Distinct transport proteins regulate the movement of waste products and xenobiotics across the blood-brain barrier (BBB). Members of the drug transporter families MDR, MRP, and OATP have been identified in the BBB, and a detailed characterization of the involved proteins is now required to target drugs more efficiently to the brain.

In the central nervous system there are two fluid barriers: the blood-brain barrier (BBB) formed by the brain capillary endothelial cells and the blood-cerebrospinal fluid barrier formed by the choroid plexus. Capillary endothelial cells that form the BBB are not fenestrated, have minimal pinocytosis, and are connected by high-resistance tight junctions. This reduces the unregulated diffusion of molecules across the BBB to a minimum (15). In addition, the tight junctions separate the plasma membrane into a luminal domain facing the blood side and an abluminal domain facing the brain side (Fig. 1). Thus capillary endothelial cells form a polarized barrier similar to the polarized barriers located in the small intestine, the renal proximal tubule, or the liver.

Functionally, the BBB actively regulates the transport of nutrients, waste products, and drugs into and out of the brain by means of distinct transport systems expressed in the luminal and/or abluminal membrane domain. This is similar to the small intestine and the liver, where multispecific transporters such as the organic anion transporting polypeptides (Oatps in rodents, OATPs in humans), the multidrug resistance protein 1 (Mdr1a/b, MDR1), as well as the multidrug resistance-associated proteins (Mrps/MRPs) have been identified and work in concert with detoxification enzymes to protect the organism from potentially harmful compounds. During the characterization of these drug transporters, individual proteins were also identified in the cells of the BBB. Their role in the transport of xenobiotics across the BBB will be summarized in this review. Drug transporters expressed in choroid plexus epithelial cells have been summarized elsewhere (7).

The ATP-dependent efflux pumps

MDRs. The MDRs belong to the ATP-binding cassette (ABC) superfamily of transporters and were first identified in malignant tumor cells, where they conferred multidrug resistance (9). In humans, there are two proteins, known as MDR1 and MDR3 (also called MDRI), whereas in rodents there are three forms, Mdr1a, Mdr1b, and Mdr2 (Table 1). The human MDR3 and the rodent Mdr2 are probably not involved in multidrug resistance. They are phospholipid flippases expressed at the canalicular membrane of hepatocytes, where they are important for the secretion of phosphatidylcholine into bile. However, human MDR1 and rodent Mdr1a/b confer multidrug resistance by actively exporting a wide variety of mainly amphipathic and hydrophobic compounds from tumor cells. Under normal physiological conditions, these efflux pumps are expressed in organs involved in the elimination of endogenous xenobiotics, such as the liver and the kidney, and in epithelial tissues that protect the organs from entry of xenobiotics, like the small intestine, testes, placenta, and BBB (17).

Schinkel and coworkers (18) demonstrated the expression of Mdr1a in the capillary endothelial cells of the BBB and its physiological importance in protecting the brain from xenobiotics by using knockout mice. By immunohistochemistry, Mdr1 was identified in capillary endothelial cells of normal but not of Mdr1a-deficient mice and later was fine-localized to the luminal membrane of these cells (3, 8), where it can mediate efflux of potentially toxic compounds. The protective function of Mdr1 was discovered because the Mdr1a-deficient mice demonstrated a much higher sensitivity to ivermectin, a neurotoxin used to treat mite infestations and normally tolerated very well because of its inability to cross the BBB. Compared with the wild-type mice, the Mdr1a-deficient mice were 50- to 100-fold more sensitive to ivermectin, and, on treatment with subtoxic amounts, the brain levels in the knockout mice were ~90-fold higher compared with normal mice. Similarly, the brain level ratio between Mdr1a-deficient and normal mice was elevated for numerous additional compounds including HIV protease inhibitors (amprenavir, nelfinavir, indinavir, and saquinavir), immune suppressants (tacrolimus and cyclosporin), anticancer drugs (vinblastine and paclitaxel) and the antiarrhythmic quinidine as well as the glucocorticosteroid dexamethasone and the cardiac glycoside digoxin (2). These results demonstrate the importance of Mdr1a for brain protection from drug toxicity but also suggest that coadministration of a specific Mdr inhibitor (also called a reversal agent) could enhance penetration of the BBB by certain compounds.

To test this possibility, Mayer and coworkers (12) used a very effective reversal agent, PSC833, a nonimmunosuppressive cyclosporin A analog, and determined brain levels of digoxin (12). Oral administration of PSC833 to wild-type mice 2 h before an intravenous injection of [3H]digoxin increased brain digoxin levels 19-fold compared with wild-type mice not receiving PSC833. However, brain digoxin levels in Mdr1a-knockout mice were even higher, suggesting that PSC833 administration resulted in a significant but not com-
plete inhibition of Mdr1a-mediated digoxin export across the BBB. These results confirm that 1) Mdr1a plays an important role in protecting the brain from various compounds by preventing their penetration of the BBB and 2) that specific reversal agents might be promising compounds to overcome this limitation and increase the brain levels of certain drugs.

