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Multidrug Resistance-Associated Proteins: Expression and Function in the Central Nervous System

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***Abstract*——Drug delivery to the brain is highly restricted, since compounds must cross a series of structural and metabolic barriers to reach their final destination, often a cellular compartment such as neurons, microglia, or astrocytes. The primary barriers to the central nervous system are the blood-brain and blood-cerebrospinal fluid barriers. Through structural modifications, including the presence of tight junctions that greatly limit paracellular transport, the cells that make up these barriers restrict diffusion of many pharmaceutically active compounds. In addition, the cells that comprise the blood-brain and blood-cerebrospinal fluid barriers express multiple ATP-dependent, membrane-bound, efflux transporters, such as members of the multidrug resistance-associated protein (MRP) family, which contribute to lowered drug accumulation. A relatively new concept in brain drug distribution just beginning to be explored is the possibility that cellular components of the brain parenchyma could act as a "second" barrier to brain permeation of pharmacological agents via expression of many of the same transporters. Indeed, efflux transporters expressed in brain parenchyma may facilitate the overall export of xenobiotics from the central nervous system, essentially handing them**

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off to the barrier tissues. We propose that these primary and secondary barriers work in tandem to limit overall accumulation and distribution of xenobiotics in the central nervous system. The present review summarizes recent knowledge in this area and emphasizes the clinical significance of MRP transporter expression in a variety of neurological disorders.

I. Introduction

Neurologically based diseases remain difficult to treat, and are often refractory to currently available medications despite recent advances in our understanding of their underlying pathophysiological mechanisms. The reasons for the observed pharmacotherapeutic failures are multifactorial, but increasingly the presence of membrane-bound, active transport carriers has been implicated. Several ATP-dependent transport proteins have overwhelmingly emerged as the main culprits including P-glycoprotein (P-gp)[1], the breast cancer resistance protein (BCRP), and multiple isoforms of multidrug resistance-associated proteins (MRPs). The importance of P-gp and BCRP within the central nervous system (CNS) have both been recently reviewed elsewhere (Doyle and Ross, 2003; Lee and Bendayan, 2004; Bauer et al., 2005). Here we provide a comprehensive review of studies examining CNS expression, function, and localization of MRP transporters undertaken to date. Given the complexity of the MRP family, we first provide a summary of the larger superfamily to which MRP proteins belong, as well as an overview of MRP expression, function and localization in peripheral tissue compartments.

II. The ATP-Binding Cassette Transporter Superfamily

The ATP-binding cassette (ABC) superfamily of proteins contains a number of membrane-bound, ATP-driven transporters that pump drugs, drug metabolites, and endogenous metabolites out of cells. Members of the ABC family are classified as such according to the presence of several consensus sequences including two ATP binding motifs (Walker A and Walker B), as well as the ABC signature C motif (ALSGGQ) (Leslie et al., 2005). At present there are 49 known human ABC family members belonging to 7 different subfamilies. A comprehensive list of currently known human ABC transporters compiled by Dr. M. Müller (Wageningen University, The Netherlands) and divided by subfamilies can be found at http://nutrigene.4t.com/humanabc.htm. Mutations in some of the ABC genes result in the generation of genetic disorders such as cystic fibrosis, anemia, Dubin Johnson's syndrome, and retinal degeneration (Dean et al., 2001; Stefkova et al., 2004). Moreover, P-gp, BCRP, and several MRP isoforms in particular are important determinants of drug accumulation in target cells (e.g., tumor cells) and of overall drug uptake, distribution, and excretion. Collectively, they have all been implicated in the development of the multidrug resistance (MDR) phenotype. Underlying this type of MDR is active efflux from cells of a large number of structurally and functionally unrelated pharmacological agents. The development of MDR is associated with poor clinical outcome in several neurological disorders (Loscher and Potschka, 2005b).

At present the cystic fibrosis transmembrane conductance regulator/MRP family (ABC subfamily C) contains 13 members, including one ion channel (cystic fibrosis transmembrane regulator gene), two cell surface receptors [sulfonylurea 1 and 2 (SUR1 and 2)], and a truncated protein that does not mediate transport (ABCC13) (Haimeur et al., 2004). These proteins, which show no capacity for drug transport, will not be discussed further. The remaining nine MRP members can be further divided into two types based on putative membrane topology (Kruh and Belinsky, 2003). MRP1 to MRP3 and MRP6 and MRP7 contain three transmembrane domains, TMD_0, TMD_1, and TMD_2, which show a 5 + 6 + 6 configuration in transmembrane helices (Fig. 1). Nucleotide binding domains 1 and 2 are located between TMD_1 and TMD_2 and between TMD_2 and the carboxyl terminus, respectively. A cytoplasmic linker (L_0) located between the first two TMDs is essential for a functional protein (Bakos et al., 1998). MRP4, MRP5, MRP8, and possibly MRP9 are considered to be "short" MRPs, as they do not contain TMD_0 but do retain the cytoplasmic linker. Not surprisingly, the many MRP isoforms show differences with respect to tissue distribution, substrate specificity, and proposed physiological function. Table 1 provides a brief summary of the nomenclature and general substrate specificities of MRP1 through MRP9.

A. MRP1

The *MRP1*[2] gene was first cloned in 1992 from a human small cell lung cancer cell line (H69AR) that

[1] Abbreviations: P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; MRP, multidrug resistance-associated protein; CNS, central nervous system; ABC, ATP-binding cassette; MDR, multidrug resistance; TMD, transmembrane domain; GSH, reduced glutathione; DNP-SG, 2,4-dinitrophenyl-*S*-glutathione; $E_2 17\beta G$, estradiol-17β-glucuronide; GSSG, oxidized glutathione; LTC4, leukotriene C4; MDCK, Madin-Darby canine kidney; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; MK571, (3-(3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl) ((3-dimethyl amino-3-oxo propyl)thio)methyl)thio propanoic acid; OAT, organic anion transporter; OATP, organic anion transporter polypeptide; BBB, blood-brain barrier; CP, choroid plexus; CSF, cerebrospinal fluid; HAD, HIV-associated dementia; GFAP, glial fibrillary acidic protein; AED, antiepileptic drug; HAART, highly active antiretroviral therapy.

[2] Nomenclature used throughout this review: human MRP proteins are denoted by capital letters; human *MRP* genes are designated by italics. For mammalian Mrp proteins, the first letter is capitalized followed by lower case letters. Mammalian *Mrp* genes are also italicized.

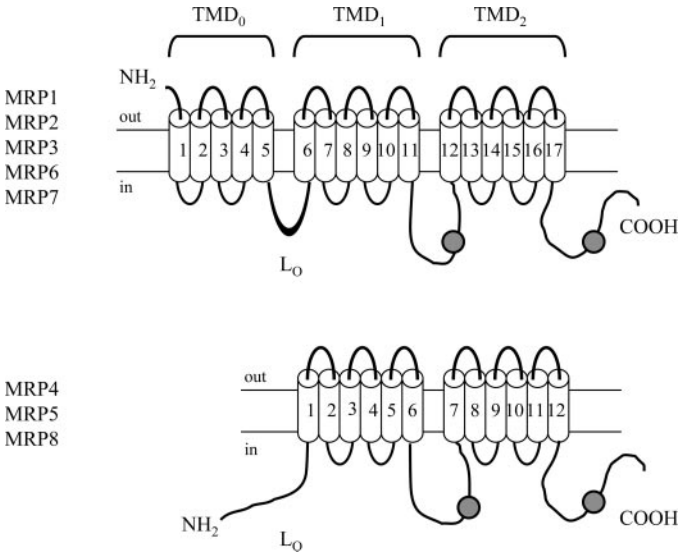

FIG. 1. Membrane topology of MRP proteins. MRP1, MPR2, MRP3, MRP6, and MRP7 contain an extra N-terminal extension (TMD_0) with five transmembrane helixes connected to a cytoplasmic linker (L_0). MRP4, MRP5, MRP8, and possibly MRP9 contain only TMD_1 and TMD_2 as well as L_0. Nucleotide binding domains (NBD) are shown as circles.

demonstrated an MDR phenotype without concomitant expression of P-gp (Cole et al., 1992). *Mrp1* was subsequently cloned and sequenced in several other species including mouse, rat, dog, monkey, and cow (Stride et al., 1996; Ma et al., 2002; Taguchi et al., 2002; Yang et al., 2002; Godinot et al., 2003). In humans, MRP1 is widely expressed, with highest levels observed in the kidney, lung, testes, and peripheral blood mononuclear cells (Haimeur et al., 2004). Similar tissue distribution of Mrp1 has been noted in rats, dogs, mice, and cows (Stride et al., 1996; Conrad et al., 2001; Taguchi et al., 2002; Nunoya et al., 2003). In polarized epithelia of the intestine, kidney, liver, and lung, MRP1/Mrp1 is localized to basolateral plasma membranes of all species tested (Mayer et al., 1995; Peng et al., 1999; Pei et al., 2002; Scheffer et al., 2002b).

Cells that highly express MRP1 confer resistance to a variety of natural product anticancer drugs including vinca alkaloids, anthracyclines, and epipodophyllotoxins (Cole et al., 1994; Zaman et al., 1994; Breuninger et al., 1995). In contrast to P-gp, MRP1 shows preferential transport of anionic compounds such as glucuronide, glutathione (GSH), and sulfate conjugates (Leier et al., 1994, 1996; Jedlitschky et al., 1996, 1997; Loe et al., 1996). Typical conjugated substrates include 2,4-dinitrophenyl-*S*-glutathione (DNP-SG), estradiol-17β-glucuronide ($E_2$17βG), and estrone 3-sulfate. MRP1 also has the capacity to transport metalloids as oxyanions, such as antimony and arsenic, acetanalide pesticides, and various dietary constituents including bioflavanoids and tobacco-derived carcinogens (Leslie et al., 2005). MRP1 transports certain cationic compounds such as vincristine and etoposide, but only in the presence of the antioxidant GSH, probably via cotransport (Rappa et al., 1997; Loe et al., 1998). Interestingly, the expression patterns of MRP1 and γ-glutamyl cysteine synthetase, the rate-limiting enzyme of GSH synthesis, may be coordinately regulated by oxidative stress and heavy metals (Ishikawa et al., 1996; Yamane et al., 1998; Lin-Lee et al., 2001). Transport of conjugated compounds and the oxidized form of GSH (GSSG), in addition to possible up-regulation of GSH-synthesizing enzymes, strongly suggests a role for MRP1 in detoxification and "phase III" elimination of toxic endogenous metabolites (Leslie et al., 2001). In addition, MRP1 shows high affinity ($K_m \cong 100$ nM) for the inflammatory mediator leukotriene C_4 (LTC4) and probably also plays a significant role in mediating immune responses (Haimeur et al., 2004). Indeed, Mrp1-deficient mice are viable and fertile but show decreased response to inflammatory stimuli, presumably due to decreased LTC4 efflux (Lorico et al., 1997; Wijnholds et al., 1997).

