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MINIREVIEW

Toward a Systems Level Understanding of Organic Anion and Other Multispecific Drug Transporters: A Remote Sensing and Signaling Hypothesis

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ABSTRACT

Organic anion transporters (Oats) are located in the barrier epithelia of diverse organs, where they mediate the absorption and excretion of a wide range of metabolites, signaling molecules, and xenobiotics. Although their interactions with a broad group of substrates have been extensively studied and described, the primary physiological role of Oats remains elusive. The presence of overlapping substrate specificities among the different Oat isoforms, together with recent metabolomic data from the Oat1, Oat3, and renal-specific transporter (RST/URAT1) knockout mice, suggests a possible role in remote signaling wherein substrates excreted through one Oat isoform in one organ are taken up by another Oat isoform located in a different organ, thereby mediating communication between different organ systems, or even between different organisms. Here we further develop this "remote sensing and signaling hypothesis" and suggest how the regulation of SLC22 subfamily members (including those of the organic cation, organic carnitine, and unknown substrate transporter subfamilies) can be better understood by considering the organism's broader need to communicate between epithelial and other tissues by simultaneous regulation of transport of metabolites, signaling molecules, drugs, and toxins. This systems biology perspective of remote signaling (sensing) could help reconcile an enormous array of tissue-specific data for various SLC22 family genes and, possibly, other multispecific transporters, such as those of the organic anion transporting polypeptide (OATP, SLC21) and multidrug resistance-associated protein (MRP) families.

The transport of xenobiotics and endogenous metabolites across the epithelia of organs is mediated by a complex array of membrane transport systems, one of which is the organic anion transport (Oat) system. Members of the Oat family, which is characterized by a high structural and sequence homology, handle a diverse array of drugs that include loop and thiazide diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting enzyme inhibitors, β-lactam and sulfonamide antibiotics, and antiviral agents (Burckhardt and Burckhardt, 2003). When Oat1, the prototypical member of the Oat family was originally cloned and identified as novel kidney transporter (Lopez-Nieto et al., 1997), it was proposed that it, along with Oct1, was part of a larger subfamily of solute carrier (SLC) transporters, later designated SLC22 transporters. The SLC22 transporters have come to include the Oats, Octs, organic carnitine transporters, unknown substrate transporters, and urate transporters (Sweet et al., 2001; Eraly and Nigam, 2002; Eraly et al., 2004b; Wu et al., 2009).

Members of the Oat family continue to be identified and characterized, and include Oat2 [originally cloned as novel liver transporter in rat (Simonson et al., 1994)], Oat3 [origi-

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ABBREVIATIONS: Oat, organic anion transporter; NSAID, nonsteroidal anti-inflammatory drug; SLC, solute carrier; Oct, organic cation transporter; PAH, *para*-aminohippurate; TMD, transmembrane domain; PKC, protein kinase C; IS, indoxyl sulfate; HNF, hepatocyte nuclear factor; HEK, human embryonic kidney; PDZ, postsynaptic density 95/disc-large/zona occludens; Urat, uric acid transporter.

nally cloned as ROCT (Brady et al., 1999; Kusuhara et al., 1999)], Oat4 (Cha et al., 2000), renal-specific transporter (RST)/URAT1 (Mori et al., 1997; Enomoto et al., 2002), Oat5 (Youngblood and Sweet, 2004), Oat6 (Monte et al., 2004), Oat7 (Shin et al., 2007), and Oat10 [originally identified as hORCTL3 (Bahn et al., 2008)]. Oats function mostly as exchangers, coupling entry of an organic anion to exit of another organic anion. Oat1 and Oat3, localized to the basolateral membrane of the renal proximal tubule (Kojima et al., 2002; Motohashi et al., 2002), couple organic anion entry into the cell with dicarboxylate exit (Sweet et al., 1997). This transport system is driven by concentration gradients produced by the Na^+/K^+-ATPase and the sodium-dicarboxylate cotransporter (NaDC3), which maintains high intracellular concentrations of dicarboxylates, especially α-ketoglutarate. Despite extensive characterization of the interaction between the Oats and a wide range of substrates, the primary physiological role of the Oats remains unclear. Oat1 knock-out mice were observed to manifest no apparent morphological or physiological abnormalities although reduced renal excretion of the prototypical substrate *para*-aminohippurate (PAH) and altered levels of endogenous organic anions such as benzoate, 3-hydroxybutyrate, 4-hydroxyphenyllactate, and *N*-acetylaspartate were observed in the plasma and/or urine (Eraly et al., 2006). Oat3 and RST knockout mice have also demonstrated alterations in levels of endogenous metabolites (Sweet et al., 2002; Eraly et al., 2008).

