British Journal of Pharmacology (2004) 141, 915–924
www.nature.com/bjp

α_1-Adrenoceptor subtypes involved in vasoconstrictor responses to exogenous and neurally released noradrenaline in rat femoral resistance arteries

[1]Joseph Zacharia, [1]Chris Hillier & *,[1]Allan MacDonald

[1]Department of Biological & Biomedical Sciences, Glasgow Caledonian University, Glasgow G4 0BA, U.K.

1 The α_1-adrenoceptor subtypes involved in responses to exogenous and neurally released noradrenaline in rat femoral resistance arteries were characterised using a small vessel myograph, with antagonists prazosin (nonsubtype selective), 5-methyl-urapidil (α_{1A}-selective), BMY 7378 (α_{1D}-selective) and the alkylating agent chloroethylclonidine (preferential for α_{1B}-).

2 Prazosin and 5-methyl-urapidil produced rightward shifts of the exogenous noradrenaline concentration – response curve (CRC) with pA_2 values of 9.2 and 9.1 respectively, in agreement with the presence of α_{1A}-adrenoceptors. BMY 7378 (1 μM) shifted the noradrenaline CRC with an apparent pK_B of 6.7, in agreement with the presence of α_{1A}-, but not α_{1D}-, adrenoceptors. Chloroethylclonidine at 1 μM had no effect and at 10 μM produced only a small reduction (c. 20%) in the maximum response to noradrenaline, indicating little, if any, contribution from α_{1B}-adrenoceptors.

3 Responses of the rat femoral resistance arteries to electrical field stimulation (EFS) at 5–30 Hz for 10 s and 0.05 ms pulse width were principally due to α_1-adrenoceptor stimulation. Prazosin and 5-methyl-urapidil inhibited EFS-mediated responses with pIC_{50}s of 9.3 and 8.2, respectively, consistent with the α_{1A}-adrenoceptor being the predominant subtype. Responses to EFS at 10–30 Hz were relatively insensitive to BMY 7378 (pIC_{50}, 6.5–6.7), while responses to 5 Hz were inhibited with a significantly higher pIC_{50} of 8.02, suggesting the contribution of α_{1D}-adrenoceptors. Chloroethylclonidine had no effect on responses to EFS, ruling out the contribution of an α_{1B}-subtype.

4 In the presence of cocaine, the predominant subtype involved in responses to EFS was the α_{1A}-adrenoceptor, with a contribution from α_{1D}-adrenoceptors at low frequency, as seen in the absence of cocaine. However, there was also a significant increase in the sensitivity to BMY 7378 at higher frequencies, suggesting that a further small α_{1D}-adrenoceptor component may be uncovered in the presence of cocaine.

5 The present study has shown a predominant role of the α_{1A}-adrenoceptor in contractions due to exogenous noradrenaline and to neurally released noradrenaline in rat femoral resistance arteries. 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 and at higher frequencies in the presence of neuronal-uptake blockade.

British Journal of Pharmacology (2004) **141,** 915–924. doi:10.1038/sj.bjp.0705690

Keywords: α_1-Adrenoceptors; α_1-adrenoceptor subtypes; α_{1A}-adrenoceptor; α_{1B}-adrenoceptor; α_{1D}-adrenoceptor; noradrenaline; electrical field stimulation; rat femoral artery; resistance artery

Abbreviations: CRC, concentration – response curve; EFS, electrical field stimulation; ERTP, effective resting transmural pressure; PSS, physiological saline solution

Introduction

α_1-Adrenoceptors located in vascular smooth muscle play an important role in the regulation of peripheral resistance and systemic arterial blood pressure. It is now well accepted that there are three functional α_1-adrenoceptor subtypes, 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; Bylund *et al*., 1998). These three subtypes display high affinity for prazosin (pK_B > 9) in functional and radioligand binding experiments and can be designated as α_{1H}-adrenoceptor subtypes. α_1-Adrenoceptors with low affinity for prazosin (pA_2 < 9) have also been identified in functional studies and classified as the α_{1L}-subtype (Flavahan & Vanhoutte, 1986; Muramatsu *et al*., 1990a, b). The α_{1L}-adrenoceptor subtype has not been cloned and evidence suggests that it is not a separate gene product but a low-affinity state of the α_{1A}-adrenoceptor (Ford *et al*., 1997).

The relative importance of the different subtypes in regulation of peripheral resistance and systemic arterial blood pressure is not clear, as the contribution of different subtypes to vasoconstriction varies with species and vascular bed (Vargas & Gorman, 1995; Guimarães & Moura, 2001). A further factor that may influence the subtypes involved in vasoconstriction physiologically is the manner of receptor activation, that is, whether by circulating catecholamines or by neurally released noradrenaline. Honner & Docherty (1999)

*Author for correspondence; E-mail: a.macdonald@gcal.ac.uk
Advance online publication: 23 February 2004

have shown that responses of rat vas deferens to exogenous noradrenaline involve α_{1A}-adrenoceptors while responses to nerve stimulation involve non-α_{1A}-adrenoceptors, resembling the α_{1D}-adrenoceptor. Yang & Chiba (2001) have recently shown that in canine splenic artery exogenous noradrenaline produces contraction *via* α_{1A}-adrenoceptors while noradrenaline released by nerve stimulation produced contraction *via* α_{1B}- and α_{1D}-adrenoceptors. This may suggest that circulating catecholamines produce vasoconstriction *via* extrajunctional α_{1A}-adrenoceptors while sympathetic nerve stimulation produces vasoconstriction *via* junctional α_{1B}- and α_{1D}-adrenoceptors in this artery. In contrast, other studies have shown no difference in the receptor subtype involved in responses to exogenous and endogenous, neurally released noradrenaline (Williams & Clarke, 1995).

The aim of the present study was therefore to compare the α_1-adrenoceptor subtypes involved in responses to exogenous and endogenous, neurally released, noradrenaline in rat femoral resistance arteries, as the skeletal muscle vascular bed is a major vascular bed with a large contribution to the peripheral vascular resistance. We previously reported that small branches of the rat femoral artery showed a predominance of the α_{1A}-adrenoceptor in responses to exogenous α_1-adrenoceptor agonists (Jarajapu *et al*., 2001b). Preliminary experiments with these branches showed that they did not all respond to electrical field stimulation. We therefore carried out a direct comparison of responses to exogenous and endogenous noradrenaline, using only branches which responded to both exogenous noradrenaline and to electrical field stimulation.

Preliminary accounts of this work have been presented to the British Pharmacological Society (Zacharia *et al*., 2002; 2003).

Methods

Male Wistar rats (200–250 g, 10–13 weeks) were killed by stunning and exsanguination. Hindlimbs were removed and transported to the lab in physiological saline solution (PSS) under ice-cold conditions. Second - and third-order femoral 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 (normalised diameter, $IC_{0.9}$, 224 ± 5, $n = 129$) 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 normalised, that is, the resting tension – internal circumference relation was determined for each vessel segment (Mulvany & 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 mmHg (13.3 kPa). ERTP was calculated from the Laplace equation (ERTP = wall tension/(internal circumference/2π)). The Myodaq-Myodata software was used for data acquisition. At 30 min after normalisation, the vessels were exposed to 123 mM potassium solution twice followed by 10 μM noradrenaline in the presence of 123 mM potassium solution. The arteries were considered viable if the ERTP produced by 123 mM potassium was greater than 100 mmHg (13.3 kPa). The presence of functional endothelium was checked with 1 μM carbachol after precontracting with 1 μM noradrenaline. All vessels studied produced greater than 60% relaxation. Vessels were allowed to equilibrate for a further 30 min before beginning experimentation.

