131 DIFFERENCES IN PROPERTIES BETWEEN THE NAD-DEPENDENT AND O_2-DEPENDENT TYPES OF RAT LIVER XANTHINE DEHYDROGENASE

Tatsuya Saito, Takeshi Nishino, Keizo Tsushima
Yokohama City University School of Medicine
Department of Biochemistry, Yokohama, Japan

Rat liver xanthine dehydrogenase exists as NAD-dependent type(type-D) in the freshly prepared sample but through the procedure of purification most of type-D enzyme is converted to O_2-dependent type(type-O). This conversion occurs either reversibly through the oxidation of sulfhydryl groups or irreversibly through the proteolysis of enzyme molecule. In the present experiment using purified type-D enzyme 8 moles of sulfhydryl groups per mole of FAD were modified with 4,4'-dithiodipyridine for the complete conversion. 2 moles of 4-thiopyridone were formed by addition of 1 mole of 4,4'-dithiodipyridine, suggesting that these sulfhydryl groups were located closely to each other. Modification with other sulfhydryl reagents showed that sulfhydryl groups essential for the conversion existed in the hydrophobic environments. This enzyme is known to consist of two identical subunits. Each subunit contains one FAD, two iron-sulfur centers and one molybdo-pterine as cofactors. Anaerobic titration of both types of enzyme showed that the redox properties of FAD of type-D enzyme was different from that of type-O enzyme, but that the former became similar to the latter in the presence of NAD. This suggests that the binding of NAD modulates the reactivity of type-D enzyme. The steady state kinetics of both types of enzyme showed that K_m value for O_2 of type-D enzyme was considerably higher than that of type-O and that V_{max} of xanthine-O_2 activity of type-D enzyme was about one-fourth of that of type-O enzyme.

132 CLINICO-BIOCHEMICAL AND MOLECULAR STUDIES OF PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY

Takeshi Sakiyama, Munenori Iwase, Kenichi Horinouchi, Akira Akatsuka, Yasuyoshi Yoshida, Takanobu Kikuchi*, Hiroyuki Shimatake*, Steven R. Williams** and Teruo Kitagawa, Nihon University School of Medicine, Dept. Pediatrics, *Toho University School of Medicine, Dept. of Molecular Biology, Tokyo Japan, **Genentech Inc., South San Francisco U.S.A.

Purine nucleoside phesphorylase (PNP) deficiency is characterized by severe combined immunodeficiency. Although cases of adenosine deaminase deficiency have been reported, this first case has been the only one ever reported in Japan. The patient was a 7-year-old girl who had developed recurrent respiratory infections since 3 years old and died from varicella infection. She had significantly low levels of serum uric acid and reduced T-lymphocytes function, which lead us to examine the PNP activity in erythrocytes. The activity was only 4.8% of the controls. In addition, PNP activity in the parents showed only about 50% of normal controls. The residual PNP in liver was immunologically cross reactive to the antibody which was determined by Ochtalony and Western blotting. The physico-chemical characteristics of PNP activities from the patient's liver showed no remarkable difference when compared to that of the controls except for the slightly different optimal pH. To determine the reason why the PNP activity in the patient was deficient, the molecular approach was necessary. For this purpose the m RNA was extracted from the patient's as well as control livers and Northern blotting method was used. The result showed that the patient had the same molecular RNA size and the intact volume of m RNA. These strongly suggests that the patient has point mutation in PNP-DNA.

133 INFLUENCE OF THE TEMPERATURE ON HYPOXANTHINE TRANSPORT THROUGH HUMAN ERYTHROCYTE MEMBRANES

C.Salerno, E.Capuozzo, A.Giacomello, C.Crifò
Laboratory of Biochemical and Clinical Analysis, University of Chieti, and Department of Biochemical Sciences, University of Rome.

In phosphate-free isotonic media, 14-C hypoxanthine is taken up by human erythrocytes but less than 5% of the purine base is metabolized to IMP. The kinetics of uptake is strongly dependent on temperature and on initial hypoxanthine concentration in the suspending medium. At 0-5°C, but not at higher temperatures, the addition of cold hypoxanthine to the incubation mixture leads to a decrease of the amount of labeled base taken up by the cells. Experiments performed at equilibrium show that the total amount of radioactive material taken up by erythrocytes is a hyperbolic function of the total (labeled plus cold) hypoxanthine concentration. A decrease in the rate of 14-C hypoxanthine uptake is observed when the cells are preincubated in the presence of proteinase K. By contrast, when the uptake is studied at 20-37°C, the time course of hypoxanthine uptake by protease-treated cells appears to be very similar to that observed using native cells. At these temperatures, hypoxanthine is taken up by the cells until its intracellular concentration becomes approximately equal to the extracellular one.

