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ANTI-5'-NUCLEOTIDASE ANTIBODIES CAUSE HUMAN PERIPHERAL BLOOD T CELLS TO PROLIFERATE. Linda F. Thompson, Julie M. Ruedi, and Martin G. Low. Scripps Clinic and Research Foundation, Dept. of Immunology, La Jolla, CA, and Columbia University, Dept. of Physiology and Cellular Biophysics, New York, NY USA.

Human peripheral blood T cells were stimulated to proliferate when cultured with submitogenic doses of phorbol myristate acetate (PMA) and goat antibodies to ecto-5'-nucleotidase (ecto-5'-NT). The degree of proliferation, as measured by ^{3}H-thymidine incorporation on day 3, was similar to that achieved by stimulation with phytohemagglutinin (PHA). Anti-5'-NT antibodies had no effect on PHA-induced proliferation. Maximal stimulation was achieved with 0.6-1.0 ng PMA/ml and 150 µg/ml of IgG isolated from a goat anti-5'-NT antiserum. Both intact IgG and $F(ab')_2$ fragments were stimulatory. IL-2 receptor expression and IL-2 secretion were also induced by anti-5'-NT antibodies and PMA. As expected, anti-5'-NT antibodies and PMA did not induce the proliferation of ecto-5'-NT$^-$ T cells isolated by cell sorting. Pretreatment of total T cells with phosphatidylinositol (PI)-specific phospholipase C removed 80% of the ecto-5'-NT activity from the cell surface and also inhibited the ability of the cells to proliferate in response to anti-5'-NT antibodies and PMA by 84%. Thus, the activation signal provided by anti-ecto-5'-NT antibodies is apparently transduced by a form of the enzyme which is attached to the membrane via PI-linkage. These data suggest that ecto-5'-NT may play a role in lymphocyte activation as has been proposed for other PI-linked lymphocyte surface proteins including Thy-1, T cell activating protein, TAP, and the rat alloantigen RT-6.

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PURINE NUCLEOTIDES SYNTHESIS DURING TERMINAL DIFFERNTIATION

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Changes of PRPP-related enzyme activities and incorporation rates of glycine, Hx and Ad into acid soluble fraction (ASF) of HL60 cells were studied during terminal differentiation induced by incubation with 1.6% DMSO for 48 hours. Activities of HPRT, APRT, and PRPP synthetase were determined in homogenate treated with activated chacoal. H(A)PRT activities were measured based on the determination of conversion of ^{14}C-labeled Hx (Ad) to IMP (AMP). PRPP synthetase activities were measured as follows; PRPP, which was formed by interaction of R-5-P, ATP and enzyme solution, was measured by HPRT assay system. Incorporation rates of Ad, Hx, and glycine into purine nucleotides were measured after incubation at 37 °C for 20 min in the presence of the respective ^{14}C-labeled compound in HB101 medium. ASFs of cell pelletes, which were obtained by silicon oil procedure, were chromatographed. Radioactivities of all purine nucleotides were counted. HPRT and APRT activities increased 3.3 folds (4.69 to 15.70 nmol/min/10^6cells) and 1.5 folds (4.28 to 6.26) higher, respectively, but no increase in PRPP synthetase activities was shown. The incorporation of glycine decreased 2.2 folds (215 to 99 pmol/min/10^6cells) lower in rate, but those of Ad and Hx showed no remarkable changes. It was concluded that the decreased rate of de novo synthesis was a major change during terminal differntiation.

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Transport and intracellular metabolism of fluorinated pyrimidines.

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Transport and intracellular metabolism of the three fluorinated pyrimidines, FU, FUR and FUdR in normal human RBCs, L-1210, HL-60, CCRF-CEM and Hela cells were studied. The cells were suspended in HB 101 medium and incubated at 37°C in the presence of each labelled compound. At the desingated time, the cell suspension was put onto silicone oil layer (specific gravity:1.034) and centrifuged at 12,000xg in 7 sec. The radioactivity of cell pellet obtained was counted following lysis with NCS tissue solubilyzer. The acid-soluble fraction from cell pellet was applied onto silica gel TLC plate and chromatographed. The plate was cut into 0.5cm strips and their radioactivities were counted. All three compounds showed the same velocity of transport by RBCs. FUR transport velocity was, however, markedly high in case of L-1210, HL-60, CCRF-CEM cells and not in Hela cells. FU and FUdR transports remained at low level. These results showed that intracellular phosphorylation of the compound resulted in the acumulation in cells since FUR was preferentially converted to nucleotide form, while FU and FUdR were not in all cells studied. The chromatogram of FUdR treated CCRF-CEM and Hela cells revealed that the main radioactive peak coinsided with FU. It was concluded that FUR acumulated as its nucleotide form, while FUdR was mainly converted to FU in these cells.

