S02-07

THE AVIAN BRAIN $GABA_A$ RECEPTOR γ4 SUBUNIT: EXPRESSION AND FUNCTIONAL PHARMACOLOGY.

I.C. Forster[1], R.J. Harvey[3], M.G. Darlison[3] and J.A. Benson[2].
Institutes of [1]Physiology and [2]Pharmacology, University of Zurich, CH-8057 Zürich and [3]Institute of Cell Biochemistry and Clinical Neurobiology, University of Hamburg, D-20246 Hamburg.

The γ subunit of heteropentameric $GABA_A$ receptors contributes to a high-affinity benzodiazepine binding site and is therefore an important determinant of $GABA_A$ receptor pharmacology. In this study we characterize the recently cloned γ4 subunit (Harvey et al., 1993, FEBS Lett., 331,211-216), which in avian species replaces the mammalian γ3 subunit. We co-expressed γ4 subunits with rat α3 and β2 subunits and demonstrated functional expression of GABA-activated receptor channels in transfected HEK-293 cells. Expression was confirmed by nuclear injection in *Xenopus* oocytes which were then used to determine the pharmacology of the recombinant α3β2γ4 receptor. The evoked current for GABA was potentiated by sodium pentobarbital, suppressed by picrotoxin and blocked by Zn^{2+}, in a dose-dependent manner. The benzodiazepine full agonists flunitrazepam and triazolam strongly potentiated the GABA-evoked current, whereas the partial agonists bretazenil and abecarnil enhanced the response less strongly and the β-carboline agonists DMCM and β-CCM had inhibitory effects. However, the inverse agonist Ro15-4513 enhanced the GABA response and the positive agonist zolpidem was inactive at α3β2γ4 receptors. Our results show that the avian γ4 subunit confers on $GABA_A$ receptors a novel pharmacology which differs from that imparted by mammalian γ subunits.

S02-08

LASER-FLASH PHOTOLYSIS OF CAGED GLUTAMATE

C. Lüscher, H.-R. Lüscher, E. Niggli. Dept. of Physiology, University of Bern, Switzerland.

Flash photolysis of "caged" compounds provides a means to rapidly change the concentration of biologically active products. We used *CNB-L-Glutamate* ($t_{1/2} \approx 21\mu s$, quantum yield ≈ 0.14) to investigate the kinetics of non-NMDA glutamate receptors in cultured hippocampal neurons. Membrane currents were recorded in the whole-cell mode of the patch-clamp technique in the presence of bicuculline, strychnine and TTX. A Nd:YAG laser (flash duration ≈ 7 ns at 355 nm) was used to epi-illuminate a ≈ 250 µm field. At a holding potential of -60 mV the currents induced by a flash showed a rapid activation ($t_{1/2}$ < 7 ms) followed by a slower inactivation, but the amplitude was much smaller than expected (< 100 pA). Surprisingly, rapid superfusion ($t_{1/2} \approx 200$ ms) of the cells with 0.5 mM CNB-L-Glutamate led to a slow inward current, which desensitized partially after several hundred ms. Similar currents but with ≈ 2 fold larger amplitude were elicited with 0.5 mM free glutamate. Both effects were reversibly blocked by CNQX (10 µM). These findings suggest that CNB-L-Glutamate, as sold, may not be biologically inert. Instead, the caged compound may exhibit an intrinsic action on AMPA receptors or may have become contaminated with glutamate. In both cases the photolytic dynamic range of CNB-L-Glutamate (without further purification) would be reduced.

Nitric Oxide

S03-01

Inducible nitric oxide synthase deficient mice

FY Liew
Department of Immunology , University of Glasgow, Glasgow, G11 6NT, UK.

To directly define the physiological role of inducible nitric oxide synthase (iNOS) we constructed a strain of mice deficient in iNOS. These mice are viable, fertile and without evidence of histopathological abnormalities. However, in contrast to wild-type and heterozygous mice, which are highly resistant to the protozoa parasite Leishmania major infection, mutant mice are uniformly susceptible. The infected mutant mice developed a significantly stronger Th1 type of immune response than the wild-type or heterozygous mice. The mutant mice showed reduced non-specific inflammatory response to carrageenin and were resistant to lipopolysaccharide-induced mortality.

