POSTER PRESENTATIONS

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Allergic sensitization to ovalbumin in mouse model of food allergy is accompanied by stimulation of enterocyte brush border alkaline phosphatase activity

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Introduction The main egg protein ovalbumin (OVA) belongs to the six most frequent food allergens and its allergenicity is intensively studied in an experimental models.

Aim In our study we focused not only on immune responses but also on changes in allergenicity of ovalbumin after the thermal treatment (boiling) in mouse model of Type I food allergy.

Methods BALB/c mice were divided into three groups and sensitized twice i.p. with native OVA or boiled OVA (b-OVA) with alum as adjuvant. Third group injected with alum only was used as control. Mice were challenged ten times intragastrically with either native or b-OVA. Allergen-specific serum IgG1and IgG2a levels were determined by ELISA. Allergen-specific IgE levels in sera were quantified by degranulation of rat basophile leukemia (RBL-2H3) cells. Levels of serum mast cell protease-1 (MCPT-1) enzyme were determined by ELISA. Isolated brush border membranes from jejunal scrapings were used for estimation of alkaline phosphatase, gamma-glutamylphosphatase (GGT), dipeptidyl peptidase IV (DPP IV), lactase and sucrase activities.

Results OVA-sensitized mice significantly increased OVA-specific IgE compared to b-OVA sensitized group, while b-OVA sensitized mice increased OVA-specific IgG2a. MCPT-1 level of both OVA and b-OVA increased significantly compare to controls; the level of MCPT-1 of OVA-treated mice increased more than twice compared to levels found in b-OVA-treated mice. Specific activity of alkaline phosphatase was significantly stimulated in mice treated with both forms of OVA when compared with controls. However, in mice treated with native form of OVA the enzyme specific activity was significantly higher compared with mice treated with b-OVA. On the other hand, there were no significant differences in levels of GGT, DPP IV, sucrase and lactase.

Conclusion We observed that allergic response in experimental model of food allergy to OVA is strongly influenced by its thermal processing. Activity of jejunal alkaline phosphatase has a pivotal role in intestinal homeostasis and is dependent on the form of administrated OVA. (Supported by grants IAA500200710 and IAA500200801 from GA AS CR).

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Clostridium tyrobutyricum protects mouse colon from DSS-induced colitis associated with elevated levels of IL-18 in BALB/c and TNF-α in SCID mice

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Introduction Cytokines IL-18 and TNF-α have been implicated in the pathogenesis of inflammatory bowel disease due to their overproduction in inflamed intestinal mucosa.

Aim The aim of this study was to evaluate the effect of Gram-positive bacterium *Clostridium tyrobutyricum* on the development of dextran sulfate sodium (DSS)-induced colitis associated with inflammatory cytokine production in colonic mucosa of immunocompetent BALB/c and immunodeficient SCID mice (absence of T and B lymphocytes).

Methods Two-month-old BALB/c and SCID mice reared in specific-pathogen-free conditions were used. Acute ulcerative colitis was induced after receiving 2.5% DSS in drinking water for 7 days. Another group of mice received daily 2 x 10^8 CFU of *Clostridium tyrobutyricum* by intrarectal tubing one week prior to DSS exposure and during DSS drinking. Clinical and histological scores were evaluated. Mucin production, tight junction protein ZO-1 and inflammatory cell marker CD11b were detected. IL-18 was detected histochemically by rabbit polyclonal antibody and secondary goat IgG with attached fluorophore Cy3. TNF-α production *ex vivo* of colonic organ cultures was analyzed by ELISA.

Results Administration of DSS led to an appearance of clinical symptoms, infiltration of inflammatory cells in lamina propria , decreased production of mucins and ZO-1. Elevated levels of TNF-α in the colon of SCID mice and of IL-18 in BALB/c mice were observed. *Clostridium tyrobutyricum* prevented DSS-induced colitis with mild changes in colon morphology and maintenance of barrier integrity. Both TNF-α in SCID mice and IL-18 in BALB/c mice in the colon descendens were significantly reduced.

Conclusion In DSS model the severity of inflammatory symptoms of colon epithelium depends largely but not exclusively on host immune functions. IL-18, a Th1 cytokine, played key role in immunocompetent BALB/c mice while TNF-α expression, a typical product of macrophages, increased significantly in the colon of immunodeficient SCID mice. *Clostridium tyrobutyricum* suppressed high levels of both cytokines.

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Hydrogen sulfide in inflammatory bowel diseases

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Introduction*:* One of the most important functions of the intestinal epithelium is to absorb and to secrete water and electrolytes. Changes in the relation between absorption and secretion are relevant for many gastrointestinal diseases. The switch between both transport directions is regulated by different pathways involving classical neurotransmitters and hydrophilic hormones. Recently it has been demonstrated that small gaseous molecules are also able to regulate epithelial functions. These so called gasotransmitters are for example nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S). The last one was in the focus of this study. H_2S is produced during a reaction of the amino acid L-cysteine catalysed by the enzymes cystathion-β-synthase and cystathion-γ-lyase. Furthermore, sulphate-reducing bacteria are able to produce H_2S from sulfate contained in nutrients. Previous studies have shown that exogenous H_2S induces anion secretion in rat colon by stimulating enteric neurons and a direct effect on epithelial cells, depending on the activation of K^+ channels and a change in the mitochondrial membrane potential. To date, there are no data about the involvement of H_2S in inflammatory bowel diseases.

Aim*:* The aim of the study was to investigate, how the gasotransmitter H_2S influences inflammatory bowel diseases with the help of two models of diarrhoea, an acutely induced diarrhoea and a chronic colitis.

Methods*:* To induce acute diarrhoea, rats are treated with castor oil. Rectal application of TNBS (trinitrobenzolsulfone acid) is used for inducing chronic colitis. One group of rats is fed with a sulphate-reduced diet before starting experiments, whereas others are treated with inhibitors of cystathion-β-synthase and cystathion-γ-lyase. Ussing chamber experiments are used to investigate changes in ion transport in these models. A myeloperoxidase assay and hematoxylin eosin (HE) staining is used to obtain information about the inflammation level of the tissue.

Results*:* In Ussing chambers the effect of secretagogues and different concentrations of NaHS were tested. There were no significant differences between the group of dietary fed rats and the control group in the model of acute diarrhoea. Similarly there were no significant differences in the enzyme assay and the HE staining of these groups. Potential changes in the model of chronic colitis are going to be investigated.

Conclusion*:* These preliminary results do not yet allow a clear conclusion about the pathophysiological role of H_2S for the development of diarrhoea.

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Sodium-dependent inhibition of β-Ala uptake by leptin in Caco-2 cells

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Introduction: It is now well documented that leptin is a multifunctional hormone involved in a variety of functions, including regulation of the nutrients absorption. We previously demonstrated that apical leptin is implicated in a short term regulation of intestinal absorption of sugars and glutamine in rodents by modulating the expression of the corresponding transporters in the brush-border membrane (BBMV). β-Ala is absorbed in the small intestine by the high affinity (Km ~20 μM) Na^+ and Cl^--dependent TAUT transporter and the low affinity (Km ~3 mM) H^+-dependent amino acid transporter PAT1 which is highly dependent on the Na^+/H^+ exchanger NHE3 activity.

Aim: To extend our previous studies and investigate the possible effect of leptin on PAT1-mediated β-Alanine uptake in the human model of intestinal epithelium Caco-2 cells which has been shown to express this transporter.

Methods: Uptake of 1mM β-Ala from the apical membrane was measured in sodium and sodium-free buffer at pH 6 in Caco-2 cells grown on plates (apical leptin) or permeable supports (apical or basal leptin), after 5 and 30 min incubation in the absence and presence of leptin (0.2 and 8 nM). After incubation of Caco-2 cells for 30 min in the presence of 1mM β-Ala with or without 8 nM leptin. BBMV were prepared and expression of PAT1 was analyzed by Western blot using a polyclonal antibody raised against 107-119 residues of human PAT-1 (Abyntek, Spain).

