The effect of a selective α7-nicotinic acetylcholine receptor antagonist on endothelium-dependent relaxation in rat mesenteric arteries

P.S. Chadha,[1,2] J.D. Moffatt[3] and R. Lever,[1] [1]Department of Pharmacology, The School of Pharmacy, University of London, WC1N 1AX, UK, [2]School of Medical Sciences, University of New South Wales, NSW 2052, Australia and [3]Department of Physiology, St. George's, University of London, SW17 0RE, UK.

Neuronal type nicotinic acetylcholine receptors (nAChR), in particular of the α7 subtype, are expressed on vascular endothelial cells where their function remains unclear. Endothelial cells have also been shown to possess all the components of an endogenous cholinergic system including acetylcholinesterase (AChE), choline acetyltransferase (ChAT) and the vesicular ACh transporter (VAChT) (Reviewed by Kirkpatrick *et al*., 2001). Previously, we have found that nicotine exposure has differential effects in mesenteric and pulmonary arteries where it leads to improved maximum endothelium-dependent relaxation in mesenteric vessels and an impaired response in pulmonary vessels (Chadha *et al*., 2005). In the present study, we have investigated acetylcholine-induced relaxation in rat mesenteric arteries, following exposure of vessels to the α7 nAChR-selective antagonist methyllycaconitine (MLA).

Male Wistar rats (240-260g) were killed by CO_2 asphyxiation. Third order mesenteric arteries were isolated and treated with 6-hydroxydopamine (2 mM) and capsaicin (0.1 mM) for 30 minutes, in order to remove neuronal influences. Following this, some vessels were incubated in DMEM containing MLA (10^{-7} M) both alone and in the presence of nicotine (10^{-7} M) or the VAChT inhibitor vesamicol (100 μM) and AChE (0.1 units/ml). Following 48 hours incubation, 2 mm segments of artery were mounted on a wire myograph under normalised tension in oxygenated (95% O_2/5% CO_2) Krebs' buffer maintained at 37°C. Maximum contraction to KCl (120 mM) was initially determined and ~50% maximal tone subsequently induced using phenylephrine, in the presence of nifedipine (0.3 μM), before assessment of ACh-induced relaxation. Maximal responses to ACh (E_{max}) are expressed as percent relaxation of active tone (mean ± SEM) and differences determined by ANOVA, followed by Dunnett's post test ($p < 0.05$). n = 6-7 per group.

Nicotine exposure enhanced subsequent maximal endothelial-dependent relaxation of mesenteric arteries, in response to ACh (87.2 ± 3.1% *vs.* 73.7 ± 5.4% vehicle control), an effect that was abolished by co-exposure to MLA (60.2 ± 9.3%). Interestingly, exposure of rat mesenteric arteries to the α7 nAChR-selective antagonist MLA alone augmented endothelium-dependent relaxation in response to ACh compared to vehicle control (E_{max}: 87.4 ± 2.1% *vs.* 69.5 ± 5.1%; pEC_{50}: 8.0 ± 0.1 *vs.* 7.1 ± 0.2, respectively). Moreover, the effects of MLA treatment were completely abolished following co-exposure to vesamicol and AChE (E_{max}: 65.6 ± 5.9%; pEC_{50}: 7.1 ± 0.1), whilst vesamicol/AChE treatment alone was without effect.

These data suggest that endothelial nAChRs may possess a role in control of vascular tone in rat mesenteric arteries and, furthermore, an autocrine effect of endogenous ACh may be in place, mediated in part by α7 nAChR.

Chadha PS, Moffatt JD & Lever R. (2005) *Proceedings of the British Pharmacological Society*, http://www.pa2online.org/abstracts/Vol3Issue2abst057P.pdf

Kirkpatrick CJ, Bittinger F, Unger RE, Kriegsmann J, Kilbinger H & Wessler I. (2001) *Japanese Journal of Pharmacology*, **85:** 24-28.