One possible important physiological role for the Oats is suggested by the overlapping substrate specificities between the different Oat isoforms and their localization in diverse organ systems. Oats and other multispecific transporters of the SLC22 subfamily (and possibly other SLC subfamilies as well as those of the multidrug resistance-associated protein, MRP, transporter family) may be involved in the transport of molecules between different tissue or fluid compartments, thereby participating in a "remote signaling" network between organ systems. This broader framework that considers diverse SLC22-expressing tissues and corresponding fluid compartments together with the demands placed on the whole organism can render the more fruitful interpretation of emerging data on tissue-specific expression and complex regulation of transporter expression and function during pre- and postnatal development and under conditions of stress and injury. Here, we explore the structure and tissue distribution of Oats, their potential role in chemosensation between organs and organisms, their function in maintaining whole body homeostasis, and their regulation by various factors.

Structure and Tissue Distribution of Oats

The Oats are generally 535 to 568 amino acids long and are considered to possess 12 transmembrane domains (TMD); the amino and carboxyl termini are located intracellularly (Sweet and Pritchard, 1999; Burckhardt and Wolff, 2000; Eraly et al., 2004a). A long hydrophilic extracellular loop is present between the first and second TMD and contains several *N*-linked glycosylation sites. Studies with hOat1 and hOat4 have shown that glycosylation is required for transporter trafficking to the plasma membrane (Tanaka et al., 2004; Zhou et al., 2005). A second long intracellular loop, present between the sixth and seventh TMDs, contains potential protein kinase C phosphorylation sites (Lopez-Nieto et al., 1997). Protein kinase C (PKC) activation has been reported to inhibit Oat activity (You, 2002; Terlouw et al., 2003) through a mechanism other than direct phosphorylation; PKC activation resulted in decreased mOat1 transport with no evidence of direct phosphorylation of the transporter (You et al., 2000), whereas mutation of canonical PKC consensus sites did not affect down-regulation of hOat1 transport by PKC activation (Wolff et al., 2003). In the case of hOat4, PKC activation resulted in the redistribution of the transporter from the cell surface to intracellular compartments (Zhou et al., 2007). Recent evidence also suggests that PKC inhibits Oat1 activity by regulating Oat1 internalization into recycling endosomes and that this internalization occurs partly through a dynamin- and clathrin-dependent pathway (Zhang et al., 2008).

Several conserved amino acids also seem to be crucial for transport function. Site-directed mutagenesis of histidine in TMD 1, lysine in TMD 8, and arginine in TMD 11 resulted in decreased transport of PAH in rat Oat3 and flounder renal Oat (Feng et al., 2001; Wolff et al., 2001). Leucine in TMD1 for hOat1 (Hong et al., 2004) and glycine in TMDs 5 and 8 of hOat4 (Zhou et al., 2004) have been implicated in directing of the transporters to the plasma membrane. Moreover, several amino acid residues in TMD7 have been shown to be essential for hOat1 transport activity and transporter stability (Hong et al., 2007).

The Oats are widely distributed in the epithelia of various organs (Table 1) (Anzai et al., 2006; Rizwan and Burckhardt, 2007; Zhou and You, 2007). Oat1 has been immunolocalized to the basolateral membrane of the proximal tubule in the human (Hosoyamada et al., 1999; Motohashi et al., 2002), rat (Tojo et al., 1999; Kojima et al., 2002) and mouse (Bahn et al., 2005) kidneys. Moreover, human Oat1 has also been identified in the brain and placenta (for review, see Ahn and Bhatnagar, 2008). Oats 2, 5, and 7 are expressed mainly in the liver (Simonson et al., 1994; Sun et al., 2001; Shin et al., 2007). Oat2 has also been localized to the basolateral membrane of human renal proximal tubule cells and to the apical membrane of the cortical collecting duct and the thick ascending limb in rats (Sweet, 2005). Oat3 is found in the basolateral membrane of the human, rat, and mouse proximal tubule and is distributed in the thick ascending limb of Henle's loop, distal convoluted tubule, and collecting duct in rats (Rizwan and Burckhardt, 2007). It is also expressed in the brain, specifically the choroid plexus, where it probably mediates the transport of drugs and metabolites from the cerebrospinal fluid to the blood (Sweet et al., 2002). Oat4 is found in both the placenta and the apical membrane of the proximal tubule (Cha et al., 2000). Oat5 has been localized to the apical membrane of rat proximal tubule cells (Sweet, 2005). The distribution of the Oats in diverse organs and the presence of the same Oat isoform in different tissues suggest that the Oats, by transporting signaling molecules and metabolites, may participate in a broader communication network between organs.

Oat1 and Oat6—A Potential Role in Olfactory Sensing?

