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Pharmacological characterization of α_1-adrenoceptors in mouse isolated femoral small arteries

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Abstract

Arteries were isolated from male DBA/2 mice and mounted on a small vessel wire myograph for isometric recording. Responses to exogenous noradrenaline were inhibited with high affinity by prazosin (pK_B, 9.3) and 5-methyl-urapidil (pK_B, 9.2) and with low affinity by 8-[2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione (BMY 7378) (pA_2, 6.7). Chloroethylclonidine (10 μM) produced only a small reduction in the maximum response to noradrenaline. Responses to electrical field stimulation were also inhibited with high affinity by prazosin (pIC_{50}, 9.3–9.5) and 5-methyl-urapidil (pIC_{50}, 8.0–8.3). Responses were sensitive to BMY 7378 at low frequencies of stimulation (pIC_{50} at 2 Hz, 8.2) but not at high frequencies (pIC_{50} at 20 Hz, 6.5). In conclusion, contractions to exogenous and endogenous noradrenaline in mouse femoral small arteries are mediated mainly by α_{1A}-adrenoceptors. α_{1D}-adrenoceptors are not involved in responses to exogenous noradrenaline but appear to be activated by neurally released noradrenaline at a low frequency of stimulation.

Keywords: α_1-Adrenoceptor; Noradrenaline; Electrical field stimulation; Femoral small artery, mouse

1. Introduction

α_1-Adrenoceptors are a heterogeneous family of G-protein-coupled receptors, which are of particular therapeutic interest because of their importance in maintaining peripheral resistance and systemic arterial blood pressure. Current classification differentiates α_1-adrenoceptors into three subtypes, namely α_{1A}, α_{1B} and α_{1D}, corresponding to the three cloned α_1-adrenoceptors, designated α_{1a}, α_{1b} and α_{1d} (Hieble et al., 1995). These three subtypes display high affinity for prazosin (pK_B>9) and are known as α_{1H}-adrenoceptors. A fourth subtype (α_{1L}-adrenoceptor), based on low affinity for prazosin (pK_B<9) in functional experiments (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990a; Muramatsu et al., 1990b), has not been cloned and evidence suggests that it is not a separate gene product but a functional phenotype of the α_{1A}-adrenoceptor (Ford et al., 1997). Pharmacological characterization of the different subtypes is subject to the limitations of availability of highly subtype selective ligands. Recently, knock out (KO) mouse models have become available and these provide a pharmaco-genetic approach to characterize subtype functions (Rokosh and Simpson, 2002; Cavalli et al., 1997; Tanoue et al., 2002; O'Connell et al., 2003). However, little previous work has been carried out to date on α_1-adrenoceptor subtypes in mouse small arteries and therefore it is important that more background information be gained in this species. The aim of the present study therefore was to characterize the α_1-adrenoceptors involved in responses to exogenous noradrenaline and neuronally released noradrenaline in mouse small femoral resistance arteries, using the α_1-adrenoceptor selective antagonist prazosin (Cambridge et al., 1977), the α_{1A}-adrenoceptor selective antagonist 5-methyl-urapidil (Gross et al., 1988), the α_{1D}-adrenoceptor selective antagonist BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione) (Goetz et al., 1995) and the preferential α_{1B}-

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adrenoceptor alkylating agent, chloroethylclonidine (Han et al., 1987).

2. Materials and methods

2.1. Myography

Experiments were carried out in accordance with the European Community guidelines for the use of experimental animals. Male DBA/2 mice (28–32 g, 20–23 weeks) were killed by stunning and exsanguination. Hind limbs were removed and transported to the lab in physiological saline solution (PSS) under ice-cold conditions. First and second order femoral small arteries were dissected out under a microscope (Zeiss) within an hour. The vessel segments were incubated in PSS of composition (mM): NaCl (119), KCl (4.5), $NaHCO_3$ (25), KH_2PO_4 (1.2), $MgSO_4.7H_2O$ (1.2), (+) glucose (11) and $CaCl_2$ (2.5), at 37 °C and gassed with carbogen.

Arterial segments of 2 mm length (normalised diameter, $IC_{0.9}$, ca. 190 μm) were mounted in a four-channel wire myograph (Danish Myotech, Aarhus, Denmark) for isometric tension measurement. The vessels were incubated in the PSS for 1 h after mounting. The vessels were then normalized, i.e. the resting tension-internal circumference relation was determined for each vessel segment (Mulvany and Halpern, 1977). The resting tension was set to a normal internal circumference of $IC_{0.9}$, where $IC_{0.9}$=0.9 IC_{100} and IC_{100} is the internal circumference of the vessel under an effective resting transmural pressure (ERTP) of 100 mm Hg (13.3 kPa). ERTP was calculated from the Laplace equation (ERTP=wall tension/(internal circumference/2π)). Myodaq-Myodata software was used for data acquisition. Thirty minutes after normalization the vessels were exposed to 123 mM K^+ solution twice followed by 10 μM noradrenaline in the presence of 123 mM K^+ solution. The arteries were considered viable if the transmural pressure produced by 123 mM K^+ was greater than 100 mm Hg (13.3 kPa). Vessels were allowed to equilibrate for a further 30 min before beginning experimentation.

