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SPECIAL REPORT

Mechanisms of noradrenaline-induced vasorelaxation in isolated femoral arteries of the neonatal rat

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Isolated arteries from the femoral circulation of Wistar rats mounted on a small vessel myograph demonstrated age related tension development to noradrenaline (NA, $1\times10^{-8}-5\times10^{-5}$ M) day 20 greater than day 10 ($P<0.005$); day 100 greater than day 20 ($P<0.001$) and depolarizing potassium (125 mM) buffer day 20 greater than day 10 ($P<0.001$). NA evoked dilatation in femoral arteries from neonatal rats (10 days) when added to unstimulated vessels or to those preconstricted with the thromboxane mimetic, U46619. Relaxation to NA was inhibited by L-NAME (0.1 mM) ($P<0.001$), endothelial removal ($P<0.001$) and the α_2-adrenoceptor antagonist, yohimbine (0.1 μM) ($P<0.001$). α_1- or β-adrenoceptor antagonism was without effect. Relaxation was evoked in femoral arteries of the 10-day-old rats by the α_2-adrenoceptor agonist UK14304 ($1\times10^{-8}-5\times10^{-5}$ M). This relaxation was also abolished by L-NAME (0.1 mM) ($P<0.001$) or endothelial removal ($P<0.001$). α_2-adrenoceptor-mediated vasorelaxation was the predominant response to NA stimulation in femoral arteries of the neonatal rat. These responses were endothelium-dependent and were NO-mediated.

Keywords: Noradrenaline; vasorelaxation; rat; femoral artery; endothelium; nitric oxide; adrenoceptors

Abbreviations: ICI118551, 1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol; KPSS, equimolar substitution of NaCl with KCl in physiological salt solution; L-NAME, N^{ω}-nitro L-arginine methyl ester; NA, noradrenaline; NO, nitric oxide; pEC_{50}, the $-$log of molar concentration producing 50% of maximum responses; PSS, physiological salt solution; U46619, 9,11-Dideoxy-11_{α},9_{α}-epoxy-methanoprostaglandin $F_{2\alpha}$; UK14304, 5-Bromo-N-[2-imidazolin-2-yl]-6-quinoxalinamine

Introduction Catecholamines are known to play a role in foetal and neonatal cardiovascular control and in cardiovascular adaptations at birth (Slotkin & Seidler, 1988; Agata *et al*., 1995). Most previous studies examining responses of the foetus or neonate to catecholamines have been *in vivo* investigations of systemic blood pressure or of regional and organ blood flow. However, effects of catecholamines on isolated blood vessels of the foetus or neonate have not been investigated in depth. Our preliminary studies (Ozaki *et al*., 1998) revealed the surprising observation that arteries from the skeletal muscle circulation of the neonatal rat dilate rather than constrict to noradrenaline, whilst showing tension development to potassium and other agonists. In view of the novelty of this observation, we have now investigated in detail the mechanisms of noradrenaline-induced vasorelaxation in the neonatal rat skeletal muscle circulation.

Methods *Preparation of vessels* 10, 20, 100 and 200-day-old Wistar rats were killed by an overdose of pentobarbitone (200 mg kg^{-1}, i.p.). Femoral arteries (10 and 20-day-old rats) and second order branches of the femoral artery (100 and 200-day-old rats) were dissected and mounted on a small vessel wire myograph as a ring preparation (Mulvany & Halpern, 1977). The arteries were bathed in physiological salt solution (PSS: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026 and glucose 5.5 mM), pH 7.4 at 37°C and gassed with 5% carbon dioxide in air. The passive tension-internal circumference characteristics of the arteries were determined by stretching to achieve an internal circumference equivalent to a transmural pressure of 50 mmHg (10-day-old rats) or 60 mmHg (20-day-old rats), pressures at which the arteries had been shown, in preliminary experiments, to produce maximal contraction to depolarizing potassium solution (125 mM KPSS, equimolar substitution of NaCl with KCl in PSS). Arteries from 100 and 200-day-old rats were stretched to 90% of the circumference, which would be attained when relaxed *in situ* under a transmural pressure of 100 mmHg (Mulvany & Halpern, 1977). To confirm viability of the arteries, the vessels were subjected to a standard run-up procedure involving contractions to 10 mM phenylephrine, KPSS and 10 mM phenylephrine in KPSS. Arteries which produced tension equivalent to less than 100 mmHg pressure in response to KPSS were rejected from the study.