Recently, Nunoya et al. (2003) directly compared the resistance profiles and transport characteristics of three species of MRP1 commonly used in transport studies (i.e., rat, mice, and humans) and found substantial differences. In MRP1/Mrp1-transfected human embryonic kidney cell lines (HEK293), resistance conferred to vincristine by rat Mrp1 (7-fold) was slightly lower than for mouse Mrp1 (12-fold) and human MRP1 (11-fold). Rat and mouse Mrp1-expressing cells also showed little or no resistance to doxorubicin, daunorubicin, or epirubicin, compared with human MRP1. Bovine Mrp1 does not seem to transport doxorubicin efficiently either (Taguchi et al., 2002). Although LTC4 affinity is similar for rat Mrp1, mouse Mrp1, and human MRP1 ($K_m \sim 50$–100 nM), human MRP1 transports $E_2$17βG with greater than 10-fold efficiency compared with the mouse and rat homologs (Nunoya et al., 2003). These results clearly highlight the importance of using caution when making cross-species comparisons.

B. MRP2

MRP2/*Mrp2* has been cloned from various species including humans, dogs, mice, rats, and rabbits (Buchler et al., 1996; Taniguchi et al., 1996; van Kuijck et al., 1996; Fritz et al., 2000; Conrad et al., 2001). MRP2/Mrp2 protein is localized to the apical membrane of polarized cells from a variety of human and rat tissues including enterocytes of the small intestine (Mottino et al., 2000; Rost et al., 2002), hepatocytes (Buchler et al., 1996; Keppler et al., 1997a), and renal proximal tubules (Schaub et al., 1997, 1999). In this respect, MRP2 colocalizes with P-gp and BCRP (Schinkel and Jonker, 2003). High expression of MRP2 is found in liver, intestine, and kidney, with little or no expression observed in other tissues (Kool et al., 1997). The tissue distribution of Mrp2 in dogs, mice, and rats is very similar to that in humans with one notable exception: dogs show lower levels of Mrp2 in the liver compared with the kidney

TABLE 1
MRP substrate specificity

Protein/Gene	Alternative Names	Substrates	References
MRP1/*ABCC1*	MRP; GS-X	Leukotriene C_4; oxidized glutathione; vincristine; daunorubicin; etoposide; methotrexate; glutathione, glucuronide, and sulfate conjugates	Jedlitschky et al. (1996); Loe et al. (1996); Keppler et al. (1997); Rappa et al. (1997); Loe et al. (1998); Hooijberg et al. (1999); Leslie et al. (2005)
MRP2/*ABCC2*	cMOAT; cMRP	Similar to MRP1; cisplatin; methotrexate	Jedlitschky et al. (1997); Evers et al. (1998); Suzuki and Sugiyama (1998); Cui et al. (1999); Hooijberg et al. (1999); Kawabe et al. (1999)
MRP3/*ABCC3*	MOAT-D cMOAT2 MLP-2*	Monoanionic and conjugated bile acids; etoposide; methotrexate	Hirohashi et al. (2000); Zelcer et al. (2001); Meier and Stieger (2002)
MRP4/*ABCC4*	MOAT-B	Cyclic nucleotides (cAMP, cGMP); nucleotide analogs (PMEA, azidothymidine-monophosphate); prostaglandins; methotrexate	Schuetz et al. (1999); Reid et al. (2003a,b); Wielinga et al. (2003)
MRP5/*ABCC5*	MOAT-C SMRP	Cyclic nucleotides (cAMP, cGMP); nucleotide analogs (PMEA, stavudine-monophosphate)	Jedlitschky et al. (2000); Wijnholds et al. (2000b); Wielinga et al. (2003); Reid et al. (2003a)
MRP6/*ABCC6*	MOAT- E; MLP-1[a]	Small peptides (BQ123); glutathione conjugates	Madon et al. (2000); Belinsky et al. (2002); Iliás et al. (2002)
MRP7/*ABCC10*		Estradiol-17β-glucuronide; leukotriene C_4; docetaxel	Chen et al. (2003); Hopper-Borge et al. (2004)
MRP8/*ABCC11*		Nucleotide analogs (PMEA), DHEAS, fluoropyrimidines	Guo et al. (2003); Chen et al. (2005b)
MRP9/*ABCC12*		N.A.	Bera et al. (2002)

MOAT, multispecific organic anion transporter; N.A., not available; SMRP, short MRP; DHEAS, dehydroepiandrosterone 3-sulfate.
[a] The MLP-1 and MLP-2 proteins were subsequently identified as rat orthologs of MRP6 and MRP3, respectively.

(Conrad et al., 2001; Cherrington et al., 2002; Ninomiya et al., 2005).

The substrate specificity and resistance profile of MRP2 are similar to those for MRP1 and include various conjugated and unconjugated organic anions and cations, i.e., methotrexate, LTC4, DNP-SG, $E_2$17βG, vincristine, etoposide, and bilirubin glucuronosides (Jedlitschky et al., 1997; Evers et al., 1998; Cui et al., 1999; Hooijberg et al., 1999). Unlike MRP1, MRP2 also confers resistance to cisplatin (Koike et al., 1997; Cui et al., 1999; Borst et al., 2000). In several instances, MRP2 transports these compounds with lower affinity than MRP1 (Konig et al., 1999a). For example, compared with MRP1, MRP2 has exhibited 10- and 4-fold lower affinities for LTC4 and $E_2$17βG, respectively (Cui et al., 1999). No difference was observed in the affinity of human and rat MRP2/Mrp2 for both LTC4 and $E_2$17βG (Cui et al., 1999). Given its location and substrate specificity, MRP2/Mrp2 probably plays an important role in excreting metabolites into the bile. This conclusion is supported by the observation that absence of MRP2 from the canalicular membrane results in impaired efflux of bilirubin glucuronide into the bile and manifests clinically as Dubin-Johnson syndrome (Leslie et al., 2001).

C. MRP3

The *MRP3*/*Mrp3* gene has been cloned from human, rat and mouse (Hirohashi et al., 1998; Kiuchi et al., 1998; Belinsky et al., 2005). MRP3/Mrp3 is highly expressed in the intestine and kidney (Uchiumi et al., 1998; Cherrington et al., 2002; Maher et al., 2005). It is localized to the basolateral side of hepatocytes (Konig et al., 1999b; Kool et al., 1999b), cholangiocytes (Kool et al., 1999b; Soroka et al., 2001), and intestinal epithelial cells (Hirohashi et al., 2000; Rost et al., 2002). Only low levels of MRP3 are found normally in the liver. However, MRP3 expression in liver is higher in patients with Dubin-Johnson syndrome, probably as compensation for the absence of MRP2. This has been demonstrated in rodent models of Dubin-Johnson syndrome (e.g., the Eisai hyperbilirubinemic rat), in which Mrp3 mRNA and protein expression in liver and kidney are increased significantly (Kuroda et al., 2004). Furthermore, induction of MRP3/Mrp3 also occurs in cholestatic rat (Hirohashi et al., 1998) and human livers (Kool et al., 1999b; Konig et al., 1999b), which further supports up-regulation of MRP3/Mrp3 as a protective mechanism (i.e., bilirubin and metabolite removal) when MRP2/Mrp2 is either absent or nonfunctional.

MRP1, MRP2, and MRP3 have similar substrate profiles with some notable differences. Murine fibroblast cells transfected with MRP3 show high levels of resistance to etoposide and teniposide but not to doxorubicin, vincristine, or cisplatin (Zelcer et al., 2001). Although all three proteins transport etoposide, MRP3 does so in a GSH-independent manner (Zelcer et al., 2001). Unlike MRP1 (Loe et al., 1998) and MRP2 (Evers et al., 2000), MRP3-overexpressing cells do not transport GSH (Zelcer et al., 2001). Glucuronide and sulfate conjugates of bile salts are substrates of both MRP1 and MRP3, but MRP3 also mediates transfer of monovalent bile salts including glycocholate (Zeng et al., 2000). Glucuronide conjugates (i.e., $E_2$17βG) seem to be preferentially transported by MRP3/Mrp3 compared with GSH conjugates such as DNP-SG and LTC4 (Hirohashi et al., 1999; Zeng et al., 2000).

The human and rat MRP3/Mrp3 orthologs generally have similar substrate specificities, although some ki-

netic differences have been described. In Sf9 transfected vesicles, the affinities of $E_2 17\beta G$ by human and rat MRP3/Mrp3 are comparable, i.e., 42.9 and 33.4 μM, respectively (Akita et al., 2002). However, uptake of $E_2 17\beta G$ by MRP3 is inhibited by methotrexate at concentrations 11-fold lower than those for rat Mrp3 (Akita et al., 2002). Conversely, a 4-fold higher concentration of DNP-SG is required to inhibit uptake of $E_2 17\beta G$ by human MRP3 versus rat Mrp3 (Akita et al., 2002). Finally, the monovalent bile salt taurocholate is only transported by rat Mrp3 (Akita et al., 2002). Given its location and substrate profile, MRP3/Mrp3 is proposed to play an important role in enterohepatic circulation of endogenous compounds such as bile salts (Rost et al., 2002).

D. MRP4

MRP4 is expressed at low levels in a variety of human tissues, with high levels occurring in the prostate and kidney (Kool et al., 1997; Lee et al., 1998). The rat and murine Mrp4 orthologs show greater than 83% amino acid identity with human MRP4 (Chen and Klaassen, 2004). Membrane localization of MRP4/Mrp4 in polarized cells remains unresolved. In human and rat kidney proximal tubule epithelia, MRP4/Mrp4 was located in the apical membrane (van Aubel et al., 2002; Leggas et al., 2004). However, when transfected into Madin-Darby canine kidney II (MDCKII) cells, MRP4 routed to the basolateral membrane (Lai and Tan, 2002). Immunocytochemical studies by Lee et al. (2000) localized MRP4 to the basolateral membrane of tubuloacinar cells of the prostate. Finally, MRP4/Mrp4 was localized to the sinusoidal membrane of human, rat, and mouse hepatocytes, as well as in the human hepatoma cell line HepG2 (Rius et al., 2003; Zelcer et al., 2003).

Unlike MRP1–3, MRP4 contains only two membrane-spanning domains (Fig. 1). MRP family members displaying this topology seem to show a unique capacity to transport and confer resistance to a variety of monophosphorylated compounds. The ability of MRP4 to transport cyclic nucleotides (cAMP and cGMP), nucleotide analogs such as 9-(2-phosphonylmethoxyethyl)adenine (PMEA) and azidothymidine monophosphate and purine analogs (6-mercaptopurine and 6-thioguanine) has been well described (Schuetz et al., 1999; Chen et al., 2001; Lai and Tan, 2002; Wielinga et al., 2002, 2003; Reid et al., 2003a). MRP4 does not confer resistance to typical unconjugated substrates of MRP1 and MRP2 such as doxorubicin, etoposide, vincristine, or taxol (Lee et al., 2000) but does retain the capacity to efflux conjugated compounds including $E_2 17\beta G$ (Zelcer et al., 2003).