2.2. Functional studies using exogenous noradrenaline

After equilibration, three to four concentration response curves (CRC) to noradrenaline were obtained in each arterial segment (30 min between each concentration–response curve). No significant time-dependent changes in sensitivity (pEC_{50} values, n=5: 1st CRC, 6.56±0.11; 2nd CRC, 6.51±0.12; 3rd CRC, 6.46±0.10; 4th CRC 6.48±0.13) or maximum responses (mN/mm, n=5: 1st CRC, 1.55±0.10; 2nd CRC, 1.54±0.09; 3rd CRC, 1.53±0.08; 4th CRC, 1.47±0.10) were found. The first concentration response curve was taken as control and subsequent curves were obtained after incubating the vessels with different concentrations of antagonists for 30 min. For chloroethylclonidine treatment, arterial segments were incubated with chloroethylclonidine for 30 min at 37 °C followed by washing for 60 min (each wash every 15 min). To characterize α_1-adrenoceptors, (8a*R*, 12a*S*, 13a*S*)-5,8,8a,9,10,11,12,12a, 13a-decahydro-3-methoxy-12-(ethylsulphony)-6*H*-isoquino[2,1-g][1,6]-naphththyrine (RS 79948) (100 nM, α_2-adrenoceptor blocker), propranolol (1 μM, β-adrenoceptor blocker), cocaine (3 μM, neuronal uptake blocker) and corticosterone (3 μM, non-neuronal uptake blocker) were added to the PSS before each concentration response-curve. 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., n being the number of animals. Agonist potency is expressed as the pEC_{50} (the negative logarithm of the concentration required to produce 50% of the maximum response, E_{max}). The EC_{50} and E_{max} values were calculated using the Graphpad Prism software program, which fits CRCs to the four parameter logistic equation below:

$$Y = Bottom + \left[(top - bottom)/\left(1 + 10^{(\log EC_{50} - X)P}\right)\right]$$

where X is the logarithm of the molar concentration of agonist, Y is the response and P is the Hill slope.

Antagonist affinity was 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 $\log(r-1)$ vs. $\log(B)$, where r is the ratio of the agonist EC_{50} in the presence and absence of antagonist and B is the molar concentration of antagonist (Arunlakshana and Schild, 1959). If the antagonism met the criteria of competition (Schild slope of unity), then affinity

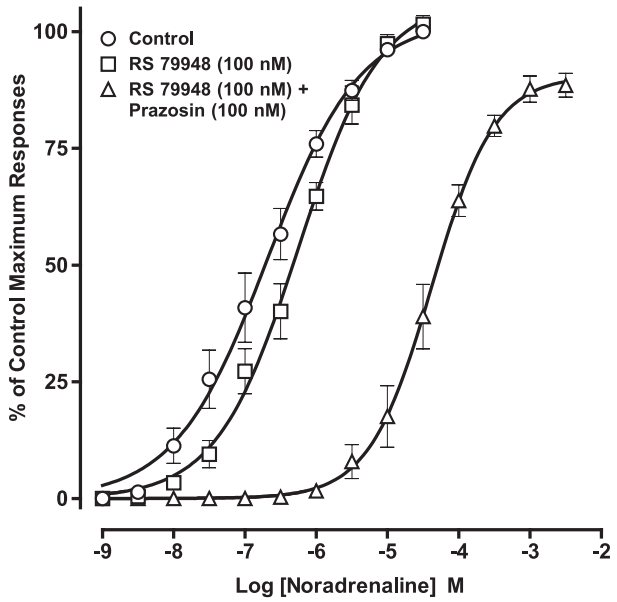

Fig. 1. Effect of RS 79948 and prazosin on responses of mouse femoral small arteries to exogenous noradrenaline (n=7).