The Oats are predominantly expressed in the kidney, choroid plexus, and liver (Eraly et al., 2004b; Robertson and Rankin, 2006). However, Oat6 (slc22a20) was found to be

TABLE 1
Gene loci and organ distribution of organic anion transporters (OATs)

Oat Isoform	Gene	Gene Locus	Amino Acid Length	Endogenous Substrates	Tissue and [Membrane] Localization	Other Identified Homologs
hOAT1	SLC22A6	11q13.1–13.2	506–563	α-Ketoglutarate, urate, PGE_2, $PGF_{2\alpha}$, folate, nicotinate, xanthine, hypoxanthine, neurotransmitter metabolites (5-methoxyindole-3-acetate, homovanillate, vanilmandelate, 3,4-dihydroxyphenyl acetate, 5-hydroxyindole-3-acetate, *N*-acetyl-5-hydroxytryptamine, melatonin)	Kidney [BL] Brain [U] Placenta [U]	Rat, mouse, flounder, pig, rabbit, monkey, *Caenorhabditis elegans*
hOAT2	SLC22A7	6p21.2–21.1	546 and 548	cAMP, propionate, DHEAS, ES, PGE_2, $PGF_{2\alpha}$, l-ascorbate, α-ketoglutarate	Kidney [BL] Liver [SM]	Mouse, rat
hOAT3	SLC22A8	11q11.7	542	α-Ketoglutarate, cAMP, cortisol, PGE_2, $PGF_{2\alpha}$, DHEAS, ES, taurocholate, urate, estradiol-17β-glucuronide	Kidney [BL] Brain [LM] Adrenals [U] Skeletal muscle [U] Developing bone [U]	Rat, mouse, pig, rabbit, monkey
hOAT4	SLC22A11	11q13.1	550	ES, DHEAS, estrone, 17β-estradiol-3-sulfate, PGE_2, $PGF_{2\alpha}$, octanoate, succinate, cholate, urate, taurocholate	Kidney [LM] Adrenals [U] Placenta [BL]	
mOat5	slc22a19	19	551	ES, DHEAS	Kidney [LM]	Rat
mOat6	slc22a20	19	556	ES, propionate, methylbutyrate, benzoate, heptanoate, 2-ethylhexanoate, pyruvate, PGE_2	Olfactory mucosa [U] Testis [U]	Human, rat
hOAT7	SLC22A9	11q13.1	553	ES, β-estradiol sulfate, DHEAS, butyrate, propionate, valerate, caproate, lactate, nicotinate, acetate	Liver [SM]	
hOAT10	SLC22A13	3p21.3	551	Nicotinate, urate, succinate, L-lactate, glutarate	Kidney [LM] Brain [U] Heart [U] Colon [U]	
hURAT1	SLC22A12	11q13.1	332 and 553	Urate, acetoacetate, succinate, β-hydroxybutyrate, lactate, nicotinate, α-ketoglutarate	Kidney [LM]	Mouse

hOAT, human organic anion transporter; mOat, mouse organic anion transporter; PGE_2, prostaglandin E_2; PGF2α, prostaglandin F2α, DHEAS, dehydroepiandrosterone sulfate; ES, estrone-3-sulfate; BL, basolateral membrane; U, undetermined; SM, sinusoidal membrane; LM, luminal membrane.

primarily expressed in the olfactory epithelium in mice, and to a lesser degree in testis and early embryonic tissues (Monte et al., 2004). It shares 40 and 60% sequence homology with Oat1 and Oat3, respectively, and contains the signature sequence motifs that are found in the slc22 family (Monte et al., 2004). Through in situ hybridization, it was observed that Oat6 was expressed in the olfactory epithelium but not in the neurons of the main olfactory epithelium or the vomeronasal organ (Kaler et al., 2006). Oat6 was found to possess a significant affinity for small volatile compounds that have been previously identified as odortype molecules in mouse urine (Singer et al., 1997; Willse et al., 2005). These odortype molecules have also been found at increased levels in the plasma of Oat1 knockout mice, suggesting that odortype substances excreted through an Oat1-mediated mechanism in the kidney may be taken up by the olfactory mucosa though an Oat6-mediated mechanism (Fig. 1). The possibility that a path like this may constitute part of a signaling mechanism between organisms is supported by the observation that mammals use odor cues in urine for identity recognition (Sherborne et al., 2007; Bates et al., 2008). Furthermore, Oat6 could conceivably modulate signaling from the olfactory epithelium to distant organs such as the brain, where other members of the SLC22 family, such as Oat1, Oat3, OCTN2. and RST are expressed (Kido et al., 2001; Imaoka et al., 2004; Kusuhara and Sugiyama, 2005). Although it remains unclear whether humans possess the Oat6 homolog, this alternate transport pathway that circumvents the blood-brain barrier raises the possibility of designing intranasally administered drugs targeted toward specific Oats and other SLC22 or multispecific transporters expressed in the olfactory mucosa and the brain.

