IMPORTANCE OF ABC TRANSPORTERS IN EARTHWORM *EISENIA FETIDA* CAN BE COMPROMISED BY ENVIRONMENTAL INFLUENCES

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In many organisms, the multixenobiotic resistance mechanism (MXR) is mediated by the activity of ABC transporters that bind and actively efflux different toxic substrates from cells. This study attempted to identify MXR-related genes in the earthworm *Eisenia fetida* and compare the impact of the selected modulators, including verapamil, cyclosporine A, MK571, probenecid, orthovanadate, bisphenol A, and snow samples on the earthworm's MXR. To confirm the presence of MXR-reversing agents in *E. fetida*, we measured the accumulation of model substrates rhodamine B and rhodamine 123 in the body tissue of an adult earthworm. Experiments performed using the filter paper contact test showed that all of the tested modulators and environmental samples significantly inhibited transport activity of the model substrates. Furthermore, partial mRNA sequence coding for P-glycoprotein (P-gp/Abcb1) was identified and showed a high amino acid identity (up to 78 %) with homologues from different organisms. We could say that the ABC transporters are involved in various specific mechanisms that help earthworms to survive in a polluted environment and that their efficiency can become compromised by environmental contamination.

KEY WORDS: *bisphenol A, efflux transporters, modulators, multixenobiotic resistance (MXR), P-glycoprotein, snow samples*

DEFENSIVE ROLE OF MXR TRANSPORTERS IN ADRIATIC SEA URCHIN SPECIES

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The multixenobiotic resistance (MXR) mechanism mediated by ATP-binding cassette (ABC) transport proteins is an efficient chemical defence in many aquatic organisms. The primary role of MXR is the protection of the cell (organism) from a wide spectrum of natural and man-made toxic compounds and/or their metabolites, including xenobiotics by providing them with robustness. Several ABC transporters take part in the MXR phenotype: P-glycoprotein (ABCB1; P-gp), MRP (ABCC1-5; multidrug resistance-associated) and BCRP (ABCG2; breast cancer resistance) proteins. MXR transporters are very important for the embryos of aquatic organisms, including sea urchins, which develop in direct contact with water. Although Mediterranean Sea urchins are frequently used as models in ecotoxicology studies, their MXR mechanism has so far been poorly explored. This study presents the first identification of ABC transporter genes involved in the MXR mechanism of two Adriatic sea urchins: black (*Arbacia lixula*) and rocky (*Paracentrotus lividus*). A potential embrotoxicity test was established based on the ABC exporter efflux activity during the first cell division of sea urchin zygotes. Furthermore, the impact of exposure to sub-lethal doses of specific contaminants (inorganic mercury, trybutiltin, bisphenol A) with regard to MXR transporter activity and expression during embryonic development was characterized. Overall, our results represent the first step toward a full characterization and understanding of the ecotoxicological role of the MXR mechanism in these two Mediterranean Sea urchin species.

KEY WORDS: *ABC transporters,* Arbacia lixula*, embrotoxicity test, embryonic development, MXR transporter activity,* Paracentrotus lividus

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THE ROLE OF DRUG TRANSPORTER POLYMORPHISMS IN CLINICAL PRACTICE

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Drug transporters play an important role in carrying substrates across different barriers, thus contributing to drug bioavailability. They can act as uptake or efflux transporters. Most efflux transporters belong to the ATP-binding cassette (ABC) superfamily of membrane proteins, which may influence the intracellular concentration of numerous compounds in a variety of cells and tissues. They are expressed in the apical membrane of many barrier tissues such as the intestine, liver, blood-brain barrier, kidney, and placenta, and contribute to plasma, cerebrospinal fluid, and intracellular drug disposition. Particularly interesting for drug dispositions are ABCB1 (for digoxin, HIV protease inhibitors, some antiepileptics, antidepressants, antipsychotics, immunosuppressants), ABCC2 (anticancer drugs such as methotrexate, cisplatin, irinotecan, antibiotics), and ABCG2 (anticancer drugs, fluvastatin, cimetidin). The variability in their expression and activity caused by genetic polymorphisms may lead to treatment failures and adverse drug reactions. The main uptake carrier systems are organic anion transporting polypeptides (OATP). OATP1B1 (coded by the *SLCO1B1* gene) is a polymorphic influx transporter expressed on the sinusoidal membrane of human hepatocytes, where it mediates the uptake of different endogenous and xenobiotic compounds. SNP c.521T>C in the *SLCO1B1* decreases the transporting activity of OATP1B1, resulting in increased plasma concentrations of drug substrates such as statins. This polymorphism enhances the risk of statin-induced myopathy and even rhabdomyolysis. Some SLCO1B1 variants can also influence the clearance of methotrexate and increase the risk of gastrointestinal toxicity. Other drugs recognized as OATP1B1 substrates include mycophenolic acid, sirolimus, valsartan, enalapril, torsemide, rifampin, cephalosporins, HIV protease inhibitors, methotrexate and irinotecan.