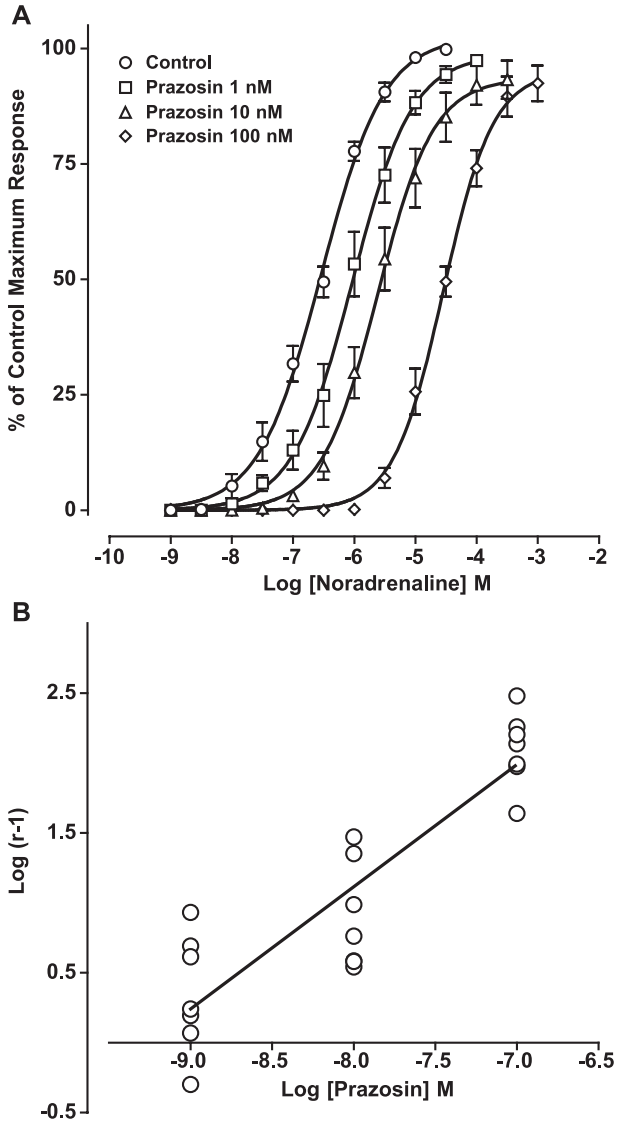

Fig. 2. (A) Effect of prazosin on noradrenaline induced contractions in mouse femoral small arteries (n=7). (B) Schild plot for antagonism of noradrenaline by prazosin in mouse femoral small arteries (n=21).

was expressed as pK_B. When one concentration of antagonist was used to obtain the affinity, pA_2 values were calculated from the Schild equation (Schild, 1949):

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

2.3. Electrical field stimulation

Vessels were placed between platinum electrodes and stimulated every 5 min at 20 V and 0.09 ms pulse width applied for 10 s at frequencies of 2–20 Hz using a Harvard stimulator. Up to five frequency–response curves were obtained in each arterial segment (15 min and thorough washing with PSS between each frequency–response curve). No significant time-dependent changes were seen in responses, e.g responses at 10 Hz, mN/mm, n=6: 1st curve, 1.01±0.12; 2nd curve, 0.97±0.13; 3rd curve, 0.98±0.07; 4th curve, 1.03±0.11; 5th curve, 0.98±0.09. In experiments to characterize α_1-adrenoceptors, the vessels were incubated with RS 79948 (100 nM, α_2-adrenoceptor blocker) for 15 min before recording frequency–response curves. The first frequency–response curve was taken as control and subsequent curves were obtained after incubating the vessels with different concentrations of antagonists for 15 min. Antagonist potencies were expressed as mean pIC_{50} values (the negative logarithm of the concentration of antagonist producing 50% inhibition of the prazosin-sensitive component of the response to field stimulation). For chloroethylclonidine treatment, arterial segments were incubated with chloroethylclonidine for 30 min at 37 °C followed by washing for 60 min (each wash every 15 min).

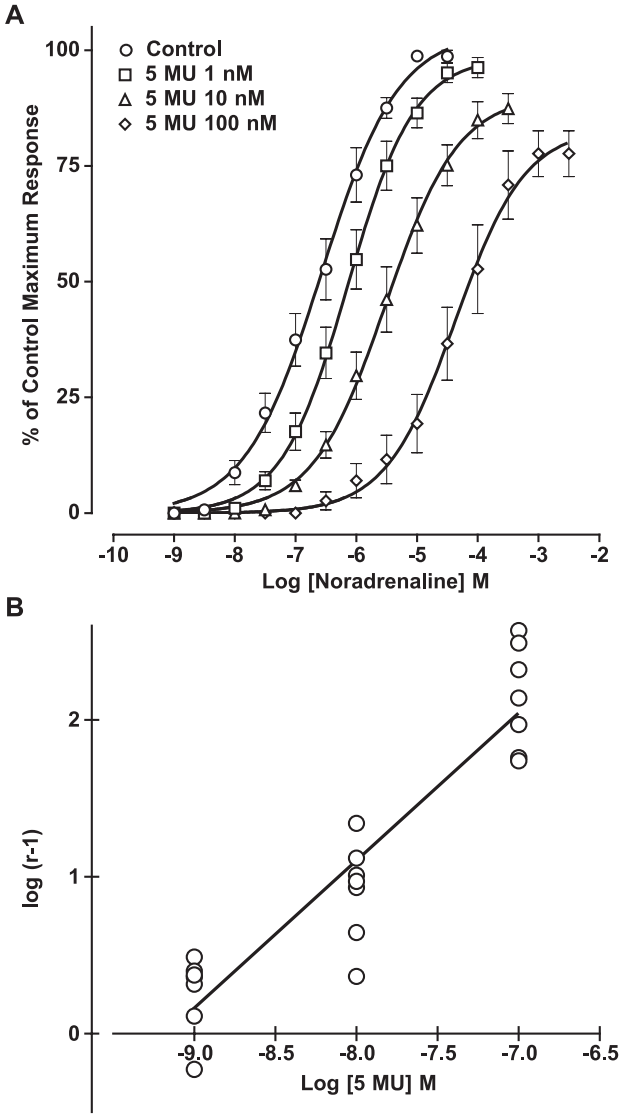

Fig. 3. (A) Effect of 5-methyl-urapidil (5 MU) on noradrenaline induced contractions in mouse femoral small arteries (n=7). (B) Schild plot for antagonism of noradrenaline by 5 MU in mouse femoral small arteries (n=21).

