121 THE EXCRETION OF OXALIC ACID IN GOUTY PATIENTS

Enrico Marinello, Adriana Casini^o, Roberto Leoncini,
Roberto Marcolongo^{oo}, Maria P. Picchi^o & Daniela

Vannoni

University of Siena - Departments of Biological Chemistry, ^oChem-
istry, ^{oo} Rheumatology - Italy

Utilizing the very satisfactory HPLC procedure recently report-
ed by Hughes et al. (1), we have determined the urinary excre-
tion of oxalic acid in control subjects (aged 20 to 70 years) and
in gouty patients (from 35 to 73 years), before and after allopur-
inol treatment. The urinary excretion in normal subjects was
27.5 ± 1.89 mg/24h, in gouty patients 31.3 ± 3.08 mg/24h before al-
lopurinol, 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 ribonucleoti-
des, this increase suggests that the glycine might be channeled
toward the formation of glyoxylate and oxalic acid in gouty pa-
tients (2). Results suggest that the behavior of urinary oxalic ac-
id 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

G. Marone, M. Tamburini, M. Triggiani, A. Kagey-Sobotka,
L.M. Lichtenstein

Department of Medicine, University of Naples II
School of Medicine - 80131 Naples and Division of
Clinical Immunology - Department of Medicine, The
Johns Hopkins University - Baltimore, MD 21239

5'-N-ethylcarboxamide (NECA) > 2-chloroadenosine (2-ClA) >
adenosine (A) > (-)-N⁶-(R-phenylisopropyl)-adenosine {(-)-R-PIA} >
(+)-S-PIA, in μ M concentrations, inhibited the release of
histamine and immunoreactive leukotriene C₄ (LTC₄) from human
basophils challenged with anti-IgE or antigen. The effect of
NECA, 2-ClA and A was potentiated by dipyrindamole and inhibited
by low concentrations of theophylline (T).

In contrast, NECA, 2-ClA, A and (-)-R-PIA, in μ M
concentrations, enhanced the release of both histamine and LTC₄
from mast cells purified from human lung. The effect of A and
its analogs was not blocked by dipyrindamole and partially
inhibited by T.

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

123 ADENOSINE RECEPTORS ON HUMAN LYMPHOCYTES

G. Marone, R. Petracca, S. Vigorita, M. Plaut

Department of Medicine, University of Naples II School of
Medicine - 80131 Naples and The Johns Hopkins University,
Department of Medicine, Division of Clinical Immunology
Baltimore, MD 21205

5'-N-ethylcarboxamideadenosine (NECA) > 2-chloroadenosine (2-
ClA) > adenosine (A) > {(-)-N⁶-(R-phenyl-isopropyl)-adenosine} (-)
-R-PIA > (+)-S-PIA, in μ M 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 PGE₁, 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 x 10⁻⁴
M), a selective agonist of the P-site, and high concentrations
of A (10⁻⁴-10⁻³ M) inhibit the effect of PGE₁, 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
A₂/R_a receptor. Human L also possess an inhibitory adenosine
A₁/R_i receptor and a P-site.

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

Diana L. Marquardt and Harry E. Gruber, Univ. of California,
San Diego School of Medicine, Department of Med., San Diego, CA.

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 (25-250 μ M) 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 adeno-
sine, nor did a 60-min preincubation with AICAR. However, MMCs
cultured for 6 days in the presence of 10-100 μ M 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 < 0.005)
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 triphos-
phate (ZTP), has been identified in 6-day cultures of AICAR-
treated MMCs at a concentration of 0.065 ± 0.002 nmoles/10⁶ cells
(controls < 0.005 nmoles ZTP/10⁶ 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.

M.E. Marshall, L.K. Riley,
M.S. Coleman. Univ. of KY Medical Center, Depts.
of Hematology/Oncology and Biochemistry,
Lexington, KY, U.S.A.

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 ³²P
labeled probes. Probe:mRNA hybridization was detected
colorimetrically with streptavidin and biotin-alkaline
phosphatase or biotin-horseradish peroxidase. The biotin and
³²P 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.

Hector Martinez-Valdez,* Thomas C. Long, Andrew J. Andres, and
Milton W. Taylor. Indiana University, Department of Biology,
Bloomington, IN 47405.* Universidad Nacional Autonoma de Mexico,
Facultad de Medicina, Departamento de Bioquimica, 04510 Distrito
Federal MEXICO.

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.