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Evidence for involvement of α_{1D}-adrenoceptors in contraction of femoral resistance arteries using knockout mice

[1,5]Joseph Zacharia, [1,6]Chris Hillier, [2]Akito Tanoue, [3]Gozoh Tsujimoto, [4]Craig J. Daly, [4]John C. McGrath & *,[1]Allan MacDonald

[1]Vascular Biology Group, Department of Biological & Biomedical Sciences, School of Life Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA; [2]Department of Pediatric Pharmacology, National Children's Medical Research Center, Taishido 3-35-31, Setagaya-ku, Tokyo 154-8509, Japan; [3]Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Kyoto University Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan and [4]Autonomic Physiology Unit, Division of Neuroscience and Biomedical Systems, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ

1 The role of α_{1D}-adrenoceptors in vasoconstrictor responses to noradrenaline in mouse femoral resistance arteries was investigated using wire myography in α_{1D}-adrenoceptor knockout (α_{1D}-KO) and wild-type (WT) mice of the same genetic background.

2 α_{1D}-KO mice were 2.5-fold less sensitive than WTs to exogenous noradrenaline and BMY 7378 was significantly less potent against noradrenaline in α_{1D}-KO mice than in WTs, showing a minor contribution of α_{1D}-adrenoceptors in response to noradrenaline.

3 Prazosin and 5-methyl-urapidil were equally effective against noradrenaline in α_{1D}-KO and WT mice. Chloroethylclonidine produced a significantly greater attenuation of the response to noradrenaline in α_{1D}-KO mice than in WTs.

4 Responses to electrical field stimulation (EFS), at 2–20 Hz for 10 s and 0.09 ms pulse width were significantly smaller overall in α_{1D}-KOs than in WTs although no significant differences were seen at the different frequencies.

5 BMY 7378 produced significantly greater inhibition of responses at 2 and 5 Hz than at higher frequencies in WTs. In α_{1D}-KOs, this greater sensitivity to BMY 7378 at lower frequencies was not apparent, confirming that the effect of BMY 7378 was due to blockade of α_{1D}-adrenoceptors.

6 Prazosin and 5-methyl-urapidil had similar inhibitory effects on responses to EFS in α_{1D}-KO and WT mice. Chloroethylclonidine inhibited responses to EFS to a significantly greater extent in α_{1D}-KO mice.

7 The present study with α_{1D}-KO mice shows that α_{1D}-adrenoceptors contribute to vasoconstrictor responses to exogenous and neurally released noradrenaline in femoral resistance arteries.
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Abbreviations: CRC, concentration–response curve; EFS, electrical field stimulation; ERTP, effective resting transmural pressure; IC, internal circumference; KO, knockout; PSS, physiological saline solution; WT, wild type

Introduction

α_1-Adrenoceptors play critical roles in the regulation of blood flow in the peripheral vascular system. Molecular cloning studies have identified three distinct genes coding α_1-adrenoceptors (α_{1A}-, α_{1B}- and α_{1D}-subtypes) that are structurally homologous. All three receptor subtypes are expressed in blood vessels (Piascik *et al*., 1994; 1995; 1997; Miller *et al*., 1996) but the relative importance of the different subtypes to vasoconstriction is not clear as the contribution of different subtypes varies with species and vascular bed (Vargas & Gorman, 1995; Guimarães & Moura, 2001). In resistance arteries, most important in the regulation of peripheral resistance and systemic arterial blood pressure, the α_{1A}-adrenoceptor appears to be dominant (Kong *et al*., 1994; Blue *et al*., 1995; Williams & Clarke, 1995; Chen *et al*., 1996; Ipsen *et al*., 1997; Lachnit *et al*., 1997; Stassen *et al*., 1997; Jarajapu *et al*., 2001a, b, c; Daly *et al*., 2002). Our own previous studies in femoral resistance arteries of the rat (Zacharia *et al*., 2004a) and mouse (Zacharia *et al*., 2004b) are in agreement, showing that the α_{1A}-adrenoceptor is predominant in mediating responses to exogenous and neurally released noradrenaline. However, in these studies there also appeared to be a contribution from α_{1D}-adrenoceptors in responses to neurally released noradrenaline at low frequencies of stimulation, but

*Author for correspondence; E-mail: a.macdonald@gcal.ac.uk
[5]Current address: School of Medicine, University of Maryland, 525 Howard Hall, 660 West Redwood Street, Baltimore, MD 21201, U.S.A.
[6]Current address: Biopta Ltd., CIDS, Thompson Building, Gilmorehill, Glasgow G12 8QQ, U.K.

not in responses to higher frequencies or to exogenous noradrenaline. Therefore, in the present study, the role of α_{1D}-adrenoceptors in responses of mouse femoral small arteries to exogenous noradrenaline and to electrical field stimulation (EFS) was further examined using α_{1D}-knock out mice (Tanoue *et al*., 2002) in conjunction with the α_1-adrenoceptor-selective antagonist, prazosin (Cambridge *et al*., 1977), the α_{1D}-adrenoceptor-selective antagonist BMY 7378 (Goetz *et al*., 1995), the α_{1A}-adrenoceptor-selective antagonist, 5-methylurapidil (Gross *et al*., 1988) and the preferential α_{1B}-adrenoceptor-alkylating agent, chloroethylclonidine (Han *et al*., 1987).

Methods

Myography

Breeding pairs of wild-type (WT) and α_{1D}-adrenoceptor knockout (α_{1D}-KO) mice of the same genetic background of 129Sv and C57Black/6J strains were kindly supplied by Professor Gozoh Tsujimoto (National Children's Medical Research Center, Tokyo, Japan). All mice were bred in the University of Glasgow. Mice were maintained on 12:12-h light/dark schedule at 22–25°C and 45–65% humidity and fed *ad libitum* on a standard rodent diet and tap water. The generation and background of WT and α_{1D}-KO mice have been described previously (Tanoue *et al*., 2002).

Male mice (28–35 g, 16 weeks) were asphyxiated with CO_2. 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), $MgSO_4 \cdot 7H_2O$ (1.2), (+) glucose (11) and $CaCl_2$ (2.5), at 37°C and gassed with carbogen.

