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EVIDENCE FOR A MEMBRANE ADENOSINE RECEPTOR IN *LEISHMANIA MEXICANA MEXICANA* (WR224). Brian D. Hansen, J. Perez-Arbelo and Peter K. Chiang.

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Plasma membranes from the promastigote and amastigote forms of *Leishmania mexicana mexicana* were examined for the presence of an adenosine receptor. Specific binding of selected adenosine receptor ligands was tested for modulation of membrane associated adenylate cyclase activity. Time course experiments utilizing amastigote and promastigote membranes demonstrated that total binding of all adenosine receptor ligands equilibrated within 20 min. All incubations of membranes were conducted for 30 min. Specific, nonspecific and total binding of ³H-cyclohexyladenosine (CHA) and ³H-methyl-2-phenylethyladenosine (PIA) to promastigote and amastigote membranes over ligand concentrations of 8 to 400 nM were determined. Scatchard plot analysis of the specific binding data indicated single binding sites for both CHA and PIA with K_d values of 75 nM and 200 nM respectively for promastigotes and 55 nM and 175 nM respectively for amastigotes. Membranes were also found to possess adenylate cyclase activity which could be readily inhibited in the presence of increasing concentrations of adenosine, PIA, and CHA (0 to 1 mM). The rate of promastigote replication and transformation to the amastigote form and transformation of the amastigote to the promastigote stage could all be significantly increased in the presence of the adenosine receptor ligands. This receptor mechanism may therefore act to modulate the rate of cell replication and life cycle transformation.

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PURIFICATION OF MAMMALIAN GLYCINAMIDE RIBONUCLEOTIDE (GAR) SYNTHETASE. Robert G. Hards, Sharon L. Graw and David Patterson. Eleanor Roosevelt

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GAR synthetase, the third enzyme of *de novo* purine biosynthesis, has been purified to homogeneity from rat, and partially from human and hamster sources. Physicochemical characteristics of the purified proteins were determined and compared. There is mounting evidence that GAR synthetase is part of a multifunctional protein which includes 5-aminimidazole ribonucleotide (AIR) synthetase and GAR transformylase activities. In order to answer this question, the activities of these latter two enzymes were monitored during the purification of GAR synthetase. In addition to being part of a multifunctional protein, GAR synthetase is also thought to be a component of a multienzyme complex. Antibodies raised to the purified enzyme were used in conjunction with Western blots to study the molecular weight of GAR synthetase under conditions that would maintain the integrity of any complex present and then under conditions that would lead to the dissociation of the complex into its various components. Finally, antibodies raised to rat liver GAR synthetase were used to select cDNA clones of the gene from a λgt11 expression library. These clones will be subsequently used to characterize the structure of the gene encoding GAR synthetase in mammalian cells. This work was supported by grants NIH AG 00029 and NIH HD 13432. This is ERICR contribution #564.

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SOURCE AND FATE OF CIRCULATING PYRIMIDINES

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Experiments were undertaken to investigate the working hypothesis that the liver is a source of pyrimidine nucleotide precursors for utilization by peripheral tissues, that the precursor is orotate, and that the erythrocytes act as an intermediary buffer, and convert the orotate to uridine, which is more readily utilized by peripheral tissues than orotate. Differentiated human hepatoma cells were labelled with ¹⁴C aspartic acid and labelled orotate was removed from the medium by selective precipitation techniques. Acid precipitable radioactivity was low in lymphoblasts, fibroblasts or erythrocytes labelled alone with ¹⁴C orotate but were enhanced tenfold if erythrocytes were co-cultured with the nucleated cells. Factors influencing orotate metabolism in the erythrocyte include phosphate, bicarbonate and other factors operating in a time dependent manner. Orotate and many pyrimidine nucleotides are directly quantifiable on high pressure liquid chromatography of extracts from lymphoblasts, fibroblasts and erythrocytes and their measurement provide a ready means for diagnosis of inherited disorders of pyrimidine metabolisms such as orotic aciduria and pyrimidine 5' nucleotidase deficiency.

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PURINE NUCLEOTIDES IN HUMAN HEARTS DURING OPEN HEART SURGERY

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In order to evaluate myocardial nucleotide metabolism tissue samples were taken during open heart surgery. Nucleotides were extracted and determined by means of isotachopheresis. Concentrations of hypoxanthine, inosine, and adenosine in coronary sinus' blood were measured with HPLC.

In human hearts the concentrations of nucleotides and creatine phosphate (CP) were: CP 19.9 ± 7.4, ATP 20.8 ± 13.5, ADP 11.1 ± 5.6, AMP 12.0 ± 6.4, IMP 3.4 ± 2.1, NAD⁺ 9.2 ± 3.0, and NADH/H⁺ 5.9 ± 2.8 nmoles/mg protein. Potassium induced ischemic heart arrest resulted in a significant decrease of 71% CP. In contrast an increase of 20% AMP, 16% IMP and 44% NADH/H⁺ was observed. During reperfusion a further decrease of all nucleotides was observed. Concomitant with the decrease of tissue nucleotides an increase of hypoxanthine, inosine, and adenosine in the coronary sinus occurred. These ischemic degradation products probably might be reutilized by the heart.

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Inhibitor of 2',5'-oligoadenylate synthetase induced in human T lymphoblastoid cell line treated with deoxyadenosine, deoxycytosine and interferon

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2',5'-oligoadenylate(2-5A) synthetase is an interferon (IFN)-induced enzyme, which has been considered to play a central role in antiviral activity. In general, the increased susceptibility to viral infections are common in severe combined immunodeficiency. Therefore, using human T lymphoblastoid cell line treated with deoxycytosine as an in vitro model of adenosine deaminase deficiency, we investigated the effect of deoxyadenosine (dAdo) on the 2-5A synthetase activity induced by IFN. To measure 2-5A synthetase activity, we used two different assay systems, Sokawa's and Baglioni's methods. In the former, the net 2-5A synthetase activity was determined after semipurification of the enzyme through poly(I):poly(C) agarose gel, whereas in the latter method, the enzyme activity in the crude extract was detected. The enzyme activity was constant regardless of changes of dAdo concentration by Sokawa's method. However, in Baglioni's method, the activity decreased as the dAdo concentration increased. The activity of 2'-phosphodiesterase in the crude extract, which is also induced by IFN and degrades 2-5A, was similar in spite of changes of dAdo concentration. Inhibitor of 2-5A synthetase which clearly differs from dATP (a competitive inhibitor of the enzyme) accumulated in dAdo-treated cells could be induced in our system.

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DETERMINATION OF S-ADENOSYLHOMOCYSTEINE IN TISSUES FOLLOWING PHARMACOLOGICAL INHIBITION OF S-ADENOSYLHOMOCYSTEINE

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The amount of S-adenosylhomocysteine in liver increases 2 to 3 fold during the first minute following death of the animal (Hoffman, D.R. et al. (1979) Can. J. Biochem. 57, 56-65), and postmortem increase in AdoHcy should be avoided when determining the physiological level of AdoHcy. Injection of mice with the drug combination ara-A plus dCF caused a massive increase in the AdoHcy content in several tissues of mice. A large fraction of the amount of AdoHcy in liver and to a lesser degree in kidney, heart and lung of mice so treated, could be attributed to a rapid postmortem accumulation of AdoHcy. This could be prevented by freezing the organs in vivo using liquid nitrogen.