181 THE EFFECT OF DEOXYGUANOSINE ON THE IN VITRO PROLIFERATION AND DIFFERENTIATION OF NORMAL HUMAN B LYMPHOCYTES.

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The effect of dGuo - being the toxic metabolite in PNP-deficiency - on normal B lymphocytes was studied: purified B cells were cultured with formalinized Staphylococcus aureus Cowan I during 18 hours, leading to activation of resting B cells which then become sensitive to the action of B cell growth and differentiation factors present in the supernatant of Con-A-activated T cells. In this system both proliferation and differentiation of the B cells do occur and can be inhibited by dGuo in a dose-dependent way. Subsequently the mechanism of this inhibitory phenomenon was studied, e.g. by addition of hypoxanthine, adenine, deoxycytidine and the PNP-inhibitor 8-aminoguanosine. The results indicate that the accumulation of dGTP is not the cause of dGuo toxicity for in vitro function of normal B cells. Toxicity of dGuo requires the presence of active PNP and most probably is based on a product of the salvage of guanine (i.e. GMP, GDP or GTP). We conclude that proliferation and differentiation of normal B cells is inhibited by dGuo through a mechanism independent of dGTP accumulation. Consequently B cells of PNP deficient patients escape dGuo toxicity because PNP is lacking.

182 THE INHIBITORY EFFECT OF DEOXYADENOSINE AND DEOXYGUANOSINE ON IN VITRO LYMPHOCYTE FUNCTION ARE EXPRESSED AT DIFFERENT STAGES OF LYMPHOCYTE ACTIVATION.

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In earlier studies we showed that deoxyguanosine(dGuo) which confers immunodeficiency in purine nucleoside phosphorylase (PNP) deficient patients, inhibits the in vitro proliferation of normal human T and B lymphocytes. Deoxyadenosine (dAdo) being the toxic metabolite in adenosine deaminase (ADA) deficiency, also inhibits the in vitro proliferation of normal human T lymphocytes under conditions where ADA activity is inhibited. We analyzed the kinetics of the inhibition of T cell proliferation by dAdo and dGuo respectively and found significant differences. Whereas dAdo has to be present from the initiation of the culture onwards, dGuo can be added as late as 48 hrs. after the initiation of the culture to exert its toxic effect. This finding points towards an interference of dAdo with early lymphocyte activation events which preceed the onset of DNA synthesis. Subsequently, the effect of dAdo and dGuo on a number of events associated with lymphocyte activation was investigated like e.g. on mitogen-induced Ca-influx, on Ca-ionofoor-mediated expression of the receptor for Interleukin 2 (IL-2) and on the IL-2 dependent proliferation of receptor carrying T lymphocytes. The results provide evidence for different mechanisms underlying the toxic effects of dAdo and dGuo for lymphocytes.

183 EVIDENCE AGAINST THE ADENOSINE-CATECHOLAMINE ANTAGONISM IN THE IN SITU DOG HEART.

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Adenosine has been reported to attenuate the positive inotropic effects of catecholamines in in vitro-heart preparations of rodents. These results were not confirmed in anesthetized dogs during intracoronary infusion of isoproterenol. However, these experiments did not study the effects of adenosine on the inotropic effects of endogenously released catecholamines. Therefore we performed cardiac sympathetic nerve stimulation (CSNS) in 5 anesthetized, vagotomized dogs. The left circumflex coronary artery was perfused at a constant pressure of 130±4 mmHg. The contractile function of the circumflex-perfused myocardium was analyzed by sonomicrometry. CSNS at 1, 2, 5, 10 and 20 Hz increased systolic segment shortening (SS_{cx}) in a frequency-dependent manner from 10.9±2.6 at control to 15.3±3.4 % at 20 Hz. During intracoronary infusion of adenosine (50 µg/kg/min) CSNS still increased SS_{cx} frequency-dependently from 9.9±3.5 to 16.3±4.5 %. The lack of any adenosine-catecholamine antagonism in our experiments was not due to the sequence of procedures, nor to the marked flow increase induced by adenosine, since SS_{cx} at rest was rather reduced with adenosine infusion and since during intracoronary infusion of papaverine and sodium-nitroprusside similar results were obtained. It seems that the observation of adenosine-catecholamine antagonism in rodents is a species dependent phenomenon.

Supported by SFB 30 - Kardiologie, Düsseldorf

184 ENZYME ACTIVITIES OF PURINE CATABOLISM AND SALVAGE IN HUMAN MUSCLE.

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Since there is little information about the enzyme distribution of purine catabolism and salvage available, it was reasonable to determine activities of some key enzymes in human tissue samples. This might be of importance for purine metabolism of human muscle of different origin. Using radiochemical tests the following enzyme activities were detected: adenosine kinase (AK), adenine phopshoribosyltransferase (APRT), hypoxanthin guanine phosphoribosyltransferase (HGPRT), adenosine deaminase (ADA), and purinenucleoside phosphorylase (PNP).

The following activities were determined:

Enzyme	Myocardium	M. rectus abdominis	M. quadriceps
AK	156 ± 38	85 ± 21	140 ± 42
APRT	242 ± 73	383 ± 69	266 ± 79
HGPRT	134 ± 40	175 ± 45	280 ± 56
ADA	5408 ± 513	1742 ± 414	521 ± 76
PNP	4375 ± 646	2381 ± 466	2552 ± 328

Enzyme activites in nmol/g protein/min.

185 PURINE SALVAGE IN RAT HEART MYOBLASTS.

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The reutilization of purine bases and purine nucleosides was investigated in rat heart myoblasts. The cells were cultivated in Eagle's medium. Incubations were performed at 37° for 1 hour, 14C-labeled adenine, hypoxanthine, adenosine and inosine were used as substrates (5.2 µmol/l). After the incubation cells were harvested, purine metabolites extracted with perchloric acid and separated by thin-layer chromatography. The radioactivity of metabolites was measured.

The total incorporation into rat heart myoblasts' purines was for adenine, hypoxanthine, adenosine and inosine was 1.4 ± 0.77, 0.64 ± 0.22, 4.95 ± 1.49, 1.55 ± 0.68 nmoles/mg protein/hr respectively. Thin-layer chromatographic separation showed that most of the radioactivity has been incorporated into adenine nucleotides; the amount of labeled adenine nucleotides was 52%, 21%, 30% and 34% of total intracellular radioactivity using the 4 substrates. Phosphate stimulated incorporation in all experiments.

186 INTERMITTENT CONTROL OF HYPERURICEMIA IN THE TREATMENT OF GOUT

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The conventional treatment of those patients with gout in whom urate-lowering medication is considered desirable is to administer such drugs continuously for an indefinite period.

It has been shown that dissolution rates of urate crystals in normouricemic or hyperuricemic solutions *in vitro* are very fast compared with rates of crystal growth in supersaturated solutions. Our previous clinical studies have shown that after discontinuing treatment with allopurinol the serum uric acid level returns rapidly to pre-treatment values, but long periods may pass before the return of gouty arthritis. In the light of these observations it seems logical to assess the effect of intermittent control of hyperuricemia in the treatment of gout.

In a preliminary trial, six patients have been taking allopurinol for only two months in a year. The biochemical response has been as anticipated - normouricemia during treatment, with hyperuricemia resumed once the drug is stopped. However, to date the clinical response seems to have been comparable to that normally obtained with continuous therapy. The results are sufficiently encouraging to justify setting up a formal trial comparing continuous with intermittent treatment.