Results: Leptin acting from the apical membrane, at the two concentrations and incubation times assayed, reduced β-Ala uptake by ~40-50%., Leptin also inhibited uptake of the amino acid when it was present in the basal compartment. In both cases the effect was reversible. Interestingly, this inhibitory effect was abolished when sodium in the medium was replaced by choline, suggesting inhibition of NHE3 activity. On the other hand, expression of PAT1 in BBMV was not significantly modified after leptin treatment. .

Conclusion: Taken together, these results indicate that leptin controls β-Ala transport in a model of human intestinal cells. Mechanism may involve inhibition of NHE3 exchanger which would in turn, decrease the H^+ gradient necessary for PAT1 activity.

Regulatory influences on monocarboxylate transporter 1 (MCT1) in ovine ruminal epithelial cells

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Introduction: MCT 1 is a cotransporter of monocarboxylates and protons in a variety of mammalian cell types. In the rumen epithelium, monocarboxylates arise from intracellular catabolism of short chain fatty acids (SCFA), which are a major source of energy for ruminants, and have to be transported into the blood. Previous studies in humans, rodents and pigs suggested that gene expression of MCT1 is regulated by peroxisome proliferator-activated receptor α (PPARα), a ligand-activated nuclear receptor.

The aim of our study was to examine if the MCT 1 in ruminal epithelial cells is regulated by PPARα.

Methods: Ruminal epithelial cells of sheep were cultivated according to methods previously established. After subcultivation, WY 14643, a synthetic and selective ligand of PPARα, was applied to the culture medium in two different concentrations (25µM; 100µM) and remained for 48 hours. After processing the specimens, the relative amount of mRNA of MCT1 and acyl-CoA oxidase were analyzed by semiquantitative RT-PCR and RT-qPCR. Functional quantification was made by measuring the intracellular pH (pH_i) of cells by using BCECF/AM, a cell-penetrating pH-sensitive fluorescent dye. Cells were exposed to 20mM lactate for 45 minutes. Extracellular lactate was removed and recovery of pH_i was determined. We compared the pH_i recovery of untreated cells with cells incubated with 100µM WY 14643. To inhibit MCT1-dependent pH_i recovery we applied 600µM p-hydroxymercuribenzoic acid (pHMB), an inhibitor of MCT1.

Results: By RT-PCR it could be demonstrated that the gene expression of MCT1 in the ruminal epithelial cells was increased by WY 14643 in comparison to untreated cells. This effect was dose-dependent. Acyl-CoA oxidase, which has been shown to be regulated by PPARα, increased by WY 14643 treatment, too. The pH_i measurement revealed that the ruminal epithelial cells showed a rapid pH_i recovery after preloading with lactate. The recovery could effectively be inhibited by pHMB. WY 14643 treated cells tended to show an increase in ability of pH_i recovery.

In conclusion, it could be demonstrated, that MCT1 in ruminal epithelial cells is regulated by PPARα. Furthermore the amount of lactate-dependent proton export is probably increased after WY 14643 treatment. It seems that the effect depends on MCT1, because pHMB inhibited the pH_i recovery.

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Impact of oxidative stress on *pept-1* gene expression

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Introduction: The intestinal proton-coupled transporter PEPT-1 is responsible for the uptake of di- and tripeptides as well as of peptidomimetics like ß-lactam antibiotics. In *Caenorhabditis elegans* loss of PEPT-1 leads to major metabolic changes with growth retardation, lipid accumulation and impairments in oxidative stress response. Thus, expression of *pept-1* seems to be crucial for maintaining normal metabolic functions.

Aim: Up-to-date little is known about transcriptional regulation of *pept-1*.Therefore, we aimed to elucidate signaling cascades and corresponding transcription factors (TF) regulating expression of *pept-1* in *C. elegans*.

Methods: Using Genomatix, TRANSFAC and TESS software we determined putative TF binding sites by analysis of the *pept-1* promoter region. The prime candidate TF was down-regulated via RNA interference (RNAi) followed by Western Blot analysis to determine PEPT-1 level. As the TF is known to be activated by oxidative stress animals were treated with 2 % NaN_3 and 10 mM H_2O_2 in combination with Western Blot analysis to validate the input of ROS-dependent signaling cascades on the TF.

Results: Analysis of *pept-1* promoter region revealed two binding sites for SKN-1, the worm homolog of NRF2 via CTAAATCATTTT and TCTTGTCATGCT motifs. A high conservation of these sites between different *Caenorhabditis* species provides first evidence on their biological relevance. Down-regulation of WDR-23 as well as GSK-3, both repressors of SKN-1, resulted in a 3- to 4- fold elevated PEPT-1 protein level, while down-regulation of SKN-1 did not change the PEPT-1 content significantly. Induction of oxidative stress enhanced the PEPT-1 in Western Blot analysis.

Conclusion: Basal transcription of *pept-1* is not mediated by SKN-1, while oxidative stress induces a up-regulation of *pept-1* expression in *C. elegans.* Further experiments need to elucidate whether this effect is SKN-1 dependent. The increase of PEPT-1 function could result in higher absorption rates of the dipeptide cysteinyl-glycine, the precursor of glutathione from the gut, linking peptide transport to oxidative stress response.

Ruminal SCFA-transport via SMCT1: a new player in the game

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Introduction: In the reticulorumen of ruminants, huge amounts of short-chain fatty acids (SCFA) are produced by microbial fermentation of carbohydrates. The main part of these substrates is absorbed directly across the ruminal wall. The underlying mechanisms of this absorption are still under discussion. Since sodium coupled monocarboxylate transporter 1 (SMCT1) is known to transport SCFA in the human intestinal tract, we studied if SMCT 1 is involved in SCFA-transport across the ruminal epithelium.

Methods: Expression of SMCT1 in sheep rumen epithelium was investigated by reverse transcription polymerase chain reaction (RT-PCR) with chimerical (human/sheep) SMCT1 primers and subsequent sequencing of PCR products. Functional studies were carried out on sheep rumen epithelia mounted in Ussing chambers under short-circuit conditions. On both, mucosal and serosal side of the chamber, the same chloride- and bicarbonate free buffer solution adjusted to pH 7.4 and gassed with oxygen was applied. Epithelia were incubated with the SMCT1-inhibitor fenoprofen (1mM) on mucosal or serosal side for 45 min. After this time, acetate, propionate or butyrate (20 mM each) was added on inhibitor incubated side. Short-circuit current (I_{sc}) and transepithelial conductance (G_t) were continuously measured.

Results: The nucleotide sequence of the ruminal PCR product shared 94.6 % homology with human SMCT1 and 86.8 % with mouse SMCT1. In Ussing chamber experiments, addition of acetate, propionate or butyrate to the mucosal buffer solution initially led to a significant decrease of I_{sc}. Thereafter, an I_{sc}-increase followed. The magnitude of this increase was substrate dependent in the order acetate < propionate < butyrate. Pre-incubation with fenoprofen significantly reduced initial I_{sc}-decrease. Subsequent I_{sc}-increase was reduced after fenoprofen pre-incubation in epithelia treated with propionate or butyrate. Serosal propionate or butyrate application induced an I_{sc} increase that was significantly reduced after fenoprofen treatment. Changes of I_{sc} after serosal acetate addition were not affected by fenoprofen.

Conclusions: SMCT1 is expressed in sheep rumen epithelium. The fenoprofen sensitivity of I_{sc} indicates an involvement of SMCT1 in SCFA-transport across the ruminal wall. Differences in fenoprofen sensitivity between the three SCFA suggest a substrate dependence of this mechanism.

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Repeated measures ANOVA as a tool for statistical comparison of Ussing chambers data

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Introduction: Responses to various chemical agents or their different combinations measured in Ussing chamber as a short-circuit current (SCC) are usually compared by Student's t-test and one-way ANOVA using maximal or minimal deviation from baseline SCC. But it means that each SCC tracing is characterized by only one number (ΔSCC).

Although in most cases above mentioned approach is sufficient to distinguish significantly different responses, there are cases in which two responses have similar ΔSCC, but have different shapes.

