Modulation of the purinergic signaling by extracellular purine-converting enzymes under basal and experimental pathophysiological conditions

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High levels of Hypoxanthine (HYPO) and Guanine (GUA) may be detected in the external milieu of different cell types and several lines of evidence suggest that these compounds do not seem to be released as such from the cells but presumably derive from the metabolism of corresponding adenine- and guanine-based nucleotides and nucleosides. In fact, a broad spectrum of membrane-bound and soluble purine-converting enzymes is known to contribute to the extracellular purine homeostasis. However, the mechanisms underlying the release of soluble purinergic enzymes and potential interplay between extra- and intracellular purine homeostasis remain largely unknown. To contribute at elucidating these pathways, we evaluated whether cells are able to actively release enzymes metabolizing purine nucleosides at basal and also some experimental pathophysiological conditions, such as activation of purinergic receptors or hypoxia. Furthermore, since the extracellular and intracellular purine-converting pathways are in tight functional connection, we evaluated whether the intracellular spectrum of purine compounds could also be affected during modulation of extracellular purine turnover.

Cultured rat C6 glioma cells were incubated for 45 min with ^{3}H-labelled Adenosine or Guanosine as tracers, followed by HPLC analysis of both intra- and extra-cellular spectra of generated nucleotide and nucleoside metabolites by using a combined UV and radiochemical detections. The results indicate that, in resting conditions, the intracellular levels of nucleotide triphosphates were about three fold higher than those of the corresponding nucleosides or purine bases. Conversely, outside the cells, the levels of Hypoxanthine (HYPO), Xanthine (XAN) and Guanine (GUA) exceed by many times those of the nucleotides. Therefore, the spectrum of the extracellular purines, released from C6 cells in resting conditions, is substantially different from the intracellular one.

We also found detectable amounts of Purine Nucleoside Phosphorylase (PNP) and GUA deaminase released from cells in the culture medium where no detectable release of cellular LDH was found. The stimulation of some purinoceptors ($P2X_7$, $P2Y_1$ or A_{2A}) increased PNP activity in extracellular medium, and magnitude and time-course of this stimulated enzyme release depended on particular receptor subtype activated. In addition, receptor stimulation was accompanied by concurrently elevated extracellular levels of purine bases without any changes in the intracellular quantities of purine compounds. Cell pre-treatment with selective antagonists of adenosine-selective (P1) and nucleotide-selective (P2) receptors prevented the increase in the release of both PNP and purines caused by the corresponding agonists. In contrast, the addition of exogenous ADO or GUO (40-100 μM) significantly modified the amounts of the intracellular, but not extracellular, nucleotides and nucleosides.

The exposure of C6 cells to acute hypoxia for 2 hours markedly diminished their ability to convert the intracellular ^{3}H-nucleosides into the corresponding nucleotides, and this impaired nucleotide-regenerating capability was partly recovered during subsequent 1-hour re-oxygenation. Among the intracellular enzymes whose activity was evaluated by a phospho-array, hypoxia restrained the AMP-Kinase activity thus reducing the amounts of triphosphate nucleotides and favouring an accumulation of purine nucleosides. As expected, hypoxia induced a strong increase in the levels of extracellular nucleosides not accompanied by an equivalent increase of the purine base production.

Collectively, these results indicate the existence of a highly dynamic turnover between extracellular and intracellular nucleotides, nucleosides and also purine-converting enzymes. The balanced homeostasis can be selectively modulated by different stimuli, including shifts in extracellular purine levels, activation of certain purinergic receptors or acute hypoxia.