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SPECIAL REPORT

Influence of contractile agonists on the mechanism of endothelium-dependent relaxation in rat isolated mesenteric artery

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This study demonstrates directly that the relative contribution of nitric oxide (NO) and an NO synthase-independent repolarization to acetylcholine-evoked relaxation in rat isolated mesenteric resistance arteries is determined by the processes which mediate pre-contraction. Noradrenaline-induced contractions were reversed by acetylcholine via both NO and NO synthase-independent smooth muscle repolarization. In contrast, reversal of contractions to the thromboxane-mimetic, U46619, by acetylcholine was entirely mediated by the actions of NO, independently of a change in membrane potential.

Keywords: Nitric oxide; endothelium-dependent hyperpolarization; vascular smooth muscle

Introduction Endothelium-dependent relaxation to acetylcholine in rat isolated mesenteric resistance arteries is mediated by both nitric oxide (NO) and an NO-independent repolarization (Garland & McPherson, 1992). However, the influence which different contractile mechanisms may have on the relative contribution of these two pathways for relaxation has not been investigated.

In this study, the possibility that the mechanisms mediating relaxation to acetylcholine in segments of mesenteric artery may vary with different contractile agonists was examined in segments pre-contracted with either noradrenaline or U46619 (9,11-dideoxy-9α,11α-methanoepoxyprostaglandin $F_{2\alpha}$).

Methods Male Wistar rats (250-350 g) were stunned and killed by cervical dislocation. Segments (2 mm in length) of third order branches of the superior mesenteric artery (D_{100} 300±11 μm; $n=20$) were removed and mounted in a Mulvany-Halpern myograph (model 400A, J.P. Trading, Denmark) under normalized tension for simultaneous recording of smooth muscle membrane potential and tension as previously described (Waldron & Garland, 1994). All tissues were initially pre-contracted with noradrenaline and relaxed to cumulative concentrations of acetylcholine. Following this, each tissue was contracted with either noradrenaline or U46619 for all subsequent relaxation curves. The concentrations of contractile agonist used was adjusted to give the same level of tension under all conditions.

All data are expressed as mean±s.e.mean and the significance of differences between mean values was calculated by Student's t test.

All drugs were from Sigma.

Results The resting membrane potential of smooth muscle cells in segments of mesenteric artery was −58.1±3.9 mV ($n=20$ cells from 12 tissues). Noradrenaline (1-3 μM) stimulated depolarization and contraction (mean responses: 32.6±2.5 mV and 14.3±1.0 mN; $n=12$) and subsequent application of acetylcholine (0.01-5 μM) stimulated concentration-dependent repolarization and relaxation (Figure 1a). Pre-incubation with the two NO synthase inhibitors, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NOARG; both 100 μM; 30 min), did not alter either the resting membrane potential or the tone of arterial segments. In the