Our aim was to adopt new statistical method more sensitive to changes in shape of SCC responses.

Methods: We did not use fitting a function to obtained data because we wanted to compare complete responses and there was not a single function usable to carbachol responses of distal and proximal colon in the presence of different inhibitors. Instead, we divided SCC responses to 30 s intervals and estimated area under the curve (AUC) for each interval. AUC was calculated as sum of measured currents (ΔSCC) multiplied by time step (1 s). Each SCC response was characterized by 9 AUCs corresponding to 270 s tracing. Responses were compared by repeated measures ANOVA using AUCs as dependent variable and interaction between used drug combination and AUCs was tested. The 30 s interval was selected as a compromise between number of parameters describing each SCC response and resolution of the method. Due to the violation of sphericity assumption, Hyun-Feldt correction for violations of sphericity was applied when significance was calculated.

Results and conclusions: We conclude that we found an easy way how repeated measures ANOVA can be used as a meaningful tool for statistical comparison of Ussing chambers data rather than ΔSCC alone.

The study was supported by grant from Charles University (GA UK 25410).

Inhibition of the sodium-coupled glucose cotransporter SGLT1 by plant extracts and metabolites derived from apple and grape vine

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Introduction: The intestinal absorption of glucose from dietary carbohydrates is mainly mediated by the sodium-dependent glucose transporter SGLT-1. Inhibition of SGLT1 may provide a smoothening of glucose appearance in plasma, reduce overshoots in insulin levels and thus may particularly useful in insulin resistant type 2 diabetics.

Aims: For identifying potential hypoglycemic plant derived compounds we screened a large number of diverse plant extracts as well as numerous individual plant compounds of plant secondary metabolism (mainly flavonoids) for their ability to inhibit SGLT1.

Methods: The human SGLT-1 transporter was heterologously expressed in Xenopus oocytes and transport activity was determined under voltage clamp conditions. Current responses to 1-O-methyl-alpha-D-glucopyranoside (αMDG), a non-metabolizable analogue of glucose were determined in the absence and the presence of either extracts or isolated polyphenols. Preselected compounds were also applied in small intestine of mice for inhibition of uptake of radiolabeled αMDG into tissue using "gut ring" and for transepithelial flux analysis with the "everted sac" techniques.

Results: Amongst the tested plant extracts only an apple and a grape vine extract showed promising inhibition of SGLT-1 in oocytes. *A*nalysis of these extracts revealed Phlorizin as the major compound in the apple extract. Trans-resveratrol and its oligomeric form ε-viniferin were found as major components of the grape vine extract Vineatrol®. Whereas phlorizin revealed in oocytes an IC_{50} value of 0.42 μM for transport inhibition, trans-resveratrol did not inhibit SGLT-1 whereas ε-viniferin was reduced transport of αMDG with an IC_{50} of 9.4 μM. The EC-50 value for phlorizin in mice jejunum was 67 μM suggesting either pronounced hydrolysis by lactase-phlorizin-hydrolase with release of phloretin and/or marked unstirred layer effects.

Conclusion: Despite the fact that we did not yet find any flavonoid more potent than phlorizin, the inhibition of SGLT-1 by a large spectrum of structural related compounds should help to better define structural requirements for more potent inhibitors.

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Electrogenic properties of the human organic anion-transporting polypeptides OATP1B1 and OATP1B3

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Introduction: Organic anion-transporting polypeptides (OATPs) are involved in the liver uptake of many endogenous and xenobiotic compounds, such as bile acids and drugs, by mechanisms not fully understood. Whereas rat Oatp1a1 behaves as an electroneutral transporter, the existence of an electroneutral or electrogenic mechanisms in the human isoforms OATP1B1 or OATP1B3-mediated transport has not been elucidated yet. Recently, studies of our group using CHO cells and *Xenopus laevis* oocytes expressing these transporters have demonstrated their sensitivity to changes in plasma membrane potential.

Aim: To investigate the possible electrogenic transport mechanism of OATP1B1 and OATP1B3.

Methods: OATP1B1 and OATP1B3 were expressed in *X. laevis* oocytes. Oocytes membrane potential was held at -50 mV and electrogenic properties were investigated using the two-electrode voltage clamp technique.

Results: Oocytes were perfused with Na^+ buffer. Replacement of Na^+ buffer by Na^+-free buffer induced an outward deflection of the current trace in oocytes expressing OATP1B1 or OATP1B3, being its magnitude markedly higher in the latter, indicating the existence of a Na^+ leakage. Perfusion of 100 μM taurocholic acid, estradiol-17β-D-glucuronide or estrone-3-sulfate also resulted in an outward deflection of the current trace, which was similar in Na^+ buffer and Na^+-free buffer although smaller for OATP1B3 in both cases. These results indicated hyperpolarization of the plasma membrane associated to the net entrance of negative charge. Non-injected and Oatp1a1 expressing oocytes did not show Na^+ leakage or substrate induced current.

Conclusion: OATP1B1 and OATP1B3 are electrogenic transporters showing a net anion influx associated to the substrate transport and a Na^+ leakage not coupled to substrate transport.

Intestinal explants as a useful tool for transintestinal cholesterol excretion measurement

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Introduction: So far, the liver has been the main target for cholesterol elimination. Several recent studies have described a new route of cholesterol excretion, the Trans-Intestinal Cholesterol Excretion or TICE. TICE allows direct elimination of plasma cholesterol into the feces directly via the intestine. Its activity is mainly localized in the proximal part of the small intestine. TICE is is inducible nutritionally and pharmacologically. Until now, TICE has been only studied *in vivo* in mice using intestinal perfusion in bile-diverted mice or with kinetic studies involving stable isotopes.

The aim of this study was to develop a new approach to explore *in vitro* the enterocytic cholesterol transport (basolateral uptake, intracellular processing, apical eflux).

Methods: Intestinal segments from C57Bl/6 mice were harvested and mounted in Ussing chambers. Mucosal explants were stretched between an apical and a basolateral chamber filled respectively with mannitol or glucose krebs buffer. Both media are oxygenated and heated at 37°C for the duration of the experiment unless specified. Paracellular fluorescent markers (4,4kDa FITC-dextran) were added to the apical chamber to verify the explant integrity. Various concentrations of ^{3}H-cholesterol (GE Healthcare) were added in the basolateral chamber. At regular interval of 30 minutes, medium of the apical and basolateral sides were collected and the concentrations of FITC-dextran and cholesterol were determined respectively by spectrophotofluorometry and by liquid scintillation counting.

Results: After 30 minutes of incubation, we observed ^{3}H cholesterol appearance (TICE) at the apical side. TICE was significantly induced (+100%) when cholesterol acceptors were added at the apical side (Taurocholate/phosphatidylcholine; 10/2mM). TICE was reduced by 80% when measurement was performed at 4°C. TICE was strongly oxygen-dependent.

In conclusion, we developed an innovative technique to measure TICE *in vitro*. This method would be very useful to screen rapidly pharmacological or nutritional TICE modulators in mouse but also putatively in human intestinal explants. Finally, it will allow us to better characterize the involvement of basolateral, intracellular or apical proteins in the cholesterol enterocytic transport.

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Influence of Ovalbumin on functional characteristics of IPEC-J2 monolayer

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Introduction & Aim: The functional properties of the IPEC-J2 Monolayer have been used to determine the risks of incorporation of genetically modified products. Ovalbumin was used as an example of an unknown protein mixture. The examination of possible influences of this mixture on the physiological und functional qualities of IPEC-J2 Monolayer was done by testing the transport of [^{3}H]-mannitol, [^{3}H]-lysin and the reaction on a sodium addition.

Methods: Diffusion of [^{3}H]-mannitol and transport rates of [^{3}H]-lysin has been determined by flux measurement across the cell monolayer. The analyses of the electro-physiological characteristics were determined by Ussing-Chamber technique. The reaction on sodium addition under the influence of ovalbumin was checked. The protein mixture was added serosal, mucosal and simultaneous on both sides.

