Membrane Transport

MT01

Purification and Functional Analysis of CopB, the *Enterococcus hirae* Copper Export ATPase

P. Duda, A. Odermatt, M. Solioz

Copper is an essential element for life as cofactor of important enzymes. However, excessive intracellular copper concentrations are toxic due to the capacity of copper to oxidize biomolecules directly by transition from Cu^{++} to Cu^{+} or indirectly via radical formation. Thus cells are forced to control their copper content tightly.

We are investigating copper homeostasis and transport in the Gram-positive bacterium *E. hirae*. The *cop*-operon of *E. hirae* encodes two regulatory proteins, CopY and CopZ, and two copper transport ATPases, CopA and CopB. The predicted amino acid sequences of the latter porteins contain all the conserved motifs of P-type ATPases.

Analyses of inside-out membrane vesicles in *E. hirae* have lead to the first demonstration of copper transport across a cellular membrane *in vitro*: vesicles of CopB deficient strains do not, in contrast to wild type vesicles, accumulate copper in an ATP-dependent manner. Thus CopB has been shown to be the copper exporter of *E. hirae*.

To further study CopB function, we purified it from membranes of an overexpressing *E. hirae* strain by Ni-NTA agarose and MonoQ-sepharose columns. Purified CopB is active when reconstituted into liposomes. It is inhibited by vanadate and forms an acylphosphate reaction intermediate as expected of a P-type ATPase.

MT02

A MERCURY BINDING SITE ON THE EXTRACELLULAR SIDE OF THE NA,K-ATPASE.

Wang X. and Horisberger J.-D., Institut de Pharmacologie et de Toxicologie, Université de Lausanne.

Inorganic mercury inhibits the Na,K-ATPase. The K^+ activated Na,K-pump current measured in *Xenopus* oocytes was inhibited with first order kinetics (K_{on} 5.10^3 $M^{-1}.s^{-1}$ and an estimated K_d of 160 nM). To study the hypothesis that C_{113} is the Hg^{2+} binding site, we applied 5 µM $HgCl_2$ for 1 min on oocytes expressing wild type, $C_{113}S$ and $C_{113}Y$ mutant Na,K pumps and observed an inhibition of 0.43±0.07, 0.12±0.02 and 0.05±0.03, respectively. Since C_{113} is a component of the cardiac steroid binding site, we studied the interaction of strophanthidin with mercury. Na,K-pump inhibition due to a 2-min exposure to 5 µM $HgCl_2$ was reduced from 0.68±0.05 to 0.30±0.07 by strophanthidin. These results suggest that C_{113} is an extracelullar binding site of mercury on the Na,K-ATPase.

MT03

INTERACTION OF CALNEXIN WITH NA,K-ATPase SUBUNITS

Beggah A.T. and K. Geering, Institut de Pharmacologie et Toxicologie de l'Université, rue du Bugnon 27, CH-1005 Lausanne

Assembly of the α(Sα) and β (Sβ) subunits in the ER is essential for the maturation of Na,K-ATPase. When expressed alone in *Xenopus* oocytes, Sα and Sβ are retained in the ER and degraded. In this study, we investigated whether calnexin, a glycoprotein-specific chaperone plays a role in the posttranslational processing of Sα and Sβ. A PCR fragment from a *Xenopus* heart library and a full lenght clone from a kidney library showed 90% and 76% identity, respectively, with mammalian calnexin, indicating the existence of calnexin isoforms. Both endogenous, and overexpressed *Xenopus* calnexin transiently associated with glycosylated as well as non-glycosylated Sβ and with the non-glycosylated Sα of Na,K-ATPase expressed in *Xenopus* oocytes. The interaction time coincided with the half life of the individual subunits and was abolished after subunit assembly. Thus, these data indicate that calnexin not only plays a role in the early maturation of glycoproteins but might be of general importance in initial protein processing.

