217 ADENYLOSUCCINASE DEFICIENCY. G. Van den Berghe and J. Jaeken. Laboratory of Physiological Chemistry, ICP and University of Louvain, Brussels, and Department of Pediatrics, University of Leuven, Belgium.

Adenylosuccinase (ASase) deficiency was suspected in 3 mentally retarded autistic children by the discovery of succinyladenosine (S-Ado) and SAICAriboside in their body fluids and confirmed in case A by the finding of an absence of enzyme activity in the kidney and of a partial deficiency in the liver (Jaeken & Van den Berghe, Lancet 2:1058, 1984). Further studies of ASase (nmol/min/mg protein vs means ± SEM in 5-8 controls) in cases B and C have shown a partial deficiency in kidney (0.18 and 0.21 vs 1.08 ± 0.10), liver (0.14 and 0.11 vs 0.72 ± 0.18) and muscle (0.18 and 0.66 vs 2.61 ± 0.34). In the lymphocytes and fibroblasts of the 3 cases, mean ASase activity was respectively 37 and 63 % of control. HPLC measurements of metabolites in quick-frozen kidney of cases A & B showed an accumulation of S-AMP (0.2 mM vs undetectable in controls) whereas no SAICAribotide could be found. In case A there was an approx. 4-fold increase of the adenine, guanine and uridine nucleotides; only the latter were similarly increased in case B.In quick-frozen liver of the 3 cases S-AMP was comparable to controls, no SAICAribotide could be detected but the concentration of an unknown compound, migrating just prior to the nucleotide 3-phosphates was decreased by approx. 50 %. ASase deficiency seems to induce a secondary increase of the synthesis of nucleotides by the PRPP transferases and/or a decrease of their degradation in the kidney and to hinder the formation of a derivative of the "de novo" pathway in the liver. Supported by FRSM, FWGO & CST 17645.

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INHIBITION OF ^{3}H-THYMIDINE INCORPORATION BY ADENOSINE AND DEOXYADENOSINE IN HUMAN PERIPHERAL LYMPHOCYTES AND MALIGNANT LYMPHOID CELL LINES.

Children with ADA deficiency have an impairment of both cellular and humoral immunity. We performed a detailed study of the relative toxicities of the ADA substrates adenosine and deoxyadenosine with regard to ^{3}H-thymidine incorporation in mitogen-stimulated human peripheral blood lymphocytes and malignant lymphoid cell lines. Lymphocytes, isolated by Percoll gradient centrifugation and counterflow elutriation, were stimulated with various mitogens (PHA, ConA, PWM, SpA, StA) known to activate different lymphocyte subpopulations. Both lymphocytes and lymphoid cell lines were incubated with various adenosine and deoxyadenosine concentrations in presence of the ADA inhibitor deoxycoformycin. Combinations of deoxycoformycin and adenosine or deoxyadenosine gave inhibition of ^{3}H-thymidine incorporation in both lymphocytes and cell lines. Adenosine affected ^{3}H-thymidine incorporation of mitogen-stimulated lymphocytes to a similar extent as deoxyadenosine. However, to the malignant T cell line Molt 4 deoxyadenosine was more toxic than adenosine. This was not observed with the cell lines REH (nonBnonT), KM-3 (nonBnonT) and RAJI (B).
Lymphocytes stimulated with B- or T cell mitogens were affected in their thymidine incorporation to a comparable extent by adenosine and deoxyadenosine. The cell line Molt was more sensitive to deoxyadenosine than both stimulated lymphocytes and the cell lines REH, KM-3 and RAJI.

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BIPHASIC EFFECT OF ADENOSINE ON CELL GROWTH AND CELL CYCLE OF HUMAN LYMPHOID CELL LINES.

Accumulation of adenosine is detected in plasma of ADA-deficient children(1). We incubated human lymphoid cell lines with adenosine in presence of the ADA inhibitor deoxycoformycin. The malignant T cell line Molt4and the non-malignant B cell line RPMI 1788 were both more affected by adenosine than the malignant B cell line RAJI was. However, adenosine had a remarkable biphasic effect on cell growth of all three cell lines. After 48 hours incubation with 40μM adenosine growth of all three cell lines was severely inhibited (Molt4 93%, RPMI 1788 87%, RAJI 63%). When 100μM adenosine was used the inhibitory effect was less severe (Molt4 58%, RPMI 1788 72%, RAJI 49%). This biphasic effect was also reflected in the cell cycle. After 24 hours incubation addition of 20μM adenosine to the cell cultures resulted in accumulation of cells in the G_1 phase while addition of 50μM adenosine resulted in a decrease of cells in the G_1 phase. Drawing conclusions about the inhibitory effects of adenosine should be done with caution.
Reference: Mills, G.C., Schmalstieg, F.C., Newkirk, K.E., Goldman, A.S. and Goldblum, R.M. (1976). Proc. Natl. Acad. Sci. USA, 73, 2867.