Results: The diffusion of [^{3}H]-mannitol was not affected by ovalbumin (Fig.1). In contrast the transport of [^{3}H]-lysin was higher after a serosal addition (Fig.2). Ovalbumin has an influence on the current, because the value decreases about 50-75% in comparison to control (Fig.3).

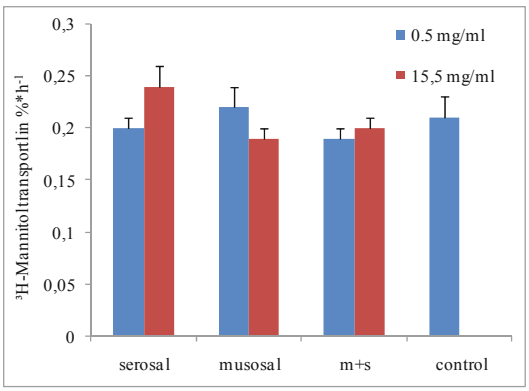

Fig. 1: Transport of [^{3}H]-mannitol by IPEG-J2 monolayer in the presence of 0.5 and 15.5 mg/ml Ovalbumin. (n=9)

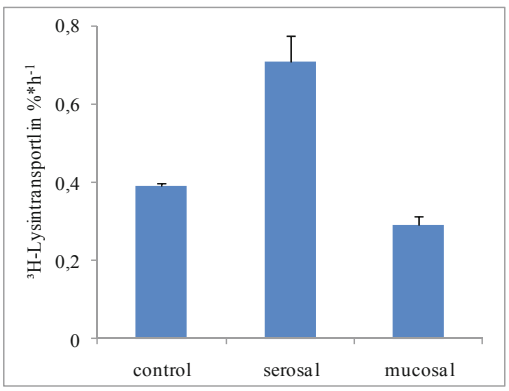

Fig.2: Transport of ^{3}H-Lysin by IPEG-J2 monolayer in the presence of 15.5 mg/ml Ovalbumin (n=6)

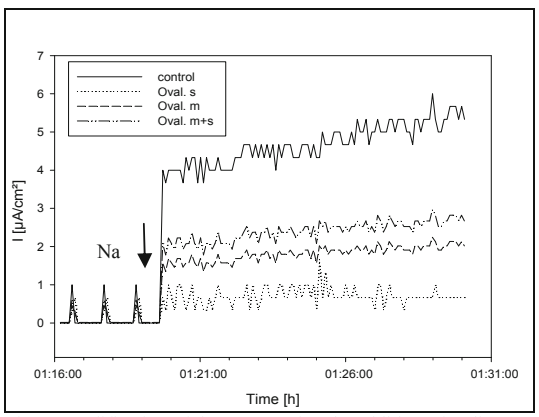

Fig. 3: Modification of the current after the addition of sodium (Oval.- ovalbumin, s - serosal, m - mucosal, m+s – both sides)

Conclusions: The addition of ovalbumin has no effect on the passive (paracellular) characteristics of the monolayer. The first results show

- an increase of the active [^{3}H]-lysin transport after a serosal ovalbumin addition and
- ovalbumin affects the electro-physiological characteristics of the IPEC-J2 monolayer.

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Influence of *Enterococcus faecium* as a probiotic feed additive on transport properties of the small intestine of piglets at weaning

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Introduction: The pathogenesis of many diseases of the gastrointestinal tract involves changes in its barrier and transport functions. Weaning affects mucosal structure and function and the bacterial composition of the gut flora, which can lead to post-weaning diarrhea. Supplementation with probiotics shows positive effects on diarrhea incidence and performance parameters in piglets (1). Studies regarding the mechanisms of probiotics in the intestine have demonstrated effects on transepithelial transport functions and an activation of immunologic mechanisms of the gut, such as the release of cytokines. Cytokines can influence transport mechanisms of the gastrointestinal tract (2).

Aim: We therefore tested the hypothesis as to whether *Enterococcus faecium* NCIMB 10415 (*E. faecium*) changes the absorptive and secretory transport properties within the piglet small intestine. Furthermore, we examined whether interleukin-1α (IL-1α) induces a change in ion transport, and whether this change is attributable to a change in Cl transport.

Methods: Piglets (days 14, 28, 35, and 56) and sows were randomly assigned to a control and a probiotic group. The mid jejunum was stripped of its outer muscle layers and mounted into conventional Ussing chambers. To examine absorption, the epithelia were stimulated with various concentrations of glucose, and to study secretion, they were stimulated with prostaglandin E_2 (PGE_2). Mannitol flux rates were measured as a parameter of paracellular permeability. IL-1α was added to the serosal side at 100 ng/ml after the addition of various inhibitors.

Results: The short circuit response (ΔI_{sc}) tended to be higher after the addition of glucose in the probiotic compared with the control group (days 28 and 56). The PGE_2 response was higher in the probiotic group than in the control group on day 35. Hence, effects of age were observed. Mannitol flux rates were numerically lower in the probiotic group, and the flux rates decreased with increasing age of the piglets. IL-1α induced an increase in I_{sc}. Part of this increase could be inhibited by bumetanide.

Conclusion: Thus, *E. faecium* changed the transport properties within the small intestine of piglets around weaning, possibly with a protecting effect on barrier function. The underlying mechanisms are still not clear; the effects of cytokine secretion (IL-1α) should be further characterized.

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Structural change of enterocyte and Caco2-cell cytoskeleton during hexose absorption.

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Introduction and aims: Hexose transport by SGLT1 and GLUT2 across a cell is of primary interest for investigation of carbohydrate absorption. Moreover, it still remains unknown how the transporter movement is realized in the enterocyte cytoplasm. Perhaps the transporters are organized into endosomes connected with microtubules.

The distribution of fibrillar actin and α-tubulin in the enterocyte of the rat small intestine and Caco2-cell as enterocyte model has been studied. The investigation has been performed by means of confocal and electron microscopy in a wide range of carbohydrate loads.

Methods: To study the samples using a confocal microscope, pieces of the isolated loop after perfusion with maltose solution (12.5 mM/l) were fixed with 2% formaldehyde in PBS and frozen. Sections of 7–10 μm thickness were obtained using a Leica cryostat. The actin was stained by rhodamine-phalloidin. The tubulin was revealed by the method of indirect immunofluorescence. The polyclonal antibody to α-tubulin was used as primary antibody, the immunoglobulin conjugated to Alexa Fluor 488 was used as secondary antibody. The sections were analyzed using a confocal microscope Leica TCS SL with argon and helium-neon lasers. Caco2-cells, previously cultivated to confluence, were incubated with 2.5 or 25 mM/l glucose, fixed with 2% formaldehyde, and stained as enterocytes. Electron microscopy of the enterocyte and Caco2-cell on JEOL 100U microscope and preparation for that were made by standard way.

Results and conclusions: Fibrillar actin in the enterocyte and Caco2-cell is localized at the microvilli and close to cell membrane. α-Tubulin distribution in the enterocyte depends on carbohydrate concentration. A label to α-tubulin is localized in the enterocytes of the villus top and colocalized with actin close to apical membrane after perfusion of the isolated loop with the Ringer solution (control). The label is revealed in the enterocytes of the whole villus length after the perfusion with maltose solution. In contrast to the control no colocalization with actin was detected. The label to α-tubulin prevails in apical part of Caco2-cell and close to lateral membrane. It is colocalized with actin label at the tight junction region. No difference of α-tubulin distribution depending on glucose concentration has been found.

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Effect of dexamethasone on digestive enzymes and glucose absorption in the rat small intestine

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Introduction: Glucocorticoid hormones mediate the effects of stress in mammals, but their effects on the digestive and absorptive functions of the small intestine are not clear.

The aim of the work is to study the effects of dexamethasone on activities of digestive enzymes and absorptive capacities of the intestine at different time intervals after its injection.

