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PERSPECTIVE

Polyspecific Organic Cation Transport: Insights into the Substrate Binding Site

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ABSTRACT

Positively charged endogenous and exogenous organic compounds of diverse chemical structures are transported by polyspecific organic cation transporters (OCT). In two contributions to the May 2005 issue of *Molecular Pharmacology*, amino acid residues within the fourth and tenth transmembrane helices of rat OCT1 are described that contribute to cation and corticosterone binding. In a three-dimensional model based on the structure of the lactose permease, these residues are located in a large grove, the binding site for biogenic amines and cationic drugs.

Many widely used pharmaceuticals carry a positive or negative charge and hence are organic cations or anions. The charge renders these compounds hydrophilic, greatly facilitating their solubility in gastrointestinal fluids, plasma, and in the extra- and intracellular aqueous spaces. However, the charge largely decreases the solubility of drugs in lipids and efficiently slows uptake into or release from cells by simple diffusion across cell membranes. Rapid transport of charged drug molecules into hepatocytes for metabolism and biliary excretion, or across small bowel and proximal tubular epithelia for intestinal absorption and renal excretion, requires the presence of transporters (carriers, permeases). Given the large number of drugs and other xenobiotics, these intestinal, hepatic, and renal transporters face the formidable task of efficiently handling chemically unrelated compounds. To do so, these transporters cannot be specific for a single compound or close congeners, as in the majority of Na^+-coupled transporters, but must be polyspecific, showing wider recognition properties.

Meanwhile, several families of polyspecific transporters for organic cations and anions exist: the ATP-driven multidrug resistance transporters [e.g., MDR, P-glycoprotein; ABCB1 (Ambudkar et al., 1999)] and multidrug resistance-associated proteins [MRPs; ABCC family (König et al., 1999; Borst et al., 2000)], as well as the ATP-independent families of organic anion transporting polypeptides [OATPs; solute carrier family SLC21 (Hagenbuch and Meier, 2004)] and organic cation and anion transporters [OCTs, OCTNs, OATs; solute carrier family SLC22 (Koepsell and Endou, 2004)]. The members of the SLC22 family belong to the major facilitator superfamily of uniporters, symporters, and antiporters, which occur in bacteria, lower eukaryotes, plants, and mammals. All members of the SLC22 family are polyspecific and interact with a vast number of endogenous and exogenous compounds including frequently used drugs. In this issue of *Molecular Pharmacology*, Hermann Koepsell and his associates offer novel insights into the molecular basis of polyspecificity of the rat organic cation transporter 1 (OCT1) (Gorboulev et al., 2005; Popp et al., 2005).

The substrate specificity of the organic cation transporters has been studied in detail previously (Koepsell et al., 2003; Koepsell, 2004). The transporters OCT1 and/or OCT2, for instance, interact not only with endogenous compounds, such as choline, dopamine, histamine, and 5-hydroxytryptamine, but also with receptor antagonists (e.g., phenoxybenzamine, cimetidine), receptor agonists (clonidine, *O*-methylisoprenaline), ion channel blockers (procainamide, quinidine, mepiperphenidol, verapamil), psychoactive drugs (desipramine), antivirals (acyclovir, ganciclovir), antidiabetic agents (metformin, phenformin), antimalarial agents (quinine), and other drugs (Koepsell, 2004). A detailed comparison between rat OCT1 and rat OCT2 performed earlier (Arndt et al., 2001)

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ABBREVIATIONS: OCT1, organic cation transporter 1; TEA, tetraethylammonium; MPP, 1-methyl-4-phenylpyridinium.

laid the basis for the present contribution by Gorboulev et al. (2005) in this issue. Using electrophysiological and tracer techniques, Arndt et al. (2001) found a series of substances for which OCT1 and OCT2 had considerably different affinities. These substances included cyanine 863, quinine, procainamide, mepiperphenidol, and *O*-methylisoprenaline, which showed 5 to 70 times higher IC_{50} values (i.e., had lower affinities) for OCT2 than for OCT1, and corticosterone, which, in contrast, inhibited OCT2 at lower concentrations than OCT1 (IC_{50} of 4 μM versus an IC_{50} of 151 μM).