KEY WORDS: *adverse drug reactions, apical membrane, drug bioavailability, genetic polymorphism, multidrug-resistant proteins, organic anion transporters*

MOAT3 (SLC22A8) AND MOAT1 (SLC22A6) EXPRESSION IN MOUSE KIDNEYS; LOCALISATION ALONG THE NEPHRON AND SEX-DIFFERENCES

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In mouse kidneys, various endogenous and exogenous organic anions are eliminated by the organic anion transporters mOat1 (Slc22a6) and mOat3 (Slc22a8), which reside in the basolateral membrane (BLM) of epithelial cells of different nephron segments. The exact localisation of renal mOat1 in the proximal tubule (PT) has been previously verified in *Oat1* knock-out (KO) mice. In contrast, mOat3 has been localised to the BLM of PT, the thick ascending limb of Henle, *macula densa*, distal tubules, and cortical collecting ducts, but its cell localisation has not been properly validated in *Oat3* KO mice. Furthermore, the sex-dependent expression of these transporters has been studied extensively at the mRNA level, while their protein expression has not been examined. Using *Oat3* KO mice, we verified the specificity of anti-mOat3-antibodies and reinvestigated the localisation of mOat3 along the mouse nephron by using a specific antibody. We also analysed the sex-dependent expression of renal mOat3 and mOat1 proteins and defined the hormone(s) responsible for these sex differences using adult wild-type males and females, and castrated males treated with various sex hormones. Cell localisation and relative protein expression of mOat1 and mOat3 was analysed by Western blotting and fluorescence immunocytochemistry. The results indicate that mOat3 is localised exclusively to the BLM of PT, where it colocalises with mOat1, exhibiting sex-dependent expression with an opposite pattern; mOat3 expression is female-dominant due to androgen inhibition, while mOat1 expression is male-dominant due to androgen stimulation.

KEY WORDS: *androgens, gender differences, immunocytochemistry, knock-out mice, membrane transporter, organic anions, proximal tubule, Western blotting*

EXPRESSION OF HEPATIC AND RENAL SULFATE ANION TRANSPORTER SAT-1 (SLC26A1) IN RATS TREATED WITH ETHYLENE GLYCOL

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Oxalate (Ox) urolithiasis is a systemic disorder that affects ~10 % of the adult human population with a male prevalence. It occurs ~2.5 times more often in men than in women. A trigger for urolithiasis is urine oversaturation with a crystal-forming material such as CaOx, where Ox originates from the liver metabolism and/or absorption from food. The Slc26 family of multifunctional anion exchangers, among which Sat-1 (Slc26a1) and chloride/formate exchanger CFEX (Slc26a6) are prime examples, is responsible for Ox trafficking in the organism. The aim of this work was to test the role of hepatic and renal Sat-1 in the generation of Ox urolithiasis in a rat model of this disease, following treatment with ethylene glycol (EG). Three-month-old male (M) and female (F) Wistar rats were divided into control (drank tap water) and EG-treated (drank 0.75 % EG in tap water for one month) groups. Urine and blood serums were sampled and tested for Ox crystals and concentration, while liver and kidney tissue samples were used in morphological, immunocytochemical, Western-blotting, and real-time RT-PCR studies. In EG-treated animals, M but not F exhibited hyperoxalemia, hyperoxaluria and large Ox crystals in their urine. However, the expression of Sat-1 protein was upregulated in both the liver and kidneys of EG treated F but not M. The expression of Sat-1 mRNA remained unchanged in all of the groups, indicating an involvement of a post-transcriptional regulation of protein expression. We conclude that Sat-1 does not contribute significantly to the generation of Ox urolithiasis in rats.

KEY WORDS: *gender differences, immunocytochemistry, kidney, liver, nephrolithiasis, oxalemia, oxaluria, real time RT-PCR, urolitiasis, Western blotting*

THE EFFECTS OF NATRIURETIC PEPTIDES ON THE BRADYKININ SIGNALING PATHWAY AFTER ISCHEMIC MOUSE BRAIN INJURY

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Among the consequences of a stroke are brain edemas, which additionally increase brain damage. This study is focused on cerebral edemas and bradykinin as their direct cause. It is known that natriuretic peptides decrease cerebral edema after ischemic brain injury through mechanisms that are still unknown. We examined the effects of natriuretic peptides and the bradykinin signaling pathway *in vitro* in HEK293 cells, primary isolated neurons, and astrocytes using the whole cell patch clamp technique and by measuring the intracellular calcium concentration. In a mouse model of ischemic brain injury (MCAO), we determined the degree of neurological damage in the mouse brain *in vivo* after applying only bradykinin or a combination of bradykinin with natriuretic peptides. We measured the size of the ischemic lesion and edema by micro CT and performed histological staining by Nissl. In HEK293 cells, ligands of guanylat cycalse A, but not guanylat cyclase B, inhibited the bradykinin signaling pathway (bradykinin receptor type 2-dependent). Our preliminary results showed that the same inhibition exists in primary isolated mouse neurons. When natriuretic peptides were applied, no brain damage was detected. As shown in our previous study, when only bradykinin was applied, brain damage increased in comparison to control animals through an increase in the brain edema. However, the combination with natriuretic peptides decreased the size of the lesion and the brain edema. The results indicate the existence of an endogenous antagonist of the bradykinin signaling pathway and a possible protective role for natriuretic peptides in humans during strokes.