Functional studies using exogenous noradrenaline

After equilibration, three to four cumulative concentration – response curves (CRCs) to noradrenaline were obtained in each arterial segment (30 min between each CRC). Preliminary experiments showed that repeated CRCs were reproducible and no correction for time-dependent changes was required. The first CRC 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 (10 μM) for 30 min followed by washing for 60 min (each wash every 15 min). RS 79948 (0.1 μM, 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 30 min 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 vessels. 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 that 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). pK_B values were used when one concentration of antagonist was used to obtain the affinity. It was calculated from the Schild (1949) equation:

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

where K_B is the equilibrium dissociation constant.

Electrical field stimulation

For electrical field stimulation (EFS), vessels were placed between platinum electrodes and stimulated every 5 min at 20 V and 0.05 ms pulse width applied for 10 s at frequencies of 5–30 Hz using a Harvard stimulator. Three to four frequency – response curves were obtained in each arterial segment (15 min between each frequency – response curve). After each frequency – response curve the vessels were thoroughly washed with PSS. Preliminary experiments showed that repeated frequency – response curves were reproducible over a period of 2 h and no correction for time – dependent changes was

required. 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. In experiments with cocaine, vessels were incubated with cocaine (3 μM) for the 15-min period before the frequency – response curve. The effects of cocaine were reproducible over five stimulation periods in the absence of antagonist. Antagonist potencies were expressed as mean pIC_{50} or pIC_{30} values (the negative logarithm of the concentration of antagonist producing 50 or 30% inhibition respectively of the prazosin-sensitive component of the response to field stimulation).

Drugs

The following drugs were used: (−)- noradrenaline (arterenol) bitartrate, propranolol hydrochloride, corticosterone acetate, guanethidine mono sulphate, suramin Na salt and prazosin hydrochloride (Sigma, Dorset, U.K.); cocaine HCl (Thornton and Ross, U.K.); (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) UK 14304 (brimonidine) and tetrodotoxin (Tocris, Bristol, U.K.); 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, U.S.A.).

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. All drug dilutions were made using PSS.

Statistics

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

Results

Vasoconstrictor responses to exogenous noradrenaline

Noradrenaline produced concentration-dependent contractile responses that were unaffected by the α_2-adrenoceptor antagonist RS 79948 (0.1 μM) (pEC_{50}s: control, 6.11 ± 0.02 ; RS 79948, 6.05 ± 0.02, $n = 7$, $P > 0.05$). UK 14304 (up to 30 μM) had no contractile effect.

Prazosin (1–100 nM) produced a parallel rightward shift of the noradrenaline CRC (Figure 1a). Maximum responses were unaffected by 1 nM but were significantly reduced by 10 and

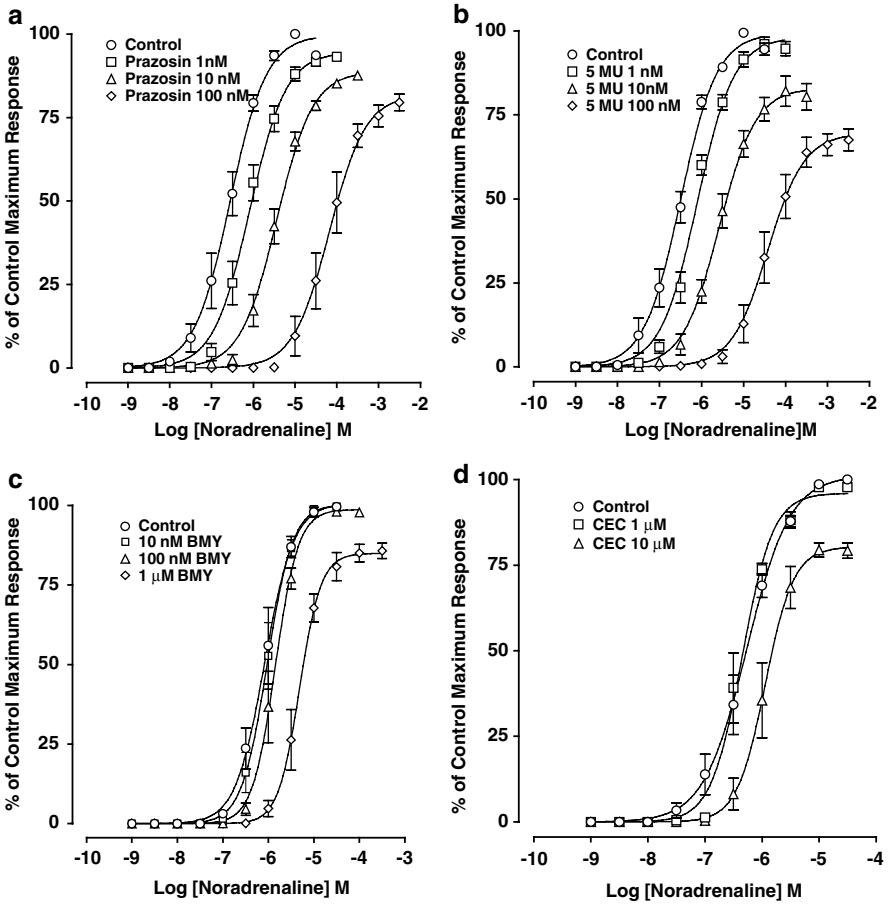

Figure 1 Effects of antagonists on responses of rat femoral resistance arteries to exogenous noradrenaline; (a) prazosin ($n = 9$); (b) 5-methyl-urapidil (5 MU) ($n = 6$); (c) BMY 7378 (BMY) ($n = 5$); (d) chloroethylclonidine (CEC) ($n = 4$).

100 nM prazosin (E_{max} %, $n=9$: control, 100±2; 1 nM prazosin, 95±2, $P>0.05$; 10 nM prazosin, 89±2, $P<0.01$; 100 nM prazosin, 82±3, $P<0.01$). A Schild plot gave a pA_2 value of 9.2 with a slope of 1.09 (95% CL: 0.92–1.26), not significantly different from unity.

5-Methyl-urapidil (1–100 nM) also produced a parallel rightward shift of the noradrenaline CRC (Figure 1b). Maximum responses were unaffected by 1 nM but were significantly reduced by 10 and 100 nM 5-methyl-urapidil (E_{max}, %, $n=6$: control, 99±2; 1 nM 5-methyl-urapidil, 98±1, $P>0.05$); 10 nM 5-methyl-urapidil, 83±2, $P<0.01$; 100 nM 5-methyl-urapidil, 69±2, $P<0.01$). The Schild plot gave a pA_2 value of 9.1 with a slope of 0.94 (95% CL: 0.71–1.18), not significantly different from unity.