MT04

AN AMINO ACID SUBSTITUTION IN A GLUTAMATE GATED CHLORIDE CHANNEL PORE ENABLES THE COUPLING OF LIGAND BINDING TO CHANNEL GATING.

Etter,A.,Cully,D.F.,Schaeffer,J.M.,Liu,K.K.,Arena,J.P. (Merck Research Laboratories, Department of Cell Biochemistry and Physiology P.O. Box 2000, Rahway, N.J. 07065-0900.

We recently reported the cloning of two subunits (GluClα + GluClβ) of a glutamate gated chloride channel (1). Homomeric GluClα channels show only a glutamate response after opening of the channels by ivermectin. This result suggests that ivermectin promotes coupling of ligand binding to channel gating. A chimera was created with the N-terminal of GluClα (the putative ligand binding domain) and the C-terminal of GluClβ (the presumed pore). The chimera showed an ivermectin independent glutamate current indicating that glutamate binds to GluClα and suggesting that coupling of ligand binding to channel gating is impaired in this subunit. A series of amino acid substitutions were performed in the second membrane spanning domain. An ivermectin independent glutamate response was observed for T308P, T308G and T308A consistent with GluClα being deficient in coupling. Our results suggest that the lack of responsiveness of other ligand gated ion channel subunits as homomeric channels may result from poor coupling of ligand binding to channel gating rather than from poor expression, poor assembly, or lack of an agonist binding domain.(1) Cully, D.F. *et al.*, *Nature* **371**, 707 (1994).

MT05

STEREOLOGICAL ANALYSIS OF ENDOCYTOSIS IN RAT LIVER CELLS IN SITU

Rahner, C., Weber, E., Landmann, L., Dept. of Anatomy, 4056 Basel

Current concepts of endocytosis depend almost exclusively on data from cultured cells. We therefore examined the uptake of the bulk-phase marker horseradish peroxidase (HRP) by hepatocytes in the rat liver in situ. Animals were injected through the jugular vein with HRP (100 mg / kg) and subsequently infused continuously with HRP (3 mg / kg·min) for various time intervals up to 60 min. After fixation by perfusion, visualization of HRP by the DAB technique, and systematic random sampling thin sections were analyzed using standard stereological methods. HRP labeled compartments (small vesicles, tubules, and with a delay multivesicular bodies) started to appear at the sinusoidal cell pole and increased in number up to 10 min. At this time the tracer reached multivesicular bodies as well as endocytic structures in the Golgi-lysosomal region, the number of which increased continuously up to 60 min. Stereological estimation indicated that HRP-positive structures show two rapid volume increases in the first 2.5 min and between 5 and 12.5 min. Thereafter a moderate increase up to 60 min was observed resulting in the labeling of appr. 2% of hepatocellular volume. Conclusion: The data are compatible with models of endocytosis comprising uptake of bulk-phase marker at the basolateral membrane in small vesicles that deliver their contents to early, sorting endosomes and subsequent transfer to late endosomes and lysosomes. They indicate that multivesicular bodies are part of the early as well as of the late endosomal compartment.

Supported by SNF grant # 32-40486.94

MT06

METABOLIC SUPPORT OF Na-PUMP FUNCTION IN APICALLY PERMEABILIZED A6 KIDNEY CELL EPITHELIA

Guerrero, L., *Beron, J., Wallimann, T. and *Verrey, F., Institute for Cell Biology, ETH Zürich and *Institute of Physiology, University of Zürich