KEY WORDS: *HEK293,* MCAO, *micro CT*, *primary culture of neurons and astrocytes, whole cell patch clamp technique*

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EXPRESSION OF WATER CHANNEL AQP 1 IN RAT KIDNEYS IS REGULATED BY SEX HORMONES

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The physiological function of aquaporin 1 (AQP1) has been thoroughly characterized in the mammalian kidneys, where it is responsible for constitutive water reabsorption. The AQP1 protein is found in the apical and basolateral plasma membranes of the proximal tubules and descending thin limbs. In the membrane, it takes two forms: non-glycosylated (NG, ~28 kDa) and glycosylated (G, 40 kDa to 50 kDa). The factors influencing renal AQP1 expression in (patho)physiological conditions are poorly understood. The aim of our work was to investigate the possible effects of sex hormones on renal AQP1 expression in rats, using immunocytochemistry, Western blotting, and real time RT-PCR. The effects were extensively studied in prepubertal and adult, gonadectomised and sex hormone-treated gonadectomised rats. The AQP1 protein expression in various kidney zones was higher in males than in females and observed sex differences were present in both forms of AQP1 (NG and G) in adult rats. The AQP1 mRNA expression was in concordance with the protein expression. Castration reduced and ovariectomy increased the abundance of AQP1 protein in isolated renal total cell membranes. Furthermore, the treatment of castrated animals with testosterone upregulated, whereas treatment with estradiol and progesterone had no significant effect on AQP1 protein expression. The abundance of AQP1 protein and mRNA in the kidneys of prepubertal rats was similar in both sexes. We conclude that sex differences exist in the expression of AQP1 along the nephron of adult rats; AQP1 is more abundant in males due to upregulating effect of androgens.

KEY WORDS: *aquaporin 1, castration, immunocytochemistry, ovariectomy, real time RT-PCR, sex steroids, Western blotting*

THE ROLE OF SEROTONIN TRANSPORTER SLC6A4 IN BEHAVIORAL DISORDERS

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Serotonin (5-hydroxytryptamine, 5HT) is a biologically active molecule with many physiological functions in mammals, including regulation of brain development and neurotransmission. Alterations in brain 5HT homeostasis have been indicated as biological substrates in several behavioural and psychiatric disorders. The 5HT transporter (5HTt) mediates the reuptake of serotonin into the presynaptic neuron, thus regulating the magnitude and duration of 5HT synaptic action. The *5HTt* gene contains two functional polymorphisms: *5-HTTLPR*, a 22 bp-VNTR in the promoter (16-repeat "long" (L) allele is more efficient in transcription than the 14-repeat "short" (S) variant), and *VNTR-2*, a 17 bp-VNTR in the second intron repeated 9, 10 or 12 times (the 12-repeat allele has stronger enhancer-like properties). In order to examine the possible roles of the *5HTt* gene in the predisposition to alcoholism, schizophrenia, suicidal behaviour, and autism, we performed case-control or family-based association studies. The lack of association of *5HTt* gene polymorphisms with autism suggests that either *5HTt* is not involved in development of autism or that the *5HTt* action mechanism does not involve the examined polymorphisms. On the other hand, the preferential transmission of the *5-HTTLPR* allele S in families with alcoholic probands, the preferential transmission of the *VNTR-2* allele 12 in families with schizophrenic probands, and the significant excess of haplotype L10 among suicide victims in comparison to healthy controls, point to an association of the *5HTt* gene with these three disorders, indicate a possible involvement of 5HTt in respective serotonergic imbalances, and suggest focusing on 5HTt in the use of 5HT-directed therapy.

