11

CHARACTERIZATION OF NOVEL HIGH AFFINITY PURINE BASE TRANSPORT SYSTEMS IN S49 CELLS.

Joanne Beck and Buddy Ullman,
The Oregon Health Sciences University, Department of Biochemistry, Portland, Oregon, U.S.A.

Mutant cell lines have been isolated from wild type S49 cells that possess a novel high affinity purine base transport system that is not found in parental cells or any other mammalian cell line. These cells are capable of transporting purine bases at a rate 10 to 30-fold greater than wild type cells. The Km value of this novel nucleobase transporter for purine bases is exceptionally low, approximately 10-100 micromolar. The high affinity transporter does not recognize nucleosides or pyrimidine nucleobases and can be distinguished from other previously described nucleobase transporter in mammalian cells on the basis of its high affinity for ligands and its sensitivity to inhibition by DPA and NBMPR. That the expression of this high affinity purine base transport system is independent of the NBMPR- and DPA-sensitive nucleoside transport system has been demonstrated by the insertion of the high affinity nucleobase transport mutation into nucleoside transport-deficient S49 cells. Two classes of high affinity purine base transport mutants were generated that can be differentiated on the basis of their substrate specificities, affinities for purine bases, and sensitivities to inhibitors of nucleoside transport. The data suggest that the expression of the high affinity transport probably requires the unmasking or alteration of a specific genetic locus that is silent or different in wild type cells.

12

PRPP AND PURINE NUCLEOTIDE METABOLISM IN HUMAN LYMPHOBLASTS WITH BOTH PRPP SYNTHETASE SUPERACTIVITY AND HGPRT DEFICIENCY. M.A. Becker, M. Kim, K. Husain. University of Chicago, Chicago, Illinois, USA.

PRPP and purine nucleotide synthesis and culture growth in response to medium additions were compared in cloned human B lymphoblast lines selected for resistance to growth inhibition by 6-thioguanine (TG) and in the respective normal and PS-superactive parental cell lines. TG-resistant cell lines were severely deficient in HGPRT (<0.2% normal activity). Cells with both HGPRT deficiency and PS superactivity retained the kinetic defect in PS of the parental cells but had PRPP concentration 20-fold greater than normal cells and 6- and 5-fold greater, respectively, than the parental PS-superactive cells and cells defective in HGPRT alone. Nevertheless, PRPP generation in HGPRT-deficient lines was minimally increased (<8%) compared with parental cell lines and purine synthesis de novo in the cell line with both enzyme defects was comparable to that in the PS-superactive parental line.

Regardless of the status of PS activity, all HGPRT-deficient cell lines showed growth patterns typical of HGPRT deficiency in HAT medium and in medium with 8-azaguanine. However, growth of HGPRT-deficient cell lines, including the line with PS superactivity, was more sensitive than that of parental lines to inhibition of purine synthesis de novo by 6-methylthioinosine (MMPR), especially when adenine was provided, most likely reflecting compromised guanine nucleotide synthesis in HGPRT-deficient cells when purine synthesis de novo is blocked and adenine is the sole source of purine for salvage.

13

INHERITED SUPERACTIVITY OF PRPP SYNTHETASE (PS): ASSOCIATION OF PURINE OVERPRODUCTION AND SENSORINEURAL DEAFNESS. M.A. Becker, J.G. Puig, F.A. Mateos, M.L. Jimenez, M. Kim, H.A. Simmonds. Univ. of Chicago, Chicago, IL, USA; Cuidad Sanitaria La Paz, Madrid, Spain; Guy's Hospital, London, England.

In fibroblasts from an 8 year-old male (VRG) with tophaceous gout, uric acid overproduction, and sensorineural deafness, and from his gouty mother, superactivity of PS due to purine nucleotide inhibitor-resistance was found. Compared with normal cells, VRG fibroblasts showed increases in the following: PRPP concentration (3-fold) and generation (2-fold); purine synthesis de novo (2.5-fold); purine base salvage (2.6-fold); ATP and GTP concentrations (1.4-fold); and excretion of newly synthesized purines (5-fold). Derangements in PS and in PRPP and purine synthesis were attenuated in cells from the mother, suggesting that VRG is a hemizygote for an X chromosome-linked defect and his mother is a heterozygote. Comparison was made of affected males in 8 families with PS superactivity. VRG was intermediate in range of expression of PS superactivity and in severity of the enzyme defect as measured by the degree of aberration of PRPP and purine nucleotide synthesis in fibroblasts. Metabolic abnormalities were more severe in VRG than in most patients (in whom expression is limited to early adult-onset gout with uric acid overproduction), but less severe than in 2 patients with more complex defects in PS associated with uric acid overproduction and neurodevelopmental abnormalities, including deafness in hemizygous male children and heterozygous women. Within the spectrum of defects resulting in PS superactivity, certain derangements may be causally related to neurological impairment, most commonly sensorineural deafness.

14

LONG TERM EXPRESSION OF HUMAN ADENOSINE DEAMINASE IN MURINE HEMATOPOIETIC CELLS. JW Belmont, GR MacGregor, KA Moore, FA Fletcher, D Hawkins and CT Caskey. Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of medicine, Houston, Texas 77030 USA.

