

Modulation of P-Glycoprotein at the Blood-Brain Barrier: Opportunities to Improve Central Nervous System Pharmacotherapy

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Abstract—Pharmacotherapy of central nervous system (CNS) disorders (e.g., neurodegenerative diseases, epilepsy, brain cancer, and neuro-AIDS) is limited by the blood-brain barrier. P-glycoprotein, an ATP-driven, drug efflux transporter, is a critical element of that barrier. High level of expression, luminal membrane location, multispecificity, and high transport potency make P-glycoprotein a selective gatekeeper of the blood-brain barrier and thus a primary obstacle to drug delivery into the brain. As such, P-glycoprotein limits entry into the CNS for a large number of prescribed drugs, contributes to the poor success rate of CNS drug candidates, and probably contributes to patient-to-patient variability in response to CNS pharmacotherapy. Modulating P-glycoprotein could therefore improve drug delivery into the brain. Here we review the current understanding of signaling mechanisms responsible for the modulation of P-glycoprotein activity/expression at the blood-brain

barrier with an emphasis on recent studies from our laboratories. Using intact brain capillaries from rats and mice, we have identified multiple extracellular and intracellular signals that regulate this transporter; several signaling pathways have been mapped. Three pathways are triggered by elements of the brain's innate immune response, one by glutamate, one by xenobiotic-nuclear receptor (pregnane X receptor) interactions, and one by elevated β -amyloid levels. Signaling is complex, with several pathways sharing common signaling elements [tumor necrosis factor (TNF) receptor 1, endothelin (ET) B receptor, protein kinase C, and nitric-oxide synthase], suggesting a regulatory network. Several pathways include autocrine/paracrine elements, involving release of the proinflammatory cytokine, TNF- α , and the polypeptide hormone, ET-1. Finally, several steps in signaling are potential therapeutic targets that could be used to modulate P-glycoprotein activity in the clinic.

I. Introduction

More than 98% of drug candidates for CNS¹ disorders never make it to the clinic (Pardridge, 2007a). For most of

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¹ Abbreviations: CNS, central nervous system; MDR, multidrug resistance; HIV, human immunodeficiency virus; PSC833, valspodar; ABC, ATP-binding cassette; h, human; PXR, pregnane X receptor; AP-1, activator protein-1; NF- κ B, nuclear factor- κ B; MRP/Mrp,

these drugs, the major confounding issue is their inability to cross the blood-brain barrier at sufficient levels to have a therapeutic effect. This barrier resides within the brain's capillary endothelium, and it has been an object of study for more than 100 years. Research on the blood-brain barrier has occurred in several stages. Initial work focused on the barrier's physiological properties (i.e., the ability to prevent movement of solutes between blood and CNS). The morphological basis of the barrier was determined to be primarily the tight junctions that connect the endothelial cells. The molecular basis for the barrier's properties was explored as well as the involvement of specific transporters that increased or decreased solute permeability. Over the past several years, research on all of these aspects has continued within the context of the barrier as a dynamic tissue responding to changes in its environment and as part of a more complex neurovascular unit in which endothelial cells, astrocytes, pericytes, and neurons interact.

The present review was written in this context. It is focused on P-glycoprotein, the one blood-brain barrier transporter that is considered to be the major obstacle to CNS entry of therapeutic drugs and is thus seen as the molecular basis for preclinical and clinical drug failure. Our emphasis in the present review is on the underlying mechanisms that modulate P-glycoprotein at the blood-brain barrier. We posit that an understanding of these mechanisms is important to provide new strategies for improving CNS pharmacotherapy and to recognize how barrier properties change in disease.

II. The Blood-Brain Barrier

Although the vascular system penetrates every tissue of the body, blood vessels display a remarkable range of phenotypes with regard to structure, gene expression, function, cellular ultrastructure, and blood-tissue exchange properties (Aird, 2007a,b). Indeed, even within a single organ the range of endothelial heterogeneity can be quite wide. This is certainly seen with regard to barrier properties of vessels within the CNS, in which pial (surface) vessels present at most a moderate barrier, but cerebral microvessels (3- to 8- μ m diameter) present a formidable barrier to macromolecules, small organic drugs, and ions. These small vessels within the brain parenchyma constitute the blood-brain barrier. In man, their total length is estimated to be more than 600 km with a surface area of 10 to 30 m² (Pardridge, 2003). This makes the blood-brain barrier the third largest discrete surface area for solute and water exchange after intestine and lung. However, as the name indicates, compared with capillaries in peripheral tissues, solute

exchange between blood and brain is severely restricted and thus this barrier is a major impediment to CNS pharmacotherapy (Pardridge, 2007a). The mechanistic basis for restricted access of drugs to the CNS lies within the special properties of the cells that make up the brain capillary endothelium.

A. The Structural/Physical Barrier

The blood-brain barrier reflects the properties of two components (Begley, 2004; Hawkins and Davis, 2005; Löscher and Potschka, 2005). One forms a structural/physical barrier, comprising the endothelial cells themselves and the extremely tight, intercellular junctional complexes that connect one cell to another. The structural barrier limits diffusion of solutes between blood and brain. For many solutes permeability is inversely related to size (most macromolecules have extremely low permeability) and directly related to lipophilicity. Indeed, for many small, uncharged molecules, in vivo blood-brain barrier passive permeability increases with the octanol/water partition coefficient, probably indicating diffusion through the lipid-like core of cellular surface membranes (Smith, 2003).

Current research is focused on several strategies designed to circumvent the physical barrier: hyperosmotic blood-brain barrier opening, barrier opening using alkyl glycerols, and drug delivery using surface-modified liposomes and nanoparticles, which hijack endothelial cell transcytotic mechanisms (Tiwari and Amiji, 2006). All have been shown to work in proof-of-principle experiments, and some have increased the efficacy of therapeutic agents that normally only poorly enter the brain (see, e.g., Tiwari and Amiji, 2006; Kumar et al., 2007; Pardridge, 2007b). However, in general, two factors have limited use of these promising approaches: toxicity and an inability to deliver enough drug to the CNS. At present, none of the above approaches is in routine clinical use for treating CNS disorders.

B. The Selective/Biochemical Barrier

Although many solutes follow the consensus relationship between blood-brain barrier permeability and octanol/water partition coefficient, there are a substantial number of outliers, indicating that one cannot think of the blood-brain barrier as being solely physical in nature. These outliers emphasize the higher than expected blood to brain permeability of certain highly hydrophilic solutes (e.g., glucose and amino acids), and the lower than expected permeability of certain hydrophobic foreign chemicals (xenobiotics), including therapeutic drugs and CNS toxicants. This phenomenon is explained by the second component of the barrier, which is both biochemical and selective. Its molecular basis is a group of specific transport proteins expressed on the luminal (blood-facing) and abluminal (brain-facing) plasma membranes of the endothelial cells. Some of these transporters selectively increase barrier permeability to es-

multidrug resistance-associated protein; BCRP/ABCG2, breast cancer resistance-associated protein; CAR, constitutive androstane receptor; PCN, pregnenolone-16 α -carbonitrile; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; ET, endothelin; NOS, nitric-oxide synthase; PKC, protein kinase C; Oatp, organic anion-transporting polypeptide; AED, antiepileptic drug; COX, cyclooxygenase; NMDA, *N*-methyl-D-aspartic acid.

essential nutrients, whereas others selectively prevent effective entry of xenobiotics (Löscher and Potschka, 2005; Dallas et al., 2006).

III. P-glycoprotein, a Critical Element of the Selective/Biochemical Barrier

Multiple drug transporters have been localized to brain capillary endothelial cells and several efflux transporters are positioned to play a gatekeeper role in the active, selective blood-brain barrier (Fig. 1). However, based on three critical defining criteria (specificity, energetics, and location), it is clear why P-glycoprotein is such an important element of the blood-brain barrier. P-glycoprotein was originally discovered more than 30 years ago as an overexpressed gene (*MDR1/ABCB1*) in multidrug-resistant human tumor cells (Juliano and Ling, 1976) that was subsequently shown to code for a plasma membrane protein that functions as an ATP-driven efflux pump (Gottesman and Pastan, 1988; Hennessey and Spiers, 2007). This pump handles a remarkably wide range of substrates, from approximately 300 to 4000 Da in mass. The list of substrates includes commonly prescribed drugs from many classes, such as calcium channel blockers, statins, opioids, chemotherapeutic agents, HIV protease inhibitors, antibiotics, immunosuppressive agents, and β -adrenergic antagonists (Ford and Hait, 1993). This broad substrate spectrum explains the ability of P-glycoprotein to provide cross-

resistance to multiple classes of chemotherapeutic agents and to underlie drug-drug interactions involving structurally dissimilar classes of drugs (e.g., cyclosporines and cardiac glycosides) (Marchetti et al., 2007).

P-glycoprotein is not only highly expressed in multidrug-resistant tumor cells, but is also physiologically present in a number of nontumor cells, with the highest levels of expression in barrier and excretory tissues (Fojo et al., 1987; Thiebaut et al., 1987). Of these normal tissues, brain capillaries express particularly high levels of P-glycoprotein protein (Fig. 2A) (Cordon-Cardo et al., 1989). In brain capillaries in situ, in isolated capillaries ex vivo, and in brain capillary endothelial cell cultures, the transporter has been localized to the luminal (blood side) plasma membrane (Fig. 2B). This is the ideal location to limit diffusion of drugs from blood to endothelial cell cytoplasm and thus to the brain parenchyma. Recent

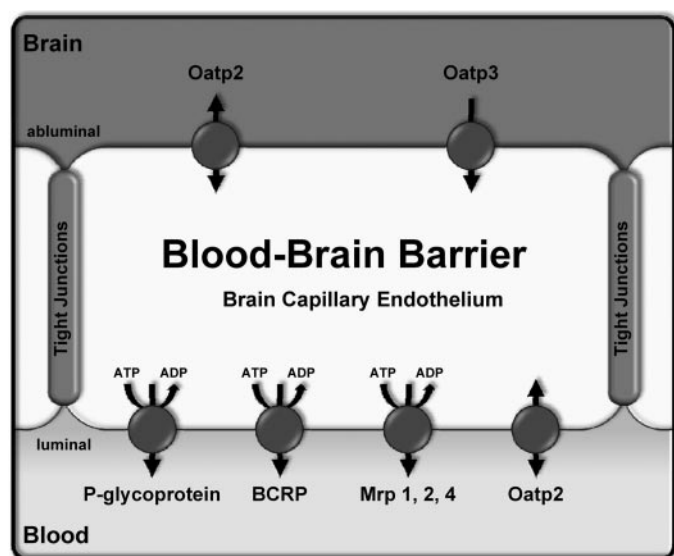


FIG. 1. Localization of efflux transporters at the blood-brain barrier. Transporters shown have been demonstrated to be expressed in the brain capillary endothelium at the protein level. Arrows indicate direction of substrate transport; ABC transporters are marked with ATP/ADP hydrolysis. P-glycoprotein, Mrp isoforms 1, 2, and 4, and BCRP are expressed in the luminal membrane. However, localization of Mrps within the brain capillary endothelium is still controversial (see, e.g., Dallas et al., 2006; Löscher and Potschka, 2005). We have localized Mrp2 and Mrp4 to the luminal membrane of rat brain capillaries (Bauer et al., 2008) and thus placed them there in the diagram. The organic anion-transporting polypeptide 2, Oatp2, is localized to both luminal and abluminal membranes (Gao et al., 1999); Oatp3 has been detected in the abluminal membrane (Ohtsuki et al., 2004).

