Control of enteric neuromuscular functions by purinergic P2X7 receptors in normal rat distal colon and experimental bowel inflammation

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Introduction: Purinergic signalling plays a pivotal role in the physiological regulation of several enteric functions, as well as in the modulation of immune/inflammatory cell activity. Recent evidence has shown an active involvement of the purinergic P2X7 receptor (P2X7R) in the fine tuning of immune functions, as well as its critical role in driving enteric neuron apoptosis under intestinal inflammation. However, the participation of this receptor pathway in the regulation of enteric neuromuscular functions remains undetermined.
Aims: This study investigated the role of P2X7Rs in the control of colonic motility, both under normal conditions and in the presence of experimental colitis.
Methods: Colitis was induced by intrarectal administration of 2,4-dinitrobenzenesulfonic acid (DNBS) in adult male Sprague-Dawley rats. Six days after colitis induction, colonic longitudinal muscle strips (LMS), obtained from normal or inflamed rats, were suspended in organ baths, containing Krebs solution additioned with NK 1, 2 and 3 receptor antagonists, and connected to isometric transducers to record atropine-sensitive cholinergic motor activity. The effects of A804598 (selective P2X7R antagonist; 0.001-100 μM) and BzATP (selective P2X7R agonist; 0.001-10 μM) were tested on contractions evoked by electrical stimulation (ES: 0.5 ms, 28 V, 10 Hz), delivered as single train (sES) or repeated every 60 s (rES), or by carbachol (1 μM) in the presence of tetrodotoxin.
Results: In normal LMS, A804598 induced a negligible enhancing effect on sES-induced contractions (+7.8±3.5% at 0.1 μM), while a significant increase in the contractile responses elicited by sES was recorded in LMS from inflamed animals (+42.5±3.9% at 0.1 μM). Incubation of LMS with the adrenergic blocker guanethidine (10 μM) did not affect the enhancing effect exerted by A804598 in the presence of colitis. Upon incubation with N^{w}-propyl-L-arginine (NPA, inhibitor of neuronal nitric oxide synthase), which *per se* increased the sES-induced contractions in both normal and inflamed LMS preparations (+15.1±5.5% in normal and +143.5±9.5% in inflamed animals), the A804598 effects were lost. The pharmacological activation of P2X7Rs with BzATP did not significantly affect the contractions to rES in normal LMS (E_{max}= -10.2±1.9%), while a marked reduction was recorded in LMS from animals with colitis (E_{max}= -30.5±2.2%). The inhibitory effect of BzATP was antagonized by A804598, and it was also markedly blunted by NPA. Both P2X7R ligands did not affect carbachol-induced contractions.
Conclusions: The purinergic system contributes to functional neuromuscular changes associated with bowel inflammation through the involvement of P2X7Rs, which modulate the activity of excitatory cholinergic nerves via a facilitatory control on inhibitory nitrergic pathways.