**MDR-associated proteins.** Besides the MDRs, there are other drug efflux pumps that also belong to the ABC transporters. The first member was originally cloned from a multidrug-resistant human lung cancer cell line and therefore named multidrug resistance-associated protein (MRP). The MRP family contains at least seven members, MRP1 to MRP7 (4), with MRP1 and MRP2 being the best characterized (Table 1).

The ubiquitously expressed MRP1 is the major leukotriene C4 (LTC4) transporter. When overexpressed, MRP1 confers resistance to different antitumor agents such as vincristine and daunorubicin. Under normal physiological conditions, however, MRP1 helps to protect the organism against toxic compounds, as was demonstrated by the Mrp1-knockout mice that are hypersensitive to the anticancer drug etoposide (16).

MRP2, also known as canalicular multispecific organic anion transporter (cMOAT), is strongly expressed in liver, kidney, and small intestine. Deficiency of MRP2 results in the Dubin-Johnson syndrome, a genetically inherited disease that is characterized by impaired excretion of glucuronidated bilirubin, which results in conjugated hyperbilirubinemia (10). So far, no Mrp2-knockout mice have been generated. However, Mrp2-deficient rat strains, like the transport-negative TR rats and the Eisai hyperbilirubinemic rats, are useful animal models and have been extensively studied to determine the substrate specificity of the canalicular Mrp2. It turned out that Mrp2 is an important component of the detoxification system of hepatocytes and that it excretes anionic glucurononides as well as glutathione conjugates of endo- and xenobiotics, including many drugs and drug conjugates but also unconjugated organic anions, into bile (11).

Recently, Miller and coworkers (14) were able to show that Mrp2 is expressed at the BBB in isolated rat brain capillaries by using functional experiments and confocal microscopy. They demonstrated that luminal accumulation of the fluorescent organic anion and Mrp substrate sulforhodamine was inhibited by coincubation of the capillaries with other Mrp substrates such as LTC4 and that MDR1-specific inhibitors like the reversal agent PSC833 had no effect. Using an anti-Mrp2 antibody, confocal immunolocalization studies detected Mrp2 at the luminal surface of the endothelium of normal rats, but no staining was obtained with capillaries isolated from the mutant TR rats. Thus Mrp2 is clearly expressed at the rat BBB and, given the expression of the drug-metabolizing enzymes in the endothelial cells, it might play a similar important role in the detoxification and protection of the brain as in hepatocytes. In addition, the effect of the HIV protease inhibitors saquinavir and ritonavir on Mdr1- and Mrp2-mediated transport was tested, and it could be demonstrated that both agents interact with both ABC transporters and therefore might be helpful reversing agents for drug resistance caused by both ABC transporters.

**The OATPs**

The Oatps/OATPs are a group of membrane transporters classified within the solute carrier family 21A (rodents, Slc21a; human, SLC21A) (Table 2). They exhibit a wide spectrum of substrates and are involved in the uptake and export of many substrates, including many drugs and drug conjugates. The transporter proteins shown in bold have been identified at the blood-brain barrier.
amphipathic transport substrates. Some members are selectively expressed in the liver, where they are involved in the hepatic elimination of endo- and xenobiotics. Most Oatp/OATPs however, are expressed in multiple tissues, including the BBB, choroid plexus, lung, heart, intestine, kidney, placenta, and testis (20). Many Oatp/OATPs represent polyspecific organic anion transporters with partially overlapping and partially distinct substrate specificities for a wide range of amphipathic organic solutes, including bile salts, organic dyes, steroid conjugates, thyroid hormones, neuroactive peptides, numerous drugs, and other xenobiotics (13). Two of the best-characterized members, human OATP-A (SLC21A3) and rat Oatp2 (Slc21a5), have recently been localized to the BBB by using immunolocalization techniques (6, 8).

Human OATP-A was the first human OATP isolated from human liver. Later it turned out that its strongest expression is in brain, followed by kidney, liver, lung, and testis. An OATP-A-specific antibody recognized a ~60-kDa protein in human frontal cortex homogenates and stained brain microvessels and capillaries, whereas astrocytes and neurons were immunonegative (6). Similarly, rat Oatp2, which was isolated from rat brain, was exclusively localized to endothelial cells of cerebral capillaries by using in situ hybridization and immunolocalization techniques (6, 8). Unlike Mdr1, which is expressed only in the luminal membrane of the endothelium, Oatp2 was detected in both the luminal and the abluminal membranes.

These two Oatp/OATPs, which share 73% amino acid identities, have been extensively characterized functionally in different in vitro systems. It was demonstrated that they transport similar compounds, including sulfated and glucuronidated steroids [dehydroepiandrosterone sulfate (DHEAS), estrone-3-sulfate, and estradiol-17β-glucuronide], thyroid hormones (T₃ and T₄), drugs like fexofenadine, cationic compounds (ADP-ajmalinium, rocuronium), and neuroactive peptides ([D-penicillamine 2,5]enkephalin (DPDPE)). In addition, there are compounds that are only transported by either OATP-A (e.g., deltorphin II and the cyanobacterial toxin microcystin) or Oatp2 (e.g., Leu-enkephalin and digoxin) (13).

