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PHORBOL ESTERS ABROGATE MAST CELL ADENOSINE RESPONSIVENESS. Diana L. Marquardt and Linda L. Walker. University of California San Diego, School of Medicine, Department of Medicine, San Diego, California, USA.

Adenosine potentiates the release of preformed mediators such as β-hexosaminidase (β-hex) from stimulated mast cells by a mechanism that is poorly understood. Mouse bone marrow-derived mast cells incubated for 2 hours with nanomolar concentrations of 4β-phorbol 12β-myristate 13acetate (PMA) exhibited a marked suppression of the ability of adenosine to augment β-hex release induced by specific antigen or the calcium ionophore, A23187. Antigen-induced mediator release itself was also suppressed by PMA, whereas PMA and A23187 caused a synergistic enhancement of β-hex release. Overnight PMA treatment of mast cells produced a similar hyporesponsiveness to adenosine as well as a striking inhibition of the secretory response to antigen or A23187. The generation of leukotriene C_4 was unaffected by overnight PMA. The ability of adenosine to augment mast cell cyclic AMP concentrations was only modestly inhibited by 2-hour or overnight PMA exposure. PMA induced an enhancement of antigen-stimulated intracellular free calcium levels as determined utilizing fura-2, but the ability of adenosine to potentiate this calcium response was completely abrogated. Either protein kinase C activation or a PMA effect on adenosine receptor expression or recycling in some way abolishes the ability of adenosine to augment mediator release. The inability of PMA to affect LTC_4 generation underscores the dissociation between preformed and newly generated mediator releases. These data suggest that protein kinase C activity may be a key component of the mechanism of action of adenosine in mast cells.

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ENHANCED ADENINE NUCLEOTIDE DEGRADATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD): THE EFFECT OF OXYGEN THERAPY. F Mateos, P Gómez, J Puig, M Jiménez, T Ramos, and J Mantilla. 'La Paz' Hospital, Department of Clinical Biochemistry and Internal Medicine. Universidad Autónoma, Madrid, Spain.

Hypoxemia may result in tissue hypoxia and increased production and excretion of adenine triphosphate (ATP) degradation intermediates and uric acid (UA). If this hypothesis is correct, then adenine nucleotide degradation should diminish following increased delivery of oxygen to hypoxic tissues. Five patients with clinically stable COPD were infused with [8-^{14}C]adenine to radiolabel the adenine nucleotide poool. Cumulative 3 days radioactivity excretion and urinary hypoxanthine (Hx), xanthine (X), UA and total purines (TP=sum of Hx, X and UA) were measured to assess purine nucleotide degradation before and after inhalation of oxygen (FiO_2, 24%) for 4 days. Results (mean±SEM) were compared to those obtained in 4 normal subjects

		PaO_2	Cumulative ^{14}C excretion	Hx	X	UA	TP
		(mm Hg)	(% dose)	(μmol/g cr.)		(mmol/g cr.)	
Controls			3.46±0.28	24±4	18±1	2.00±0.10	2.04±0.1
COPD	Basal	50±2	5.01±0.34*	47±13*	30±8*	4.23±0.78*	4.31±0.8*
	Oxygen	65±1*	3.09±0.26+	26±5+	15±5	3.44±0.72+	3.48±0.7+

*P<0.05 vs controls; +P<0.05 vs basal

The increased basal excretion of ^{14}C and urinary purines in COPD patients suggest that hypoxemia accelerates adenine nucleotide degradation. The decrease in these parameters elicited by oxygen therapy lends further support to the hypothesis.

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ERYTHROCYTE ATP (iATP) AS AN INDICATOR OF NEONATAL HYPOXIA. F Mateos, J Puig, T Ramos, R Carranza, ME Miranda, and R Gasalla. 'La Paz' Hospital, Departments of Clinical Biochemistry and Internal Medicine, Universidad Autónoma, Madrid, Spain.

Reliable parameters of neonatal hypoxia have long been sought to identify infants with risk of brain damage. Increased hypoxanthine (Hx) concentrations have been correlated with clinical and laboratory signs of perinatal asphyxia. Hypoxia stimulates erythrocyte 2,3-diphosphoglycerate synthesis and may diminish iATP. In this study we compared pH, iATP and plasma Hx concentrations in the umbilical cord blood of 38 full-term neonates. Subjects were asigned to the following groups: 10 controls (I), 10 delivered by caesarean section on maternal indications (II), 9 by vaginal route with meconium-stained amniotic fluid (III) and 9 with sustained bradicardia (< 100/min) defined by cardiotocographic monitoring (IV). Results (mean±SEM) were as follows:

Group	Apgar score (1 min)	Weight (g)	pH	Hx (μM)	ATP ($nmol/10^9$ cel)
I	8±0	3400±310	7.33±0.03	3.6±0.3	170±7
II	8±0	3375±270	7.33±0.03	1.9±0.1*	165±5
III	6±2	3405±328	7.26±0.26+	6.2±1.6+	128±4+
IV	4±1	3344±136	7.17±0.12+	10.8±5.4+	130±1+

*P<0.05 vs group I; +P<0.01 vs groups I and II

Some neonates with clinical signs of asphyxia (groups III and IV) had normal pH and Hx levels, but all evidenced subnormal iATP concentrations. We conclude that umbilical cord iATP discriminates between normal and asphyxiated infants and thus may be a usefull indicator of hypoxia in the full-term newborn.

