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ADENOSINE RECEPTORS IN HPRT-DEFICIENT NEUROBLASTOMA CELLS AND IN LYMPHOBLASTOID CELLS FROM LESCH-NYHAN PATIENTS. Roberta M. Palmour, Kathy

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Previously we found alterations of adenosine (AR) receptors in HPRT deficient murine neuroblastoma cells characterized by increased cAMP accumulation after AR stimulation, decreased sensitivity to methylxanthines and lower levels of purines and nucleosides relative to parental HPRT+ cells. Using ³H-N-ethyl-carboxamide (NECA) as a radioligand in a receptor binding method we have now shown altered number and affinity of AR receptors. At all stages of the cell growth curve HPRT- cells have more AR receptors/mg protein, but affinity varies from HPRT+ cells in a complex fashion, which may depend partly on accumulation of purine metabolites in the medium.

Recently we have found A2 type AR receptors on the surface of long-term human lymphoblastoid cells, which respond to NECA stimulation with an increase in cAMP. One lymphoblastoid cell line from a Lesch-Nyhan patient had increased AR binding sites and increased cAMP response. If confirmed with other cell lines these data would suggest that alterations of AR receptors may be a result of HPRT deficiency and may play a role in symptoms of Lesch-Nyhan disease (Supported in part by grants from the Medical Research Council of Canada).

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DOPAMINE DEPLETION AND SELF MUTILATION IN THE RAT: A MODEL FOR LESCH-NYHAN DISEASE. Roberta M. Palmour, Kathy Schucher, Christopher Lipowski and Frank R.

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Because dopamine depletion occurs in specific brain regions in Lesch-Nyhan disease, we have looked at the effect of pemoline on self mutilation in rats with regional brain dopamine depletion produced with 6-hydroxydopamine (6OHDA). The efficacy and correct positioning of all lesions were confirmed postmortem by HPLC analysis of catecholamines and their metabolites. Locomotion, rearing, licking, gnawing, biting, severity of self mutilation and latency to self mutilation were scored quantitatively each h after an 18-h observation period.

By comparison to controls, animals lesions in the caudate or amygdala showed increased locomotion, while rats given icv 6OHDA showed decreased locomotion. Although temporal patterns of stereotypy varied, there were no significant variations in incidence of various stereotypics between groups. Controls in these series showed 50% incidence of self mutilation with a latency (lat) of 5.7 h and a mean severity (ms) of 2. Incidence of self mutilation was 100% for caudate lesions (lat=8.3h, ms=1.8), 61% for nucleus accumbens lesions (lat=6.1 h, ms=1.4), 88% for amygdala lesions (lat=5.8 h, ms=2.7), and 82% for ventricular lesions (lat=5.5 h, ms=1.4). More detailed studies show that a lesion in the lateral amygdala promotes self mutilation, while a lesion in the medial amygdala inhibits it. Thus dopamine depletion may contribute to behavioral sequelae of Lesch-Nyhan disease (Supported in part by grants from the Medical Research Council of Canada).

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ANALYSIS OF THE TRANSCRIPTIONAL REGULATORY SEQUENCES IN THE HAMSTER

APRT GENE. Joo Hung Park, De-chu Tang, and Milton W. Taylor. Department of Biology, Indiana University, Bloomington, Indiana 47405

The 5' region of the hamster aprt gene has been sequenced and the transcriptional initiation site determined by S1 nuclease mapping. No canonical TATAA sequence is found upstream of the hamster aprt gene. A series of deletion mutants have been constructed and their expression in CHO or mouse aprt⁻ cells have been analyzed by S1 mapping and enzymatic activity. We have also found that the hamster aprt gene is resistant to increased expression due to an adjacent retroviral enhancer. We are currently trying to dissect out the signals responsible for such enhancer resistance.