Oat3 as a Potential Blood Pressure Regulator

Oat1 knockout mice, despite showing accumulation of several endogenous organic anions in the plasma as well as diminished responsiveness to diuretics under basal conditions (Vallon et al., 2008b), do not demonstrate apparent physiological abnormalities (Eraly et al., 2006). On the other hand, Oat3 knockout mice manifest a 10 to 15% lower blood pressure than wild-type mice, suggesting possible involvement of Oat3 in blood pressure regulation (Vallon et al., 2008a). Metabolomic analyses in Oat3 knockout mice revealed increased plasma levels of potential Oat3 substrates that may serve as endogenous blood pressure regulators. These compounds included thymidine, which when administered to mice in vivo resulted in a 10 to 15% reduction in blood pressure and was also found to be transported by Oat3 in vitro. Other known inhibitors of Oat3 (i.e., eosin-Y and probenecid) also resulted in reduction of blood pressure when administered to mice. Additional proposed modulators of blood pressure that are

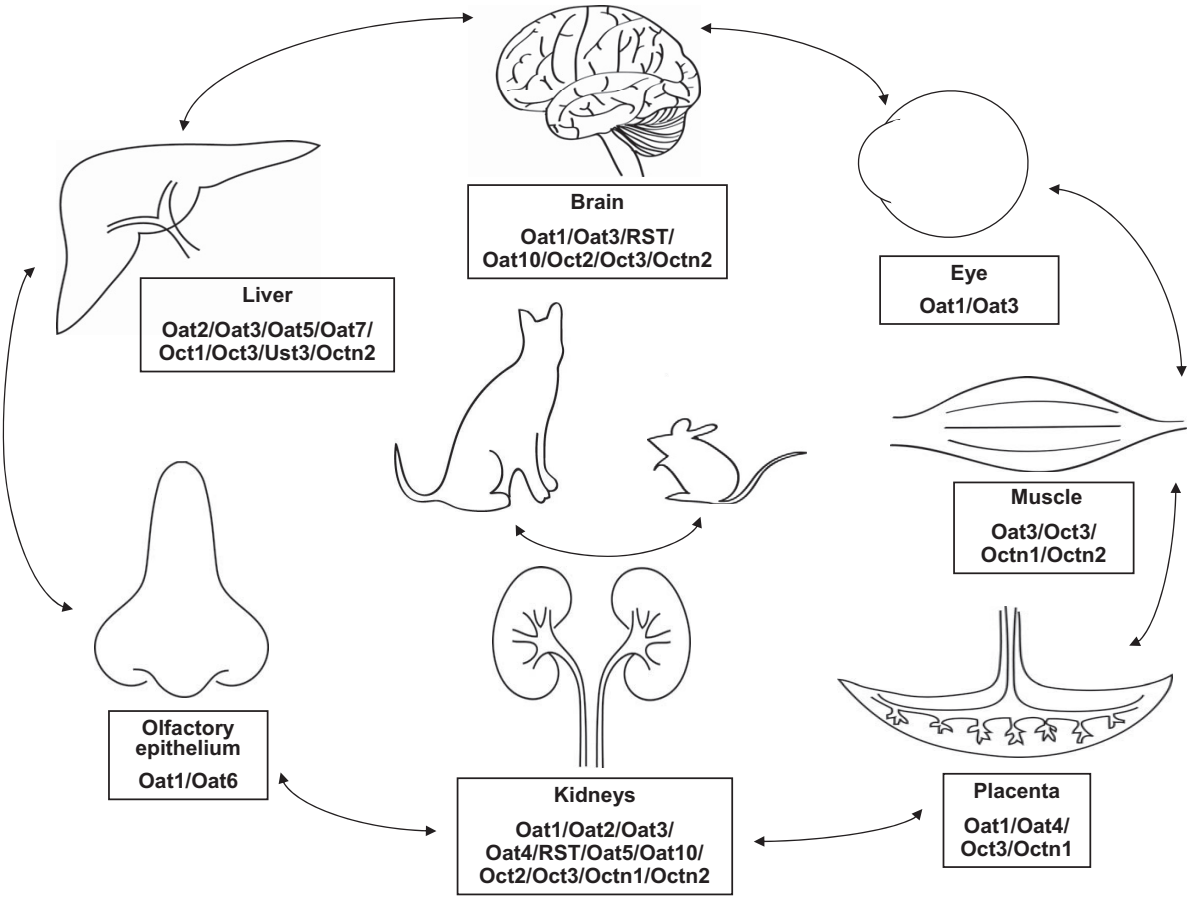

Fig. 1. Chemosensation between organs/organisms mediated by SLC22 family members. Organic anion transporters (Oats), organic cation transporters (Octs), organic carnitine transporters (Octns), and unknown substrate transporters (Usts) located in the barrier epithelia of certain organs may take up substrates excreted by transporters in other organs (arrows), thus mediating potential remote signaling and therefore communication between organs. This method of communication via the SLC22 transporters can also occur between organisms; for example, odor-type molecules excreted through Oat1 in the urine of cat may be taken up by the Oat6 in the olfactory mucosa of mouse.

Oat3 substrates include cGMP and cAMP (Eraly, 2008), cyclic nucleotides that cause vasodilation.