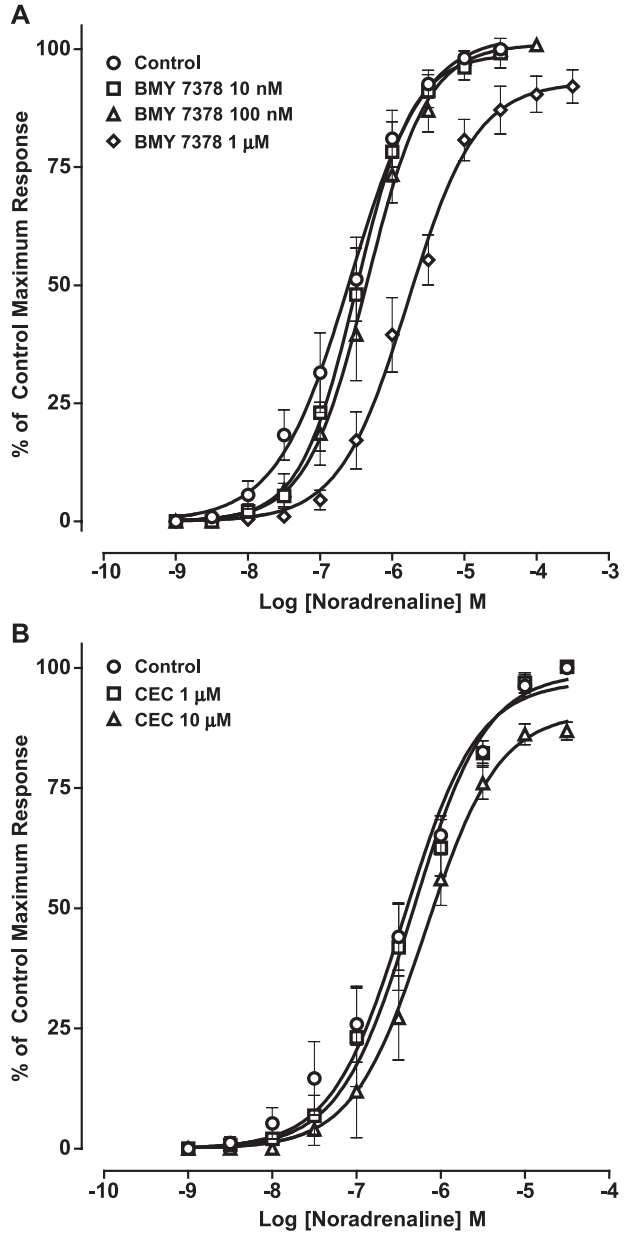

Fig. 4. (A) Effect of BMY 7378 on contractile responses to noradrenaline in mouse femoral small arteries (n=4). (B) Effect of chloroethylclonidine (CEC) on contractile responses to noradrenaline in mouse femoral small arteries (n=4).

2.4. Drugs

The following drugs were used: (−)-noradrenaline (arterenol) bitartrate, (−)-propranolol hydrochloride, corti-

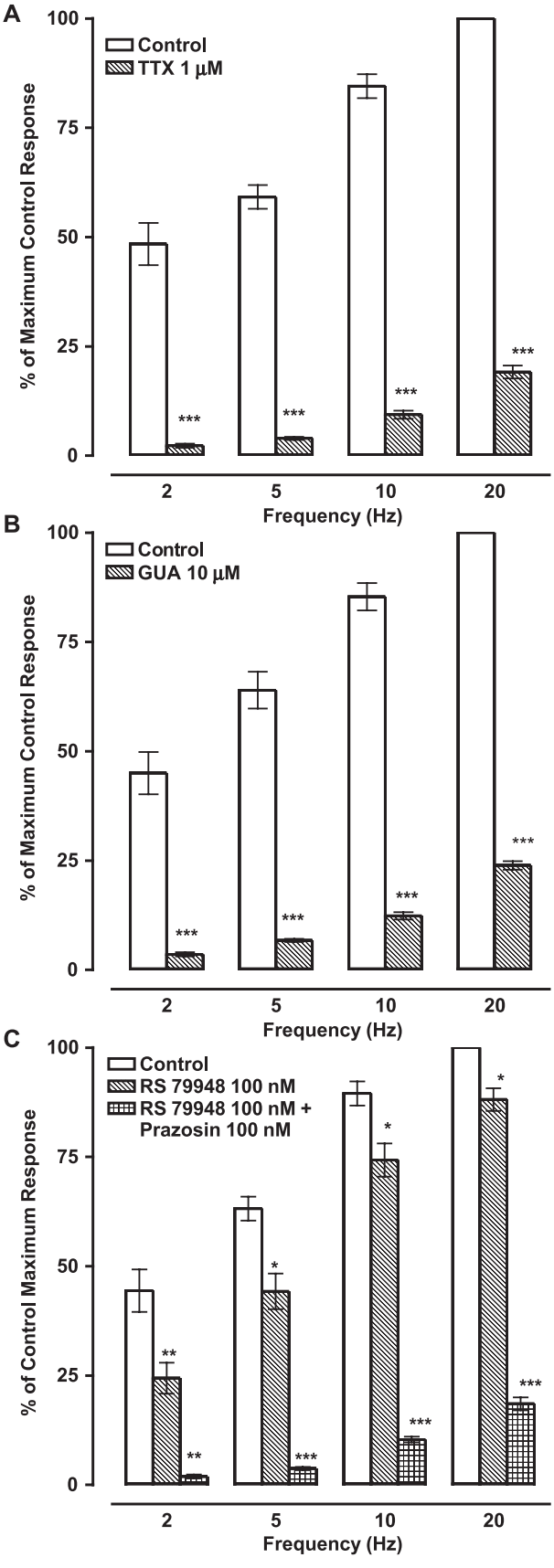

Fig. 5. Effects of drugs on the responses of mouse femoral small arteries to electrical field stimulation at different frequencies (2–20 Hz) for 10 s and 0.09 ms pulse width. (A) Tetrodotoxin (TTX, 1 μM) (n=5), (B) guanethidine (GUAN, 10 μM, n=5), significance of difference from control, ***P<0.001 (paired t-test) and (C) RS 79948 (0.1 μM) and RS 79948 (0.1 μM) + Prazosin (0.1 μM). Significance of difference from control, *P<0.05, **P<0.01, ***P<0.001 (repeated measures ANOVA with post tests, n=4).