KEY WORDS: *case-control study,* 5HTt, 5-HTTLPR, *preferential transmission,* VNTR-2

DISTRIBUTION AND SEX DIFFERENCES IN EXPRESSION OF P-GLYCOPROTEIN (P-GP/MDR1/ABCB1) IN RAT LIVER, KIDNEYS, AND GASTROINTESTINAL TRACT

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P-gp is an ATP-dependent transmembrane efflux transporter constitutively expressed in the membrane of hepatocyte bile canaliculi (BC), brush border membrane (BBM) of the renal proximal tubule (PT) and intestinal epithelium, blood-tissue barriers, maternal-fetal barriers, and hematopoietic cells. Sex steroids regulate P-gp expression and function in the rat liver, exhibiting female (F) dominant sex differences. However, the hormone(s) responsible for these differences in the rat liver, and possibly in other P-gp expressing organs, have not been resolved. Here we used the commercial monoclonal antibody (clone C219) to study effects of sex and gonadectomy on the localisation and abundance of P-gp by immunocytochemistry (IC) and Western blotting (WB) in the liver, kidneys, and gastrointestinal tract of adult male (M) and F Wistar rats. We confirmed sex differences (M<F) in the P-gp expression in BC, and found that gonadectomy in M increases this expression. In the kidneys, the antibody stained BBM of the PT S2 segments in the M cortex (CTX) and S3 segments in medullary rays and outer stripe (OS) in both M and F. Gonadectomy in M, but not in F, increased the P-gp expression in OS and decreased in CTX. In the small intestine, the P-gp abundance increased from proximal to distal segments, but possible sex differences or effects of gonadectomy could not be detected due to high variability of the P-gp expression. The data indicate that in rats, androgens inhibit the expression of P-gp in BC and PT S3 segmenst, and stimulate it in the PT S2 segments.

KEY WORDS: *bile canaliculi, castration, immunocytochemistry, ovariectomy, proximal tubule, sex steroids, Western blotting*

IMMUNOCHEMICAL LOCALISATION OF THE CHLORIDE-FORMATE EXCHANGER SLC26A6 (CFEX, PAT-1) IN RAT ORGANS

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In mice, the chloride-formate exchanger (CFEX, Slc26a6), also known as the putative anion transporter-1 (PAT-1), has been localised to the brush-border membrane (BBM) of renal proximal tubule (PT) and small intestine in mice. Its localisation and protein expression in rat organs is unknown. Here, an anti-CFEX polyclonal antibody (CFEX-Ab) was used in Wistar rats to investigate the cell localisation and abundance of CFEX protein in 1) kidneys, liver, gastrointestinal tract, and pancreas of adult intact males, 2) kidneys of adult male and female rats, and 3) kidneys of gonadectomised rats of both sexes. The studies were performed by immunocytochemistry in tissue cryosections and by Western blotting (WB) in isolated total cell membrane. In the kidneys, CFEX-Ab exclusively stained the BBM of PT with heterogeneous intensity (S1~S2>S3). In the liver, stained was the hepatocyte canalicular membrane, while the bile ducts were CFEX-negative. In the gastrointestinal tract, the antibody strongly stained the enterocyte BBM in duodenum and jejunum (duodenum>jejunum); ileum, cecum, and colon remained unstained. In pancreas, CFEX protein was localised to the pancreatic duct apical membrane. A CFEX-related protein band of ~100 kDa was detected in the membranes isolated from the above-mentioned organs, exhibiting zonal differences in the kidneys (cortex<outer stripe) and gastrointestinal tract (duodenum>jejunum). Renal expression of CFEX protein was male-dominant, downregulated by castration, and unaffected by ovariectomy. We conclude that in rats, CFEX protein is expressed in the kidneys, liver, pancreas, and intestine, but only in the kidneys its expression exhibits stimulation by androgens.

KEY WORDS: *enterocytes, hepatocytes, immunocytochemistry, intestine, kidney, membrane transporters, pancreas, proximal tubules, sex differences, Western blotting*

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THE GENETIC DIVERSITY, PROTEIN GLYCOSYLATION, AND FUNCTION OF MEMBRANE TRANSPORTERS

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Nearly all proteins can be modified by a covalent addition of complex oligosaccharides called glycans. Glycan parts are as important for the structure and function of proteins as polypeptide parts, but contrary to polypeptides, glycans are not defined by a firm genetic template. Instead, glycans are products of a complex dynamic interaction between (i) genetic polymorphisms, gene expression, and the regulation of hundreds of genes and (ii) past and present environmental factors. Due to their structural complexity and technological limitations, knowledge on glycans lags significantly behind knowledge on proteins and DNA, but this is changing rapidly. The first population studies on the glycome revealed significant inter-individual variations in protein glycosylation. Genome-wide association studies of the glycome are currently mapping the complex network of genes that regulate protein glycosylation. Large scale studies of the glycome in several major diseases are underway and are starting to reveal the role of protein glycosylation in personalized disease risks and disease courses. The glycosylation of membrane transporters is particularly interesting and there is increasing evidence that altered glycosylation and the consequential malfunction of membrane transporters may be relevant to a number of diseases, including diabetes and cancer.