S03-03

Nitric oxide and the Cardiovascular System

Thomas F. Lüscher, Cardiology, Cardiovasc. Res. University Hospital, Bern

The circulation is controlled by the central nervous system, hormones and local vascular mechanism. The endothelium is in a strategic anatomical position in the blood vessel wall between the blood (and platelets and monocytes) and vascular smooth muscle. Endothelial cells are stimulated by mechanical and hormonal signals and it release mediators modulating contraction and proliferation of vascular smooth muscle, platelet function, coagulation and monocyte adhesion. Nitric oxide (NO) and prostacyclin (PGI_2) and a putative hyperpolarizing factor (EDHF) mediate relaxation. NO inhibits smooth muscle proliferation and (with PGI_2) platelet function. Bradykinin induced NO production is regulated by angiotensin converting enzyme on the endothelium; this enzyme converts angiotensin I into angiotensin II, and inactivates bradykinin. Endothelin-1, thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGI_2) are endothelium-derived contracting factors. In contrast to TXA_2 and PGH_2 which activate platelets, endothelin-1 has no platelet effects, but proliferative properties in vascular smooth muscle. Under physiological conditions, the endothelium plays a protective role as NO prevents adhesion of circulating blood cells, keeps the vasculature in a vasodilated state and inhibits vascular smooth muscle proliferation. In disease states, decreased NO release contributes to enhanced vasoconstriction, adhesion of platelets and monocytes and proliferation of vascular smooth muscle, all events which are involved in cardiovascular disease.

S03-02

THERAPEUTIC STRATEGIES FOR THE INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE

Pfeilschifter, J., Dept. Pharmacology, Biozentrum, University of Basel, CH-4056 Basel

In recent years, NO, a gas previously considered a potentially toxic chemical, has become established as a diffusible universal messenger mediating cell-cell communication throughout the body. In mammals, NO is a recognized mediator of blood vessel relaxation that helps to maintain blood pressure. In the central nervous system NO acts as a non-conventional neurotransmitter and participates in the establishment of long-term plasticity required for memory formation. In addition, NO is responsible for some parts of the host response to sepsis and inflammation and contributes to certain disease states. A number of strategies have emerged with regard to a pharmacological control of pathological NO overproductions. I will discuss these novel therapeutic approaches that may provide new means for clinical medicine.

S03-04

The Role of Nitric Oxide (NO) in Apoptosis

B. Brüne, U.K. Meßmer and K. Sandau
University of Erlangen-Nürnberg, Faculty of Medicine, Erlangen, Germany

Nitric oxide (NO) is recognized as a ubiquitous messenger throughout physiology and pathophysiology. Cyclic GMP-independent signaling pathways comprise cytostasis and/or cytotoxicity related to NO. Activation of the cytokine/lipopolysaccharide inducible NO-synthase (iNOS) causes nitrite accumulation in the cell supernatant and apoptotic cell death of macrophages (RAW 264.7 cells) or ß-cells (RINm5F cells). Apoptosis is characterized by morphological (chromatin condensation) and biochemical criteria (DNA fragmentation), while iNOS-inhibitors (N^G-monomethyl-L-arginine) are used to trace back NO-generation to cell death. Prior to DNA laddering we observed the accumulation of the tumor suppressor p53. Stabilization of p53 was correlated to the extend of DNA damage, initiated by a chemically heterogeneous group of NO-releasing compounds. As potential protective modulators of apoptosis we focused on Bcl-2 overexpression and superoxide production. Regulation of NO-production, activation or inhibition of specific intracellular signaling pathways modulate the cellular response to a potentially cytotoxic molecule like nitric oxide.

S03-05

ROLE OF NOS2 ACTIVITY IN THE PROLIFERATION OF THE HUMAN COLON CELL LINE SW480

Vecchini F, E. Pringault* and E. Felley-Bosco, Institut de Pharmacologie et de Toxicologie, Lausanne and *Institut Pasteur, Paris.

