# IN RAT ILEUM ADENOSINE DEAMINASE IS NOT ONLY AN ECTO- BUT ALSO AN EXO-ENZYME

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In the intestine, adenosine (Ado) affects motility, secretion and permeability and exerts tissue protection under conditions of stress, e.g. ischemia and inflammation. Metabolism of Ado by adenosine deaminase (ADA) and its transport into the cells are key mechanisms for the control of Ado concentration at its receptor sites. The aim of this study was to evaluate ADA involvement in the removal of Ado from the incubation medium of rat ileum in vitro. For this purpose longitudinally cut strips (3 cm) from distal ileum of male Wistar rats were incubated in vials containing 2 ml of aerated (95% $O_2$, 5% $CO_2$) and warmed (36.5 °C) Tyrode solution. The ADA inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) and the blocker of equilibrative nucleoside transporters (ENT), S-(4-nitrobenzyl)-6-thioinosine (NBTI) were added into the medium 15 min before Ado. Conditioned medium was obtained by removing the untreated tissue after incubation of 30 min. An HPLC method (modified from (1)) was used to quantify Ado and its metabolites in the samples. The reported data are means±SEM of the values obtained in 3 to 8 experiments. ADA expression was examined on formaldehyde-fixed tissue sections, using an anti-ADA antibody. The staining was detected by confocal fluorescent microscopy.

**Results** Time-course studies show that exogenous Ado (50 μM) is rapidly cleared from the incubation medium of rat ileum ($t_{1/2}$= 0.5 min), and inosine (Ino) simultaneously rises. At 1 min, when 16.3±1.0 μM Ado is still detectable, EHNA (1 μM) allows the recovery of 45,0±0.6 μM of this nucleoside and reduces Ino from 20.5±1.5 to baseline level. In the presence of EHNA, the $t_{1/2}$ of Ado is 15 min. By contrast NBTI (10 μM) does not affect Ado and Ino concentrations. In conditioned medium Ado falls with a $t_{1/2}$ of 1.2 min. No significant loss occurs within 60 min of incubation in fresh medium. Immunohistochemical analysis reveals that ADA is constitutive in the ileum tissue, with an intense staining on mucosal layer and its cellular localization seems to be mainly citoplasmic. **Conclusions** In rat ileum Ado removal from the extracellular environment is primarily due to metabolic conversion by ecto-ADA, not requiring Ado transport into the cells. ADA is abundant in the tissue and is also released as an exo-enzyme. Pharmacologic control of its activity using hydrophilic, membrane impermeable drugs, can be regarded as a possible means for affecting intestinal Ado levels in pathological conditions such as those originating from ischemic and inflammatory insults.

**References:** (1) Childs K.F et al. (1996) J. Chromatogr. B Biomed. Appl. 668:181-186.