Arterial segments of 2 mm length (normalized diameter, $IC_{0.9}$, c. 190 μm) were mounted in a four-channel wire myograph (Danish Myotech, Aarhus, Denmark) for isometric tension measurement and were maintained in PSS at 37°C gassed with carbogen. After incubation for 1 h, the vessels were then normalized, that is, the resting tension–internal circumference (IC) relation was determined for each vessel segment (Mulvany & Harpen, 1977). The resting tension was set to a normal IC of $IC_{0.9}$, where $IC_{0.9} = 0.9IC_{100}$ and IC_{100} is the internal circumference of the vessel under an effective resting transmural pressure (ERTP) of 100 mmHg (13.3 kPa). ERTP was calculated from the Laplace equation (ERTP = wall tension/(IC/2π)). Myodaq-Myodata software was used for data acquisition. At 30 min 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 equivalent transmural pressure produced by 123 mM K^+ was >100 mmHg (13.3 kPa). Vessels were allowed to equilibrate for a further 30 min before beginning experimentation.

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 CRC). Preliminary experiments showed that no significant time-dependent changes in sensitivity (pEC_{50} values, $n = 3$: 1st CRC, 6.43 ± 0.14; 2nd CRC, 6.38 ± 0.11; 3rd CRC, 6.31 ± 0.10; 4th CRC 6.40 ± 0.13) or maximum responses (mN/mm, $n = 3$: 1st CRC, 1.74 ± 0.13; 2nd CRC, 1.75 ± 0.12; 3rd CRC, 1.74 ± 0.10; 4th CRC, 1.77 ± 0.12) were found in α_{1D}-KO. The first CRC was taken as control and subsequent curves were obtained after incubating the vessels with increasing concentrations of the same antagonist 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, 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 CRC. 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 pEC_{50} and E_{max} values were calculated using the Graphpad Prism software program, which fits CRCs to the four parameter logistic equation below:

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

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 & Schild, 1959). If the antagonism met the criteria of competition (Schild slope of unity), then affinity was expressed as pK_B. When one concentration of antagonist was used to obtain the affinity, estimated pK_B values were calculated from the Schild equation (Schild, 1949):

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

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, for examples, responses at 10 Hz, mN/mm, $n = 6$: 1st curve, 0.89 ± 0.14; 2nd curve, 0.92 ± 0.12; 3rd curve, 0.84 ± 0.14; 4th curve, 0.86 ± 0.15; 5th curve, 0.86 ± 0.12. In experiments to characterize α_1-adrenoceptors, the vessels were incubated with RS 79948 (100 nM) 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).

Drugs

The following drugs were used: (−)-noradrenaline (arterenol) bitartrate, (−)-propranolol hydrochloride, corticosterone acetate, and prazosin hydrochloride (Sigma, Dorset, U.K.); cocaine HCl (Thornton and Ross, U.K.); (8a*R*, 12a*S*, 13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6*H*-isoquino[2,1-g][1,6]-naphthyridine (RS 79948), U.K. 14304 (5-bromo-*N*-[2-imidazolin-2-yl]-6-quinoxalinamine) and tetrodotoxin (Tocris, Bristol, U.K.); 5-methylurapidil, chloroethylclonidine 2HCl and (8-[2-[4-(2-methoxyphenyl)-1 piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione) (BMY 7378) (RBI, Natick, U.S.A.).

Corticosterone was dissolved in 20% absolute ethanol. Stock solutions of all other drugs were prepared in distilled water.

Statistics

Best fit pEC_{50} and E_{max} values obtained from nonlinear regression of CRC (described above) and other mean values were compared by an unpaired *t*-test for two groups or by repeated measures one-way analysis of variance (ANOVA) followed by Dunnet's post-test (three or more groups) after checking for normality (Kolmogorov–Smirnov test). Comparison of responses to EFS at different frequencies of stimulation in WT and α_{1D}-KO mice was carried out using two-way ANOVA followed by a Bonferroni post test. Two-tailed *P*-values were used and $P<0.05$ was considered to be significant throughout.

Results

Vasoconstrictor responses to exogenous noradrenaline

α_{1D}-KO mice showed a 2.5-fold decrease in sensitivity to exogenous noradrenaline with no significant difference in the maximum response compared to WTs (pEC_{50}s: WT, 6.8 ± 0.1; α_{1D}-KO, 6.4 ± 0.1, $P<0.001$, *t*-test, $n=14$. Max responses: WT, 1.7 ± 0.1; α_{1D}-KO, 1.8 ± 0.1 mN/mm, $P>0.05$, *t*-test, $n=14$, Figure 1).

BMY 7378 at low concentrations (10 and 100 nM) appeared to have slightly less effect in α_{1D}-KO than in WT mice (Figure 2). The difference in shifts was not significant at 10 nM (WT, 2.4 ± 0.7; α_{1D}-KO, 1.2 ± 0.2, $P>0.05$, *t*-test, $n=4$) but was significant at 100 nM (WT, 4.9 ± 1.1; α_{1D}-KO, 1.1 ± 0.2,

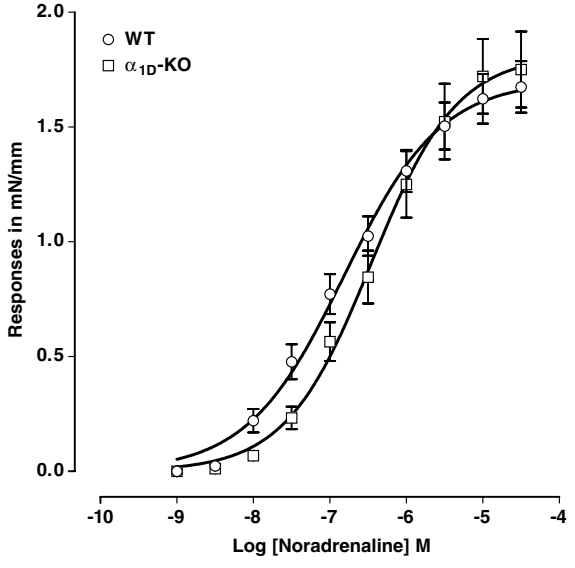

Figure 1 Effect of exogenous noradrenaline on WT (○) and α_{1D}-KO (□) mouse femoral small arteries. Contractile responses are measured in mN/mm and are means ± s.e.m. ($n=14$).