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EFFECTS OF GUANOSINE AND DEOXYGUANOSINE ON ^{3}H-THYMIDINE INCORPORATION OF HUMAN LYMPHOID CELLS.

Guanosine and deoxyguanosine are detected in urine of PNP-deficient patients (1). We have studied the effects of these compounds on ^{3}H-thymidine incorporation in human peripheral blood lymphocytes and malignant lymphoid cell lines. Peripheral blood lymphocytes, isolated by Percoll gradient centrifugation and counterflow elutriation were stimulated with various mitogens (PHA, Con A, PWM, SpA, StA) known to activate different lymphocyte subpopulations.
In all cell cultures tested deoxyguanosine inhibited ^{3}H-thymidine more strongly than guanosine. Stimulated lymphocytes and cell lines were cultured in presence of different concentrations of guanosine and deoxyguanosine. Lymphocytes stimulated with B cell mitogens were as sensitive to guanosine and deoxyguanosine as lymphocytes stimulated with T cell mitogens were. Incorporation of ^{3}H-thymidine in the cultured cell line Molt 4 by guanosine and deoxyguanosine was inhibited more severe than in the cultured RAJI (B) and REH (nonBnonT) cell lines and in stimulated peripheral blood lymphocytes. When possibilities for antileukemic chemotherapy using deoxyguanosine or guanosine are considered, deoxyguanosine seems to be a better candidate than guanosine.
Reference: Stoop, J.W.; Zeger, B.J.M.; Hendrickx, G.F.M.; Siegenbeek van heukelom, L.H.; Staal, G.E.J.; De Bree, P.K.; Wadman, S.K. & Ballieux, R.E. (1977). N. Engl. J. Med. 296, 651.

221 DEAMINATION OF GUANINE TO XANTHINE: A METABOLIC PATHWAY OF UNDERESTIMATED IMPORTANCE IN HUMAN PURINE CATABOLISM?

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The high xanthine (X) plasma concentration and urine excretion relatively to hypoxanthine (H) in xanthinuric patients, as well as the strong rise of X plasma concentration directly after i.v. allopurinol injection in healthy subjects, might reflect considerable guanine (G) breakdown.

Urate (U), H and X were measured in consecutive plasma and urine specimens before and after an i.v. allopurinol bolusinjection (17.1 μmol/kg body mass) in 8 healthy individuals. The relative importance of the G-X pathway as compared to the H-X pathway in normal unperturbated purine catabolism was estimated from the initial slope of the concentration-time curves and the excretion data. The calculations include the following assumptions: I) an average extrarenal urate elimination rate equal to 35% of the total urate elimination rate, II) a distribution volume equal to 65% of body mass for H and X and III) complete inhibition of the conversion of H to X and of X to U from 5 to 20 min after allopurinol injection, due to high allopurinol and oxypurinol plasma concentrations.

The deamination of G to X was this way estimated to stand for 85 ± 30% ($\bar{x}$ ± SD) of the X production in unperturbated metabolic state. This does not exclude, however, that the conversion of H to X can be of dominant importance in a state with fast purine catabolism and H accumulation, e.g. hypoxia.

222 KINETICS OF ALLOPURINOL TURNOVER AFTER I.V. INJECTION IN MAN AND ITS USE AS A MEANS TO ASSESS LIVER FUNCTION.

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Intravenous allopurinol loadings (17.1 μmol/kg body mass) have been performed in 12 healthy subjects and 22 patients with different liver diseases. Plasma and urine concentrations of allopurinol and oxypurinol were monitored during 180min. The concentration curves were analysed with the aid of a computer-based 3-compartment biodynamic model and the estimated model parameters compared to a "liver function score".

This test was primarily set up in order to achieve a liver function test more sensitive than other tests at hand. The best diskriminating parameter in the model was the fractional rate constant for transport of allopurinol from the plasma to the liver (k_{31}). Good correlation was obtained between this parameter and the liver function score (r=-0.85, n=34). In a group of healthy subjects, a group of patients with slightly to moderately reduced overall liver function, and in a group with severely reduced overall liver function, k_{31} (min^{-1}) was estimated to 0.141 ± 0.039 ($\bar{x}$ ± SD, n=12), 0.072 ± 0.023 (n=14) and 0.025 ± 0.015 (n=8), respectively.