costerone acetate, guanethidine mono sulphate, and prazosin hydrochloride (Sigma, Dorset, UK); cocaine HCl (Thornton and Ross, UK); (8a*R*, 12a*S*, 13a*S*)-5,8,8a,9,10,11,12,12a, 13a-decahydro-3-methoxy-12-(ethylsulphony)-6*H*-isoquino[2,1-g][1,6]-naphththyrine (RS 79948), UK 14304 (5-bromo-*N*-[2-imidazolin-2-yl]-6-quinoxalinamine) and tetrodotoxin (Tocris, Bristol, UK); α,β methylene ATP, 5-methyl-urapidil, chloroethylclonidine 2HCl and (8-[2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione) (BMY 7378) (RBI, Natick, USA).

UK 14304 was dissolved in 10% dimethyl sulphoxide and corticosterone in 20% absolute ethanol. Stock solutions of all other drugs were prepared in distilled water.

2.5. Statistics

Significances of differences were obtained by using paired and unpaired Student's *t* test to compare two groups and repeated measures one-way analysis of variance (ANOVA) followed by post-tests for multiple group comparisons.

3. Results

3.1. Vasoconstrictor responses to exogenous noradrenaline

Noradrenaline produced concentration-dependent contractile responses of the mouse femoral small arteries (pEC_{50}, 6.68±0.08, *n*=7) (Fig. 1). RS 79948 (100 nM), produced a small (2.7 fold) but significant shift in the noradrenaline concentration–response curve (pEC_{50}, 6.25±0.07, *n*=7, *P*<0.001) (Fig. 1). Subsequent addition of prazosin (100 nM) produced a further 74-fold shift in the noradrenaline CRC (pEC_{50}, 4.38±0.05, *n*=7, *P*<0.001) (Fig. 1). UK 14304 produced only small contractions with a maximum response of 15±1% (*n*=5) of the maximum noradrenaline response. These responses were not inhibited by RS 79948 (100 nM) (pEC_{50}s, control, 6.08±0.08; RS 79948, 6.00±0.08, *n*=5, *P*>0.05) but were completely abolished by prazosin (100 nM).

Prazosin (1–100 nM, *n*=7) produced a parallel rightward shift of the noradrenaline CRC without affecting the maximum response (Fig. 2A). A Schild Plot gave a pK_B value of 9.3 with a slope of 0.87 (95% CL: 0.66–1.09), not significantly different from unity (Fig. 2B).

5-Methyl-urapidil (1–100 nM) also produced a parallel rightward shift of the noradrenaline CRC (Fig. 3A). Maximum responses were unaffected by 1 nM but were significantly reduced by 10 and 100 nM 5-methyl-urapidil (E_{max}, %, *n*=7: control, 98±2; 1 nM 5-methyl-urapidil, 99±2 (*P*>0.05); 10 nM 5-methyl-urapidil, 90±3 (*P*<0.001); 100 nM 5-methyl-urapidil, 83±4 (*P*<0.001)). The Schild Plot gave a pK_B value of 9.2 with a slope of 0.94 (95% CL: 0.76–1.12), not significantly different from unity (Fig. 3B).

BMY 7378 had no significant effect on the noradrenaline CRC at concentrations of 10 and 100 nM (Fig. 4A, *n*=5). BMY 7378 (1 μM) produced a 6.3-fold shift