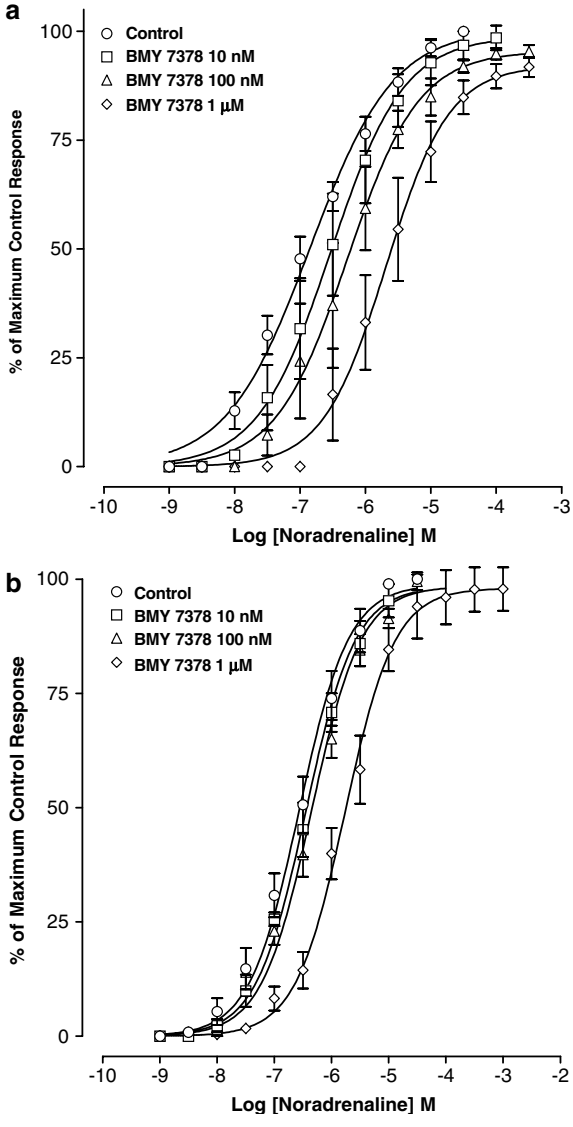

Figure 2 Effect of BMY 7378 on contractile responses to noradrenaline in mouse femoral small arteries. Values are mean ± s.e.m. (a) WT ($n=4$) and (b) α_{1D}-KO mouse ($n=4$).

$P<0.05$, t-test, $n=4$). At 1 μM BMY 7378 the difference in shifts was not significant (WT, 15 ± 6; α_{1D}-KO, 7 ± 2, $P>0.05$, t-test, $n=4$). pK_B values of BMY 7378 estimated from the shifts produced by 1 μM were 7.1 ± 0.2 in WT and 6.6 ± 0.1 in α_{1D}-KO ($P>0.05$, t-test, $n=4$).

The effects of prazosin (1–100 nM) on noradrenaline CRCs were similar in WT and α_{1D}-KO mice. Prazosin produced parallel rightward shifts of the noradrenaline CRC with a slight lowering of maximum responses at the higher concentrations (Figure 3a and b). Schild plots were not significantly different from unity (slope$\pm$95% CL: WT, 0.88 ± 0.18; α_{1D}-KO, 0.81 ± 0.27), yielding pK_B values of 9.3 (Figure 3c) and 9.1 (Figure 3d), respectively.

The effects of 5-methyl-urapidil (1–100 nM) on noradrenaline were also very similar in WT and α_{1D}-KO mice, with a parallel rightward shift of the noradrenaline CRC (Figure 4a and b). Maximum responses to noradrenaline were also slightly lowered in both WT and α_{1D}-KO by 5-methyl-urapidil at the higher concentrations. Schild plots were close to unity (slope$\pm$95% CL: WT, 0.86 ± 0.16; α_{1D}-KO, 0.86 ± 0.18) with pK_B values of 9.1 (Figure 4c) and 9.0 (Figure 4d), respectively, for WT and α_{1D}-KO.

Chloroethylclonidine had a greater effect in α_{1D}-KO mice (Figure 5b) than in the WT (Figure 5a). Maximum responses to noradrenaline were reduced to a greater extent in α_{1D}-KO mice (E_{max} % of control, $n=4$, ANOVA followed by Dunnet's post-test: 1 μM chloroethylclonidine, 84 ± 1, $P<0.01$; 10 μM chloroethylclonidine, 43 ± 2%, $P<0.001$,) than in WT (E_{max} % of control, $n=4$, ANOVA followed by Dunnet's post-test: 1 μM chloroethylclonidine, 102 ± 3, $P>0.05$; 10 μM chloroethylclonidine, 79 ± 2, $P<0.001$).

Vasoconstrictor responses to EFS

EFS (2–20 Hz) produced frequency-dependent contractile responses of the femoral small arteries. Tetrodotoxin (1 μM, not shown) significantly reduced, but did not completely abolish responses.

Responses to EFS overall tended to be smaller in α_{1D}-KOs than in WTs, with a significant difference overall detected