An intriguing finding is the expression of Oat3 and Urat1 in the vascular smooth muscle cell (Hediger et al., 2005; Yamamoto et al., 2006). Oat3 and Urat1 transport uric acid, which has long been linked to hypertension (Hediger et al., 2005; Feig et al., 2008). Although the exact role of Oat3, and possibly Urat1, in blood pressure regulation remains unknown, a potential mechanism may involve regulation of their transport of endogenous blood pressure modulators. A remote sensing circuit may be present wherein changes in blood pressure sensed by the kidney are transmitted into changes in Oat3 expression or function (through regulatory mechanisms such as phosphorylation or glycosylation.) Changes in Oat3 expression or function, in turn, would result in altered excretion of blood pressure modulators. For example, elevated blood pressure sensed by the kidney could lead to decreased Oat3 expression or function and therefore reduced renal excretion of vasodilators. The vasodilator would accumulate in the body and subsequently be taken up by Oat3 expressed in the vascular smooth muscle, resulting in lowered blood pressure. This potential involvement of Oat3 in blood pressure regulation supports a central role for the Oats in responding to changes in the internal and external environment and thereby maintaining whole body homeostasis.

Role of Oats in Homeostasis—The Effect of Toxins, Ischemia and Substrate Interactions

Organisms are constantly exposed to environmental toxins and stressors that perturb whole-body homeostasis and can ultimately lead to tissue injury or death; therefore, the ability to eliminate these toxins and counteract the stressors is vital for the organism's survival. The Oats play an important role in this process by mediating the clearance of a vast array of toxins that include drugs (e.g., methotrexate, NSAIDs, antiviral agents, cephalosporins), environmental toxins (e.g., mycotoxins, mercuric conjugates) and some products of endogenous metabolism (e.g., uremic toxins such as indoxyl sulfate) (Sweet, 2005). Impaired elimination of these toxins, subsequent accumulation in the body and thus perturbation of homeostasis can conceivably be a result of either reduced Oat function or competitive inhibition of binding to the Oats by other substrates (drugs, environmental toxins, or metabolites) (Fig. 2). Furthermore, a broader consideration of the action of exogenous toxic substrates in the context of a remote sensing system involving communication between cells and tissues via small endogenous molecules may lead to the development of a new view on the effects of drugs and toxins that are Oat substrates on whole-organism physiology as well as cell and tissue-specific toxicities.