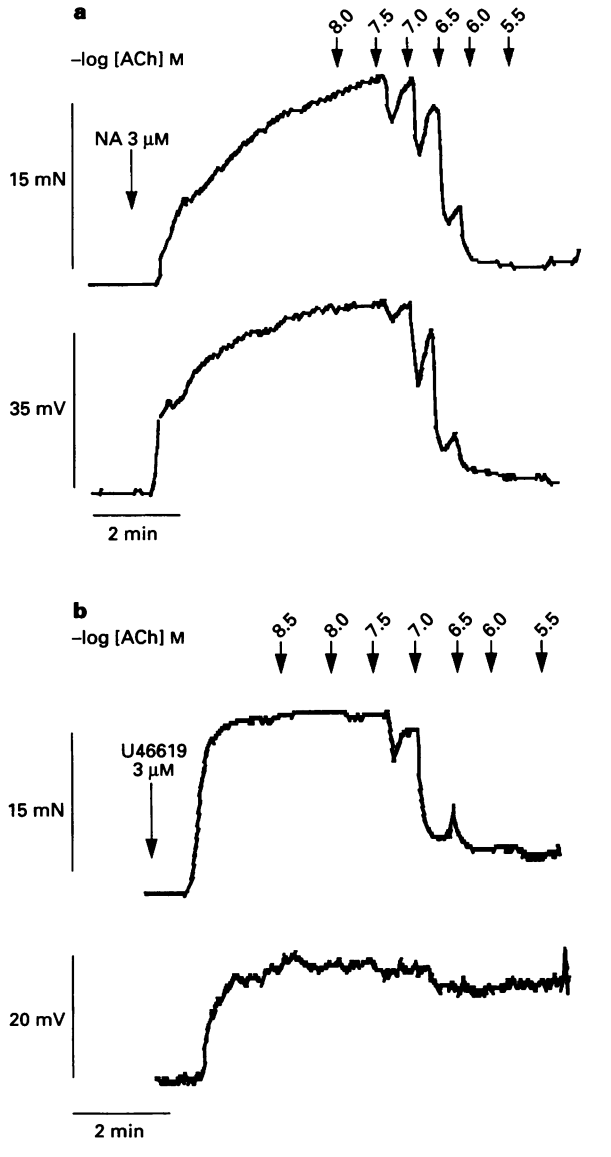

Figure 1 Representative traces showing simultaneous recording of acetylcholine-evoked changes in smooth muscle membrane potential and tension in tissues stimulated with noradrenaline (a) and U46619 (b).

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presence of these inhibitors, the pD_2 values for repolarization and relaxation were significantly increased from 7.32 ± 0.08 and 7.16 ± 0.12, respectively to 6.55 ± 0.27 and 6.53 ± 0.11, respectively ($n=4$; $P<0.01$) although the maximum responses were not altered (Figure 2a).

Nifedipine (1 μM; 10 min), abolished contractions evoked by increases in extracellular potassium (data not shown) and significantly depressed noradrenaline-evoked contraction (data not shown) so that a higher concentration of noradrenaline was required to induce the same level of tone as in controls. In the presence of nifedipine, acetylcholine-evoked relaxation of noradrenaline-stimulated tissues was significantly attenuated and in the presence of both nifedipine and the two NO synthase inhibitors, the acetylcholine-evoked relaxation was abolished (Figure 2a).

U46619 (0.5–1.0 μM) stimulated contraction of the same magnitude (mean contraction 15.0 ± 0.5 mN; $n=10$; $P>0.05$), but the accompanying depolarization was only half that with noradrenaline (16.6 ± 5.2 mV; $n=9$; $P<0.01$). Acetylcholine caused concentration-dependent relaxation of U46619-stimulated tone which was not significantly different from the relaxation observed in noradrenaline-stimulated tissues (pD_2 value and maximum relaxation; 7.2 ± 0.09 and $97.4 \pm 2.3\%$, respectively; $n=10$; $P>0.05$). However, acetylcholine had little effect on the membrane potential of U46619-stimulated tissues (Figure 1b). Futhermore, exposure to the NO synthase inhibitor L-NAME (100 μM; 30 min), significantly inhibited acetylcholine-evoked relaxation of U46619 contracted tissues (Figure 2b).

Nifedipine (1 μM), had little effect on the contraction to U46619 at the concentrations used in this study or on the acetylcholine-evoked relaxation of U46619-stimulated tone (Figure 2b).