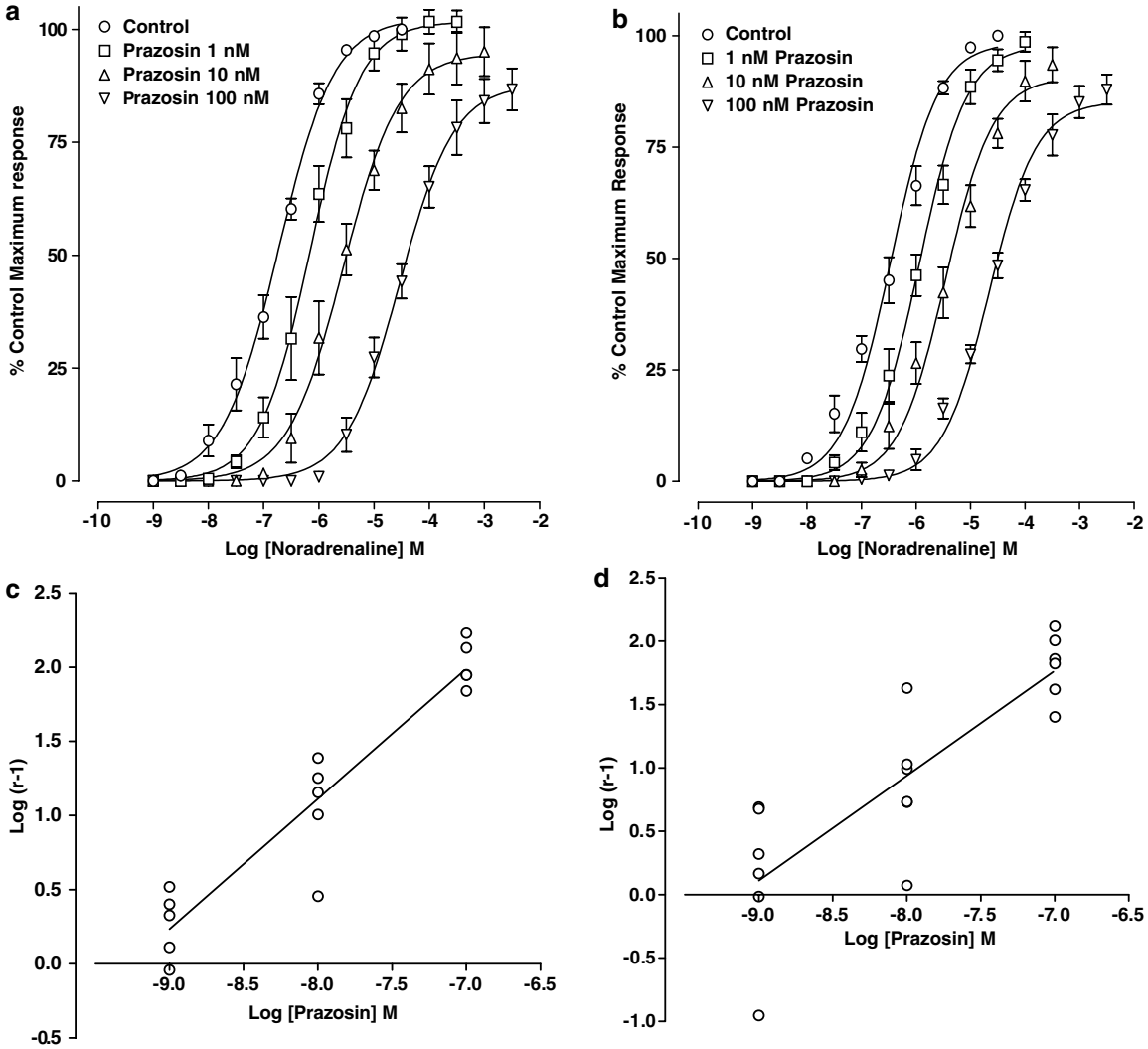

Figure 3 Effect of prazosin on noradrenaline-induced contractions in mouse femoral small arteries. Values are means$\pm$s.e.m. (a) WT ($n=5$) and (b) α_{1D}-KO ($n=6$). Schild plot for antagonism of noradrenaline by prazosin in mouse femoral small arteries (c) WT ($n=15$) and (d) α_{1D}-KO ($n=18$).

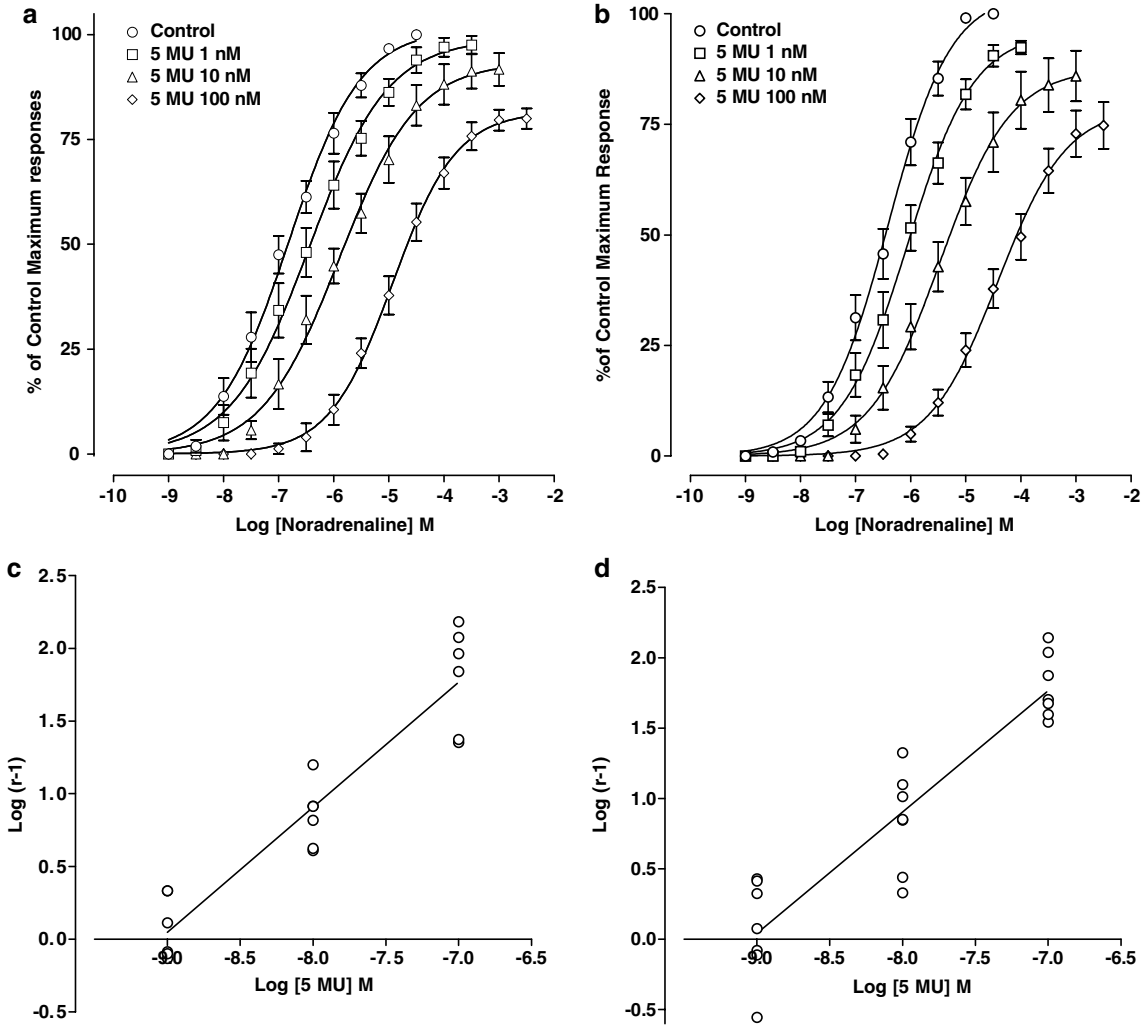

Figure 4 Effect of 5-methyl-urapidil (5 MU) on noradrenaline-induced contractions in mouse femoral small arteries. Values are mean ± s.e.m. (a) WT ($n=6$) and (b) α_{1D}-KO ($n=7$). Schild plot for antagonism of noradrenaline by 5 MU in mouse femoral small arteries (c) WT ($n=18$) and (d) α_{1D}-KO ($n=21$).

by two-way ANOVA ($P<0.05$) (Figure 6). No significant differences between WTs and α_{1D}-KOs were seen at the different frequencies ($P>0.5$, Bonferroni post-test).