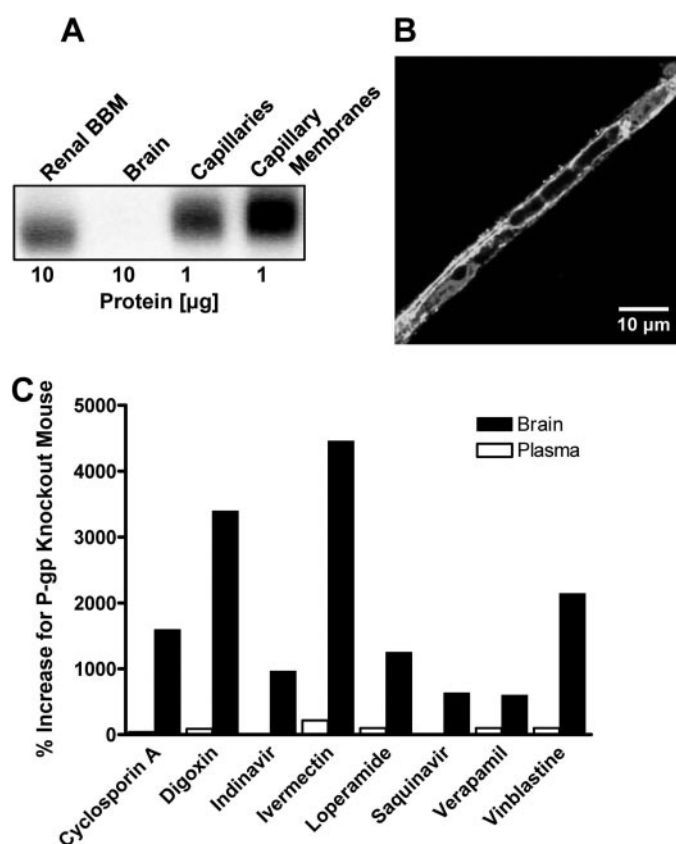


FIG. 2. Expression of P-glycoprotein (P-gp) at the blood-brain barrier. A, Western blot showing enrichment of P-glycoprotein from whole rat brain homogenate to capillary lysate to isolated capillary membranes. Note that P-glycoprotein expression levels are highest in capillary membranes (1 μ g of total protein loading) compared with brain and renal brush border membranes (BBM) (10 μ g of total protein loading). B, immunostaining of an isolated rat brain capillary for P-glycoprotein (green). Nuclei are stained with propidium iodide (red). Note the "railroad track"-like P-glycoprotein staining of the luminal membrane that defines the capillary lumen and outlines trapped red blood cells. C, genetically knocking out P-glycoprotein increases drug levels in the brain. Plasma levels are not changed substantially, indicating a key role for P-glycoprotein at the blood-brain barrier. Data shown for each drug is the percentage increase in the two compartments calculated from data for wild-type and P-glycoprotein-null mice (graphed from data generated by Schinkel and coworkers and compiled in Mizuno et al., 2003).

studies using electron microscopy have also detected P-glycoprotein expression in intracellular membranes within brain capillary endothelial cells (e.g., the endoplasmic reticulum, vesicles, and nuclear envelope) (Bendayan et al., 2006; Babakhanian et al., 2007). P-glycoprotein can be associated with elements of the trafficking machinery of the cell (e.g., lipid rafts and caveolae) (Bé langer et al., 2004; Orlowski et al., 2006; Barakat et al., 2007) and, at least in tumor cells, internalized transporter seems to play some role in multidrug resistance (Molinari et al., 2002). At this time it is not clear what role this pool of internalized P-glycoprotein plays in endothelial cell function, although one could envision it providing a transporter for rapid insertion into the plasma membrane, protecting cytoplasmic function by sequestering xenobiotics in vesicles, and possibly limiting nuclear exposure to xenobiotics.

Evidence for the importance of P-glycoprotein comes from its remarkable ability to restrict drug access to the CNS. This is best seen in comparisons of drug distribution in wild-type and P-glycoprotein-null mice (genetic knockout) and in studies in which transporter function is abolished by P-glycoprotein-specific and potent inhibitors (chemical knockout). These show 10- to 50-fold increases in brain levels of a number of drugs (many highly lipophilic) when P-glycoprotein activity is abolished (Fig. 2C). Moreover, despite the fact that P-glycoprotein is expressed in liver, gut, and kidney, for many drugs P-glycoprotein knockout mice show only modest increases in plasma levels. This finding probably reflects redundancy of transport function and the relative importance of other excretory transporters in liver and kidney. These results further highlight the important role P-glycoprotein plays as a specific gatekeeper of the CNS. It is noteworthy that they also provide a scientific basis to modulate P-glycoprotein transport activity as a way to potentially improve CNS pharmacotherapy.

This potential has been investigated using animal models for specific diseases. Consider a recent study with the chemotherapeutic agent, paclitaxel (Taxol), a highly lipophilic antimitotic drug used to treat solid tumors (Fellner et al., 2002). Although paclitaxel is very potent *in vitro* against a variety of tumors, including glioblastomas, it is ineffective against brain tumors *in vivo*, because it does not cross the blood-brain barrier. In nude mice, specific inhibition of P-glycoprotein by the second-generation inhibitor, PSC833, increased paclitaxel accumulation in brain by an order of magnitude; these increased brain paclitaxel levels were sustained for at least 24 h. When given to nude mice with a cerebrally implanted human glioblastoma, paclitaxel was ineffective. However, pretreating mice with PSC833 before paclitaxel therapy substantially reduced tumor volume by 90% (drugs given twice over a 5-week period) (Fellner et al., 2002). These *in vivo* experiments using an animal model suggest one strategy for overcoming

P-glycoprotein-based CNS pharmacoresistance: direct inhibition of transporter activity.

Similar results showing increased drug penetration into the brain have since been obtained with several other P-glycoprotein inhibitors, suggesting one general strategy to overcome CNS multidrug resistance based on P-glycoprotein (Kemper et al., 2003, 2004). These studies demonstrate dramatically how P-glycoprotein can be an overriding impediment to CNS chemotherapy. In addition to the long list of failed CNS drugs that are P-glycoprotein substrates and, therefore, do not cross the blood-brain barrier, these studies further indicate that the ability to control the activity/expression of this transporter in brain capillaries could be of substantial benefit in the treatment of a number of CNS diseases. Ideally, one would want to specifically and transiently reduce P-glycoprotein activity (as in the mouse study) before administering a normally impermeant therapeutic drug, which then could access the CNS during the window in time when efflux pump activity is reduced.

IV. Modulation of P-Glycoprotein Transport Activity

A. In the Periphery

Because P-glycoprotein greatly influences CNS pharmacotherapy, it is important to know the extent to which transporter activity is pathophysiologically modulated. Currently, there is convincing evidence that disease processes (e.g., inflammation) can influence the expression of multiple hepatic ABC transporters, including P-glycoprotein (McRae et al., 2003; Ho and Piquette-Miller, 2006). At the blood-brain barrier emerging evidence shows that P-glycoprotein expression is also changed during disease, e.g., in epilepsy and stroke (see below). Because the brain capillary endothelium is the interface between the periphery and the CNS, it is exposed to normal and pathological signals from both the blood and the CNS. The extent to which these signals change P-glycoprotein-mediated transport is likely to be a significant factor in devising treatment regimens. Thus, it is also important to know how signals derived from both CNS and the periphery influence transporter expression and overall blood-brain barrier function. Because of its role in multidrug resistance in cancer, there is a substantial body of literature on signals that modulate P-glycoprotein expression in tumor cells. Transcriptional regulation of *MDR1* gene expression through the binding of several *trans*-acting proteins to consensus *cis*-elements in the promoter region is complex and not yet fully understood. For the *hMDR1* gene, several promoter elements have been identified, including, a GC-box, a Y-box, a p53 element, a pregnane X receptor (PXR) element, an inverse MED1 element, an AP-1 element, a NF- κ B element, and a heat shock protein element. Detailed descriptions can be found in (Labialle et al. (2002, 2004) and Scotto (2003). These promotor ele-

ments are binding sites for transcription factors that respond to environmental cues, such as oxidative stress, inflammation, hypoxia, xenobiotics including drugs and toxicants, heavy metal salts, and others.

hMDR1 gene expression can also be substantially affected by epigenetic mechanisms, including DNA methylation and histone acetylation (Baker and El-Osta, 2003, 2004; Baker et al., 2005). Finally, a number of intracellular signals were found to affect transporter activity without changing transporter protein expression, suggesting multiple post-translational mechanisms of modulation, including transporter trafficking (Kipp and Arias, 2002), degradation (Zhang et al., 2004), protein phosphorylation/dephosphorylation (Chambers et al., 1990; Sachs et al., 1999), and specific association with other membrane proteins (e.g., caveolin) (Schlachetzki and Pardridge, 2003).

B. At the Blood-Brain Barrier

Until a few years ago, it was uncertain whether P-glycoprotein activity in brain capillaries could be influenced by any of these signals. Now it is clear that both transporter activity and expression can be modulated by a number of physiological and pathological signals to either increase or decrease P-glycoprotein transport activity. The remainder of this review is focused primarily on recent studies in intact brain capillaries and in vivo animal models demonstrating how signaling alters P-glycoprotein expression and activity at the blood-brain barrier. Use of freshly isolated brain capillaries provides a means of investigating mechanisms of transporter function and regulation in an intact, integrated tissue without the uncertainties inherent in use of cells in culture (primary cells in culture and cell lines). These capillaries retain the ability to support for many hours specific, ATP-driven transport mediated by P-glycoprotein, Mrp2, and BCRP, providing a platform to study both rapid and transcriptional regulation at the functional, protein, and mRNA levels (Bauer et al., 2004, 2008a; Hartz et al., 2004). Changes in tight junction function and specific junctional protein expression can also be assessed in these isolated capillaries (Hartz et al., 2004). Although adherent pericytes are retained by the capillaries, connections with astrocytes and neurons are broken during isolation; thus, potentially important interactions within the neurovascular unit are not present. As described below, we have used these capillaries along with pharmacological tools and knockout and transgenic animals to examine multiple signaling pathways in detail in vitro and to verify several pathways in vivo.