KEY WORDS: *diabetes, genetics of protein glycosylation, personalized medicine, protein glycosylation*

FUNCTIONAL CHARACTERIZATION OF MATE (SLC47A) TRANSPORTERS IN ZEBRAFISH (*DANIO RERIO*)

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Among the 50 different solute carrier (SLC) protein families, multidrug and toxin extrusion proteins (MATE/SLC47A) are especially important. They are known to excrete cationic endogenous substances and xenobiotics. However, unlike ATP-dependent ABC transporters, MATEs use the proton gradient as the driving force for the substrate efflux. MATE-related genes are widely distributed throughout all domains of life, and mammalian MATE transporters are classified into three phylogenetic subgroups. The goal of our study was to evaluate the presence and function of MATE transporters in zebrafish (*Danio rerio*), a widely used model in biomedical and environmental research. Through phylogenetic analysis, we identified 5 MATE orthologs. Using qRT-PCR, we determined the tissue distribution and sex differences in the mRNA expression of MATE genes. Four out of five genes discovered were cloned and transiently expressed in HEK293 cells. Their membrane localisation was confirmed by confocal microscopy, and their functional characteristics evaluated using the DAPI uptake assay. K_m values for DAPI uptake mediated by zebrafish MATE transporters were 4.65 μmol L^{-1}, 0.71 μmol L^{-1}, 0.34 μmol L^{-1}, and 1.85 μmol L^{-1}, respectively - in the same range as K_m values previously determined for two human MATEs. To further characterize zebrafish MATEs, we determined the inhibition potencies for a number of model compounds. We also identified, cloned, and expressed four functionally active MATE transporters in zebrafish, and using the DAPI uptake assay, obtained K_i values for model substrates and inhibitors. Our current research focus is on a more detailed functional characterization and evaluation of the *in vivo* relevance of zebrafish MATE orthologs.

KEY WORDS: *DAPI assay, MATE proteins, zebrafish*

MEMBRANE TRANSPORTERS OF ORGANIC ANIONS, CATIONS, AND WATER IN EXPERIMENTAL CISPLATIN NEPHROTOXICITY

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Antineoplastic cis-diaminodichloroplatinum (cisplatin, CDDP) is a highly nephrotoxic drug that causes hypoosmotic polyuria and decreased renal secretion of organic anions (OA) and cations (OC). The aim of this work was to investigate the expression of membrane proteins responsible for the transport of water (water channels/aquaporins, AQP1 and AQP2), OA (Oat1 and Oat3), and OC (Oct1 and Oct2) along the nephron in a rat model of cisplatin nephrotoxicity. Adult male Wistar rats were injected with a single dose of cisplatin (5 mg kg^{-1} b.m., i.p.) or 0.9 % NaCl (control), and tested for five consecutive days for urine excretion and standard biochemical parameters in urine. The animals were then sacrificed, the kidneys were sampled, and the platinum tissue content was determined by atomic absorption spectrometry. The expression of various transporters was studied using protein-specific polyclonal antibodies by Western blotting (WB) in isolated membranes and by immunofluorescence cytochemistry in tissue cryosections. In comparison with control animals, the cisplatin-treated rats exhibited hypoosmolar polyuria and a high accumulation of platinum in the kidney tissue. The abundance of AQP1, AQP2, Oat1, Oat3, Oct1, and Oct2 proteins in isolated renal membranes from the cisplatin-treated animals was strongly diminished. These WB findings were fully supported by immunocytochemical data. We conclude that the increased excretion of water (urine) OA and OC in cisplatin nephrotoxicity is caused by the downregulation of respective membrane transporters in epithelial cells along the nephron.

KEY WORDS: *aquaporins, CDDP, immunocytochemistry, organic anion transporters, organic cation transporters, rats, Western blotting*

AGE-RELATED EXPRESSION OF ORGANIC COMPOUND AND WATER TRANSPORTERS IN RAT KIDNEYS

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Ageing is characterized by an impaired functional and structural integrity of mammalian kidneys. A diminished handling of organic compounds, drugs, and water, as observed in the kidneys of older humans and experimental animals, indicates that ageing may affect the expression and/or activity of the renal transporters that mediate excretion and/or reabsorption of organic anions and cations, glucose, and water. We compared the expression of relevant transporters in the kidneys of 3-month-old and 24-month-old male Wistar rats, including Na/K-ATPase, organic anion transporters Oat1 (Slc22a6), Oat2 (Slc22a7), Oat3 (Slc22a8), and Oat5 (Slc22a19), organic cation transporters Oct1 (Slc22a1) and Oct2 (Slc22a2), glucose transporters Sglt1 (Slc5a1) and Sglt2 (Slc5a2), and water channels AQP1 and AQP2. These transporters/channels reside in specific cell membrane domains along the nephron. Protein expression was studied by immunofluorescence cytochemistry (IC) in tissue cryosections and Western blotting (WB) in isolated membranes, whereas expression of mRNA in the tissue was tested by RT-PCR. The protein and mRNA expression of Oat1, Oat3, Oct1, Oct2, and AQP1 was significantly weaker in senescent animals. However, the expression of Na/K-ATPase, Oat2, Oat5, Sglt1, Sglt2, and AQP2 at both protein and/or mRNA levels was not affected by age. Our findings imply that the cellular uptake and excretion of endogenous and/or exogenous compounds in the kidneys can be compromised due to the ageing-related downregulation of certain renal transporters. This can lead to an impaired ability to maintain the homeostasis of specific compounds and an increased susceptibility to drug interaction and toxicity in kidneys and other organs.

