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HERPES SIMPLEX VIRUS (HSV-1) MEDIATED HUMAN HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE (HPRT) GENE TRANSFER INTO NEURONAL CELLS.

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Complete deficiency of HPRT results in a devastating neurological disease, Lesch-Nyhan syndrome. We have constructed two vectors derived from HSV-1 containing expressible human HPRT cDNA, designated HSV-HP40 and HSV-HP87. HPRT deficient cultured rat neuronal cells infected with these vectors express human HPRT at levels comparable to those in wild type rat neuronal cells. Stable transformation to the HPRT^{+} phenotype was observed in 10^{-3} to 10^{-4} of infected cells. These vectors were slightly less cytopathic than wild-type HSV-1 (strain KOS). We are attempting to reduce viral cytopathic effect by UV-irradiation of the recombinant virions which renders HSV-1 replication defective while maintaining transforming capacity. These experiments demonstrate the feasibility of HSV-1 mediated HPRT gene transfer into neuronal cells. In vivo infection of mice is currently in progress.

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HYPOXANTHINE ACCUMULATION AND DOPAMINE DEPLETION IN LESCH-NYHAN DISEASE: ANIMAL MODELS AND HUMAN STUDIES

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In LND, Hx is elevated, especially in brain and CSF. Since Hx accumulation may promote lipid peroxidation and since similar mechanisms may subserve 6-OHDA neurotoxicity, we considered a simple relationship between Hx accumulation and DA depletion in patients with LND. Cannulas packed with solid Hx were implanted in caudate nucleus of rats; turning behavior was stimulated by injection of apomorphine. Treated animals show pronounced time and dose-dependent ipsilateral turning, which peaks 14-18 days after cannula implantation. Turning is blocked or reduced by haloperidol, a general DA antagonists and by SCH 23390, a selective D1 antagonist. Uric acid, purines, nucleosides, catecholamines and metabolites and 5HIAA were monitored by HPLC of brain regions. Depletion of DA was inversely related to purine concentration at peak times of behavioral effect. NE was not reduced in limbic or corticol forebrain. DA receptors were increased 1.3-1.7x in treated caudate. Allopurinol did not block the development of DA depletion. In fact, cannulas packed with allopurinol elicited similar behaviors.

In a preliminary clinical trial, we tested the ability of encapsulated and immobilized xanthine oxidase to reduce circulating purines. A single HPRT-null patient (age 18 monghs) stopped allopurinol therapy 3 days before admission to the study. Over a 10 day period of microcapsule administration, his UA/Creat ratio returned to 1.9 and his plasma Hx level fell from 190 uM to 100 uM.

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COORDINATE REGULATION OF A1 AND A2 ADENOSINE RECEPTOR IN CULTURED NEUROBLASTOMA AND LYMPHOBLASTOID CELLS

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In a variety of tissues, both A1 and A2 receptors have been demonstrated; in several instances they facilitate opposing physiological effects. We have found both A1 and A2 receptors on the surface of cultured murine neuroblastoma (NB) cells. A1 receptors predominate in B and A2 receptors in T lymphoblastoid (LB) cells. Many studies have shown upregulation of A1 receptors by methylxanthines (MXs), while a few have suggested that carbamazepine (CBZ) acts as an A1 antagonist. The present investigation demonstrates that A2 receptors are also upregulated in NB cells and in T LB cells either by acute (16 hr) or chronic (1-3 week) exposure to MX. The effects were time and dose dependent, and are stimulated as well by CPT, a presumed A1 antagonist. Binding studies show an increase in number of receptors, but no change in affinity. The physiological balance between A1/A2 effects is thus primarily a function of the concentration of agonist present in these isolated cell systems.

There is also evidence to suggest that drugs may shift the balance of A1/A2 actions in clinical situations. We show that ethanol acutely increases the sensitivity of A2 receptors; chronic ethanol diminishes it. Chronic effects are not reflected in increased binding sites. Contrary to published reports, CBZ (a widely used anticonvulsant) facilitates actions of ADO at A2 receptors, but inhibits A1 response.

