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The effect of the cannabinoid antagonists on responses to electrical field stimulation in intact longitudinal segments taken from the human colon

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Cannabinoids have been used for centuries for a variety of gastrointestinal problems such as infection, inflammation and disturbed pattern of motility, emesis and pain (Izzo & Cout, 2005). It is known that CB1 receptors are located on the enteric nervous system which modulate the release of the neurotransmitters. CB2 receptors on the other hand are mainly concerned with the immune function (Aviello et al., 2008). The aim of the present study was to investigate if cannabinoid receptors are involved in mediating a response to electrical field stimulation in intact human colon.

Specimens of human colon were prepared following a resection in bowel cancer patients. The samples were obtained from macroscopically normal regions within the colon not involved with malignancy, and were not affected by colitis, fibrosis or inflammation as assessed by pathologists. Specimens of full thickness (intact) were placed in ice-cold carbogenated (95% O2, 5% CO2) Kreb's solution of the following composition (mM): NaCl 118, NaHCO3 25, KCl 4.6, MgSO4 1.2, NaH2PO4 1.3, glucose 11, CaCl2 2.5. Intact tissues were cut into strips in the direction of longitudinal muscles of dimensions 3 mmx 15 mm and mounted in organ baths containing Kreb's at 37°C and continuously bubbled with carbogen. Each strip was suspended between 2 parallel platinum electrodes in tissue baths and placed under 1g tension for isometric recording. Electrical field stimulation (EFS) at different frequencies were applied every 1 min (0.5, 1.0, 5.0, 10.0 and 20.0 Hz, 0.5ms pulse width, 50V, 10s). Some strips were pre-contracted with carbachol (1.0 µM) prior to the application of EFS. All experiments were repeated in the same strips and in the presence of 1.0 µM of atropine, AM251 (a CB1 receptor antagonist) and AM630 (a CB2 receptor antagonist). Drugs were applied non-cumulatively. Mean $\pm$ s.e.mean were expressed as the percentage of maximum contraction response to EFS or carbachol; n=2 (minimum of 8 strips were taken from each sample).

The study had been approved by the NHS Research Ethics Service Committee, Yorkshire and the Humber. EFS induced frequency-dependent contraction responses in most intact longitudinal strips. In the presence of 1.0 µM atropine the EFS-induced contraction responses were significantly ($p<0.001$) reduced to 1.15 $\pm$ 1.0 at 1 Hz, 0.58 $\pm$ 0.4 at 5 Hz, 0.88 $\pm$ 0.4 at 10 Hz and 0.073 $\pm$ 0.073 at 20 Hz from 21.53 $\pm$1.0, 2.64 $\pm$ 9.5, 60.98 $\pm$ 8.2 and 97.63 $\pm$ 2.3, respectively. The contraction responses to EFS were not modified by 1.0 µM of AM251 and AM630, CB_1 and CB_2 receptor antagonists, respectively. However, EFS induced a relaxation response in pre-contracted tissues with carbachol (1.0 µM) which were significantly ($p<0.05$) attenuated by pretreatment with 1.0 µM of AM251 but not AM630. The relaxation responses to EFS in precontracted tissues with carbachol were 63.2 $\pm$ 5.5 at 1Hz, 52.35 $\pm$ 8.5 at 5 Hz and 35.2 $\pm$ 11.08 at 10 Hz which were reduced by the application of AM251 to 3.15 $\pm$ 3.0,

29 ± 5.1 and 8.9 ± 7.2, respectively. The results suggest a tonic inhibitory effect exerted by endocannabinoids via CB1 receptors in the human colon.

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