179 RENAL EXCRETIONS OF OXYPURINOL AND OXYPURINES - EFFECTS OF PYRAZINAMIDE, PROBENECID AND BENZBROMARONE -

Tetsuya Yamamoto, Yuji Moriwaki, Sumio Takahashi, Toshikazu Hada and Kazuya Higashino
The Third Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

The renal handlings of oxypurinol and oxypurines by normal subjects after intake of allopurinol were investigated by studies on the influences of uricosuric agents (probenecid and benzbromarone) and pyrazinamide. Pyrazinamide decreased the fractional clearance of oxypurinol, hypoxanthine and xanthine 0.46-, 0.52- and 0.40-fold, respectively. Probenecid increased the fractional clearance of oxypurinol 1.8-fold, decreased that of xanthine 0.61-fold and did not affect that of hypoxanthine. Benzbromarone increased the fractional clearance of oxypurinol 2.17-fold but did not affect those of hypoxanthine and xanthine. These results suggest that oxypurinol is reabsorbed at least at a postsecretory site of the renal tubules, that the main renal transport of xanthine is secretory when conversion of oxypurines to uric acid is decreased and that the main renal transport of hypoxanthine that is not affected by these three agents may occur when conversion of oxypurines to uric acid is decreased.

180 MODULATION OF DIPHTHAMIDE SYNTHESIS BY METHYLTHIOADENOSINE.

Hisashi Yamanaka* and Dennis A. Carson**, *Tokyo Women's Medical College, Japan, **Research Institute of Scripps Clinic, USA

Exogenous addition of methylthioadenosine (MeSAdo) inhibits proliferation of cells which are deficient in MeSAdo phosphorylase. A series of evidences has shown that the target of toxic mechanism of MeSAdo is not totally dependent on polyamine synthesis. Eukaryotic cells contain unique modified amino acid in elongation factor 2 (EF-2), designated as diphthamide. This residue is the specific target of mono(ADP-ribosyl)ation catalyzed by diphtheria toxin (DT) or Pseudomonous toxin. The structure of diphthamide is 2- [3-carboxyamido-3-(trimethylammonio)propyl]histidine, and the first reaction to modify histidine involves the transfer of an aminocarboxypropyl group from S-adenosylmethionine. MeSAdo should be the nucleoside product of this reaction. By these reasons, we have analyzed the effect of MeSAdo on the biosynthesis of diphthamide. A mutant cell line H3 which is deficient in MeSAdo phosphorylase and resistant to MeSAdo, has been isolated from murine lymphoma cell line R1.1. for the study. As measured by susceptibility to DT induced ADP-ribosylation, MeSAdo inhibits the formation of diphthamide in a dose dependent manner. In addition, MeSAdo substantially protected H3 cells from the lethal effect of DT. These results suggest that the modulation of diphthamide synthesis can be, at least a part of, the mechanism of MeSAdo toxicity toward eukaryotic cells.

181 CHARACTERIZATION OF POLY(ADP-RIBOSE) POLYMERASE WITH HUMAN AUTOANTIBODIES.

Hisashi Yamanaka*, Carol A. Penning** and Dennis A. Carson***, *Tokyo Women's Medical College, **Cambridge Medical Hospital, ***Research Institute of Scripps Clinic

Poly(ADP-ribose) polymerase (ADPRP) is highly activated by DNA with strand breaks and transfer poly (ADP-ribose)chains outo nuclear proteins. Poly (ADP-ribosyl)action rapidly consumes cellular NAD and leads cells to die. This reaction has been implied to be involved in the deoxyadenosine toxicity toward resting human lymphocytes, and could be an important mechanism in the toxicity of all DNA-damaging agents. To analyze the characteristics of ADPRP, we have utilized human autoantibodies to ADPRP which we have identified in the sera of rheumatic patients. cDNA clones encoding ADPRP were isolated from human libraries and used in these studies. The results of these studies indicate that ADPRP is an abundant nuclear enzyme. Eukaryotic cells synthesize ADPRP throughout S-phase, and keep the cellular ADPRP level constantly in proportion to the amount of DNA. Interestingly more than 99% of ADPRP is present in cells as inactive form. These data may suggest the importance of ADPRP as a structural protein in chromatin conformation. In addition, these results are consistent with the hypothesis of "suicide mechanism" of ADPRP. Eukaryotic cells have approximately 100-fold more enzyme than that is required for the regular cell growth, and keep it for an emergency event. When cellular DNA is severely damaged, ADPRP is activated and eliminates the cell. This mechanism could be applicable to the toxicity of any DNA damaging agents, including many purine and pyrimidine analogs.