NOS2 gene expression is associated with chronic human inflammatory diseases (e.g. ulcerative colitis) which can lead to cancer. However, its implication in the promotion of colon cancer is not established yet. In order to understand the role of NOS2 gene expression in intestinal cells dedifferentiation, we generated a stably-transfected anti-NOS2 SW480 cell line. A human NOS2 cDNA fragment was expressed in an antisense orientation under the control of CMV promoter in the SW480 clone B6, which constitutively expresses NOS2. The antisense B6 population (B6 cmv/NOS 0.8) showed a 75% reduction in NOS2 activity, measured by ^{3}H-Arg conversion to citrulline, and a lower rate of cell proliferation than control cells, measured by ^{3}H-thymidine incorporation. This inhibitory growth effect was accompanied by a 50% decrease in villin, and glutathione S-transferase α (GSTα) expression assessed by Western blot analysis. Villin is a structural protein involved in the morphogenesis of intestinal cells while GSTα is an antioxidant enzyme. The expression of Bcl-2, a protooncogene with antioxidant properties, did not change. These results suggest that NOS2 gene expression modulated the growth and some properties of human colon cells in culture.

S03-06

ACTIVATION OF GLIAL GLUTAMATE RECEPTORS MEDIATES THE REPLENISHMENT OF THE NO PRECURSOR POOL IN NEURONS

G.Grima, B.Benz and K.Q.Do. Brain Research Institute, University of Zurich

Arginine (Arg), the nitric oxide (NO) precursor, is predominantly localised in glial cells, whereas NO synthase, the NO producing enzyme, is mainly found in neurons. A transfer of Arg from glia to neurons is necessary to replenish the neuronal pool. This is further supported by the finding that Arg is released upon selective pathway stimulation both in vitro (Hansel et al., 1992) and in vivo (Do et al.; 1994). We have investigated the mechanism underlying this process by application of glutamate (Glu) agonists to cerebellar slices and cortical astrocytes which were preincubated with [^{3}H]-Arg. In cerebellar slices, Glu, in a TTX-insensitive manner, AMPA and kainate increased significantly the extracellular level of labelled Arg. Moreover, tACPD induced a delayed rise of Arg level. In astrocytes in culture, Glu, AMPA, kainate and the Ca ionophore A23187 also caused an increase in Arg concentration. NMDA was ineffective in the latter system. Thus, activation of ionotropic non-NMDA and metabotropic Glu receptors, localised on astrocytes, mediate this Arg release in order to supply NO synthase with its substrate. NO transmission may be based on the transfer of Arg from glial cells to neurons which is dependent on activation of excitatory amino acid receptors on glial cells.

S03-07

Interleukin 1β- induced expression of nitric oxide synthase in rat renal mesangial cells is suppressed by Cyclosporin A

Gaby Walker, Dieter Kunz and Josef Pfeilschifter

Department of Pharmacology, Biozentrum, University of Basel, Klingelbergstr. 70, CH-4056 Basel, Switzerland

The expression of nitric oxide synthase (iNOS, EC 1.14.13.39) is induced in rat renal mesangial cells by exposure to the inflammatory cytokine interleukin 1β (IL-1β). Here we report that Cyclosporin A (CsA), the most potent immunosuppressive drug, inhibits IL-1β dependent iNOS expression in renal mesangial cells. Addition of CsA dose dependently suppresses IL- 1β-induced nitrite formation (IC_{50} = 0.9 μM). Western- and Northern - blot analyses of mesangial cell extracts reveal that the inhibition of IL-1β - induced nitrite formation by CsA is due to decreased iNOS protein and iNOS mRNA steady state levels. Using nuclear run on experiments we show that the transcription rate of the IL-1β-induced iNOS gene is reduced. Furthermore, by electrophoretic mobility shift analysis we demonstrate reduced DNA-binding of the nuclear factor NF κB, an essential component of the IL-1β-dependent up-regulation of iNOS gene transcription. The data presented in this report suggest that the cellular machinery involved in the IL-1β dependent transcriptional up- regulation of the iNOS gene in mesangial cells is a target for the action of CsA.

