139 PURINE SYNTHETIC CAPACITIES OF DE NOVO AND SALVAGE PATHWAYS IN RAT HEPATOMA 3924A CELLS.

Yutaka Natsumeda, Tadashi Ikegami and George Weber. Indiana University School of Medicine, Laboratory for Experimental Oncology, Indianapolis, IN, U.S.A.

The purpose was to elucidate whether the failure of anti-tumor action of purine de novo synthetic inhibitors might be due to the high capacity of salvage synthesis in neoplastic cells. Hepatoma 3924A cells in monolayer culture were used to compare enzymic activities and metabolic fluxes of de novo and salvage pathways. The specific activity of the rate-limiting enzyme for IMP de novo synthesis, amidophosphoribosyltransferase, was 21, whereas the activities of the salvage enzymes, adenine, hypoxanthine and guanine phosphoribosyltransferase, were 38, 34 and 99 μmol/hr/g cells, respectively. Moreover, the K_m value for the shared substrate, PRPP, of the salvage enzymes was orders of magnitude lower than that of amidophosphoribosyltransferase. In the hepatoma cells the initial rate kinetics of [^{14}C] formate, [^{14}C] adenine, [^{14}C] hypoxanthine and [^{14}C] guanine incorporation into acid-soluble nucleotides and acid-insoluble nucleic acids followed Michaelis-Menten kinetics. The apparent Vmax of de novo synthesis was 213, whereas those of salvage pathways from adenine, hypoxanthine and guanine were 2,200, 530 and 590 nmol/hr/g cells, respectively. The higher capacity of purine salvage than that of the de novo pathway indicates the important role purine salvage synthesis might play in circumventing the action of inhibitors of purine de novo synthesis in cancer chemotherapy.

(Supported by NCI grants CA-13526 and 05034).

140 Modulation of Polymorphonuclear leukocyte function by adenosine analogues

Y.Nishida, A.Takeuchi, and T.Miyamoto
Faculty of Medicine, University of Tokyo

There are many reports indicating that in humans, many cells have adenosine receptors. In this experiment we studied the effects of methylthioadenosine(MTA), naturally occuring nucleosides, N^6-phenylisopropyladenosine(PIA), a R site adenosine receptor agonist, and 2',5'-dideoxyadenosine(DDA), a P site adenosine receptor agonist, on polymorphonuclear leukocyte (PMN) function. PMN chemotaxis was examined by the modified Boyden Chamber method. PMN incubated with PIA showed no change in chemotaxis. In contrast, MTA and DDA reduced chemotaxis in a dose-dependent manner. The inhibition rate were 43.2% at 200 μM MTA and 20% at 200 μM DDA. Phagocytosis was determined by microscopic examination of PMN exposed to latex beads for 60 min at 37°C. PIA had no effect on phogocytic function over concentrations ranging from 20 μM to 1000 μM. In contrast, phagocytic function was markedly suppressed by DDA and MTA to 11.4% and 59.7% respectively at 500 μM. Superoxide(O_2^-) production by PMN, induced by 150 μg Con A and 1 g cytochalasin E, was measured by the reduction of cytochrome C. Cytochrome C reduction by PMN incubated with 100 μM MTA was reduced to 30% of the control value. 300 μM of PIA showed a slight decrease to 80% of the control value in cytochrome C reduction. These findings suggested that many functions of PMN are influenced by adenosine analogues.

141 Suppression by metylthioadenosine of histamine or leukotriene-induced contraction in isolated guinea pig tracheal rings.

Y. Nishida, S. Suzuki and T. Miyamoto
Faculty of Medicine, University of Tokyo

Methylthioadenosine(MTA) is a naturally occurring purine nucleoside which has been shown to have numerous effects on cell metabolism and function. We studied the action of MTA on tracheal smooth muscle, using isolated guinea pig tracheal rings. The tracheal rings were suspended in a 10 ml organ bath, containing atropinized Krebs-Henseleit solution. When MTA was added to the medium, relaxation of isolated guinea pig tracheal rings occurred after a latent period and was dose-dependent over concentrations ranging from 50 to 1000 μM. Pretreatment of the tracheal rings with 100 μM MTA inhibited the contraction produced by varying concentrations of histamine or leukotriene D_4. The inhibition caused by 100 μM MTA was dependent on histamine or leukotriene D_4 concentrations, ie 51% at 0.25 μg/ml histamine, 42.5% at 1.0 μg/ml histamine, 22% at 2.0 μg/ml histamine, and 81% at 0.5 μg LTD_4, 50% at 1 μg LTD and 46.2% at 2 μg LTD_4. The tracheal contractions induced by a standard concentration of histamine(1 μg/ml or leukotriene D_4(1 μg) were inhibited dose-dependently by pretreatment with MTA, ie 22% (histamine), 8.4%(leukotriene D_4) at 10 μM, and 58%(histamine), 25%(leukotriene D_4) at 100 μM. 500 μM of MTA blocked completely the contraction induced by histamine or leukotriene D_4. It seems that MTA is an important modulator of tracheal smooth muscle cell function.