As expected, MRP4-transfected cells show increased efflux of monophosphorylated nucleotides and nucleotide analogs such as cAMP, cGMP, and PMEA. However, the full substrate spectrum of MRP4 seems to be much broader than initially presumed. Recently Reid et al. (2003b) showed transport of prostaglandins E_1 (K_m = 2.4 μM) and E_2 (K_m = 3.4 μM), in MRP4-transfected HEK293 cells, but not in cells expressing MRP1, MRP2, MRP3, or MRP5. Low-affinity transport of methotrexate (K_m = 0.22–1 mM) by MRP4 has also been reported (Chen et al., 2002; van Aubel et al., 2002). In membrane vesicles prepared from MRP4-transfected Sf9 insect cells, ATP-dependent uptake was observed for $E_2 17\beta G$ (K_m = ~30 μM) and dehydroepiandrosterone 3-sulfate (K_m = 2 μM), but not LTC4 or DNP-SG (Chen et al., 2001; van Aubel et al., 2002; Zelcer et al., 2003). In contrast to MRP1, MRP4-mediated transport of dehydroepiandrosterone 3-sulfate is GSH-independent (van Aubel et al., 2002). Interestingly, GSH stimulates transport of unconjugated bile acids (cholyltaurine, cholylglycine, and choline) from hepatocytes, suggesting that some MRP4 substrates may require GSH for efficient transport (Rius et al., 2003).

E. MRP5

The *MRP5* gene is ubiquitously expressed with the highest levels of expression found in skeletal muscle and the brain (Kool et al., 1997; Belinsky et al., 1998). Mouse and rat Mrp5 orthologs show high levels of amino acid identity with MRP5, i.e., >94% (GenBank accession no. AB020209; Suzuki et al., 2000). The tissue expression of mouse Mrp5 is reported to be similar to that in human and rat (Suzuki et al., 2000; Maher et al., 2005, 2006); Mrp5 knockout mice are healthy and fertile and do not show any observable physiological dysfunctions (Wijnholds et al., 2000b).

Similar to MRP4, MRP5 lacks the TMD_0 domain and does not interact with typical substrates of MRP1, MRP2, or MRP3 such as vincristine, LTC4, etoposide, or daunorubicin (McAleer et al., 1999; Jedlitschky et al., 2000). However, the substrate profile of MRP5 seems to be much narrower than MRP4. Both MRP4 and MRP5 transport monophosphorylated compounds, such as PMEA (Wijnholds et al., 2000b; Reid et al., 2003a; Weilinga et al., 2003), but differences do exist with respect to sensitivities to MRP inhibitors. For example, the inhibitory concentration for probenecid is 10-fold higher for MRP4-mediated PMEA transport than for MRP5 (Reid et al., 2003a). In contrast, MK571 inhibits MRP4-mediated transport of PMEA at 4-fold lower concentrations than it does for MRP5 (Reid et al., 2003a). Studies in MRP4- and MRP5-overexpressing cells have shown that the anti-HIV agent stavudine monophosphate is one monophosphorylated compound transported by MRP5 but not by MRP4 (Reid et al., 2003a).

Membrane localization of MRP5 in polarized cells, including MRP5-transfected MDCKII cells, seems to be basolateral (Wijnholds et al., 2000b). Interestingly, in nonpolarized MRP5-transfected HEK293 cells, much of the MRP5 protein is located intracellularly, with little expression occurring in the plasma membrane. Whether this intracellular expression is an artifact of the transfection process or endogenous nonpolarized cells show a

similar pattern of intracellular MRP5 expression is unknown.

F. MRP6

Mrp6 was initially cloned from rat liver (Hirohashi et al., 1998) and has been subsequently cloned in humans and mice (Belinsky and Kruh, 1999; Madon et al., 2000; Beck et al., 2003). The mouse and rat Mrp6 orthologs show greater than 78% amino acid identity with human MRP6. Mutations in the MRP6 gene have been implicated in the etiology of pseudoxanthoma elasticum, a hereditary connective tissue disorder characterized by loss of tissue elasticity (Bergen et al., 2000; Hu et al., 2003).

Human, rat, and mouse MRP6/Mrp6 is predominantly expressed in the liver and kidney, with low levels detected in most other tissues (Kool et al., 1999a; Madon et al., 2000; Maher et al., 2005, 2006). Initial immunocytochemical studies localized rat Mrp6 to both the basolateral (strong staining) and canalicular (weaker staining) plasma membranes of hepatocytes (Madon et al., 2000). In a subsequent study, MRP6 was only present in the basolateral membrane of human hepatocytes (Scheffer et al., 2002a). A basolateral orientation was also demonstrated in human and mouse kidney proximal tubules and in MRP6-transfected MDCKII epithelial cells (Beck et al., 2003; Sinko et al., 2003).

Chinese hamster ovary cells transfected with *MRP6* cDNA show increased resistance to a variety of anticancer agents including etoposide, doxorubicin, daunorubicin, and cisplatin but not to vincristine or vinblastine (Belinsky et al., 2002). In these same cells, MRP6 mediated transport of GSH conjugates (LTC4 and DNP-SG) and the cyclic pentapeptide endothelin receptor inhibitor BQ123 but not of glucuronide conjugates (i.e., $E_2 17\beta G$), methotrexate, or cyclic nucleotides (Belinsky et al., 2002). Likewise, in MRP6 transfected Sf9 insect cells, MRP6 transported LTC_4 well (K_m = 600 nM) but showed only low level transport of *N*-ethylmaleimide *S*-glutathione (K_m = 282 μM) (Ilias et al., 2002). In contrast, under different conditions, Madon et al. (2000) reported that rat Mrp6 transports the endothelin receptor antagonist BQ-123 (K_m ~17 μM) but not LTC4, or DNP-SG. Little or no *MRP6* expression is detectable in human tumor specimens (e.g., intestine, testis, prostate, lung, adrenal gland, cervix, ovary, kidney, and melanoma) or human tumor cell lines, which suggests that MRP6 does not play an important role in tumor MDR (Kool et al., 1999a; Scheffer et al., 2002a).

G. MRP7

The MRP7 protein exhibits a membrane topology similar to that of MRP1, MRP2, MRP3, and MRP6 (Hopper et al., 2001). MRP7 mRNA was detected in a variety of tissues, with relatively higher levels reported in colon, skin, and testes (Hopper et al., 2001). Two *Mrp7* genes have also been identified in mice (*Mrp7A* and *Mrp7B*), and these show >80% amino acid similarity with their human counterparts (Kao et al., 2003).

The substrate specificity and resistance profile of MRP7 have been examined in MRP7-transfected HEK293 cells (Chen et al., 2003; Hopper-Borge et al., 2004). Drug resistance to docetaxel, and to a lesser degree paclitaxel, vincristine, and vinblastine, was reported (Hopper-Borge et al., 2004). MRP7 did not transport methotrexate, DNP-SG, monovalent bile salts (glycocholic acid and taurocholate) or cyclic nucleotides (cAMP and cGMP) (Chen et al., 2003); only modest LTC4 transport was noted. Likewise, MRP7 mediated low-affinity transport of $E_2 17\beta G$ (K_m = 58 μM) (Chen et al., 2003). Further studies examining substrate specificity, subcellular localization, and physiological function are needed to clarify the role, if any, that MRP7 may play in development of the MDR phenotype.

H. MRP8

MRP8 is the third MRP isoform without a third transmembrane domain in the amino-terminal portion of the protein. *MRP8* mRNA transcript is highly expressed in breast cancer but also shows a low level of expression in a variety of other human tissues including breast, testes, and the brain (Bera et al., 2001; Tammur et al., 2001; Yabuchi et al., 2001). In transfected MDCKII and HepG2 cells, MRP8 is localized at the apical pole (Bortfeld et al., 2006). Despite extensive searches within the mouse genome, a murine Mrp8 ortholog was not found (Shimizu et al., 2003), and MRP8 has yet to be identified in any other species. In MRP8-transfected LLC-PK1 cells, MRP8 confers resistance to the pyrimidine analogs 5′-fluoro-5′-deoxyuridine, 5′-fluorouracil, and 5′-fluoro-2′-deoxyuridine, but not to typical MRP1 substrates such as vincristine, doxorubicin, or etoposide. MRP8-transfected cells also showed increased resistance to PMEA, but not to other purine analogs such as 6-thioguanine (Guo et al., 2003).

The ability of MRP8 to actively extrude compounds and contribute to MDR was examined in human MRP8-transfected cells. Compared with non-MRP8-expressing controls, efflux of PMEA and cAMP was significantly higher in MRP8-overexpressing cells (Guo et al., 2003). The ability of MRP8 to mediate transport of the monophosphorylated metabolite of 5′-deoxy-5′-fluorouridine might represent a general mechanism for MRP8-mediated resistance to fluoropyrimidines (Guo et al., 2003). Because MRP8-transfected cells show no resistance to vincristine, doxorubicin, etoposide, or taxol, it is unlikely these compounds are MRP8 substrates. In contrast, MRP8 mediates transport of $E_2 17\beta G$, dehydroepiandrosterone 3-sulfate, as well as LTC4 and the monoanionic bile acids taurocholate and glycocholate, but not prostaglandin E_1 or E_2 (Chen et al., 2005b; Bortfeld et al., 2006). These studies indicate that the resistance profiles of MRP4, MRP5, and MRP8 are certainly similar, but not identical.

I. MRP9

Little is known about the newest member of the MRP family. Multiple transcript variants of the human *MRP9* gene have been independently described, ranging in size from 1.3 to 4.5 kilobases (Tammur et al., 2001; Yabuuchi et al., 2001; Bera et al., 2002). *MRP9* mRNA is expressed in a variety of adult tissues including brain, testes, and primary breast tumors, as well as the breast carcinoma cell line, GI-101 (Yabuuchi et al., 2001; Bera et al., 2002). *MRP9* mRNA is also widely expressed in fetal tissues such as liver, spleen, kidney, and lung (Yabuuchi et al., 2001). Recently Mrp9 was cloned in the mouse, and low levels of mouse *Mrp9* mRNA were detected in the brain, prostate, uterus, and stomach (Shimizu et al., 2003). Results from Northern blotting studies indicate that only testes shows significant expression of the mouse ortholog (Shimizu et al., 2003). Rats also seem to express *Mrp9* mRNA; however, functional characteristics of the rat ortholog remain to be examined (GenBank accession no. NM_199377).

The function and substrates of MRP9 have yet to be studied. Based on chromosomal location (16q12.1), both MRP8 and MRP9 are proposed to play a role in the pathogenesis of paroxysmal kinesigenic choreoathetosis (Tammur et al., 2001; Yabuuchi et al., 2001), a movement disorder characterized by abnormal involuntary movements (Bhatia, 2001). With the recent identification of the murine ortholog, generation of a knockout mouse is probably underway (Shimizu et al., 2003). This model should provide valuable information regarding the physiological significance of MRP9.

III. Multidrug Resistance-Associated Protein Expression and Function in the Central Nervous System

Over the last decade, studies examining the expression of MRPs in the brain have produced contradictory and often controversial results. Overall transporter function reflects several factors: location (within a tissue, as well as within cells), level of expression, substrate and inhibitor specificity, and functional kinetics. At first glance, the first two factors would seem to be cell-dependent and the second two transporter-dependent. However, molecular-level interactions with lipids, proteins, and small signaling molecules can influence transporter specificity and kinetics, making those aspects of function at least partially cell-dependent.

Ideally, one would want to assess all aspects of transporter expression and function in the intact tissue in situ. This is rarely practical, because of limited access and experimental tools that can be less than optimal for the job at hand (below). Simpler experimental systems, i.e., isolated tissue, cultured cells (primaries and cell lines), can help to overcome some of the limitations. However, as one moves farther away from the in situ situation one may acquire a false picture of transporter function as a result of altered expression levels and changed membrane environment.