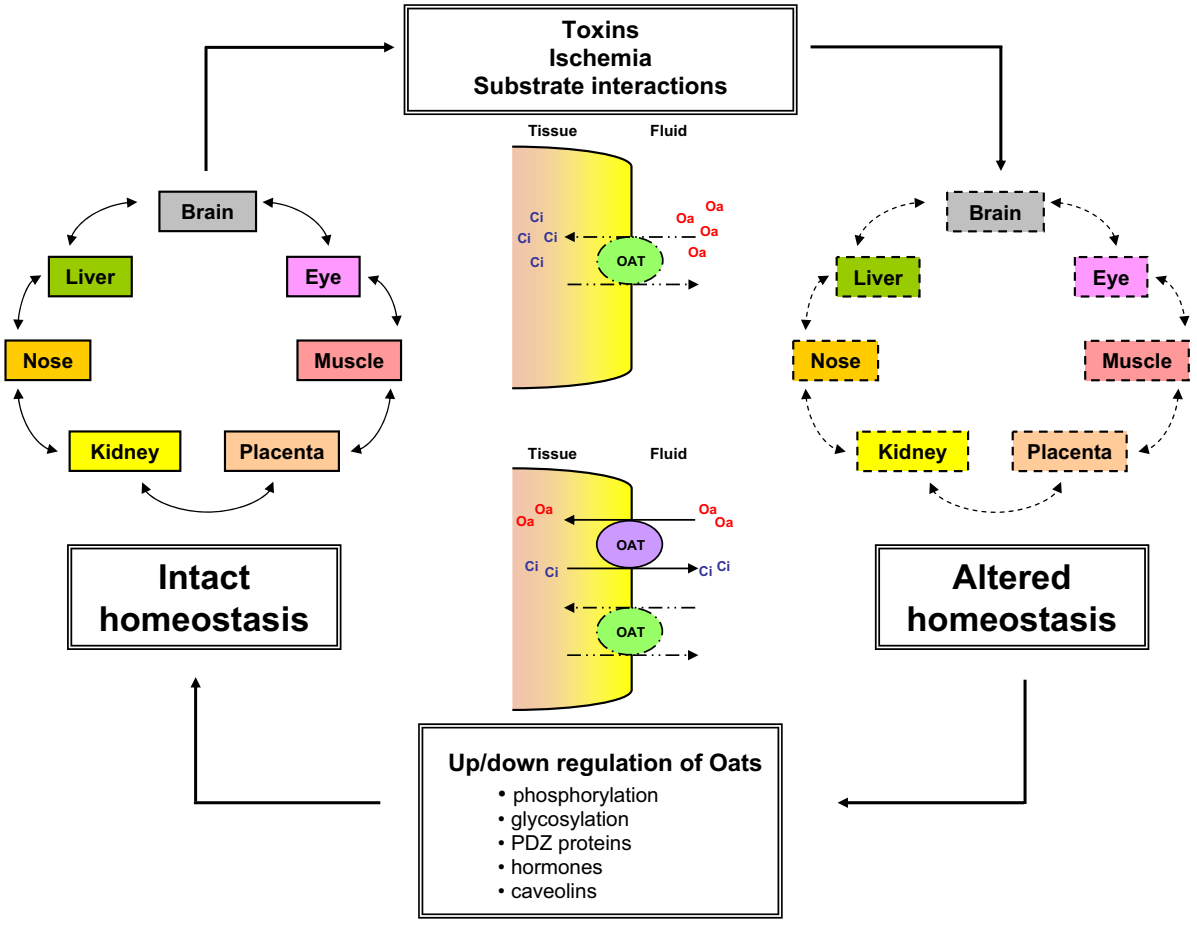

Fig. 2. Potential role of organic anion transporters in homeostasis. By eliminating both endogenous and exogenous toxins, the Oats play a potentially critical role in maintaining whole-body homeostasis. Factors that impair the clearance of substrates by the Oats, such as toxins, ischemia, or competitive inhibition by other substrates, can disrupt Oat function and therefore the efficient exchange of organic anions (Oa) and their counter-ions (Ci) across the membrane. This can eventually lead to perturbed homeostasis. To compensate for the loss of function of other Oats and thereby restore homeostasis, enhanced expression and/or function of the Oats can occur on the transcriptional, translational or post-translational level in either the injured tissues or in other tissues participating in a remote-sensing network.

Endogenous and Exogenous Toxins

Decreased Oat function can result from the cytotoxicity conferred by the same toxins that they transport; significant tissue injury can result from these toxins because they are taken up by the Oats and accumulate within the cell. NSAIDs such as acetylsalicylate, salicylate, and ibuprofen, substrates of Oat1 and Oat3, have been associated with renal papillary necrosis (Muhalwas et al., 1981; Shah et al., 1981). Methotrexate, transported by hOAT2 (expressed in liver) and hOAT3 (expressed in kidney), has been linked to both hepatic and renal toxicity (Dubin and Harrell, 1969; Frei et al., 1975). Antiviral agents, such as adefovir and cidofovir, which are transported by hOAT1, induce renal proximal tubular injury that can be prevented by coadministration of NSAIDs (Cihlar et al., 1999; Mulato et al., 2000). Products of endogenous metabolism can also cause cytotoxicity and reduced Oat function. Indoxyl sulfate (IS), a product of dietary protein metabolism that interacts with Oat1, Oat3, and Oat4, accumulates within the proximal tubule cell during renal failure and triggers free radical production and eventually nephrotoxicity (Enomoto and Niwa, 2007). Furthermore, it has been shown that IS may also exert toxic effects on bone metabolism through its uptake by Oat3 expressed in bone osteoblasts (Nii-Kono et al., 2007). Accumulation of IS in bone osteoblasts has been associated with free radical generation and reduced levels of parathyroid hormone receptor expression (Nii-Kono et al., 2007).

Ischemia/Reperfusion Injury

Ischemia is another important cause of tissue injury and hence altered Oat function. PAH clearance has been reported to be decreased in recipients of cadaveric renal allografts 3 to 7 days after transplant; immunohistochemical analyses of these renal allografts have shown altered distribution of hOat1 from the basolateral membrane of proximal tubule cells to the cytoplasm (Kwon et al., 2007). In rat kidneys subjected to ischemia/reperfusion injury, reduced clearance of PAH was also observed, together with a decreased expression of both Oat1 and Oat3 protein and mRNA levels (Matsuzaki et al., 2007; Schneider et al., 2007; Di Giusto et al., 2008). It is interesting to consider the possibility that Oats or other multispecific transporters (e.g., OATPs, multidrug resistance-associated proteins) in other epithelial tissues may, through a remote signaling network, compensate in an effort to preserve organismal homeostasis until the injured tissue recovers.

Substrate Interactions

Competition for binding and transport to the Oats has been demonstrated by numerous drug-drug interactions, among the more frequently reported of which is the inhibition by probenecid, a well known substrate of Oat1 and Oat3, of the renal excretion of several drugs including methotrexate, cidofovir, and NSAIDs (Aherne et al., 1978; Schild and Roch-Ramel, 1988; Cundy et al., 1996). This competitive inhibition has been used to advantage in some clinical settings, such as coadministration with probenecid to reduce the nephrotoxic effects and prolong the serum half-life of β-lactam antibiotics or antiviral agents (e.g., adefovir and cidofovir).