BMY 7378 (10 nM–1 μM) had no significant effect on the noradrenaline CRC at concentrations of 10 and 100 nM (Figure 1c). BMY 7378 (1 μM) produced an eight-fold shift of the noradrenaline CRC, giving an estimated pK_B of 6.7. The maximum response to noradrenaline was also significantly reduced (E_{max} %, $n=5$: control, 101±4; 1 μM BMY 7378, 85±2 ($P<0.05$)).

Chloroethylclonidine had no significant effect on the noradrenaline CRC at a concentration of 1 μM (Figure 1d). However, 10 μM chloroethylclonidine shifted the noradrenaline CRC to the right with a reduction in the maximum response (E_{max} %, $n=4$: control 101±3%; 10 μM chloroethylclonidine, 80±3% ($P<0.01$)).

Vasoconstrictor responses to EFS

EFS (5–30 Hz) produced frequency-dependent contractile responses of the femoral resistance arteries. Responses to 30 Hz were approximately 40% of the size of those elicited by 1 μM noradrenaline. Tetrodotoxin (1 μM, Figure 2a) and guanethidine (10 μM, Figure 2b), significantly reduced, but did not completely abolish responses. RS 79488 (0.1 μM) potentiated responses at all frequencies, particularly at 10 Hz (Figure 2c). Prazosin (1 μM) abolished the tetrodotoxin-sensitive contractile responses due to nerve stimulation (Figure 3a). Subsequent addition of 10 μM α, β-methylene ATP produced a small, nonsignificant, additional blockade (Figure 3a). On addition of 10 μM α,β-methylene ATP first, a potentiation of the nerve-induced responses was obtained (Figure 3b). Subsequent addition of prazosin (0.1 μM) reduced the responses to the size of the tetrodotoxin-resistant component (Figure 3b). Suramin (500 μM) also potentiated contractile responses to EFS, although the size of the potentiation was less than that obtained with α,β-methylene ATP (e.g. % potentiation at 30 Hz: α,β-methylene ATP, 40±7, $n=4$; suramin, 20±6, $n=4$, $P<0.05$).

Prazosin (0.1 nM – 1 μM) produced concentration-dependent inhibition of the responses to field stimulation with no significant differences in the sensitivity to prazosin at different frequencies (Figure 4a, Table 1). 5-Methyl-urapidil also produced a concentration-dependent inhibition of responses

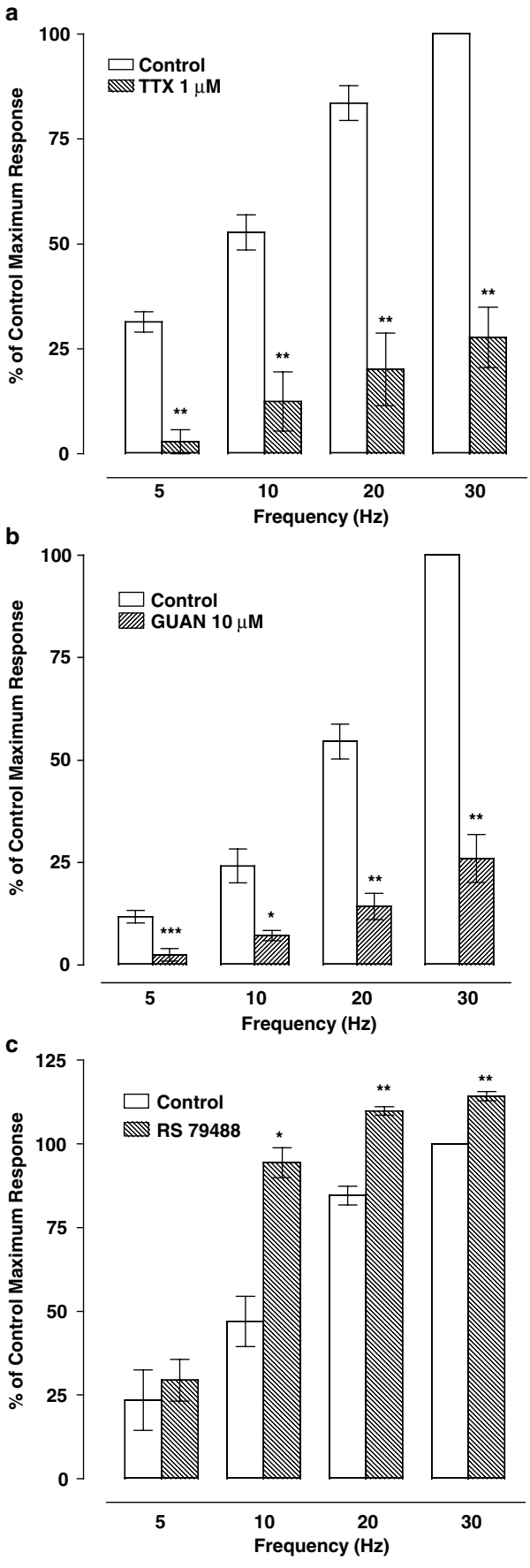

Figure 2 Effects of drugs on the responses of rat femoral resistance arteries to electrical field stimulation at different frequencies for 10 s and 0.05 ms pulse width. (a) Tetrodotoxin (TTX, 1 μM) ($n=5$); (b) guanethidine (GUAN, 10 μM) ($n=4$); (c) RS 79488 (0.1 μM) ($n=4$). Significance of difference from control, $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (paired t-test.)

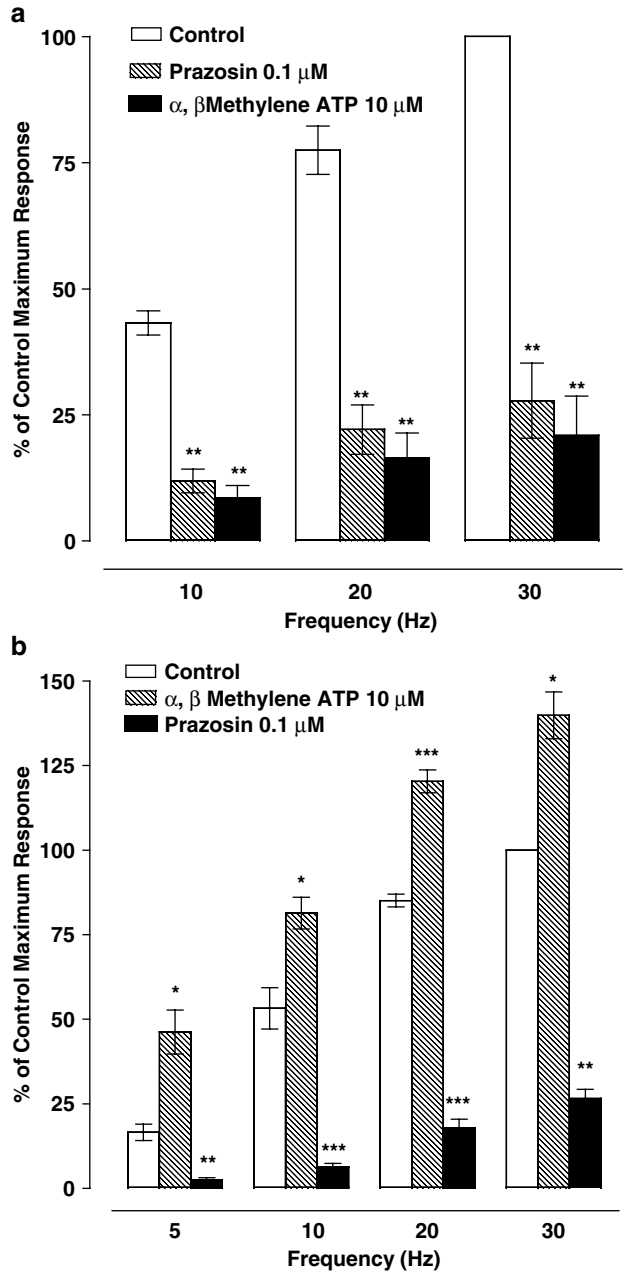

Figure 3 Effects of prazosin and α, β-methylene ATP on the responses of rat femoral resistance arteries to electrical field stimulation at different frequencies for 10 s and 0.05 ms pulse width. (a) Prazosin (0.1 μM) followed by α, β-methylene ATP (10 μM) ($n=5$); (b) α,β-methylene ATP (10 μM) followed by prazosin (0.1 μM) ($n=4$). Significance of difference from control, $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (paired t-test.)