Protocol Cumulative concentration responses to noradrenaline (NA; $1\times10^{-8}-5\times10^{-5}$ M) were first examined in 10, 20, 100 and 200-day-old rats. Then, in 10-day-old rat pups, the following protocol was conducted. A cumulative concentration response to the thromboxane A_2 mimetic U46619 (9,11-Dideoxy-11_{α},9_{α}-epoxy-methanoprostaglandin $F_{2\alpha}$) ($1\times10^{-10}-5\times10^{-5}$ M) was carried out. Arteries were then preconstricted with a submaximal concentration of U46619 (1 μM) and a cumulative response to NA ($1\times10^{-8}-5\times10^{-5}$ M) performed. To determine the role of nitric oxide (NO) synthase and the endothelium in dilator responses observed to NA, this protocol was repeated after preincubation for 20 min and in the continued presence of the NO synthase inhibitor L-NAME (N^{ω}-nitro L-arginine methyl ester, 0.1 mM), and again after removal of the endothelium. Endothelial removal was achieved by passing a human hair through the lumen of the mounted vessel several times. Successful endothelial removal was verified by lack of the dilator response to acetylcholine (1 μM). To determine the

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adrenoceptor involved in the NA-induced relaxation, responses to NA were again examined after preincubation for 20 min and in the presence of the α_1-adrenoceptor antagonist prazosin (0.1 μM), the α_2-adrenoceptor antagonist yohimbine (0.1 μM), the β_1-adrenoceptor antagonist atenolol (5 μM) or the β_2-adrenoceptor antagonist ICI118551 (1-[2,3-(Dihydro-7-methyl - 1 H-inden-4-yl) oxy] - 3- [(1-methylethyl) amino] -2-butanol, 0.1 μM). The order of addition of these antagonists was randomized. Responses to α_1, α_2 and β agonists were also determined by performing cumulative concentration responses to (a) the α_1-adrenoceptor agonist phenylephrine ($1\times10^{-8}-5\times10^{-5}$ M), and (b) to the α_2-adrenoceptor agonist UK14304 (5-Bromo-N-[2-imidazolin-2-yl]-6-quinoxalinamine) ($1\times10^{-8}-5\times10^{-5}$ M) or the mixed β-adrenoceptor agonist isoprenaline ($1\times10^{-9}-5\times10^{-5}$ M) in preconstricted arteries. To determine any role of NO and of the endothelium in α_2-adrenoceptor agonist responses, responses to UK14304 ($1\times10^{-8}-5\times10^{-5}$ M) were examined in preconstricted arteries before and after treatment with L-NAME (0.1 mM) or endothelial removal, using the protocol described above.

Chemicals The chemicals used in this study were noradrenaline tartrate (Winthrop, Guildford, U.K.); U46619, L-NAME hydrochloride, acetylcholine chloride, prazosin hydrochloride, yohimbine hydrochloride, atenolol, ICI118551 hydrochloride, UK14304, L-phenylephrine hydrochloride and (−)-isoproterenol hydrochloride (Sigma, Poole, U.K.). All drugs were dissolved in distilled water.

Statistical analysis Values are given as mean ± s.e.mean with the exception of pEC_{50} values, which are expressed as geometric means with 95% confidence limits. Tension is expressed as mN mm^{-1} artery length, as percentage maximum response to KPSS for relaxation, or as a percentage of initial pre-constriction. The pEC_{50} was calculated as the −log of molar concentration producing 50% of maximum responses. Maximum tension and maximum relaxation values were calculated by least squares nonlinear regression analysis (GraphPad Prism 2.0, GraphPad Software Inc., U.S.A.). Differences between means were assessed by Student's *t*-test. Concentration-response curves were compared by two-way repeated-measures analysis of variance (ANOVA) (StatView J-