Competitive binding to the Oats extends beyond xenobiotics to include endogenous substrates. In patients with renal failure, the accumulation of uremic toxins may inhibit the clearance of neurotransmitter metabolites from the brain by Oat1 and Oat3, leading to neurologic impairment (Sweet, 2005). In fact, homovanillic acid, a substrate of Oat1 and Oat3, has been detected at elevated levels in the cerebrospinal fluid of patients with uremic encephalopathy (Moe and Sprague, 1994; Alebouyeh et al., 2003). This interaction between substrates and Oats expressed in different organs is made more complex by the presence of overlapping substrate specificity among the Oats. Although substrate discrimination is present among the Oat isotypes as manifested by the presence of important differences in the structural binding determinants among Oat1, Oat3, and Oat6 (Kaler et al., 2007; Truong et al., 2008), a significant substrate overlap can still be appreciated among the different Oats. Furthermore, overlapping substrate specificity with other transporters such as the Octs (Ullrich et al., 1993) is also present. Thus, not only organic anions but also organic cations (especially in disease states leading to altered pH of body fluids) may compete for binding to the Oats, adding to the complexity of the interaction between Oat systems expressed in multiple organs and a vast array of both endogenous and exogenous substrates.

Reacting to perturbations in homeostasis brought about by the factors mentioned above and bringing the whole body system back to balance may be another important role played by the Oats. This restoration of homeostasis can potentially occur through up-regulation of the Oats on the transcriptional or translational level to compensate for decreased Oat function in the same cell, related cells, or distant cells participating in a remote signaling network. For instance, up-regulation of Oats in the intact proximal tubule cells may be one mechanism whereby the organism compensates for the loss of Oats in damaged proximal tubule cells. Indeed, it has been observed that proximal tubule cells exposed to ischemic insult initially show decreased expression of Oat1 and Oat3, but later on demonstrate up-regulated Oat1 and Oat3 expression accompanied by recovery of PAH clearance (Schneider et al., 2007). Furthermore, rats that had sustained liver injury by bile duct ligation demonstrated increased expression of Oat1 in the kidney cortex together with a higher urinary excretion of PAH, suggesting the presence of a compensation mechanism for the impaired hepatic route of substrate elimination (Brandoni et al., 2003).

Other Physiological Roles

Oat2 was initially cloned as novel liver-specific transporter from rat (Simonson et al., 1994) and since then has been cloned from human and mouse and functionally characterized (Sun et al., 2001; Kobayashi et al., 2002b). Oat2 substrates include prostaglandin E_2, PAH, tetracycline, and salicylate (Anzai et al., 2006). Propionate, a 3-carbon short-chain fatty acid, was also found recently to be transported by Oat2 (Islam et al., 2008). Propionate is eventually converted to succinyl-CoA, an intermediate of the tricarboxylic acid cycle, and also serves as a ligand for GPR41 (Brown et al., 2003), a G-protein-coupled receptor that stimulates leptin release (Xiong et al., 2004). Therefore, through modulation of propionate transport, Oat2 may regulate cellular metabolism in different organs. A role in remote signaling for Oat2 is also

supported by the observation that it avidly transports cGMP, the second messenger that modulates intracellular responses to external stimuli in diverse cell types (Cropp et al., 2008).

The association of serum uric acid levels with multiple disorders, including hypertension, the metabolic syndrome, coronary artery disease, cerebrovascular disease, and kidney disease (for review, Feig et al., 2008) has placed urate metabolism and transport at the center of much interest. Urate excretion through the kidneys seems to be mediated in part by the Oats. Oat1 and Oat3 knockout mice showed slightly decreased urine urate levels, whereas RST (murine ortholog of the human uric acid transporter URAT1) knockout mice demonstrated elevated urine urate levels, suggesting that Oat1 and Oat3 mediate the renal secretion of urate, whereas RST mediates the renal reabsorption of urate (Eraly et al., 2008). It is noteworthy that recent genome-wide association studies failed to show an association between URAT1 and serum urate levels; instead, a link was demonstrated with the locus encoding GLUT9 (Li et al., 2007; Döring et al., 2008; Vitart et al., 2008; Wallace et al., 2008), indicating that other transporters may be involved in urate excretion.

Regulation of Oats

The vital role that the Oats play in whole-body homeostasis through toxin clearance and their potential role in mediating an intricate communication network between organ systems by transporting "signaling" molecules between tissues may explain the presence of complex regulatory mechanisms that control Oat activity. These regulatory mechanisms are apparent from embryogenesis, where Oat1 and Oat3 expression begins at midgestation in concert with proximal tubule differentiation in murine kidneys and slowly increases through nephron development (Lopez-Nieto et al., 1997; Pavlova et al., 2000; Sweet et al., 2006). Oat expression was also detected in extrarenal tissues during embryogenesis with Oat1 transcripts detected in the murine brain and Oat2 in liver, lung, intestine, and developing bone and cartilage (Pavlova et al., 2000).

