143

DeoxyGTP ACCUMULATES IN THYMOCYTES, BUT NOT IN T OR B LYMPHOCYTES IN SIMULATED PNP DEFICIENCY

H Anne Simmonds, Anna Taddeo, Lynette D Fairbanks, John A Duley.
Purine Research Laboratory, Guy's Hospital London,UK; Istituto di Reumatologia, Siena,Italy.

Deoxyguanosine (dGR) and deoxyadenosine (dAR) accumulate in PNP and ADA deficiency, associated respectively with T cell immunodeficiency or SCID. Conflicting hypotheses have been invoked from *in vitro* studies to explain this differential toxicity. It was originally proposed that T cells, but not B cells, could accumulate high dGTP and dATP levels because of a much higher kinase, but lower 5'-nucleotidase in T cells. *In vitro* studies of dGR toxicity have frequently used very high substrate levels and been complicated by the lack of an effective PNP inhibitor. Several reports have shown that the toxicity demonstrated related to GTP rather than dGTP accumulation.

We used guanosine (GR) to inhibit PNP and have investigated the metabolism of [^{14}C]dGR in peripheral blood cells *in vitro* at levels approximating those found *in vivo* in PNP deficiency. We found that thymocytes accumulated some dGTP even without inhibitor and high levels of dGTP following PNP inhibition. Moreover, they were the only cells to do so to any significant degree. This contrasts with our studies using dAR which showed dATP accumulation by both thymocytes and B cells. The exclusive ability of thymocytes to accumulate dGTP provides a logical explanation for the selective toxicity to T cells in PNP deficiency. The rapid metabolism of dGR to GTP in the absence of inhibition, and subsequent effects of GTP on cell metabolism, may also account for the differing results reported in toxicity studies using low *versus* high dGR concentrations.

144

LESCH NYHAN SYNDROME AND HPRT VARIANTS:Study of heterogeneity at the gene level

Surjit Singh, I.Willers, K.held, H.W.Goedde
Institute of Human Genetics, Univ. of Hamburg Butenfeld 32, D-2000 Hamburg 54. F.R.G

A sample of 15 from our collective of 30 patients with lesch nyhan syndrome and its variant forms, which were previously studied for enzyme kinetics,immuno CRM profiles and selection in various growth media (Singh et al 1985-previous meeting) were further examined by molecular genetic techniques. DNA and RNA from cultured fibroblasts and blood were subjected to southern or northern blot analysis with full length cDNA of HPRT.§. Four patients showed abnormal patterns with PstI in the southern blots(enzymes used included TaqI,HindIII,PstI, BglII,BamHI,EcoRI) which could be interpreted as deletions of various exons of the HPRT gene.Two of these are interesting novel mutations involving exon 2 and3, and 6 to 9. In some other mRNA positive cases the RNase A mapping was attempted with success revealing a small deletion or a point mutation. The results point to a large heterogeneity of lesions in the HPRT gene.

§Thanks are due to Prof. C.T.Caskey and Dr.J.T.Stout and Dr.R.Gibbs (Dept.of Molecular Genetics, Houston) for the initial help and the Deutsche Forschungsgemeinschaft for the financial support .

145

MYCOPHENOLIC ACID INDUCED AMPLIFICATION AND MUTATION OF INOSINATE DEHYDROGENASE IN MOUSE NEUROBLASTOMA CELLS.

Floyd F. Snyder, Stephen D. Hodges and Ernest Fung. University of Calgary, Departments of Pediatrics and Medical Biochemistry, Calgary, Alberta T2N 4N1, Canada.

Mouse neuroblastoma cells (NB) have been adapted for growth in 1 mM mycophenolic acid (NB-myco) by incremental increases in drug concentration with each passage without mutagenesis. 50% growth inhibition occurred at 0.1 uM and 1 mM for NB and NB-myco cells respectively representing a 10,000-fold increase in resistance. There were increases in IMP dehydrogenase activity, 25-fold, and a 56.7K dalton protein, 200-500 fold, in NB-myco as compared to NB cells. The resistant phenotype was also unstable. In the absence of drug there were decreases in the 56.7K dalton protein, 4-fold over 90 days, and IMP dehydrogenase activity, 3-fold over 80 days. These findings are characteristic of amplification, others are indicative of a mutation. The substrate Km's were unchanged but the Ki's have increased in the NB-myco cells: 2400-fold for mycophenolic acid, and 4-fold for XMP. The kinetic studies are consistent with an ordered bi bi reaction where IMP binds first and XMP is released last; mycophenolic acid and NAD$^+$ exhibit uncompetitive inhibition with IMP. Guanine nucleotide synthesis was also perturbed. The ATP concentration was unchanged, GTP and UTP were increased 2- and 1.5-fold respectively in NB-myco cells as compared to NB cells. Upon removal of mycophenolic acid, ATP did not change, but within hours GTP increased to 4.5-fold that of NB cells.

Supported by the Medical Research Council of Canada grant MT-8665.

146

MOUSE MODELS OF PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY. CHARACTERIZATION OF PARTIALLY AND SEVERELY ENZYME DEFICIENT MUTANTS.

Floyd F. Snyder, Ellen Mably and Ernest Fung. University of Calgary, Departments of Pediatrics and Medical Biochemistry, Calgary, Alberta T2N 4N1, Canada.