S03-08

MOLECULAR MECHANISMS OF DEXAMTHASONE INHIBITION OF NITRIC OXIDE SYNTHASE EXPRESSION IN INTERLEUKIN 1β-STIMULATED MESANGIAL CELLS: EVIDENCE FOR TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL REGULATION

Dieter Kunz, Gaby Walker, Wolfgang Eberhardt and Josef Pfeilschifter
Dept. Pharmacology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

Inducible nitric oxide synthase (iNOS) is expressed in rat glomerular mesangial cells upon exposure to the inflammatory cytokine interleukin 1β (IL-1β). Nanomolar concentrations of dexamethasone suppress IL-1β-induced iNOS protein expression and production of nitrite, the stable endproduct of NO formation. However, IL-1β-triggered iNOS mRNA steady state levels are only less affected by the action of the drug, suggesting that Dexamethasone may act at different levels of iNOS expression. Several experiments were performed to address the mechanism underlying the steroid modulation of IL-1β- triggered iNOS expression. Nuclear run on-assays demonstrate that Dexamethasone drastically reduces IL-1β-induced iNOS gene transcription. This effect is due to decreased binding of NF κB to its cis-regulatory element within the rat iNOS promoter. However, treatment with Dexamethasone results in a prolongation of the half-life of iNOS mRNA from 1h to 2.5h, thus counteracting the decreased transcriptional activity of the iNOS gene. Moreover, Dexamethasone dramatically reduces iNOS protein levels by reduction of iNOS mRNA translation even after iNOS was already expressed. Furthermore, Dexamethasone drastically increases degradation of iNOS protein. Thus, Dexamethasone may exert its antiinflammatory effects on iNOS expression by different mechanisms: decreased gene transcription and translation of iNOS mRNA, and increased degradation of iNOS protein. These results may have important implications for clinical treatment of diseases associated with pathological NO overproduction and strongly argue for the effectiveness of glucocorticoid therapy even after the initiating events of inflammatory processes have taken place.

S03-09

IDENTIFICATION AND QUANTIFICATION OF ENDOGENOUS S-NITROSOGLUTATHIONE IN RAT CEREBELLUM

I.M. Kluge[1], U. Gutteck-Amsler[1], Ph. Christen[2], M. Zollinger[3] and K.Q. Do[1]. [1]Brain Research Institute, [2]Institute of Biochemistry, University of Zurich, [3]Current address: Sandoz Pharma Ltd. Basel.

The form in which the messenger nitric oxide (NO) is stored, delivered and transported in the central nervous system has not been studied directly. We have investigated whether S-nitrosoglutathione (GSNO) may be a potential "package form" of NO. To identify and quantify GSNO in rat brain, we developed an analytical procedure based on tissue extraction, HPLC purification, derivatization and micro HPLC coupled to continuous-flow fast atom bombardment mass spectrometry. The fluorenylmethoxycarbonyl-(Fmoc-) derivative of endogenous GSNO was clearly identified on the basis of its retention time and mass spectrum by comparison with authentic Fmoc-GSNO. Using selected ion monitoring mass spectrometry and $GS^{15}NO$ as internal standard we determined the levels of endogenous GSNO in rat cerebellum. They lie in the μM range and show age-dependency (P4: 267±33; P10: 50±3; adult: 356±31 pmol/mg protein). The packaging of NO in the form of GSNO might serve to facilitate its transport, prolong its half-life, target its delivery to specific effectors and may provide a means to control the toxicity of the free radical NO.