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STUDIES ON THE ORGANIZATION AND STRUCTURE OF GENES AND ENZYMES OF PURINE SYNTHESIS IN ANIMALS AND MAN. David Patterson¹, Steven Henikoff²,

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We have reported the isolation of mutants of Chinese hamster cells defective in 11 of the 12 steps of AMP synthesis which have been used to assign genes coding for enzymes of purine synthesis to various human chromosomes. We have now obtained direct evidence that in animals and probably in humans, 3 of the purine biosynthetic enzymes, phosphoribosylglycineamide (GAR) synthetase, GAR formyltransferase, and phosphoribosylaminoimidazole (AIR) synthetase, previously reported as defective in Ade⁻C, Ade⁻E, and Ade⁻G mutants respectively, are encoded by a single genetic locus and contained on a single polypeptide. In humans, this locus appears to be on chromosome 21. GAR synthetase and AIR synthetase, and probably GAR formyltransferase, are genetically linked in organisms as diverse as Drosophila and human. Ade⁻E mutants of CHO cells, originally reported as unable to carry out GAR formyltransferase activity, are defective in the trifunctional protein required for 10-formyl FH₄ synthesis and not GAR formyltransferase activity. We are attempting to isolate the human genes coding for these enzyme activities and to purify the proteins to understand their structure, function, and regulation. This work was supported by NIH AG 00029 and NIH HD 13432. This is ERICR contribution #562.

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CYTIDINE DEAMINASE DEFICIENCY AND IMMUNODEFICIENCY: MORE THAN A COINCIDENCE? Jean-Louis Pérignon, Françoise Le Deist, Fernando Arenzana-Seisdedos, Laure

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A 9 month-old boy, the fourth and the only surviving child of consanguineous parents of tunisian origin, was investigated for severe immunodeficiency associated with autoimmune anemia and thrombocytopenia. Increased serum immunoglobulin levels contrasted with an absence of antibody response after sensitization and blood T lymphocytes increased in number were incapable of proliferating normally in the presence of mitogens, antigens or of OKT3 antibody. Defective secretion of IL2 and of IFN_γ by patient's leukocytes was observed whatever the inducer used. Proliferative response as well as IFN_γ secretion were restored by incubation in the presence of exogenous IL2. Enzyme study disclosed a profound deficiency in cytidine deaminase (CDA) in the patient's lymphocytes (10-21-23 pmol.min⁻¹.10⁻⁶ cells, normal value 130 ± 30, n = 39). Low values were found in the father (20-21) and the mother (18-55). Cytidine (100 μM) did reverse the inhibitory effect of pyrazofurin on parents' lymphocytes PHA-induced proliferation but not that of patient's lymphocytes. During a 6-months survey lymphocyte CDA activity of the patient, his father and mother rose to 63, 140 and 130 pmol.min⁻¹.10⁻⁶ cells, respectively. The fact that CDA deficiency has been transitory in the parents rules out an hereditary enzymatic defect. Nevertheless, our results suggest that CDA activity should be systematically determined in immunodeficient patients.

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MODULATION OF CYTOTOXICITY AND METABOLISM OF 5-FLUOROURACIL (FU) IN TWO INTESTINE CELL LINES.

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Initial FU metabolism is catalyzed by either pyrimidine phosphorylase (PNP) or orotate phosphoribosyltransferase (OPRT). The human colon carcinoma cell line WiDr is 3 times more sensitive to FU and 5 times more sensitive to 5'deoxy-5-fluorouridine than the intestinal cell line Intestine 407. However, in both cell lines PNP activity with either ribose-1-P (Rib-1-P) or deoxyRib-1-P (dRib-1-P) is comparable with OPRT activity (1-2 nmol/hr per 10⁶ cells). Modulation of the availability of co-substrates with purine nucleosides might elucidate the mechanism of action of FU. PNP activity with inosine as co-substrate was 30 and 86% in WiDr and Intestine 407, and with deoxyinosine 7 and 19%, respectively, of the activity with Rib-1-P and dRib-1-P. Inosine increased the Rib-1-P levels 2-4-fold, while deoxyinosine enhanced dRib-1-P levels several fold. Inosine did not show a synergism with FU in growth-inhibition. Deoxyinosine at 0.1-1 mM showed synergism with FU at non-toxic concentrations (0.1-0.5 μM). Deoxyinosine itself was not toxic. The synergism was greater with Intestine 407 cells than with WiDr. Examination of the medium at 24 hr showed that in both cell lines deoxyinosine was rapidly broken down to hypoxanthine (at 0.1 mM for 80% in Intestine 407 and 50% in WiDr). Growth inhibition could be reversed by 2-10 μM thymidine in Intestine 407, but only partly in WiDr cells. The results show that low non-toxic concentrations of FU can be made toxic by supplementary dRib-1-P. The effect of thymidine shows that inhibition of thymidylate synthase differs in the cell lines.