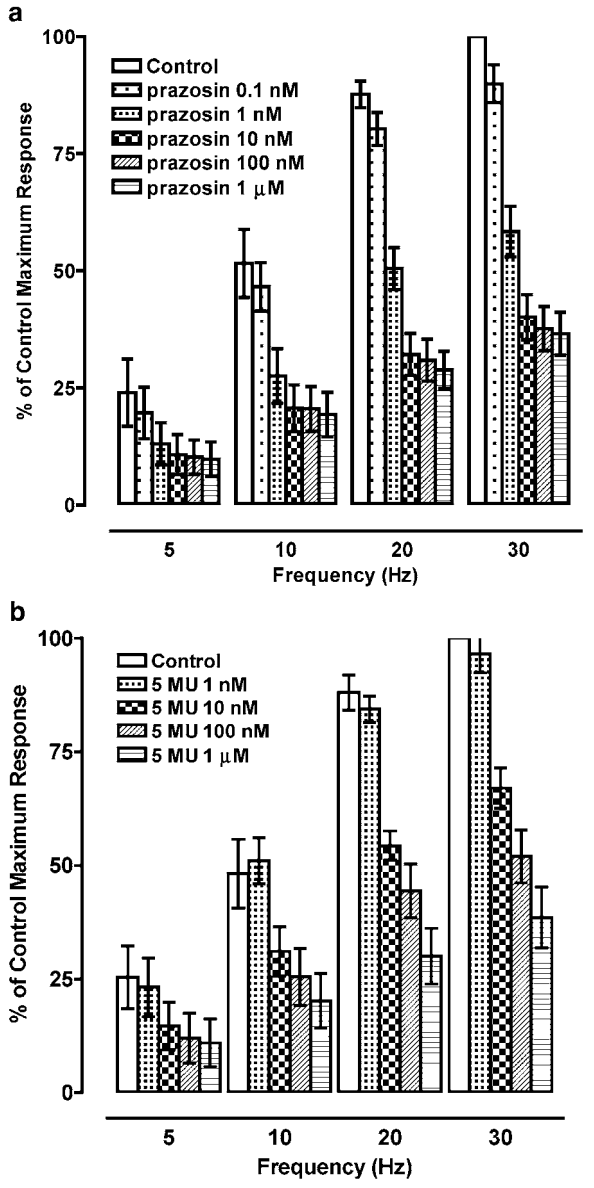

Figure 4 (a) Effect of different concentrations of prazosin on responses of rat femoral resistance arteries to electrical field stimulation at different frequencies for 10 s and 0.05 ms pulse width. Significances of difference from control have been omitted for clarity. All treatment values were significantly different from control ($P<0.001$) except for 0.1 nM prazosin ($P>0.5$) at all frequencies (repeated measures ANOVA with post tests, $n=8$). (b) Effect of different concentrations of 5-methyl-urapidil (5 MU) on responses of rat femoral resistance arteries to electrical field stimulation at different frequencies for 10 s and 0.05 ms pulse width. Significances of difference from control have been omitted for clarity. All treatment values were significantly different from control ($P<0.001$) except for 1 nM 5 MU ($P>0.5$) at all frequencies, (repeated measures ANOVA with post-tests, $n=5$).

to field stimulation, with a potency around ten times less than that of prazosin at all frequencies (Figure 4b, Table 1). BMY 7378 at concentrations of 10 and 100 nM had no effect on responses to field stimulation at 10–30 Hz (Figure 5a). Responses to field stimulation were more sensitive to BMY 7378 at 5 Hz, with significant inhibition by 10 and 100 nM (Figure 5a), giving a higher pIC_{50} than at higher frequencies (Table 1). Chloroethylclonidine (1 and 10 μM) had no significant effect on responses at any frequency (data not shown).

Cocaine (3 μM) increased the size of responses to electrical field stimulation (Figure 5b). The potencies of prazosin and 5-methyl-urapidil were unaffected by cocaine (Table 1). Responses at 10–30 Hz were more sensitive to BMY 7378 in the presence of cocaine, with significant inhibition of responses at 100 nM BMY 7378 (Figure 5b). Although this increase in sensitivity to BMY 7378 in the presence of cocaine was not reflected in a significant difference in pIC_{50} values (Table 1),

Table 1 Inhibition of responses to electrical field stimulation in rat femoral resistance arteries

	n	pIC_{50} values 5 Hz	10 Hz	20 Hz	30 Hz
Absence of cocaine					
Prazosin	8	9.30±0.07	9.26±0.19	9.23±0.13	9.26±0.10
5-MU	5	8.26±0.15	8.22±0.10	8.17±0.17	8.05±0.19
BMY 7378	5	8.06±0.07	6.61±0.05[a]	6.50±0.06[a]	6.50±0.07[a]
Presence of cocaine					
Prazosin	6	9.51±0.07	9.31±0.15	9.32±0.07	8.95±0.10
5-MU	5	8.17±0.15	7.96±0.09	7.74±0.13	7.62±0.14
BMY 7378	7	7.34±0.07[b]	6.85±0.15	6.78±0.12	6.79±0.19

pIC_{50} represents the negative logarithm of the concentration required to produce 50% inhibition of the noradrenergic response.
[a]Significantly different from value at 5 Hz ($P<0.001$), repeated measures ANOVA with post test.
[b]Significantly different from value in absence of cocaine ($P<0.001$), unpaired *t*-test.