For MRPs, functional assessment is further confounded by overlapping specificities and similar tissue expression profiles among MRP family members and between MRPs and members of other transporter families, particularly organic anion transporters (OATs) and organic anion transporting polypeptides (OATPs). Moreover, we lack inhibitors and substrates that are specific for certain MRPs or even for multiple family members. Thus, although one can establish expression of an MRP isoform within a tissue and localize the protein to certain cells and to a region of the plasma membrane, the contribution the transporter makes to transport of a specific substrate may be difficult to determine. In contrast, one research tool can provide a means to overcome this problem. That is, generation and use of animal models with altered expression of single MRP family members, e.g., Mrp1-null mice, Mrp4-null mice, and TR^- rats (natural mutation; these animals do not express Mrp2). In the absence of specific compensation, these can provide unambiguous evidence of loss of transport function. But, it is important to remember that in many tissues, transport of MRP substrates may be also distributed over multiple transporters, including some OAT and OATP members.

Despite these difficulties, a large body of evidence does indicate that all of the functionally characterized MRP isoforms (1–8) are expressed in at least one CNS compartment and that they probably play a role in transport of drugs and metabolites (Table 2). However, it should not be surprising that there is substantial controversy in the field about the localization and function of certain family members. The following sections provide a comprehensive summary of current knowledge with respect to MRP expression and function in the different cellular compartments of the CNS.

A. The Blood-Brain Barrier

The cells of the CNS are particularly sensitive to chemical injury and thus require a protected and highly regulated extracellular environment. It has been known for over a century that exchange of solutes between blood and brain is restricted, and known for approximately 40 years that the site of the barrier is in the brain capillary endothelium. It has also been long appreciated that the functional unit of the blood-brain barrier (BBB) includes more than just capillary endothelial cells. Several other cell types are in constant and intimate contact with the endothelium and development and maintenance of the brain capillary phenotype (electrically tight, nonfenestrated endothelium with characteristic tight junctions and high expression of xenobiotic transporters) seems to be critically dependent on interactions with other cells found closely associated with brain capillaries, e.g., pericytes and astrocytes.

TABLE 2
Brain tissue distribution of MRP family members

Protein and/or gene expression of MRP/Mrp isoforms in various brain compartments was compiled from reverse transcription-polymerase chain reaction, immunohistochemical, and immunoblotting studies (see text for details and references); (−) indicates negative or negligible expression reported.

Isoform	Peripheral Sites of Expression	CNS Expression				
		WB	BBB	CP	MG	AST
MRP1	Ubiquitous (high expression in lungs, kidney, testes)	D, H, M, P, R	B, H, M, P, R	H, M, R	R	H, M, R
MRP2	Liver, kidney, gut	P, R	F, H, P, R	M, R (−)	R (−)	H, R (−)
MRP3	Small and large intestine, kidney, pancreas, prostate, placenta	R	B	M, R	R	R
MRP4	Prostate, kidney, lungs, pancreas, testis, ovary	H, M, R	B, H, M	H, M, R	R	H, R
MRP5	Ubiquitous (high levels in skeletal muscle, heart)	H, M, R	B, H, M	M, R	R	H, R
MRP6	Kidney, liver (low levels in most other tissues)	H, M (−)	B	M, R (−)	R (−)	H, R (−)
MRP7	Skin, colon, testes, spleen	H, M	N.D.	N.D.	N.D.	N.D.
MRP8	Breast, testes, liver, placenta	H	N.D.	N.D.	N.D.	H (−)
MRP9	Testes, skeletal muscle, ovary	H, M (−)	N.D.	N.D.	N.D.	N.D.

WB, whole brain homogenate; BBB, blood-brain barrier, CP, choroid plexus; D, dog; MG, microglia; AST, astrocytes; H, human; M, mouse; R, rat; B, bovine; P, porcine; F, fish; R, rat; N.D: not determined.

Two elements have been traditionally considered responsible for the barrier function of the brain capillary endothelium: very tight, tight-junctions (nonfenestrated endothelium), which form an effective seal to paracellular diffusion, and the cells themselves, which exhibit a low rate of endocytosis. Over the past decade it has become increasingly evident that superimposed upon this passive barrier is a selective, metabolism-driven barrier that largely reflects expression and function of ABC transporters (Begley, 2004). Among these transporters, P-gp is the best studied example. High levels of expression, luminal membrane localization, high transport potency, and affinity for a large number of commonly prescribed drugs make this ABC transporter a formidable element of the selective BBB. Consistent with these findings, experiments with P-gp-null mice show order of magnitude or larger increases in brain accumulation of a large number of drugs (Schinkel et al., 1996).

P-gp is not the only important contributor to the selective barrier. As discussed below, there is certainly evidence for participation of several MRP isoforms. MRPs are clearly expressed at the BBB (Fig. 2). However, for most MRP isoforms, there is still considerable discussion about at least one of the following: mRNA and protein expression levels, subcellular localization of the protein and its involvement in transport of specific drugs (Begley, 2004; Fricker and Miller, 2004; Graff and Pollack, 2004; Loscher and Potschka, 2005a). The lack of consistent data may reflect species differences in sub-

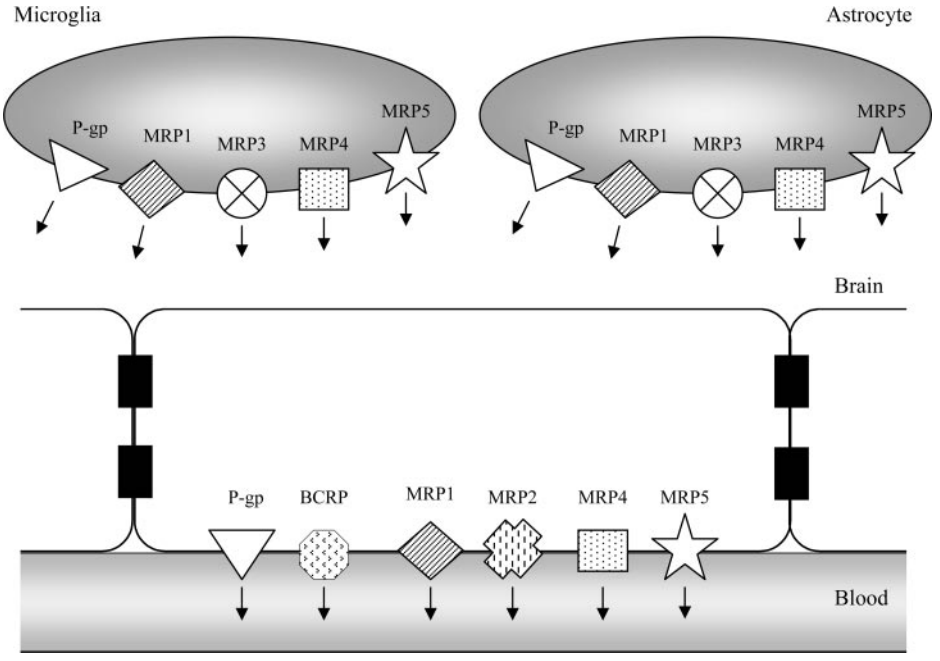

FIG. 2. Proposed localization of P-gp, BCRP, and MRP isoforms in BBB endothelium, microglia, and astrocytes. P-gp, BCRP, MRP1/Mrp1, MRP2/Mrp2, MRP4/Mrp4, and MRP5/Mrp5 are present in the luminal (apical) membrane of brain endothelial cells of various species. In glial cells (i.e., astrocytes and microglia), gene and/or protein expression of P-gp, MRP1/Mrp1, Mrp3, MRP4/Mrp4 and MRP5/Mrp5 has been confirmed by our group and others. Glial expression of MRP2/Mrp2, MRP6/Mrp6, and BCRP is probably negligible. References are indicated in the text.

strate specificity, metabolism and/or transporter expression, use of insensitive or less than specific antibodies, and substantial differences in expression levels between brain capillaries in in situ and in vitro systems, e.g., isolated brain capillaries, endothelial cells in primary culture, and endothelial cell lines (Regina et al., 1998; Torok et al., 2003).

Because MRPs pump substrates out of cells, i.e., they are ATP-driven efflux transporters, only those MRP proteins localized to the luminal plasma membrane of brain capillary endothelial cells will contribute to the barrier and excretory functions of the tissue. Thus, although a case can be made for expression of mRNA and perhaps protein for MRP1 through MRP6 in brain capillary endothelial cells from at least one species (see Graff and Pollack, 2004; Loscher and Potschka, 2005b), firm evidence for luminal membrane localization has been obtained only for MRP1/Mrp1 (in cow and human), Mrp2 (in rat, but not detected in cow, human, or rats which do not express Mrp2), MRP4/Mrp4 (in mouse, cow, and human), and MRP5/Mrp5 (in human and cow) (Miller et al., 2000; Zhang et al., 2000, 2004; Leggas et al., 2004; Nies et al., 2004; Bronger et al., 2005).

Functional evidence for involvement of MRPs in the barrier and excretory functions of the BBB is hard to come by, and this certainly reflects the lack of isoform-specific substrates and inhibitors. Organic anion transport inhibitors that are also MRP inhibitors, e.g., probenecid and MK571, have been shown to increase drug accumulation in brain or to inhibit efflux from endothelial cell monolayers (Gutmann et al., 1999; Potschka et al., 2001; Potschka and Loscher, 2001; Sun et al., 2001). Consistent with this finding, Sugiyama et al. (2003) found reduced efflux of the endogenous metabolite $E_2 17\beta G$ in Mrp1 knockout mice after intracerebral microinjection. However, other experiments with Mrp1 knockout mice show no increase in brain penetration of the organic anion fluorescein (Sun et al., 2001) or the efflux of etoposide (Cisternino et al., 2003) or morphine-6 β-D-glucuronide (Bourasset et al., 2003), all transported by Mrp1, although some poorly.

For Mrp2 there is a naturally occurring knockout in two strains of rats (TR^- and Esai hyperbilirubinemic rats), which are models for human Dubin-Johnson syndrome. Potschka et al. (2003b) found increased brain accumulation of the antiepileptic agent, phenytoin, in TR^- rats compared with normal rats. In those experiments, plasma phenytoin levels were the same in both groups of animals, so it seems that Mrp2 is an important determinant of phenytoin penetration into the brain. From these findings, it is clear that further studies are needed to clarify the location and functional significance of the various MRP isoforms in the BBB.

In addition to MRPs, several drug-metabolizing enzymes have been shown to be expressed in the barrier including epoxide hydrolase, glutathione *S*-transferase, and various isoforms of the cytochrome P450 and UDP-glucuronosyltransferase families (Ghersi-Egea et al., 1994; Lawrenson et al., 1999; Miksys and Tyndale, 2002; Granberg et al., 2003). Biotransformation of foreign compounds via these phase I and II enzymes might result in metabolites that can then be removed from the brain by efflux transporters such as MRPs. In this way, metabolic enzymes and active transporters can act in concert as a biochemical barrier to remove potentially harmful compounds from the brain environment. The pharmacological significance of the metabolic barrier remains to be determined.

