EXOGENOUS CAMP METABOLISM AND ITS PHARMACOLOGICAL MODULATION IN RAT ILEUM


**Background**

The second messenger cyclic AMP (cAMP) plays a pivotal role in signal transduction and an integral role in several physiological processes, e.g. smooth muscle relaxation and control of ion channel conductance, cell proliferation and various metabolic processes\(^1,2\). Therapeutic agents or endogenous substances, such as adenosine, capable of simulating adenylyl cyclase through membrane receptors linked to Gs proteins, enhance the formation of cAMP from ATP. Extrusion of the formed cAMP into the extracellular space is an ubiquitous process mediated by a unidirectional anion transport system\(^2,3\). A variety of cells and tissues endowed with ecto-phosphodiesterases (ecto-PDEs) and ecto-5'-nucleotidases (ecto-5'-NUs) are capable of degrading extracellular cAMP to 5'-AMP and hence to adenosine\(^4,6\). Rosenberg (1992) hypothesized that the release of cAMP may be an important source of physiologically active adenosine\(^5\). This pathway has been identified in several tissues and cells including the whole kidney, cultured renal and aortic smooth muscle cells, cultured cardiac fibroblasts and intact cerebral vessel\(^1-5\).

Since this nucleoside plays a relevant role in the control of intestinal motility and secretion, in the present study the accumulation of AMP, adenosine and inosine formed by ileum strips during incubation in vitro with exogenous cAMP was monitored. The purpose of the present investigation was to evaluate the hypothesis that also in intestinal tissue a functioning extracellular cAMP-adenosine pathway exists. To test this hypothesis we evaluated the influence of inhibition of ecto-phosphodiesterases and of ecto 5'-nucleotidases on the accumulation of adenosine, inosine and AMP in the medium and tissue upon incubation of rat ileum strips with exogenous cAMP.

**Methods**

Male Harlan Wistar rats (250-270 g b.w.) were killed by cervical dislocation and bled. Distal ileum (30 cm) was removed and cut into 2 cm segments. Each segment was divided into four longitudinal strips, that were placed in vials (2 for each vial) containing 2 ml of warmed (36.5°C) aerated (95% O\(_2\), 5% CO\(_2\)) Tyrode solution and allowed to equilibrate for 45 minutes. cAMP (50 µM) was then added to the incubation medium. Dipropylsulfophenylxanthine (DPSPX 0.1 and 1 mM), isobutylmethylxanthine (IBMX 0.1 mM), α,β-methylene ADP (AOPCP 0.2 mM), erythrononyladenosine (EHNA 1 µM) and nitrobenzyladenosine (NBTI 1 µM) were added to the incubation medium 15 min before cAMP. In each experiment control tissue samples were included. After 1 h, medium aliquots (1 ml) were taken and the reaction was terminated by adding HClO\(_4\) (1 M, final concentration). The tissue samples were rapidly immersed in liquid N\(_2\) and homogenized in 0.5 ml of 1 M HClO\(_4\). After centrifugation the tissue residue was used to determine protein content by Lowry protein assay\(^5\). The clear tissue extracts and the medium samples were frozen at –20°C and later analyzed by an HPLC method\(^6\) to quantify cAMP, AMP and its metabolites (adenosine and inosine). The reported data are mean±SEM of the values obtained from 8 to 15 incubations. Significance of the differences between means was evaluated by the Student’s t test.
Results During tissue exposure to 50 µM cAMP, its measured concentration in the medium linearly fell from 47.2±2.2 to 29.7±2.1 µM within 60 min. AMP, adenosine and inosine concomitantly increased to 1.90±0.24 and 3.2±0.4 µM and 5.23±0.53 µM, respectively (control values: 0.6±0.3 and 2.2±0.3 and 2.6±0.4 µM, respectively). Addition of exogenous cAMP caused minor changes in AMP, adenosine, inosine levels in the tissue that only in some experiments reached statistical significance. Pretreatment with IBMX (0.1 mM), a broad spectrum PDE inhibitor, increased medium cAMP from 30.9±2.8 to 45.7±3.5 µM; in the same experiments the membrane impermeable PDE inhibitor, DPSPX significantly enhanced cAMP only at a concentration of 1 mM (9.0±3.0 µM).

IBMX (0.1 mM) lowered cAMP-induced accumulation of AMP in the medium by 52%. Similarly, DPSPX (0.1 mM) reduced AMP level by 49%. DPSPX added to the medium at a higher concentration (1 mM) reduced AMP level by 84%. Purine levels in the tissue were not affected by these PDE inhibitors.

When 200 µM AOPCP, an ecto 5'-NU inhibitor, was combined with cAMP, it enhanced AMP in the medium from 0.47±0.08 to 2.73±0.29 nmol/mg protein while lowering adenosine levels by 44%. In the tissues the pretreatment with AOPCP reduced both AMP and adenosine levels to 33% and 53 %, respectively.

After tissue incubation with 50 µM AMP, the medium nucleotide was hardly detectable, but reached 10.6±1.83 µM (2.5±0.36 nmol/mg protein) in the presence of AOPCP, that concomitantly decreased adenosine and inosine level by 65% and 45%, respectively. In the tissue exposed to exogenous AMP, pretreatment with AOPCP lowered adenosine levels from 0.78±0.10 to 0.34±0.10 nmol/mg protein.

EHNA (1 µM), an adenosine deaminase inhibitor, enhanced the basal levels of adenosine in tissue from 1.35±0.34 to 2.63±0.52 nmol/mg protein but did not significantly modify purine content in the incubation medium. EHNA, when combined with cAMP, induced an accumulation of adenosine from 0.26±0.73 to 3.49±0.46 nmol/mg protein and lowered inosine level by 56% in the medium. After tissue incubation with 50 µM adenosine, the medium nucleoside rose from 0.1±0.4 µM (0.2±0.1 nmol/mg protein) to 2.4±0.6 µM (0.6±0.1 nmol/mg protein) and inosine level from 0.39±0.08 to 1.99±0.45 nmol/mg protein). Under these conditions EHNA increased adenosine level in the medium to 4.3±0.5 µM (1.3±0.2 nmol/mg protein) without affecting the purine pool in the tissue. NBTI (1 µM), an inhibitor of the equilibrative nucleoside transporter, did not cause significant changes in adenosine or inosine accumulation due to exogenous cAMP or adenosine.

Conclusions Exogenous cyclic AMP is degraded by rat ileum strips to AMP, adenosine and inosine. Since exogenous cAMP does not cross the plasma membrane, these results indicate that its degradation by rat intestine leads to the formation of inosine in the extracellular compartment via a sequential action of ecto-phosphodiesterase, ecto 5'-nucleotidase and adenosine deaminase. Indeed, the conversion of exogenous cAMP into AMP by rat ileum is reduced by IBMX as well as by DPSPX. Thus ecto-PDE plays a key role in the metabolic degradation of the cyclic nucleotide.

Inhibition of ecto-5'-NT by AOPCP hinders cAMP metabolism to adenosine and inosine, while increasing the recovery of AMP. Thus the cAMP-adenosine pathway occurs in the extracellular space.

The conversion of cAMP into AMP by ecto-PDEs appears to be the rate-limiting step for further catabolism to adenosine. In fact exogenous AMP is completely cleared from the medium, suggesting that the rate of AMP formation from cAMP may control further
catabolism. Adenosine originating from cAMP is then converted into inosine rather than taken up by the tissue through equilibrative nucleoside transporters.

References


SIF – Società Italiana di Farmacologia
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