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REDUCTION OF ARA-C CYTOTOXICITY IN HL 60 CELLS BY ADDITION OF DEOXYCYTIDINE, CYTIDINE OR INCREASED LEVELS OF CYTIDINE DEAMINASE.
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Ara-C closely resembles the natural nucleosides cytidine and deoxycytidine. The deamination of the three compounds are catalyzed by the enzyme cytidine deaminase (CDD). Tetrahydrouridine (THU), an inhibitor of the enzyme, was found to increase the cytoxicity of Ara-C in 3 days suspension cultures of HL 60 cells. THU alone was found without any effect. The activity of CDD in extracts of HL 60 cells could be induced to increase, when the cells were exposed to the differentiation inducer 1,25 dihydroxy D_3. This was not seen with retinoic acid another inducer of differentiation. Corresponding to these findings, the cytotoxicity of Ara-C was found to be antagonized by 1,25 dihydroxy D_3, while retinoic acid increased the cytotoxicity of Ara-C, supporting the assumption, that CDD activity is relevant for the Ara-C effect.

Cytidine added together with Ara-C reduced the Ara-C effect. However, the combination of cytidine and uridine was able to neutralize the effect of cytidine on the Ara-C effect, probably by competetive inhibition of uridine cytidine kinase.

Similarly, deoxycytidine could neutralize the Ara-C effect, which then could be restored by the addition of THU. An explanation for this could be a higher degree of Ara-C phosphorylation compared to deoxycytidine phosphorylation, both catalyzed by deoxycytidine kinase, which has been observed in acute myeloid leukemia cells.

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NAD SYNTHESIS BY ERYTHROCYTES IN PHOSPHORIBOSYL -PYROPHOSPHATE SYNTHETASE (PPRPs) SUPERACTIVITY
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Extremely low erythrocyte NAD levels (14μM-control mean 70μM) were a repeated finding in a patient with PPRPs superactivity and neurological abnormalities, including inherited nerve deafness. The biochemical basis for this was studied using (^{14}C) labelled NAD precursor, Nicotinic acid (NA)+glutamine, or Nicotinamide (Nam) and intact erythrocytes as an in vitro model. Various incubation times (1 min-48hrs) and substrate levels (1μM-3mM) were used, at physiological phosphate (1mM Pi) and PRPP stimulating conditions (18mM Pi). NAD formation was higher from Nam than NA at low substrate levels in controls and patient. The patient showed faster NAD production, but a slower incorporation of Nam and NA into NMN, deNMN, with no stimulation by Pi. NAD was the predominant metabolite from either route after 24 or 48 hrs, with no decomposition in patient or controls. Some NA and deNMN were found at low and high Nam concentrations in the patient, indicating deamination of unmetabolized Nam. At high precursor concentrations 10-fold higher levels of NAD were produced by NA than by Nam. In the patient synthesis was less than 10% of controls from both precursors and although endogenous NAD levels were doubled by NA, the results suggest they could not be normalized by therapy with either NA or Nam. NAD stability was not altered in the patient. Thus, lower substrate utilization for nucleotide synthesis, or alternative metabolism must be implicated in the low erythrocyte NAD level. Higher substrate levels did not increase production rate, suggesting that maximal activity and not substrate affinity of the committed enzymes may be involved.

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MYOGENIC HYPERURICEMIA: A COMPARATIVE STUDY BETWEEN TYPE V AND TYPE VII GLYCOGENOSIS.
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Excess purine degradation in exercising muscle due to impaired glycolysis causes hyperuricemia in muscle glycogenoses (myogenic hyperuricemia). However, hyperuricemia has been seen more frequently in Type VII than in Type V. This study was designed to clarify a metabolic basis for the different frequency of hyperuricemia between the diseases. 5 patients (2 males, 3 females) with Type V and 4 (3 males, 1 female) with Type VII participated in the study. 1) In every patient, semiischemic forearm exercise caused no increase in lactate but exaggerated increases in ammonia, inosine and hypoxanthine in cubital venous blood. The ammonia increase was not different between Type V and Type VII, but it was greater in male than female patients. 2) Semiischemic forearm exercise performed after glucagon injection showed that lactate production was induced by exercise in Type V but not in Type VII. 3) Saline or glucose solution was infused in a patient with Type V during exercise on a bicycle ergometer. With saline infusion, ammonia and hypoxanthine in systemic circulation increased greatly after exercise. Conversely, with glucose infusion there were no increases in these metabolites. These findings indicated that blood glucose can be available as metabolic fuel through muscle glycolysis in Type V but not in Type VII. Thus, muscle purine degradation may be accelerated more in Type VII than in Type V, leading to a higher frequency of hyperuricemia.