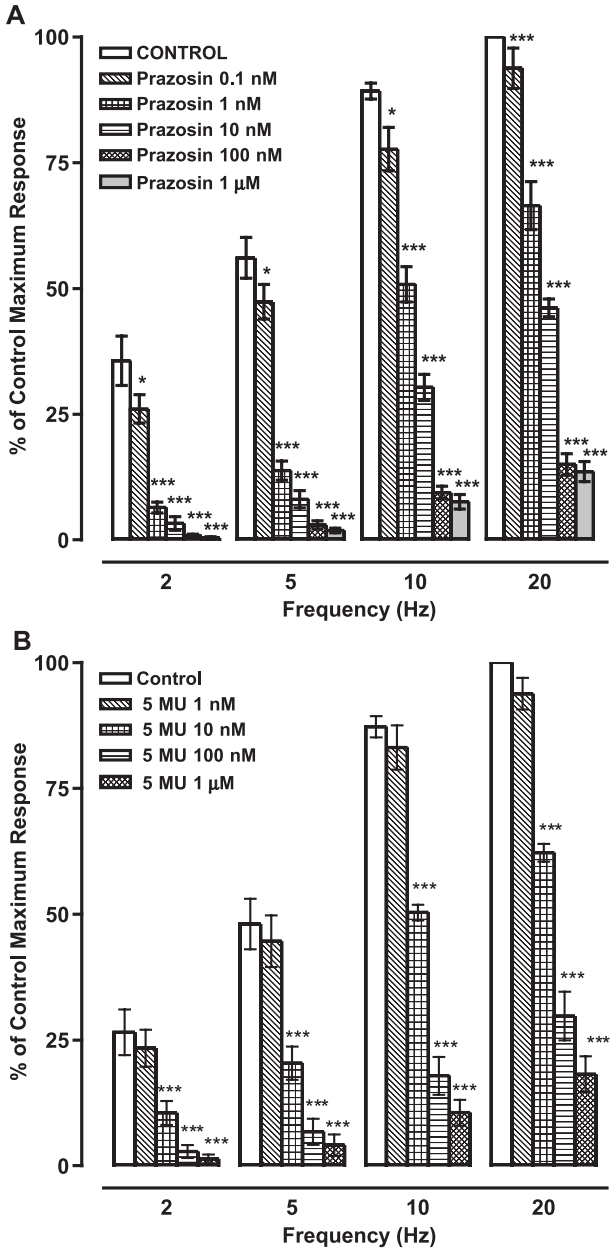

Fig. 6. (A) Effect of different concentrations of prazosin on responses of mouse femoral small arteries to electrical field stimulation at different frequencies for 10 s and 0.09 ms pulse width. Significance of difference from control, **P*<0.05, ****P*<0.001 (repeated measures ANOVA with post tests, *n*=6), (B) Effect of different concentrations of 5-methyl urapidil on responses of mouse femoral small arteries to electrical field stimulation at different frequencies for 10 s and 0.09 ms pulse width. Significance of difference from control, ****P*<0.001 (repeated measures ANOVA with post tests, *n*=6).

of the noradrenaline CRC, giving an estimated pA_2 of 6.7 (n=5).

Chloroethylclonidine (1 μM) had no significant effect on the noradrenaline CRC (Fig. 4B). Chloroethylclonidine (10 μM) reduced the maximum response to noradrenaline (E_{max}%, n=4: 10 μM chloroethylclonidine, 89±4%, P<0.05) although pEC_{50} values were not significantly altered (pEC_{50}s: control, 6.30±0.11; CEC 10 μM, 6.20±0.07, P>0.05, n=4).

3.2. Vasoconstrictor responses to electrical field stimulation

Electrical field stimulation (2–20 Hz) produced frequency-dependent contractile responses of the femoral small arteries. Tetrodotoxin (1 μM, n=5, Fig. 5A) and guanethidine (10 μM, n=5, Fig. 5B), significantly reduced, but did not completely abolish responses. RS 79948 (100 nM, n=4) inhibited responses at all frequencies with greater inhibition at lower frequencies (Fig. 5C). Subsequent addition of prazosin (100 nM, n=4) further reduced the contractile responses due to nerve stimulation to sizes similar to those after tetrodotoxin and guanethidine. Addition of α, β methylene ATP (10 μM, n=4) after prazosin had no significant effect on responses due to electrical field stimulation (not shown).

Prazosin (0.1 nM–1 μM, n=6) produced concentration-dependent inhibition of the responses to field stimulation (Fig. 6A). The pIC_{50} values at 2 and 5 Hz were slightly higher (P<0.05) than the pIC_{50} values at 10 and 20 Hz (Table 1).

5-Methyl-urapidil (1 nM–1μM, n=6) also produced a concentration-dependent inhibition of responses to field stimulation, with potency around twenty times less than that of prazosin at all frequencies (Fig. 6B, Table 1). The pIC_{50} values at 2 and 5 Hz were slightly higher (P<0.05) than the pIC_{50} values at 10 and 20 Hz (Table 1).

BMY 7378 (10 nM–1 μM, n=7) produced concentration-dependent inhibition of responses at 2 and 5 Hz, giving a pIC_{50} of around 8.2 (Fig. 7A, Table 1). Responses to field stimulation at 10 and 20 Hz were less sensitive to BMY 7378, with no significant inhibition by 10 nM and lower pIC_{50} values than at lower frequencies (Table 1).

Chloroethylclonidine (1 and 10 μM, n=3) inhibited responses at frequencies of 2 and 5 Hz, but had little or no effect on responses at higher frequencies (10 and 20 Hz) (Fig. 7b).

Table 1
Inhibition of responses to electrical field stimulation in mouse femoral small arteries

	n	pIC_{50} values			
		2 Hz	5 Hz	10 Hz	20 Hz
Prazosin	6	9.51±0.02	9.47±0.04	9.30±0.04[a]	9.27±0.04[a]
5-MU	6	8.26±0.15	8.22±0.07	7.98±0.03[a]	7.95±0.04[a]
BMY 7378	7	8.16±0.05	8.17±0.08	6.67±0.04[c]	6.54±0.04[c]

The pIC_{50} represents the negative logarithm of the concentration required to produce 50% inhibition of the noradrenergic response. Significance of difference from 2 Hz, [a]P<0.05, [c]P<0.001 (repeated measures ANOVA followed by post tests for multiple group analysis).

