CANNABINOID MODULATION OF NICOTINE RESPONSES IN GUINEA-PIG ILEUM MYENTERIC NEURONS

W.R. Sones, D.G. Demuth, R. Makwana, M.E. Parsons and A. Molleman. School of Life Sciences, University of Hertfordshire, C.P. Snow Building, Hatfield, AL10 9AB.

Within the enteric nervous system, the action of acetylcholine at nicotinic acetylcholine receptors (nAChRs) has been shown to be the predominant mechanism for excitatory neurotransmission (Galligan, 2002). The non-selective cannabinoid receptor agonist, CP55940, has been shown to inhibit electrically evoked contractions in the guinea-pig myenteric plexus-longitudinal muscle (MPLM) preparation through stimulation of CB_1 receptors. A putative direct interaction between endogenous cannabinoids and nAChRs has previously been reported by Oz *et al*. (2003). The aim of the present study was to examine cannabinoid modulation of contraction of guinea-pig ileum evoked by nicotine, and to investigate whether modulation is due to interaction with nAChRs through whole-cell patch clamp recordings from myenteric neurons grown in primary culture.

Segments of whole ileum and MPLM, derived from adult Heston-2 guinea-pigs of either sex (300-500g; Pertwee *et al*., 1996), were mounted in isolated organ baths containing Krebs solution, under an initial tension of 0.5 g. A repeatable response to 100 µM nicotine with a 30 min interval between doses was established. Cannabinoids were added 20 min prior to the addition of the fourth dose of nicotine. Data are presented as mean ± S.E.M. inhibition as a percentage of the size of the untreated response. Myenteric cultures were prepared from segments of ileum using a technique adapted from Barajas-Lopez *et al*. (1993). Nicotinic currents were measured using whole-cell patch clamp at a holding potential of -60 mV and are expressed as pA /pF membrane capacitance to compensate for cell size. Data are presented as mean ± S.E.M.

In the whole ileum, CP55940 (1 µM) inhibited nicotine evoked contractions by 41.2 % (S.E.M. = 6.7 %, $P < 0.001$ Student's t-test). This was not attenuated by the selective CB_1 receptor antagonist SR141716 (1 µM). In the MPLM preparation, CP55940 also inhibited nicotine evoked contractions (EC_{50} 415 ± 88.8 nM). Again, inhibition was not reversed by SR141716. In contrast, SR141716 demonstrated in electrically evoked contractions, which were more sensitive to inhibition by CP55940 (EC_{50} 14.6 ± 1.97 nM, $P < 0.01$ ANOVA), attenuation of the cannabinoid agonist inhibition. In the presence of tetrodotoxin, which restricts nicotinic responses to the presynaptic nerve terminal within longitudinal muscle (Galligan, 1999), both CP55940 (300 nM) and SR141716 (300 nM) significantly inhibited nicotine evoked contractions. In cultured myenteric neurons, nicotine (1 mM) induced an inward current with a mean peak amplitude of 29.5 ± 5.2 pA/ pF, which was significantly inhibited by both CP55940 (1 µM) and the endocannabinoid anandamide (1 µM). Inhibition was not attenuated by SR141716 (300 nM), which significantly reduced nicotinic currents when added alone. Palmitoylethanolamide, an analogue of anandamide which does not bind to CB_1 and CB_2 receptors (Lambert *et al*., 1999), significantly reduced nicotinic currents.

These results suggest cannabinoids interact with nicotinic receptors in the gut through a non-CB_1/CB_2 receptor mediated mechanism.

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