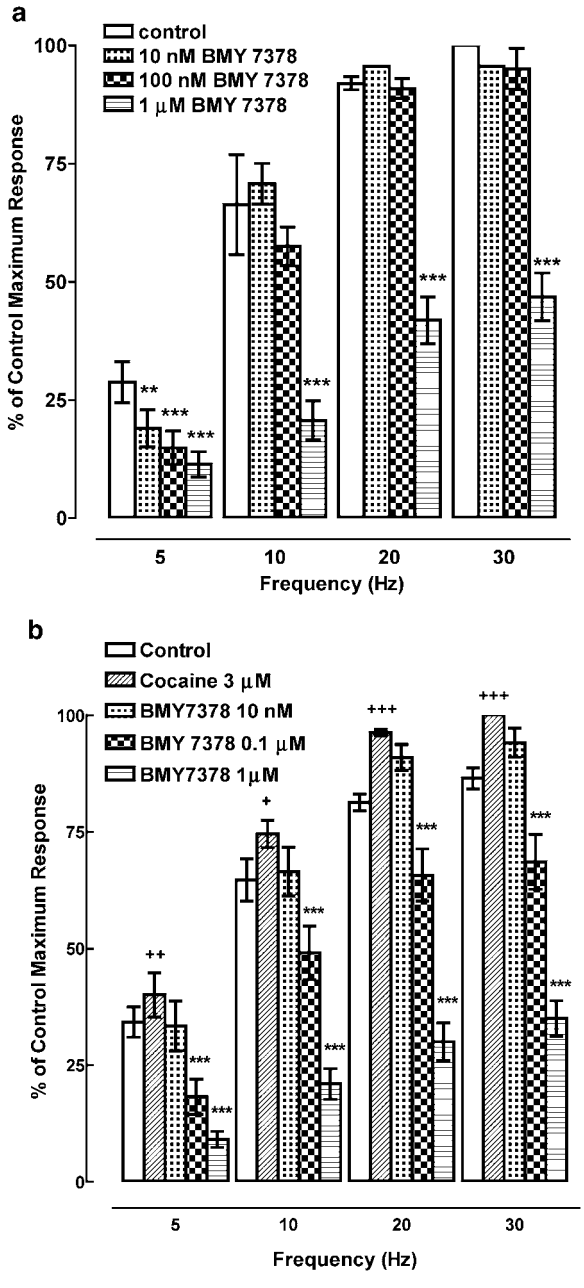

Figure 5 (a) Effect of BMY 7378 on responses of rat femoral resistance arteries to electrical field stimulation at different frequencies for 10 s and 0.05 ms pulse width ($n=5$). Significance of difference from control, $^{**}P<0.01$, $^{***}P<0.001$ (repeated measures ANOVA followed by post-tests. (b) Effect of BMY 7378 on responses of rat femoral resistance arteries to electrical field stimulation at different frequencies for 10 s and 0.05 ms pulse width in the presence of cocaine, 3 μM ($n=7$). Significance of difference from control, $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ (paired *t*-test). Significance of difference from cocaine, $^{***}P<0.001$ (repeated measures ANOVA with post-test).

Table 2 Inhibition of responses to electrical field stimulation in rat femoral resistance arteries by BMY 7378

	n	BMY 7378 pIC_{30} values 10 Hz	20 Hz	30 Hz
Absence of cocaine	5	6.90±0.08	6.63±0.06	6.64±0.09
Presence of cocaine	7	7.47±0.13**	7.40±0.14**	7.50±0.17**

The pIC_{30} represents the negative logarithm of the concentration required to produce 30% inhibition of the noradrenergic response.
**Significantly different from value in absence of cocaine ($P<0.01$), unpaired *t*-test.

pIC_{30} values in the absence and presence of cocaine were significantly different (Table 2). As in the absence of cocaine, the pIC_{50} of BMY 7378 was significantly higher at 5 Hz than at greater frequencies (Table 1). In the presence of cocaine, chloroethylclonidine had no effect on responses (data not shown), similar to the results obtained in the absence of cocaine.

Discussion

Exogenous functional studies

In the present study, responses to exogenous noradrenaline were unaffected by the α_2-adrenoceptor antagonist, RS 79948, indicating a lack of contribution of postjunctional α_2-adrenoceptors to the responses. This was confirmed by the

lack of a contractile response to the α_2-adrenoceptor agonist, UK 14304, in agreement with our previous study (Jarajapu *et al*., 2001b).

Prazosin produced concentration-dependent, parallel rightward shifts in the sensitivity of noradrenaline, consistent with competitive antagonism, although there was a small reduction in the maximum responses at 10 and 100 nM prazosin. An effect of prazosin on maximum responses to noradrenaline has previously been reported in rat aorta (Godfraind & Alosachie, 1988) and in human skeletal muscle resistance arteries (Jarajapu *et al*., 2001a). The Schild slope, close to unity, is also consistent with reversible competitive antagonism. The pK_B value of 9.2 from the Schild plot is consistent with an action of noradrenaline at α_{1H}-adrenoceptor subtypes and rules out the presence of the α_{1L}-subtype (Flavahan & Vanhoutte, 1986; Muramatsu *et al*., 1990a, b).

5-Methyl-urapidil also produced a parallel rightward displacement of the noradrenaline CRC although there was a reduction in maximum responses at the higher concentrations of 5-methyl-urapidil. A Schild slope of 0.94 was observed, consistent with simple competitive antagonism. The pA_2 value of 5-methyl-urapidil of 9.1 is in agreement with the reported affinity at the cloned mammalian α_{1a}-adrenoceptor expressed in rat fibroblasts (Ford *et al*., 1996).

BMY 7378 produced no significant effect on the noradrenaline CRC at 10 and 100 nM, indicating the absence of a contribution from α_{1D}-adrenoceptors. At 1 μM BMY 7378 there was a significant shift in the sensitivity to noradrenaline giving an estimated pK_B value of 6.7, corresponding to the affinity of BMY 7378 for α_{1A}-adrenoceptors (6.6, Goetz *et al*., 1995).

Chloroethylclonidine (1 μM) had no significant effect on maximum responses or sensitivity of noradrenaline indicating a lack of the α_{1B}-adrenoceptor subtype. Chloroethylclonidine (10 μM) did produce a small reduction in the sensitivity and maximum responses of noradrenaline and this may indicate the presence of a small population of α_{1B}- and/or α_{1D}-adrenoceptors. However, chloroethylclonidine has also been shown to alkylate cloned α_{1a}-adrenoceptors of several species, including rat (Büscher *et al*., 1996) and native α_{1A}-adrenoceptors of murine tissues (Yang *et al*., 1998) and therefore its effect may represent alkylation of the predominant α_{1A}-subtype in rat femoral resistance arteries.

Thus, the present study has confirmed our previous functional studies in different branches of the rat femoral artery (Jarajapu *et al*., 2001b): responses to exogenous noradrenaline are predominantly mediated by the α_{1A}-adrenoceptor, with little or no evidence for the α_{1B}- or α_{1D}- subtypes or for α_2-adrenoceptors.

Electrical field stimulation

In the present study, frequencies between 5 and 30 Hz were chosen because these frequencies fall within the physiological range for sympathetic nerve firing activity, based on studies on human skin nerves (Hallin & Torebjörk, 1974). In the presence of tetrodotoxin (1 μM) contractile responses to EFS were greatly reduced but were not completely abolished, suggesting that most of the EFS-induced contraction was due to activation of excitatory nerve fibres. Guanethidine, a sympathetic neurone blocker, inhibited nerve-mediated responses to a similar extent as tetrodotoxin, suggesting that a small amount of direct muscle stimulation may be responsible for the tetrodotoxin-resistant response.

The α_2-adrenoceptor antagonist RS 79448 was used to determine the role of α_2-adrenoceptors in the response to endogenous noradrenaline. RS 79448 potentiated the EFS responses, therefore indicating the presence of inhibitory prejunctional α_2-adrenoceptors. A negative feedback mechanism may play an important role in determining the relative contributions of α_1-adrenoceptors, α_2-adrenoceptors and purinoceptors in vasoconstriction responses (MacDonald *et al*., 1992). The experiments do not, however, rule out the activation of postjunctional α_2-adrenoceptors by neurally released noradrenaline, as effects of postjunctional α_2-adrenoceptors may be masked by the prejunctional effects. However, the experiments with RS 79488 and exogenous noradrenaline and the lack of effect of UK 14304 in this and our previous study (Jarajapu *et al*., 2001b) suggest a lack of postjunctional α_2-adrenoceptors in this preparation.

