121 THE EXCRETION OF OXALIC ACID IN GOUTY PATIENTS

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Utilizing the very satisfactory HPLC procedure recently reported by Hughes et al. (1), we have determined the urinary excretion of oxalic acid in control subjects (aged 20 to 70 years) and in gouty patients (from 35 to 73 years), before and after allopurinol treatment. The urinary excretion in normal subjects was 27.5 ± 1.89 mg/24h, in gouty patients 31.3 ± 3.08 mg/24h before allopurinol, and 43.22 ± 4.66 mg/24h after treatment. It is evident that there is no substantial difference between normal subjects and gouty patients, while allopurinol significantly increases ($p < 0.05$) the urinary excretion of oxalic acid. Since allopurinol inhibits the incorporation of glycine into purine ribonucleotides, this increase suggests that the glycine might be channeled toward the formation of glyoxylate and oxalic acid in gouty patients (2). Results suggest that the behavior of urinary oxalic acid should be under constant control during allopurinol treatment.

(1) Hughes H. et al. (1982) Anal. Biochem. 119, 1-3
(2) Dean D.M. et al. (1968) Clin. Sci. 35, 325-335

122 ADENOSINE RECEPTORS ON HUMAN BASOPHILS AND LUNG MAST CELLS

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5'-N-ethylcarboxamide (NECA) > 2-chloroadenosine (2-ClA)> adenosine (A) > (-)-N^6-(R-phenylisopropyl)-adenosine {(-)-R-PIA}> (+)-S-PIA, in μM concentrations, inhibited the release of histamine and immunoreactive leukotriene C_4 (LTC_4) from human basophils challenged with anti-IgE or antigen. The effect of NECA, 2-ClA and A was potentiated by dipyridamole and inhibited by low concentrations of theophylline (T).

In contrast, NECA, 2-ClA, A and (-)-R-PIA, in μM concentrations, enhanced the release of both histamine and LTC_4 from mast cells purified from human lung. The effect of A and its analogs was not blocked by dipyridamole and partially inhibited by T.

These results support the hypothesis that adenosine and its analogs produce distinct biological effect in the modulation of mediator release from human basophils and lung mast cells.

123 ADENOSINE RECEPTORS ON HUMAN LYMPHOCYTES

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5'-N-ethylcarboxamideadenosine (NECA) >2-chloroadenosine (2-ClA) >adenosine (A)> {(-)-N^6-(R-phenyl-isopropyl)-adenosine } (-)-R-PIA > (+)-S-PIA, in μM concentrations, increase the intracellular level of adenosine 3',5'-monophosphate (cAMP) of human B and T ($OKT4^+$ and $OKT8^+$ phenotype) lymphocytes (L). Low concentrations of A (10^{-9}-10^{-6} M) and (-)-R-PIA (10^{-9}-10^{-6} M) inhibit the stimulating effect of PGE_1, isoproterenol (ISO), histamine (H), NECA, cholera toxin (CT) and forskolin (FOK) on cAMP metabolism of L. This effect is inhibited by low doses of methylxanthines. 2',5'dideoxyadenosine (DDA) (10^{-6} - 2×10^{-4} M), a selective agonist of the P-site, and high concentrations of A (10^{-4}-10^{-3} M) inhibit the effect of PGE_1, ISO, H, CT and FOK in B and T L. The effect of DDA is not blocked by methylxanthines. These results support the hypothesis that human B and T ($OKT4^+$ and $OKT8^+$) L possess a membrane adenosine A_2/R_a receptor. Human L also possess an inhibitory adenosine A_1/R_i receptor and a P-site.

124 INHIBITION OF MAST CELL MEDIATOR RELEASE BY 5-AMINO-4-IMIDAZOLECARBOXAMIDE RIBOSIDE (AICAR).

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Stimulated mast cells produce and release adenosine, and the release of mast cell mediators is potentiated by adenosine, yet very little is known regarding mast cell purine metabolism. Because AICAR has been shown to alter metabolism of adenosine and accelerate the repletion of ATP pools in other tissues, its effect on mast cell function was examined. Simultaneous addition of AICAR (25-250μM) with A23187 or specific antigen did not alter mouse bone marrow-derived mast cell (MMC) β-hexosaminidase (β-hex) release in the absence or presence of exogenous adenosine, nor did a 60-min preincubation with AICAR. However, MMCs cultured for 6 days in the presence of 10-100μM AICAR demonstrate a slightly increased spontaneous release of β-hex and histamine, and a markedly decreased mediator release response to A23187 or antigen equal to 45.2±0.1% of release from control cells ($p < .005$) with or without the additional presence of adenosine (10^{-4}-10^{-10} M). Exposure to these concentrations of AICAR had no effect on MMC viability, rate of division, or basal β-hex concentrations. An unusual ribonucleotide triphosphate previously identified as a regulatory molecule in formyl-depleted cells, AICAR triphosphate (ZTP), has been identified in 6-day cultures of AICAR-treated MMCs at a concentration of 0.065 ± 0.002nmoles/10^6 cells (controls < 0.005nmoles ZTP/10^6 cells). A modest enhancement (~15%) of intracellular ATP levels is also seen in the AICAR MMCs. This global inhibition of MMC mediator release may prove to be important in the treatment of allergic diseases.

125 DETECTION OF mRNA FOR ADENOSINE DEAMINASE USING BIOTINYLATED PROBES.

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Adenosine deaminase (ADA), a purine salvage pathway enzyme, is a useful biomarker of disease activity in certain human leukemias. To study the mechanisms controlling ADA expression in leukemic cells we have developed an assay for quantitating ADA mRNA. mRNA is selectively immobilized on nitrocellulose and subsequently hybridized to DNA probes as described by Bresser et al. (Bressner J, Hubbell HR and Gillespie D., Proc Natl Acad Sci USA (1983) 80, 6253 ff.). We have modified the technique to allow use of biotinylated rather than ^{32}P labelled probes. Probe:mRNA hybridization was detected colorimetrically with strepavidin and biotin-alkaline phosphatase or biotin-horseradish peroxidase. The biotin and ^{32}P assays were equally sensitive.

With this technique we have compared ADA enzyme activity and mRNA levels in lymphoblastoid cell lines and lymphocytes from leukemic patients. Our data indicate that there may be both transcriptional and post-transcriptional control mechanisms for ADA. The use of biotinylated DNA sequences as hybridization probes provides a sensitive, stable, and rapid method for quantitating specific mRNA levels, and should be applicable to other systems.

126 LOW ADENINE NUCLEOTIDE POOLS IN BLOOM'S SYNDROME MAY REFLECT A DEFECTIVE SALVAGE PURINE BIOSYNTHESIS.

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A nucleotide analysis of Bloom's syndrome lymphocytes and fibroblasts has revealed a partial depletion in adenine nucleotide pools. ATP/ADP ratio in these cells was at least three-fold lower than in primary cultures of normal human cells. Fractionation of each adenine nucleotide by high performance liquid chromatography showed that differences did not reflect an excess in ADP but rather low levels of ATP in Bloom's syndrome cultures. Moreover, such low energy state has been correlated with their low growth. Recent data from our laboratory indicate that Bloom's syndrome cells may reflect an impaired salvage pathway of purine biosynthesis since these cells appear to be defective or even lack adenine phosphoribosyl transferase (APRT) activity. Our work mainly focuses on the kinetics of purine salvage pathway in Bloom's syndrome cells, as well as on drug resistance (cytotoxic purine analogs), in order to characterize the extent of the defect.