B. The Blood-Cerebrospinal Fluid Barrier

The choroid plexuses (CPs) are highly vascularized, leaf-like structures that protrude into the lateral, third, and fourth ventricles and form the major interface between cerebrospinal fluid (CSF) and the blood (Segal, 2000). The CP epithelium is composed of fenestrated capillaries, surrounded by a single layer of epithelial cells joined by tight junctions. Like the BBB, tight junctions between the epithelial cells restrict movement through the paracellular route, although these are not nearly as tight as those in brain capillaries. The CPs secrete CSF into the ventricles, thereby providing a fluid "cushion" for the brain (Segal, 2000). Additional functions of the CSF include nutrient supply, regulation of osmolarity, provision of neuroactive peptides, and metabolic waste removal (Strazielle et al., 2004). The composition of the CSF is rigorously maintained, and thus entry and exit of substances into the CSF are tightly regulated. Given its role in maintaining CSF homeostasis, it is not surprising that a variety of transport proteins are present in CP epithelium including ion channels, carriers of nonelectrolytes, nutrients, and neurotransmitters (Lee et al., 2001a; Graff and Pollack, 2004).

The CPs also express several drug transporters. CP expression of P-gp has been confirmed (Fig. 3), but this transporter has been localized mainly to intracellular compartments (Rao et al., 1999). An assortment of transporters belonging to the solute-carrier superfamily are also expressed in the apical and basolateral membranes of the CP epithelium including oatp/OATPs, OATs, and organic cation-organic cation/carnitine transporters (Ghersi-Egea and Strazielle, 2002; Graff and Pollack, 2004; Kusuhara and Sugiyama, 2004). Like the BBB, various drug-metabolizing enzymes have been identified in the CP including glutathione *S*-transferase, UDP-glucuronosyltransferase, and several isoforms of the cytochrome P450 family (Ghersi-Egea et al., 1994; Miksys and Tyndale, 2002). The activity of these enzymes is very high, so the CP has been postulated to be a major site of xenobiotic metabolism in the brain (Ghersi-Egea et al., 1995). As with the BBB, the extent to which metabolism contributes to the blood-CSF barrier remains to be established.

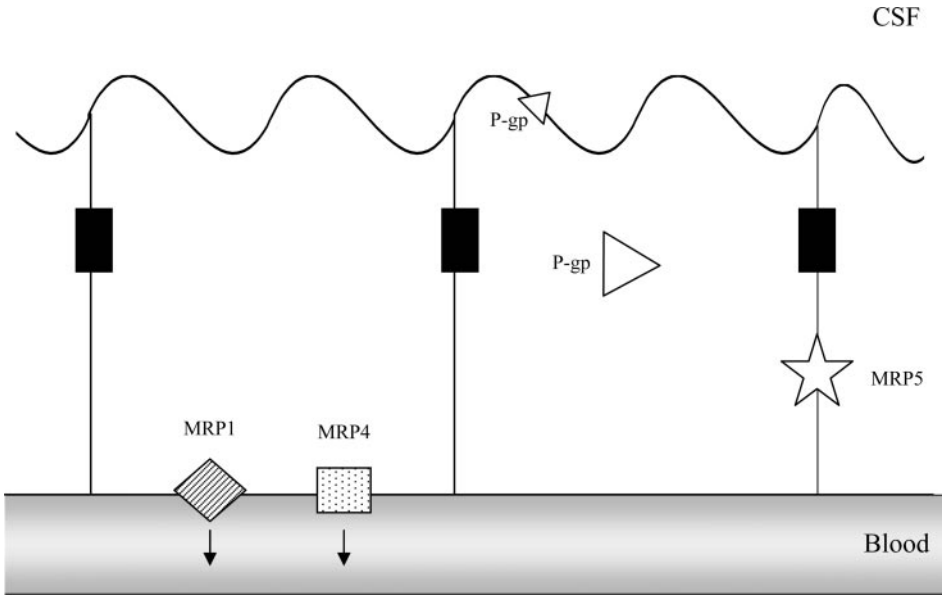

FIG. 3. Localization of P-gp and MRP isoforms in the CP epithelia. MRP1/Mrp1 and MRP4/Mrp4 are localized to the basolateral membranes of rat, mouse, and human CP epithelia. Mrp5 gene expression has been confirmed in mouse and rat CP, but subcellular protein localization has yet to be determined. Based on its location in polarized epithelia of hepatocytes and kidney proximal tubules, one might speculate that Mrp5 will be present on the blood side along with MRP1/Mrp1 and MRP4/Mrp4. P-gp seems to be mainly found within intracellular vesicular compartments but is also found to a small degree on the apical pole. Expression of the remaining MRP isoforms is either negligible or unexamined. Tight junctions located between epithelial cells are shown as black bars. References are indicated in the text.

MRP1/Mrp1 gene expression has been detected in human, mouse, and rat CP (Nishino et al., 1999; Rao et al., 1999; Sisodiya et al., 2001; Wijnholds et al., 2000a; Choudhuri et al., 2003; Mercier et al., 2004; Soontornmalai et al., 2006). By using immunocytochemical studies, Mrp1 was localized to the basolateral membranes of cultured rat CP cells and in mouse brain slices (Rao et al., 1999; Wijnholds et al., 2000a; Soontornmalai et al., 2006). When grown in vitro as confluent monolayers, rat CP cells show increased basal-to-apical transepithelial flux of ^{99m}Tc-sestamibi (a nonspecific MRP1 substrate) and accumulate more probe in the presence of the general MRP inhibitor MK571 (Rao et al., 1999). After intravenous dosing, triple knockout mice (Mrp1$^{-/-}$/Mdr1a$^{-/-}$/Mdr1b$^{-/-}$) show a 10-fold increase in etoposide CSF concentrations, compared with double knockout mice (Mdr1a$^{-/-}$/Mdr1b$^{-/-}$) (Wijnholds et al., 2000a), further demonstrating the presence of a functional Mrp1 isoform on the basolateral side, which drives organic anion efflux into the blood.

After intracerebroventricular administration, CSF concentrations of DNP-GS and $E_2$17βG were comparable in Mrp1 knockout (Mrp1$^{-/-}$) and wild-type mice (Mrp1$^{+/+}$) (Lee et al., 2004). Elimination of both substrates from the CSF was, however, probenecid-sensitive, suggesting that organic anion transporters other than Mrp1 mediate their efflux from the CSF (Lee et al., 2004). Mrp2, Mrp3, and Mrp6 were not considered candidates since expression of these MRP isoforms in rodent CP is negligible (Choudhuri et al., 2003; Lee et al., 2004). On the other hand, CP *Mrp4* and *Mrp5* mRNA levels are high (Choudhuri et al., 2003; Lee et al., 2004), and both isoforms have shown some ability to transport conjugated organic anions (Wijnholds et al., 2000b; Zelcer et al., 2003). One likely explanation for the lack of effect of knocking out Mrp1 is that the substrates examined are primarily handled by Oat and Oatp family members, e.g., apical Oat3 and Oatp3, and basolateral Oatp2. Another possibility is specific compensation. The ability of one MRP isoform to compensate for the absence of another has been reported previously in the liver and kidney for human, mice, and rats deficient in MRP2/Mrp2, i.e., up-regulation of MRP3/Mrp3 and/or Mrp4 (Konig et al., 1999b; Kuroda et al., 2004; Chen et al., 2005a; Chu et al., 2006).

Although expression of MRP4/Mrp4 in CP is certain, the exact location of the proteins has yet to be conclusively established. Recently MRP4/Mrp4 was localized to the basolateral (blood) side of intact mouse, rat, and human CP using a monoclonal antibody that recognizes amino acids 372 through 431 of the human MRP4 protein (Leggas et al., 2004). Since MRP4 has been localized to the apical pole of human brain endothelial cells and human, rat, and mouse kidney proximal tubules (van Aubel et al., 2002; Leggas et al., 2004; Nies et al., 2004), but the basolateral side of tubuloacinar cells (Lee et al., 2000), these studies would seem to support the hypothesis that the polarity of MRP4/Mrp4 expression is cell-specific. The expression of MRP4/Mrp4 in the apical and basolateral membranes of the BBB and CP, respectively, indicates a role for this transporter in limiting organic anion influx from blood and in driving organic anion efflux from the brain to blood.

C. Microglia

First described by the Spanish neuroanatomist del Rio-Hortega (1932), microglia represent up to 20% of the total CNS glial population (Lawson et al., 1990; Raivich et al., 1999). Microglia exist in the CNS in several morphologically distinct forms, including ramified (resting), spheroid (activated), and phagocytic (reactive) types (Thomas, 1992). Normally, microglia are in a quiescent resting state, acting as a "sensor" of the brain microenvironment. Ramified microglia possess a small cell body and are highly branched. After injury or infection, microglia are activated, which results in retraction of processes, proliferation, and up-regulation of several cell surface factors (Hanisch, 2002; Liu and Hong, 2003). Progression of activated microglia to a phagocytic state is dependent on the severity of brain injury. In this way, microglia show remarkable functional plasticity (Streit et al., 1988). For example, following reversible axotomy (crushing of the nerve), microglia proliferate and surround the nerves while secreting soluble trophic factors such as basic fibroblast growth factor and nerve growth factor (Heumann et al., 1987; Gomez-Pinilla et al., 1990; Araujo and Cotman, 1992). Increased expression of various integrins and major histocompatibility complex class I markers also occurs. In this scenario microglia seem to play a neuroprotective role in the spheroid or activated stage and aid in the recovery of reversibly damaged neurons. Conversely, ricin-induced degeneration of neurons (an irreversible and lethal event) results in microglia becoming fully activated phagocytes. This stage of activation is characterized by a significant increase in the expression of phagocytic stage markers including several integrins ($\alpha5\beta1$, $\alpha6\beta1$, and $\alpha M\beta2$), and major histocompatibility complex class I and II antigens. Excessive microglial activation is implicated in the pathogenesis of a variety of neurodegenerative diseases including HIV-associated dementia (HAD), Parkinson's disease, and Alzheimer's disease (McGeer and McGeer, 1998; Akiyama et al., 2000; Xiong et al., 2000; Liu and Hong, 2003; Block and Hong, 2005).

The ability of microglia to metabolize and transport compounds is not well characterized. Compared with cultured astrocytes and neurons, cultured microglia express higher intracellular levels of GSH, higher specific activity of GSH reductase and peroxidase, but limited catalase activity (Hirrlinger et al., 2000). Microglia also express a variety of ion channels (e.g., potassium and sodium) and receptors (Gottlieb and Matute, 1997; Eder, 1998; Noda et al., 2000). Microglial gene expression of Mrps (Fig. 2) was first demonstrated in 2002 (Ballerini et al., 2002; Hirrlinger et al., 2002a). Subsequent studies by our group confirmed expression of Mrp1, Mrp4, and Mrp5 mRNA and protein within a cultured rat microglia cell line, MLS-9 (Dallas et al., 2003, 2004b). These cells also express a functional P-gp protein (Lee et al., 2001b). In agreement with Hirrlinger et al. (2002a), we could not detect mRNA or protein for Mrp2 or Mrp6 in primary cultures of rat microglia or in the microglial cell line.