182 PURIFICATION OF RAT LIVER AMIDOPHOSPHORIBOSYLTRANSFERASE (ATase)

TAKASHI YAMAOKA, FUMIKO KAGITA, HIROKO YOSHIKAWA, MITSUO ITAKURA, AND KAMEJIRO YAMASHITA
University of Tsukuba, Institute of Clinical Medicine, Tsukuba-shi, Ibaraki 305, JAPAN

Purification of mammalian ATase is essential for the understanding of the regulation of de novo purine synthesis in mammals. The basic properties of rat liver ATase after step 4 of purification methods described below includes a) feedback inhibition by adenosine or guanosine mono-, di- and tri-phosphates, with the decreasing order of magnitude, b) Inhibition by 1,10-orthophenanthroline as a Fe^{++} chelator, but not by 1,7-metaphenanthroline, and c) Inactivation by exposure to oxygen with a half life of 15 min at 37°C with the stable activity under argon. In this study ogygen inactivation of ATase was overcome by the use of oxygen-free buffer and HPLC methods. The purification procedures include the following 8 steps.
1. 55,000xg supernatant of rat liver homogenate, 2. Heat treatment to 56°C, 3. Acid precipitation to pH 5.1, 4. Ammonium sulfate fractionation between 30 and 55%, 5. Gel filtration by Toyopearl HW55F, 6. DEAE-5PW HPLC column, 7. Hydroxyapatite HPLC column, 8. SDS polyacrylamide gel and electroelution. After step 7, ATase was purified to 958-fold with 4.1% recovery. After step 8, ATase was finally purified to homogeneity proved by a single protein band with silver stain. The monomer size of ATase was 62K on SDS polyacrylamide gel. The amount of ATase in rat liver is estimated as about 10 μg/g liver. The methods of purification of mammalian ATase open the research direction to molecular cloning and monoclonal antibody production.

183 Purification and regulation of cytosolic 5'-nucleotidase I from rabbit heart

Yukiko Yamazaki, Albert R. Collinson, Vu L. Truong, and John M. Lowenstein, Graduate Dept of Biochemistry, Brandeis University, Waltham MA 02254, USA

Rabbit heart contains two cytosolic 5'-nucleotidases, termed N-I and II, which can be separated using phosphocellulose chromatography. N-I prefers AMP over IMP as a substrate, in contrast to N-II which prefers IMP over AMP. N-II closely resembles the soluble enzyme found in heart and liver of chicken and rat with respect to substrate specificity (Itoh and coworkers, Comp. Biochem. Biophys. **81B**, 159-163 (1985); Biochim. Biophys. Acta **657**, 402-410 (1981); Biochem. J., **235**, 847-851 (1986); and Naito, Biochim. Biophys. Acta **438**, 159-168 (1976)). N-I has been purified approximately 1000-fold to a specific activity of 90 μmol/mg protein/min. The enzyme is strongly activated by ADP; it is not activated by ATP. Gel filtration indicates a molecular weight for N-I of about 255,000. At low [AMP], the activity of N-I is stimulated by a regulatory protein (R) in the presence of ATP. R was also purified to homogeneity. Gel electrophoresis in the presence of sodium dodecyl sulfate shows a subunit molecular weight for R of about 25,000. N-I is most likely the enzyme responsible for the release of adenosine from AMP under conditions of hypoxia or increased work load.

184 PROLIFERATIVE EFFECT OF INSULIN BY SELECTIVE GENE EXPRESSION IN PURINE METABOLISM IN RAT HEPATOCYTES

HIROKO YOSHIKAWA, MASAMI SATO, TAKASHI YAMAOKA, MITSUO ITAKURA, AND KAMEJIRO YAMASHITA
University of Tsukuba, Institute of Clinical Medicine, Tsukuba-shi, Ibaraki 305, JAPAN

To study the mechanism of gene expression during cell proliferation, the effect of insulin as a growth factor on purine metabolism was studied in primary cultued rat hepatocytes. Hepatocytes were prepared by the collagenase perfusion methods of Berry and Friend from seven-week-old male Wistar rats. After the first plating culture for 4 hours, hepatocytes were cultured in Williams E medium without fetal calf serum or insulin for 20 hours. Then insulin was administered. Both the rates of DNA and de nove purine synthesis, assayed respectively by [^{3}H] thymidine and [^{14}C]formate incorporation, increased dose- and time-dependently to the maximum of 260 and 250%, respectively, with the same range of insulin concentrations between 10^{-7} and 10^{-5} M at 24 and 8 hours. Insulin at 10^{-7} M at 8 hours increased the specific activities of amidophosphoribosyltransferase, hypoxanthine guanine phosphoribosyltransferase and adenine phosphoribosyltransferase, respectively, to 180, 130 and 200%, whereas that of xanthine oxydase remained unchanged at 80%. The selective increases in the specific activities of anabolic enzymes of purine metabolic pathway were due to the induction of enzyme proteins shown by increased Vmax and unchanged Km and by the dissappearance of the increases by Actinomycin D or cycloheximide. It is concluded that the proliferative effect of insulin, associated with the increased rate of purine synthesis, is a result of selective gene expression of anabolic enzymes.