S03-10

STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF MUTATIONS IN 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE

Oppliger, T., Thöny, B., Bürglisser, D., Heizmann, C. W. and Blau, N. Division of Clinical Chemistry, Department of Pediatrics, University of Zürich, Steinwiesstrasse 75, 8032 Zürich, Switzerland

Four mutants with single amino acid alterations in the H4-biopterin biosynthetic enzyme 6-pyruvoyl-tetrahydropterin synthase (PTPS) were over-expressed and characterized *in vitro*. The corresponding DNA mutations were found in patients with hyperphenylalaninemia and monoamine neurotransmitter insufficiency due to lack of H4-biopterin, a cofactor required also for nitric oxide synthases. One mutant (ΔV57) is incorrectly folded and thus unstable *in vitro* and *in vivo*, while a second mutant (P87L) has substantial activity but enhanced sensitivity to local unfolding. Two other mutants, R16C and R25Q, form stable homomultimers and exhibit significant activity *in vitro*, but no activity in COS-1 cells. *In vivo* ^{32}P labeling showed that wild-type PTPS, P87L, and R25Q are phosphorylated, while R16C is not modified. This strongly suggests that the serine 19 within the consensus sequence RXXS, is the site of modification. Our results demonstrate that PTPS undergoes protein phosphorylation and requires additional, post-translational modification(s) for its *in vivo* function.

S03-11

NITRIC OXIDE PRODUCTION IN EXPERIMENTAL GRAM NEGATIVE PERITONITIS.

Le Roy, D., Heumann, D., Eugster, H.-P.,Glauser, M-P., Mauël, J., Smith, J. and Corradin S. Division of Infectious Diseases, CHUV, Lausanne, Institute of Biochemistry, Epalinges, and Institute of Toxicology, Zurich.

Overproduction of nitric oxide (NO) may be responsible for refractory hypotension in septic shock. We recently reported that septic shock patients exhibited mean plasma nitrate levels of 90 µM at entry compared to 30 µM in either normal or cardiogenic shock controls. We have now characterized the production of NO in experimental murine peritonitis. Importantly, plasma nitrate accumulation was found to resemble results with shock patients. We then began to examine the role of various cytokines in the regulation of NO production in this model. Surprisingly, mice with a disrupted gene for the IFN-γ receptor exhibited similar levels of NO production and lethality as control animals challenged i.p. with *E. coli*. Studies are currently in progress with both TNF receptor 55 (CD120a) and TNF-α/β double knockout mice.

S03-12

CONSTITUTIVE EXPRESSION OF MURINE iNOS GENE IN HUMAN MONOCYTIC LINES THP-1 AND U937.EFFECT ON NO PRODUCTION

*Bertholet S., #Felley-Bosco E.,*Smith J. and *Mauël J. *Institute of Biochemistry and #Institute of Pharmacology and Toxicology, University of Lausanne.

Nitric oxide (NO) is an important mediator of numerous processes including smooth muscle relaxation, neurotransmission, and cell cytotoxicity. NO is synthesized from L-arginine by NO synthases (NOS). After activation with various agents, murine macrophages express high levels of inducible NOS (iNOS) mRNA and protein, and produce high amounts of NO. In contrast, human monocytes and macrophages have been shown to express iNOS mRNA and protein under certain circumstances in vitro, but they produce only low levels of NO. Whether human cells possess the complete machinery to produce NO is still unknown. The purpose of this study is to determine if human monocytic lines THP-1 and U937, constitutively expressing the murine iNOS gene, may produce NO levels comparable to activated murine macrophages.

S03-13

ORGANIZATION AND LOCATION OF THE HUMAN 6-PYRUVOYL-TETRAHYDROPTERIN (PTPS) GENE AND ITS PSEUDOGENE

C. Kluge, L. Brecevic, N. Blau, C.W. Heizmann, B. Thöny
Division of Clinical Chemistry, Department of Pediatrics, University of Zürich, Steinwiesstr. 75, CH - 8032 Zürich

PTPS is involved in the biosynthesis of tetrahydrobiopterin, which is an essential cofactor for aromatic amino acid hydroxylases and nitric oxide synthases. Inherited PTPS deficiency causes hyperphenylalaninemia and severe neurological disorders in newborns. Using the cDNA as a probe, we determined the structure of the human gene encoding the PTPS after isolation and characterization of lambda phages and P1 clones. Southern blotting and FISH to metaphase chromosomes revealed a single gene (*PTS*) and one pseudogene (*PTS-P1*), located on chromosome 11q22.3-q23.3 and 9p13, respectively. So far, 6 exons spread over a region of about 7 kb could be determined for the *PTS* gene, whereby the exon/intron boundary sequences conform to consensus acceptor and donor sequences. The 5'-upstream region is currently under investigation for characterization of the promoter.