The basolateral Na,K-ATPase (Na-pump) provides the driving force for the transepithelial transport of Na across kidney epithelial cells. To study *in situ* the contribution of different ATP generating systems to the pump function, we have apically permeabilized *X. laevis* A6 kidney cell epithelia with digitonin. This allows to introduce unpermeant inhibitors and substrates into the cells, to control the intracellular Na concentration and to measure, by voltage-clamp, the ouabain-sensitive electrical current generated by the Na-pumps (Ip). Confocal fluorescence microscopy after apical addition of S-NHS-biotine (M.W. 443) and secondary labeling with streptavidin-Texas red showed that all cells were permeabilized. Endogenous LDH and CK, however, were released to a small extent into the apical medium (<6%). With Na and K concentrations which allow a near maximal function of the pumps, the ouabain-inhibitable Ip was ~5 µA/cm² in the presence of D-glucose. Blocking glycolysis with 2-deoxy-D-glucose decreased the Ip by ~50%. This decrease was fully compensated by the addition of exogenous ATP. Blocking of oxidative phosphorylation by Antimycin A inhibited the Ip to the same extent but was only partially compensated by exogenous ATP. A6 cells were shown to contain a CK activity of 1.2 IU/mg. Exogenously added phospho-creatine restored the pump activity to a large extent during inhibition of either of the two ATP synthesis pathways, supporting the hypothesis that the CK system may play a role in the ATP supply to the Na pump of kidney epithelial cells. In conclusion, apically digitonin-permeabilized A6 epithelia are a useful model to test the effects of exogenously added substrates on Na-pump function.

MT07

ADAPTATION OF RAT RENAL DISTAL CONVOLUTED TUBULE TO ALTERED ELECTROLYTE TRANSPORT RATES. EFFECT OF BLOCKING APICAL NACL ENTRY BY SEGMENT SPECIFIC DIURETICS.

Loffing J, Loffing-Cueni D, Hegyi I, Kaplan MR*, Hebert SC*, Le Hir M, Kaissling B; Institute of Anatomy Zurich, CH; *Brigham and Women's Hospital, Boston, USA

We studied in wistar rats the effects of NaCl transport inhibition by hydrochlorothiazide (HCTZ 40 mg/kg BW/24 h) or metolazone (0.04 to 40 mg/kg BW/24 h) on distal convoluted tubule (DCT) structure, and immuno- and mRNA-expression of the thiazide-sensitive NaCl-cotransporter (TSC). - After three days of treatment kidneys were studied by light and electron microscopy, and immunohistochemistry using a polyclonal antiserum against the TSC. - In controls TSC-immunoreactivity was seen in the luminal membrane and in small apical vesicles of DCT cells exclusively. - In treated animals we found dose-dependent epithelial damages in all DCT profiles, consisting in loss of mitochondria, appearence of heterophagosomes, apoptotic and necrotic cell death. TSC-related immunostaining decreased at the luminal membrane and appeared in vesicles, distributed throughout the DCT cells, in basal intercellular spaces and even in the interstitium. Northern blot analysis revealed an important decline in renal TSC-mRNA expression. - Thus, blocking apical NaCl entry by segment specific diuretics triggers in rat distal convoluted tubule a striking epithelial degeneration associated with an important decline in the NaCl transporting machinery.

MT08

PURIFICATION OF AN INHIBITORY FACTOR OF CHOLESTEROL UPTAKE IN BBMV

D. Boffelli, F.E. Weber, M. Werder and H. Hauser, Eidgenössische Technische Hochschule Zürich

It is known from the literature that human plasma contains a protein, which strongly inhibits transfer of neutral lipids and phospholipids mediated by plasma lipid transfer proteins. We found that sheep serum contains an activity that inhibits cholesterol uptake into brush border membrane vesicles. Based on the hypothesis that this activity is related to the human Lipid Transfer Inhibitor Protein, we purified the inhibitory activity from sheep serum with a modified version of the protocol used for h-LTIP. The purified protein showed a molecular weight and isoelectric point similar to the h-LTIP.

MT09

DIFFERENTIAL LOCALIZATION OF TWO LACTATE TRANSPORTER mRNAs IN CORTICAL NEURONS AND ASTROCYTES. G. Pellegri, J.-L. Martin and P.J. Magistretti. Laboratoire de Recherche Neurologique du CHUV and Institut de Physiologie, Université de Lausanne, Switzerland.