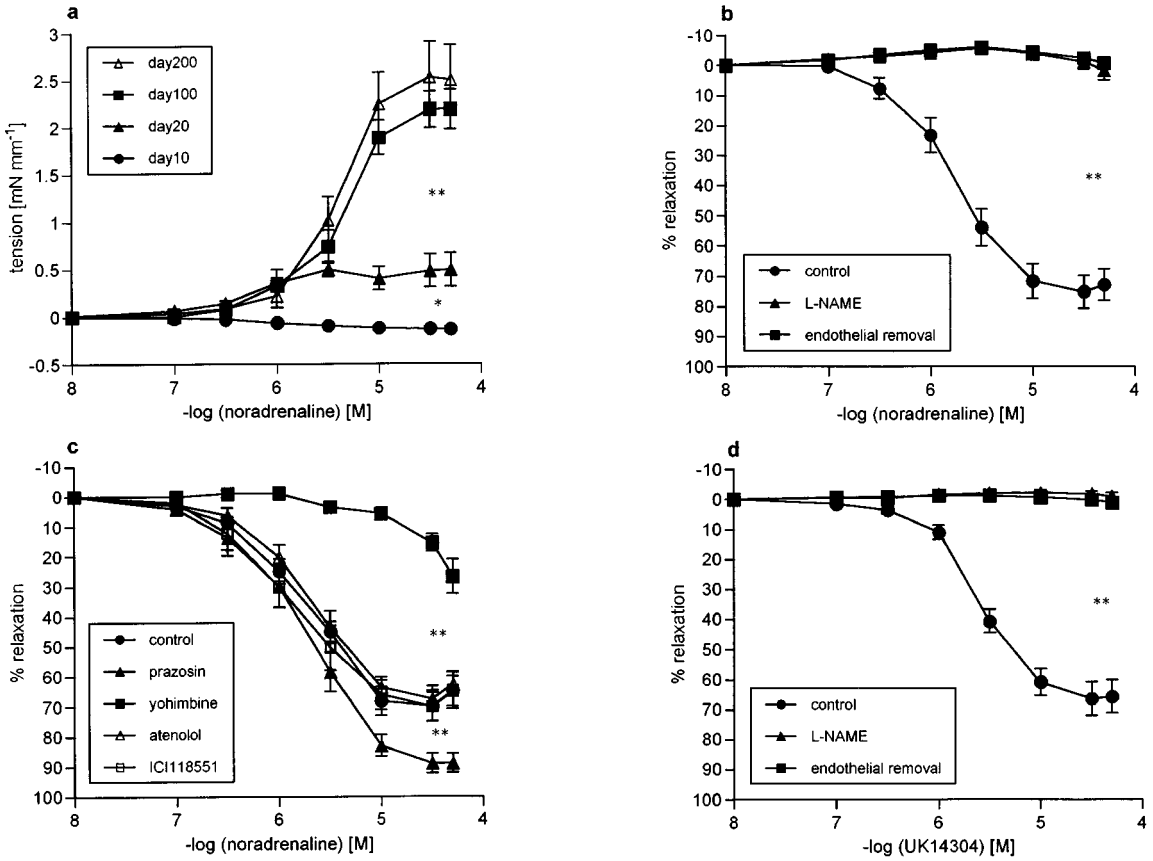

Figure 1 (a) Tension development to noradrenaline (NA) in femoral arteries (day 10 and 20) and branches of the femoral artery (day 100 and 200). Values are given as mean ± s.e.mean. $*P<0.005$ for maximum tension, day 10 *vs* day 20; $**P<0.001$ for maximum tension, day 20 *vs* day 100. (b) The effect of N^{ω}-nitro L-arginine methyl ester (L-NAME, 0.1 mM) and endothelial removal on NA-induced vasorelaxation in isolated femoral arteries of 10-day-old rats. Data are expressed as percentage relaxation of preconstricted tone induced by 9,11-Dideoxy-$11_{\alpha},9_{\alpha}$-epoxy-methanoprostaglandin $F_{2\alpha}$ (U46619, 1 μM). Values are given as mean ± s.e.mean. $**P<0.001$ by ANOVA; control *vs* L-NAME and control *vs* endothelial removal. (c) The effect of adrenoceptor antagonists on NA-induced vasorelaxation in isolated femoral arteries of 10-day-old rats. Prazosin: 0.1 μM; Yohimbine: 0.1 μM; Atenolol: 5 μM; ICI118551 (1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol); 0.1 μM. Data are expressed as percentage relaxation of pre-constricted tone induced by U46619 (1 μM). Values are given as mean ± s.e.mean. $**P<0.001$; control *vs* yohimbine (by ANOVA) and control *vs* prazosin (maximum relaxation, by *t*-test). (d) Vasorelaxation to 5-Bromo-N-[2-imidazolin-2-yl]-6-quinoxalinamine (UK14304) in isolated femoral arteries of 10-day-old rats and the effect of L-NAME (0.1 mM) and endothelial removal on UK14304-induced vasorelaxation. Data are expressed as percentage relaxation of preconstricted tone induced by U46619 (1 μM). Values are given as mean ± s.e.mean. $**P<0.001$ by ANOVA; control *vs* L-NAME and control *vs* endothelial removal.

4.5, Abacus Concepts Inc., U.S.A.). Significance was assumed if $P<0.05$.