By using immunogold cytochemistry at the electron microscope level, Mrp1 protein was found primarily in the plasma membrane of the MLS-9 cells (Dallas et al., 2004a). The novel finding that Mrp1 also localized in smooth membrane caveolae and clathrin-coated vesicles in the plasma membrane of cultured microglia cells is particularly interesting (Dallas et al., 2004a). In a variety of cell types, caveolae and clathrin-coated vesicles are associated with endocytotic and pinocytotic transport as well as cell signaling (Gonzalez-Gaitan and Stenmark, 2003). Caveolins (1, 2, and 3) are the structural proteins associated with caveolae. In rat brain capillaries and the rat brain endothelial cell line RBE4, we previously reported P-gp localization in caveolae and clathrin-coated vesicles using immunogold cytochemistry and electron microscopy (Bendayan et al., 2002). Applying confocal microscopy, Virgintino et al. (2002) also observed colocalization of P-gp and caveolin-1 in the luminal side of isolated human microvessels. Likewise, Demeule et al. (2000) and Jodoin et al. (2003) reported colocalization of P-gp and caveolin-1 in isolated rat brain microvessels and in a bovine brain microvessel endothelial cell/astrocyte coculture system. Furthermore, the expression of caveolin-1 directly modulated the functional activity of P-gp (Jodoin et al., 2003). Recently, we have confirmed the expression of caveolin-1 and colocalization with P-gp in primary cultures of rat astrocytes, suggesting that caveolae represent an important membrane domain for transporter localization in brain parenchyma (Ronaldson et al., 2004a). Additional studies are needed to establish whether caveolin-1 also colocalizes with Mrp1 and other Mrps in brain cellular compartments including microglial cells. The functional relevance of these findings remains to be determined.

The Mrp proteins present in cultured rat microglia seem to be functional and display transport properties comparable with those reported previously in other cells types (Dallas et al., 2003, 2004b). That is, the cells displayed saturable, ATP-dependent uptake of vincristine, which was stimulated by a variety of MRP/organic anion inhibitors (i.e., MK571, genistein, probenecid, and sulfinpyrazone). As expected, intracellular depletion of GSH using an irreversible inhibitor of γ-glutamyl cysteine synthetase, i.e., 25 μM buthionine sulfoximine, resulted in a significant increase in vincristine accumulation by the cells. In addition, efflux of the acyclic nucleoside analog PMEA by microglia was rapid, ATP-dependent, but GSH-independent. PMEA efflux was also inhibited significantly by genistein, indomethacin, probenecid, sulfinpyrazone, and zidovudine monophosphate, suggesting the involvement of Mrp4/Mrp5 (Dallas et al., 2004b). Taken together, these studies support functional expression of at least four different ABC transporters in microglia (i.e., Mrp1, Mrp4, Mrp5, and P-gp), cells that are particularly important in pharma-

coresistance development in HIV patients (discussed below).

D. Astrocytes

First named for their star-shaped appearance, astrocytes display a variety of morphologies and account for 40% of the cells present in the brain. These cells are generally divided into two distinct groups based on morphology and location within the CNS (Privat and Rasaboul, 1986). Protoplasmic astrocytes, found in gray matter regions, are spheroid in shape, contain clumped chromatin, and have many highly branched processes. Fibrous astrocytes, found in the white matter, are characterized by oval nuclei with evenly dispersed chromatin and have a less complex branching pattern. Astrocytes perform a number of essential "housekeeping" functions in the brain including glutamate uptake and release, free radical scavenging, water transport, and ion buffering (Chen and Swanson, 2003). In situ, astrocyte foot processes are in close contact with endothelial cells that form the BBB and provide both functional and structural support to these cells (Goldstein, 1988). Like microglia, astrocytes can become reactive (reactive astrogliosis) following brain injury or infection (Eddleston and Mucke, 1993). Reactive astrocytes show morphological changes, increased production of soluble factors (e.g., growth factors, proteases, inflammatory cytokines, and metabolic enzymes), and increased proliferation. Reactive astrocytes may contribute to the pathogenesis of a variety of neurodegenerative diseases, including Alzheimer's, epilepsy, and HAD (D'Ambrosio, 2004; Kramer-Hammerle et al., 2005; Mrak and Griffin, 2005). Metabolically, astrocytes are very active, expressing a number of enzymes, including glutathione *S*-transferase, catalase, and several cytochrome P450 isoforms (Moreno et al., 1995; Sagara and Sugita, 2001; Miksys and Tyndale, 2002).

Several studies have demonstrated expression of Mrp1 mRNA transcript and protein in primary rat astrocyte cultures (Decleves et al., 2000; Hirrlinger et al., 2001, 2002a). Immunocytochemical studies confirmed that in many cases cells that were immunoreactive for Mrp1 protein also expressed the astrocyte marker glial fibrillary acidic protein (GFAP) (Hirrlinger et al., 2001). GFAP is an astrocyte-specific marker commonly used for identification in mixed glial cultures (Eng et al., 2000). Mrp1 staining of GFAP-negative cells probably represents contaminating microglial cells (up to 10%), generally found in astrocyte cultures. The Mrp protein(s) present in rat astrocytes may be functional since fluorescein accumulation increased considerably in the presence of the nonspecific organic anion transport inhibitors, indomethacin, probenecid, and sulfinpyrazone (Decleves et al., 2000).

Following generation of a hydrogen-peroxide induced oxidative stress, astrocytes release high levels of GSSG, a known MRP1 substrate (Hirrlinger et al., 2001). Addition of 20 μM MK571 to astrocyte cultures decreases GSSG efflux by 50%, suggesting the involvement of Mrp1 and/or another Mrp isoform. Efflux of reduced GSH by astrocytes is also inhibited by MK571 at concentrations >10 μM (Hirrlinger et al., 2002c). Given these results, Mrp1 may play an essential role in maintaining GSH concentrations and redox balance of astrocytes during oxidative stress (Hirrlinger et al., 2001, 2002c). Incomplete inhibition of GSH and GSSG efflux from primary astrocyte cultures in the presence of high concentrations of MK571 (50 μM) suggests that other transporters (e.g., Mrp4) might also contribute to thiol removal (Hirrlinger et al., 2002c).

Recently Gennuso et al. (2004) have implicated regulation of astroglial Mrp1 protein expression in the etiology of neonatal bilirubin encephalopathy. Modest levels of hyperbilirubinemia seem to be neuroprotective for infants; however, severely jaundiced newborns accumulate high levels of unconjugated bilirubin in astrocytes and neurons, leading to disruption of cellular functions and neuronal cell death (Ostrow et al., 2004). In cultured mouse astrocytes, concentrations of unconjugated bilirubin below the compound's aqueous saturation point (i.e., <70 nM) up-regulates Mrp1 protein expression and promotes trafficking of the protein from intracellular compartments to the plasma membrane (Gennuso et al., 2004). Presumably this trafficking prevents accumulation of unconjugated bilirubin within the astrocytes; MRP1 is confirmed to mediate transport of unconjugated bilirubin by MRP1-overexpressing cells and in $Mrp1^{-/-}$ knockout mice (Rigato et al., 2004; Calligaris et al., 2006). In contrast, bilirubin concentrations modestly above the solubility cutoff (i.e., 145 nM), resulted in the absence of Mrp1 trafficking and promoted a loss in astrocyte plasma membrane integrity, decreased mitochondrial function, and increased cell apoptosis (Gennuso et al., 2004).

Studies examining expression of the remaining Mrp isoforms in cultured astrocytes have been somewhat contradictory. Hirrlinger et al. (2002a) detected expression of *Mrp1*, *Mrp3*, *Mrp4,* and *Mrp5*, but not *Mrp2* mRNA in astrocytes from neonatal Wistar rat pups. In contrast, Ballerini et al. (2002) reported detection of *Mrp1* through *Mrp6* mRNA in astrocytes isolated from rat fetuses. Differences in prenatal versus postnatal expression of some Mrp isoforms might explain these discrepancies (Kao et al., 2002; Yabuuchi et al., 2002). Human astrocytes maintained in culture show considerable amounts of MRP1 protein expression when probed with the MRP1-specific monoclonal antibody MRPr1 (Spiegl-Kreinecker et al., 2002). However, MRP1 expression in resting astrocytes in situ has yet to be established (Aronica et al., 2003; Nies et al., 2004). Recently Nies et al. (2004) showed strong MRP4 and MRP5 staining in both resting and reactive astrocytes present in resected perilesional glioma and cerebral hemorrhage biopsy samples. In contrast, no MRP2, MRP3, or MRP6

staining was noted in the same tissue samples (Nies et al., 2004). Further studies are needed to clarify the expression patterns and physiological function of MRPs in normal, healthy astrocytes and how these transporters may be regulated in various disease states.

E. Neurons and Oligodendrocytes

Neurons form the basic structural and functional component of the CNS. The primary function of neurons is to respond to stimuli by conducting electrical signals along conductive processes, i.e., the axon. The conduction of electrical impulses results in the release of neurotransmitters that further regulate (positively and negatively) nearby neuronal responses (Ludwig and Pittman, 2003). In this way, the brain maintains a complex communication network. In the CNS, oligodendrocytes are responsible for the formation of myelin around the axons of neurons, which aid in the propagation of neuronal impulses and maintain this communications array (Jessen, 2004). Neurodegenerative diseases are characterized by a progressive loss of neurons due to apoptosis, often as a direct result of inflammatory events mediated by astrocyte or microglial activation (Jellinger, 2003; Block and Hong, 2005).

Neurons and oligodendrocytes express a variety of metabolic enzymes including GSH reductase, GSH peroxidase, catalase, and various cytochrome P450 isoforms (Cammer et al., 1991; Ravindranath et al., 1995; Hirrlinger et al., 2002b). Not surprisingly these cells also express an assortment of transport proteins including those for lipids (Schmitz and Kaminski, 2002; Tanaka et al., 2003), glutamate (Domercq et al., 1999; Kanai and Hediger, 2003), and amino acids (Braissant et al., 2001; Mackenzie and Erickson, 2004). In particular, transport of neurotransmitters by neurons in the CNS has been extensively examined (Raiteri et al., 2002). In general, the transport characteristics of pharmacological agents by uptake (e.g., organic cation transporters and OATs) or efflux (e.g., MRPs, P-gp, and BCRP) transporters in "healthy" neurons and oligodendrocytes have not been thoroughly examined.

Primary cultures of rat striatal and mouse cortical neurons may express an Mrp-like transporter (DeCory et al., 2001). Bimane-glutathione efflux by these cultures was decreased significantly by MK571 and probenecid. However, the authors failed to detect Mrp1 protein in the cells using polyclonal antibodies recognizing either the carboxyl terminus of human and mouse MRP1/Mrp1 or the aminoproximal portion of mouse Mrp1 (DeCory et al., 2001). This finding might indicate that another Mrp isoform is present in mouse and rat neuronal cultures that also has the capacity to transport bimane-glutathione or that the specificity of the antibodies is poor. Studies by Hirrlinger et al. (2002a) have detected multiple *Mrp* mRNA transcripts in primary cultures of embryonic rat brain neurons and oligodendrocytes including *Mrp1*, *Mrp3*, *Mrp4,* and *Mrp5*, but not *Mrp2* or *Mrp6*. Immunohistochemical studies in neurons from normal human tissue adjacent to dysembryoplastic neuroepithelial tumors, glioblastomas, and cerebral hemorrhages support the presence of MRP4, MRP5, and MRP8, but not MRP2 protein in human brain sections (Nies et al., 2004; Vogelgesang et al., 2004; Bortfeld et al., 2006). In contrast, neurons adjacent to MRP1-staining dysplastic neurons (from resected epileptic or glioma tissue samples) are devoid of MRP1 (Sisodiya et al., 2001; Aronica et al., 2003; Nies et al., 2004). Likewise, perilesional tissue obtained from surgical resections of gliomas and cerebral hemorrhage showed no MRP3 expression and inconsistent MRP6 staining in pyramidal neurons (Nies et al., 2004). Neuronal MRP6 mRNA and protein were noted in normal tissue arrays (Beck et al., 2005). The apparent discrepancies observed between the in vitro and in situ studies suggests that neuronal expression of MRPs may be 1) species specific, 2) disease-dependent (i.e., presence and stage of disease) (see sections IV.A. and IV.B.), 3) low in healthy tissue, and 4) altered during in vitro culture.