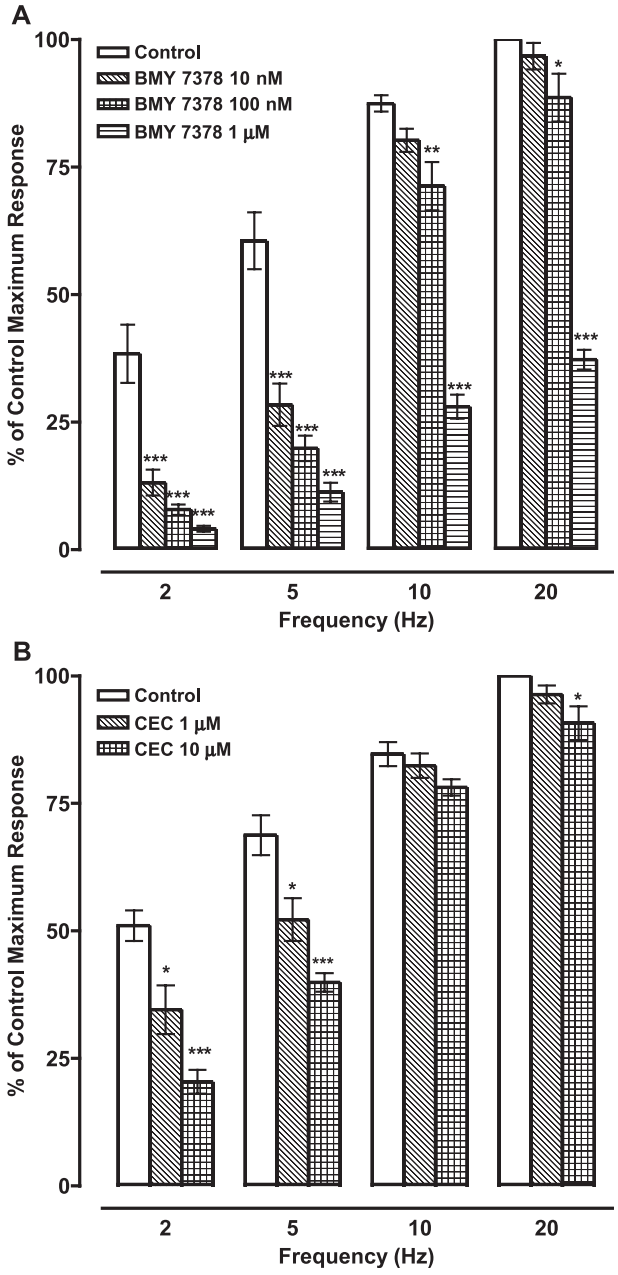

Fig. 7. (A) Effect of BMY 7378 on responses of mouse femoral small arteries to electrical field stimulation at different frequencies for 10 s and 0.09 ms pulse width. Significance of difference from control, *P<0.05, **P<0.01, ***P<0.001 (repeated measures ANOVA followed by post tests, n=7). (B) Effect of chloroethylclonidine (CEC) on responses of mouse femoral small arteries to electrical field stimulation at different frequencies for 10 s and 0.09 ms pulse width. Significance of difference from control, *P<0.05, ***P<0.001 (repeated measures ANOVA followed by post tests, n=3).

4. Discussion

4.1. Vasoconstrictor responses to exogenous noradrenaline

The response to exogenous noradrenaline was predominantly mediated by α_1-adrenoceptors with a minor contribution from postjunctional α_2-adrenoceptors. The small contraction produced by the α_2-adrenoceptor agonist, UK 14304, was mediated by α_1-adrenoceptors, consistent with the partial α_1-adrenoceptor agonist activity of UK 14304 (Daly et al., 1988). The lack of α_2-adrenoceptor-mediated contraction to UK 14304 is not unexpected, since preconstriction is generally required to observe this in arteries (e.g. Dunn et al., 1991).

The pK_B value of 9.2 obtained for prazosin against exogenous noradrenaline is consistent with an action of noradrenaline at the α_{1H}-adrenoceptor and rules out the presence of the α_{1L} subtype (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990a; Muramatsu et al., 1990b). The pK_B value for 5-methyl-urapidil of 9.2 is in agreement with the reported affinity (9.2) at the cloned mammalian α_{1a}-adrenoceptor expressed in CHO-K1 cells (Ford et al., 1996), suggesting that the α_{1A}-adrenoceptor is predominant in mediating responses to exogenous noradrenaline. This is supported by the estimated pA_2 value of 6.7 for BMY 7378, similar to the reported affinity of BMY 7378 for α_{1A}-adrenoceptors (6.6) (Goetz et al., 1995). The small reduction in the maximum response of noradrenaline produced by the high concentration of chloroethylclonidine may indicate the presence of a small population of α_{1B}-adrenoceptors and/or α_{1D}-adrenoceptors (based on the findings that chloroethylclonidine has been shown to alkylate α_{1D}-adrenoceptors under some conditions (Schwinn et al., 1991)). However, murine α_{1A}-adrenoceptors also appear to be alkylated by chloroethylclonidine (Yang et al., 1998) and therefore the effect of choloroethylclonidine in mouse femoral small arteries may represent alkylation of the predominant α_{1A}-subtype.