The following text is organized into eight sections, seven dealing with specific signals and diseases followed by a perspectives section that describes challenges and certain emerging areas of research.

1. *Ligand-Activated Nuclear Receptors.* Geick et al. (2001) discovered a complex regulatory cluster of several

binding sites for the ligand-activated nuclear receptor, the PXR (*NR1I2*) in the 5'-upstream region of *hMDR1*. Three DR4 motifs [direct repeats of a AG(G/T)TCA motif with a spacer of four nucleotides between the binding motif], one DR3 motif, and one ER6 motif (everted repeat) were identified at approximately -8 kilobase pairs. Electrophoretic mobility shift assays further revealed that PXR binds as a heterodimer with the retinoid X receptor α to all DR4 motifs (Geick et al., 2001). In addition, reporter gene assays confirmed that this cluster of response elements is responsible for PXR-mediated *hMDR1* induction.

PXR is a member of a superfamily of ligand-activated transcription factors, the so-called orphan nuclear receptors. It is activated not only by naturally occurring steroids, such as pregnenolone and progesterone, and synthetic glucocorticoids and antiglucocorticoids but also by a wide range of xenobiotics including dietary compounds, toxicants, and a large number of commonly prescribed drugs (e.g., steroids, chemotherapeutic agents, HIV protease inhibitors, glucocorticoids, and anticonvulsants). Studies in liver and gut have shown that PXR targets genes that are responsible for xenobiotic metabolism (phase I and phase II) and efflux. Therefore, PXR is considered to be a "master regulator" of defenses against xenobiotics at the cellular and molecular levels (Dussault and Forman, 2002). Efflux transporters known to be regulated by PXR include organic anion-transporting polypeptide isoform 2 (*SLCO1A4*), bile salt export pump (*ABCB11*), multidrug resistance-associated proteins isoforms 2 and 3, Mrp2 and Mrp3 (*ABCC2* and *ABCC3*), and P-glycoprotein (*ABCB1*, *MDR1*) (Kliwer et al., 1998; Lehmann et al., 1998; Geick et al., 2001; Teng et al., 2003). Certainly in hepatocytes, PXRs, other nuclear receptors (e.g., CAR, xenobiotic-metabolizing enzymes, and efflux transporters) seem to comprise a regulated network of core defense mechanisms (Rosenfeld et al., 2003; Hartley et al., 2004).

Initial studies with whole-brain homogenates showed no evidence of PXR expression (Kliwer et al., 1998; Zhang et al., 1999; Jones et al., 2000). However, we reasoned that capillaries comprise less than 1% of brain volume and if PXR expression were restricted to those structures, mRNA levels in whole brain might be too low to detect (Bauer et al., 2004). Using reverse transcriptase-polymerase chain reaction, we detected PXR mRNA in freshly isolated rat brain capillaries; immunostaining confirmed receptor expression within the capillary endothelial cells (Bauer et al., 2004). Consistent with this finding, two PXR ligands, pregnenolone 16 α -carbonitrile (PCN) (specific for rodent PXR) and dexamethasone (ligand for PXR and glucocorticoid receptor), both increased levels of P-glycoprotein protein expression (Western blots and quantitative immunostaining) and increased P-glycoprotein-mediated transport of a fluorescent cyclosporin A derivative into isolated rat brain capillary lumens (Bauer et al., 2004). It is noteworthy

that dosing rats with PCN and dexamethasone increased P-glycoprotein expression in plasma membranes from liver and brain capillaries and up-regulated P-glycoprotein-specific transport in the capillaries. Expressions of Mrp2, another drug efflux pump expressed at the blood-brain barrier, and of glutathione transferase π , both PXR target genes, were similarly up-regulated by PCN and dexamethasone in vivo and in vitro (Bauer et al., 2004, 2008a). These in vitro and in vivo dosing experiments provided the first evidence for PXR expression in brain and for regulation by nuclear receptors of xenobiotic efflux pumps at the blood-brain barrier. As noted by Bauer et al., one cannot rule out the possibility that at least some of the effects of dexamethasone in these experiments were mediated by the glucocorticoid receptor.

Experiments with transgenic mice expressing hPXR further emphasized the clinical implications of these findings (Bauer et al., 2006). Although the DNA-binding domain of PXR is highly conserved across species, the ligand-binding domain is not. Thus, there are substantial species differences in ligand affinities for rodent versus human PXR. For example, PCN is a ligand for rodent PXR but not for hPXR, and rifampin, an antibiotic, and hyperforin, a component of the herbal remedy St. John's wort, are high-affinity ligands for hPXR but not for rodent PXR. Consistent with these differences in receptor specificity, rifampin and hyperforin increased P-glycoprotein expression in brain capillaries from hPXR transgenic mice but PCN did not (Bauer et al., 2006). Conversely, PCN increased P-glycoprotein expression in brain capillaries from wild-type mice, but rifampin and hyperforin did not. In additional experiments, hPXR transgenic mice were given rifampin at a dose adjusted to result in free plasma drug levels equivalent to those seen in patients undergoing a course of rifampin treatment. In rifampin-pretreated hPXR transgenic mice, P-glycoprotein expression in liver, intestine, and brain capillaries was substantially increased. Moreover, the antinociceptive effects of injected methadone, a CNS-acting analgesic and weak P-glycoprotein substrate, were reduced by 70% compared with those in hPXR transgenic mice not pretreated with rifampin (Bauer et al., 2006). Plasma methadone levels were not affected by rifampin pretreatment, indicating that P-glycoprotein is not a major determinant of plasma methadone levels; it is likely that other hepatic and renal transporters are more important for drug excretion. However, methadone antinociception was shown to be a simple function of brain drug levels (Bauer et al., 2006). These studies with an animal model indicate that clinically relevant decreases in the efficacy of CNS-acting drugs that are P-glycoprotein substrates would be one consequence of PXR activation at the blood-brain barrier.

These findings have several important implications for the clinic. First, because of the large number of

xenobiotics that are PXR ligands, these studies suggest that one basis for patient-to-patient variation in the effectiveness of CNS pharmacotherapy could be variable levels of blood-brain barrier efflux transporter induction by therapeutic drugs (polypharmacy) and dietary constituents. Second, blood-brain barrier P-glycoprotein expression has been shown to be elevated in epilepsy and stroke (see below), but it is not clear to what extent increases in transporter expression reflect activation of PXR or other nuclear receptors by prescribed drugs and endogenous ligands. Third, a recent report shows that PXR activation provides increased neuroprotection in a mouse model of Niemann-Pick type C1 disease, a fatal lipid storage disorder (Langmade et al., 2006). This finding suggests that disease-induced, progressive neurodegeneration could be delayed by therapy designed to activate PXR. Choosing to target PXR in a disease with substantial neurological complications could have unintended consequences, especially if other CNS-acting drugs are prescribed.

Finally, PXR is only one member of the family of ligand-activated nuclear receptors that regulates expression of drug-metabolizing enzymes and transporters (Francis et al., 2003). Our preliminary studies with rat brain capillaries show mRNA expression of CAR, farnesoid X receptor, liver X receptor, vitamin D receptor, and additional target drug efflux transporters and phase I and phase II drug-metabolizing enzymes (Bauer et al., 2008a). Each of these receptors recognizes a somewhat different set of ligands and activates a different pattern of genes coding for enzymes and transporters. However, in other tissues, these nuclear receptors form an interacting, regulatory network in which cross-talk among receptors is the rule rather than the exception (Pascucci et al., 2008). Thus, both the identity of the receptor ligands that enter brain capillary endothelial cells and the structure of the regulatory network will significantly determine the range of chemicals that alter the blood-brain barrier, the range of drugs for which barrier function is modified, and the nature and extent of the change in the barrier seen by each specific CNS-acting drug.

2. Inflammation. The acute inflammatory response is a complex immunological reaction that is triggered by a wide variety of stimuli, including infection, trauma, and cell stress. Inflammation is a major factor in a number of diseases, including diseases of the brain, many of which have a major inflammatory component that aggravates therapy. Inflammation results in the release of a number of proinflammatory cytokines, which can have systemic effects and signal changes in multiple tissues. The complexity of the acute response is reflected in differences in the extent and time courses of cytokine release, immune cell infiltration, and individual tissue responses to the various triggering events. In experimental studies, this complexity can make generalizations difficult because patterns of responses can be dose-, time-, context-, and model-dependent. With these

caveats in mind, it is interesting to note that expression of P-glycoprotein seems to be tied in with the innate immune response. First, P-glycoprotein expression is up-regulated in inflamed bowel (Farrell et al., 2000), and mild colitis is one of the few phenotypes other than altered sensitivity to xenobiotics that is associated with the P-glycoprotein-null mouse (Wilk et al., 2005). Second, a number of studies in liver and intestine show that inflammation, usually induced experimentally by injection of lipopolysaccharide (LPS) (bacterial endotoxin), reduces expression (mRNA and protein) of certain drug-metabolizing enzymes and drug efflux transporters, including P-glycoprotein (McRae et al., 2003; Ho and Piquette-Miller, 2006). In the LPS model, changes in the disposition of a number of xenobiotics are consistent with the effects on enzyme and transporter expression.

A similar hepatic response is seen with treatments that induce cholestasis, for example, experimental bile duct ligation (McRae et al., 2003; Ho and Piquette-Miller, 2006). The proinflammatory mediators responsible probably include IL-6, IL-1 β , and TNF- α . These cytokines down-regulate transporter expression, possibly by suppressing nuclear receptors (e.g., PXR, hepatocyte nuclear factor-1 α , and CAR) from transcriptional activation of genes coding for luminal drug efflux transporters. However, signals generated by bile salts, proinflammatory cytokines, oxidative stress, retinoids, drugs, and hormones are involved, and the mechanisms responsible for the profound changes in hepatic enzyme and transporter expression in cholestasis are expected to be complex.