IV. Clinical Relevance of Multidrug Resistance-Associated Proteins in the Central Nervous System

Treatment of neurological disorders requires penetration of pharmacological agents through the BBB and/or blood-CSF barriers and access to the appropriate brain parenchymal target(s). In patients, development of cellular drug resistance or the MDR phenotype occurs through a variety of mechanisms including increased metabolism, alteration of target proteins, increased CNS elimination, and decreased cellular drug accumulation (Dean et al., 2001). In the CNS, several members of the ABC transporter family including P-gp, BCRP, and MRPs could certainly contribute to the MDR phenotype. The following sections summarize the potential role of MRP proteins in the pathophysiology and pharmacological treatment of several neurological disorders.

A. Epilepsy

Epilepsy defines a group of chronic neurological disorders characterized by recurrent seizures. It is one of the most commonly diagnosed neurological disorders, affecting 1 to 2% of the world's population according to the World Health Organization (http://www.who.int). Approximately 30% of epileptic patients are nonresponsive to current treatment regimens (Regesta and Tanganelli, 1999). Although the reasons for the observed resistance to antiepileptic drugs (AEDs) is probably multifactorial (Sisodiya et al., 2002), increasingly evidence supports increased expression of various MRP isoforms as one contributory mechanism [see Loscher and Potschka (2002) for a detailed account of the role of drug transporters in AED pharmacoresistance]. Immunocytochemical studies have positively identified the MRP1

protein in dysplastic neurons, reactive astrocytes, and balloon cells (glial elements of focal cortical dysplasia) of malformations commonly observed in refractory epilepsy, i.e., human focal cortical dysplasia, dysembryoplastic neuroepithelial tumors, and hippocampal sclerosis samples (Sisodiya et al., 2001, 2002; Aronica et al., 2003). Generally, the MRP1 staining was more prominent in the epileptic lesions, compared with surrounding normal tissue samples. Using "small-number" cDNA arrays, *MRP2*, and *MRP5* genes were shown to be up-regulated in temporal lobectomies of treatment-experienced epileptic patients compared with nonepileptic control tissues: human aneurysm domes or umbilical vein vessels (Dombrowski et al., 2001). It is notable that gene expression of *MRP1* and *MRP3* in this same study was not significantly different between the epileptic and nonepileptic tissues; nonetheless, in these tissue samples absolute levels of *MRP1* were higher than those of all other efflux transporters examined, including P-gp. Finally, dysembryoplastic neuroepithelial tumors from patients undergoing AED treatment with various combinations of carbamazepine, oxcarbazepine, tiagabine, and lamotrigine, also exhibit increased MRP2 and MRP5 protein expression, compared with peritumoral tissue or samples obtained from patients diagnosed with arteriovenous malformations (Vogelgesang et al., 2004). Given that some AED medications are known to up-regulate transporter expression in peripheral compartments [e.g., carbamazepine-induced induction of intestinal MRP2 mRNA and protein expression (Giessmann et al., 2004)], it is unclear whether the altered transporter expression observed in these various studies is due to the underlying disease, the therapies used to treat the disease, or a combination of the two.

It is important to note that in brain microdialysis studies in rats and rabbits, probenecid (a general organic anion inhibitor) enhanced the extracellular concentrations of the AEDs carbamazepine, phenytoin, and valproate, which could suggest involvement of at least one Mrp isoform in the CNS distribution of these compounds, probably Mrp1 and/or Mrp2 (Scism et al., 2000; Potschka and Loscher, 2001; Potschka et al., 2001). Interestingly, by using the same methodology, brain extracellular concentrations of carbamazepine, lamotrigine, and felbamate were not found to be significantly different between Mrp2-deficient TR^-, and age-matched control Wistar rats (Potschka et al., 2003a). Given the redundant nature of transporter expression, a compensatory up-regulation of another known, or yet to be discovered transporter, cannot be excluded as one possible explanation for the observed lack of effect. Alternatively, species differences in substrate affinity, metabolism, and/or transporter expression may also contribute to the observed differences. In vivo studies using transgenic models, as well as in vitro studies in MRP/Mrp-overexpressing cell lines, are certainly warranted to further clarify the role of MRPs in AED brain distribution and pharmacoresistance.

B. Brain Cancer

Brain tumors are among the most difficult cancers to treat effectively. Even in instances when chemotherapeutic agents can penetrate the BBB in sufficient quantities, the tumors themselves provide further drug resistance through a variety of cellular mechanisms, including alterations in drug-metabolizing enzymes, alterations in drug target specificity, and expression of various drug transporters (Bredel and Zentner, 2002). Expression of the MRP1 protein specifically has been verified in multiple human brain tumor types including astrocytomas, glioblastomas, meningiomas, neuroblastomas, and oligodendrogliomas (Norris et al., 1996; Abe et al., 1998; Goto et al., 2000; Mohri et al., 2000; Tews et al., 2001; Benyahia et al., 2004). Mohri et al. (2000) observed both MRP1 mRNA and protein expression in 50 and 90% of chemotherapy-naive grade III anaplastic astrocytomas and grade IV glioblastomas, respectively (see Kleihues et al., 1993, for a review of the World Health Organization tumor classification system). Expression of MRP1 in low-grade astrocytomas has not been consistently observed, suggesting that MRP1 expression in grade II gliomas is probably near the detection limit of the assays used (Abe et al., 1998; Mohri et al., 2000; Haga et al., 2001). The apparent induction of MRP1 protein expression noted in late-stage tumors would seem to support this notion; i.e., 90% of tumor cells showed positive MRP1 staining in glioblastoma multiform (grade IV) versus less than 10% staining in grade II oligodendrogliomas (Benyahia et al., 2004). Likewise, significant up-regulation of *MRP1* and *MRP3* has also been demonstrated in malignant gliomas (anaplastic astrocytomas and glioblastomas), compared with epileptic control tissue or low-grade astrocytomas (Haga et al., 2001; Spiegl-Kreinecker et al., 2002). Interestingly, at least one immunohistochemical study failed to detect significant amounts of MRP3 protein in human glioma samples, despite the presence of high *MRP3* gene levels (Bronger et al., 2005). This finding highlights an important problem associated with the *MRP* genes: mRNA levels do not always reflect absolute protein levels observed in a given cell or tissue (Mottino et al., 2000; Slitt et al., 2003). An intrinsic increase in MRP1 expression in high-grade tumors could explain, in part, the lack of therapeutic efficacy observed in patients despite receiving aggressive chemotherapeutic regimens. In addition, the ability of the regimens themselves to induce MRP1 probably contributes to overall tumor cell resistance. Indeed, Abe et al. (1998) reported that 70% of gliomas from chemotherapy-naive patients express MRP1 protein, whereas aggressive chemotherapy resulted in 100% of gliomas obtained from these same patients showing MRP1-positive cell expression, post-therapy. Recently, expression of several other MRP iso-

forms was reported in resected human glioma samples. MRP3, MRP4, MRP5, and MRP8 mRNA and protein were confirmed to be present in astrocytic and oligodendroglial tumors, as well as mixed gliomas, whereas MRP2 and MRP6 were undetected (Bronger et al., 2005; Calatozzolo et al., 2005; Bortfeld et al., 2006). In contrast to the above-mentioned studies, Bronger et al. (2005) failed to detect significant amounts of MRP1 protein in their glioma samples. The reasons for the discrepancies are unclear; however, it should be noted that all but four of the samples from this particular study were treatment-naïve.

Multiple MRP isoforms have also been identified in cell lines derived from human glioblastomas, anaplastic astrocytomas, neuroblastomas, and medulloblastomas (Goto et al., 2000; Haga et al., 2001; Decleves et al., 2002). Goto et al. (2000) consistently observed *MRP1* mRNA expression in 21 different neuroblastoma cell lines representative of three differing disease phases: treatment-naive, chemotherapy-treated, and relapsed patients following chemotherapy. Greater than 50% of the chemotherapy-treated cell lines were drug-resistant, whereas all of the drug-naive cell lines were drug-sensitive. Furthermore, the cell lines generated following chemotherapy tended to show higher *MRP1* expression than lines established prior to treatment (Goto et al., 2000). With one exception, seven different human glioma cell lines were shown to express *MRP1* and *MRP3*, but not *MRP2* mRNA transcript (Haga et al., 2001). The glioblastoma cell lines GL15 and 8MG cells were also positive for *MRP4* and *MRP5* (Decleves et al., 2002). It is notable that in four different glioblastoma cell lines MRP1 expression correlated well with relative resistance profiles of the anticancer drugs doxorubicin, etoposide, and cisplatin (Mohri et al., 2000); that is, increased MRP1 expression resulted in greater drug resistance.

Studies examining the correlation of *MRP1* gene expression with patient survival in some cancers have produced dissimilar results. In untreated pediatric neuroblastomas, Goto et al. (2000) showed that positive tumor expression of *MRP1* was correlated with a lowered probability of survival compared with *MRP1*-negative tumor samples. However, tumors expressing intermediate levels of *MRP1* did not show greater survival probabilities than those expressing high levels, indicating that higher levels of *MRP1* at diagnosis did not necessarily indicate a worse prognosis (Goto et al., 2000). Norris et al. (1996) observed that high *MRP1* mRNA expression was correlated with unfavorable clinical tumor stages and poor clinical outcome in 60 pediatric neuroblastoma patients (Norris et al., 1996). Whereas *MRP1* expression was not significantly associated with patient survival in a study by Matsunaga et al. (1998), *MRP1* expression was generally higher in patients with an unfavorable outcome. Multiple factors other than MRP1 expression are associated with the development of drug resistance in neuroblastomas (i.e., MYCN oncogene, Cyclin A, and Topoisomerase II gene expression) and in neoplasms in general (Bader et al., 1999), some of which (e.g., MYCN oncogene) are now known to regulate the expression of the transporters themselves (Pajic et al., 2005). Thus, it is likely that differing patient populations and methodologies used in the collection and interpretation of data may explain, in part, the discrepancies reported in these three studies. Further considerations include concurrent administration of differing chemotherapy regimens and use of other medications and/or nutrients that can alter transporter expression (i.e., grapefruit juice and complementary and alternative medicines).