There is ample experimental evidence indicating that lactate and pyruvate are adequate substrates for brain tissue and that lactate is produced by astrocytes and utilized by neurons. This metabolic exchange requires the presence of a lactate transport system. In vitro kinetic studies in cultured neurons and astrocytes suggested that lactate release by astrocytes and uptake into neurons, could be mediated by a lactate/proton cotransport, by analogy with the monocarboxylate transporter (MCT) of pheripheral tissues. The recent cloning of two MCT in peripheral tissues, i.e. MCT1 and MCT2, has allowed for the regional and cellular localization of these two transporters in the brain. In *situ* hybridization revealed the presence of both MCT1 and MCT2 in the adult mouse brain. Expression of MCT1 and MCT2 was already detected at E15 and reached maximal levels at PN15. Cellular localization of MCT1 and MCT2 revealed striking differences. Thus, MCT1 mRNA was predominantly localized in astrocytes, while MCT2 was more abundant in neurons. This heterogeneous distribution of lactate transporters, suggests distinctive and cell-specific functional and metabolic roles for each transporter.

MT10

CELLULAR LOCALIZATION AND CHARACTERIZATION OF GLUTAMATE TRANSPORTERS IN CORTICAL NEURONS AND ASTROCYTES. G.Pellegri, L.Pellerin, N.Stella, J.-L.Martin and P.J. Magistretti. Laboratoire de Recherche Neurologique du CHUV and Institut de Physiologie, Université de Lausanne, Switzerland.

In addition to its receptor-mediated actions on neuronal excitability, glutamate (Glu) stimulates in a concentration-dependent manner glucose uptake, glycolysis and lactate release in astrocytes (L.Pellerin and P.J.Magistretti, PNAS 91:10625-10629,1994). Glu-evoked glycolysis in astrocytes, which provides a simple mechanism to couple neuronal activity to energy metabolism, is mediated by Glu transporters. The recent cloning of Glu transporters (GLT-1, EAAC1, and GLAST), has opened the possibility to localize the different transporter mRNAs. Northern blot analysis shows that Glu transporters are, in murine primary cultures of astrocytes and neurons, differentially distributed, with GLT1 mRNA exclusively expressed in astrocytes and EAAC1 mRNA only in neurons. GLAST mRNA is localized both in neurons and astrocytes. Experiments, conducted to characterize the affinity and kinetic of Glu transporters using ^{3}H-D-Aspartate, confirm the presence of functional Glu transporters differentially expressed in cultured astrocytes and neurons.

MT11

TRANSFECTION OF OK-CELLS WITH RAT RENAL Na/Pi-COTRANSPORTER(NaPi-2): FUNCTIONAL AND MORPHOLOGICAL STUDIES

M. Pfister, S. Quabius, J. Forgo, M. Lötscher, J. Biber and H. Murer; Institute of Physiology, University Zürich.

A cDNA related to rat proximal tubular Na/P_i-cotranpsort (NaPi-2) has been stably transfected into OK-cells by the use of a dexamethasone inducible vector resulting in an approx. two-fold increase of Na-dependent P_i-transport. Parathyroidhormone inhibited the intrinsic as well as the exogenous Na/P_i-cotransport. Localization of dexamethasone induced NaPi-2 protein by immunofluorescence revealed expression of the NaPi-2 protein in distinct clusters at the apical membrane. Double immunofluorescence studies revealed that the NaPi-2 protein is colocalized together with actin within the microvilli at the apical membrane of OK-cells, suggesting that the insertion of the NaPi-2 protein into the apical membrane of OK-cells is dependent on the interaction of cytoskeletal elements such as actin or (an) associated protein(s) thereof.

MT12

UNCONVENTIONAL MYOSINS AS MOLECULAR MOTORS FOR MEMBRANE STRUCTURE AND TRAFFICKING.