Severe inflammation has profound effects on the blood-brain barrier (Huber et al., 2001). Inflammatory mediators (e.g., TNF- α , IL-1 β , and interferon- γ) can increase junctional permeability, and recent evidence shows that inflammation alters P-glycoprotein expression in the brain and in brain capillary endothelial cells (Tan et al., 2002; Theron et al., 2003; Seelbach et al., 2007). However, both increases and decreases in transporter expression have been observed, and it is not clear from these studies what mechanisms are responsible and whether the changes in tight junction structure and function are somehow linked to altered P-glycoprotein expression.

Consider two examples that show opposite effects of inflammation on P-glycoprotein expression. First, Goral-ski et al. (2003) showed that intracranial ventricle injection of LPS in rats both reduced expression of P-glycoprotein in the brain and increased accumulation of the P-glycoprotein substrate, digoxin, in brain tissue. Similar effects on digoxin disposition were found in wild-type mice but not in P-glycoprotein knockout mice. However, it is not clear from these initial studies what mechanisms were responsible for decreased pump expression and whether changes in tight junctional permeability can also be linked to altered P-glycoprotein expression. Second, using a well characterized experimental model of chronic, peripheral inflammatory pain in rats, Seel-

bach et al. (2007) found increased P-glycoprotein expression in Western blots of brain capillary membranes, reduced uptake of injected morphine into the brain, and reduced antinociceptive effects of injected morphine in a tail-flick assay. These changes came at a time when tight junction permeability to sucrose had increased (Wolka et al., 2003). Thus, for the weak P-glycoprotein substrate, morphine, the inflammation-induced increase in efflux trumped the increase in junctional leakiness. Certainly, changes in the expression of other transporters could be involved. Note that because a large part of the general population suffers from chronic pain, this study has important implications for CNS pharmacotherapy. At present, the signals that underlie this "action at a distance" have not been characterized, but elements of the peripheral and CNS inflammatory responses are likely participants.

Recent studies with isolated rat brain capillaries show both a complex time course of P-glycoprotein response to proinflammatory mediators and a complex chain of signaling (Fig. 3, A and B). Exposure of capillaries to low levels of LPS, TNF- α , or ET-1 causes a rapid, reversible loss of P-glycoprotein transport function with no change in protein expression; inhibitors of protein synthesis are without effect, and tight junctional permeability is not affected (Hartz et al., 2004, 2006). Signaling is complex, involving ligand binding to Toll-like receptor 4, TNF receptor 1, and ET_B receptors followed by activation of NOS and PKC (Fig. 3B). A minor component of LPS signaling bypasses all steps in the main pathway except NOS activation; this might be initiated by LPS acting through, for example, a scavenger receptor. All steps in the main signaling sequence occur on or in the capillary endothelial cells in a single sequence of events. Thus, signaling by TNF- α and ET-1 is downstream of LPS binding to Toll-like receptor 4, which, in turn, causes release of TNF- α and ET-1 by the endothelial cells (Fig. 3B).

In this brain capillary model, experiments with microtubule disruptors suggest that changes in P-glycoprotein trafficking underlie both loss of transport function and its restoration (unpublished data). This is very much like the situation in hepatocytes in which one mechanism that drives changes in bile flow is the rapid, regulated trafficking of ABC transporters (P-glycoprotein, MRP2, MDR2, and the bile salt export pump) between intracellular compartments and the canalicular plasma membrane (Kipp et al., 2001). Both microtubules and the products of phosphoinositide 3-kinase have been implicated in membrane vesicle trafficking. Striking movies of hepatocytes expressing a P-glycoprotein-green fluorescent protein show intermittent, tubulovesicular movement of the fusion protein between the pericanalicular region and the bile canalicular membrane (Kipp and Arias, 2000; Kipp et al., 2001).

Note that the ability to manipulate P-glycoprotein trafficking suggests exciting possibilities with regard to

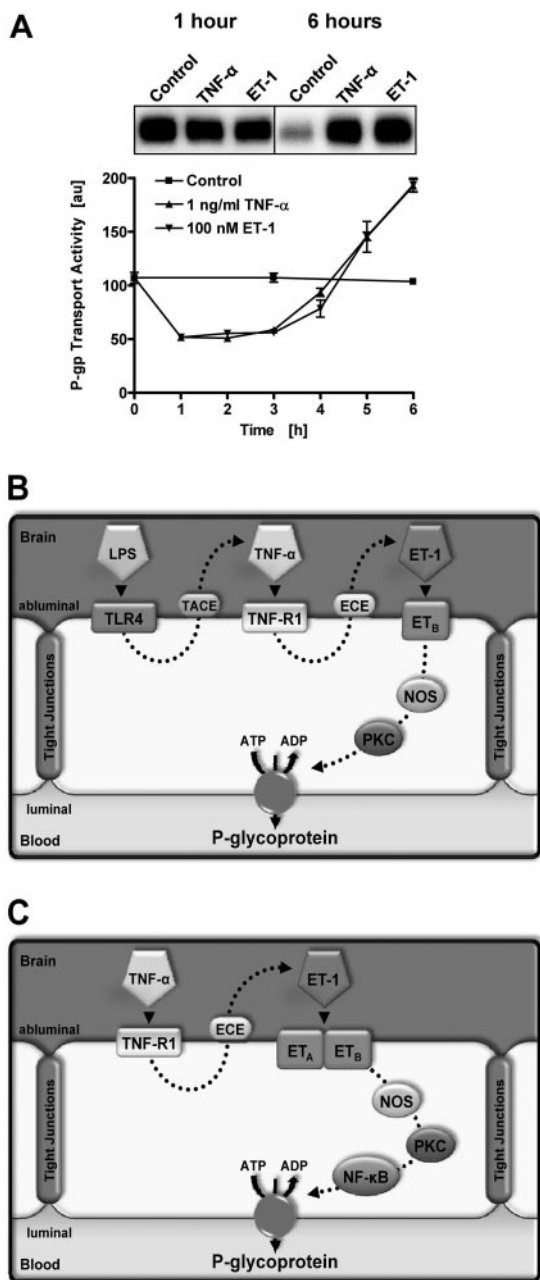


FIG. 3. Proinflammatory signaling to P-glycoprotein (P-gp) in rat brain capillaries. A, time course showing changes in P-glycoprotein-mediated transport activity in isolated rat brain capillaries exposed to TNF- α and ET-1. Note the rapid reduction in activity and the delayed increase over control levels. The inset shows Western blots of capillary membranes after 1 and 6 h of exposure. Note the absence of change in P-glycoprotein expression after 1 h and the dramatic change after 6 h (Bauer et al., 2007). These blots were processed using different exposure times and expression levels cannot be compared across blots. We showed constant P-glycoprotein expression in capillaries over 6 h of incubation in control medium (Bauer et al., 2007). B, LPS, TNF- α , and ET-1 signaling to P-glycoprotein in the short-term (rapid reduction of transport activity) (Hartz et al., 2004, 2006). C, TNF- α and ET-1 signaling to P-glycoprotein in the long-term (increased transport activity and transporter expression) (Bauer et al., 2007). TLR4, Toll-like receptor 4; TACE, tumor necrosis factor- α -converting enzyme; ECE, endothelin-converting enzyme.

substrates to the CNS with only minor disruption of protection. At present this mechanism of modulation has only been demonstrated in vitro. Whether this will happen in an in vivo model remains to be seen.

In contrast to short-term exposure, longer-term exposure of the capillaries to low levels of TNF- α or ET-1 eventually causes increased P-glycoprotein expression signaled through TNF receptor 1, ET_B and ET_A receptors, NOS, PKC, and the transcription factor, NF- κ B (Fig. 3C) (Bauer et al., 2007). After 6 h of exposure, P-glycoprotein transport activity and protein expression are approximately twice that of controls (which do not change over the time course of the experiment). Inhibition of protein synthesis blocks these increases. As one might expect, expression of other proteins also changes. At the same time P-glycoprotein expression is increased, expression of Mrp2 and Mrp4 is decreased, expression of glucose transporter 1 and Na⁺/K⁺-ATPase is increased, and expression of BCRP and tight junctional proteins does not change (Bauer et al., 2007). It is not clear whether the same signaling pathway is responsible for the changes observed in all transporters, but it is clear that the effects of TNF- α and ET-1 exposure are not restricted to P-glycoprotein.

Taken together, these data for brain capillaries show that certain proinflammatory stimuli first decrease P-glycoprotein transport activity with no change in expression and then increase expression and activity (Fig. 3A). A similar overall pattern of change in drug efflux transporter activity and expression (Mrp2 and P-glycoprotein) was previously demonstrated in renal proximal tubule, in which ET_B receptor, NOS, and PKC signaling first decreased transporter activity and then increased expression and activity (Miller, 2002; Terlouw et al., 2003). In these tubules, signaling was initiated by low levels of tubular nephrotoxins. One consequence of long-term exposure of tubules to low concentrations of ET-1 or the nephrotoxic aminoglycoside, gentamicin, was reduced sensitivity to acute gentamicin toxicity. It was suggested that the initial decrease in transporter activity was an attempt to preserve ATP in the face of acute injury and that the longer-term increase in transporter expression was protective. One could invoke the same explanation for TNF- α action in our experiments with rat brain capillaries.

3. Oxidative Stress/Ischemia. Cellular stress (e.g., exposure to heavy metals, hypoxia, reactive oxygen species, and some chemotherapeutic agents as well as heat stress) up-regulates expression of P-glycoprotein and some MRP isoforms in tumor cells and some epithelial tissues (Sukhai and Piquette-Miller, 2000; Thévenod et al., 2000, 2003). Increases in pump expression, along with increases in expression of heat shock proteins can be seen as a response designed to repair cellular damage and to remove from the cells both the stress-causing agents and the products of their actions.

drug delivery. Rapid and reversible loss of specific transport activity in the capillary endothelium could provide the window in time needed to deliver P-glycoprotein

In stroke, ischemia/hypoxia followed by reperfusion leads to generation of a complex cocktail of reactive oxygen species, proinflammatory cytokines, and chemokines. Damage to the infarct region is calamitous and damage to the peri-infarct region can be variable, determining largely long-term functional losses and thus the full extent of recovery. In brain capillary endothelial cells, exposure to hydrogen peroxide over a period of 1 to 2 days, increases both P-glycoprotein expression and P-glycoprotein-mediated transport (Felix and Barrand, 2002). This *in vitro* exposure is followed by activation of intracellular signaling through protein kinases [extracellular signal-regulated kinase 1/2 and stress-activated protein kinase, PKC, and Akt (protein kinase B)] and transcription factors (c-Jun, a component of AP-1), which in turn activate NF- κ B and transcription (Nwaozuzu et al., 2003). All of these signals are indeed up-regulated in animal models of stroke. Consistent with these results on oxidative stress, glutathione depletion causes similar up-regulation of P-glycoprotein expression in rat brain capillary endothelial cells in primary culture (Hong et al., 2006). These effects are reversed by the reactive oxygen species scavenger, *N*-acetylcysteine, suggesting that loss of glutathione leads to elevated reactive oxygen species, which induces P-glycoprotein expression.

