Role of the molecular complex A2B receptor-adenosine deaminase in the alterations of colonic motility associated with experimental colitis

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Introduction. Adenosine A_{2B} receptors ($A_{2B}R$) are involved in the regulation of several enteric functions. However, the role played by these receptors in the pathophysiology of intestinal dysmotility associated with inflammation remains less characterized. For these reasons, the purpose of this study was to investigate the expression of $A_{2B}Rs$ in rat colon and their involvement in the control of colonic cholinergic motility in the presence of bowel inflammation.
Methods. Colitis was induced by 2,4-dinitrobenzenesulfonic acid in male Sprague-Dawley rats (220-250 g body weight). Colonic $A_{2B}R$ expression and localization were examined by RT-PCR and immunofluorescence. The molecular interaction between $A_{2B}R$ and adenosine deaminase was assayed by immunoprecipitation. The contractile activity of longitudinal muscle preparations (LMP) was recorded *in vitro*. To this aim, colonic LMP were set up in organ baths containing Krebs solution and connected to isotonic transducers. The effects of an $A_{2B}R$ antagonist (MRS 1754) and agonist (N-ethylcarboxamidoadenosine, NECA) were recorded on atropine-sensitive cholinergic contractions evoked by electrical stimulation (10 Hz, 30 mA, 0.1 ms), obtained upon incubation of colonic tissues with guanethidine, N^{ω}-nitro-L-arginine methylester (L-NAME) and L-732,138 (NK_1 receptor antagonist). $A_{2B}R$ ligands were also tested on contractions evoked by carbachol in the presence of tetrodotoxin.
Results. In normal colon, RT-PCR revealed the presence of $A_{2B}R$ mRNA, while in inflamed tissues a significant increase in $A_{2B}R$ receptor expression was detected. Immunofluorescence displayed a predominant localization of $A_{2B}Rs$ in the colonic neuromuscular compartment. In the presence of colitis, $A_{2B}R$ expression was enhanced at muscular level, but reduced in myenteric ganglia. In functional experiments, the $A_{2B}R$ antagonist MRS 1754 enhanced both electrically evoked and carbachol-induced cholinergic contractions in normal LMPs, while it was less effective in inflamed tissues. The $A_{2B}R$ agonist NECA decreased the colonic cholinergic motility, with an increased efficacy in inflamed LMP. Immunoprecipitation and functional tests revealed a link between $A_{2B}Rs$ and adenosine deaminase. In particular, the increase in endogenous adenosine availability, obtained through the pharmacological blockade of adenosine deaminase with EHNA (0.5 mM), resulted in a restoration of the A_{2B}-mediated inhibitory activity in the presence of bowel inflammation.
Conclusions. Under normal conditions, endogenous adenosine modulates the cholinergic colonic motility via $A_{2B}Rs$ located in the neuromuscular compartment. In the presence of colitis, this inhibitory control is impaired, due to a molecular link between $A_{2B}R$ and adenosine deaminase, which catabolises adenosine thus preventing $A_{2B}R$ activation.