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EFFECT OF FRUCTOSE ON THE CONCENTRATION OF PRPP IN ISOLATED RAT HEPATOCYTES. F. Vincent, G. Van den Berghe & H.G. Hers. Laboratory of Physiological Chemistry, International Institute of Cellular and Molecular Pathology & University of Louvain, Brussels, Belgium.

Fructose loads induce a depletion of the hepatic purine nucleotides, followed by an increase of their "de novo" synthesis, which has been attributed to a conversion of glutamine-PRPP amidotransferase from its large inactive to its small active form (Itakura et al. J. Clin. Invest. 67: 994, 1981). Addition of 5 mM fructose to isolated rat hepatocytes provoked, after a 10 min latency and within 1-2 min, a 6-10 fold increase of the concentration of PRPP above its basal value of 2-5 nmol/g of cells, which lasted for approx. 30 min. Fructose decreased hepatocyte ATP from 2 to 0.4 mM and Pi from 3.7 to 1.4 mM after 2 min. Thereafter both compounds increased gradually, reaching respectively 0.8 and 2.6 mM at 30 min. Fructose also increased progressively the concentration of ribose-5-P from 0.1 to 0.2 mM at 25 min. Under control conditions, approx. 5 % of 10 μ M hypoxanthine (HX) added to the cell suspension was incorporated into the purine nucleotides. This value was not modified when HX was added 2 min after fructose but it increased approx. 5-fold when HX was added 30 min after fructose. The regeneration of the adenine nucleotides following fructose loads involves thus also an increased concentration of PRPP and an increased capacity for purine salvage. This occurs notwithstanding a depletion of ATP, one of the substrates of PRPP synthetase, and of Pi, its major stimulator. Supported by FRSM.

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DETECTION OF INHERITED ADENYLOSUCCINASE DEFICIENCY BY 2-DIM. THIN LAYER CHROMATOGRAPHY OF URINARY IMIDAZOLES. S.K. Wadman, P.K. de Bree, M. Duran, H. Fabery de Jonge. University Children's Hospital 'Het Wilhelmina Kinderziekenhuis', Utrecht, The Netherlands.

Inherited adenylosuccinase deficiency, a 'new' defect of de novo purine synthesis and nucleotide interconversion has been associated with psychomotor retardation and autistic behavior (1). Biochemical features were: excessive urinary excretion of succinylaminoimidazole carboxamide riboside (SAICAR) and succinyladenosine (SAdo). We found that SAICAR can easily be detected by our 2-dim. tlc in use for the screening of abnormal urinary imidazoles. The method consists of 1: isolation of imidazoles (together with aminoacids and purines with a cation exchange resin; 2: tlc on cellulose plates, solvent I: isopropanol - ammonia 10% (4:1) and II: butanol - acetic acid - water (4:1:1); detection with Pauly reagent. SAICAR gives rise to an isolated spot with a characteristic bluish-brown color. Xanthinuria and PNP deficiency can also be detected with this system. Confirmative quantitative analysis of SAICAR and SAdo can be done by HPLC.

Three new cases could be diagnosed: a one-year-old girl with neurological problems, her elder brother with psychomotor retardation and epilepsy and a ten-year-old girl with severe mental retardation and neurological problems. Chemical and clinical data will be presented.

1. J. Jaeken, G. Van den Berghe: Lancet II(1984) 1058-1061.

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CARRIER MEDIATED UPTAKE OF DEOXYGUANOSINE IN RAT LIVER MITOCHONDRIA. Linda Watkins and Roger A. Lewis, University of Nevada School of Medicine, Department of Biochemistry, Reno, NV 89557

It has been known for a number of years that mitochondria contain enzymes important for the synthesis of DNA. However, detailed studies on mitochondrial purine and pyrimidine nucleotide metabolism are lacking. This report describes the uptake of deoxyguanosine by rat liver mitochondria and indicates that this deoxynucleoside is taken up by a carrier mediated mechanism. Using the ATP structural analogues AMPPNP and AMPPCP as inhibitors, it was shown that ATP is required for the uptake of deoxyguanosine. Furthermore, ADP is inhibitory to the uptake process. When nucleosides were tested as competitors to the uptake of deoxyguanosine (10 μ M), deoxyinosine (100 μ M) was inhibitory (55%), but neither deoxyadenosine, deoxycytidine, deoxythymidine nor guanosine (all at 100 μ M) were significantly effective in altering the process. Likewise, 6-(2-hydroxy-5-nitrobenzyl)-thioinosine (NBTI) (5 μ M) and cytochalasin B (50 μ M) were not highly inhibitory, 5 and 17%, respectively. The observations that deoxyguanosine uptake requires ATP, is specific for deoxyguanosine (or deoxyinosine), is not inhibited by NBTI or cytochalasin B and is inhibited by ADP indicates that the process for uptake of deoxyguanosine by mitochondria is quite different from that by the plasma membrane.

