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KINETIC PARAMETERS OF DOPAMINE UPTAKE BEFORE AND AFTER ALCOHOL EXPOSURE IN THE RAT

P. Olshausen, T. Reum, D. Schönfuß, R. Morgenstern

Institute of Pharmacology and Toxicology, Charité, Humboldt-University Berlin

Dopaminergic mechanisms play a key role in the development of addiction in general, one being the addiction to alcohol involving specifically the mesolimbic/mesocortical dopaminergic system, the so called reward system. Alcohol has several CNS effects of which possible changes in the dopamine re-uptake mechanisms via the dopamine transporter (DAT) are of interest in this study. Apart from the acutely induced CNS changes caused by alcohol, theories exist linking the activity of the dopaminergic system with a possible predisposition to dependence. The 'deficiency hypothesis' assumes that an attenuated activity of the reward system might be compensated for by the intake of certain addictive substances, whereas the 'surfeit hypothesis' claims a heightened activity to be responsible for an extremely pronounced effect of the drug. In previous experiments with laboratory animals it was shown that there are individual differences in alcohol preference, as in humans, with some animals preferring alcohol more than others. With this in mind, the following questions arise for this study: 1. Do preferring and non-preferring rats already differ in their *in vivo* dopamine re-uptake kinetics before the first

exposure to alcohol? 2. How do these uptake kinetics change in the two groups (preferring/non-preferring) after the exposure to alcohol?

The method of *in vivo* voltammetric detection was used to measure the time-concentration curves of dopamine in individual rats before and after exposure to alcohol. The basic principle of voltammetry is to use the oxidation of dopamine on the surface of a carbon fibre electrode to measure dopamine levels; the freed electrons are proportional to the number of oxidized dopamine molecules and can be measured as a current in an electrochemical system. With the help of this method we studied the kinetics of dopamine uptake in the nucleus accumbens. The voltammetric method employed was the continuous amperometry. The medial forebrain bundle was stimulated with a stimulation electrode and the release and uptake of dopamine were measured with the carbon fibre electrode implanted in the nucleus accumbens. The uptake was the important variable for calculating kinetic parameters such as the dopamine transporter's maximum velocity (V_{max}) or dopamine's half-life in the extracellular space ($t_{1/2}$) with the help of a mathematical model employing partial differential equations. Three groups of animals were measured. The control group ($n = 7$) did not receive any alcohol. The other rats ($n = 15$) were offered 5% and 10% alcohol *ad libitum* for three months and were then conditioned to press a lever to obtain 5% alcohol during a 4 week period with an ensuing progressive ratio schedule to test for possible dependence. These rats were categorised according to the amount of alcohol they drank during the *ad libitum* phase. Using this method of categorisation we were not able to detect a statistically significant difference (ANOVA) between the groups in any of the kinetic parameters before or after alcohol exposure nor were there any statistically significant changes (t-test) in

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the individual groups after drinking alcohol. Therefore, these findings alone do not suggest a correlation between DAT activity and alcohol preference. Further analyses will be carried out focusing on the rats' performance in the progressive ratio schedule.

DC-SIGN, A NOVEL RECEPTOR ON DENDRITIC CELLS INVOLVED IN A NEW PATHWAY FOR HIV-1 INFECTION

T.B.H. Geijtenbeek, J.F.M. Jacobs, S.J. van Vliet, G.C.F. van Duijnhoven, Y. van Kooyk, C.G. Figdor

Department of Tumor Immunology, University Hospital Nijmegen, Nijmegen, The Netherlands.

