ESPR—Abstracts for Oral Presentations

1 DEGRADATION KINETICS OF HIGH ENERGY PHOSPHATES IN THE RABBIT BRAIN USING NMR-SPECTROSCOPY

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Others have shown by NMR spectroscopy that ischaemia produces a degradation of phosphocreatine (PCr) and ATP. We tested the feasibility of NMR spectroscopy for gaining kinetic data on the degradation of PCr and ATP at different body temperatures. Anaesthesized rabbits were ventilated and cooled externally to 24° C and 21° C respectively. NMR spectra of 31P phosphates were recorded using a 1.9 Tesla, 24 cm bore superconducting magnet. Degradation kinetics of the compounds were measured after cardiac arrest at 35°, 24° and 21°.

The decay of PCr and ATP followed a single exponential function, indicating first order kinetics. The half time values (T/2) are given in the table (4 animals per temperature; SD)

	35°C	24°C	21°C
T/2 PCr (min)	19 ± 8	48 ± 6	63 ± 19
T/2 ATP (min)	35 ± 10	69 ± 10	112 ± 19

Temperature dependency follows the Arrhenius law for chemical kinetics. From the Arrhenius plot the activation energies were calculated to be 16.7 ± 2.8 kcal/mol for PCr and 14.6 ± 2.1 kcal/mol for ATP. These energies are typical for enzyme catalyzed reactions. Our data show that in situ NMR spectroscopy can be used to study the kinetics of degradation of high energy phosphates. This method could be used to investigate how far hypothermia can protect the brain during operations in circulatory arrest and deep hypothermia.

2 ATRIAL NATRIURETIC PEPTIDE IN CHILDREN - THE HEART AS AN ENDOCRINE ORGAN.

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Cardiocytes of human atria possess granules similar to those of endocrine glands. These granules contain polypeptides with potent natriuretic and vasorelaxing properties (atrial natriuretic peptide = ANP). The presence of ANP in circulating blood has not yet been demonstrated in human peripheral blood.

Using a specific and sensitive radioimmunoassay, we were able to detect and quantitate ANP in plasma of normal children (n=51) and of children with cardiovascular diseases (n=18). In normal children, the mean ANP plasma concentration was 27.5 fmol/ml (range 1 - 45.5 fmol/ml). There was no significant difference of ANP-levels between infants (n=12) and older children (n=39). However, in patients with cardiovascular diseases such as congenital heart malformations and bronchopulmonary dysplasia, ANP-levels were up to 2o times higher (mean 162.7 fmol/ml; range 37 - 537 fmol/ml) when compared with the mean value in normal children. 15 out of 18 patients exceeded the upper range of ANP-levels in controls. Furthermore, in critically ill children (n=3), ANP-levels were markedly higher at the time of hospital admission (range 92.5 - 266.6 fmol/ml) than after therapy (range 25.8 - 55.1 fmol/ml). In accordance to observations in experimental animals, our findings suggest that atrial distension caused the increased ANP-release in our patients with cardiac diseases. Measurement of ANP-levels may be a useful tool to assess circulatory status in cardiac diseases.

3 Abnormalities at a cellular level in relation to the presence of bronchial hyperresponsiveness (BHR) and asthma.

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We compared in vitro responses of leucocytes (study I) in asthmatic children with various degrees of BHR and in healthy children. Cell responses were assessed by the generation of superoxide anion (O_2^-) and the release of histamine after challenge with calcium ionophore A 23187 plus Ca^{2+}, and with deuterium oxide (D_2O). BHR was graded on the basis of responses to inhaled histamine and to exercise. The mean generation of O_2^- and the mean release of histamine by the Ca ionophore and by D_2O were significantly greater in the asthmatics than in the healthy subjects ($P<0.05$ and $P<0.025$ respectively). Between patients with pronounced and limited BHR the histamine release but not the O_2^- generation was significantly different ($P<0.025$). In study II calcium influx, change in membrane potential and histamine release after anti-IgE, Ca ionophore and D_2O were measured in a suspension of basophils, selected by a fluorescence activated cell sorter. (Purity 60%, recovery 20%, high reproducibility). Results of study I and preliminary results of study II suggest a basic intracellular abnormality in asthma and BHR.