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BIOCHEMICAL GENETIC ANALYSIS OF NUCLEOSIDE TRANSPORT IN LEISHMANIA DONOVANI PROMASTIGOTES

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A rapid sampling kinetic technique has been used to evaluate the nucleoside transport functions of Leishmania donovani. The results indicate that Leishmania donovani promastigotes possess two independent purine nucleoside transporters of broad but non overlapping substrate specificity. The first transports inosine, guanosine and their analogs, while the second recognizes adenosine, analogs of adenosine, and the pyrimidine nucleosides, uridine, cytidine, and thymidine. Mutant strains of Leishmania have been generated that are genetically deficient in their expression of either of the two nucleoside transport systems. The apparent K_m values of the two nucleoside permeases for their purine nucleoside substrates were 0.3 - 0.6 micromolar, approximately two orders of magnitude lower than the Km values of the mammalian nucleoside transporter for these nucleosides. Wild type Leishmania were capable of concentrating purine nucleosides from the medium and converting them to the nucleotide level with great efficiency and rapidity. Inosine and adenosine transport could be distinguished by different sensitivities to sulphydryl reagents suggesting structural differences between the two carriers. Both nucleoside transporters were virtually refractory to inhibition by NBMPR and DPA, two potent inhibitors of nucleoside entry into mammalian cells. This latter observation has important chemotherapeutic implications for the treatment of diseases of parasitic origin.

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GENETIC ANALYSIS OF 2',3'-DIDEOXYCYTIDINE METABOLISM IN HUMAN CEM T LYMPHOBLASTS

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2',3'-Dideoxycytidine (ddC) is known to inhibit the in vitro infectivity and growth of HTLV-III/LAV, the causative agent of acquired immunodeficiency syndrome (AIDS). The cellular determinants that mediate the action of ddC in cultured human CEM thymic lymphoblasts were analyzed by somatic cell genetic approaches. Whereas ddC at a concentration of 5 micromolar inhibited the growth of wild type CEM cells by 50%, two nucleoside transport-deficient clones were 4-fold resistant to the pyrimidine analog. A deoxycytidine kinase-deficient CEM cell derivative was completely refractory to growth inhibition by 1 millimolar ddC. An 80% diminished rate of ddC influx into the two nucleoside transport-deficient cell lines could account for their resistance to the dideoxynucleoside, while the resistance of the deoxycytidine kinase-deficient cells to ddC toxicity could be explained by a complete failure to incorporate ddC in situ. NBMPR and DPA, two potent inhibitors of nucleoside transport in mammalian cells, mimicked the effects of the genetic deficiency in nucleoside transport with respect to ddC toxicity and ddC incorporation. These data indicate that the intracellular metabolism of ddC in CEM cells is initiated by the nucleoside transport system and deoxycytidine kinase and has important implications for the design of biochemically rational chemotherapeutic regimens exploiting antiretroviral nucleoside analogs in the treatment of AIDS.

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INFLUENCE OF SUCCINYLPURINES ON THE BINDING OF ADENOSINE TO A PARTICULATE FRACTION OF RAT CEREBRAL CORTEX. M. Françoise Vincent & Georges Van den Berghe, International Institute of Cellular and Molecular Pathology, Laboratory of Physiological Chemistry, Brussels, Belgium.

Adenylosuccinase (ASase) deficiency provokes accumulation in body fluids of succinyl-adenosine (S-Ado) and SAICAriboside, the dephosphorylated derivatives of the two substrates of the enzyme. The concentration of both compounds reaches about 100 µM in cerebrospinal fluid, i.e. 10- to 20-fold that in plasma. As an attempt to elucidate the mechanisms of the psychomotor retardation, accompanied by autistic features, recorded in ASase deficiency, interference of S-Ado and SAICAriboside with the binding of adenosine (Ado) to its cerebral receptors was investigated. The binding of 1-2.5 nM [2-^{3}H]Ado to a particular fraction of rat cerebral cortex, in the presence of 0.25-1 µM deoxycoformycin, was diminished by 56 ± 4 % (mean ± SEM, n=6) by unlabelled Ado (2.5-100 µM). Diminution of binding by ligands for A_1 receptors was 24 % with 2.5 µM 2-chloroadenosine, 32 % with 25 µM R-N^6-phenylisopropyl-Ado (PIA), 29 % with 25 µM N^6-cyclohexyl-Ado (CHA) and 36 % with 25 µM N^6-cyclopentyl-Ado (CPA). 200 µM 5'-N-ethylcarboxamide-Ado (NECA), a ligand for A_2 receptor sites, and 100 µM dipyridamole, a ligand for uptake sites, decreased binding by, respectively, 39 and 33 %.

In contrast, neither S-Ado nor SAICAriboside, both at up to 200 µM concentrations, influenced the binding of [2-^{3}H]Ado. These results indicate that the psychomotor defects observed in ASase deficiency are probably not due to the occupation of cortical membrane sites for Ado by the succinylpurines.