Characterisation of the responses to field stimulation using prazosin (1 μM) followed by the P_{2x}-purinoceptor-desensitising agent, α,β-methylene ATP (10 μM) (Kasakov & Burnstock, 1983), provided a clear picture that the tetrodotoxin-sensitive responses were due to α_1-adrenoceptor activation and that P_{2X}-purinoceptors did not contribute significantly to the response. When the vessels were incubated with α,β-methylene ATP (10 μM) first, responses to EFS were potentiated. As α,β-methylene ATP first activates and later desensitises P_{2X}-purinoceptors (Kasakov & Burnstock, 1983) the mechanism of the potentiating effect has been attributed to residual depolarisation caused by α,β-methylene ATP stimulation of postjunctional P_{2X}-purinoceptors (Neild & Kotecha, 1986). Alternatively, α,β-methylene ATP may be blocking prejunctional inhibitory purinoceptors that inhibit the release of noradrenaline, as shown in rat mesenteric arteries (Shinozuka *et al*., 2001). The fact that suramin, a competitive P_{2X}-purinoceptor antagonist (Leff *et al*., 1990), also produced potentiation suggests that potentiation involves blockade of prejunctional P_{2x}-purinoceptors. Suramin produced less potentiation than with α,β-methylene ATP however and this may also suggest that part of the potentiating effect of α,β-methylene ATP is postjunctional.

Prazosin produced concentration-dependent inhibition of nerve-mediated responses to EFS with a pIC_{50} of around 9.3 at all the frequencies recorded. The pIC_{50} is not a direct measure of affinity for the α_1-adrenoceptors activated by EFS, since equilibrium conditions do not apply, but would be expected to be related to it. In fact, the pIC_{50} values obtained ($\sim$9.3) are almost identical to the pK_B values (9.2) of prazosin obtained in these arteries using exogenous noradrenaline and are consistent with the activation of high-affinity α_{1H}-adrenoceptors (Flavahan & Vanhoutte, 1986). Inhibition of neurogenic contractions by low concentrations of prazosin is in agreement with studies in other nerve-stimulated vascular preparations for example rat tail artery (Papanicolaou & Medgett, 1986), isolated perfused rat mesentery (Williams & Clarke, 1995), rat hepatic mesentery (Phillips *et al*., 1998).

5-Methyl-urapidil also produced concentration-dependent inhibition of nerve-mediated responses to EFS. A pIC_{50} of around 8.2 was recorded at all frequencies. In contrast to prazosin where the pIC_{50} (9.3) and pK_B (9.2) values were identical, the pIC_{50} for 5 MU (8.2) against EFS was around 10 fold smaller that the pK_B value (9.1). Thus the prazosin/

5-methyl-urapidil potency ratio was approximately 1.0 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. Both ratios, however, are consistent with the α_{1A}-adrenoceptor being the predominant subtype.

Responses to EFS were not affected by BMY 7378 (10 and 100 nM) at 10–30 Hz showing a lack of contribution of the α_{1D}-adrenoceptor subtype at these frequencies. A higher concentration of BMY 7378 (1 μM) did inhibit responses to EFS at 10–30 Hz, giving pIC_{50} values of 6.5–6.7, consistent with the affinity of BMY 7378 at the α_{1A}- subtype. At 5 Hz, low concentrations of BMY 7378 (10 and 100 nM) produced concentration-dependent inhibition of responses with a pIC_{50} of 8.02, suggesting a significant contribution of α_{1D}-adrenoceptors to the response.

Chloroethylclonidine had no significant effect on nerve-mediated contractions making the contribution of an α_{1B}-adrenoceptor subtype unlikely.

Cocaine increased the size of the responses to EFS, as would be expected from inhibition of neuronal uptake of noradrenaline. The predominant subtype involved in responses to EFS in the presence of cocaine was the α_{1A}-adrenoceptor as found in the absence of cocaine. As in the absence of cocaine, the pIC_{50} of BMY 7378 was higher at the lowest frequency of 5 Hz, suggesting a contribution of α_{1D}-adrenoceptors to the response at this frequency. There was also a significant difference in the sensitivity to BMY 7378 at higher frequencies, suggesting that a further small α_{1D}-adrenoceptor component may be uncovered in the presence of cocaine. This suggests that there are extrajunctional α_{1D}-adrenoceptors which can be activated by neurally released noradrenaline in the presence of cocaine, but surprisingly are not activated by exogenous noradrenaline. The physiological significance of this is not clear and further studies are required to elucidate the role of α_{1D}-adrenoceptors in vasoconstriction.

This study has shown that, in rat femoral resistance arteries, responses to exogenous noradrenaline and to endogenous noradrenaline, released by nerve stimulation, are mediated predominantly by α_{1A}-adrenoceptors. The predominance of the α_{1A}-adrenoceptor in responses to an exogenous α-adrenoceptor agonist is in agreement with several other studies in rat small arteries for example mesenteric (Kong *et al*., 1994; Williams & Clarke, 1995; Chen *et al*., 1996; Ipsen *et al*., 1997), renal (Blue *et al*., 1995), caudal (Lanchit *et al*., 1997), femoral (Jarajapu *et al*., 2001b) and hind limb (Zhu *et al*., 1997) arteries. In a few studies in rat mesenteric resistance arteries, evidence for an α_{1L}-subtype was presented (Chen *et al*., 1996; Van der Graaf *et al*., 1996; Stam *et al*., 1999). Since it has been proposed that the α_{1L}-adrenoceptor subtype represents a low-affinity state of the α_{1A}-adrenoceptor (Ford *et al*., 1997), then it is possible that different experimental conditions may account for this discrepancy. α_{1A}-Adrenoceptors have also been shown to be predominant in human skeletal muscle (Jarajapu *et al*., 2001a) and subcutaneous (Jarajapu *et al*., 2001c) resistance arteries. Canine subcutaneous resistance arteries also contain an α_{1A}-/α_{1L}- adrenoceptor (Argyle & McGrath, 2000).

There are a few exceptions to the general finding of α_{1A}- or α_{1L}-adrenoceptors mediating contraction to exogenous α_1-adrenoceptor agonists in resistance arteries. Leech & Faber (1996) found an 'α_{1D}-like' adrenoceptor in rat cremaster muscle arterioles, although the low affinity of BMY 7378 (pK_B 6.86) casts doubt on this conclusion. One study has suggested that the receptor mediating contraction to phenylephrine in rat mesenteric resistance vessels is the α_{1B}-adrenoceptor (Piascik *et al*., 1997). This conclusion was based on the indirect evidence of a lack of receptor protection by BMY 7378 (α_{1D}-adrenoceptor ligand) and A-61603 (α_{1A}-adrenoceptor ligand) from inactivation by alkylating agents, phenoxybenzamine and chloroethylclonidine. An α_1-adrenoceptor 'resembling the α_{1B}' has also been reported in rabbit cutaneous resistance arteries (Smith *et al*., 1997).