Gorboulev et al. (2005) ascertained which regions in the OCT1 and OCT2 proteins might be responsible for the differential affinity toward corticosterone. They fragmented rat OCT2 into 16 pieces containing individual transmembrane helices or intra-/extracellular loops connecting the helices and inserted each of these pieces into rat OCT1. It turned out that the tenth transmembrane domain of OCT2 was responsible for the high affinity for corticosterone. The authors went on to identify which amino acids within the tenth transmembrane helix are contributing to this high affinity and found three: isoleucine 443, tyrosine 447, and glutamate 448. The respective amino acids in OCT1 are alanine 443, leucine 447, and glutamine 448. Replacement of alanine, tyrosine, and glutamate of OCT1 by the respective amino acids of OCT2 increased the affinity of the mutant OCT1 for corticosterone, and, conversely, insertion of the three critical OCT1 amino acids into OCT2 decreased the affinity of the mutant OCT2 for corticosterone. These experiments revealed for the first time components of the binding site for glucocorticoids on OCT1 and OCT2, and, since glucocorticoids interfere with organic cation transport, revealed that transmembrane helix 10 contributes to the organic cation binding site.

In the contribution by Popp et al. (2005), the authors mutated eighteen amino acids in the fourth transmembrane helix of rat OCT1, and tested each mutant for its ability to translocate the organic cations tetraethylammonium (TEA) and 1-methyl-4-phenylpyridinium (MPP). The replacement of two residues, tryptophan 218 by tyrosine, and tyrosine 222 by leucine, increased the affinity for both TEA and MPP, whereas the threonine 226 to alanine (T226A) mutant had a higher affinity for MPP alone. These "hot spots" were investigated in further detail using additional organic cations for uptake. Other amino acid replacements (e.g., tryptophan 218) had an impact on the selectivity or preference of OCT1 for different cations. It is interesting that tryptophan 218, tyrosine 222, and threonine 226 are located on one side of the fourth transmembrane domain, together with lysine 215 and valine 229. These five amino acids are conserved in all OCTs and therefore are most probably of great functional importance.

Many articles dealing with structure-function relationships of transporters end with the statement that further conclusions necessitate X-ray analysis of crystallized transporters. Popp et al. provide an alternative: they used the previously published structure of the lactose permease of *Escherichia coli* (Abramson et al., 2003) as a template, and computed the three-dimensional structure of rat OCT1. The calculations revealed a large cleft of about 20 $\times$ 60 Å that OCT1 exposes to the cytosolic side of the cell membrane. The above-mentioned three amino acids of the fourth transmembrane helix binding TEA and MPP, the three residues in the tenth transmembrane helix binding corticosterone, and aspartate 475 [identified previously as contributing to organic cation binding (Gorboulev et al., 1999)] are all located in the large cleft and are therefore easily accessible for organic cations. The clusters of functionally important residues turned out to be spatially separated, offering various niches for binding of organic cations. Nonidentical but overlapping binding sites for organic cations have been postulated earlier on the basis of the first mutational studies on OCT1 (Gorboulev et al., 1999) and are strongly supported by the present findings.

Why are the articles by Gorboulev et al. (2005) and Popp et al. (2005) an important development in the field? First, they provide an example of a thorough and analytical characterization of transporters and mutants thereof, using several control experiments, an investigation that took the authors 3 years to complete. Second, the strategy to use differential affinities of two transporters for the detection of amino acids involved in the binding of corticosterone can be adopted to other transporters of the SLC22 family, and possibly to members of other transporter families. Third, we are learning from these two contributions how nature makes transporters polyspecific (e.g., by offering large sites with various anchor points for binding). Subsequent experiments exploiting further substrates will refine the present picture.

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