S03-14

DIRECT ACTIVATION OF CYCLIC NUCLEOTIDE GATED CHANNELS OF OLFACTORY RECEPTOR NEURONS BY NITRIC OXIDE.

Broillet, M.-C. and Firestein, S.
Department of Biology, Columbia University, New York, NY 10027

In vertebrate olfactory neurons the final step of the transduction cascade is mediated by an ion channel activated by cAMP. Nitric oxide (NO) is best known to act as an intercellular messenger via guanylyl cyclase, but it has also been reported to have direct effects on some ion channels. To test the direct effect of NO on cyclic nucleotide gated (CNG) channels, we used excised inside-out patches from olfactory receptor neurons isolated from the nasal epithelium of adult-phase tiger salamanders (*Ambystoma tigrinum*). The nitric oxide donors SIN 1 or S-nitrosocysteine (SNC) were added to the cytosolic solution, resulting in single channel openings and long bursts of openings. The single channel conductance was not affected, but a significant increase in the open probability was observed. Rp-cAMPS, a competitive antagonist of the CNG channels, inhibited the action of cAMP but had no apparent effect on channel openings due to SNC, suggesting that NO activation of the channel does not occur via the CN binding site. The application of iodoacetamide, a SH binding reagent, induced an immediate activation of CNG channels apparently identical to that of NO, suggesting that NO effects resulted from a chemical modification of SH groups. The NO-induced activity of the channel, in contrast to that induced by cAMP, showed no desensitization by calcium-calmodulin (Ca-CaM). Thus it appears that the effects of Ca-CaM cannot be entirely on gating efficacy but affect in some way the binding reaction directly. Our results demonstrate that NO can directly activate the olfactory CNG channels independently from the intracellular regulatory cascade probably via the chemical modification of SH groups.

S03-15

LOCALISATION OF NITRIC OXIDE (NO) SYNTHASE (NOS) IN THE OVIDUCT, EFFECT OF NO ON HUMAN SPERM MOTILITY:IMPLICATION OF NO IN THE FERTILIZATION PROCESS.

M.Rosselli,B.Imthurn,E.Macas,PJ.Keller,*RK.Dubey. Obstetrics and Gynecology, Endocrinology Clinic, University Hospital Zurich,*Center for Clinical Pharmacology, University of Pittsburgh, USA.

To evaluate if NO is implicates in fertilization process, we analyzed the direct effect of NO, chemically derived from S-nitroso-Nacethylpenicillamine and sodium-nitroprusside on the motility and viability of human spermatozoa.We tested whether inhibition of NOS prevents sperm motility and viability by incubating washed total semen cells with N-nitro-L-arginine-methyl-ester.Moreover,we analyzed if NOS is present in the oviduct:-^{3}H-L-Citrulline formation in oviduct segments-Nitrite/Nitrate from cultured oviduct epithelial cell and-localization of NOS with NADPH-diaphorese. Our results suggest: excessive NO causes human sperm toxicity and negatively affect sperm motility.Moreover by identifying NOS activity in the oviduct we speculate that NO is implicated in fertilization process.

S03-16

CHARACTERIZATION OF REACTIVE OXYGEN SPECIES (ROS)-INDUCED GLUTAMATE UPTAKE INTO PRIMARY ASTROCYTE CULTURES.

O. Sorg[1], T. Horn[2], and F.E. Bloom[2]. [1]Institut de Physiologie, Université de Lausanne, and [2]Department of Neuropharmacol., The Scripps Research Institute, La Jolla (USA).