In general, MRP1 expression is heterologous and diffusely scattered in tumor tissue originating from primary and secondary glioblastomas (Tews et al., 2000, 2001). MRP1 clusters have also been observed surrounding necrotic areas of atypical and malignant meningiomas (Tews et al., 2001). The precise cellular location of MRP1 also seems to be heterologous within differing tumor cell types. For example, MRP1 was primarily found in the cell membrane of benign meningiomas and neuroblastomas (Tews et al., 2001; Spiegl-Kreinecker et al., 2002), whereas cytoplasmic staining was predominant in tumor cells from oligodendrogliomas, astrocytomas, and grade IV glioblastomas (Tews et al., 2000; Benyahia et al., 2004). Generally, these various studies showed both cytoplasmic and membrane staining. A cytoplasmic location of MRP1 suggests that in at least some tumor cells, nuclear removal of chemotherapeutic agents via intracellular sequestration plays a role in the development of tumoral resistance (Duvvuri and Krise, 2005). This has been shown, in part, for other drug resistance transporters such as P-gp and the human major vault protein (Molinari et al., 2002; Mossink et al., 2003).

C. HIV/AIDS

Greater than 50% of HIV/AIDS patients will experience a debilitating neurological abnormality during the course of their disease (Sacktor, 2002). HAD, also known as HIV encephalopathy, is one such abnormality characterized by an array of neurological disturbances including cognitive, behavioral, and motor dysfunction. Several classes of antiretroviral medications are approved clinically for the treatment of HIV/AIDS including nucleoside reverse transcriptase inhibitors and protease inhibitors. Current treatment guidelines advocate the use of multiple antiretroviral agents concurrently, i.e., so-called highly active antiretroviral therapy (HAART) regimens. Before the introduction of HAART in 1996, approximately 20% of HIV/AIDS patients developed HAD (Sacktor, 2002). Although HAART has effectively decreased peripheral viral replication and has reduced the incidence of HAD by ~50% in industrialized countries (Bagasra et al., 1996; McArthur et al., 2003),

the prevalence of neurocognitive abnormalities continues to increase over time as HAART increases patient lifespan (Anderson et al., 2002; Valcour et al., 2004). Poor brain permeability of antiretroviral medications probably contributes to the ability of the CNS to remain highly resistant to current HAART regimens. The presence of ABC transporters including P-gp and MRPs, in concert with P450 enzymes (i.e., CYP3A4) represents one mechanism for this pharmacological resistance, particularly for protease inhibitors that undergo extensive metabolism.

Interactions of protease inhibitors with multiple MRP/Mrp isoforms are well documented in transfected cells and in peripheral viral compartments such as lymphocytes (Srinivas et al., 1998; Jones et al., 2001a,b; Olson et al., 2002; Williams et al., 2002). Studies in MRP1/Mrp1- and MRP2/Mrp2-overexpressing cell lines indicate that saquinavir, ritonavir, nelfinavir, and indinavir act as both transporter substrates and inhibitors. Clinically, lower amounts of saquinavir and ritonavir accumulate in peripheral blood mononuclear cells of HIV-infected patients who demonstrate greater MRP1 expression (Meaden et al., 2002).

Expression of MRP1 may also be altered by concomitant protease inhibitor therapy and the presence of HIV-1 infection. Ritonavir exposure causes a concentration-dependent induction in MRP1 protein and decreased ritonavir accumulation by LS-180V intestinal carcinoma cells (Perloff et al., 2001). It is notable that an overexpression of MRP1 in CEM cells is associated with a considerable increase in productive HIV-1 infection (Speck et al., 2002). By actively effluxing compounds from cellular targets of the virus, MRPs would contribute directly to therapeutic failure and result in suboptimal intracellular drug concentrations. Furthermore, the lowered intracellular antiretroviral concentrations allow the virus a sanctuary site to continue to replicate (Meaden et al., 2001). Given that there is no difference between MRP1 expression in lymphocytes from HIV-infected and noninfected control patients, the clinical significance of these findings, particularly in relation to the CNS, remains to be determined (Meaden et al., 2001).

Non-nucleoside reverse transcriptase inhibitors interact with MRP4, MRP5, and MRP8. Schuetz et al. (1999) first demonstrated interaction of reverse transcriptase inhibitors with MRP4 using the acyclic nucleoside analog PMEA. In PMEA-resistant human T-lymphoid cells, up-regulation of *MRP4* mRNA and enhanced PMEA efflux were reported (Schuetz et al., 1999). Similar results were observed with the monophosphorylated form of the thymidine analog zidovudine, confirming the ability of MRP4 to interact with phosphorylated nucleosides. Using MRP5-transfected MDCKII and HEK293 cells, Wijnholds et al., (2000b) also showed reduced accumulation and increased efflux of PMEA. Sulfinpyrazone, a typical organic anion inhibitor, increased accumulation and decreased efflux of PMEA in these same cell systems. Initial rates of PMEA efflux from MRP4- and MRP5-transfected HEK293 cells are identical (Reid et al., 2003a) Nevertheless, the two proteins showed some differences in substrate specificity of nucleoside analogs. HEK293-MRP4 cells show high-level resistance to PMEA and abacavir, whereas HEK293-MRP5 cells show high-level resistance to PMEA and stavudine (Reid et al., 2003a). In addition, MRP8-transfected LLC-PK1 cells show increased resistance to PMEA and zalcitabine but not to zidovudine or lamivudine (Guo et al., 2003). MRP8-overexpressing cells also show greater PMEA efflux than nonparental control cells, further confirming nucleoside analogs, such as PMEA, as MRP8 substrates.

Taken together, these studies support a role for multiple MRPs in CNS disposition of several classes of antiretroviral drugs. Nonetheless, few studies have been undertaken in actual brain compartments. Using confocal microscopy, Miller et al. (2000) showed that ritonavir and saquinavir significantly decrease the transport of the fluorescent organic anion sulforhodamine 101, an Mrp1 and/or Mrp2 substrate, in isolated pig brain capillaries. Using in vivo brain perfusion, Park and Sinko (2005) showed brain uptake of saquinavir was significantly increased (>4-fold) following MK571 administration, suggesting the involvement of Mrp1 and/or Mrp2. Hayashi et al. (2005) reported increased Mrp1 mRNA and protein expression in both cultured mouse astrocytes and brain microvessel endothelial cells following exposure to the HIV-1 protein tat. The regulation of MRP1 expression in each case occurred via multiple mitogen-activated protein kinase signaling pathways (Hayashi et al., 2005). Recent studies from our group also support the role of several Mrp isoforms in the distribution of protease inhibitors in the primary cellular targets of HIV in the brain, i.e., in rat brain microglia (Dallas et al., 2004a,b). Within the CNS, the primary target cells of HIV infection are perivascular macrophages and resident microglial cells (Bagasra et al., 1996; Anderson et al., 2002) and, to a lesser extent, astrocytes (Anderson et al., 2002; Albright et al., 2003). These hard-to-reach cellular compartments provide sanctuaries for the virus and continue to hamper effective eradication of the virus from the CNS. In cultured rat microglia, saquinavir accumulation and efflux are significantly altered in the presence of general Mrp inhibitors such as MK571, genistein, and sulfinpyrazone (Dallas et al., 2004a). Because these cells do not express Mrp2, this is presumably via an Mrp1-mediated process (Dallas et al., 2003). The expression of multiple MRP isoforms in microglia with vastly different substrate specificities has clear implications for HAD pharmacotherapy. In particular, a functional form of MRP1, in conjunction with P-gp, could contribute to decreased cellular accumulation of protease inhibitors such as saquinavir and ritonavir. Indeed, cultured microglia accumulate increased concentrations of saquinavir and indi-

navir in the presence of the P-gp blocker PSC833 (Ronaldson et al., 2004b). Glial expression of Mrp4 and Mrp5 (Dallas et al., 2004b) can further alter cellular accumulation of the monophosphorylated forms of nucleoside analogs, including zidovudine and stavudine. Lowered parenchymal uptake of multiple antiretroviral agents from a single HAART regimen may therefore contribute to an overall decrease in therapeutic efficacy and development of drug resistance in patients and facilitate the development of a particularly hard-to-treat viral sanctuary site.

D. Parkinson's and Alzheimer's Diseases

Studies to examine the influence and importance of efflux transporters in the development and treatment of Parkinson's and Alzheimer's disease are only now beginning (Lam et al., 2001; Furuno et al., 2002; Vogelgesang et al., 2002). Recently, Sultana and Butterfield (2004) observed a slight increase in MRP1 protein expression in frozen hippocampal samples from Alzheimer's patients versus age-matched control subjects. The Alzheimer's samples also showed a concomitant increase in adducts of MRP1 or glutathione *S*-transferase bound to 4-hydroxy-2-transnonenal, a lipid peroxidation product. Given that microglia and astrocytes 1) express several functional Mrp isoforms (Dallas et al., 2003, 2004b; Decleves et al., 2000), 2) are implicated in the pathogenesis of both Parkinson's and Alzheimer's diseases (Nagele et al., 2004; Teismann and Schulz, 2004), and 3) represent a possible novel therapeutic target for these disorders (Kitamura and Nomura, 2003; Liu and Hong, 2003; Monsonego and Weiner, 2003), a pivotal role for parenchymal MRPs in treatment of these two neurodegenerative disorders is inferred.

V. Concluding Remarks

It has been 14 years since Cole and Deeley cloned the first MRP transporter, MRP1 (Cole et al., 1992). In that time the MRP family has grown by eight isoforms, and we have amassed a great deal of information regarding the physiological function and importance of MRPs in drug/solute absorption, distribution, and elimination. Indeed, 30 years after its discovery, researchers continue to struggle to uncover the physiological role of P-gp. Nonetheless, our understanding of the importance of many of the MRP isoforms specifically in the CNS remains rudimentary, at best. This is due, in part, to the inherent difficulty in studying transport mechanisms in a compartment where multiple cell types interact in a complicated and yet to be fully defined manner. Within our own groups, questions of where, why, and how ABC transporters are regulated in the CNS are of particular importance and interest (Bauer et al., 2004; Hartz et al., 2004; Bendayan et al., 2005). Paramount to the success of these types of studies will be the generation and availability of well-characterized and highly specific antibodies. Although the information we gather in isolated cellular systems provides important clues to the functional significance of MRPs in the CNS, more integrated and physiologically complex models will also be needed to fully comprehend the impact of these carriers in the brain. Use of organotypic slices, knockout and knockdown mammals, and multiple cell-type culture systems are a few examples of models that may help us in that regard.

Technical problems aside, brain expression of multiple MRP isoforms with vastly different substrate specificities has clear implications for the pharmacotherapy of neurological disorders. The primary brain barriers severely restrict the capacity of pharmacological agents to penetrate the brain in sufficient quantities to reach their therapeutic target and exert an intended effect. The ability of cellular components of the brain parenchyma to act as a secondary barrier to brain permeation of pharmacological agents probably contributes further to the overall problem of effective pharmacotherapy. It is likely that these transporters facilitate an overall exit of compounds from the CNS because once extruded from glial cells into the brain interstitium, compounds become more readily available for removal via the BBB or the blood-CSF barrier. Effective treatment of neurological disorders will certainly require the development of novel compounds that can both readily penetrate into the brain across both of these barriers and ultimately reach their target sites in sufficient quantities. Sadly, the redundant nature of CNS transporters with a wide spectrum of overlapping substrate profiles (i.e., MRPs, P-gp, and BCRP) will probably continue to present clinicians with an immense therapeutic challenge for some time to come.

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