Results Normalized internal diameters of femoral vessels were not significantly different between the age groups (day 10: 294.8±5.2, $n=26$; day 20: 287.8±15.9, $n=7$; day 100: 323.5±20.8, $n=10$; day 200: 272.0±20.5 μm, $n=10$). The maximum tension to KPSS increased from day 10 (1.264±0.102 mN mm^{-1}) to day 20 (2.353±0.235 mN mm^{-1}, $P<0.001$) but was similar at day 100 (2.319±0.243 mN mm^{-1}) and day 200 (2.609±0.396 mN mm^{-1}). Basal tension decreased in response to NA at day 10, but NA stimulated constriction in older animals (Figure 1a). Maximum tension, at day 10 (−0.138±0.027 mN mm^{-1}, $n=8$), was less than that at day 20 (0.558±0.188 mN mm^{-1}, $n=7$, $P<0.005$) and the latter less than at day 100 (2.646±0.287 mN mm^{-1}, $n=10$, $P<0.001$). Tension at day 100 was similar to that at day 200 (2.947±0.432 mN mm^{-1}, $n=10$, n.s.). In day 10 animals, U46619 led to constriction of arteries in a concentration-dependent manner (maximum contraction: 124.2±5.1%K; pEC_{50}: 7.10 (6.84–7.37), $n=8$). Pronounced concentration dependent relaxation to NA occurred in arteries pre-constricted with U46619 (Figure 1b) (maximum relaxation: 75.9±5.4% of initial pre-constriction; pEC_{50}: 5.79 (5.55–6.02), $n=8$). L-NAME prevented NA-induced relaxation, as did removal of the endothelium (Figure 1b). The effect of the selective adrenoceptor antagonists on NA-induced relaxation is illustrated in Figure 1c. Yohimbine substantially inhibited the relaxation to NA ($P<0.001$). In the absence of functional α_1-adrenoceptor activity (in the presence of prazosin) relaxation to NA was significantly greater ($P<0.001$) but sensitivity was unaffected (pEC_{50}: 5.77 (5.48–6.05), $n=11$). Atenolol and ICI118551 had no significant effect on NA-induced vasorelaxation. The α_2-adrenoceptor agonist UK14304 also produced a pronounced relaxation in preconstricted arteries similar to that of NA (maximum relaxation: 74.3±6.2%, pEC_{50}: 6.57 (6.32–6.82), $n=7$) (Figure 1d). Similarly to NA-induced relaxation, L-NAME and endothlium removal inhibited UK14304-induced vasorelaxation (Figure 1d). Phenylephrine induced only a meagre constriction (maximum contraction: 15.9±2.6%K, pEC_{50}: 5.86 (5.36–6.35), $n=8$) (Figure 2a). Mixed β-adrenoceptor agonism with isoprenaline (10^{-8}–10^{-4} M) induced relaxation of preconstricted arteries in a concentration-dependent manner (maximum relaxation: 75.0±2.9%, pEC_{50}: 6.69 (6.31–7.06), $n=8$) (Figure 2b).