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MOLECULAR GENETICS OF MAMMALIAN UMP SYNTHESIS

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To study regulation of pyrimidine biosynthesis in mammalian cells we isolated a series of Chinese hamster ovary(CHO) cell mutants defective in the enzymatic steps required for UMP synthesis. Urd-A mutants are deficient in CAD, the multifunctional protein catalyzing the first 3 activities of the pathway, Urd-B mutants are deficient in the 4th step of the pathway, DHO dehydrogenase, and Urd-C mutants are deficient in UMPS, the multifunctional protein catalyzing the last two steps of UMP synthesis. Urd-C mutants are functionally equivalent to cells from human patients with orotic-aciduria. The human Urd-A locus maps to human chromosome 2 and Urd-C to chromosome 3. To analyze mutations in these genes we isolated separate CHO cell mutants in which the CAD and UMPS genes are amplified by ca. 25-fold. We fused these two mutants to create a hybrid cell line in which both CAD and UMPS are amplified. We used these amplified lines to construct both cDNA (in lambda gt 10) and genomic (in lambda EMBL-3) libraries. From these libraries we isolated a clone which contains a 1.9 kb UMPS cDNA, a clone of greater than 3.0 kb which appears to be a CAD cDNA, and in addition several genomic clones which probably contain at least part of the CHO UMPS gene. We have initiated experiments using these clones to study regulation of UMP synthesis and the nature of the defects in our various mutants. In one Urd-A mutant, mRNA levels are markedly reduced, but transcriptional elongation rate as measured by nuclear run on is not reduced.

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QUALITATIVE AND QUANTITATIVE STUDIES ON THE NUCLEOTIDES OF INTESTINAL MUCOSA.

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The conversion of hypoxanthine and xanthine to urate via xanthine oxidase (Xanthine: oxygen oxidoreductase (E.C. 1.1.3.22.) may when ischaemic tissues are re-oxygenated generates radical oxygen species. Such ischaemia may be involved in the adverse effects of non-steroidal anti-inflammatory drugs (NSAIDS) on the human intestine where high levels of xanthine oxidase are known to be present. However little qualitative or quantitative information concerning the nucleotide content of intestine is available.

We have determined the nucleotide profiles in freeze-clamped sections of rat intestine (stomach, jejunum, ileum and colon) and for comparison liver and heart by anion-exchange HPLC and quantified the individual nucleotides. The mean of 10 analyses are summarised below

	ATP	ADP	AMP	GTP	GDP	UTP	CTP	nmol/g wet wt
Jejunum	1936	616	252	444	213	150	30	
Liver	2930	960	222	400	90	260	99	
Heart	3210	690	120	115	42	trace		

The results suggests that the intestine is a major site of nucleotide metabolism with nucleotide concentrations equivalent to liver rather than heart. A slight concentration gradient down the gut was observed. During short-term absorption studies using saline, glucose, acetate and citrate neither energy charge nor absolute levels of nucleotides changed significantly. Rapid degradation of nucleotides was induced by warm ischaemia and NSAID's.

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OPTIMISED CONDITIONS FOR THE ROUTINE HPLC SEPARATION OF NUCLEOTIDES IN CELL EXTRACTS

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High Performance Liquid Chromatography is the accepted method for the study of nucleotides in cell and tissue extracts. A variety of chromatographic separations have been described but to date the use of microparticulate anion exchange procedures remains the most popular. However the accurate analysis of cell extracts requires careful attention to both sample preparation and chromatographic operation. This study records the results of some 14 years experience and many thousands of analyses.

Optimal conditions for the acid extraction of cellular nucleotides to maximise recovery, minimise nucleotide interconversions, maximal removal of protein and excess acid extractant have been defined. For RBC's these are 1 part cells to 2.5 parts 12% TCA with acid removal with ether or Alamine /Freon. Protein removal was 99.8% efficient Cyclic nucleotides e.g cXMP are excellent internal standards. For the reliable separation of standard, normal and abnormal cell extracts by anion exchange chromatography over time with any one column, adherence to the following conditions was critical. Buffer pH needs to be adjusted to maintain difficult separations e.g. ADP/NADP, GMP/IMP, buffer molarity should be regularly reduced to counter column ageing and maintain resolution and potential interferences such as EDTA, Ficol-hypaque should be avoided.