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PURINE METABOLISM BY GUINEA-PIG ILEUM

In guinea-pig ileum adenosine and adenosine analogs act presynaptically to inhibit cholinergic transmitter release and this has been shown to be due to actions at A₁ (R₁) receptors. The I.C.₅₀ of adenosine on the twitch response of this tissue is about 2 μ M. The purine content of the medium and tissues was measured throughout the pharmacological studies. The tissue released purines into the medium at 7.8 $\pm$ 1.0 SEM pmol/mg/min and this was mostly uric acid. NAD (2 μ M) was rapidly hydrolysed to 5'-AMP (2.8 $\pm$ 0.3 pmol/mg/min). Both 5'- and 2'-AMP formed adenosine (2.8 $\pm$ 0.6 and 3.2 $\pm$ 0.6 pmol/mg/min). Adenosine was deaminated (3.2 $\pm$ 0.2 pmol/mg/min) then oxidised to uric acid. Deoxycoformycin (dCF, 2 μ M) increased adenosine to 1-2 μ M in the medium from all substrates but the effect of adenosine on the twitch was not simply related to the increased adenosine concentration except when adenosine itself was the substrate, when a linear correlation was found between the inhibition of twitch response and the concentration of adenosine in the medium.

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MECHANISM OF URATE PRODUCTION BY GUINEA-PIG ILEUM

Previous work has shown considerable purine (mostly uric acid) production by isolated guinea-pig ileum preparations used in studies of adenosine receptor pharmacology. This tissue was stimulated at 0.2 Hz and the purine efflux studied by HPLC. The following drugs were added to the medium (alone or in combination) 20 min before the study and were present throughout the study: deoxycoformycin (dCF, 10 μ M), 6-(2-hydroxy-5-nitrobenzylthioguanosine (HNBTR, 1 μ M) allopurinol (10 μ M and 50 μ M) and oxipurinol (10 μ M). Allopurinol and oxipurinol increased the proportion of hypoxanthine and xanthine and did not reduce purine efflux, but this was reduced when dCF and/or HNBTR was present. The results show about one quarter of the uric acid is preformed and the rest synthesised via adenosine in the course of the experiment, in both the tissue and the medium. Since endogenous purine concentrations are in excess of the I.C.₅₀ of adenosine in this tissue they are an important consideration in the interpretation of experiments with exogenous purines and purine analogs.

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FREQUENCY OF INTRAARTICULAR MONOSODIUM URATE (MSU) CRYSTALS IN ASYMPTOMATIC HYPERURICEMIC SUBJECTS. A. Weinberger, C.A. Agudelo, H.R. Schumacher, J. Boner, J. Pinkhas, Tel Aviv Univ. Israel; Bowman Gray Sch. Med. North Carolina and University of Pennsylvania, USA.

In previous studies we have been able to demonstrate crystals in asymptomatic 1st metatarsophalangeal (MTP) joints never clinically involved in 66% of gouty patients. In small group of subjects with asymptomatic hyperuricemia (AHU) crystals could not be identified by using this technique. Recent studies have displayed the presence of MSU crystals in asymptomatic hyperuricemic individuals without an history of gout. Since it is a controversial issue whether subjects with AHU are susceptible to develop gout than those with normouricemia, we extended the study. Aspiration of asymptomatic 1st MTP joint was attempted in 31 subjects with AHU with no history of gout or pseudogout. Of them 12 had psoriasis, 6 with chronic renal failure (CRF) with 3.2 yrs mean duration of hyperuricemia, and 11 subjects had various types of associated diseases. Two subjects had AHU with no other associated diseases. Serum uric acid levels were 8.0-14.5 mg/dl, mean 9.8 mg/dl. Serum creatinine levels were 0.8-7.5 mg/dl, mean 1.7 mg/dl. Negatively birefringent rod crystals typical of MSU was found only in the aspirated fluid of one patient who suffered from psoriasis. Our finding that no patient with CRF had positive MSU crystals in the aspirates is in contrast to previously published reports of about 30% prevalence of crystalline deposits in these patients' toe. We confirm previous studies in which crystals may only rarely be found in hyperuricemic patients who have never had symptoms of arthritis.