM chloroethylclonidine, the maximum response was decreased to $78 \pm 5\%$ of the control (Fig. 7A, $P < 0.05$) with a 6-fold decrease in the potency (pEC_{50} of noradrenaline decreased from 6.3 ± 0.1 to 5.6 ± 0.1).

Chloroethylclonidine (10 μM) did not significantly affect the contractile responses to A-61603 (Fig. 7B). Incubation of arterial segments with chloroethylclonidine had no effect on base line tension, ruling out any agonist action at α_1-adrenoceptors (Ibarra et al., 2000; Docherty and O'Rourke, 1997).

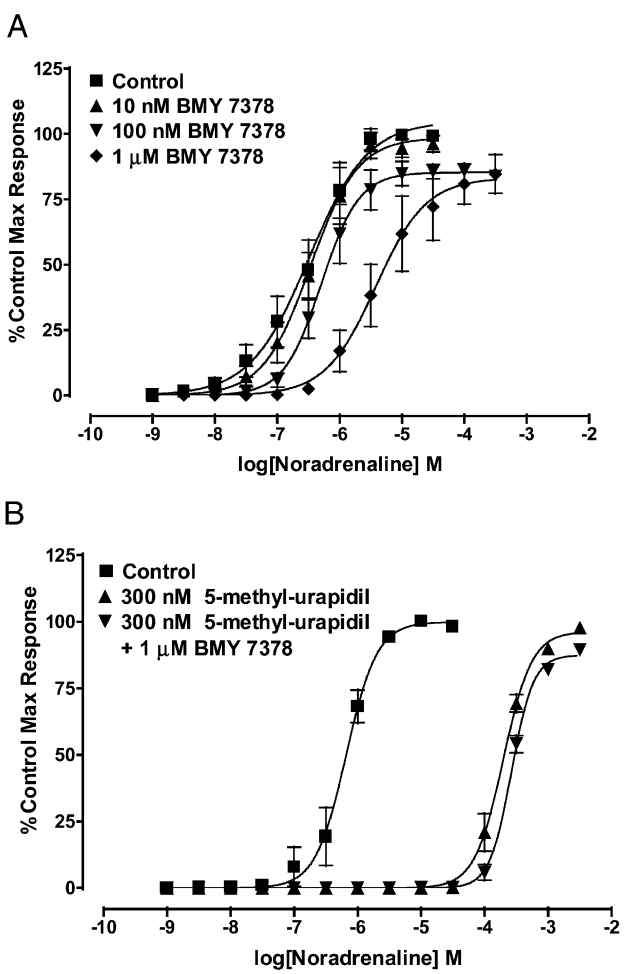

Fig 8. (A) Effect of BMY 7378 on the contractile responses to noradrenaline in rat femoral resistance arteries ($n = 8$). (B) Effect of 1 μM BMY 7378 on the contractile responses to noradrenaline in the presence of 300 nM 5-methyl-urapidil ($n = 6/4$). Given are means ± S.E.M.

The potency of noradrenaline was not affected by 10 and 100 nM of BMY 7378 but was shifted 12-fold rightwards by 1 μM BMY 7378 giving a pK_B of 7.2 ± 0.3 (Fig. 8A). Maximum responses were significantly decreased to 83% of the control ($P < 0.05$) by 1 μM BMY 7378. In the presence of 300 nM 5-methyl-urapidil which produced a 365-fold shift in the potency of noradrenaline, no further shift was produced by 1 μM BMY 7378 (Fig. 8B).

4. Discussion

The present study shows that the contractile responses to noradrenaline in rat femoral resistance arteries are predominantly mediated by the α_{1A}-adrenoceptor subtype. This study also shows that there is no functional contribution of post-junctional α_2-adrenoceptors to noradrenaline-mediated responses in these arteries.

Results with agonists show that noradrenaline-mediated contractile responses are predominantly mediated by α_1-adrenoceptors since phenylephrine, a selective α_1-adrenoceptor agonist, was found to be equi-potent and equi-efficacious with noradrenaline, whereas brimonidine, a selective and a full agonist at α_2-adrenoceptors (Cambridge, 1981; Thaina et al., 1999), did not evoke any contractile responses. Since endothelium is known to influence responses to α_2-adrenoceptor stimulation (Carrier and White, 1985; Godfraind and Alossachie, 1988) experiments were also carried out in endothelium-denuded arterial segments. Little or no contractions to α_2-adrenoceptor agonist were observed in the endothelium-denuded arterial segments, showing that the lack of responses in intact preparations was not due to the presence of endothelium. Similar results were previously obtained in human skeletal muscle resistance arteries where removal of endothelium failed to uncover an α_2-adrenoceptor-mediated contraction (Jarajapu et al., 2001a).

Studies with selective α_1- and α_2-adrenoceptor antagonists support the predominance of α_1-adrenoceptors in these arteries as the sensitivities of noradrenaline and phenylephrine were not affected by the selective α_2-adrenoceptor antagonist RS 79948 but well shifted by the selective α_1-adrenoceptor antagonist prazosin (Cambridge et al., 1977).

The lack of involvement of α_2-adrenoceptors in the contractile responses to noradrenaline in these arteries is in contrast to findings in the perfused rat hind limb where the α_2-adrenoceptor antagonist, yohimbine, antagonised responses to noradrenaline (Zhu et al., 1997). However, at higher concentrations yohimbine may also antagonise α_1-adrenoceptors (Drew and Whiting, 1979) and studies with a more selective α_2-adrenoceptor antagonist are indicated. The α_2-adrenoceptor antagonist used in the present study,

RS 79948, is highly selective for α_2-compared to α_1-adrenoceptors (Brown et al., 1993; Uhlen et al., 1998) and so is a more reliable tool to identify the involvement of α_2-adrenoceptors.