That Oatp2 indeed is functional at the BBB has been demonstrated by different in vivo studies in which transport of the Oatp2 substrates DHEAS, estradiol-17β-glucuronide, and DPDPE across the rat and mouse BBB was determined either by injection of radio labeled compound directly into the cortex or by in situ brain perfusion technique. Asaba et al. (1) studied transport of the neuroactive steroid DHEAS across the rat BBB and could show that apparent efflux was 10-fold higher than influx and was saturated, with an apparent $K_m$ value of 33 μM, which is similar to the 17 μM determined for Oatp2-mediated DHEAS transport in vitro. This net DHEAS efflux, which is physiologically the right transport direction since DHEAS concentrations in rodent brain by far exceed the concentrations in the periphery, could be inhibited by several Oatp2 substrates. Furthermore, by using immortalized mouse brain capillary endothelial cells in culture, uptake of DHEAS ($K_m$ ~ 34 μM) and digoxin could be demonstrated. By using RT-PCR

<table>
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<tr>
<th>Human Protein</th>
<th>Gene Symbol</th>
<th>Mouse Protein</th>
<th>Gene Symbol</th>
<th>Rat Protein</th>
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<tr>
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Only human OATP-A and rat Oatp2 have been identified so far at the blood-brain barrier on the protein level. PGT, prostaglandin transporter.
and sequencing, an Oatp2-like transcript was identified, suggesting that BBB Oatp2 mediates efflux of DHEAS also in the mouse. A similar study by Sugiyama et al. (19) characterized estradiol-17β-glucuronide efflux across the rat BBB. With specific inhibitors for the different organic anion transporters, they deduced that the major part of estradiol-17β-glucuronide efflux was mediated by Oatp2. In a third study, Dagenais et al. (5) investigated transport of the δ-opioid receptor agonist DPDPE across the BBB of normal and Mdr1α-knockout mice by using a brain perfusion technique. Due to Mdr1α-mediated efflux, DPDPE exhibited poor BBB permeability in wild-type mice, whereas in the Mdr1α-knockout mice uptake of DPDPE could readily be determined. The obtained results suggest that Oatp2 is responsible for saturable uptake of DPDPE and potentially other opioid peptides across the BBB into the brain.

Together, these results show that Oatp2 and OATP-A are expressed at the BBB and functionally can mediate either 1) efflux of compounds that are synthesized in the brain and active in the periphery or waste products that need to be secreted from the brain, as exemplified by estradiol-17β-glucuronide or DHEAS, as well as an estradiol-17β-glucuronide efflux across the rat BBB. With specific inhibitors for the different organic anion transporters, they deduced that the major part of estradiol-17β-glucuronide efflux was mediated by Oatp2. In a third study, Dagenais et al. (5) investigated transport of the δ-opioid receptor agonist DPDPE across the BBB of normal and Mdr1α-knockout mice by using a brain perfusion technique. Due to Mdr1α-mediated efflux, DPDPE exhibited poor BBB permeability in wild-type mice, whereas in the Mdr1α-knockout mice uptake of DPDPE could readily be determined. The obtained results suggest that Oatp2 is responsible for saturable uptake of DPDPE and potentially other opioid peptides across the BBB into the brain.

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Summary and perspectives

Of the three drug transporter families MDR, MRP, and OATP, so far only four individual members have been localized in the BBB at the protein level (Tables 1 and 2). The only protein shown to be expressed in the human BBB is OATP-A. In the rat, immunostaining of capillary endothelial cells has been obtained with antibodies against Mdr1, Mrp2, and Oatp2. Whereas Mdr1 and Mrp2 are located exclusively in the luminal membrane of the endothelial cells (Fig. 2), Oatp2 is expressed in both the luminal and the abluminal membrane (Fig. 2). Under normal physiological conditions, this arrangement of transporters with a bidirectional Oatp2 in both membranes and the unidirectional Mdr1 and Mrp2 in the luminal membrane results functionally in an efficient efflux system for compounds that need to be secreted from the brain, as exemplified by estradiol-17β-glucuronide or DHEAS, as well as an efficient protection system for the brain from uptake of potentially toxic solutes such as, for example, digoxin.

What does this mean for transport drugs across the BBB? Given that many drugs are substrates for the discussed drug transporters, it is evident that efficient efflux systems prevent certain drugs from reaching the brain. To overcome the tight BBB, all involved transport proteins expressed in the human BBB need to be unequivocally identified and characterized in detail. By modeling compounds to be specific substrates, e.g., for Oatp2 but not recognized by Mrp2, and coapplication of reversal agents to inhibit Mdr1, it might be possible in the future to target drugs with higher efficiency to the brain and thus to better treat the numerous disorders of the central nervous system, where currently no or only inefficient drugs are available.

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References