Dendritic cells (DCs) are professional antigen presenting cells which capture pathogens anywhere in the body. The DCs migrate towards lymphoid organs and present the processed antigens to T cells. A specific subset of T cells will recognise the antigen on the DC and will be triggered to initiate an immune response. In this study we present DC-SIGN, a novel adhesion molecule on DCs involved in the initial contact between DCs and T cells. DC-SIGN recognises and binds to its ligand ICAM-3 on naive T cells, which brings the DC close to the T cell. The interaction between DC-SIGN and ICAM-3 is transient, but lasts just long enough for the T cell to scan the surface of the DC for specific antigens. We further demonstrated that DC-SIGN binds to the HIV-1 envelope glycoprotein gp120. A kinetics experiment revealed that DC-SIGN can be internalised within one hour after binding its ligand. These results led us to the proposal of a new model for HIV-infection. The DC encounters and binds to HIV-1 in mucosal tissues. After binding of the virus to DC-SIGN, the complex is first rapidly internalized (protecting it from a fatal immune response). The virus is subsequently presented to resting CD4⁺ T lymphocytes. Within T cells the virus will replicate which causes a severe suppression of the immune system. This process will ultimately lead to the lethal disease AIDS.

INVESTIGATION OF FOLLICULAR PENETRATION

N. Otberg, J. Lademann, W. Sterry, H.J. Weigmann

Department of Dermatology, Humboldt-University of Berlin

Topically applied substances are widely used in the field of medical science and cosmetics. In medical science, the investigation of percutaneous absorption for

any topical drug application is necessary for the optimization of effects and the reduction of side effects. In cosmetic research, the studies of sunscreens are of great importance. The main attention in the investigation of percutaneous absorption pathways has been given to the stratum corneum and its appendages. Permeation through the stratum corneum (transcorneal permeation) occurs through the intercellular lipid domain or through the corneocytes themselves (transcellular route). Furthermore, sweat glands and hair follicles are an important pathway for topically applied substances. Preliminary tests have shown that some vellus hair follicles are functionally 'closed' while some other follicles are 'open' allowing transfollicular penetration. As a result of these studies a new procedure was designed for the detection of transfollicular penetration to identify parameters of vellus hair physiology that influence follicular absorption. Different methods for the investigation of these influencing parameters on transfollicular penetration were combined. Cyanoacrylate follicular biopsies, a sebum sensitive tape (Sebutape[®]) and a modified phototrichogram as well as light and laser scan microscopy were used. Two skin areas of 2.25 cm² on the backs of six healthy volunteers were observed for a period of 21 days. Sebum excretion and hair growth of any single hair follicle were measured by this procedure. A correlation between sebum excretion, hair growth and the phenomenon of open and closed vellus hair follicles was found. In additional experiments, the vellus hair follicle density in six different regions of the body was studied in cyanoacrylate follicular biopsies and the volume and surface to the hair follicle infundibulum were measured. These experiments have shown the contribution of the follicular surface to the application area.

TARGETING ACTIVE NEOVASCULARIZATION WITH INTEGRIN ANTAGONISTS IN THE MURINE MODEL OF RETINAL NEOVASCULARIZATION AND SUCCESS OF TOPICAL APPLICATION

B. Riecke^a, E. Chavakis^a, A. Alt^a, K.T. Preissner^b, M. Brownlee^c, H.-P. Hammes^a

^a III. Medical Department, Justus-Liebig-University, Germany

^b Institute for Biochemistry, Justus-Liebig-University, Germany

^c Diabetes Research Departments of Medicine and Pathology, Albert Einstein College of Medicine, New York, USA

Purpose: In the murine model of hypoxia induced retinopathy, small peptide antagonists of the vitronectin type integrin receptor (VNR) inhibit retinal