H. Geißler, E. Schwarz and T. Soldati. Dept. of Molecular Cell Research, MPI for Medical Research, Heidelberg, Germany.

Cytosolic macromolecular crowding opposes strong resistance to diffusion. Thus, particles of proteins and RNA, vesicles and organelles have to be powered along cytoskeletal tracks by molecular motors. Despite identification of unconventional myosins in a wide variety of organisms, little is known about their molecular functions. Therefore, we are investigating transport processes in *Dictyostelium discoideum*, a powerful, genetically and biochemically tractable eukaryotic model organism. In combination with a biochemical study, prelimi-nary results obtained both by screening PCR-generated libraries of myosin fragments, and by testing antibodies to known myosins for cross-reactivity with *D. discoideum* myosins show that this organism expresses previously unidentified myosins. Investigation of their expression patterns, intracellular localization and functional involvement is in progress.

MT13

The mammalian endocrine pancreas is a source of guanylin.

I. David[1], D. Loffing-Cueni[1], WG. Forssmann[2] and M. Reinecke[1]. Institute of Anatomy, University of Zürich[1], Lower Saxony Institute for Peptide Research, Hannover, Germany[2]

The peptide guanylin, recently isolated from the intestine, and localized to entero-endocrine cells, is involved in the paracrine regulation of epithelial electrolyte/water transport. Since high amounts of guanylin are present also in the systemic circulation, we investigated the endocrine pancreas as a potential endocrine source. Immunohistochemical localization using region-specific guanylin antisera and antisera against the classical islet hormones revealed that in the pancreas of rat, mouse, guinea pig and dog guanylin-immunoreactivity (-IR) is exclusively present in islet A cells. On the ultrastructural level, guanylin-IR was confined to the A-cell granules. By RT-PCR and hybridization with an internal oligonucleotide designed for rat guanylin at the length of 514 bp specific signals were obtained in rat duodenum (control) and pancreas. The results indicate that mammalian A cells express the entire guanylin molecule and suggest that islet-derived guanylin may be secreted as hormonal factor. However, an additional paracrine action of A cell-derived guanylin on islet B cells is also conceivable.

MT14

FUNCTIONAL RECONSTITUTION OF THE N-ACETYL-GLUCOSAMINE TRANSPORTER OF *E. COLI*

Mukhija, S., Steinmann, L. and Erni, B. Institut für Biochemie der Universität Bern, Freiestr. 3, 3012 Bern

The transporter for GlcNAc (II^{GlcNAc}) of the bacterial phosphotransferase system couples vectorial translocation to phosphorylation of the substrate. II^{GlcNAc} containing a carboxy-terminal affinity tag was purified by Ni^{2+}- chelate affinity chromatography and reconstituted into phospholipid vesicles by detergent-dialysis followed by freeze/thaw sonication. II^{GlcNAc} was oriented randomly in the vesicles as inferred from phosphorylation studies. Import and phosphorylation of GlcNAc was measured with proteoliposomes preloaded with Enzyme I, HPr (His-containing phosphocarrier protein) and phosphoenolpyruvate (PEP). Uptake and phosphorylation occured in a 1:1 stochiometry . The Km and kcat for vectorial phosphorylation were 66.6±8.2 μM and 6.2±0.7 s^{-1}. The Km and kcat for non-vectorial phosphorylation were 750±19.6 μM and 15.8±0.9 s^{-1}. Active extrusion of GlcNAc entrapped in vesicles was also measured. External GlcNAc inhibited the extrusion of GlcNAc in a concentration dependent manner. Comparison with the transporters for mannose ($IICD^{Man}$) and glucose ($IICB^{Glc}$) reconstituted by the same method indicates that their transport mechanisms must be different.