Method. In the first set of experiments 4 groups of rats (Wistar, males) were investigated. Group 1: 1 hr after dexamethasone injection to the rats, previously fasted for 24 hrs**;** group 3: 24 hrs after dexamethasone injection to the rats and the beginning of their fasting; groups 2 and 4: controls (similar as in groups 1 and 3, respectively, but with injection of propylene glycol instead of dexamethasone). Enzyme activities (maltase, aminopeptidase M, and alkaline phosphatase) were measured in preparations of mucosa taken from different parts of the small intestine. In the second set of the experiments we were recording a dynamics of glucose solution (200 g/l) consumption by rats who previously fasted for 24 hours and got injections of dexamethasone or propylene glycol (control) 1 and 24 hrs before the experiment.

Results. In all experimental groups body weight of the animals decreased relative to the initial weights, but in the most extent - in the group 3. In this group of rats, corticosteron concentration in the blood plasma and small intestinal mass, as well as protein concentration in the distal part of the small intestine, were lower than in the control rats (group 4). Maltase activity increased by 25% in the distal part of the small intestine in group 1 as compared with group 2, and by 41% in the proximal small intestine in group 3 as compared with group 4. Activity of aminopeptidase M reduced by 25% in the colon in group 3 as compared with group 4. The rate of free consumption of glucose solution may be regarded as indicator of capacity of the small intestine for glucose absorption. This rate was higher in the rats which got an injection of dexamethasone 1 hr before the experiment, but significantly lower in the rats which got this injection 24 hrs before the experiment (in comparison with the corresponding controls).

Conclusion. The effect of dexamethasone on the digestive enzymes and glucose absorption varies (in magnitude or direction) depending on the time elapsed since its injection

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The flavonol quercetin does not influence the toxicokinetics of the mycotoxin ochratoxin A in rats

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Introduction: Previous studies indicate that intestinal absorption of the nephrotoxic mycotoxin ochratoxin A (OTA) occurs mainly through passive diffusion of the undissociated form. However, several *in vitro* studies have shown that OTA is partly resecreted into the intestinal lumen by the multidrug resistance associated protein (MRP-2) and breast cancer resistance protein (BRCP). *In vitro* studies using Caco-2-cells have shown that polyphenols (quercetin, genistein, resveratrol) may impair OTA-efflux through competitive inhibition of the MRP-2, possibly resulting in an increased systemic availability of OTA (Sergent et al. 2005). Therefore, the aim of the present *in vivo* study was to investigate possible effects of quercetin on the toxicokinetics of OTA in rats.

Material and Methods: Eighteen growing male F344 Fisher rats with an average body weight of 200 g were allocated to two dietary treatments consisting of 1) a commercial, flavonoid-free balanced diet containing 10 mg OTA per kg derived from wheat inoculated with *Aspergillus ochraceus* and 2) the same diet supplemented with 100 mg quercetin per kg. The animals were fed restrictively (~0.7 of *ad libitum* intake, 13 g/d) to ensure equal OTA intake. Animals were kept in metabolism crates to allow total urine and faeces collection. After six days on trial, rats were euthanized and blood, liver, kidney, muscle and brain samples were taken from each animal. Faeces, urine and tissue samples were analysed for OTA and OTalpha by HPLC using fluorescence detection.

Results and Conclusions: Quercetin supplementation had no effect ($p>0.05$) on feed consumption (12.7 *vs.* 12.5 g/d), OTA-intake (137.1 vs. 130.0 µg/d), water intake (13.8 vs. 13.3) and body weight gain (1.9 vs. 1.7 g/d). Faecal and urinary excretion of OTA and OTalpha of rats was similar in both treatment groups. Concentrations of ochratoxin A in plasma (11.3 vs. 10.9 µg/ml), kidney (1.4 vs. 1.4 µg/g) , liver (1.1 vs. 1.1 µg/g) , muscle (0.48 vs. 0.53 µg/g) and brain (0.13 vs. 0.14 µg/g) were not affected by quercetin supplementation as well**.** Based on the total excretion and tissue concentrations of OTA, it is concluded that the polyphenol quercetin has no impact on the toxicokinetics of OTA *in vivo.*

SGLT1 and GLUT2: which prevails in glucose transport across the apical membrane of the enterocytes under normal conditions?

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Introduction: It has been widely accepted that dietary glucose crosses the apical membrane of the enterocyte via the Na^+/glucose co-transporter (SGLT1), while the GLUT2 transports glucose from cytosol to the blood. But, recent evidence indicates that GLUT2 can be rapidly inserted into the apical membrane after a meal. Some authors assume that intestinal glucose absorption by the apical GLUT2 pathway may be 3 to 5-times greater than by SGLT1 at high concentrations of sugars. However, it remains unclear, is it true under physiological conditions?

Methods: We studied kinetics of membrane hydrolysis of maltose and absorption of glucose in isolated loop of the rat small intestine during its perfusion in chronic experiments with maltose or isocaloric glucose solutions in a wide range of initial concentrations of the substrates (25–200 mM of glucose). Experimental data were analyzed by means of mathematical models which took into account current views about mechanisms of hydrolysis and transport of nutrients, the pre-epithelial («unstirred») layer, trans-epithelial fluid fluxes, and geometric peculiarities of intestinal surface.

Results: A complete saturation of maltose hydrolysis and glucose absorption (both free, and released from maltose hydrolysis) was not reached even at the highest substrate concentration used. Good agreement with experimental data (both for maltose hydrolysis, and glucose absorption) was observed only on the model which imitated a complex geometry of the intestinal surface. The results of modeling show that the facilitated diffusion plays, perhaps, a certain role in glucose transfer across the apical membrane of the enterocytes. However, under physiological conditions, even in the range of high carbohydrate loads, glucose transport via SGLT1 seems to be several times higher than that via the apical GLUT2.

Conclusion: In order to evaluate correctly relative capabilities of various mechanisms of glucose transport across the intestinal epithelium under normal conditions, one should take into account diffusion characteristics of the pre-epithelial layer of the small intestine, trans-epithelial fluid fluxes, and geometric peculiarities of its absorptive surface.

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The short-chain fatty acid butyrate is a substrate of breast cancer resistance protein (BCRP) efflux transporter

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Introduction: Colorectal cancer (CRC) is one of the most common solid tumours worldwide. Although the causes of CRC are multifactorial, a diet rich in dietary fibre and low in saturated fats is associated with a reduced risk of CRC. Butyrate (BT) is one of the main end products of anaerobic bacterial fermentation of dietary fibre in the human colon and is known to play a key role in colonic epithelium homeostasis, being able to prevent/inhibit colon carcinogenesis.

Human multidrug resistance ATP-binding cassete (ABC) transporters are ubiquitous membrane proteins responsible for the efflux of multiple, endogenous or exogenous, compounds out of the cells.

Aim: Because nothing was known concerning the interaction of BT with ABC transporters, the aim of this work was to investigate the possibility of BT being transported by P-glycoprotein (MDR1), multidrug resistance proteins (MRPs) or breast cancer resistance protein (BCRP).

Methods: Uptake and efflux of ^{14}C-BT and ^{3}H-FA (folic acid) were investigated in Caco-2, IEC-6 or MDA-MB-231 cell lines. The expression of BCRP mRNA was detected by RT-PCR. The effect of drugs upon cell viability, proliferation and differentiation was quantified with the lactate dehydrogenase assay, sulforhodamine B assay and alkaline phosphatase activity assay, respectively.

Results: In both IEC-6 cells and Caco-2 cells, no evidence was found for the involvement of either MDR1 or MRPs in ^{14}C-BT efflux from the cells. By contrast, several lines of evidence support the conclusion that BT is a BCRP substrate. First, BCRP inhibitors reduced ^{14}C-BT efflux in IEC-6 cells, and IEC-6 cells were found to express rBCRP mRNA. Second, both BT and BCRP inhibitors significantly decreased the efflux of the known BCRP substrate ^{3}H-FA, in IEC-6 cells. Third, BCRP inhibitors reduced ^{14}C-BT efflux in the BCRP-expressing MDA-MB-231 cell line. So, the interaction between BT and BCRP does not seem to be IEC-6 cell-specific, and BT appears to be a substrate of both rat and human BCRP. Fourth, although BCRP is not involved in ^{14}C-BT efflux in Caco-2 cells, treatment of these cells with BT induced BCRP-mediated ^{14}C-efflux; so, BT appears to induce BCRP expression in Caco-2 cells. Finally, in IEC-6 cells, combination of BT with a BCRP inhibitor significantly potentiated the effect of BT upon cell proliferation.