neovascularization (RNV) by 76% when subcutaneous application is initiated prior to the onset of angiogenesis (Nature Medicine 1996; 2: 529). Clinically relevant questions remain whether this approach is effective on preexisting and active retinal neovascularization and whether VNR-antagonists can be topically administered as long as systemic applications carry the risk of side effects and repeated intraocular injections are impractical within the clinical setting. The aim of this study was to investigate a) the dynamics of integrin and growth factor expression, b) ligation inhibition of the VNR as a secondary intervention after the onset of angiogenesis, and c) the efficacy of an RGD-antagonist in inhibiting RNV when topically applied by eye drops. Methods: Seven day old mice were exposed to reduced oxygen (75% of normal) for five days. After the return to room air, the kinetics of angiogenesis in the retina were studied by analyzing a) the expression of α_V -type integrins, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) by performing western blot analysis and b) the time course of neovascularization by performing fluorescein angiographies and quantitative histology. Based on the findings subcutaneous treatments with varying doses of RGD peptide were initiated with a delay of two or five days, respectively, after the return to room air, and were continued for five days. In a topical application trial the peptide antagonist was epicorneally administered in a specially developed eye drop solution. The effect of the treatments was evaluated by fluorescein angiographies and quantitative histology. Results: Immediately after induction of hypoxia, expression of α_V -type integrins was highest and then rapidly declined. VEGF expression was high during the initial phase of angiogenesis and then declined to baseline levels, while bFGF was continuously present throughout the neovascularization period. Maximal extent of angiogenesis was noted between day p17 and p19. Early secondary intervention treatment reduced preretinal neovascularizations in a dose dependent manner with a maximum of 56%. Delayed secondary intervention had no inhibitory effect of preretinal neovascularization, even at the maximally efficient dose of earlier treatments. No side effects have been observed by histological examination of parenchymal organs such as heart or kidney. RGD-eye drop treatment reduced preretinal endothelial cell nuclei dose dependently with a maximum of 48%. Conclusion: With low molecular weight integrin antagonists, the effective inhibition of an ongoing process of angiogenesis can be achieved without detectable side effects. Timely intervention is of crucial importance for the efficacy of this approach. The findings indicate that angiogenesis-related α_V -integrin expression is VEGF- rather than bFGF-dependent, and

the efficacy of RGD-treatment in proliferative retinopathy is only effective as long as the α_V -integrin target is predominantly expressed. The cRGDfV-antagonist used is a small pentapeptide, whose size and biochemical properties enable topical application and lead to sufficient concentrations in the retina to inhibit angiogenesis after epicorneal administration. The topical application by the means of eye drops has the potential of maximizing the therapeutic effect of angiostatic agents while minimizing possible, yet unknown, systemic side effects. It reflects a clinically feasible long term application method that circumvents systemic delivery and intraocular injections.

INFLUENCE OF UNIVENTRICULAR VERSUS BIVENTRICULAR REPAIR FOR COMPLEX CONGENITAL CARDIAC DEFECT ON PERIOPERATIVE PRODUCTION OF VASOACTIVE MEDIATORS

S. Ernst^a, A. Heath^a, J.F. Vazquez-Jimenez^b, G. von Bernuth^a, B.J. Messmer^b, M.-C. Seghaye^a

^a Dept of Paediatric Cardiology, RWTH Aachen, Germany

^b Thoracic and Cardiovascular Surgery, RWTH Aachen, Germany

Objectives: To investigate whether univentricular circulation for operative palliation of congenital cardiac defects influences release of vasoactive mediators. Methods: 25 children (15-177 months) with complex congenital cardiac malformation received either univentricular palliation with atrio-pulmonary or total cavo-pulmonary connection (group UV, $n = 15$) or anatomical biventricular correction (group BV, $n = 10$). Plasma concentrations of vasodilatory mediators such as atrial natriuretic peptide (ANP) and substance P (SP), the molar ratio cGMP/ANP as an index for the biological activity of ANP, and the potent vasoconstrictor Endothelin-1 (ET-1) were sequentially determined before, during, and after cardiac operation up to the tenth post-op. day. Results: ANP blood concentrations and ANP biological activity were similar in both groups before, during and after the operation. In contrast, during cardiac operation and up to the first 3 post-op. days, SP plasma concentrations were significantly higher and ET-1 concentrations significantly lower in group UV compared to group BV. Conclusions: Our results show a clear transient imbalance in favour of vasodilation after univentricular palliation for complex cardiac defects with increased production of SP and decreased production of ET-1. This could contribute to the frequent development of generalised capillary leak with oedema, pleural

effusions, and ascites in children after univentricular palliation. However, by decreasing the systemic and pulmonary vascular resistance, this imbalance also could be beneficial in univentricular circulation and may represent a transient adaptive mechanism.