MT15

STRUCTURAL DETERMINANTS FOR ER DEGRADATION IN THE α SUBUNIT OF NA,K-ATPase

Beguin P. and Geering K., Institut de Pharmacologie et Toxicologie de l'Université, rue du Bugnon 27, CH-1005 Lausanne

Na,K-ATPase is composed of a multimembrane spanning, catalytic α subunit (Sα) and a β subunit (Sβ) involved in the maturation of the enzyme. Sα synthesized without Sβ is subjected to the ER quality control and is rapidly degraded. To define structural determinants in Sα which are involved in the recognition by ER proteases, we produced deletion mutants and tested their degradation after expression in *Xenopus* oocytes. A mutant containing the first four transmembrane segments (M1-M4) was stably expressed in the ER while deletion mutants M1-M5, M1-M7,8, 9 or 10 were degraded. Mutants including the pair M5 and M6 were stable while mutants containing the pair M7 and M8 were only stable if Sα was expressed with Sβ. Finally, chimera between M1-M2 and M5 or M7 were degraded. These data indicate that degradation signals are located in transmembrane regions M5 and M7 of the C-terminal part of Sα. Pair formation with M6 is likely to occlude the degradation signal in M5 while interaction of Sβ with the extracytoplasmic loop between M7 and M8 is necessary to stabilize the pair M7-M8.

MT16

Down-regulation of the NaCa-exchanger protein of rat cardiac myocytes by antisense oligodeoxynucleotides.

Schwaller, B.*, Lipp, P.#, Niggli, E.# and Porzig, H.°, *Inst. of Histol. and Gen. Embryol., Univ. Fribourg, #Dept. of Physiol., Univ. Bern and °Dept. of Pharmacol., Univ. Bern

An antisense oligodeoxynucleotide (AS-ODN) directed against the 3' non-translated region of the rat cardiac NaCa-exchanger mRNA has been shown to specifically inhibit the NaCa exchange function. In cultured newborn rat heart cells the exchange current (I_{NaCa}) and Ca^{2+} transport were almost completely blocked after the addition of 3μM of AS-ODNs for 48 h. To quantify the amount of exchanger protein present before and after treatment with AS-ODNs, we measured the binding of the monoclonal antibody R3F1 recognizing an epitope on the cytoplasmic face of the exchanger. Maximal antibody binding capacity was reduced by approximately 40% in AS-ODNs treated cells, while no NaCa exchange function was detected under these experimental conditions. Confocal immunofluorescence microscopy with R3F1 in control cells showed immunofluorescence mainly in the plasma membrane region which was clearly diminished in AS-ODNs treated cells. In control cells, a polyclonal antibody against the NaCa exchanger showed highest immunofluorescence in the perinuclear zone that was almost absent in AS-ODNs treated cells. This is assumed to reflect the high synthesis rate of the protein in untreated cells. Our data suggest that the functional NaCa exchanger in the plasma membrane has a short half live and that the protein in AS-ODNs treated cells is either no longer localized in the plasma membrane or non-functional.

MT17

TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF THE EPITHELIAL SODIUM CHANNEL BY ALDOSTERONE IN A6 KIDNEY CELL LINE

Anne May, Alessandro Puoti and Bernard C. Rossier
Institut de Pharmacologie et de Toxicologie, Université de Lausanne, 1005 Lausanne, Switzerland

In the amphibian A6 kidney cell line, a high resistance epithelium, the rate limiting step for electrogenic sodium transport is the highly selective, low conductance, amiloride-sensitive sodium channel (ENaC) located at the apical membrane. Sodium transport is upregulated by aldosterone (300 nM) after a latent period of 60 min and a maximal effect at 24 hours (4-6 fold over control). The amphibian channel (*Xenopus laevis*) is made of three homologous subunits (α, β and γ xENaC). We have examined the effect of aldosterone (300nM) on mRNA abundance, rate of protein synthesis and turn-over of ENaC subunits in A6 cells grown on porous substrate. We observe: i) a slow 2 to 3 fold increase in α, β, γ mRNA abundance, ii) a rapid 3 to 12 fold increase in the rate of subunit synthesis that occurs as early as 2 hours after hormonal stimulation, iii) a surprisingly rapid turn-over of the channel subunits with no detectable effect of aldosterone. We conclude that a possible change in channel protein abundance can be primarily regulated by a rapid change in the rate of synthesis

MT18

ANALYSIS OF THE EPITHELIAL SODIUM CHANNEL (ENaC) IN VIVO.

