91 INCREASE OF AMIDOPHOSPHORIBOSYLTRANSFERASE ACTIVITY AND PHOSPHORIBOSYLPYROPHOSPHATE CONCENTRATION AS THE BASIS FOR INCREASED DE NOVO PURINE BIOSYNTHESIS IN THE REGENERATING RAT LIVER

Mitsuo Itakura, Masami Tsuchiya, Kamejiro Yamashita
The University of Tsukuba, Institute of Clinical Medicine, Sakura-mura, Niihari-gun, Ibaraki 305, JAPAN

The rate of de novo purine biosynthesis assayed by the specific activity of hepatic purines in the pulse-labeling with radioactive glycine significantly increased in the regenerating rat liver in the time course after a 70% hepatectomy and it peaked 12 hours post-surgery by 2.4 folds above sham-operated control without associated change of the glycine pool sizes. In the regenerating rat liver in comparison to sham-control, the activity of amidophosphoribosyltransferase(ATase) significantly increased with its peak 1.8 folds higher than control 18 hours post-surgery and hepatic 5-phosphoribosyl 1-pyrophosphate(PRPP) concentration significantly increased with its peak 3.0 folds higher than control 12 hours post-surgery. Although there was a 17 and 24% decrease of hepatic ATP and GTP concentration respectively in the regenerating rat liver 12 hours post-surgery, they were counter-balanced by the increased concentration of AMP, ADP, GMP and GDP. Results of these studies suggest that the increased ATase activity and PRPP concentration lead to the increased rate of de novo purine biosynthesis in the regenerating rat liver.

92 MECHANISM OF INCREASED RATE OF DE NOVO PURINE BIOSYNTHESIS IN RAT LIVER AFTER BILATERAL ADRENALECTOMY

Mitsuo Itakura, Akiko Nakane and Kamajiro Yamashita
The University of Tsukuba, Institute of Clinical Medicine, Sakura-mura, Niihari-gun, Ibaraki 305, JAPAN

In rat liver after bilateral adrenalectomy de novo purine biosynthesis was studied 24, 48 and 72 hours in comparison to sham-operated control. At these time points the rate of de novo purine biosynthesis assayed by [^{14}C]glycine incorporation to hepatic purines significantly increased by 1.61, 1.95 and 2.01 folds respectively associated with comparable free glycine concentration in plasma or liver tissue. Hepatic concentration of 5-phosphoribosyl 1-pyrophosphate(PRPP) increased by 2.91, 1.50 and 1.75 folds at these 3 time points. 24 hours after bilateral adrenalectomy ATP and GTP concentration decreased by 33 and 24% respectively which were counterbalanced by the increased concentration of AMP(245%) and GMP(38%) associated with 18% decrease of adenylate energy charge. The replacement of corticosterone acetate to adrenalectomized animals for 24 hours partially reversed these changes. The results suggest that the increased rate of de novo purine biosynthesis in adrenalectomized rat liver is mediated by the increased concentration of PRPP and that it is compensatory against increased catabolism of purine ribonucleotides as a result of increased AMP concentration.

93 REGULATION OF THE CYTOSOL 5'-NUCLEOTIDASE OF THE HEART BY ADENYLATE ENERGY CHARGE.

R. Itoh, J. Oka and H. Ozasa
The National Institute of Nutrition, Tokyo, Japan

5'-Nucleotidase was partially purified from chicken and rat heart by the similar procedure used for the purification of the cytosol 5'-nucleotidase from chicken and rat liver. Some kinetic properties of the enzymes from heart were similar to those of the cytosol 5'-nucleotidase from the liver.

The heart enzymes from both species were activated by ATP. When AMP was used as a substrate, the substrate-velocity plot of the chicken heart enzyme was sigmoidal (h=1.8). ATP at the concentration of 5 mM converted the curve into hyperbolic one and decreased $s_{0.5}$ from 40 mM to 8 mM. The substrate-velocity plot of the rat heart enzyme with AMP was hyperbolic even in the absence of ATP. ATP at the concentration of 5 mM activated the enzyme by decreasing Km from 21 mM to 7 mM.

AMP-hydrolysing activity of these two enzyme preparations from cardiac tissue was markedly increased with the decrease in adenylate energy charge [(ATP + 0.5ADP)/(ATP + ADP + AMP)] in the physiological range. This suggests that the cytosol 5'-nucleotidase is important in production of a vasodilator, adenosine, from AMP during hypoxic condition of the heart.