142 Reversible interconversion between sulfo and desulfo xanthine dehydrogenase (oxidase)

Takeshi Nishino
Department of Biochemistry, Yokohama City University School of Medicine, Yokohama 232, Japan

The catalytically inactive XDH or XO of desulfo form is known to be present in the enzyme preparations purified from milk, rat and chicken livers. This inactive form has been considered to be merely preparation or storage artifacts. However, determination of content of the active and inactive forms of XDH in rat liver crude extract by titration of the enzyme with ^{14}C-allopurinol showed that the active enzyme in crude extract was only about 0.5 mol per mol of FAD of the enzyme while demolybdoform was 0.15 mol. Furthermore, the enzyme purified from the livers of chickens fed a high protein diet has a higher specific activity than that from normal livers and the molecular activity in crude extract changes with the diet. These suggest that in vivo the mechanism exists whereby interconversion between the active and inactive forms may be effected. Successful separation of the active and inactive enzymes by the affinity chromatography on Sepharose 4B/folate gel prompted us to investigate the interconversion between the sulfo and desulfo forms by an enzymatic system. Reversible interconversion between two forms was known to occure by the systems of rhodanese or mercaptopyruvate sulfur transferase. This reversible reaction was considered to be mediated by sulfide anion formed by the enzymatic systems. In fact sodium sulfide reactivated the desulfo enzyme as shown by Massey and at the same time inactivated the sulfo enzyme.

143 THE PREVALENCE OF PURINE METABOLIC DISORDERS IN JAPAN

Tsutomu Nobori, Hisashi Yamanaka, Naoyuki Kamatani, Kusuki Nishioka, and Kiyonobu Mikanagi Institute of Rheumatology, Tokyo Women's Medical College, Tokyo, Japan.

The patients with purine metabolic disorders have been registered at our institute from about 1000 various clinical institutes in overall Japan. This survey was done from 1982 to 1984. Results were summarized in Table 1. One hundred six patients were registered and the patients with disorders of purine salvage enzymes were frequent.

Table 1. Prevalence of purine metabolic disorders

Name of disorders	No. of patients	Prevalence (in a population of one million)
G-6-Pase deficiency	31	0.26
PRPP synthetase mutant	4	0.03
HGPRT complete deficiency	25	0.21
HGPRT partial deficiency	2	0.017
APRT complete deficiency	10	0.08
APRT partial deficiency	16	0.13
ADA deficiency	4	0.03
PNP deficiency	0	---
5'-NT deficiency	2	0.017
Muscle AMP deaminase deficiency	0	---
Xanthine oxidase deficiency	10	0.08
others	2	0.017
Total	106	0.89

144 EFFECTS OF METHOTREXATE ON INTRACELLULAR NUCLEOTIDE METABOLISM AND ITS ROLE IN COMBINATION CHEMOTHERAPY

Tsutomu Nobori, Toshiki Ohkubo, Hajime Kawasaki, Hitoshi Kamiya, and Minoru Sakurai Dept. of Pediatrics, Mie Univ. School of Med., Tsu, Japan

In the combination chemotherapy of MTX and Ara-C or MTX and 5-FU, pretreatment with MTX elevated intracellular Ara-CTP and FUTP levels, respectively. The enhanced phosphorylation of Ara-C and 5-FU might be one of the mechanisms for the synergistic interaction between them. Ara-CTP and FUTP levels in L1210 leukemic cells after treatment with Ara-C and 5-FU were measured by high pressure liquid chromatography(HPLC). When MTX was preceded 3hrs to Ara-C, Ara-CTP levels markedly elevated to about 170% of that of Ara-C alone. At 12hr after Ara-C, intracellular Ara-CTP levels in all of groups, in which MTX preceded Ara-C, elevated 2.5- to 4-fold as compared with that of Ara-C alone. The changes in nucleotide pools after MTX were also analyzed by HPLC. ATP, GTP, and dTTP levels were decreased by 40, 30, and 50%, respectively, in the cells at 3hr after MTX. And also dCTP levels markedly decreased by 40% at 3hr. It was concluded that the decreased dCTP activated deoxycytidine kinase and as a result, enhanced the Ara-C phosphorylation in the cells. On MTX and 5-FU combination chemotherapy, FUTP levels elevated 1.6-fold as compared with that of 5-FU alone when MTX was preceded 2 or 6hrs to 5-FU. dTTP levels in the cells treated with MTX or 5-FU were decreased to 23% of that in the untreated cells. There was, however, no significant difference in dTTP levels between MTX-5-FU and 5-FU alone.