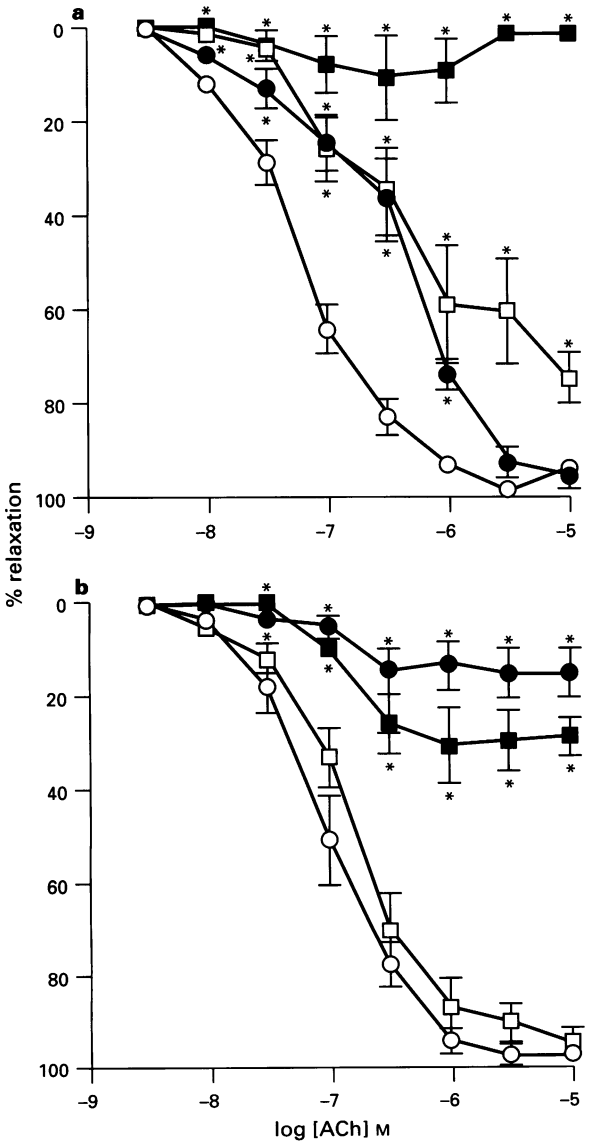

Figure 2 Mean concentration-response curves for acetylcholine-evoked relaxation of arterial segments stimulated with noradrenaline (a) or U46619 (b) in the absence (○) and presence of NO synthase inhibitors (●), nifedipine (□) or nifedipine and NO synthase inhibitors (■). All values are means from 4 to 8 experiments with s.e.means. *$P<0.01$ compared to control values.

Discussion These data demonstrate that the relative contribution of voltage-dependent and independent mechanisms to endothelium-dependent relaxation may be determined by the processes mediating contraction. Furthermore, they show that, in noradrenaline-stimulated tissues, acetylcholine-evoked relaxation and repolarization persist in the presence of high concentrations of a combination of two NO synthase blockers.

In rat isolated mesenteric arteries, noradrenaline-evoked smooth muscle contraction which was accompanied by marked depolarization. The contraction was depressed in the presence of nifedipine, a blocker of voltage-dependent calcium channels, indicating that, as previously reported (Nelson *et al.*, 1988), calcium entry via nifedipine-sensitive channels plays a major role in contraction to this agonist.

Application of acetylcholine caused concentration-dependent relaxation and repolarization of arterial segments stimulated with noradrenaline. Previous studies have shown both of these responses to be largely insensitive to the NO synthase inhibitor L-NOARG (Garland & McPherson, 1992). However, a recent study in the carotid artery has suggested that exposure to one NO synthase inhibitor alone may not be sufficient to block NO production in some arteries (Plane *et al.*, 1996). In this study, relaxation and repolarization persisted in the presence of a high concentration of both L-NOARG and L-NAME, indicating that full relaxation can be attained by NO-independent pathways, probably via smooth muscle hyperpolarization (Garland & McPherson, 1992).

In the presence of nifedipine, acetylcholine-evoked relaxation was attenuated and in the additional presence of two NO synthase blockers, acetylcholine-evoked relaxation was abolished. Thus, in these resistance arteries, the action of acetylcholine can be explained by both a NO and an NO-independent nifedipine-sensitive pathway, presumably hyperpolarization. This is in contrast to the bovine coronary artery, in which NO-independent relaxation to bradykinin appears to be via a nifedipine-insensitive pathway (Drummond & Cocks, 1996).

U46619-evoked smooth muscle contraction was accompanied by a much smaller depolarization than that seen with noradrenaline, and acetylcholine-evoked relaxation was not accompanied by significant smooth muscle repolarization. As in the bovine coronary artery (Drummond & Cocks, 1996), U46619-evoked contractions were resistant to nifedipine, indicating that the contraction was mediated by pathways other than calcium entry through voltage-dependent calcium channels. With U46619, nifedipine did not alter the acetylcholine-evoked relaxation indicating that relaxation could be fully explained by the voltage-independent actions of endothelium-derived NO.

In conclusion, this study provides the first direct evidence that the relative importance of voltage-dependent and -independent mechanisms for smooth muscle relaxation is influenced by the contractile agonist. This has important implications for the conclusions drawn from studies on the role of NO and NO-independent pathways in endothelium-dependent relaxation, particularly in the absence of measurements of smooth muscle membrane potential.

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