The predominance of α_{1A}-adrenoceptors in responses to exogenous noradrenaline in mouse femoral small arteries is in agreement with our previous studies in rat femoral resistance arteries (Jarajapu et al., 2001b; Zacharia et al., 2004) and in human skeletal muscle resistance arteries (Jarajapu et al., 2001a). Little previous work has been carried out in characterising α_1-adrenoceptors in mouse isolated arteries and several of these studies have been carried out in conduit vessels (Cavalli et al., 1997; Tanoue et al., 2002; Yamamoto and Koike, 2001; Shibano et al., 2002). Previous studies in isolated small arteries from mouse tail (Daly et al., 2002) and mesentery (Daly et al., 2002; Hedemann and Michel, 2002) have shown that the α_{1A}-adrenoceptor is predominant in mediating responses to an exogenous agonist. Expression of the α_{1A}-adrenoceptor in murine small arteries (including the femoral artery) but not the thoracic aorta or its main branches was also shown by β-galactosidase staining in α_{1A}-adrenoceptor knockout mice in which the α_{1A}-adrenoceptor coding sequence was replaced by the LacZ gene (Rokosh and Simpson, 2002). Thus it would appear that in mice, as in other species, the α_{1A}-adrenoceptor is dominant in small arteries contributing to resistance.

4.2. Vasoconstrictor responses to electrical field stimulation

Frequencies between 2 and 20 Hz were chosen because these frequencies fall within the physiological range for sympathetic nerve firing activity (Hallin and Torebjörk, 1974). Most of the electrical field stimulation-induced contraction was due to activation of excitatory nerves and was predominantly mediated by α_1-adrenoceptors, although post-junctional α_2-adrenoceptors also contributed to the response. The contribution of α_2-adrenoceptors may have been even greater than apparent since RS 79948 would have been likely to have produced potentiation of noradrenaline release via blockade of pre-junctional α_2-adrenoceptors. Block of pre-junctional α_2-adrenoceptors may also explain why less inhibition was obtained with RS 79948 at higher frequencies of stimulation since pre-junctional effects would be greater. No evidence for a purinergic component of the response was found.

In the presence of RS 79948 to block pre- and post-junctional α_2-adrenoceptors, prazosin inhibited nerve-mediated responses to electrical field stimulation with a pIC_{50} of around 9.3–9.5, similar to the pK_B value (9.3) of prazosin obtained in these arteries using exogenous noradrenaline and consistent with the activation of high affinity α_{1H}-adrenoceptors (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990a; Muramatsu et al., 1990b). In contrast to prazosin, the pIC_{50} values for 5-methyl-urapadil (8.0–8.3) against electrical field stimulation were around ten fold lower that the pK_B value (9.2) against exogenous noradrenaline. Thus the prazosin/5-methyl-urapidil potency ratio was approximately 1 against exogenous noradrenaline and 10 against endogenous noradrenaline. This may suggest that not all of the prazosin-sensitive α_1-adrenoceptors activated by endogenous noradrenaline are of the α_{1A}-subtype, since 5-methyl-urapidil is relatively less potent. At the higher concentrations of 5-methylurapidil it is likely that blockade of α_{1D}-adrenoceptors may be contributing to the effects on electrical field simulation. The pIC_{50} values around 8.2 for BMY 7378 at 2 and 5 Hz suggest a significant contribution of α_{1D}-adrenoceptors to the response. At the higher frequencies (10 and 20 Hz), responses were less sensitive to BMY 7378 and the pIC_{50} values of 6.5–6.7 were consistent with the affinity of BMY 7378 at the α_{1A}-subtype. These results suggest that α_{1D}-adrenoceptors may be preferentially activated by low frequency stimulation. The effects of chloroethylclonidine at 2 and 5 Hz may reflect a contribution of α_{1B}-adrenoceptors to the response at low frequencies. However, chloroethylclonidine has also been shown to alkylate α_{1D}-adrenoceptors in some situations (Schwinn et al., 1991), and this would be consistent

with the greater sensitivity of responses to BMY 7378 at 2 and 5 Hz.

Thus the present study has shown that responses to endogenous noradrenaline, released by electrical field stimulation, are also mediated predominantly by α_{1A}-adrenoceptors although there is also a contribution from α_{1D}-adrenoceptors at low frequencies of stimulation. We were unable to find other studies characterising responses to neuronally released noradrenaline in mouse small arteries although the involvement of the α_{1A}-adrenoceptor in nerve-mediated responses is in agreement with rat femoral resistance arteries (Zacharia et al., 2004). A contribution from α_{1D}-adrenoceptors in responses to nerve stimulation but not to exogenous noradrenaline was also seen in canine splenic arteries (Yang and Chiba, 2001) and in rat femoral resistance arteries (Zacharia et al., 2004). The explanation for activation of more subtypes by endogenous noradrenaline than by exogenous noradrenaline is not clear. It is possible that the α_{1D}-adrenoceptors are junctional and that their population is so small that they do not contribute to the response to exogenous noradrenaline. In addition, the time of exposure to and peak concentration of noradrenaline may be important so that junctional receptors may respond only to a brief pulse of a high concentration of noradrenaline. Studies in α_{1D}-adrenoceptor knock-out mice have directly shown that the α_{1D}-adrenoceptor participates in the regulation of systemic blood pressure in this species (Tanoue et al., 2002), suggesting that the current observations may have relevance to the physiological response in vivo.

5. Conclusions

In conclusion the present study has shown a dominant role of the α_{1A}-adrenoreceptor in contractions due to exogenous noradrenaline and to neuronally released noradrenaline in mouse femoral small arteries. α_{1D}-adrenoceptors do not appear to be involved in responses to exogenous noradrenaline but may be activated by neuronally released noradrenaline at a low frequency of stimulation.

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