BMY 7378 produced greater inhibition of responses at 2 and 5 Hz than at higher frequencies in WTs, reflected in significantly higher pIC_{50} values at the lower frequencies (Figure 7a, Table 1). In α_{1D}-KOs, this greater sensitivity to BMY 7378 at lower frequencies was not apparent, with pIC_{50} values at all frequencies similar to the values obtained in WTs at the higher frequencies (Figure 7b, Table 1).

Prazosin produced concentration-dependent inhibition of the responses to field stimulation with no significant differences between wild types (Figure 7c, Table 1) and α_{1D}-KO (Figure 7d, Table 1).

5-Methyl-urapidil also produced a concentration-dependent inhibition of responses to field stimulation in WT and α_{1D}-KO mice (Figure 8a and b, Table 1). The pIC_{50} values for 5-methyl-urapidil-induced inhibition were greater in α_{1D}-KO mice than WT at 2 and 5 Hz (Table 1).

In WTs, chloroethylclonidine (1 and 10 μM) inhibited responses at frequencies of 2 and 5 Hz, but had no significant effect on responses at higher frequencies (10 and 20 Hz) (Figure 8c). In the α_{1D}-KOs, chloroethylclonidine (1 and 10 μM) inhibited responses at all frequencies (Figure 8d).

Discussion

Responses to exogenous noradrenaline

The reduction in sensitivity of the femoral small arteries to exogenous noradrenaline in α_{1D}-KO mice suggests that α_{1D}-adrenoceptors make a significant, if small, contribution to the contractile response to noradrenaline. This is supported by the results with BMY 7378, which at 100 nM produced a larger shift in WT than in α_{1D}-KO mice. The complete ineffectiveness of 100 nM BMY 7378 in the α_{1D}-KO mice validates this as a selective antagonist treatment for removal of α_{1D}-adrenoceptors. However, the shifts produced by 1 μM BMY 7378 in WTs and α_{1D}-KOs were not significantly different, presumably because BMY 7378 is blocking the predominant α_{1A}-adrenoceptor at this concentration. The pK_B values obtained from the shift produced by 1 μM BMY 7378 were not significantly different in WT and α_{1D}-KO mice and were close to the

reported affinity of BMY 7378 for α_{1A}-adrenoceptors (6.6) (Goetz *et al*., 1995), supporting the predominance of α_{1A}-adrenoceptors in mediating the response to noradrenaline. It should be noted that, in a previous study using WT DBA/2 mice, we were unable to detect any contribution of α_{1D}-adrenoceptors in response to exogenous noradrenaline in the femoral arteries (Zacharia *et al*., 2004b). This is not surprising since the effect of BMY 7378 described here is very small and was detectable only at 100 nM. The small difference from the previous study may also reflect a strain difference since the WT mice used in the present study were of the same genetic background of 129Sv and C57Black/6J strains as the α_{1D}-KO mice.

The effects of prazosin against noradrenaline in WTs and α_{1D}-KOs were very similar, as expected of a nonselective antagonist, and showing that this relative resistance to BMY 7378 is not a general phenomenon.

5-Methyl-urapidil also produced very similar effects in WTs and α_{1D}-KOs and the pK_B of $\geqslant$9.0 is in agreement with the reported affinity (9.2) at the cloned mammalian α_{1a}-adrenoceptor expressed in rat CHO-K1 cells (Ford *et al*., 1997). This suggests that the actions of 5-methyl-urapidil in WTs can be explained entirely by antagonism of α_{1A}-adrenoceptors. Thus,

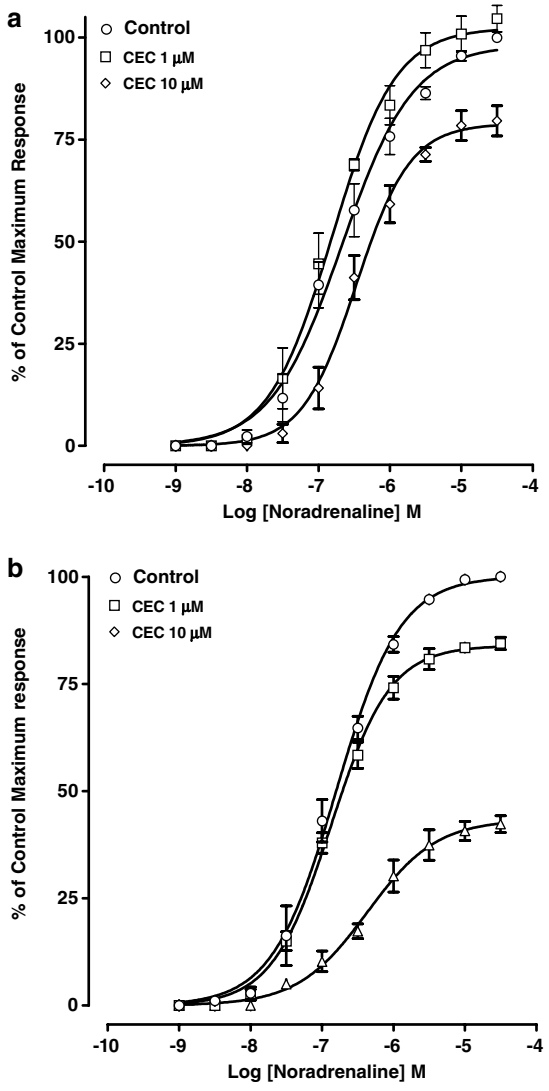

Figure 5 Effect of chloroethylclonidine (CEC) on contractile responses to noradrenaline in mouse femoral small arteries. Values are means$\pm$s.e.m. (a) WT ($n=4$) and (b) α_{1D}-KO ($n=4$).