The involvement of the α_{1A}-adrenoceptor in responses of resistance arteries to neurally released noradrenaline is also in agreement with other studies in resistance arteries of the rat (Kong *et al*., 1994; Williams & Clarke, 1995; Phillips *et al*., 1998). However, the present study also shows a significant contribution from α_{1D}-adrenoceptors to neurally released noradrenaline. A contribution from α_{1D}-adrenoceptors (and α_{1B}-) in responses to nerve stimulation but not to exogenous noradrenaline was also seen in canine splenic arteries (Yang & Chiba, 2001). The significance of this is not clear: the fact that the α_{1D}-adrenoceptor contribution is most apparent at a low frequency of stimulation in the presence of a normal uptake mechanism and at higher frequencies with uptake inhibited and is not seen in responses to exogenous noradrenaline suggests that the explanation is not simply location of the receptors in relation to the neurovascular junction as suggested by Yang & Chiba (2001) and that other factors such as the time of exposure and peak concentration of noradrenaline may also be important. *In vivo* studies in the rat have 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), suggesting that the current observations may have relevance to the physiological response *in vivo*. Studies in α_{1D}- knockout mice have also directly shown that the α_{1D}-adrenoceptor participates in the regulation of systemic blood pressure in this species (Tanoue *et al*., 2002).

In conclusion, the present study has shown a dominant role of the α_{1A}- adrenoreceptor in contractions due to exogenous noradrenaline and to neurally released noradrenaline in rat femoral resistance arteries. α_{1D}-Adrenoceptors do not appear to be involved in responses to exogenous noradrenaline but are activated by neurally released noradrenaline at a low frequency of stimulation and at higher frequencies of stimulation in the presence of uptake blockade.

References

ARGYLE, S.A. & McGRATH, J.C. (2000). An α_{1A}/α_{1L}-adrenoceptor mediates contraction of canine subcutaneous resistance arteries. *J. Pharmacol. Exp. Ther.*, **295,** 627–633.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.*, **14,** 48–58.

BLUE JR, D.R., BONHAUS, D.W., FORD, A.P.D.W., PFISTER, J.R., SHARIF, N.A., SHIEH, I.A., VIMONT, R.L., WILLIAMS, T.J. & CLARKE, D.E. (1995). Functional evidence equating the pharmacologically-defined α_{1A}- and cloned α_{1C}-adrenoceptor: studies in the isolated perfused kidney of rat. *Br. J. Pharmacol.,* **115,** 283–294.

BÜSCHER, R., HEEKS, C., TAGUCHI, K. & MICHEL, M.C. (1996). Comparison of guinea-pig, bovine and rat α_1-adrenoceptor subtypes. *Br. J. Pharmacol.,* **117,** 703–711.

BYLUND, D.B., BOND, R.A., CLARKE, D.E., EIKENBURG, J.P., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO, R.R., STROSBERG, A.D & TRENDELENBURG, U.G. (1998). Adrenoceptors, IUPHAR Receptor Compendium: London.

CASTILLO, E.F., LÓPEZ, R.M., RODRÍGUEZ-SILVERIO, J., BOBADILLA, R.A & CASTILLO, O. (1998). α_{1D}-Adrenoceptors contribute to the neurogenic vasopressor response in pithed rats. *Fund. Clin. Pharmacol.,* **12,** 584–589.

CHEN, H., FETSCHER, C., SCHÄFERS, R.F., WAMBACH, G., PHILIPP, T. & MICHEL, M.C. (1996). Effects of noradrenaline and neuropeptide Y on rat mesenteric microvessel contraction. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **353,** 314–323.

FLAVAHAN, N.A. & VANHOUTTE, P.M. (1986). α_1-Adrenoceptor subclassification in vascular smooth muscle. *Trends Pharmacol. Sci.,* **7,** 347–349.

FORD, A.P.D.W., ARREDONDO, N.F., BLUE JR, D.R., BONHAUS, D.W., JASPER, J., KAVA, M.S., LESNICK, J., PFISTER, J.R., SHIEH, I.A., VIMONT, R.L., WILLIAMS, T.J., MCNEAL, J.E., STAMEY, T.A. & CLARKE, D.E. (1996). RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride), a selective α_{1A}-adrenoceptor antagonist, displays low affinity for functional α_1-adrenoceptors in human prostate: implications for adrenoceptor classification. *Mol. Pharmacol.,* **49,** 209–215.

FORD, A.P.D.W., DANIELS, D.V., CHANG, D.J., GEVER, J.R., JASPER, J.R., LESNICK, J.D. & CLARKE, D.E. (1997). Pharmacological pleiotropism of the human recombinant α_{1A}-adrenoceptor: implications for α_{1A}-adrenoceptor classification. *Br. J. Pharmacol.,* **121,** 1127–1135.

GODFRAIND, T. & ALOSACHIE, I. (1988). Influence of endothelium and cyclic GMP on alpha-adrenoceptors. In: *Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves and Endothelium,* ed. Vanhoutte, P.M. pp. 437–442. New York: Raven Press Ltd.

GOETZ, A.S., KING, H.K., WARD, S.D.C., TRUE, T.A., RIMELE, T.J. & SAUSSY JR, D.L. (1995). BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors. *Eur. J. Pharmacol.,* **272,** R5–R6.

GUIMARÃES, S. & MOURA, D. (2001). Vascular adrenoceptors: an update. *Pharmacol. Rev.,* **53,** 319–356.

HALLIN, R.G. & TOREBJÖRK, E.H. (1974). Single unit sympathetic activity in human skin nerves during rest and various manoeuvres. *Acta Physiol. Scand.,* **92,** 303–317.

HIEBLE, J.P., BYLUND, D.B., CLARKE, D.E., EIKENBURG, D.C., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P. & RUFFOLO, R.R. (1995). International Union of Pharmacology. X. Recommendations for nomenclature of α_1-adrenoceptors: consensus updated. *Pharmacol. Rev.,* **47,** 267–270.

HONNER, V. & DOCHERTY, J.R. (1999). Investigation of the subtype of α_1-adrenoceptor mediating contractions of rat vas deferens. *Br. J. Pharmacol.,* **128,** 1323–1331.

IPSEN, M., ZHANG, Y., DRAGSTED, N., HAN, C. & MULVANY, M.J. (1997). The antipsychotic drug sertindole is a specific inhibitor of α_{1A}-adrenoceptors in rat mesenteric small arteries. *Eur. J. Pharmacol.,* **336,** 29–35.

JARAJAPU, Y.P.R., COATS, P., McGRATH, J.C., HILLIER, C. & MACDONALD, A. (2001a). Functional characterization of α_1-adrenoceptor subtypes in human skeletal muscle resistance arteries. *Br. J. Pharmacol.,* **133,** 679–686.

JARAJAPU, Y.P.R., HILLIER, C. & MACDONALD, A. (2001b). The α_{1A}-adrenoceptor subtype mediates contraction in rat femoral resistance arteries. *Eur. J. Pharmacol.,* **422,** 127–135.