94 HYPOURICEMIA DUE TO RENAL URATE WASTING: DIFFERENT TYPES OF TUBULAR TRANSPORT DEFECTS

Manuel L. Jiménez, Juan G. Puig, Felicitas A. Mateos, María E.M. Gómez and Antonio A. Gil
Facultad Autónoma de Medicina. La Paz University Hospital. Departments of Internal Medicine and Clinical Biopathology. Madrid. Spain.

Intrarenal handling of uric acid was evaluated in five patients with diminished serum urate levels (1.4 to 2.0 mg/dl) and increased renal clearance of uric acid (22.9 to 65.3 ml/min/1.73 m^2). One patient with kappa light-chain disease and Fanconi syndrome showed a blunted uric acid excretion response to both pyrazinamide and probenecid, indicating a defective tubular reabsorption of urate. Three patients had recurrent calcium oxalate nephrolithiasis and one infant bening idiopathic hematuria. In addition to hyperuricosuria the infant and two patients with recurrent urolithiasis showed renal hypercalciuria. All evidenced normal responses to pyrazinamide. Compared to normal subjects, three patients showed a diminished and one an increased uricosuric response to probenecid. The former were considered to have a defective tubular urate postsecretory reabsorption and the latter an enhanced tubular secretion of urate. These results indicate that (1) hypouricemia may provide an important clue for the existence of an underlying disease, and (2) tubular urate transport defects may be of pathogenic importance in patients with recurrent calcium nephrolithiasis.

95 HYPERURICEMIA AS POSSIBLE DIAGNOSTIC AID TO FACTITIOUS-BARTTER'S SYNDROME

Manuel L. Jiménez, Felicitas A. Mateos, Juan G. Puig, Teresa H. Ramos, Francisco J. Barbado
Facultad Autónoma de Medicina. La Paz University Hospital. Departments of Internal Medicine and Clinical Biopathology. Madrid. Spain.

Factitious-Bartter's syndrome due to surreptitious diuretic administration may be difficult to recognize. Serum urate has been constantly reported to be elevated in factitious-Bartter's syndrome but is seldom increased in true-Bartter's syndrome. A 33-year-old woman with a serum urate level of 10.4 mg/dl and diminished fractional excretion of uric acid satisfied the criteria for Bartter's syndrome, including hypokalemic alkalosis, hyperreninemia, aldosteronism, normal blood pressure, insensitivity to the pressor effects of angiotensin infusion and hyperplasia of the juxtaglomerular apparatus. Indomethacin treatment normalized these metabolic derangements except increased serum urate. A plasma screening test for furosemide was positive. In two infants with Bartter's syndrome plasma bicarbonate concentrations were brought to normal levels with indomethacin but serum urate failed to significantly change. These data indicate that systemic alkalosis does not markedly influence serum urate. Persistent hyperuricemia after normalization of the functional abnormalities of Bartter's syndrome may be of diagnostic aid to factitious-Bartter's syndrome caused by diuretic ingestion.

96 CHARACTERISTICS OF HIGH AFFINITY AND LOW AFFINITY ADENOSINE BINDING SITES IN HUMAN CEREBRAL CORTEX.

David John and Irving H. Fox, The University of Michigan, Departments of Internal Medicine and Biological Chemistry, Ann Arbor, Michigan, USA.

Binding characteristics of human cortical membrane fractions were evaluated to test the hypothesis that there are A_1 and A_2 adenosine binding sites. Binding of chloroadenosine was time dependent, reversible and concentration dependent. The K_d for chloroadenosine was 280 nM with a Bmax of 1.6 pmoles/mg protein. The apparent K_d was estimated to be 0.74 μM for 5'-N-ethylcarboxamideadenosine, 1 μM cyclohexyladenosine, 13 μM N^6-(L-2-phenylisopropyl)adenosine, 84 μM isobutylmethylxanthine and 105 μM theophylline. Hill slope factors ranged from 0.3 to 0.6. The k_{ob} was 0.080 min^{-1}, the k_1 7.5 x 10^4 min^{-1}M^{-1}, and the k_2 .074 min^{-1}. The K_d calculated from the rate constants was 990 nM. Cyclohexyladenosine binding was concentration dependent and the K_d was 5 nM with a B_{max} of 0.35 pmoles/mg protein. Cyclohexyladenosine binding was displaced by N^6-(L-2-phenylisopropyl)adenosine (K_d 4 nM), 2-chloroadenosine (K_d 10 nM), 5'-N-ethylcarboxamideadenosine (K_d 6 nM), isobutylmethylxanthine (4 μM) and theophylline (8 μM). Hill slope factors ranged from 0.5 to 0.8.

Our data support the existence of two adenosine binding sites in human cortex compatable with low affinity (A_2) and high affinity (A_1) adenosine receptors.