KEY WORDS: *aquaporins, immunocytochemistry, membrane transporters, mRNA expression, organic anions, organic cations, senescence, RT-PCR, Western blotting*

THE ROLE OF ORGANIC CATION TRANSPORTERS (OCTS, *SLC22A*) IN ZEBRAFISH *(DANIO RERIO)*

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Polyspecific organic cation transporters (OCTs) of the *SLC22* family play an important role in the distribution and elimination of cationic drugs and toxins in mammals. Our study was aimed at the identification, molecular characterization, and physiological role of zebrafish OCTs. Phylogenetic analysis identified two zebrafish OCT co-orthologs: OCT1/2a and OCT1/2b. qRT-PCR revealed a high expression of OCT1/2b in the testes and a moderate one in kidneys. OCT1/2a showed an extremely high expression in kidneys and a somewhat lower in the liver and testes of male zebrafish. To determine the substrate specificity of zebrafish OCTs, we transiently expressed them in the HEK293 cell line. The OCT1/2a transfectants transported fluorescent cations with high affinity: 4-(4-Dimethylaminostyryl)-N-methylpyridinium (ASP^+; K_m=0.14 μmol L^{-1}), ethidium bromide (0.11 μmol L^{-1}), amiloride (206 μmol L^{-1}), berberine (0.13 μmol L^{-1}) and 4',6-diamidino-2-phenylindole (DAPI; 4.17 μmol L^{-1}). Using ASP^+ as a fluorescent probe, we tested 50 compounds known to interact with human OCT1-3. Among the tested physiological substrates, high affinity was found for hormones androstenedione (K_i=1.6 μM) and progesterone (2.8 μmol L^{-1}), while moderate affinity was found for β-estradiol (29 μM) and testosterone (16 μmol L^{-1}). Among the tested xenobiotics, a high interaction was found in organotines tri-*n*-butyltin-chloride, di-*n*-butyltin-dichloride, and tri-*n*-propyltin-chloride, with K_i values of 3.9 μmol L^{-1}, 3.1 μmol L^{-1}, and 9.5 μmol L^{-1}, respectively. Our results imply a pivotal role for zebrafish OCT1/2a in the uptake of hormones in the testes, their clearance from blood through the kidneys and liver, and in the metabolic response to organotines, harmful marine contaminants whose interaction with OCT1/2a is yet to be fully elucidated.

KEY WORDS: *organic cation transporters, organotines, zebrafish*

IDENTIFICATION AND CHARACTERIZATION OF ZEBRAFISH UPTAKE TRANSPORTERS

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The *SLCO/Slco* (OATP/Oatp, organic anion transporting polypeptides) and *SLC22/Slc22* (OCT, organic cation transporters; OAT, organic anion transporters; OCTN, organic carnitine transporters) transporter families are groups of polyspecific transporters that mediate the transport of endogenous and foreign compounds across eukaryote cell membranes. Despite their importance in ADME (Absorption, Distribution, Metabolism, and Excretion), they have not been sufficiently investigated in non-mammalian organisms. The goal of our study was the molecular characterization of toxicologically relevant uptake transporters using the zebrafish (*Danio rerio*), a well-established vertebrate model in biomedicine and ecotoxicology. Through extensive research on available genome databases and phylogenetic analysis, we identified and annotated 12 and 17 members within the zebrafish *Slco* and *Slc22* family, respectively. Tissue expression profiling using qRT-PCR revealed a high expression of specific uptake transporters in detoxification tissues such as the liver (Oatp1d1, Oatp2b1, Oct1/2a), kidneys (Oatp1f2, Oatp2b1, Oct1/2a, Orctl3/4a, Orctl3/4c, Oat2d, Oat2e, Oat1/3), intestine (Oatp2b1, Oat2b), and gills (Oatp2b1). Our focus then shifted toward the functional characterization of the ubiquitously expressed but liver-predominant Oatp1d1, co-orthologs of human OAT1 and OAT3, Oat1/3 predominantly expressed in the kidneys, Oat1-like mostly expressed in the zebrafish brain, and five zebrafish co-orthologs of the mammalian OAT2/Oat2. Using transiently transfected HEK293 cells, radioactively labelled model substrates [H^3]estrone-3-sulfate and [H^3]p-aminohippurate, and fluorescent substrates Lucifer yellow and 5- and 6- carboxyfluorescein, we conducted a detailed functional characterization of zebrafish anion transporters. Our research offers novel insight into the functional evolution of *SLCO* and *SLC22* superfamilies and elucidates the toxicological role of these transporters in zebrafish.