Two mutations of purine nucleoside phosphorylase (PNP) were identified in the carrier state of the first generation progeny of male mice treated with ethylnitrosourea mated to untreated females. The variants are assigned the gene symbols $Np\text{-}1^e$ and $Np\text{-}1^f$. Both carriers have approximately half normal PNP activity in erythrocytes and $Np\text{-}1^a/Np\text{-}1^e$ was distinct from the inbred strain background, $Np\text{-}1^a/Np\text{-}1^a$ on isoelectric focusing, whereas $Np\text{-}1^a/Np\text{-}1^e$ was not distinguishable. In the homozygous state, $Np\text{-}1^e$ and $Np\text{-}1^f$ differ and determine a more basic pattern of PNP activity than the $Np\text{-}1^a$ allele. NP-1A is stable in the presence of phosphate at 55^o C whereas the half lives for the mutants were 30 and 7.5 min for NP-1E and NP-1F respectively. The substrate Michaelis constants for the variants were unchanged from controls and the maximal velocities for erythrocytes were: 16.8 ± 1.1, NP-1A; 2.16 ± 0.12, NP-1E; and 0.50 ± 0.03, NP-1F (nmole/min/mg protein). Brain, heart, kidney, liver, spleen leukocytes and thymocytes also showed reductions in activity, 4-27% of normal for NP-1E and 0.1-3.9% for NP-1F. Purine nucleoside excretion correlated with the severity of the enzyme deficiency. The substrates of PNP are not found normally in urine, < 10 uM, but inosine and guanosine were present for $Np\text{-}1^e/Np\text{-}1^e$ mice, total 150 ± 84 uM; as were inosine, guanosine, deoxyinosine, and deoxyguanosine for $Np\text{-}1^f/Np\text{-}1^f$ mice, total 1490 ± 190 uM.

Supported by the Medical Research Council grant MT-6376.

147

CONDITIONS AND LIMITATIONS FOR DIRECT mRNA SEQUENCING USING OLIGONUCLEOTIDE PRIMERS.

Rahul Joshi and Floyd F. Snyder. University of Calgary, Departments of Pediatrics and Medical Biochemistry, Calgary, Alberta T2N 4N1, Canada.

The feasibility of direct mRNA sequencing for the purpose of characterizing mutations of relatively low abundance messages was examined. A 906 base pair PstI fragment corresponding to the coding region of hypoxanthine phosphoribosyltransferase (HPRT) cDNA was inserted into a pGEM plasmid and HPRT RNA was generated in quantity. Varied amounts of HPRT RNA were diluted with 12 ug of human lymphoblast poly A+ RNA. A 20-base oligonucleotide primer, complementary to HPRT mRNA, and RNA were heated to 80^o C for 3 min, hybridized for 45 min at 54^o C and subjected to Sanger dideoxy sequencing reactions. dNTP-labelled sequencing produced reliable results at a message abundance of greater than 0.1%, but was marred by anomalous banding and high background at lower abundances. Primer-labelled sequencing produced reliable sequence at a message abundance of 0.05%. The minimal message requirement for the primer-labelled sequencing method was determined by using between 3 and 50 ug of poly A+ RNA in the sequencing of HPRT mRNA (~ 0.01% of poly A+ mRNA) and phosphoglycerate kinase (PGK) mRNA (~ 0.05% of poly A+ mRNA). Reliable sequence was obtained from 0.01 pmole of either PGK or HPRT message. The region of interest in a PGK variant previously characterized as a single amino acid substitution was sequenced. Direct sequencing is indicated to be useful for messages whose abundance is equal or greater than 0.05%.

Supported by the Medical Research Council grant MT-8665.

148

PATHWAYS OF ATP CATABOLISM IN PRIMARY RAT CARDIOMYOCYTE CULTURES.

E. Zoref-Shani*, G. Kessler-Icekson+ and O. Sperling+* Tel-Aviv Univ Med School, *Dept of Chemical Pathology, Tel-Aviv, and +Rogoff-Wellcome Med Research Inst and +Dept of Clin Biochem Beilinson Med Center, Petah-Tikva, Israel.

The pathways of ATP catabolism were investigated in cultured beating cardiomyocytes. The activity of the enzymes involved in AMP degradation was assayed in cell extracts, whereas fluxes of label from ATP to the various purine derivatives were measured in intact cells. Under physiological conditions, in accord with a 7 fold higher activity of AMP deaminase than that of AMP 5'-Nucleotidase, cells degraded AMP through deamination to IMP. Mild ATP degradation, induced by inhibition of glycolysis by iodoacetate, caused no alterations in the degradation pathways (more than 85% through deamination to IMP). However, fast ATP degradation (83% of adenine nucleotides/10 min), induced by simultaneous inhibition of glycolysis and electron transport (by antimycin A), caused increased dephosphorylation of AMP to adenosine (50% of total AMP-degradation). IMP was rapidly degraded to inosine, hypoxanthine, xanthine and uric acid, which were effluxed from the cells. The cardiomyocyte extracts were found to contain a significant activity of PNP. Despite the presence of HGPRT, salvage of hypoxanthine to IMP, both at physiological as well as at conditions associated with ATP degradation, was slow. The salvage of adenosine appeared to be efficient at physiological conditions, but not at fast rates of ATP degradation.