Hummler,E.[1],Barker,P.[3],Beermann,F.[2],Verdumo,C.[1], Gatzy,J.[3], Boucher,R.[3] and Rossier,B.C.[1] Institute of Pharmacology and Toxicology[1]; Lausanne; Swiss Institute for Experimental Cancer Research[2], Epalinges, Switzerland; University of North Carolina[3], USA

The amiloride-sensitive sodium channel, ENaC, is a heteromultimeric protein made up of three homologous subunits (α, β and γ). *In vitro*, assembly and expression of functional active sodium channels is strictly dependent on αENaC, the β and γ subunits being unable, by themselves, to induce an amiloride-sensitive sodium current. ENaC constitutes the rate-limiting step for sodium absorption in epithelial cells. The adult lung expresses α, β and γENaC, and an amiloride-sensitive electrogenic Na^+ reabsorption has been documented, but it is not established whether this sodium transport is mediated by ENaC *in vivo*. In order to assess the function of ENaC, we inactivated the mouse αENaC gene by gene targeting. This animal model will help us in understanding the molecular mechanisms involved in neonatal lung liquid clearance and in adaptation to air breathing.

MT19

OVEREXPRESSION OF THE GLYCOSYLATED HUMAN SECRETORY COMPONENT IN MONKEY CV-1 CELLS.

S. Cottet and B. Corthésy
Institut de Biologie animale de l'Université de Lausanne

Two vaccinia virus expression systems were used to direct the expression and secretion of the human secretory component (SC), a glycoprotein associated with dimeric IgA in mucosal secretion.
Our data show that whereas both vaccinia virus systems, where the target gene is regulated by different strong promoters, permit the expression of similar amounts of native SC protein, they nevertheless fail in secreting a significant proportion of the recombinant protein which accumulate within the host cell.
Accumulation of the SC protein within the secretory pathway was assayed by indirect immunofluorescence as well as enzymatic deglycosylation. In addition, in the presence of tunicamycin, a general inhibitor of N-linked glycosylation, the secretion of the nonglycosylated SC protein was very weak and delayed in time, suggesting a potential role of the sugar residues of the recombinant protein along the secretory pathway. Pulse-chase experiments were performed in order to assess the rate of the newly translated recombinant protein and whether the accumulated intracellular SC could partially be degraded in the ER compartment.

Pharmacology

P01

CHRONIC ORAL MUSK XYLENE (MX) SPECIFICALLY INDUCES CYTOCHROME P450 1A IN LONG EVANS RATS

R.Suter, U.Boelsterli[1], H.Altorfer, W.Lichtensteiger, M.Schlumpf Inst.of Pharmacology,Univ. of Zurich;[1]Inst.of Toxicology,Univ.&ETH of Zurich;[2]Dept.of Pharmacie,ETH Zurich

The widely used synthetic fragrance musk xylene, a lipophilic and highly resistant compound, bioaccumulates in fish, human fat and milk.Male and female rats fed with MX 0,1g/kg or 0,03g/kg food pellets for a minimum of 10 weeks were mated and fat concentrations of MX were determined with GC/ECD detection in parents and their 14 day old offspring. Bioaccumulation of MX in the fat of the pups was dependent on the dose: 25mg/kg lipid(MX0,03g/kg) and 130mg/kg lipid(MX0,1g/kg). In the 14 day old pups liver enzyme induction was about in the same order of magnitude as in adult rats, with different enzyme patterns:EROD (ethoxyresorufin-deethylase, Cyp1A1) activity beeing higher in pups and MROD(methoxyresorufin-demethylase, Cyp1A2) activity higher in adults.