What happens to P-glycoprotein expression after stroke *in vivo* is largely an unsettled issue. In an early study using a rat brain focal ischemia model, Samoto et al. (1994) observed a loss of P-glycoprotein expression in the ischemic lesion followed by recovery of expression during the subsequent postischemic period when angiogenesis was probably occurring. More recently Spudich et al. (2006) showed up-regulation of endothelial cell P-glycoprotein expression (mRNA and protein) 3 to 24 h after focal cerebral ischemia (medial cerebral artery occlusion) in a mouse model. It is noteworthy that this group found that inhibition of P-glycoprotein improved the efficacy of neuroprotective drugs and thus stroke outcome. In contrast, Langmade et al. (2006) surveyed several blood-brain barrier transporters in the peri-infarct region of rats after medial cerebral artery occlusion and found that although expression of endothelial BCRP and Oatp2 increased (mRNA and protein), there was no change in P-glycoprotein expression (mRNA and immunostaining of tissue sections). These disparate results may reflect differences in the models chosen, the stress applied, and the regions of the brain assayed. Certainly, because of the critical role P-glycoprotein plays in limiting access of therapeutic drugs to the CNS and the need for continued pharmacotherapy during recovery, it is important to know where, to what extent, and under what circumstances expression of this blood-brain barrier transporter is altered after stroke.

4. *Seizures.* Although a variety of drugs have proven useful in controlling seizure activity, a substantial fraction of epileptic patients do not respond to

commonly prescribed antiepileptic drugs (AEDs) that act through a range of mechanisms. Because limited drug delivery to the brain is a common cause of therapeutic failure, one suggested underlying basis for this pharmacoresistance in epilepsy is the overexpression of ATP-driven drug efflux pumps at the blood-brain barrier, including P-glycoprotein, Mrp1, Mrp2, and BCRP (Löscher and Potschka, 2005). Evidence connecting transporter overexpression with pharmacoresistance to AEDs is strongest for P-glycoprotein and can be summarized as follows: First, P-glycoprotein is overexpressed in epileptogenic brain tissue, including capillary endothelial cells, from AED-resistant patients. In rodent models, experimentally induced seizures up-regulate P-glycoprotein expression. Second, uptake of several AEDs into rodent brain is increased by specific P-glycoprotein inhibitors and in P-glycoprotein-null mice. Third, inhibition of P-glycoprotein reduces seizure severity in animal models of epilepsy. Fourth, limited clinical data suggest that adjunct therapy with a P-glycoprotein inhibitor improves seizure control in a patient with refractory epilepsy (reviewed in Löscher and Potschka, 2005; Löscher, 2007). Despite these findings, the role of P-glycoprotein in AED resistance is controversial. Indeed, it is still not clear which (if any) commonly prescribed AEDs are substrates for human P-glycoprotein (Baltes et al., 2007a,b). In addition, a case can be made that the pharmacokinetics of many of these drugs are not consistent with P-glycoprotein being a primary barrier to uptake into the CNS (Anderson and Shen, 2007). Finally, there is still substantial controversy over whether polymorphisms in the P-glycoprotein gene affect AED uptake and seizure frequency (Siddiqui et al., 2003; Tan et al., 2004b; Sills et al., 2005; Basic et al., 2008).

We have investigated the chain of events connecting seizure activity and increased P-glycoprotein expression. During seizures, the neurotransmitter, glutamate, accumulates in the brain interstitium (Holmes, 2002). It is noteworthy that glutamate has been shown to increase P-glycoprotein expression in rat brain endothelial cells (Zhu and Liu, 2004). It is known that glutaminergic signaling increases COX-2 expression and that at least in rat mesangial cells COX-2 activation leads to increased P-glycoprotein expression (Sorokin, 2004). We thus hypothesized that glutamate signaled through NMDA receptors on brain capillary endothelial cells to activate COX-2 and in turn increase P-glycoprotein expression (Bauer et al., 2008b). To test this hypothesis, we exposed isolated rat brain capillaries to glutamate for 15 to 30 min and found that specific P-glycoprotein transport activity and protein expression had roughly doubled 5.5 h later. These increases were blocked by cycloheximide, actinomycin D, NMDA receptor antagonists, and specific inhibitors of COX-2 but not COX-1. In capillaries from COX-2 knockout mice glutamate did not

increase P-glycoprotein-mediated transport or expression. In rats, intracerebral microinjection of glutamate locally increased brain capillary P-glycoprotein expression, and in an animal model of epilepsy (pilocarpine-induced status epilepticus in rats), seizure-induced increases in capillary P-glycoprotein expression were reduced by indomethacin, a nonselective COX inhibitor (Bauer et al., 2008b), and by celecoxib, a specific COX-2 inhibitor (H. Potschka et al., unpublished data). All of these findings are consistent with glutamate signaling through an NMDA receptor and COX-2 to increase P-glycoprotein expression in brain capillaries. These initial studies suggest one practical approach to increasing drug penetration of AEDs that are P-glycoprotein substrates: inhibition of endothelial cell signaling through COX-2. They show how important it is to understand at the molecular level the signaling basis for changes in transporter expression, because elements of signaling pathways are potential therapeutic targets.

5. Brain Cancer. Despite dramatic advances in the ability to treat cancers in the periphery, the CNS still remains a sanctuary for primary and metastatic disease. This situation is largely due to two elements of the blood-brain barrier, with entry of macromolecule-based drugs (antibodies and small, interfering RNA) being limited by the structural barrier and entry of lipophilic, small-molecule drugs being limited by the biochemical barrier. In fact, chemotherapeutic agents were among the earliest identified P-glycoprotein substrates (Ford and Hait, 1993), and P-glycoprotein is a major (if not *the* major) factor in the inability of these drugs to enter the CNS (Breedveld et al., 2006). Defining blood-brain barrier function within and around tumors is an active area of research that has provided evidence for both increases and decreases in tight junctional properties, perhaps suggesting opportunities for chemotherapy with certain metastatic brain tumors (Gerstner and Fine, 2007).

One can ask whether important tumor-induced changes are also seen for the elements of the biochemical barrier and what signals might underlie these changes. Gerstner and Fine (2007) summarized available literature on P-glycoprotein expression patterns in neovasculature supplying metastatic brain tumors and gliomas and found substantial differences. P-glycoprotein expression could be detected in neovasculature from both sources but at substantially reduced levels than that in normal brain. In neovasculature from metastatic brain tumors it seemed that transporter expression was consistently lower than that in gliomas. It is not clear from these studies to what extent reduced expression levels of P-glycoprotein can confer multidrug resistance, nor is it clear how tumor signals to neovasculature modulate P-glycoprotein expression. Given the apparent increased incidence of metastatic brain tumors (Palmieri et al., 2007), this is an important and evolving area of research in which an understanding of signaling to multidrug resistance transporters could be of practical benefit.

It is well documented that P-glycoprotein contributes to multidrug resistance in certain tumors and that blood-brain barrier P-glycoprotein plays a key role in limiting chemotherapeutic agents from accessing tumors within the CNS. This raises the question of why inhibitors of transporter activity are not in routine use in the clinic. Certainly, potent and specific P-glycoprotein inhibitors with acceptable toxicity profiles are now available. Moreover, as mentioned above, proof-of-principle studies in animal models have shown that specific inhibition of P-glycoprotein can both increase brain levels of chemotherapeutic agents and improve drug efficacy against implanted human tumors (Fellner et al., 2002; Kemper et al., 2003, 2004).

Early attempts to incorporate first-generation P-glycoprotein inhibitors (e.g., cyclosporin A, verapamil, and tamoxifen) into chemotherapy protocols showed some promise in patients with non-CNS tumors. However, results of later studies with first-, second-, and third-generation P-glycoprotein inhibitors have been either contradictory or disappointing (Leonard et al., 2003; Fox and Bates, 2007). Although more limited in number, studies in which P-glycoprotein inhibitors were used in the hope of improving response to chemotherapeutics in patients with brain tumors have also not shown any positive results (Chen et al., 2006; Fine et al., 2006).

There are several possible explanations for this disconnect between promising animal studies and disappointing clinical trials. Certainly there are many variables that have to be considered in choosing a dosing protocol. Thus, it is important to carefully select both the inhibitor and chemotherapeutic agent and to adjust dose levels of both to balance efficacy versus systemic and CNS toxicity. In addition, one must consider the possible contributions of other drug efflux pumps expressed on the luminal side of brain capillary endothelial cells (e.g., BCRP and MRP2), which may undergo compensatory increases when P-glycoprotein is inhibited (Deeken and Löscher, 2007).

6. HIV-1. Use of highly active antiretroviral therapy since the mid-1990s has dramatically changed the clinical picture of HIV-1 infection. However, it has also emphasized the importance of the CNS as a sanctuary for the virus. Virtually all of the drugs used to fight HIV infection penetrate the CNS poorly. Indeed, HIV protease inhibitors are substrates for P-glycoprotein and several Mrps and a number of reverse transcriptase inhibitors are substrates for BCRP (Owen et al., 2005). Both ritonavir and saquinavir are ligands for the nuclear receptor, PXR (Dussault et al., 2001), and induce P-glycoprotein expression in lymphocytes (Owen et al., 2005) and in brain capillary endothelial cells (Perloff et al., 2007), although it is not clear that this occurs solely through PXR activation. The HIV transactivator protein, TAT, can also up-regulate expression of P-glycoprotein and Mrp1 in brain capillary endothelial cells by activating NF- κ B and a mitogen-activated protein ki-

nase pathway, respectively (Hayashi et al., 2005, 2006). At this time, it is not clear to what extent the drugs used in highly active antiretroviral therapy or the viral proteins themselves affect blood-brain barrier P-glycoprotein expression in patients.

Microglia and astroglia, the cells that harbor the virus within the CNS, express P-glycoprotein (Bendayan et al., 2002). At present, we know little about regulation of ABC transporter expression in microglia, the primary HIV sanctuary within the CNS. However, recent experiments with a primary culture of rat astrocytes indicate that expression and transport function of P-glycoprotein are down-regulated after exposure to the HIV viral envelope protein, gp120 (Ronaldson and Bendayan, 2006). In these cells, signaling seemed to involve proinflammatory cytokines, with a major role for IL-6. Taken together, these findings suggest that a dynamic glial barrier behind the blood-brain barrier can further alter the ability of HIV drugs to access sites of infection within the CNS and that the glial barrier may be further strengthened by inflammation.

