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The effects of L-arginine and N^G-monomethyl L-arginine on the response of the rat anococcygeus muscle to NANC nerve stimulation

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The effect of the competitive inhibitor of L-arginine, N^G-monomethyl L-arginine (L-NMMA) on the response of the rat anococcygeus muscle to non-adrenergic, non-cholinergic (NANC) inhibitory nerve stimulation has been examined. L-NMMA causes a rise in muscle tone and inhibition of the response to nerve stimulation. The stereoisomer D-NMMA is without effect. The rise in tone and inhibition of the nerve response is reversed by L-arginine. Another analogue, L-canavanine, which is effective against L-arginine utilization in the macrophage, was without effect on the rat anococcygeus. These results provide indirect evidence for nitric oxide (NO) or a substance releasing NO as the transmitter of the NANC nerves in this tissue.

Introduction The mode of action of endothelium-derived relaxing factor (EDRF) on vascular smooth muscle and the non-adrenergic, non-cholinergic (NANC) nerves in the bovine retractor penis (BRP) and rat anococcygeus muscles are similar in so far as both act by stimulating guanylate cyclase and both are blocked by haemoglobin (Bowman *et al.*, 1982; Rapoport & Murad, 1983; Bowman & Drummond, 1984; Martin *et al.*, 1985). There is good evidence that EDRF is NO and that the precursor from which it is synthesized is L-arginine (Palmer *et al.*, 1987; 1988). Part of the evidence for the involvement of L-arginine in the synthesis of EDRF is its ability to increase the release of NO from endothelial cells cultivated in its absence and the ability of the analogue, N^G-monomethyl L-arginine (L-NMMA), to inhibit release and also to inhibit the relaxant response to agents which release EDRF. We have recently reported that L-arginine failed to potentiate and L-NMMA failed to inhibit the response to NANC nerve stimulation in the BRP in concentrations up to 3×10^{-4} M (Gillespie & Xiaorong, 1989). In that abstract we drew attention to more recent experiments in the rat anoccygeus which gave quite different results. This paper describes these later results.

Methods Experiments were performed on isolated anococcygeus muscles (Gillespie, 1972) from male rats suspended in Ag/Ag Cl ring electrodes in 10 ml organ baths and bathed in Krebs saline solution containing (mM): Na^+ 145, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 127, HCO_3^- 25, $H_2PO_4^-$ 1.2, dextrose 11, at 36°C and oxygenated with 95% O_2 plus 5% CO_2. Adrenergic motor nerves were blocked and tone raised by guanethidine sulphate (Ciba) 10^{-5} M added to the bath. Intramural nerves were stimulated by a fixed number of 20 pulses of supramaximal voltage and 0.5 ms duration at frequencies ranging from 0.1 to 10 Hz using a Grass S88 stimulator. The relaxant responses were recorded on a Grass (model 7) polygraph and expressed as a percentage reduction in the degree of tone existing at the moment of nerve stimulation. L- and D-NMMA were generously provided by Dr R.M.J. Palmer, Wellcome Research Laboratories. Solutions of NO (British Oxygen Corporation) were prepared as described by Palmer *et al.* (1987).

Results L-Arginine alone in concentrations up to 3×10^{-4} M had no effect on the response to NANC nerve stimulation. L-NMMA had two effects illustrated in Figure 1. First there was a further rise in tone almost immediately after adding the drug; secondly, there was a dose-related inhibition of the response to NANC nerve stimulation. Both effects occurred over the dose range 10^{-5} M to 3×10^{-4} M. Both effects were reversed by washing the drug out of the bath. The stereoisomer, D-NMMA, in concentrations up to 3×10^{-4} M was without effect on either tone or the response to nerve stimulation. L-Arginine, though itself unable to potentiate the response to nerve stimulation, was able to reverse the effects of L-NMMA, lowering tone and restoring the response to nerve stimulation (Figure 1). The simultaneous addition of L-arginine and L-NMMA prevented the effects of the latter on tone or the nerve response. These effects of L-NMMA are presumably prejunctional since, as Figure 1 illustrates, concentrations of the drug which inhibited the nerve response had no effect on the response to exogenously added NO. Another L-arginine analogue reported to inhibit nitrite formation by macrophages

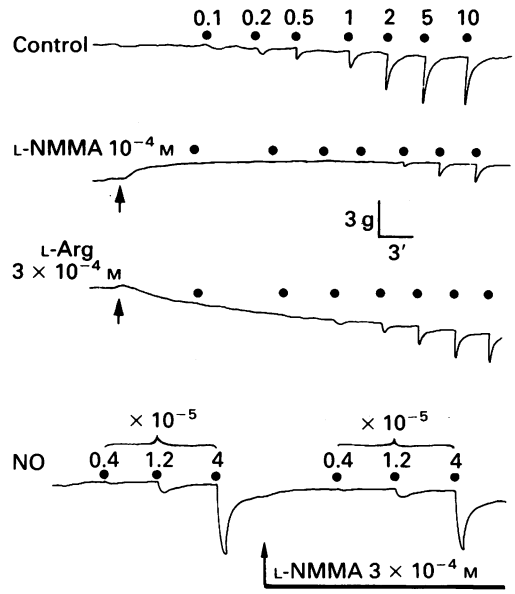

Figure 1 The effect of N^G-monomethyl L-arginine (L-NMMA) and L-arginine on the response of the rat isolated anococcygeus muscle to field stimulation of its NANC inhibitory nerves. The nerves were stimulated by 20 pulses of supramaximal voltage and 0.5 ms duration at the frequencies shown above each response. Guanethidine 10^{-5} M was present throughout to block the motor adrenergic nerves and raise tone. The top record is the control, the second, in the same tissue, shows the effect of adding L-NMMA 10^{-4} M; there was an additional rise in tone and inhibition of the response to nerve stimulation. The subsequent addition of L-arginine (L-Arg) 3×10^{-4} M with L-NMMA still present reversed both effects; tone was progressively reduced and the response to nerve stimulation partially restored. The bottom records show that L-NMMA had no inhibitory effect on the response to NO at the concentrations indicated.

(Iyengar *et al.*, 1987) is L-canavanine. This drug in concentrations up to 2×10^{-3} M had no effect on the response to NANC nerve stimulation in the rat anococcygeus muscle.

Discussion Garthwaite *et al.* (1988) were the first to describe the production of NO by neurones in the cerebellum. The present results, though indirect, suggest the NANC nerves in the rat anococcygeus may also liberate NO or a substance capable of releasing NO and that the substrate for its formation is the same as for EDRF, the guanidino-nitrogen(s) of L-arginine. The reversibility of the inhibition by L-arginine suggests that L-NMMA is acting competitively and the failure of the D-isomer indicates that the effect, as in the endothelial cell, is stereo-specific. The consistent rise in tone on adding L-NMMA suggests a constant background release of NO in concentrations capable of causing some relaxation of the tissue. Finally, the failure of L-canavanine to inhibit the nerve-evoked response suggests some difference; perhaps the enzymes for synthesis, in the endothelial cell and the NANC nerve terminal have different structural requirements from that in the macrophage.

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