KEY WORDS: *ecotoxicology, toxicology, uptake transporters, zebrafish*

MOLECULAR CHARACTERIZATION OF ZEBRAFISH OATP1D1 (*SLCO1D1*), A NOVEL ORGANIC ANION TRANSPORTING POLYPEPTIDE

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Organic anion-transporting polypeptides (OATPs) are involved in the uptake of various endogenous and foreign compounds across the plasma membrane. However, their role has not been fully investigated in non-mammalian species. The goal of our study was the molecular characterization of the physiologically and toxicologically-relevant uptake transporter Oatp1d1 in zebrafish (*Danio rerio*). Using phylogenetic analysis, we confirmed that the Oatp1d subfamily is present in teleosts and absent from amphibians and higher vertebrates. Tissue expression profiling using qRT-PCR revealed sex differences: Oatp1d1 has a higher expression in the liver, kidneys, and gonads of male fish. Using transiently transfected HEK293 cells, radioactively-labelled model substrate [H^3] estrone-3-sulfate, and the fluorescent substrate Lucifer Yellow, we performed a detailed functional characterization. We found that Oatp1d1 transports steroid hormones and their conjugates, but also the testosterone precursor dehydroepiandrosterone (DHEAS). Among thyroid hormones, Oatp1d1 transports triiodothyronine (T3), but not thyroxine (T4). Zebrafish Oatp1d1 is involved in the uptake of bilirubin and bile salts, with a preference towards taurine conjugates. Tests on a series of model substrates and inhibitors yielded a partly overlapping substrate range, but also revealed that Oatp1d1 differs from mammalian OATP1 members in terms of substrate affinities and inhibitor potencies. Furthermore, we found that Oatp1d1 transport activity depends on the inwardly directed [H^+] gradient, with a possible involvement of conserved histidine residue in the third transmembrane domain. The described characterization of Oatp1 reveals its role in the maintenance of steroid and thyroid hormones, bile acids, and bilirubin balance, and in the excretion of foreign compounds in fish.

KEY WORDS: *molecular characterization, Organic anion transporting polypeptides (OATPs), Oatp1d2, zebrafish*

BRUSH-BORDER AND BASOLATERAL TRANSPORTERS OF ORGANIC COMPOUNDS IN ACUTE AND SUBCHRONIC MODELS OF EXPERIMENTAL CADMIUM NEPHROTOXICITY IN RATS

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Cadmium nephrotoxicity (Cd-NTX) manifests itself through impaired proximal tubule (PT) reabsorptive and secretory functions. Urine symptoms (phosphaturia, proteinuria, aminoaciduria, glucosuria, increased excretion of inorganic and organic anions and cations, polyuria) indicate that Cd affects various transporters in the PT brush-border (BBM) and basolateral (BLM) membranes. In order to investigate their expression in Cd-NTX, we exploited experimental models of subchronic (treatment with $CdCl_2$ for 14 days) and acute (treatment with Cd-metallothionein 6 h to 12 h before sacrifice) Cd-NTX in rats. Immunocytochemistry, Western blotting, transmission electron microscopy, and end-point RT-PCR were applied to screen transporters located in the PT BBM (NaPi2, V-ATPase, NHE3, Sglt1, Sglt2), BLM (Na/K-ATPase, Oat1, Oat3, Oct1, Oct2), or in both membranes (AQP1). In both models, PT exhibited a loss of BBM and BLM. In the subchronic model, the expression of various transporters was strongly downregulated at both the protein and mRNA level. In the acute model we observed a time-dependent loss of BBM and BLM transporters and their redistribution in intracellular organelles, translocation of certain transporters (NHE3) from the BBM to the BLM, and minimal changes in the expression of mRNAs. The data indicate that functional defects of PT in Cd-NTX result from the loss of 1) absorptive and secretory surface, 2) transporting proteins in BBM and BLM, and 3) cell polarity. In subchronic Cd-NTX, the loss of membrane transporters is mRNA-related, whereas in acute Cd-NTX, their loss may result from the disrupted intracellular vesicle sorting and trafficking.

KEY WORDS: *heavy metal toxicity, immunocytochemistry, kidney, mRNA expression, protein expression, proximal tubule, RT-PCR, transmission electron microscopy, Western blotting*

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IDENTIFICATION OF POTENT P-GLYCOPROTEIN (P-GP, ABCB1) INHIBITORS IN COMPLEX ENVIRONMENTAL SAMPLES USING THE EFFECTS-DIRECTED ANALYSES (EDA) APPROACH