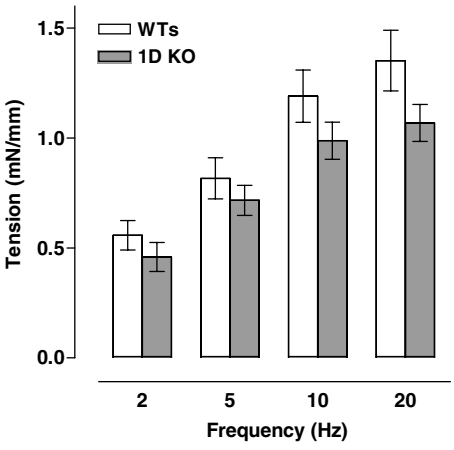

Figure 6 Responses to EFS at different frequencies for 10 s and 0.09 ms pulse width in WT and α_{1D}-KO mouse femoral small arteries. Responses measured in mN/mm and are means$\pm$s.e.m. ($n=14$). Responses in WTs were significantly greater overall than responses in α_{1D}-KOs (two-way ANOVA, $P<0.05$), although there were no significant differences at each frequency (Bonferroni post-test, $P>0.05$).

Table 1 Inhibition of responses to EFS in mouse femoral small arteries

		pIC_{50} values			
	n	*2* Hz	*5* Hz	*10* Hz	*20* Hz
Prazosin					
WT	6	9.26$\pm$0.07	9.20$\pm$0.05	9.05$\pm$0.06	9.15$\pm$0.08
1D-KO	6	9.42$\pm$0.09	9.28$\pm$0.10	9.21$\pm$0.09	9.05$\pm$0.09
5-MU					
WT	4	8.48$\pm$0.06	8.30$\pm$0.07	8.43$\pm$0.09	8.28$\pm$0.09
1D-KO	5	8.85$\pm$0.12[a]	8.83$\pm$0.15[a]	8.56$\pm$0.09	8.36$\pm$0.09
BMY 7378					
WT	5	8.04$\pm$0.11	8.08$\pm$0.08	6.43$\pm$0.11[b]	6.42$\pm$0.07[b]
1D-KO	5	6.63$\pm$0.06[c]	6.43$\pm$0.05[c]	6.13$\pm$0.13	6.16$\pm$0.15

[a]Significantly different from value in WTs ($P<0.05$), *t*-test.
[b]Significantly different from value at 5 Hz ($P<0.001$), ANOVA with Dunnet's post-test.
[c]Significantly different from value in WTs ($P<0.001$), *t*-test.
The pIC_{50} represents the negative logarithm of the concentration required to produce 50% inhibition of the noradrenergic response.

