Modulation of p-Glycoprotein Transport Function at the Blood-Brain Barrier

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The central nervous system (CNS) effects of many therapeutic drugs are blunted because of restricted entry into the brain. The basis for this poor permeability is the brain capillary endothelium, which comprises the blood-brain barrier. This tissue exhibits very low paracellular (tight-junctional) permeability and expresses potent, multispecific, drug export pumps. Together, these combine to limit use of pharmacotherapy to treat CNS disorders such as brain cancer and bacterial or viral infections. Of all the xenobiotic efflux pumps highly expressed in brain capillary endothelial cells, p-glycoprotein handles the largest fraction of commonly prescribed drugs and thus is an obvious target for manipulation. Here we review recent studies focused on understanding the mechanisms by which p-glycoprotein activity in the blood-brain barrier can be modulated. These include (i) direct inhibition by specific competitors, (ii) functional modulation, and (iii) transcriptional modulation. Each has the potential to specifically reduce p-glycoprotein function and thus selectively increase brain permeability of p-glycoprotein substrates. Exp Biol Med 230:118–127, 2005

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Roughly a century ago, Ehrlich and Goldmann demonstrated greatly restricted exchange of solutes between the blood and the central nervous system (CNS). It was subsequently shown that the blood-brain barrier resides within the brain capillary endothelium and that it has two primary elements. The first identified was a diffusional/passive element. This reflected the physical properties of both the tight junctions between brain capillary endothelial cells, which form a seal to intercellular diffusion, and the cells themselves, which exhibit a low rate of endocytosis. More recently, a second selective and metabolism-driven element has been identified. This element reflects the transport properties of solute carrier proteins (transporters) embedded in the plasma membranes of the endothelial cells (Fig. 1). Together, these elements of the barrier protect the central nervous system (CNS) from potentially toxic foreign chemicals. However, at the same time, they deny entry to therapeutic drugs that must access sites of action within the CNS. As a consequence, it has been extremely difficult to treat certain diseases of the brain, including cancer and bacterial or viral infections, because in many cases, the drugs of choice penetrate the blood-brain barrier poorly. Devising strategies to deliver therapeutic drugs into the CNS, while limiting entry of toxic chemicals and preserving an optimal extracellular environment, is a substantial challenge. Certainly, understanding how the different components of the barrier function and are modulated is an important first step to meeting this challenge.

Here we review studies from our laboratories and others focused on the multispecific, xenobiotic export pump, p-glycoprotein. For therapeutic drugs, this transporter is generally recognized to be the most important selective element of the blood-brain barrier and recent evidence suggests several ways by which p-glycoprotein activity might be modulated. In the sections that follow, we will briefly discuss the xenobiotic transporters expressed in brain capillaries, then focus on the transport properties of p-glycoprotein, and finally examine recent studies on...
Drug Transporters of the Blood-Brain Barrier

Over the past decade, it has become clear that multispecific, xenobiotic transporters play an important role in blood-brain barrier function. Such transporters are conventionally grouped into families based on molecular (genomic and protein sequence) and functional (specificity and energetics) similarities. At present, messenger RNA (mRNA) for 15 drug transporters from the organic anion transporter (OAT), multidrug resistance-associated protein (MRP), multidrug resistance protein (MDR), organic anion transporting polypeptide (OATP), organic cation transporter (OCT), concentrative nucleoside transporter (CNT), and equilibrative nucleoside transporter (ENT) subfamilies have been detected in brain capillaries or brain capillary endothelial cell lines (cow and mouse; Refs. 1–3). It is likely that this inventory of transporters is incomplete. Of the 15 transporters detected at the mRNA level, eight have been immunolocalized within brain capillary endothelial cells (Fig. 1). Four ATP-driven drug export pumps, p-glycoprotein, breast cancer resistance-associated protein (BCRP), MRP2, and MRP4 are on the luminal (blood-side) plasma membrane. Together, these transporters can handle a very wide range of anionic (MRP2 and MRP4), cationic (p-glycoprotein and BCRP), and uncharged (all four) xenobiotics (Fig. 1). Also on the luminal membrane is OATP2, another organic anion transporter that handles steroid and drug conjugates, certain opioid peptides, and the cardiac glycoside, digoxin (4). OATP2 could drive concentrative efflux from the cells if energetically coupled to the electrical potential difference across the luminal membrane. Clearly, all five transporters could contribute to the selective component of the blood-brain barrier.

Xenobiotic transporters are also present on the basolateral plasma membrane (brain side) of the brain capillary endothelial cells. These include MRP1 (5), OATP2 (6, 7), and OAT3 (Fig. 1; Ref. 7). The role of these transporters in barrier function is not well established. However, when coupled to the appropriate ion gradients, both OAT3 and OATP2 are capable of driving organic anions into the endothelial cells. Thus, basal OATP2 and OAT3 could pair with luminal MRP2 and OATP2 to drive anionic xenobiotics across the endothelium, from the CNS into blood.

**p-Glycoprotein, a Key Drug Export Pump**

Membrane proteins are important mediators of solute and macromolecular transport, hormonal signaling, regulation, and metabolism, and thus constitute one of the most important groups of proteins in cells. Among these membrane proteins, primary active xenobiotic transporters, including multidrug resistance proteins, are of special interest, because of their profound influence on the uptake, distribution, and excretion of drugs. These transporters belong to the ATP-binding cassette (ABC) superfamily of which there are 48 members in humans. Most ABC transport proteins contain two intracellular nucleotide binding domains and two transmembrane regions consisting of 12 transmembrane segments (8, 9), with the nucleotide binding sites showing far-ranging similarity among different ABC transporters (10). Some ABC transporters may have additional transmembrane domains (Mrp1), and a few are half-transporters, with a single nucleotide binding domain (BCRP). Mutations in the genes coding for these ABC proteins cause or contribute to several human disorders, including retinal degeneration, cystic fibrosis, neurological disease, cholesterol and bile transport defects, anemia, and inflammatory bowel disease, (11, 12). In addition, a growing number of preclinical and clinical studies show that polymorphisms of MDR genes can be a factor in the overall outcome of pharmacotherapy.