In **conclusion**, the results of this study are very important in the context of the high levels of BCRP expression in the human colon, where high concentrations of BT are also present. Given the anticarcinogenic role of BT at that level, the interaction of BT with BCRP and with other BCRP substrates/inhibitors is indeed of major importance.

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The primary bile salt chenodeoxycholic acid inhibits butyrate uptake in a non-tumoral intestinal epithelial cell line

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Introduction: The short-chain fatty acid butyrate (BT) plays a key role in colonic epithelium homeostasis. Studies suggest that the primary bile salt chenodeoxycholic acid (CDCA) may play a role in the etiology of colorectal cancer (McMillan et al., 2000; Reddy et al., 1997; Mahmoud et al., 1999; Tong et al., 2008).

So, our **aim** was characterize the effect of CDCA upon ^{14}C-BT uptake in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines.

Methods: ^{14}C-BT uptake by Caco-2 and IEC-6 cell lines was quantified by liquid scintillometry; the expression of the H^+-coupled monocarboxylate transporter 1 (MCT1) and of the Na^+-coupled monocarboxylate cotransporter (SMCT1) was quantified by qRT-PCR; the effects of BT and CDCA in cell viability, proliferation and differentiation were quantified by the lactate dehydrogenase assay, sulforhodamine B assay and alkaline phosphatase activity assay.

Results: A 2-day exposure to CDCA (100 μM) did not affect ^{14}C-BT uptake in Caco-2 cells. In contrast, it markedly inhibited ^{14}C-BT uptake by IEC-6 cells (to a maximum of 18% of control with 150 μM CDCA). The effect of CDCA was concentration-dependent (IC_{50}=104.2 (96.4-112.5) μM), and quantitatively similar from 1 to 7 days of exposure. CDCA acted as a competitive inhibitor of uptake at low concentrations of ^{14}C-BT, as it significantly increased the K_m, while having no effect on the V_{max}. The inhibitory effect of CDCA upon ^{14}C-BT uptake was evident both in the presence and absence of extracellular Na^+; so, CDCA inhibits both MCT1 and SMCT1. In contrast, CDCA significantly increased the mRNA expression levels of SMCT1 and had a tendency to increase MCT1.

At the molecular level, inhibition of Ca^{2+}/calmodulin (CaM) and simultaneous inhibition of protein kinase A, protein kinase C and protein kinase G reduced ^{14}C-BT uptake, and inhibition of CaM-dependent kinase II (CaMKII) increased ^{14}C-BT uptake. The inhibitory effect of CDCA upon ^{14}C-BT uptake is decreased by inhibition of CaM and of the MAP kinase pathways ERK1/2 and p38.

In IEC-6 cells, CDCA up to 100 μM (2-days) caused no significant effect upon cell proliferation and viability; however, at 200 μM, it significantly decreased cell proliferation and viability. BT (5 mM; 2 days) decreased cellular viability and proliferation and increased cell differentiation. The effect of BT upon cell proliferation and differentiation was significantly decreased by CDCA.

In **conclusion**, CDCA is an effective inhibitor of ^{14}C-BT uptake in non-tumoral (IEC-6) but not in tumoral (Caco-2) intestinal epithelial cells. Given the role played by BT in the intestine, this mechanism may contribute to the procarcinogenic effect of CDCA at this level.

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Studies on the anticarcinogenic effect of clotrimazole at the intestinal epithelial level and putative effect in energy substrate uptake

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Introduction: Butyrate (BT) is an important metabolic substrate in normal colonic epithelial cells. However, BT becomes less essential for growth of neoplastic cells, which show an increase in the rate of glucose uptake and glycolysis, producing excessive lactic acid. Clotrimazole (CTZ) is an antifungal drug that has demonstrated anticancer activity by inducing detachment of some glycolytic enzymes from cytoskeleton, thus inhibiting glycolysis.

The **aim** of this study was to investigate: (a) the anticarcinogenic effect of CTZ on tumoral and non-tumoral intestinal epithelial cell lines (Caco-2 and IEC-6 cells, respectively), by investigating its effect upon cell proliferation, viability and differentiation, alone or in conjunction with an inhibitor of mitochondrial oxidative phosphorylation (rhodamine123), and (b) the possibility of inhibition of the apical uptake of glucose or BT as a mechanism contributing to the anticarcinogenic effect of CTZ in Caco-2 cells.

Methods: We performed ^{14}C-BT, ^{3}H-deoxyglucose (^{3}H-DG) and ^{3}H-O-methylglucose (^{3}H-OMG) uptake studies in Caco-2 and IEC-6 cells; the expression of mRNA was quantified by qRT-PCR; cellular viability was quantified by the lactate dehydrogenase assay, cellular proliferation by the sulforhodamine B assay and cellular differentiation by alkaline phosphatase activity assay.

Results: In Caco-2 cells, CTZ showed anticarcinogenic activity, decreasing cellular viability and proliferation, and increasing cell differentiation. The effect of CTZ upon cell proliferation and viability was greatly potentiated in the presence of rhodamine123. In IEC-6 cells, CTZ also decreased cellular viability and proliferation, but increased cellular DNA synthesis rate and had no effect on cell differentiation. Exposure of Caco-2 cells to CTZ (10 μM) for 1 and 7 days increased (by 20-30%) the uptake of the glucose analogs ^{3}H-DG and ^{3}H-OMG, respectively, but had no effect on the uptake of ^{14}C-BT. The effect of CTZ upon ^{3}H-DG and ^{3}H-OMG uptake showed concentration-dependency and was maximal at 10 μM. CTZ did not alter the pharmacological characteristics of ^{3}H-DG and ^{3}H-OMG transport, but produced significant changes at the level of mRNA expression of facilitative glucose transporter 2 (GLUT2) and Na^{+}-dependent glucose cotransporter (SGLT1).

In **conclusion**, CTZ exhibits anticarcinogenic effect both in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines. In Caco-2 cells, the anticarcinogenic effect of CTZ was strongly potentiated in the presence of rhodamine123. Moreover, stimulation of glucose membrane uptake might be a compensation mechanism in response to the inhibition of glycolysis induced by CTZ.

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Expression of tight junction proteins in rat Peyer's patches epithelium

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Introduction: Peyer's patches (PP) are important parts of the intestinal immune system. They are characterized by presence of a follicle-associated epithelium that covers the cluster of lymphoid follicles and provides uptake and transport of antigens to immune cells. Tight junction (TJ) proteins specifically determine barrier properties along the intestine. Whereas segment-specific expression of TJ proteins has been shown in intestinal villous epithelium, information of TJ protein expression in PP epithelium is scarce.

Aim: The aim of the present study was to investigate TJ protein expression in PP epithelium in comparison with villous epithelium.

Methods: Experiments were performed on Wistar rats (200-300g). PP and contiguous parts of intestine (both 3 mm diameter) were removed from jejunum and used for comparative analysis of TJ expression by means of Western blotting. Western blots were used to investigate expression of claudin-1, -2, -4, -5, -7, -12, and occludin in PP.

Results: Compared to intestinal epithelium, different expression levels were observed. Claudin-1, -5 and occludin showed a markedly stronger expression in PP epithelium compared with neighbouring intestinal control tissue ($p<0.05$). In contrast, pore-forming claudin-2, and also claudin-7 and -12 showed a markedly lower expression compared to intestinal control epithelium ($p<0.05$). Claudin-4 expression showed variations of expression levels compared to respective controls in both directions, though.

In conclusion, expression of TJ proteins differs in PP epithelium and intestinal villous epithelium. This difference indicates different epithelial barrier function and has to be taken into account in experiments employing intestinal tissue preparations for analysis of transport and barrier functions.