134 ADENINE UPTAKE BY HUMAN ERYTHROCYTES

C. Salerno, A. Werner, W. Siems, G. Gerber, A. Giacomello, C. Crifò
Laboratory of Biochemical and Clinical Analysis, University of Chieti, and Department of Biochemical Sciences, University of Rome, Italy; Institute of Biochemistry, Humboldt University, Berlin, GDR.

Radiolabeled adenine (1-20 µM) is taken up and accumulated intracellularly above equilibrium levels by human erythrocytes suspended in phosphate-free isotonic media at 37°C. Adenine, adenosine, and adenine nucleotides are the chief labeled compounds present in the cells under these conditions. The incorporation of labeled adenine is strongly inhibited by cold adenine (5-50 µM), but not by hypoxanthine (up to 2 mM). The rate of adenine incorporation is independent of the oxygen partial tension and reaches a maximum value around physiological pH. The rate of incorporation of the purine base increases, if the cells are preincubated for 10-30 min at 37°C in the presence of 40-100 mU/ml xanthine oxidase. The same result can be obtained, using erythrocytes enriched with PRPP or with 14-C IMP. In the latter case, adenine uptake is associated with the release of equimolar amounts of hypoxanthine which results from the catabolism of intracellular IMP.

135 USE OF PURINE NUCLEOTIDE AND NUCLEOSIDE METABOLISING ENZYMES AS TOOLS TO DETERMINE THE PRESENCE OF PURINERGIC NERVE TRANSMISSION IN SMOOTH MUSCLE.

David G. Satchell, Dept. of Zoology, University of Melbourne, Parkville 3052, Victoria, Australia.

It is likely that an exogenously applied enzyme can access the synaptic region and can be used to examine the role of its substrate as a neurotransmitter provided that the end product has reduced activity in the tissue.

In guinea-pig ATP has been suggested as the neurotransmitter mediating relaxation by the non-adrenergic, non-cholinergic (NANC) nerves in the taenia coli and as a cotransmitter mediating the twitch component of the contraction in response to sympathetic nerve stimulation in the vas deferens. Nucleotide pyrophosphatase converts ATP to a much less active AMP and caused a significant reduction in the relaxations to NANC nerve stimulation in the taenia and to the twitch components of contractions to sympathetic nerve stimulation in the vas deferens. The enzyme failed to affect responses to noradrenaline and adrenergic neurotransmission in each tissue demonstrating its specificity.

In the guinea-pig trachea adenosine has been suggested as the neurotransmitter in NANC inhibitory nerves. In dipyridamole treated preparations; adenosine deaminase caused a significant reduction in inhibitory responses to NANC nerve stimulation and to adenosine wherease responses to VIP and cholinergic nerve stimulation were unaffected. Thus studies with nucleoside and nucleotide metabolising enzymes have provided support for purinergic transmission in the guinea-pig.

136 NUCLEOTIDE PYROPHOSPHATASE ANTAGONIZES CONTRACTIONS DUE TO THE TWITCH COMPONENT OF THE SYMPATHETIC NERVE RESPONSE AND TO APPLIED ATP IN THE GUINEA-PIG VAS DEFERENS.

David G. Satchell, Dept. of Zoology, University of Melbourne, Parkville 3052, Victoria, Australia.

Exogenously applied ATP causes a rapid contraction of the isolated guinea-pig vas deferens preparation and is a candidate as a cotransmitter mediating the twitch component of the response to sympathetic nerve stimulation in the preparation.

The enzyme nucleotide pyrophosphatase converts ATP to AMP which is inactive in the vas deferens preparation. In preparations treated with the enzyme, contractions to ATP were markedly reduced compared to control untreated preparations. Contractions in response to sympathetic nerve stimulation were reduced in amplitude only slightly by nucleotide pyrophosphatase treatment although the peak of the initial twitch component was abolished leaving the slower phasic component. The drug phentolamine abolished the slower phasic response to sympathetic nerve stimulation which is attributed to the noradrenaline component of the cotransmission but the initial twitch component was not reduced. In phentolamine treated preparations, nucleotide pyrophosphatase caused a marked reduction of the response to sympathetic nerve stimulation. Nucleotide pyrophosphatase failed to affect contractions due to noradrenaline in phentolamine-free preparations.

The finding that nucleotide pyrophosphatase selectively antagonised the twitch component of the response to sympathetic nerve stimulation and contractions to ATP supports the idea that ATP is a cotransmitter in the tissue.