7. Neurodegenerative Diseases. It is clear that the blood-brain barrier is affected in virtually every CNS disease. Indeed, it has been postulated in many instances that this tissue is not merely a spectator but also an active contributor to disease (Zlokovic, 2008). A role for P-glycoprotein in neurodegenerative diseases has been examined for Alzheimer's disease and to a lesser extent for Parkinson's disease. For the latter, there seem to be positive and negative associations between specific MDR1 haplotypes and disease incidence (Furuno et al., 2002; Drozdziak et al., 2003; Tan et al., 2004a, 2005), although mechanisms underlying these associations are still unclear.

In Alzheimer's disease, P-glycoprotein may play a role in the movement of β -amyloid protein from brain to blood. Unlike other ATP-driven drug efflux pumps (e.g., the Mrps), P-glycoprotein has no identified endogenous substrates and no clearly defined diseases associated with the knockout phenotype or functionally important single nucleotide polymorphisms. Given the broad substrate specificity of the transporter, the range of possible substrates should be large, but that supposition is inconsistent with reality. In this regard, recent evidence suggests that β -amyloid protein may be one such endogenous substrate, as P-glycoprotein-mediated transport has been reported in cell lines that overexpress the transporter (Lam et al., 2001; Kuhnke et al., 2007). To move from brain to blood, β -amyloid protein must cross both the basolateral and apical plasma membranes of the capillary endothelium. Current thought is that low-density lipoprotein receptor 1 is responsible for the first step in β -amyloid transport (Deane and Zlokovic, 2007). There is suggestive evidence from animal models that P-glycoprotein could mediate β -amyloid efflux to blood. That is, efflux from brain is reduced in P-glycoprotein-null mice and in mice treated with a specific P-glyco-

protein inhibitor (Cirrito et al., 2005). It should be noted, however, that in one study with rats P-glycoprotein inhibitors did not appreciably reduce β -amyloid efflux from the brain (Ito et al., 2006). Based on these few reports, there is no consensus in the literature concerning this matter.

Interestingly, brain samples taken at autopsy show a significant negative correlation between β -amyloid levels and P-glycoprotein expression (Vogelgesang et al., 2002). It is not clear whether this reflects a cause and effect relationship; however, our preliminary rodent data indicate that β -amyloid exposure signals increased P-glycoprotein degradation in brain capillaries in vitro and in vivo (unpublished data). If P-glycoprotein does indeed mediate β -amyloid efflux from the brain, our findings suggest a vicious positive feedback loop, in which increasing brain β -amyloid levels reduce P-glycoprotein expression, which leads to further elevation of β -amyloid levels. Even if this supposition is not true, the data suggest altered blood to brain transport of therapeutic drugs in patients with Alzheimer's disease. Further studies are needed to resolve the many uncertainties with regard to the relationship between β -amyloid transport and blood-brain barrier P-glycoprotein.

V. Perspectives

It is now clear that the brain capillary endothelium is not a static barrier. Rather, both the structural/physical and selective/biochemical elements of the barrier have proven to be dynamic, responding to pathophysiological signals from both the periphery and the CNS. Given its multispecificity and potency as a drug efflux pump, the importance of P-glycoprotein as a critical element of the selective blood-brain barrier is unquestioned. Indeed, the research focus on P-glycoprotein at the blood-brain barrier is beginning to shift from questions related to where the transporter is and what it does to those focusing on when and how function changes. In this regard, five important questions must be answered.

1. What extracellular and intracellular signals modulate blood-brain barrier P-glycoprotein?
2. Which signaling pathways are responsible for changes in P-glycoprotein?
3. To what extent do epigenetic mechanisms establish basal levels of transporter expression in individuals and contribute to changes seen in disease?
4. How does disease affect P-glycoprotein?
5. Will disease-induced changes in P-glycoprotein warrant a shift in how we therapeutically target the transporter?

We can now provide a partial map of important signals and their relationship to certain diseases (Fig. 4). This map shows that in brain capillaries P-glycoprotein activity changes in response to xenobiotics including therapeutic drugs, inflammation, disease, and chemical

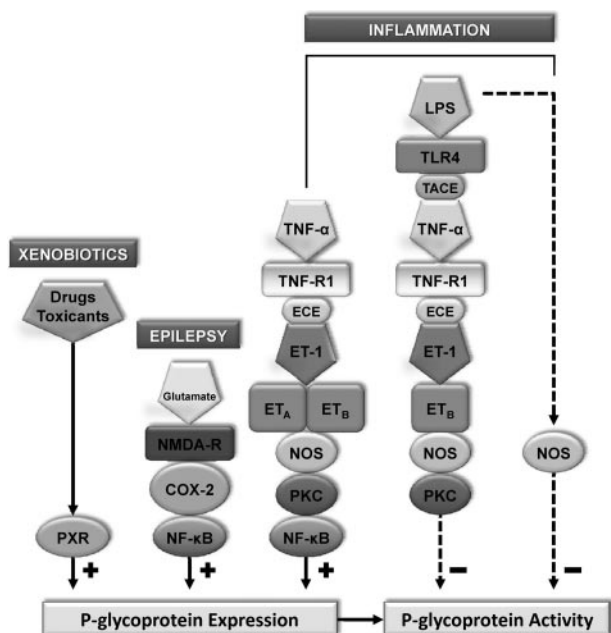


FIG. 4. Network of signaling mechanisms that modulate P-glycoprotein activity in rodent brain capillaries. Shown is a compilation of signaling pathways disclosed over the past several years by our studies on rat and mouse brain capillaries. Each step has been validated using pharmacological tools or knockout mice. At this time, two of these pathways, PXR and glutamate, have been validated in vivo (for details see Bauer et al., 2004, 2006, 2007, 2008b; Hartz et al., 2004, 2006). TLR4, Toll-like receptor 4; TACE, tumor necrosis factor- α -converting enzyme; ECE, endothelin-converting enzyme.

stress and that such changes occur through altered transporter trafficking, increased synthesis, and degradation. Signaling is complex, involving multiple surface receptors, intracellular messengers, and transcription factors. At several points in the map discrete signaling pathways intersect, suggesting divergence of signaling through PKC and NOS isoforms and possibly context-dependent signaling switches. Moreover, we now know that these same signaling pathways can modulate expression of other ABC transporters at the blood-brain barrier (e.g., Mrps and BCRP), as well as drug-metabolizing enzymes (Bauer et al., 2004, 2006, 2007, 2008a,b). Most of this work has been performed using isolated brain capillaries, but some has already been validated in vivo using transgenic and knockout animals (Bauer et al., 2004, 2006, 2008b). Certainly an emphasis on a combined in vitro/in vivo approach is needed here, especially when one wants to relate signals and mechanisms that alter transporter activity to specific diseases and therapy.

REFERENCES

- Aird WC (2007a) Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ Res* **100**:158–173.
- Aird WC (2007b) Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. *Circ Res* **100**:174–190.
- Anderson GD and Shen DD (2007) Where is the evidence that P-glycoprotein limits brain uptake of antiepileptic drug and contributes to drug resistance in epilepsy? *Epilepsia* **48**:2372–2374.
- Babakhanyan K, Bendayan M, and Bendayan R (2007) Localization of P-glycoprotein at the nuclear envelope of rat brain cells. *Biochem Biophys Res Commun* **361**:301–306.
- Baker EK and El-Osta A (2003) The rise of DNA methylation and the importance of chromatin on multidrug resistance in cancer. *Exp Cell Res* **290**:177–194.
- Baker EK and El-Osta A (2004) MDR1, chemotherapy and chromatin remodeling. *Cancer Biol Ther* **3**:819–824.
- Baker EK, Johnstone RW, Zalberg JR, and El-Osta A (2005) Epigenetic changes to the MDR1 locus in response to chemotherapeutic drugs. *Oncogene* **24**:8061–8075.
- Baltes S, Fedrowitz M, Tortos CL, Potschka H, and Löscher W (2007a) Valproic acid is not a substrate for P-glycoprotein or multidrug resistance proteins 1 and 2 in a number of in vitro and in vivo transport assays. *J Pharmacol Exp Ther* **320**:331–343.
- Baltes S, Gastens AM, Fedrowitz M, Potschka H, Kaever V, and Löscher W (2007b) Differences in the transport of the antiepileptic drugs phenytoin, levetiracetam and carbamazepine by human and mouse P-glycoprotein. *Neuropharmacology* **52**:333–346.
- Barakat S, Demeule M, Pilonet A, Regina A, Gingras D, Baggetto LG, and Beliveau R (2007) Modulation of p-glycoprotein function by caveolin-1 phosphorylation. *J Neurochem* **101**:1–8.
- Basic S, Hajnsek S, Bozina N, Filipic I, Sporis D, Mislov D, and Posavec A (2008) The influence of C3435T polymorphism of ABCB1 gene on penetration of phenobarbital across the blood-brain barrier in patients with generalized epilepsy. *Seizure*, in press.
- Bauer B, Hartz AM, Fricker G, and Miller DS (2004) Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. *Mol Pharmacol* **66**:413–419.
- Bauer B, Hartz AM, Lucking JR, Yang X, Pollack GM, and Miller DS (2008a) Coordinated nuclear receptor regulation of the efflux transporter, Mrp2, and the phase-II metabolizing enzyme, GSTpi, at the blood-brain barrier. *J Cereb Blood Flow Metab* **28**:1222–1234.
- Bauer B, Hartz AM, and Miller DS (2007) Tumor necrosis factor α and endothelin-1 increase P-glycoprotein expression and transport activity at the blood-brain barrier. *Mol Pharmacol* **71**:667–675.
- Bauer B, Hartz AM, Pekcec A, Toellner K, Miller DS, and Potschka H (2008b) Seizure-induced up-regulation of p-glycoprotein at the blood-brain barrier through glutamate and COX-2 signaling. *Mol Pharmacol* **73**:1444–1453.
- Bauer B, Yang X, Hartz AM, Olson ER, Zhao R, Kalvass JC, Pollack GM, and Miller DS (2006) In vivo activation of human pregnane X receptor tightens the blood-brain barrier to methadone through P-glycoprotein up-regulation. *Mol Pharmacol* **70**:1212–1219.
- Begley DJ (2004) ABC transporters and the blood-brain barrier. *Curr Pharm Des* **10**:1295–1312.
- Bélanger MM, Gaudreau M, Roussel E, and Couet J (2004) Role of caveolin-1 in endosome resistance development in A549 lung cancer cells. *Cancer Biol Ther* **3**:954–959.
- Bendayan R, Lee G, and Bendayan M (2002) Functional expression and localization of P-glycoprotein at the blood brain barrier. *Microsc Res Tech* **57**:365–380.
- Bendayan R, Ronaldson PT, Gingras D, and Bendayan M (2006) In situ localization of P-glycoprotein (ABCB1) in human and rat brain. *J Histochem Cytochem* **54**:1159–1167.
- Breedveld P, Beijnen JH, and Schellens JH (2006) Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. *Trends Pharmacol Sci* **27**:17–24.
- Chambers TC, McAvoy EM, Jacobs JW, and Eilon G (1990) Protein kinase C phosphorylates P-glycoprotein in multidrug resistant human KB carcinoma cells. *J Biol Chem* **265**:7679–7686.
- Chen J, Balmaceda C, Bruce JN, Sisti MB, Huang M, Cheung YK, McKhann GM, Goodman RR, and Fine RL (2006) Tamoxifen paradoxically decreases paclitaxel deposition into cerebrospinal fluid of brain tumor patients. *J Neurooncol* **76**:85–92.
- Cirrito JR, Deane R, Fagan AM, Spinner ML, Parsadanian M, Finn MB, Jiang H, Prior JL, Sagare A, Bales KR, et al. (2005) P-glycoprotein deficiency at the blood-brain barrier increases amyloid- β deposition in an Alzheimer disease mouse model. *J Clin Invest* **115**:3285–3290.
- Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, and Bertino JR (1989) Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A* **86**:695–698.
- Dallas S, Miller DS, and Bendayan R (2006) Multidrug resistance-associated proteins: expression and function in the central nervous system. *Pharmacol Rev* **58**:140–161.
- Deane R and Zlokovic BV (2007) Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* **4**:191–197.
- Deeken JF and Löscher W (2007) The blood-brain barrier and cancer: transporters, treatment, and Trojan horses. *Clin Cancer Res* **13**:1663–1674.
- Drożdżik M, Bialecka M, Mysliwiec K, Honczarenko K, Stankiewicz J, and Sych Z (2003) Polymorphism in the P-glycoprotein drug transporter MDR1 gene: a possible link between environmental and genetic factors in Parkinson's disease. *Pharmacogenetics* **13**:259–263.
- Dussault I and Forman BM (2002) The nuclear receptor PXR: a master regulator of "homeland" defense. *Crit Rev Eukaryot Gene Expr* **12**:53–64.
- Dussault I, Lin M, Hollister K, Wang EH, Synold TW, and Forman BM (2001) Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. *J Biol Chem* **276**:33309–33312.
- Farrell RJ, Murphy A, Long A, Donnelly S, Cherikuri A, O'Toole D, Mahmud N, Keeling PW, Weir DG, and Kelleher D (2000) High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. *Gastroenterology* **118**:279–288.
- Felix RA and Barrand MA (2002) P-glycoprotein expression in rat brain endothelial cells: evidence for regulation by transient oxidative stress. *J Neurochem* **80**:64–72.
- Fellner S, Bauer B, Miller DS, Schaffrik M, Fankhanel M, Spruss T, Bernhardt G, Graeff C, Farber L, Gschaidmeier H, et al. (2002) Transport of paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. *J Clin Invest* **110**:1309–1318.
- Fine RL, Chen J, Balmaceda C, Bruce JN, Huang M, Desai M, Sisti MB, McKhann