Astrocytes take up extracellular glutamate efficiently, and so prevent the potential neurotoxicity of this neurotransmitter. Recently, Volterra et al. showed that H_2O_2 induced an inhibition of glutamate uptake (Glu-up) into primary astrocyte cultures. We determined the role of four main reactive oxygen species in Glu-up inhibition, as well as the efficiency of endogenous protection mechanisms. H_2O_2 promoted an inhibition of Glu-up in a concentration-dependent manner. This effect was potentiated by the addition of $CuSO_4$ and ascorbate, a condition known to produce ·OH radical. On the other hand, superoxide and nitric oxide (NO), by themselves, had no effect on Glu-up. However, astrocytes responded to NO by an increase in cytoplasmic calcium concentration. Catalase is efficient in protecting astrocytes and surrounding neurones from the deleterous action of ROS on Glu-up, while glutathione peroxidase I is ineffective.

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S03-17

CD69 EXPRESSION BY MURINE MACROPHAGES AND NITRIC OXIDE PRODUCTION UPON CD69 STIMULATION.
Marzio R., Mauël J. and Corradin S. Institute of Biochemistry, University of Lausanne, Epalinges.
Nitric oxide (NO), produced by activated murine macrophages (mø), plays an important role in the regulation of immune function. CD69 is an early activation antigen of lymphocytes and and serves as a signal transducing molecule in many cell types. Induction of NO production by human monocytes after CD69 crosslinking was recently reported. We examined CD69 expression and function in murine mø. CD69 staining, evaluated by flow cytometry and immunohistochemistry, was negative in resting mø, but was induced by overnight LPS stimulation. CD69 upregulation was unaffected by an inhibitor of NO production or infection with *Leishmania* and was blocked by cycloheximide. Stimulation of gamma-interferon primed mø with anti-CD69 mAb in the presence of polymyxin B induced the release of high amounts of nitrite. Mø triggering was inhibited by EGTA, as shown for other CD69-dependent functions. Taken together, these results suggest that CD69 may serve as signaling receptor in murine mø.

Somatic Gene Therapy

S04-02

CYTOKINES IN SOMATIC CELL AND GENE THERAPY: PERSPECTIVES IN ONCOLOGY
R. Mertelsmann, U. Brennscheidt, A. Mackensen, F. Rosenthal, H. Veelken, D. Behringer, J. Finke, P. Kulmburg, W. Lange, A. Lindemann
University Medical Center, Department of Internal Medicine I, Hematology/Oncology, Hugstetter Str. 55, D-79106 Freiburg

The discovery and clinical evaluation of cytokines is having a rapidly increasing impact on cancer therapy by providing unprecedented opportunities for therapeutic modulation of hematopoiesis and the immune system. To facilitate chemotherapy dose intensification, colony stimulating factors (CSFs) together with peripheral blood progenitor cells (PBPCs) have been shown to shorten the period of profound pancytopenia following high-dose chemotherapy.
The second area of cancer therapy moving rapidly ahead following the discovery of cytokines and cytokine-mediated effector mechanisms is cancer immunotherapy. Given the fact that induction of anti-tumor immunity by cytokine gene transfer into tumor or bystander cells has been demonstrated by different groups in mouse tumor models, we have initiated a phase I clinical protocol for patients with refractory neoplasias. Hematopoietic and immunostimulating cytokines are beginning to have an impact on cancer treatment outcome. Not only cytokines themselves, but also cells mobilized by and grown ex vivo in the presence of cytokines as well as cell transfected with therapeutic genes of interest are being developed as novel therapeutic modalities.

S04-04

GENE THERAPY FOR NEUROLOGICAL DISEASES: FIRST CLINICAL RESULTS IN AMYOTROPHIC LATERAL SCLEROSIS

Patrick Aebischer, Centre pluridisciplinaire de thérapie génique, Centre Hospitalier Universitaire Vaudois (CHUV), 1011 Lausanne, Switzerland

