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N^G-Monomethyl-L-arginine and N^G-nitro-L-arginine inhibit endothelium-dependent relaxations in human isolated omental arteries

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Abstract—The L-arginine analogues N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} M) and N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), which specifically inhibit the synthesis of nitric oxide from L-arginine, significantly reduced acetylcholine-induced endothelium-dependent relaxations in rings of human omental arteries. The inhibitory potency of L-NMMA and L-NAME was similar. Addition of L-NMMA or L-NAME to the organ bath did not induce any significant changes in the resting tension of the tissues. The effects of L-NMMA were reversed by L-arginine (3×10^{-4} M). The L-NMMA enantiomer, D-NMMA (10^{-4} M), did not influence either the basal tone of the preparation or the relaxing effects of acetylcholine. Arterial relaxations induced by sodium nitroprusside (10^{-6} M) were not influenced by incubation with L-NMMA or L-NAME. These results suggest that endothelium-dependent relaxations in human omental arteries are mediated by the endogenous and substrate-specific generation of nitric oxide from L-arginine.

Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor (EDRF) (Palmer et al 1987; for review see Moncada et al 1989). Porcine aortic endothelial cells form nitric oxide from the terminal guanidino nitrogen atom(s) of the amino acid L-arginine (Palmer et al 1988a). This synthetic pathway is substrate specific and can be inhibited by guanidino-substituted L-arginine derivatives such as N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methylester (L-NAME), but not by the enantiomer D-NMMA (Palmer et al 1988b; Rees et al 1989a, 1990). The present study was designed to elucidate the role of nitric oxide synthesized from L-arginine in human omental arteries by analysing the actions of L-NMMA, L-NAME, L-arginine and D-NMMA on the endothelium-dependent relaxation of rings of human omental arteries.

Materials and methods

Arterial segments were taken from portions of human omentum during the course of abdominal operations (9 patients, 5 men and 4 women, aged 30–79 years). Rings (3–4 mm in length and 400–800 μm in outside diam.) were cut for isometric recording of tension. Each arterial segment was set in a 4 mL bath containing modified Krebs-Henseleit solution with the following composition (mM): NaCl 115, KCl 4·6, KH_2PO_4 1·2, $CaCl_2$ 2·5, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11·1 and disodium EDTA 0·01 mM. The solution was equilibrated with 95% O_2–5% CO_2 to give a pH of 7·3–7·4 at 37°C. To select resting tension for maximal force development, the arterial segments were exposed repeatedly to 60 mM KCl. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was 1 g. The vessels were allowed to attain a steady state of tension during a 2 h equilibration period before testing. In some experiments the endothelium was removed by inserting a roughened wire into the lumen. After each experiment the arteries were opened flat and stained with $AgNO_3$ to visualize the endothelium, and only results from vessels with more than 70% of the endothelium present were considered. Rings were contracted submaximally with noradrenaline (10^{-6} M) and cumulative relaxation curves to acetylcholine (10^{-7}–5×10^{-5} M) were obtained in each ring. After washout, and before addition of noradrenaline, the tissue was incubated for 10 min with L-NMMA (10^{-4} M, Wellcome Research Laboratories), L-NAME (10^{-4} M, Sigma), D-NMMA (10^{-4} M, Wellcome Research Laboratories) or the combination of L-NMMA + L-arginine (3×10^{-4} M, Sigma), and a second cumulative relaxation curve to acetylcholine was obtained. After a further washout, a third curve was obtained to demonstrate the reversibility of any effects observed. In some experiments the endothelium-independent vasodilator sodium nitroprusside (10^{-6} M, Sigma) was used instead of acetylcholine to relax the arterial rings.

Results are presented as mean ± s.e.m. and compared by use of Student's *t*-test with $P < 0.05$ considered as significant.

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Results

Cumulative applications of acetylcholine (10^{-7}–5×10^{-5} M) caused a concentration-dependent relaxation of rings of human omental arteries pre-contracted with 10^{-6} M noradrenaline (maximum relaxation = 66 ± 4% of the contraction induced by noradrenaline, $P < 0.001$, n = 27). This relaxation was completely absent in arteries in which the endothelium had been mechanically removed (n = 7). Incubation with L-NMMA (10^{-4} M, n = 12) or L-NAME (10^{-4} M, n = 6) neither induced changes in the resting tension of the tissues nor potentiated the contractile effects of noradrenaline. However, they significantly ($P < 0.05$) reduced the relaxant effects caused by acetylcholine (Fig. 1), with the only exception being the effect of L-NAME on relaxations induced by the highest dose of acetylcholine (5×10^{-5} M). Incubation with L-arginine (3×10^{-4} M, n = 8) reversed the inhibition by L-NMMA of relaxations induced by concentrations of acetylcholine ranging from 5×10^{-6} to 5×10^{-5} M but not by acetylcholine 10^{-7} M. Neither the resting tone nor the relaxing effects of all concentrations of acetylcholine were significantly influenced by D-NMMA (10^{-4} M, n = 6). Arterial relaxations (94 ± 4%) induced by sodium nitroprusside (10^{-6} M, n = 6) were not influenced by incubation with L-NMMA (94 ± 3%, n = 4) or L-NAME (97 ± 0·5%, n = 4).