The need to finely regulate Oat activity is also evidenced by the regulation of Oat activity, not only at the translational level but also at the transcriptional level. Computational analysis of the murine and human genomic loci of OAT1–3 have revealed several conserved binding sites for transcriptional factors important in kidney development, such as WT-1, Pbx, Tcf, and hepatocyte nuclear factor (HNF-1) (Barasch, 2001; Schnabel et al., 2001; Eraly et al., 2003b). HNF-1 regulates the transcription of other renal transporters such as type II sodium-glucose cotransporter (Pontoglio et al., 2000) and sodium/phosphate cotransporters (Soumounou et al., 2001; Cheret et al., 2002), therefore raising the possibility of its being a transcriptional regulator of the Oats (Eraly et al., 2003a). Subsequently, several studies have shown that HNF isoforms activate Oat1, Oat3, and Urat1 promoter activities. Oat1, Oat2, and Oat3 expression was found to be markedly down-regulated in kidneys of HNF-1α-null mice compared with wild type (Maher et al., 2006), and HNF-1α/β was subsequently found to transactivate human and mouse Oat1 promoters (Saji et al., 2008). Furthermore, hOat1 promoter activity is also regulated by HNF-4α, which acts through a response element consisting of an inverted repeat of hexamers separated by eight nucleotides (Ogasawara et al., 2007). The HNF-1α homodimer and the HNF-1α/β heterodimer have been shown to increase hOAT3 promoter activity in human embryonic kidney (HEK) 293 cells, whereas DNA methylation was shown to repress the promoter activity (Kikuchi et al., 2006). A similar regulatory mechanism was observed for Urat1, where mouse/human URAT1 promoter activity was activated by HNF-1α/β heterodimer and repressed by DNA methylation (Kikuchi et al., 2007).

Recent interest has focused on the role of proteins such as caveolins and PDZ proteins in regulation of OAT activity and expression (Zhou and You, 2007). PDZ proteins, which bind to PDZ consensus binding sites at the carboxyl terminus of transporter proteins, have been implicated in the selective targeting of the transporters to the plasma membrane and their retention and regulation at the membrane (Brône and Eggermont, 2005). PDZK1 was found to interact with Urat1 in yeast two-hybrid assays and transfection of Urat1 expressing HEK293 cells with PDZK1 resulted in increased surface expression and transport activity of Urat1 (Anzai et al., 2004). Further studies have shown that the PDZ domain-containing proteins PDZK1 and NHERF1 interacted with hOAT4 and that they increased estrone-3-sulfate transport in hOAT4-expressing HEK293 cells (Miyazaki et al., 2005). Moreover, hOAT4 cell surface expression in kidney LLC-PK1 cells was found to be stimulated by PDZK1 and NHERF1, whereas no effect was observed in human placenta BeWo cells, suggesting tissue-specific regulation (Zhou et al., 2008).

Oat expression also seems to be gender-dependent (Kobayashi et al., 2002a; Kudo et al., 2002; Buist and Klaassen, 2004) and may explain certain differences in pharmacokinetics between genders. Oat1 mRNA expression in the kidney is higher in male rodents compared with female rodents (Buist and Klaassen, 2004), which is consistent with previous observations that PAH transport is higher in intact male rat renal cortical slices than in those of orchiectomized male rats (Reyes et al., 1998). Oat3 mRNA expression in the liver was also observed to be higher in male rats than in female rats (Buist et al., 2002). On the other hand, Oat2 mRNA and protein levels are higher in female than in male rodent kidneys, and its expression is weakly stimulated by estradiol and progesterone and inhibited by androgens (Ljubojević et al., 2007). Human OAT4 also seems to be under hormonal regulation with progesterone exposure resulting in down-regulation (Zhou et al., 2007).

Thus, the regulation of Oat expression and function is quite complex; if the remote sensing hypothesis has merit, the data discussed above already suggest multiple points at which such a remote sensing (signaling) network can undergo dynamic regulation.

Conclusions

The development of Oat knockout mice and the use of methods such as metabolomic and computational analysis have brought great strides in our understanding of the structure and binding properties of Oats and the endogenous substrates transported by the Oats. The continuing expansion of the list of endogenous substrates transported by the Oats provides important clues to their physiological role. The overlap between substrates transported by the different Oats and their potential role as signaling molecules, suggest that the Oats may play essential roles in a broad "remote signaling" network among different organs and organisms. By con-

sidering the differing tissue expression patterns of Oats and other SLC22 as well as non-SLC22 multispecific transporters (e.g., organic anion-transporting polypeptides, Oatps, and multidrug resistance proteins) and their complex regulation in the framework of this remote signaling hypothesis and the demands placed on the whole organism in normal physiology (including pre- and postnatal development) and states of extreme stress (e.g., acute ischemic injury or toxin exposure), it may be possible to understand what currently seems like a bewildering array of data on many genes with varied tissue expression, regulation, and functional transport.

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