JARAJAPU, Y.P.R., JOHNSTON, F., BERRY, C., RENWICK, A., McGRATH, J.C., MACDONALD, A. & HILLIER, C. (2001c). Functional characterisation of α_1-adrenoceptor subtypes in human subcutaneous resistance arteries. *J. Pharmacol. Exp. Ther.,* **299,** 729–734.

KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analog, α,β-methylene ATP, to produce desensitisation of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.,* **86,** 291–294.

KONG, J., TAYLOR, D.A. & FLEMING, W.W. (1994). Functional distribution and role of *alpha*-1 adrenoceptors in the mesenteric vasculature of the rat. *J. Pharmacol. Exp. Ther.,* **268,** 1153–1159.

LANCHIT, W.G., TRAN, A.M., CLARKE, D.E. & FORD, A.P.D.W. (1997). Pharmacological characterization of an α_{1A}-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat. *Br. J. Pharmacol.,* **120,** 819–826.

LEECH, C.J. & FABER, J.E. (1996). Different α-adrenoceptor subtypes mediate constriction of arterioles and venules. *Am. J. Physiol.,* **270,** H710–H722.

LEFF, P., WOOD, B.E. & O'CONNOR, S.E. (1990). Suramin is a slowly-equilibrating but competitive antagonist at P_{2x}-receptors in the rabbit isolated ear artery. *Br. J. Pharmacol.,* **101,** 645–649.

MACDONALD, A., DALY, C.J., BULLOCH, J.M. & McGRATH, J.C. (1992). Contributions of α_1-adrenoceptors, α_2-adrenoceptors and P_{2X}-purinoceptors to neurotransmission in several rabbit isolated blood vessels: role of neuronal uptake and autofeedback. *Br. J. Pharmacol.,* **105,** 347–354.

MULVANY, M.J. & HALPERN, W. (1977). Contractile responses of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.,* **41,** 19–26.

MURAMATSU, I., KIGOSHI, S. & OSHITA, M. (1990a). Two distinct α_1-adrenoceptor subtypes involved in noradrenaline contraction of rabbit thoracic aorta. *Br. J. Pharmacol.,* **101,** 662–666.

MURAMATSU, I., OHMURA, T., KIGOSHI, S., HASHIMOTO, S. & OSHITA, M. (1990b). Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. *Br. J. Pharmacol.,* **99,** 197–201.

NEILD, T.O. & KOTECHA, N. (1986). Effects of alpha beta methylene ATP on membrane potential, neuromuscular transmission and smooth muscle contraction in the rat tail artery. *Gen. Pharmacol.,* **17,** 461–464.

PAPANICOLAOU, M. & MEDGETT, I.C. (1986). Effects of blockade of α_1- and α_2-adrenoceptors on vasoconstrictor responses to single and twin pulse stimulation in rat tail artery. *Eur. J. Pharmacol.,* **131,** 211–218.

PHILLIPS, J.K., MCLEAN, A.J. & HILL, C.E. (1998). Receptors involved in nerve-mediated vasoconstriction in small arteries of the rat hepatic mesentery. *Br. J. Pharmacol.,* **124,** 1403–1412.

PIASCIK, M.T., HROMETZ, S.L., EDELMANN, S.E., GUARINO, R.D., HADLEY, R.W. & BROWN, R.D. (1997). Immunocytochemical localization of the *alpha*-1B adrenergic receptor and the contribution of this and the other subtypes to vascular smooth muscle contraction: analysis with selective ligands and antisense oligonucleotides. *J. Pharmacol. Exp. Ther.,* **283,** 854–868.

SCHILD, H.O. (1949). pA_x and competitive drug antagonism. *Br. J. Pharmacol.,* **4,** 277–280.

SHINOZUKA, K., TANIOKA, Y., KWON, Y.M., TANAKA, N., KUBOTA, Y., NAKAMURA, K. & KUNITOMO, M. (2001). Characterization of prejunctional purinoceptors inhibiting noradrenaline release in rat mesenteric arteries. *Jpn. J. Pharmacol.,* **85,** 41–46.

SMITH, K.M., MACMILLAN, J.B. & McGRATH, J.C. (1997). Investigation of α_1-adrenoceptor subtypes mediating vasoconstriction in rabbit cutaneous resistance arteries. *Br. J. Pharmacol.,* **122,** 825–832.

STAM, W.B., VAN DER GRAAF, P.H. & SAXENA, P.R. (1999). Analysis of α_{1L}-adrenoceptor pharmacology in rat small mesenteric artery. *Br. J. Pharmacol.,* **127,** 661–670.

TANOUE, A., NASA, Y., KOSHIMIZU, H., OSHIKAWA, S., KAWAI, T., SUNADA, S., TAKEO, S. & TSUJIMOTO, G. (2002). The α_{1D}-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. *J. Clin. Invest.,* **109,** 765–775.

VAN DER GRAAF, P.H., SHANKLEY, N.P. & BLACK, J.W. (1996). Analysis of the effect of α_1-adrenoceptor antagonists on noradrenaline-mediated contraction of rat mesenteric artery. *Br. J. Pharmacol.,* **118,** 1308–1316.

VARGAS, H.M. & GORMAN, A.J. (1995). Vascular alpha-1 adrenergic receptor subtypes in the regulation of arterial pressure. *Life Sci.,* **57,** 2291–2308.

VILLALOBOS-MOLINA, R., LÓPEZ-GUERRERO, J.J. & IBARRA, M. (1999). Functional evidence of α_{1D}-adrenoceptors in vasculature of young and adult spontaneously hypertensive rats. *Br. J. Pharmacol.,* **126,** 1534–1536.

WILLIAMS, T.J. & CLARKE, D.E. (1995). Characterization of α_1-adrenoceptors mediating vasoconstriction to noradrenaline and nerve stimulation in the isolated perfused mesentery of rat. *Br. J. Pharmacol.,* **114,** 531–536.

YANG, M., REESE, J., COTECCHIA, S. & MICHEL, M.C. (1998). Murine *alpha*$_1$-adrenoceptor subtypes: 1. Radioligand binding studies. *J. Pharmacol. Exp. Ther.,* **286,** 841–847.

YANG, X.P. & CHIBA, S. (2001). Existence of different α_1-adrenoceptor subtypes in junctional and extrajunctional neurovascular regions in canine splenic arteries. *Br. J. Pharmacol.,* **132,** 1852–1858.

ZACHARIA, J., HILLIER, C. & MACDONALD, A. (2002). Neurally evoked responses of rat femoral small arteries are predominantly mediated by α_{1A}-adrenoceptors. *Br. J. Pharmacol.,* **137,** 90P.

ZACHARIA, J., HILLIER, C. & MACDONALD, A. (2003). Effect of cocaine on the α_1-adrenoceptor subtypes involved in neurally-evoked contractions of rat femoral resistance arteries. *Br. J. Pharmacol.,* **138,** 158P.

ZHOU, L. & VARGAS, H.M. (1996). Vascular α_{1D}-adrenoceptors have a role in the pressor response to phenylephirine in the pithed rat. *Eur. J. Pharmacol.,* **305,** 173–176.

ZHU, W., ZHANG, Y. & HAN, C. (1997). Characterization of subtype of α_1-adrenoceptor mediating vasoconstriction in perfused rat hind limb. *Eur. J. Pharmacol.,* **329,** 55–61.

(Received October 3, 2003
Revised December 10, 2003
Accepted January 8, 2004)