4 BIOCHEMICAL ACTION OF C3 CONVERTING FACTOR (C3 COF) ON THE THIRD COMPONENT OF HUMAN COMPLEMENT

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After preliminary characterization of C3 converting factor (C3 CoF) the question was raised whether this factor was a "true" activator of C3, i.e. generates C3a and C3b on interaction with native C3. For this purpose C3 NeF and C3 CoF activity were separated from each other by euglobulin precipitation and anion exchange chromatography using a discontinuous salt gradient. The C3 CoF containing fraction was devoid IgG. To identify the site of cleavage in the C3 molecule C3 was isolated from normal human serum to apparent homogeneity and radiolabelled with 125-iodine using the Bolten-Hunter method. Incubation of this radiolabelled C3 with C3 CoF isolated from patient's serum generated two major C3 fragments identified by autoradiography following SDS-PAGE under reducing conditions: A large fragment with 117 kD and a small fragment with approx. 10 kD. Following autoradiography proteins were transblotted on nitrocellulose and analyzed for expression of C3 antigens using the Western blot technique and monospecific antisera to C3a and C3c. The 117 kD fragment expressed C3c antigen but no C3a antigen, the 10 kD fragment expressed C3a antigen only indicating that C3 was, indeed, cleaved into C3a and C3b. We conclude that C3 CoF is a "true" activator of C3.

5 PROMOTION OF INTRATHYMIC T-CELL DEVELOPMENT BY MHC-Ia-POSITIVE THYMIC MACROPHAGES (Ia^+ Mø).

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The generation of the peripheral T-cell pool is attributed to maturation and differentiation events occurring within the thymus. Three thymic compartments, thymic epithelial cells, thymic hormons and thymic Mø are considered to contribute to the intrathymic T-cell development. While, as we have shown earlier, murine as well as human MHC-Ia-negative-Mø (Ia^- Mø) regulate thymocyte (TH) differentiation via suppressive effects (Thymus,6:295,1984) and MHC-Ia^+-Mø are involved in intrathymic tolerance induction (Pediatr Res,15:800,1984), now the contribution of Ia^+Mø to intrathymic lymphopoiesis was investigated.

An isolation method yielding cell suspensions highly enriched for Ia^+ thymic Mø was performed (BSA-gradient,antibody treatment,irradiation). Cocultivation of these Ia^+Mø with TH showed that Ia^+Mø strongly augmented the mitogen-induced proliferation of TH by about 200%. The effect was dependent on the number of Ia^+Mø added and on the degree of TH maturity: stronger augmentation occurred at higher Mø concentrations; immature TH showed highest susceptibility to the Ia^+Mø mediated effect. Cell-cell-contact was an important prerequisite for the proliferation amplifying effect as demonstrated by use of Cytochalasin B. In addition, humoral factors produced by Ia^+Mø also improved the proliferative capacity of TH, similar to results obtained using interleukin II. The results described support the view that thymic Mø populations act as a main regulatory principle in T-cell development. Ia^+Mø augment intrathymic lymphopoiesis and participate in tolerance induction,while Ia^-Mø control the number of TH finally leaving the thymus gland.

6 Deoxycoformycin (DCF) and desoxyadenosine (dADO) in vitro treatment for graft-versus-host disease (GVHD) prevention.

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Dividing as well as resting T cells are sensitive to µM concentrations of dADO in the presence of an adenosine-deaminase (ADA) inhibitor such as DCF. Therefore, this drug combination was investigated for its ability to deplete T cells from allogeneic rat bone marrow (BM) and spleen (SP) cells and for its potential to prevent GVHD in fully allogeneic rat bone marrow transplantation(BMT). - It was found that a concentration of 0.5 µM of DCF was sufficient to block ADA-activity in rat BM and SP cells completely. Concentrations as high as 100 µM were not toxic to in vitro colony forming units (CFU-GM). Increasing dADO concentrations in the presence of DCF led to increasing T cell but also increasing CFU toxicity. However, dADO concentrations below 40 µM had no significant influence on CFU growth but clearly exhibited an effect on T cells. T cell functions, as determined by mitogen and alloantigen stimulated thymidine (3HTdR) uptake, were reduced to 10-30% of normal values. Flowcytometric analysis revealed a significant T cell reduction following in vitro treatment. However, some residual T cells were detectable in treated samples. - In accordance with the described in vitro data, rat-recipients of DCF/dADO treated allogeneic BM and SP cell grafts showed a significantly decreased incidence of acute lethal GVHD. - The results indicate that a significant but incomplete depletion of T cells can be achieved through DCF/dADO in vitro treatment using concentrations which are not toxic for stem cells. Such T cell depletion results in modification of acute lethal GVHD into a chronic form in fully allogeneic rat BMT.