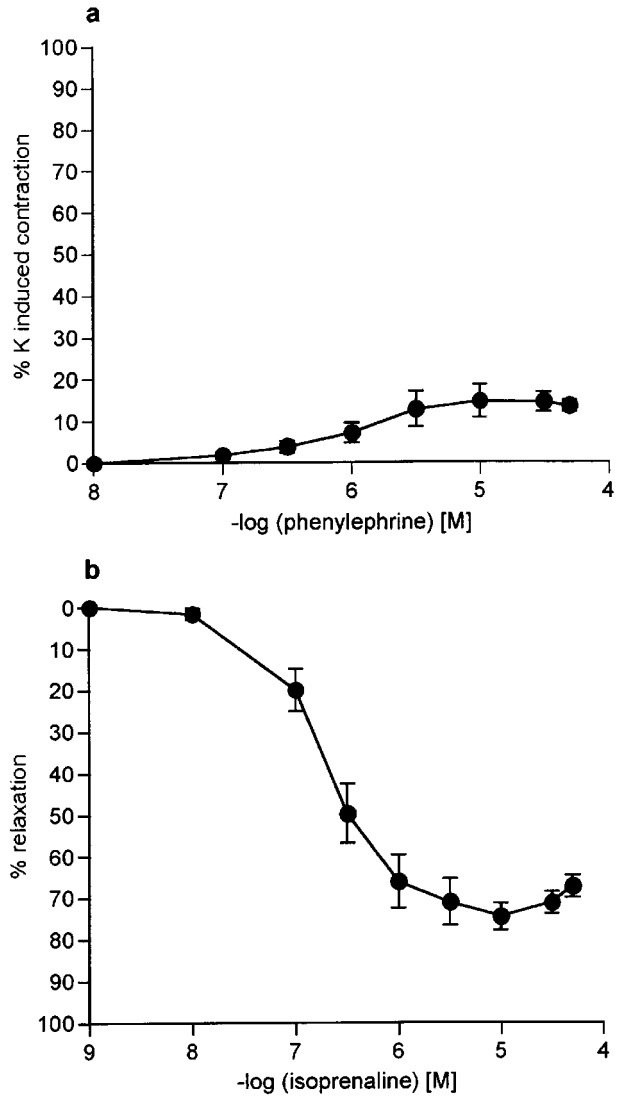

Figure 2 (a) Vasoconstriction to phenylephrine in isolated femoral arteries of 10-day-old rats. Data are expressed as a percentage of the response to 125 mM KCl. Values are given as mean±s.e.mean. (b) Vasorelaxation to isoprenaline in isolated femoral arteries of 10-day-old rats. Data are expressed as percentage relaxation of pre-constricted tone induced by U46619 (1 μM). Values are given as mean±s.e.mean.

Discussion The key observation of this study is the demonstration of relaxation to NA in systemic arteries of immature rats. NA caused relaxation of basal and agonist stimulated tone in isolated femoral vessels. To our knowledge, relaxation to NA has only been previously reported in arteries from the adult pulmonary circulation of the pig (Tulloh *et al.*, 1994). The absence of a constrictor response to NA in 10-day-old rat pups is unlikely to be due to underdevelopment of the contractile apparatus, as arteries constricted to both KPSS and U46619. After 10 days of age, there was a clear increase in the contractile response to NA and KPSS as the animals matured, a probable reflection of increasing smooth muscle mass.

The dilator response to NA was endothelium dependent and inhibited by L-NAME, suggesting that the vasorelaxation was NO mediated. Involvement of NO in NA mediated responses has been indicated previously but in experiments which have shown enhancement of NA induced tone by endothelial removal or NOS inhibition in arteries from a variety of animals and vascular beds (Egleme *et al.*, 1984; Carrier & White, 1985; Maclean *et al.*, 1993; Kaneko & Sunano, 1993; Zanzinger *et al.*, 1994; Zschauer *et al.*, 1997). The NA-induced vasorelaxation was not inhibited by either a β_1- or β_2-adrenoceptor antagonist. Thus it was clear that β-adrenoceptors were not involved in the NA mediated, NO dependent relaxation in these young rats. This contrasts with observation of the β-adrenoceptor mediated, NO dependent relaxation reported in arteries of the adult rat, including the aorta (Gray & Marshall, 1992), and vessels of the mesenteric (Graves & Poston, 1993) and pulmonary circulations (Priest *et al.*, 1997). As we found that NA-induced relaxation was blocked by yohimbine, but not by atenolol or ICI118551, α_2-adrenoceptors appeared to mediate the relaxation. This was confirmed by our observation that the α_2-adrenoceptor agonist UK14304 caused substantial concentration dependent relaxation which was similarly inhibited by L-NAME and endothelial removal. This data concurs with the evidence from a number of studies which have shown that α_2-adrenoceptor mediated NO release blunts constrictor responses to NA (Liao &

Homcy, 1992). Involvement of α_2-adrenoceptors in NO release has also been indicated in a study of pig arteries in which relaxation to NA was unmasked by α_1-adrenoceptor and β-adrenoceptor inhibition, and inhibited by NO synthase blockade (Ohgushi *et al*., 1993). We have also shown a very weak constriction to phenylephrine in the arteries of young rats which suggests immaturity of the α_1-adrenoceptor. This would provide an explanation for the overt relaxation to NA apparently mediated by the α_2-adrenoceptor.

Our data therefore suggest that maturational changes in α-adrenoceptors occur, with early development of endothelial α_2-adrenoceptor and delayed development of vascular smooth muscle α_1, which would favour dilation in response to sympathetic stimulation. One previous study (Dunn *et al*., 1989) has suggested developmental changes in α-adrenoceptors with age in the pulmonary circulation of the foetal and neonatal lamb, but to our knowledge none has suggested differential development of α_1 and α_2-adrenoceptors. The functional importance of these observations in foetal and neonatal life requires further investigation, as they have implications for our understanding of the mechanisms by which changes in foetal organ blood flow occur in response to adverse stimuli.

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