- GM, Goodman RR, Bertino JS, et al. (2006) Randomized study of paclitaxel and tamoxifen deposition into human brain tumors: implications for the treatment of metastatic brain tumors. *Clin Cancer Res* 12:5770–5776.
- Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, and Pastan I (1987) Expression of a multidrug-resistance gene in human tumors and tissues. *Proc Natl Acad Sci U S A* 84:265–269.
- Ford JM and Hait WN (1993) Pharmacologic circumvention of multidrug resistance. *Cytotechnology* 12:171–212.
- Fox E and Bates SE (2007) Tariquidar (XR9576): a P-glycoprotein drug efflux pump inhibitor. *Expert Rev Anticancer Ther* 7:447–459.
- Francis GA, Fayard E, Picard F, and Auwerx J (2003) Nuclear receptors and the control of metabolism. *Annu Rev Physiol* 65:261–311.
- Furuno T, Landi MT, Ceroni M, Caporaso N, Bernucci I, Nappi G, Martignoni E, Schaeffeler E, Eichelbaum M, Schwab M, et al. (2002) Expression polymorphism of the blood-brain barrier component P-glycoprotein (MDR1) in relation to Parkinson's disease. *Pharmacogenetics* 12:529–534.
- Gao B, Stieger B, Noe B, Fritschy JM, and Meier PJ (1999) Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. *J Histochem Cytochem* 47:1255–1264.
- Geick A, Eichelbaum M, and Burk O (2001) Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* 276:14581–14587.
- Gerstner ER and Fine RL (2007) Increased permeability of the blood-brain barrier to chemotherapy in metastatic brain tumors: establishing a treatment paradigm. *J Clin Oncol* 25:2306–2312.
- Goralski KB, Hartmann G, Piquette-Miller M, and Renton KW (2003) Downregulation of mdr1a expression in the brain and liver during CNS inflammation alters the in vivo disposition of digoxin. *Br J Pharmacol* 139:35–48.
- Gottesman MM and Pastan I (1988) The multidrug transporter, a double-edged sword. *J Biol Chem* 263:12163–12166.
- Hartley DP, Dai X, He YD, Carlini EJ, Wang B, Huskey SE, Ulrich RG, Rushmore TH, Evers R, and Evans DC (2004) Activators of the rat pregnane X receptor differentially modulate hepatic and intestinal gene expression. *Mol Pharmacol* 65:1159–1171.
- Hartz AM, Bauer B, Fricker G, and Miller DS (2004) Rapid regulation of P-glycoprotein at the blood-brain barrier by endothelin-1. *Mol Pharmacol* 66:387–394.
- Hartz AM, Bauer B, Fricker G, and Miller DS (2006) Rapid modulation of P-glycoprotein-mediated transport at the blood-brain barrier by tumor necrosis factor- α and lipopolysaccharide. *Mol Pharmacol* 69:462–470.
- Hawkins BT and Davis TP (2005) The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* 57:173–185.
- Hayashi K, Pu H, Andras IE, Eum SY, Yamauchi A, Hennig B, and Toborek M (2006) HIV-TAT protein upregulates expression of multidrug resistance protein 1 in the blood-brain barrier. *J Cereb Blood Flow Metab* 26:1052–1065.
- Hayashi K, Pu H, Tian J, Andras IE, Lee YW, Hennig B, and Toborek M (2005) HIV-Tat protein induces P-glycoprotein expression in brain microvascular endothelial cells. *J Neurochem* 93:1231–1241.
- Hennessy M and Spiers JP (2007) A primer on the mechanics of P-glycoprotein the multidrug transporter. *Pharmacol Rev* 55:1–15.
- Ho EA and Piquette-Miller M (2006) Regulation of multidrug resistance by pro-inflammatory cytokines. *Curr Cancer Drug Targets* 6:295–311.
- Holmes GL (2002) Seizure-induced neuronal injury: animal data. *Neurology* 59: S3–6.
- Hong H, Lu Y, Ji ZN, and Liu GQ (2006) Up-regulation of P-glycoprotein expression by glutathione depletion-induced oxidative stress in rat brain microvessel endothelial cells. *J Neurochem* 98:1465–1473.
- Huber JD, Eggleton RD, and Davis TP (2001) Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. *Trends Neurosci* 24:719–725.
- Ito S, Ohtsuki S, and Terasaki T (2006) Functional characterization of the brain-to-blood efflux clearance of human amyloid- β peptide (1–40) across the rat blood-brain barrier. *Neurosci Res* 56:246–252.
- Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NC, LeCluyse EL, Lambert MH, Willson TM, et al. (2000) The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol Endocrinol* 14:27–39.
- Juliano RL and Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 455:152–162.
- Kemper EM, van Zandbergen AE, Cleypool C, Mos HA, Boogerd W, Beijnen JH, and van Tellingen O (2003) Increased penetration of paclitaxel into the brain by inhibition of P-Glycoprotein. *Clin Cancer Res* 9:2849–2855.
- Kemper EM, Verheij M, Boogerd W, Beijnen JH, and van Tellingen O (2004) Improved penetration of docetaxel into the brain by co-administration of inhibitors of P-glycoprotein. *Eur J Cancer* 40:1269–1274.
- Kipp H and Arias IM (2000) Newly synthesized canalicular ABC transporters are directly targeted from the Golgi to the hepatocyte apical domain in rat liver. *J Biol Chem* 275:15917–15925.
- Kipp H and Arias IM (2002) Trafficking of canalicular ABC transporters in hepatocytes. *Annu Rev Physiol* 64:595–608.
- Kipp H, Pichetshote N, and Arias IM (2001) Transporters on demand: intrahepatic pools of canalicular ATP binding cassette transporters in rat liver. *J Biol Chem* 276:7218–7224.
- Kliwer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, et al. (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* 92:73–82.
- Kuhnke D, Jedlitschky G, Grube M, Krohn M, Jucker M, Mosyagin I, Casorbi I, Walker LC, Kroemer HK, Warzok RW, et al. (2007) MDR1-P-glycoprotein (ABCB1) mediates transport of Alzheimer's amyloid- β peptides—implications for the mechanisms of β clearance at the blood-brain barrier. *Brain Pathol* 17:347–353.
- Kumar P, Wu H, McBride JL, Jung KE, Kim MH, Davidson BL, Lee SK, Shankar P, and Manjunath N (2007) Transvascular delivery of small interfering RNA to the central nervous system. *Nature* 448:39–43.
- Labialle S, Dayan G, Gayet L, Rigal D, Gambrelle J, and Baggetto LG (2004) New invMD1 element *cis*-activates human multidrug-related MDR1 and MVP genes, involving the LRP130 protein. *Nucleic Acids Res* 32:3864–3876.
- Labialle S, Gayet L, Marthinet E, Rigal D, and Baggetto LG (2002) Transcriptional regulators of the human multidrug resistance 1 gene: recent views. *Biochem Pharmacol* 64:943–948.
- Lam FC, Liu R, Lu P, Shapiro AB, Renoir JM, Sharom FJ, and Reiner PB (2001) β -Amyloid efflux mediated by P-glycoprotein. *J Neurochem* 76:1121–1128.
- Langmade SJ, Gale SE, Frolov A, Mohri I, Suzuki K, Mellon SH, Walkley SU, Covey DF, Schaffer JE, and Ory DS (2006) Pregnane X receptor (PXR) activation: a mechanism for neuroprotection in a mouse model of Niemann-Pick C disease. *Proc Natl Acad Sci U S A* 103:13807–13812.
- Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, and Klierer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* 102:1016–1023.
- Leonard GD, Fojo T, and Bates SE (2003) The role of ABC transporters in clinical practice. *Oncologist* 8:411–424.
- Löscher W (2007) Drug transporters in the epileptic brain. *Epilepsia* 48 (Suppl 1):8–13.
- Löscher W and Potschka H (2005) Drug resistance in brain diseases and the role of drug efflux transporters. *Nat Rev Neurosci* 6:591–602.
- Marchetti S, Mazzanti R, Beijnen JH, and Schellens JH (2007) Concise review: clinical relevance of drug–drug and herb–drug interactions mediated by the ABC transporter ABCB1 (MDR1, P-glycoprotein). *Oncologist* 12:927–941.
- McRae MP, Brouwer KL, and Kashuba AD (2003) Cytokine regulation of P-glycoprotein. *Drug Metab Rev* 35:19–33.
- Miller DS (2002) Xenobiotic export pumps, endothelin signaling, and tubular nephrotoxicants—a case of molecular hijacking. *J Biochem Mol Toxicol* 16:121–127.
- Mizuno N, Niwa T, Yotsumoto Y, and Sugiyama Y (2003) Impact of drug transporter studies on drug discovery and development. *Pharmacol Rev* 55:425–461.
- Molinari A, Calcabrini A, Meschini S, Stringaro A, Crateri P, Toccaceli L, Marra M, Colone M, Cianfriglia M, and Arancia G (2002) Subcellular detection and localization of the drug transporter P-glycoprotein in cultured tumor cells. *Curr Protein Pept Sci* 3:653–670.
- Nwaazuzu OM, Sellers LA, and Barrand MA (2003) Signalling pathways influencing basal and H₂O₂-induced P-glycoprotein expression in endothelial cells derived from the blood-brain barrier. *J Neurochem* 87:1043–1051.
- Ohtsuki S, Takizawa T, Takanaga H, Hori S, Hosoya K, and Terasaki T (2004) Localization of organic anion transporting polypeptide 3 (oatp3) in mouse brain parenchymal and capillary endothelial cells. *J Neurochem* 90:743–749.
- Orlowski S, Martin S, and Escargueil A (2006) P-glycoprotein and 'lipid rafts': some ambiguous mutual relationships (floating on them, building them or meeting them by chance?). *Cell Mol Life Sci* 63:1038–1059.
- Owen A, Chandler B, and Back DJ (2005) The implications of P-glycoprotein in HIV: friend or foe? *Fundam Clin Pharmacol* 19:283–296.
- Palmieri D, Chambers AF, Felding-Habermann B, Huang S, and Steeg PS (2007) The biology of metastasis to a sanctuary site. *Clin Cancer Res* 13:1656–1662.
- Pardridge WM (2003) Blood-brain barrier drug targeting: the future of brain drug development. *Mol Interv* 3:90–105, 151.
- Pardridge WM (2007a) Blood-brain barrier delivery. *Drug Discov Today* 12:54–61.
- Pardridge WM (2007b) Blood-brain barrier delivery of protein and non-viral gene therapeutics with molecular Trojan horses. *J Control Release* 122:345–348.
- Pascucci JM, Gerbal-Chaloin S, Duret C, Daujat-Chavanieu M, Vilarem MJ, and Maurel P (2008) The tangle of nuclear receptors that controls xenobiotic metabolism and transport: crosstalk and consequences. *Annu Rev Pharmacol Toxicol* 48:1–32.
- Perloff MD, von Moltke LL, Fahey JM, and Greenblatt DJ (2007) Induction of P-glycoprotein expression and activity by ritonavir in bovine brain microvessel endothelial cells. *J Pharm Pharmacol* 59:947–953.
- Ronaldson PT and Bendayan R (2006) HIV-1 viral envelope glycoprotein gp120 triggers an inflammatory response in cultured rat astrocytes and regulates the functional expression of P-glycoprotein. *Mol Pharmacol* 70:1087–1098.
- Rosenfeld JM, Vargas R Jr, Xie W, and Evans RM (2003) Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. *Mol Endocrinol* 17:1268–1282.
- Sachs CW, Chambers TC, and Fine RL (1999) Differential phosphorylation of sites in the linker region of P-glycoprotein by protein kinase C isozymes α , β , β II, γ , δ , ϵ , η , and ζ . *Biochem Pharmacol* 58:1587–1592.
- Samoto K, Ikezaki K, Yokoyama N, and Fukui M (1994) P-glycoprotein expression in brain capillary endothelial cells after focal ischemia in rat. *Acta Neurochir Suppl (Wien)* 60:257–260.
- Schlachetzki F and Pardridge WM (2003) P-glycoprotein and caveolin-1 α in endothelium and astrocytes of primate brain. *Neuroreport* 14:2041–2046.
- Scotto KW (2003) Transcriptional regulation of ABC drug transporters. *Oncogene* 22:7496–7511.
- Seelbach MJ, Brooks TA, Eggleton RD, and Davis TP (2007) Peripheral inflammatory hyperalgesia modulates morphine delivery to the brain: a role for P-glycoprotein. *J Neurochem* 102:1677–1690.
- Siddiqui A, Kerb R, Weale ME, Brinkmann U, Smith A, Goldstein DB, Wood NW, and Sisodiya SM (2003) Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. *N Engl J Med* 348:1442–1448.
- Sills GJ, Mohanraj R, Butler E, McCrindle S, Collier L, Wilson EA, and Brodie MJ (2005) Lack of association between the C3435T polymorphism in the human multidrug resistance (MDR1) gene and response to antiepileptic drug treatment. *Epilepsia* 46:643–647.
- Smith QR (2003) A review of blood-brain barrier transport techniques. *Methods Mol Med* 89:193–208.
- Sorokin A (2004) Cyclooxygenase-2: potential role in regulation of drug efflux and multidrug resistance phenotype. *Curr Pharm Des* 10:647–657.