P02

α1-ADRENERGIC RECEPTOR ON PRE-B CELL LINES AND BIOLOGICAL OUTCOME

M. Togni, A. Conti, G.J.M Maestroni
Centre for Experimental Pathology, 6600 Locarno

In previous work we demonstrated that noradrenaline may modulate hematopoiesis via an α1b-adrenergic receptors. We had evidences that these α1b-adrenergic receptors are expressed by pre-B cells. Now we report that two human and one murine pre-B cell lines bear the same receptor with Kds which are similar to that showed in normal pre-B cells. In these cell lines activation of the receptor by noradrenaline caused inhibition of growth and cell death.
We investigated whether Ca^{++} uptake was involved in this effect and whether the α1 antagonist prazosin could reverse the action of noradrenaline. The results showed that Ca^{++} is apparently involved but not via α1-adrenergic receptor while prazosin at low concentration (1pM) counteracted the noradrenaline-induced cell death. Moreover, we found that activation of this receptor induces an overproduction of p53 protein which seems related to the expression of differentiation antigens such as sIgM and CD13.
In conclusion our findings suggest that differentiation and/or apoptosis in hematopoietic cells are not only under cytokines control but seems also to be under a neural adrenergic regulation.

P03

CYSTEINE-227 IS ESSENTIAL FOR ACTIVITY OF HUMAN CARBONYL REDUCTASE

Tinguely, J., Mäder, G., Ernst, E. and Wermuth, B. Universität Bern, Chemisches Zentrallabor, Inselspital, 3010 Bern

Carbonyl reductase (EC 1.1.1.184), a member of the short-chain oxidoreductases, catalyzes the NADPH-dependent reduction of a varierty of endogenous and xenobiotic carbonyl compounds. Incubation with one equivalent 4-hydroxymercuribenzoate decreases enzyme activity by about 70% and more than two equivalents almost completely abolish enzyme activity, suggesting the presence of one or more essential cysteine residues. In order to identify the essential residue(s) each of the five cysteines of human carbonyl reductase was converted to alanine by site-directed mutagenesis and the mutant cDNA ligated into the plasmid vector pET-11a and expressed in E.coli. Four mutants, C26A, C122A, C150A and C226A showed normal enzyme activity whereas the activity of C227A was less than 1% of that of the wild type enzyme. Similarly, replacement of Cys 227 by serine abolished enzyme activity. Cys-227 is not conserved in other short-chain oxidoreductases indicating a specific function of this residue in carbonyl reductase.

P04

LONG CHAIN FATTY ACID OXIDATION IN MITOCHONDRIA IS INHIBITED BY CHLOROACETALDEHYDE AND RESTORED BY METHYLENE BLUE

T.M. Visarius, B.H. Lauterburg, A. Küpfer, *J.W. Stucki
Departments of Clinical Pharmacology and *Pharmacology, University of Berne, Switzerland.

Chloroacetaldehyde (CAA) is a metabolite of the widely used antineoplastic agent ifosfamide (IFO) and has been implicated as a probable candidate causing the IFO associated neurotoxicity. Clinically, the redox dye methylene blue (MB) is used to prophylactically protect patients from this neurotoxicity although mechanistic information is not yet available. We therefore exposed rat liver mitochondria to CAA [500 μM] and observed a time dependent decrease [85% max.] in state 3u (uncoupling with dinitrophenol) oxidation rates for palmitoyl-L-carnitine as compared to controls. Addition of MB [2.5 μM], following CAA, increased mitochondrial respiratory rates by up to 170%. We conclude that MB is capable of shuttling electrons into the respiratory chain, thereby increasing the rate of oxidative phosphorylation following inhibition by CAA. The direct effect of MB on mitochondria intoxicated with a metabolite of IFO may aid in understanding the related toxicity.