Neuronal growth factors hold promise for providing therapeutic benefits in various neurological disorders. As a means of ensuring adequate CNS delivery of growth factors and minimizing significant adverse side effects associated with systemic delivery methods, we have developed an *ex-vivo* gene therapy approach to protein delivery using encapsulated genetically modified xenogeneic cells. One neurotrophic factor in particular, ciliary neurotrophic factor (CNTF), has been shown in various rodent models to reduce the motor neuron cell death similar to that seen in amyotrophic lateral sclerosis (ALS). The initial clinical trial focusing on the systemic administration of CNTF resulted in severe side-effects mandating the interruption of the trials, therefore preventing determination of the efficacy of the molecule. In order to deliver CNTF directly to the nervous system, we conducted a phase I study in which six patients with amyotrophic lateral sclerosis (ALS) were implanted with capsules containing genetically-engineered baby hamster kidney (BHK) cells releasing around 1.0 µg of CNTF per day *in vitro*. The CNTF-releasing implants were surgically placed within the lumbar intrathecal space. Serial CSF sampling showed CNTF ranging from 170 to 6,282 pg/ml for at least 17 weeks post-implantation whereas no CNTF was detected prior to implantation. On explant, all devices showed good BHK cell viability and CNTF output when measured *in vitro*. The patients showed no weight loss, severe coughing or response of acute phase reactants over the course of implantation which were the limiting side effects observed with systemic CNTF administration. These results demonstrate that measurable levels of human CNTF can be continuously delivered within the CSF of humans by an *ex vivo* gene therapy approach opening new avenues for the treatment of neurological diseases.

S04-05

AN OPTIMIZED PROTOCOL FOR ADENOVIRUS-MEDIATED GENE TRANSFER: EXPRESSION OF THE CHICKEN NEURONAL CELL ADHESION MOLECULE NgCAM IN RODENT NEURONS

Vogt, L., Giger, R. J., Ziegler, U., Buchstaller, A. and Sonderegger, P.
Institute of Biochemistry, University of Zürich, CH-8057 Zürich

Neuronal cell adhesion molecules of neurites and growth cones play an important role for neurite outgrowth and pathfinding in the development of the nervous system. The ability to manipulate the expression of these glycoproteins in neurons would provide a powerful tool to study their function in vitro and in vivo. Here we report the ectopic expression of chicken NgCAM in rodent neurons by adenovirus-mediated gene transfer. We constructed the replication-defective recombinant adenovirus Ad.CMVNgCAM containing the full-length cDNA of chicken neuronal glial cell adhesion molecule NgCAM under the transcriptional control of the cytomegalovirus (CMV) promoter in the early region 1 (E1) of the adenoviral genome. The transcription unit of the Ad.CMVNgCAM virus consists of more than 5 kb and, thus, is at the reported upper limit of the cloning capacity for E1-deleted recombinant adenoviruses. The size of the adenoviral genome of Ad.CMVNgCAM was 106% of the size of wild-type adenoviral genome. An optimized protocol for the construction of a replication-defective recombinant adenovirus is presented and the ectopically expressed NgCAM is structurally and functionally characterized.

S04-06

RETROVIRUS-MEDIATED GENE TRANSFER OF 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE CORRECTS H_4-BIOPTERIN DEFICIENCY IN FIBROBLASTS FROM HYPERPHENYLALANINEMIC PATIENTS

Thöny, B., Leimbacher W., Stuhlmann, H., Heizmann, C.W., and Blau, N. Division of Clinical Chemistry, Department of Pediatrics, University of Zürich, 8032 Zürich, Switzerland

H_4-biopterin (BH_4) deficiency is primarily caused by mutations in the gene encoding the 6-pyruvoyl-tetrahydropterin synthase (PTPS), and is associated with hyperphenylalaniemia and lack of dopamine and serotonin. We have previously identified and characterized several mutations in patients with PTPS deficiency. This study reports the *in vitro* correction of BH_4 deficiency by using retro-viral-mediated transfer of the PTPS cDNA into primary fibroblast cultures from patients. Following PTPS-cDNA transfer, BH_4 biosynthesis could be restored in originally defective cells, providing a direct proof that the mutations in PTPS were causative for the mutant phenotype. Moreover, sustained complementation of the metabolic defect was observed over a period of several months. This is the first step towards gene therapy as a potential approach to treat BH_4 deficiency.