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As an important part of the multixenobiotic resistance (MXR) defence in aquatic organisms, the P-glycoprotein (P-gp, ABCB1) pumps many structurally different xenobiotics out of the cell, reducing their toxicity. However, certain environmental contaminants can lead to the inhibition of P-gp-like proteins, causing an increase in the sensitivity toward toxic compounds through competitive or non-competitive blockage of the P-gp mediated efflux. Here we present the results of a study aimed at the identification of P-gp inhibitors in contaminated sediments using the effect-directed analysis (EDA) approach. The samples were collected from Gorjak creek (Zagreb, Croatia), where a nearby pharmaceutical plant discharges its wastewater effluents. Sediment samples were extracted and fractionated using the two-tiered approach. Fractions were tested for the inhibition of P-gp activity using P-gp overexpressing PLHC-1/dox cells and calcein-AM as a model fluorescent substrate. Our data revealed a high inhibitory potential of the investigated sediments. P-gp specific ATPase assay and cytotoxicity modulation experiments with colchicine indicated that most of the observed P-gp inhibition was due to the presence of non-competitive inhibitors. A detailed chemical analysis by ultrahigh-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QToFMS) revealed nonionic surfactants, including alcohol polyethoxylates (LAEOs) and polypropylene glycols (PPGs), as the major components of the most active sub-fractions. Tests on several LAEO and PPG commercial mixtures confirmed their potential to inhibit fish P-gp. Therefore, our results demonstrate a high potential of the EDA approach and indicate a need for a careful re-assessment of the ecotoxicological relevance of these ubiquitous environmental contaminants.

KEY WORDS: *complex environmental samples, effects-directed analyses (EDA), P-glycoprotein inhibitors*

ECOTOXICOLOGICAL SIGNIFICANCE OF TRANSMEMBRANE TRANSPORT PROTEINS AS INTEGRAL ELEMENTS OF CELLULAR DETOXIFICATION

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Contrary to traditional thought that most lipophilic xenobiotics enter cells by passive diffusion, it has recently become evident that uptake transport proteins from the SLC (Solute Carrier Class) superfamily play an essential role in mediating the entrance of a large number of endo- and xenobiotics into cells. Once inside the cell, however, the activity of ABC (ATP Binding Cassette) transport proteins appears to be critical for their efflux out of the cell, contributing to one of the most intriguing evolutionary defence strategies in aquatic organisms – the multixenobiotic resistance (MXR). As in mammalian tissues, the complex network of uptake and efflux transporters present in barrier and/or detoxifying tissues of aquatic animals transports a diverse group of substances and/or their metabolites in or out of the cell, enabling the proper functioning of cellular physiology and detoxification machinery in response to environmental stress. The main disadvantage of ABC transporters is their sensitivity to the presence of specific compounds, termed chemosensitizers or MXR inhibitors. If ABC transporter function is inhibited, the consequence is an increase in intracellular accumulation and toxicity of xenobiotics which are normally effluxed by corresponding transporters. Nevertheless, despite their physiological importance and role in cellular detoxification, knowledge about uptake and efflux transporters in non-mammalian species is modest, and the topic is still considered relatively exotic in the environmental context. Therefore, we will summarize the actual level of knowledge in this area, highlight critical research drawbacks, and discuss the potential environmental relevance of this research.

KEY WORDS: *ABC transporters, ecotoxicology, MultiXenobiotic Resistance, MXR inhibitors, uptake transporters*

EXPRESSION OF SODIUM-D-GLUCOSE COTRANSPORTER SGLT1 (SLC5A1) IN MURINE ORGANS

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Previous studies have demonstrated a high affinity/low capacity sodium-D-glucose cotransporter Sglt1 (Slc5a1) in the brush-border membrane (BBM) of small intestine absorptive cells and proximal tubule (PT) epithelial cells, where Sglt1 mediates glucose (re)absorption. However, the detailed localisation of mouse Sglt1 (mSglt1) along the nephron, and its cellular distribution in other organs is unknown. Here, we compared wild-type (*Sglt1*$^{+/+}$) and *Sglt1* knock-out (*Sglt1*$^{-/-}$) mice in order to describe the cell localisation of mSglt1 protein and mRNA expression in various organs. Localisation of mSglt1 protein was analysed by immunocytochemistry (IC) and Western blotting (WB) using the anti-mSglt1 antibody whose specificity was previously confirmed in *Sglt1* knock-out mice. The expression level of *mSglt1* mRNA was analysed by RT-PCR. mSglt1 protein was localised in the BBM of PT, where it exhibited segmental (S2>S3) and zonal (cortex>outer stripe) differences, in the apical domains of thick ascending limb of Henle, liver bile ducts, and pancreatic ducts. This staining was not observed in the respective organs of *Sglt1* knock-out mice. RT-PCR data were comparable to IC data. By WB, a single mSglt1-related protein band of ~75 kDa was detected in the kidney BBM. By IC and RT-PCR, mSglt1 expression was not detected in the brain, spleen and skeletal muscle. This study provides new insight into mSglt1 expression in various mouse organs, thus enabling a better understanding of the role of mSglt1 in future physiological, pathophysiological, pharmacological, and toxicological studies.

KEY WORDS: *bile duct, immunocytochemistry, intestine, kidney, knock-out mice, liver, membrane transporter, mRNA expression, pancreas, proximal tubules, RT-PCR, Western blotting*