- Spudich A, Kilic E, Xing H, Kilic U, Rentsch KM, Wunderli-Allenspach H, Bassetti CL, and Hermann DM (2006) Inhibition of multidrug resistance transporter-1 facilitates neuroprotective therapies after focal cerebral ischemia. *Nat Neurosci* **9**:487–488.
- Sukhai M and Piquette-Miller M (2000) Regulation of the multidrug resistance genes by stress signals. *J Pharm Pharm Sci* **3**:268–280.
- Tan EK, Chan DK, Ng PW, Woo J, Teo YY, Tang K, Wong LP, Chong SS, Tan C, Shen H, et al. (2005) Effect of MDR1 haplotype on risk of Parkinson disease. *Arch Neurol* **62**:460–464.
- Tan EK, Drożdżik M, Bialecka M, Honczarenko K, Klodowska-Duda G, Teo YY, Tang K, Wong LP, Chong SS, Tan C, et al. (2004a) Analysis of MDR1 haplotypes in Parkinson's disease in a white population. *Neurosci Lett* **372**:240–244.
- Tan KH, Purcell WM, Heales SJ, McLeod JD, and Hurst RD (2002) Evaluation of the role of P-glycoprotein in inflammation induced blood-brain barrier damage. *Neuroreport* **13**:2593–2597.
- Tan NC, Heron SE, Scheffer IE, Pelekanos JT, McMahon JM, Vears DF, Mulley JC, and Berkovic SF (2004b) Failure to confirm association of a polymorphism in ABCB1 with multidrug-resistant epilepsy. *Neurology* **63**:1090–1092.
- Teng S, Jekerle V, and Piquette-Miller M (2003) Induction of ABCC3 (MRP3) by pregnane X receptor activators. *Drug Metab Dispos* **31**:1296–1299.
- Terlouw SA, Masereeuw R, and Russel FG (2003) Modulatory effects of hormones, drugs, and toxic events on renal organic anion transport. *Biochem Pharmacol* **65**:1393–1405.
- Théron D, Barraud de Lagerie S, Tardivel S, Pelerin H, Demeuse P, Mercier C, Ma-bondzo A, Farinotti R, Lacour B, Roux F, et al. (2003) Influence of tumor necrosis factor- α on the expression and function of P-glycoprotein in an immortalised rat brain capillary endothelial cell line, GPNT. *Biochem Pharmacol* **66**:579–587.
- Thévenod F (2003) Nephrotoxicity and the proximal tubule: insights from cadmium. *Nephron Physiol* **93**:p87–p93.
- Thévenod F, Friedmann JM, Katsen AD, and Hauser IA (2000) Up-regulation of multidrug resistance P-glycoprotein via nuclear factor- κ B activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. *J Biol Chem* **275**:1887–1896.
- Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, and Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* **84**:7735–7738.
- Tiwari SB and Amiji MM (2006) A review of nanocarrier-based CNS delivery systems. *Curr Drug Deliv* **3**:219–232.
- Vogelgesang S, Cascorbi I, Schroeder E, Pahnke J, Kroemer HK, Siegmund W, Kunert-Keil C, Walker LC, and Warzok RW (2002) Deposition of Alzheimer's β -amyloid is inversely correlated with P-glycoprotein expression in the brains of elderly non-demented humans. *Pharmacogenetics* **12**:535–541.
- Wilk JN, Bilsborough J, and Viney JL (2005) The *mdr1a*^{-/-} mouse model of spontaneous colitis: a relevant and appropriate animal model to study inflammatory bowel disease. *Immunol Res* **31**:151–159.
- Wolka AM, Huber JD, and Davis TP (2003) Pain and the blood-brain barrier: obstacles to drug delivery. *Adv Drug Deliv Rev* **55**:987–1006.
- Zhang H, LeCulysse E, Liu L, Hu M, Matoney L, Zhu W, and Yan B (1999) Rat pregnane X receptor: molecular cloning, tissue distribution, and xenobiotic regulation. *Arch Biochem Biophys* **368**:14–22.
- Zhang Z, Wu JY, Hait WN, and Yang JM (2004) Regulation of the stability of P-glycoprotein by ubiquitination. *Mol Pharmacol* **66**:395–403.
- Zhu HJ and Liu GQ (2004) Glutamate up-regulates P-glycoprotein expression in rat brain microvessel endothelial cells by an NMDA receptor-mediated mechanism. *Life Sci* **75**:1313–1322.
- Zlokovic BV (2008) The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* **57**:178–201.