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Structural and functional changes in the small intestine in short-term anoxia and approaches to their correction

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The aim of the work is to study in experiments on rats the effect of anoxia on the structure of the small intestine and active transport of glucose and to evaluate the efficiency of some approaches to their normalization.

Methods: Active transport of glucose was determined using a technique of everted sacs of the small intestine *in vitro*. The extent of injury in the small intestinal structure was assessed on the light microscope by the standard technique.

Results: Anoxia for 15 or 30 minutes during the incubation of intestinal preparations in the Ringer solution increased an injury of the preparations and resulted in a decrease of glucose active transport in them (by ~ 90%). The presence of glucose or glutamine in the Ringer solution at the mucosal side of the sacs for the first 15 min of oxygenation (after a preliminary 15-min anoxia) reduced the damage of the sacs, while the presence of glucose also contributed to the preservation of active transport at a higher functional level.

Application of glucose in the hypertonic Ringer solution or in the inverted Ringer solution (where Na^+ was replaced by K^+) at the mucosal side of the sacs for 5-10 minutes after 15-min anoxia, significantly improved the structure of the preparations. However, glucose active transport increased only in the case of the hypertonic Ringer solution.

Conclusion: Short-term anoxia causes an injury of the small intestinal structure and significantly decreases glucose active transport. Application of glucose at the mucosal side of the intestinal epithelium after anoxia, especially in combination with the hypertonic Ringer solution, makes it possible to maintain the structure of the small intestine and a capacity of glucose active transport at higher levels.

Disabled-1 protein in the intestine

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Introduction: In rodents the small intestinal epithelium is replaced completely every two to three days. Perpetual cell renewal is fuelled by multipotent stem/progenitor cells located near the bottom of the crypts. The new cells either move downward and settle at the base of the crypts as differentiated Paneth cells or migrate upwards along the villus while differentiating into enterocytes, enteroendocrine and goblet cells. At the villus tip they undergo apoptosis or are extruded into the gut lumen. The mechanisms responsible for the fine coordination between all these processes are still largely unknown. We have reported [1] that rat intestinal mucosa expresses reelin and components of its signaling system, such as its receptors VldlR and ApoER2 and its effector protein Dab1 (disabled-1). Since crypt cells, epithelial cells and myofibroblasts express ApoER2, VldlR and Dab1 genes, whereas reelin expression was restricted to myofibroblasts, we suggested that the reelin released by the myofibroblasts might act on the crypts and epithelial cells and regulate the dynamics of the crypt-villus unit.

The aim of this work was to investigate in the rodent intestine: i) the segmental distribution and ontogeny of Dab1 mRNA abundance, ii) whether Dab1 is involved in the epithelium homeostasis and iii) the enterocyte proteins that interact with Dab1.

Methods: Normal and scrambler (natural mutant Dab1-deficient) mice and rats of different ages were killed according to the current national/local ethical guidelines and the small intestine removed. Intact intestine was used for real-time PCR and immunolabeling assays. Protein interaction assays were carried out on enterocyte extracts using glutathione-S-transferase gene fusion system.

The results reveal that the relative abundance of Dab1 mRNA is greater in the small than in the large intestine and that in the former it decreases with age. The lack of Dab1 decreases cell proliferation, migration and apoptosis. The protein interaction assays identified proteins that interact with Dab1 and/or with phosphorylated Dab1. These proteins are involved in cytoskeletal dynamics, cancer, cell proliferation, differentiation, metabolism, immune response, cell signaling and endocytosis, among others.

We conclude that Dab1 mRNA expression is affected by intestinal segment and ontogeny and suggest that Dab1 might be involved in the homeostasis of the crypt-villus unit.

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Barrier and transport characteristics of IPEC-J2, a cell line of piglet small intestinal origin

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Introduction. Intestinal dysfunction is the main reason for mortality in weaned piglets. Some feed additives are known to improve the piglets' state of health potentially due to enhanced intestinal epithelial barrier function. It is therefore desirable to study transport and barrier physiology and effects of agents using suitable epithelial cell lines. One of the few porcine intestinal epithelial cell lines available to date, IPEC-J2, was characterised in this regard.

Aim. We aimed to compare barrier and transport function of polarised IPEC-J2 with jejunal small bowel epithelia from piglets in order to assess suitability of IPEC-J2 as a model of barrier and transport function of porcine jejunal epithelial cells.

Methods. Porcine intestinal epithelial cells from jejunum (IPEC-J2, passages 65-82, Schierack et al., 2006) were grown to confluence under different culture conditions (DMEM/F12, ±PenStrep, ±fetal bovine serum; HA, PC, PCF, PET, and PTFE membrane substrates with 0.4, 0.45, 1.0, and 3.0 µm pore size and ±collagen coating; 4 to 33 days in culture. Subsequently, electrophysiological parameters and permeabilities to macromolecules were studied in Ussing chambers. Cell morphology and differentiation status were assessed by confocal microscopy. Abundance and localisation of tight junction (TJ) proteins typical for small bowel epithelium were determined on RNA and protein level as well as microscopically.

Results. Transepithelial resistance (TER) of IPEC-J2 varied with different culture conditions but was about 10 times larger than that of small bowel jejunum, already taking a jejunal apical surface multiplication by an assumed factor of 10 into consideration. Permeability to macromolecules, cell morphology, expression of the mesenchymal marker vimentin as well as certain TJ proteins (e.g. claudin-1 and -5) also depended on culture conditions. Part of the differences in barrier properties may be explained by the fact that IPEC-J2 are larger than native intestinal cells and thus have less tight junctions per apical membrane area. As a main difference in claudin expression profiles, IPEC-J2 did not express claudin-2, which may add to the observed high TER. Effects on TER as well as on short-circuit current (I_{SC}) caused by addition of various stimulating agents occurred to only a minor extent compared to native piglet epithelium.

Conclusion. The IPEC-J2 cell line differs in aspects of transport and barrier function from native small bowel epithelium of piglets.

Reelin mRNA expression is reduced in human colorectal cancer

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Introduction: Reelin is an extracellular matrix protein that plays a critical role in neuronal migration. The response of the target cells to reelin requires the presence in their membrane of either the very low density lipoprotein receptor (VldlR) or the apolipoprotein E receptor 2 (ApoER2). Reelin binding to these receptors results in phosphorylation of the Disabled-1 (Dab1) adapter protein and in activation of downstream signalling pathway (s). We recently reported that reelin and some components of its signaling system are expressed in the mucosa of the rat small intestine [1] and that reelin deficiency modifies the expression of genes related with intestinal immune response, inflammation and tumour development [2].

The aim of this study was to investigate whether the reelin-Dab1 signalling system is expressed in the human colon and whether the system is modified in colorectal cancer.

Methods: Tissue from colorectal cancer and corresponding normal tissue were obtained from patients who had undergone colon resection at Nuestra Sra de Valme Hospital. The tissues were subjected to a pathological examination to confirm the diagnosis of colorectal cancer and used for real-time PCR and immunohistochemical assays. Informed consent was obtained from all patients.

The results show that the mucosa of human colon expresses reelin, Dab1, VldlR and ApoER2 genes. Immunohistochemical analyses performed on normal colon reveal that only the pericryptal myofibroblasts present a reelin specific staining. The specific signal produced by the anti-Dab1 antibody is detected at the apical side of surface epithelial cells and crypt cells. This signal is also observed at the basement membrane located below the surface epithelium. VldlR and ApoER2 proteins are found in the cytoplasm of surface epithelium and the former is also detected in the cytoplasm of the crypt cells. The mRNA abundance of reelin is markedly decreased in human colorectal cancer, whereas that of Dab1 and of ApoER2 is increased. VldlR mRNA abundance is similar in both type of tissues.

We conclude that human colonic mucosa expresses the reelin-Dab1 signaling system and that this pathway might play a role in colorectal cancer.

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The new murine hepatic 3A cell line responds to stress stimuli by activating an efficient Unfolded Protein Response (UPR).