Twenty-two defective retrovirus vectors carrying human adenosine (ADA) coding sequences were tested for efficient transduction of human ADA enzyme activity. These vectors were designed to test functional aspects of construction including the use of internal transcriptional units, the importance of the Moloney sequences 563-1038 (gag+), and alterations in the 3' LTR. Promoters from the human ADA and cFos genes, herpes virus thymidine kinase, and cytomegalovirus immediate early region gene were tested as alternatives to the viral LTR promoter. Several vectors all containing the gag+ region allow high titer virus production. These vectors were then tested in bone marrow transplant experiments. Two vectors, one utilizing the Moloney LTR promoter and one with the HSVTK promoter, gave expression of ADA in CFU-C, CFU-S and in the blood of reconstituted animals. Infection efficiency ranged from 15-85% in CFU-S by Southern analysis. Expression efficiency was also variable. All animals had human ADA in blood 2-4 weeks post-transplant but at 10-12 weeks levels of the enzyme declined. Four of 14 long term recipients however, demonstrated expression for up to 5 months. PCR analysis on nonexpressors indicated a loss of cell with the proviral DNA suggesting that the initial expression resulted from infection of more mature progenitors. Immuno-staining is being used to characterize the lineages and distribution of ADA producing cells in the transplant recipients.

15

STIMULATION BY GLYCERATE-2,3-BISPHOSPHATE : A COMMON PROPERTY OF CYTOSOLIC PURINE 5'-NUCLEOTIDASES IN VARIOUS TISSUES. F. Bontemps, M.F. Vincent, F. Van den Bergh, G. van Waeg & G. Van den Berghe. International Institute of Cellular and Molecular Pathology, Laboratory of Physiological Chemistry, Brussels, Belgium.

Human erythrocytes contain a cytosolic purine 5'-nucleotidase (5'-Nase) (Bontemps et al., Adv. Exp. Med. Biol. 195B:283-90, 1986; Biochem. J. 250:687-96, 1988) which, similarly to other cytosolic 5'-Nases, is more active on IMP and GMP than on AMP, is stimulated by ATP and, in addition, by glycerate-2,3-bisphosphate (2,3-DPG), the main phosphate ester in human erythrocytes. Both stimulators increase activity to the same extent at saturating concentrations, but 2,3-DPG is more potent than ATP at subsaturating concentrations. To investigate if 2,3-DPG was able to stimulate cytosolic 5'-Nase in other tissues, studies were performed on high-speed supernatants, freed from small molecules by dialysis or by filtration on G-25. 5'-Nase activity was measured at pH 7.2 by release of labelled nucleosides and bases from 0.2 mM [8^{14}C]-IMP. At 5 mM, 2,3-DPG was found to stimulate the activity of cytosolic purine 5'-Nase to the same extent as 5 mM ATP, in liver, cardiac and skeletal muscle, brain, spleen and erythrocytes from rat, and in human polymorphonuclear leukocytes, mixed peripheral blood lymphocytes and platelets. Depending on the tissue, maximal stimulation varied between 2- and 14-fold. 2,3-DPG was little hydrolyzed in the various preparations and was without effect on the membranous 5'-Nase. 2,3-DPG is thus a common stimulator of cytosolic purine 5'-Nase of various tissues and can be used to identify and reveal this enzymic activity in crude preparations.

16

MECHANISM OF ATP CATABOLISM INDUCED BY DEOXYADENOSINE AND OTHER NUCLEOSIDES IN ADENOSINE DEAMINASE-INHIBITED HUMAN ERYTHROCYTES. Françoise Bontemps & Georges Van den Berghe, International Institute of Cellular and Molecular Pathology, Laboratory of Physiological Chemistry, Brussels, Belgium.

In adenosine deaminase (ADA) deficiency and during treatment with deoxycoformycin (dCF), depletion of ATP accompanies accumulation of dATP in erythrocytes and lymphocytes (Bagnara & Hershfield, PNAS, 79: 2673-77, 1982 and refs therein). Stimulation of AMP deaminase and cytosolic 5'-nucleotidase by dATP was proposed to account for this. However, both enzymes are not more stimulated by dATP than by ATP. In human erythrocytes in which ADA was inhibited with 1 µM dCF, a 3- to 30-fold stimulation of adenine nucleotide (AN) catabolism was recorded upon addition of dAdo, but also of other substrates of adenosine kinase (AK) such as Ado, Ara-A, tuberdicin and 6-MMPR. Concomitantly, there was a reproducible increase, from 10 µM up to 100 µM, of AMP and of IMP, indicating an increased activity of AMP deaminase. The effects of Ado were suppressed upon prior inhibition of AK by 10 µM iodotubercidin (ITu). Strikingly, they were also suppressed if ITu was added 2 h after dAdo, although dATP was maintained under this condition. ITu also abolished ATP catabolism induced by the other nucleosides. With all nucleosides, AMP increased during their phosphorylation and decreased after inhibition of AK. Studies with erythrocytic AMP deaminase, at low substrate and physiological concentrations of effectors, showed that, because of sigmoidal kinetics, the enzyme is very sensitive to variations of AMP between 5 and 100 µM. We conclude that elevation of AMP, secundary to phosphorylation of the nucleosides, is the main mechanism whereby they stimulate AN catabolism.