The lack of contractile responses to α_2-adrenoceptor stimulation in rat femoral resistance arteries is also in contrast to the finding that α_2-adrenoceptors are predominant in human resistance arteries (Nielson et al., 1990, 1991). However, our own studies in human skeletal muscle resistance arteries found only a small population of α_2-adrenoceptors (Jarajapu et al., 2001a), in agreement with the present results from rat femoral resistance arteries.

A-61603, a selective α_{1A}-adrenoceptor agonist, evoked contractile responses in rat femoral resistance arteries with a significantly higher potency than that of noradrenaline (55-fold) and phenylephrine (85-fold). These potency ratios are higher than the 21-fold higher potency of A-61603 over noradrenaline observed in earlier studies where stimulation of IP_3 production in mouse fibroblasts expressing bovine α_{1a}-adrenoceptors was determined (Knepper et al., 1995). The high potency of A-61603 suggests the presence of a predominant population of α_{1A}-adrenoceptors in these arteries.

Prazosin produced concentration-dependent dextral shifts in the sensitivity of noradrenaline without affecting the maximum responses. The Schild slope of 1.0 indicates the competitive nature of the antagonism. The pA_2 value (> 9) is in agreement with receptors at which prazosin shows high affinity, ruling out the presence of the α_{1L} and α_{1N} subtypes (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990).

5-Methyl-urapidil and WB 4101 also produced dextral shifts in the sensitivity of noradrenaline without affecting the maximum responses. Schild slopes of 0.9 and 1.0 observed with 5-methyl-urapidil and WB 4101 are consistent with the competitive nature of their antagonism. The pA_2 values of 9.4 and 10.4 for 5-methyl-urapidil and WB 4101, respectively, observed in this study are in agreement with the reported affinity values for these drugs at the cloned mammalian α_{1a}-adrenoceptor subtype expressed in rat fibroblasts (Ford et al., 1996). A similar affinity value ($pK_B = 9.2$) was obtained for 5-methyl-urapdil with A-61603 as an agonist, again in agreement with the presence of the α_{1A}-adrenoceptor subtype in these arteries.

The sensitivity to noradrenaline was not affected by 10 and 100 nM BMY 7378 showing the lack of contribution of the α_{1D} subtype to noradrenaline-mediated responses. The shift produced by 1 μM BMY 7378 gave a pK_B value of 7.2 which is much less than the reported affinity for BMY 7378 at the human α_{1d} subtype (9.4) expressed in rat fibroblasts but similar to the values obtained at the corresponding α_{1a} (6.6) and α_{1b} (7.2) subtypes (Goetz et al., 1995). Furthermore, in the presence of 300 nM 5-methyl-urapidil, the potency of noradrenaline was not further shifted by BMY 7378, ruling out the presence of the α_{1D} subtype in these arteries.

Chloroethylclonidine was first identified as a tool to subclassify α_1-adrenoceptor subtypes, with a preference for the α_{1B} subtype (Han et al., 1987). Later it was found that it binds to all receptor sites although the α_{1A} is relatively resistant to alkylation (Büscher et al., 1996; Hirasawa et al., 1997; Yang et al., 1998). In the present study chloroethylclonidine (1 μM) had no effect on the sensitivity or maximum responses of noradrenaline suggesting a lack of the functional α_{1B}-adrenoceptor. However, 10 μM chloroethylclonidine did produce a small reduction in the sensitivity and maximum responses to noradrenaline. This may represent alkylation of the predominant α_{1A} subtype although the lack of effect of chloroethylclonidine on the selective α_{1A} agonist, A-61603, would argue against this. The reduction might therefore be due to the presence of a small population of the α_{1B} subtype. This is in agreement with the pK_B observed with BMY 7378 which is the same as that reported for the human α_{1b} subtype expressed in rat fibroblasts (7.2, Goetz et al., 1995).

The present study shows the predominant involvement of the α_{1A} subtype in noradrenaline-mediated contractile responses in rat femoral resistance arteries. This is in agreement with other studies in mesenteric, renal and hind limb vascular beds (Kong et al., 1994; Blue et al., 1995; Williams and Clarke, 1995; Zhu et al., 1997; Salomonsson et al., 2000), which showed the involvement of the α_{1A} subtype exclusively or in conjunction with other subtypes in adrenergic contractile responses. Our studies in resistance arteries from human subcutaneous (unpublished) and skeletal muscle (Jarajapu et al., 2001b) also show the predominant involvement of the α_{1A}-adrenoceptor in noradrenaline-mediated contractile responses. These studies indicate that the α_{1A}-adrenoceptor is the predominant functional subtype in resistance vasculature in rat and human. However, as mentioned in the introduction, in vivo studies in the rat showed evidence for a significant role of α_{1D}-adrenoceptors in the regulation of arterial pressure (Zhou and Vargas, 1996; Villalobos-Molina et al., 1999). In humans, selective α_{1A}-adrenoceptor antagonists are considered to be potentially useful candidates for the treatment of benign prostatic hypertrophy, lacking in blood pressure-lowering side effects (Akiyama et al., 1999; Williams et al., 1999). The importance of the α_{1A}-adrenoceptor subtype in the maintenance of peripheral vascular resistance and the regulation of systemic arterial pressure therefore requires further study.

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