MODULATORY EFFECT OF FULLERENOL C₆₀(OH)₂₄ ON CYTOTOXICITY INDUCED BY ADRIAMYCIN, CISPLATIN, TAXOL AND THIAZOFURINE ON SELECTED HUMAN CARCINOMA CELL LINES

B. Puhaca

University of Novi Sad, Yugoslavia

Since the discovery of the hydroxyl radical trapping activity of fullerene C₆₀, much attention has been paid to the investigation of its possible application in biological systems. This research was hindered by the poor water solubility of the native C₆₀ molecule (which is soluble in a limited number of biologically unattractive solvents like toluene and benzene). Upon chemical derivatization with hydroxyl groups, conversion of C₆₀ into water-soluble derivatives was achieved which opened a wide range of possible biomedical applications.

Our research set out to investigate the activity of fullereneol C₆₀(OH)₂₄ on growth of human breast cancer cells in culture and its modulatory effect on Adriamycin (ADR), cisplatin (*cis*-Pt), Taxol and Thiazofurine induced cytotoxicity. These anti-tumor drugs have various mechanisms of action, which (except for Taxol) achieve their toxic activity by the formation of free radicals. Growth inhibition was evaluated by colorimetric SRB assay. For investigation of cell growth, two cell lines were used: MCF7 (human breast adenocarcinoma, estrogen receptor positive (ER+)) and MDA-MB-231 (human breast adenocarcinoma, estrogen receptor negative (ER-)). Cell lines were treated with fullereneol at concentrations 0.9-3.9 µg/ml. Fullereneol was given alone and in combination with cytostatics at various concentrations ranging from 10⁻⁴ to 10⁻⁸ M during 2 hours. Cytotoxic effect was evaluated 48 hours after treatment. C₆₀(OH)₂₄ mildly inhibited growth of both cell lines (3-15%). Combination of fullereneol and cytostatic, given simultaneously, resulted in various degrees of growth inhibitions depending on fullereneol concentration, type of antitumor drug, and cell line. Results showed that fullereneol in combination with ADR and *cis*-Pt inhibited cytotoxic activity of cytostatics by more than 30% for both cell lines at all applied concentrations. It

was also observed that the level of inhibition of cytotoxicity for combination of Thiazofurine and fullereneol was much lower for MCF7 (4.81-23.29%) as compared to MDA-MB-231 cell line (61% for lowest and 47% for highest fullereneol concentration). However, Taxol even in combination with fullereneol gave 100% cytotoxicity on MCF7 cells, while the same combination induced inhibition of Taxol cytotoxicity on MDA-MB-231 from 4.39 to 26.7%.

Fullereneol C₆₀(OH)₂₄ differently modulates cytotoxic effects of given cytostatics; protection was more successful for antitumor drugs whose action is based on free radical formation. Use of fullereneol to remove the excessive production of harmful reactive free radical species in the living environment may represent a new medical approach to chemotherapy.

THE CIRCALUNAR CYCLE OF SALIVARY TESTOSTERONE AND THE VISUO-SPATIAL PERFORMANCE

P. Celec, M. Skoknová, D. Ostatníková

Institute of physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Testosterone levels in saliva were determined in 53 young adult male and female subjects (mean age 20.89 ± 0.91 years). The samples of saliva were collected either once (22 subjects) or daily during a period of 30 days (31 subjects). Saliva was frozen at -20°C and radioimmunoanalyzed. Both groups of subjects were tested on their visuo-spatial performance (mental rotation and spatial visualization). All relevant information about the menstrual cycle of female subjects was also collected. This data and the results of the visuo-spatial tests as well as the circalunar cycle in their relationship to the testosterone levels were analyzed.

In this study a positive correlation of the salivary testosterone levels and the performance in visuo-spatial tests in women and a negative dependence in men was found. The outcomes showed a significant statistical difference between the results of the test during the high-testosterone (periovulatory) and the low-testosterone (menstrual) phase in women. The visuo-spatial performance in men showed significant differences in high-testosterone and low-testosterone phase as well. As far as the fluctuations of the testosterone levels during a month were concerned, the cycle with maximum peak in the periovulatory phase was confirmed in women and an analogical circalunar cycle in men was described.