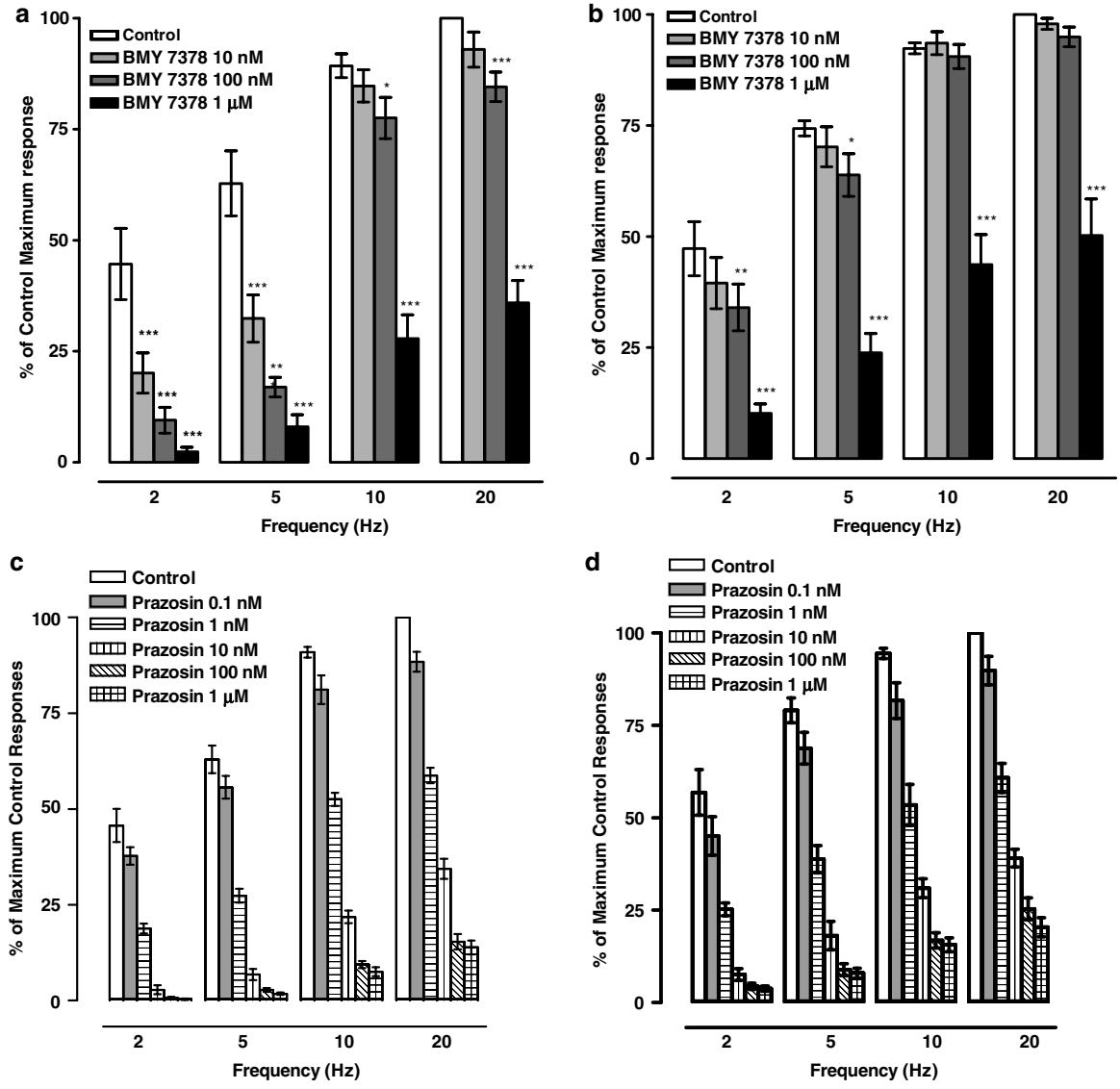

Figure 7 (a) and (b) Effect of BMY 7378 on responses of mouse femoral small arteries to EFS at different frequencies for 10 s and 0.09 ms pulse width. Values are means ± s.e.m. (a) WT ($n=5$) and (b) α_{1D}-KO ($n=4$). Significance of difference from control, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (ANOVA followed by Dunnet's post-test). (c) and (d) Effect of prazosin on responses of mouse femoral small arteries to EFS at different frequencies for 10 s and 0.09 ms pulse width in (c) WT ($n=6$) and (d) α_{1D}-KO ($n=6$). Significances of difference from control have been omitted for clarity. All treatment values were significantly different from control ($P<0.001$) except for 0.1 nM prazosin ($P>0.5$) at all frequencies (ANOVA followed by Dunnet's post-test).

although a small population of α_{1D}-adrenoceptors may be present (as suggested by the responses to exogenous noradrenaline and by the effects of BMY 7378), their loss cannot be detected with 5-methyl-urapidil. This is perhaps not surprising since 5-methyl-urapidil has moderate affinity for α_{1D}-adrenoceptors (8.0 for cloned α_{1d}-adrenoceptor (Ford *et al*., 1997)).

Chloroethylclonidine, at higher concentration (10 μM), produced a small reduction in the maximum response of noradrenaline in WTs and this 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; Schwinn *et al*., 1991). The effects of chloroethylclonidine are greater in α_{1D}-KOs, indicating an upregulation of the chloroethylclonidine-sensitive response, presumably mediated by α_{1B}-adrenoceptors.

Thus the present study in α_{1D}-KO mice has provided evidence for a minor contribution of α_{1D}-adrenoceptors in response to low concentrations of noradrenaline in femoral small arteries.

Electrical field stimulation

Tetrodotoxin-sensitive responses to field stimulation in mouse femoral resistance arteries were previously shown to be adrenergic, mainly mediated by α_1-adrenoceptors with a contribution from postjunctional α_2-adrenoceptors (Zacharia *et al*., 2004b). Experiments were therefore carried out in the presence of RS 79948 to block pre- and post-junctional α_2-adrenoceptors.

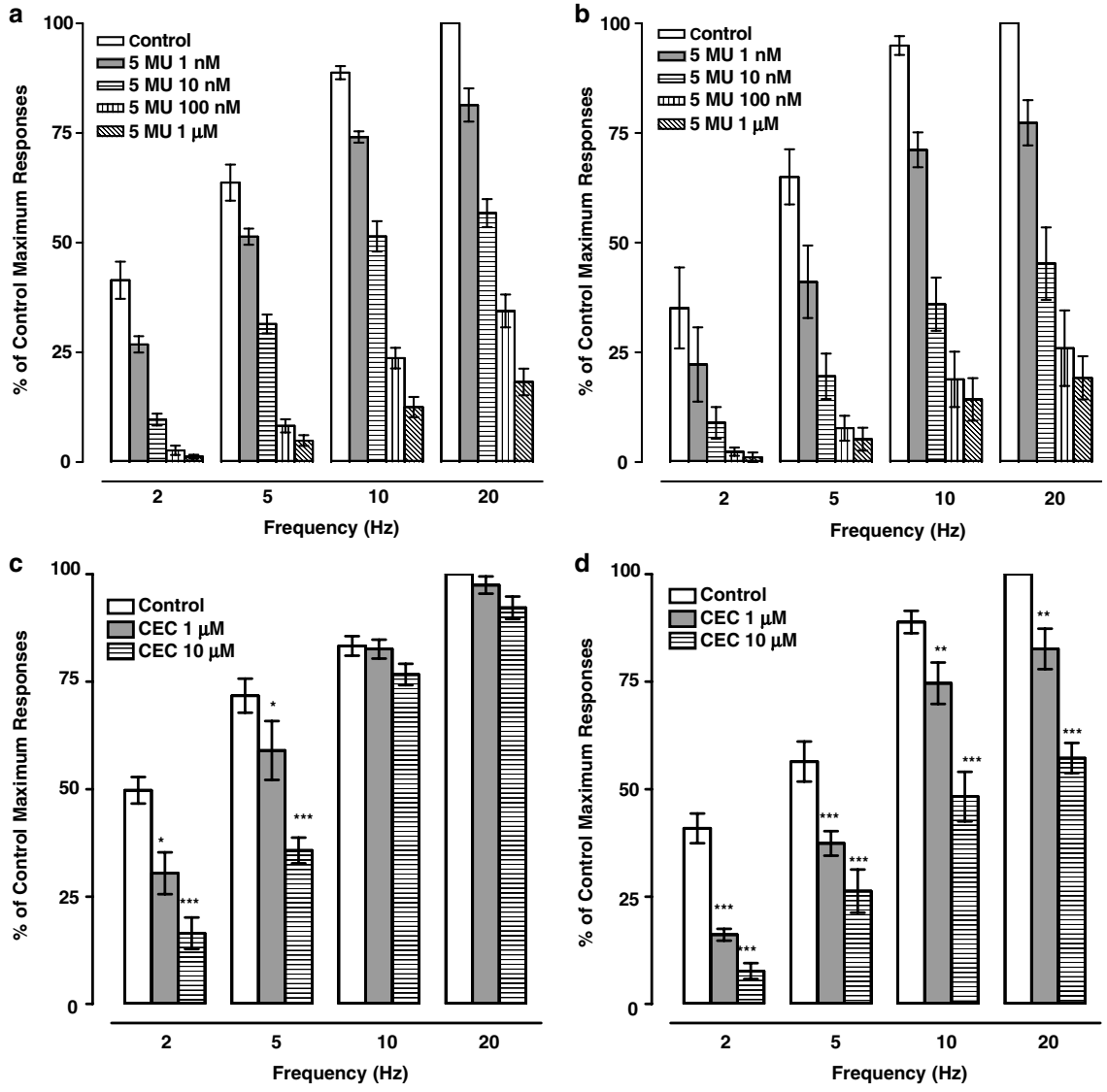

Figure 8 (a) and (b) Effect of different concentrations of 5-methyl urapidil on responses of mouse femoral small arteries to EFS at different frequencies for 10 s and 0.09 ms pulse width. Values are means ± s.e.m. (a) WT ($n=4$) and (b) α_{1D}-KO ($n=5$). Significances of difference from control have been omitted for clarity. All treatment values were significantly different from control ($P<0.001$) (ANOVA followed by Dunnet's post-test). (c) and (d) Effect of chloroethylclonidine (CEC) on responses of mouse femoral small arteries to EFS at different frequencies for 10 s and 0.09 ms pulse width (c) WT ($n=4$) and (d) α_{1D}-KO ($n=4$). Significance of difference from control, $^{*}P<0.05$, $^{***}P<0.01$, $^{**}P<0.001$ (ANOVA followed by Dunnet's post-test).