Discussion

The present study demonstrates that the L-arginine analogues L-NMMA and L-NAME significantly reduce endothelium-dependent relaxations in human omental arteries. Relaxations induced by the endothelium-independent vasodilator sodium nitroprusside were not modified. The effects of L-NMMA on acetylcholine-induced relaxations were reversed by L-arginine, thus confirming further that the inhibition induced was not the result of a nonspecific action of this compound. The effects induced by L-NMMA and L-NAME were enantiomerically-specific since D-NMMA, which does not influence the synthesis of nitric oxide by endothelial cells (Palmer et al 1988b), did not influence acetylcholine-induced relaxations. Our results therefore confirm similar observations with L-NMMA in human

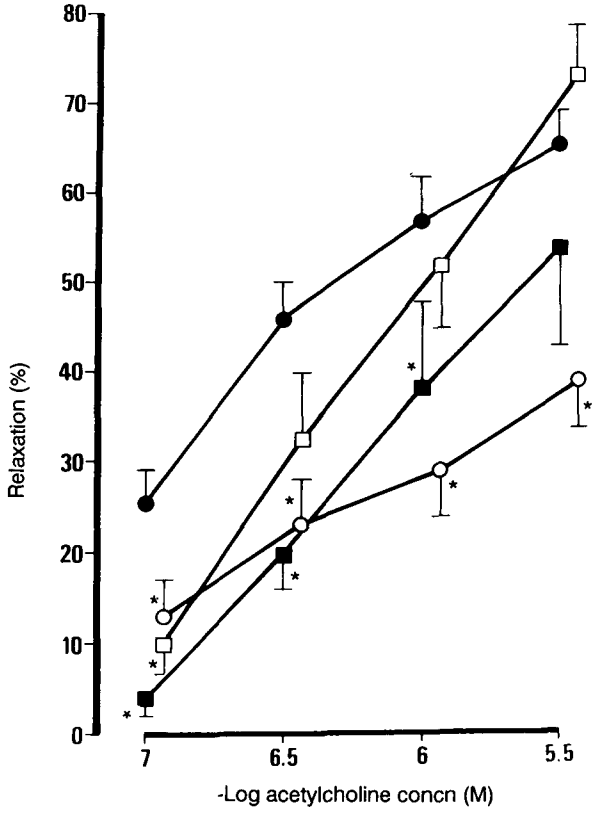

FIG. 1. Rings of human omental arteries were pre-contracted with noradrenaline (10^{-6} M), and cumulative relaxation curves were obtained by addition of acetylcholine (10^{-7}–5×10^{-5} M, n = 12) (●) to the organ bath. Incubation with L-NMMA (○) and L-NAME (■) (10^{-4} M, n = 12 and 6, respectively) inhibited the relaxation induced by acetylcholine. Co-incubation with L-arginine (3×10^{-4} M, n = 8) (□) partially reversed the inhibitory effects of L-NMMA on endothelium-dependent relaxation. Vertical bars show s.e.m. * Significantly different from control at $P<0.05$.

subcutaneous resistance arteries (Woolfson & Poston 1990) and in rings of internal mammary artery and vein (Yang et al 1990), and provide further evidence that in human vascular tissue, nitric oxide formed specifically from L-arginine mediates endothelium-dependent relaxations.

Previous studies (Moore et al 1990; Rees et al 1990) have suggested that L-NAME is a more potent inhibitor than L-NMMA of endothelium-dependent vasorelaxation in-vitro. In our experiments, however, L-NMMA and L-NAME were equally potent in their inhibitory effects on the relaxing responses to acetylcholine. Furthermore, the inhibition of acetylcholine vasodilatation produced by both L-arginine analogues was never complete. Since indomethacin pretreatment was not used in our study, this could reflect the existence of other endothelium-dependent vasodilators not related to nitric oxide such as prostacyclin. However, it could also result from an incomplete inhibition of nitric oxide generation. In the present study neither L-NMMA nor L-NAME had any effects on the resting tension of rings of omental arteries. This fact had only been previously described in human subcutaneous arteries (Woolfson & Poston 1990) and veins (Yang et al 1990) and differs from in-vitro findings in rings of various animal arteries (Rees et al 1990) and of human internal mammary artery (Yang et al 1990). Indeed, similar differences between vascular responses have been reported in-vivo since administration of L-NMMA has no direct venoconstrictor effects in man (Vallance et al 1989a) whereas it produces an increase in vascular resistance in the rabbit (Rees et al 1989a, b) and a decrease in basal arterial forearm blood flow in man (Vallance et al 1989b). The discrepancy could be the consequence of species and regional differences in the basal release of nitric oxide by vascular tissue. Thus, a low basal production of nitric oxide by omental arteries would suggest a low utilization of L-arginine, rendering the tissue less sensitive to the effects induced by the application of L-NMMA or L-NAME. Whether this is the consequence of differences in the uptake of the compounds in different vascular beds, or in the nature of the nitric oxide synthases, is not clear and further work is required to clarify these discrepancies. In conclusion, the present findings suggest that nitric oxide synthesized from L-arginine mediates acetylcholine endothelium-dependent relaxations in human omental arteries. However, it appears to play a limited role in maintaining the basal tone of this artery since inhibition of its synthesis does not induce any increases in this parameter.

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