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Introduction: the effort to establish a differentiated hepatic cell line able to express *in vitro* the most important liver functions (such as the synthesis of specific serum proteins, the metabolism of carbohydrates and lipids, and the modification and excretion of endogenous and exogenous molecules) has long been pursued in several laboratories and different model systems have been described to date. Such *in vitro* systems are essential to test a wide panel of molecules with beneficial (nutrients, bioactive molecules) or detrimental (environmental pollutants, metals, toxins) effects on the organism, and to study the mechanisms that govern their absorption, metabolism, secretion and catabolism in the liver. Primary hepatocytes are still the closest *in vitro* model for the liver. However, they have scarce and often unpredictable availability, limited growth activity and lifespan, and undergo early phenotypic alterations. Conversely, hepatoma cell lines such as HepG2, despite representing a widely used model characterized by indefinite proliferative capacity, lack several important regulatory mechanisms and crucial liver functions.

Aim: in order to have an *in vitro* model that presents the morphological and functional characteristics of hepatocytes, we have characterized a new cell line (called *3A*), isolated from 14,5 dpc embryo of a wild type mouse strain that underwent spontaneous immortalization.

Methods: we have characterized 3A cells morphology by fluorescent localization of F-actin and β-catenin, the expression of specific genes and proteins essential to liver function, the capability to excrete molecules in extracellular spaces resembling functional bile canaliculi, the glycogen storage activity and the ability to control Retinol-Binding Protein 4 (RBP4) secretion in response to retinol deprivation. We have also characterized their response to the exogenous stress stimulus induced by tunicamycin (TM) treatment, through the analysis of the Unfolded Protein Response (UPR) pathway.

Results: 3A cells display several features of hepatocytes. Furthermore, these cells activate the UPR following a typical stressful event, indicating that this model could be further exploited to investigate hepatic reaction to different injuries and toxic insults.

Conclusion: 3A cells can be considered a useful hepatocyte model that preserves several important liver characteristics, particularly suitable for studies related to ER stress response.

Caco-2 transport studies of flavan-3-ol-C-glucosides

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Introduction: Flavan-3-ol-C-glucosides belong to the group of polyphenols which are known for their positive health benefits. Since flavan-3-ol-C-glucosides occur in several plant derived foods like cocoa or rhubarb they are of great interest for the consumers as well as for research. In cocoa the compounds are formed in the alkalization process during processing. The positive health benefits of flavonoids in general are related to the antioxidative effect of these compounds which might lead to anticancerogenic, antiinflammatory and antiarteriosclerotic effects.

Aim: For any possible health impact of these compounds in humans, their bioavailability has to be characterized. Up to now there are only few data about the gastro-intestinal absorption or transport mechanisms of flavan-3-ols. A slight uptake of the flavan-3-ols catechin and epicatechin is described in the Caco-2 model and in animal models. The effect of a C-bound sugar moiety in the case of flavan-3-ol-C-glucosides has not been studied so far. Since the compounds are commercially not available, they have to be synthesized in advance.

Methods: The Caco-2 cell monolayer model is used to investigate permeability and transport of different flavan-3-ol-C-glucosides. The compounds of interest are applied to the chambers (apical or basolateral) and TEER-values (transepithelial electrical resistance values) are measured with the cellZscope® before and during incubation with the compounds. Medium at a pH of 6.5 is used during transport studies and samples are drawn at different time points up to 24 hours. The flavan-3-ol-C-glucosides on apical or basolateral side are analyzed and quantified by HPLC-FLD.

Results and Conclusion: (-)-Catechin-C-glucosides belong to the group of polyphenols. They are used for *in vitro* transport studies with the Caco-2 cell monolayer model. A transport of these compounds is of great interest because of the positive health benefit of polyphenols. The influence of the sugar moiety on the transport is reviewed.

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Inhibition of glucose uptake by tea extracts and polyphenols in vitro.

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Introduction: Tea polyphenols (e.g. catechins) inhibit luminal and brush border carbohydrate-digesting enzymes *in vivo* and *in vitro*, which is predicted to delay intestinal hydrolysis of carbohydrate, and consequently intestinal glucose absorption. In addition, *in vitro* studies have shown polyphenols, such as epicatechin gallate and quercetin, directly inhibit small intestinal glucose absorption by competitively inhibiting the sodium-glucose co-transporter-1 (SGLT-1) and non-competitively inhibiting the facilitative glucose transporter (GLUT2). By inhibiting carbohydrate digestion and absorption in the gut, polyphenols could lower postprandial glycemia, and have clinical utility in the management of diabetes.

Aims: Identify the structural motifs in polyphenols responsible for inhibition of glucose uptake.

Methods: The acute effects of tea extracts and their component polyphenols on intestinal glucose uptake were investigated using the human intestinal cell line, Caco-2.

Results: Green tea extract (GT), oolong tea extract (OT) and black tea extract (BT) inhibited glucose uptake reversibly and noncompetitively. In addition, GT extract demonstrated the greatest glucose-uptake inhibitory activity (IC_{50}:0.323mg/ml), followed by OT (IC_{50}:0.565mg/ml) and BT (IC_{50}: 1.108mg/ml). Catechins are the dominant polyphenols in tea (GT>OT>BT) and exist in either the cis- or trans- conformation. The effect of cis/trans catechins (EC, C, EGC, GC, EGCG, GCG, ECG, and CG) on glucose uptake was subsequently determined. Inhibition of glucose-uptake into Caco2 cells was detected in the following order: CG (IC_{50}=50.97μM) > ECG (IC_{50}=68.62μM) > EGCG (IC_{50}=155.29μM) > GCG (IC_{50}=176.95μM) > C> EC > GC > EGC. Theaflavins, which are present in black tea, also significantly inhibited glucose uptake, whereas the phenolic acids: Gallic acid, Chlorogenic acid and Caffeic acid, had no effect.

Conclusions: These data indicate the structure of catechins influence glucose uptake by Caco-2 cells: esterified catechins are more potent inhibitors than non-esterified catechins, and the trans-catechin, gallocatechin is a more potent inhibitor than the cis-catechin, epigallocatechin

MCT9 (SLC16A9) is a basolateral carnitine efflux pathway in epithelia

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Introduction: In the small intestine and kidney, the (re)absorption of carnitine is known to be mediated by the transporter OCTN2 (SLC22A5; Koepsell & Endou, 2004); however, until recently the basolateral exit pathway in renal proximal tubule has not been identified at the molecular level. A recent combined metabolomic and genome wide association screen identified SLC16A9 (MCT9) as being involved in carnitine metabolism, and as presented at the EITG meeting 2010, MCT9 was demonstrated as a carnitine efflux transporter when expressed in Xenopus oocytes (Suhre et al, 2011).

Aim: To investigate whether MCT9 is located in the basolateral membrane of epithelial tissues and thus can physiologically fulfil a role of an epithelial basolateral efflux pathway for carnitine

Methods: 10nm sections were cut from resin-embedded polarised Caco-2 cell monolayers grown on Transwell filters and rat kidney, and immunostained with anti-MCT9 primary antibody (Santa Cruz) and a 15nm gold-particle conjugated secondary antibody (British Biocell). Samples were viewed on a JOEL1000 electron microscope and the location and density of gold particles assessed.

Results: In the rat kidney, proximal tubule cells showed immunogold staining for MCT9 only at the basolateral membrane, and not in the apical (brush-border) membrane. Similarly, in polarised Caco-2 cells grown on Transwell filters, as a model for small intestine, MCT9 was only found in the basolateral membrane.

Conclusions: The function of MCT9 when expressed in *Xenopus* oocytes and the cellular location in rat kidney is consistent with MCT9 being the basolateral efflux pathway involved in carnitine re-absorption from the renal proximal tubule. Another carnitine transporter, OCTN3, has been reported to be present in the basolateral membrane of rat and chicken enterocytes (Duran et al, 2005) and so the relative contributions of OCTN3 (if present in human enterocytes) and MCT9 are not yet clear.

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