Antagonist potency against the α_1-adrenoceptors activated by EFS was expressed as the pIC_{50} value, not a direct measure of affinity since equilibrium conditions do not apply, but the value would be expected to be related to affinity. In fact, the pIC_{50} values obtained for the nonsubtype selective antagonist, prazosin, were very similar to the pK_B values obtained using exogenous noradrenaline, suggesting that the pIC_{50} provides a reasonable estimate of affinity under these experimental conditions. The pIC_{50} values obtained for prazosin were very similar in WTs and α_{1D}-KOs, consistent with the expectations of a nonsubtype selective antagonist.

The effects of BMY 7378 in WTs suggested a significant contribution of α_{1D}-adrenoceptors to the response at low frequencies of stimulation, as reported previously (Zacharia *et al*., 2004a, b). In α_{1D}-KOs, the pIC_{50} values for BMY 7378 were low and consistent with the affinity of BMY 7378 for α_{1A}-adrenoceptors (Goetz *et al*., 1995), confirming that the effect of BMY 7378 in WTs was due to antagonism of α_{1D}-adrenoceptors.

Although there was a significantly smaller response overall to EFS in the α_{1D}-KOs, the difference was not frequency dependent as might have been expected from the results with BMY 7378. Thus, the loss of the BMY-sensitive component at 2 and 5 Hz in α_{1D}-KOs did not significantly reduce the size of responses obtained on nerve stimulation, indicating that some compensatory process has occurred.

The pIC_{50}s for 5-methyl-urapidil (8.3–8.5 for WTs and 8.4–8.9 for KOs) against EFS were slightly lower than the pK_B value against exogenous noradrenaline (~9.0). 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 2 and 5 Hz, the pIC_{50} values for 5-methyl-urapidil were higher in α_{1D}-KOs than in WTs, consistent with a contribution of α_{1D}-adrenoceptors to the responses at 2 and 5 Hz.

The effects of chloroethylclonidine in WTs 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 (Schwinn *et al*., 1991), and this would be consistent with the greater sensitivity of responses to BMY 7378 at 2 and 5 Hz. The results with α_{1D}-KOs do not answer this question since chloroethylclonidine (1–10 μM) inhibited responses at all frequencies in α_{1D}-KOs. The increased sensitivity to chloroethylclonidine in α_{1D}-KOs may indicate the upregulation of α_{1B}-adrenoceptors, and is consistent with the increased sensitivity of exogenous noradrenaline to chloroethylclonidine (see above).

The present study confirms our previous findings in rat and mouse femoral resistance arteries that the α_{1A}-adrenceptor is dominant in mediating responses to exogenous and neurally released noradrenaline (Jarajapu *et al*., 2001b; Zacharia *et al*., 2004a, b). The predominance of the α_{1A}-adrenoceptor in mediating responses to exogenous noradrenaline or nerve stimulation is in agreement with several other studies in rat small arteries (Kong *et al*., 1994; Blue *et al*., 1995; Williams & Clarke, 1995; Chen *et al*., 1996; Ipsen *et al*., 1997; Lachnit *et al*., 1997; Stassen *et al*., 1997). α_{1A}-Adrenoceptors have also been shown to be predominant in human resistance arteries (Jarajapu *et al*., 2001a, c).

The present study also confirms our previous suggestion (Zacharia *et al*., 2004a, b) that α_{1D}-adrenceptors contribute to the effects of neurally released noradrenaline at low, but not high, frequencies of stimulation. The present results also show that there is a minor contribution of α_{1D}-adrenoceptors in response to exogenous noradrenaline. Since the role of the α_{1D}-adrenoceptor is highlighted at low concentrations of noradrenaline and at low frequencies of stimulation, its contribution may be greater than is supposed from a basic pharmacological analysis. Thus, although the α_{1A}-adrenoceptor is dominant in femoral small arteries contributing to resistance, the α_{1D}-adrenoceptor may also play a significant role.

There are few other studies in the literature that support a role for α_{1D}-adrenoceptors in resistance arteries. An 'α_{1D}-like' adrenoceptor has been reported in rat cremaster arterioles (Leech & Faber, 1996), although the low affinity of BMY 7378 casts doubt on this conclusion. A role for α_{1D}-adrenoceptors has also been proposed in rat renal resistance vessels (Salomonsson *et al*., 2000); however, the authors acknowledged that relatively high concentrations of BMY 7378 were required to attenuate the effects of noradrenaline. A 5-methyl-urapidil-resistant component of the response to noradrenaline in human skeletal muscle resistance arteries, which was likely to be due to α_{1D}-adrenoceptors, was also found in our laboratory (Jarajapu, 2001; Jarajapu *et al*., 2001a). One possible reason for the paucity of positive findings for a role for α_{1D}-adrenoceptors in resistance arteries may be related to the experimental conditions that optimally demonstrate their function: in the present study, a contribution from α_{1D}-adrenoceptors was most clearly demonstrated in experiments with nerve stimulation and with low frequencies of stimulation. That these findings may have physiological significance is supported by studies in α_{1D}-KO mice, which have directly shown that the α_{1D}-adrenoceptor participates in the regulation of systemic blood pressure (Tanoue *et al*., 2002). *In vivo* studies in the rat have also shown α_{1D}-adrenoceptors to contribute to vasopressor responses to α_1-adrenoceptor agonists (Zhou & Vargas, 1996; Villalobos-Molina *et al*., 1999) and to nerve stimulation (Castillo *et al*., 1998). Thus α_{1D}-adrenoceptors may play a more important role in the regulation of peripheral resistance and blood pressure than is currently recognized.

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