ADENOSINE RECEPTORS AND METABOLISM IN THE INTESTINAL TRACT

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Background CD73/ecto-5'nucleotidase and adenosine deaminase directly control adenosine (Ado) availability in the proximity of its receptors (ARs), through which the nucleoside exerts anti- or pro-inflammatory effects depending on its concentration, cell type and specific AR involved. The functional expression of CD73 and ARs is well established in mucosal, but not in muscular layers in the gut. The aims of this study were to determine a) the expression of A1, A2a, A2b and A3 ARs in the rat ileum, b) the distribution of CD73 and its functional role in Ado metabolism in intact ileum and intestinal longitudinal smooth muscle cells (ISMCs). **Methods** ISMCs were prepared by the primary explant technique from freshly isolated rat ileum (1). Immunohistochemistry (IHC) and immunocytochemistry (ICC) were performed on formaldehyde-fixed tissue sections and cells, respectively. ARs and CD73 immunofluorescence was detected by confocal fluorescent microscopy. For evaluating enzyme activity, ileum strips incubated in Tyrode solution or cultured ISMCs were treated with exogenous AMP (50 μM) from 0.5 to 30 min. In the incubation medium the levels of AMP, Ado and inosine were analyzed by HPLC. **Results** In full-thickness sections IHC revealed the presence of all AR subtypes in the rat ileum with more intensive staining on the mucosal layer. Mucosal and muscular layers exhibited immunopositivity to CD73, that showed a distinctive regional distribution. In ISMCs ICC confirmed CD73 staining on the surface only, mainly at the level of intercellular junctions. AMP added to the incubation medium of ISMCs was metabolized by 19% within 0.5 min and 95% after 30 min ($t_{1/2}$ = 2.9 min). At the same times AMP addition caused a marked increase in Ado without affecting inosine level. With intact ileum strips AMP degradation was more rapid (62% decrease within 0.5 min) and the nucleotide was completely cleared from the medium within 30 min ($t_{1/2}$ = 0.3 min). These changes were associated with a transient increase in Ado and significant inosine accumulation. **Conclusions** The distinctive distribution and activity of CD73 underlines its critical role in maintaining Ado levels in the gut and suggests its involvement in the control of intestinal motility. Indeed, this enzyme represents a gatekeeper for the fine activation of low and high affinity ARs. Thus, it will be important to understand how CD73 and ARs are affected by pathological conditions, since these proteins could represent potential novel therapeutic targets for the treatment of intestinal diseases.

References (1) Khan I., Blennerhassett M.G., Kataeva G.V., Collins S.M. (1995) Gastroenterology 108: 1720-1728.