The best known and most widely studied representative of the ABCB-subfamily is p-glycoprotein (p-glycoprotein ABCB1), a phosphorylated glycoprotein with an apparent molecular weight of 170 kDa. It was first identified in tumor cells (13), where overexpression conferred multidrug resistance. It is also expressed at high levels in normal barrier and excretory tissues, including brush border membrane of renal proximal tubules, apical membrane of enterocytes in the gut, bile canalicular membrane in hepatocytes, and luminal membrane of brain capillary endothelial cells (14, 15). P-glycoprotein and its isoforms have been found in numerous species, including insects, fish, amphibians, reptiles, birds, and mammals. Three isoforms of this protein have been identified in rodents: mdr1a, mdr1b, and mdr2 (16); and two isoforms in humans: MDR1 and MDR2 (17). The Mdr1 gene products confer
multidrug resistance, whereas MDR2 and mdr2 secrete phosphatidylcholine into bile at the bile canalicular membrane of hepatocytes (18). The isoforms exhibit a large structural overlap and human and rat gene products show homology of approximately 80% (19).

As an ATP-driven xenobiotic export pump, p-glycoprotein handles a remarkably wide variety of substrates ranging in mass from approximately 300 to 2000 Daltons. These include organic cations, weak organic bases, some organic anions, and some uncharged compounds, including polypeptides and polypeptide derivatives. Classes of drugs transported by p-glycoprotein include multiple types of opioids, steroids, antibiotics, calcium-channel blockers, chemotherapeutics, immunosuppressants, drugs to fight human immunodeficiency virus, and others (Fig. 2). At present, the physiological substrates of p-glycoprotein have not been identified. Mdr2 (ABCB4) has been shown to be a phospholipid translocase (18) and there is evidence also suggesting that p-glycoprotein is able to transport certain phospholipids such as sphingomyelin, phosphatidylserine, or the short-chain phospholipid 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, a platelet activating factor (20–23). Additional functions have been proposed for p-glycoprotein, such as regulatory functions in apoptosis and cell differentiation (24), indirect modulation of chloride channel activity (25), and putative involvement in cholesterol esterification (26). In addition, it is responsive to inflammatory processes (27) and immunomodulation (28). It is not clear whether these additional functions are limited to certain tissues and cells or whether they are a property of all cells expressing p-glycoprotein.

**p-Glycoprotein at the Blood-Brain Barrier**

Three factors—location, potency, and broad multispecificity—combine to make p-glycoprotein a key determinant of drug entry into the CNS (29). First, localization at the luminal membrane of cerebral microvessels is consistent with p-glycoprotein being both a barrier to entry and a drug excretory pump (Fig. 3). Second, potent ATP-driven pumping essentially prevents entry and accumulation of substrates into the cytoplasm of barrier cells. Third, the remarkably wide specificity limits of the transport protein (Fig. 2) ensure that it will handle a large number of commonly prescribed drugs.

No experiments better highlight the important contributions of p-glycoprotein to the blood-brain barrier than those involving genetic modifications in which the transporter has been knocked out. In vivo dosing studies in mdr1a-knockout mice show greatly increased brain-to-plasma ratios for a large number of drugs that are p-glycoprotein substrates and that are increased 5-fold to 50-fold (30). In addition, one well-known but unintended experiment shows the downside of manipulating p-glycoprotein function; that is, reduced protection, resulting in neurotoxicity. Treatment of mdr1a-knockout mice with ivermectin, a widely used antihelminthic, caused significantly elevated drug concentrations in the brain and dramatic neurotoxicity (31). These effects were not observed in wild-type littermates that express normal levels of p-glycoprotein.
Enhanced sensitivity to ivermectin has also been observed in certain collie dogs, with a homozygous four–base pair exonic deletion with subsequent frame shifts and a premature termination of p-glycoprotein (32). It remains to be determined whether these findings are relevant for human therapeutics. However, given the recent discovery of genetic variations in p-glycoprotein expression and function in humans (33), gene-based individual variations in sensitivity and drug access to the CNS should be expected and may have to be taken into consideration when prescribing drugs with a low therapeutic index that are p-glycoprotein substrates.

Modulation of Barrier p-Glycoprotein

Clearly, one way to enhance entry of a significant number of drugs into the CNS in a controlled manner would be to alter p-glycoprotein function at the blood-brain barrier. As discussed below, we are beginning to understand the physiological and pathological mechanisms responsible for this in other tissues, and recent studies suggest that many of the same mechanisms work in brain capillary endothelial cells. These studies show that at the cellular level, changes in p-glycoprotein activity could result from (i) direct modification of pump function by inhibitors and intracellular signals, (ii) insertion and retrieval of preformed transporter from stores in vesicular compartments, and (iii) altered transporter synthesis due to enhanced or inhibited transcription and translation. The sections that follow review what is known for brain capillaries within the context of mechanisms already described in other tissues.

**Modulation by Pump Inhibition.** One strategy with great potential for therapeutic benefit is selective inhibition of blood-brain barrier p-glycoprotein. With this objective in mind, several relatively specific p-glycoprotein inhibitors have been developed and tested (34, 35). When given to test animals, these inhibitors reduce renal and hepatic excretion of p-glycoprotein substrates and increase their brain accumulation; in some instances, direct inhibition of p-glycoprotein at the blood-brain barrier has been shown to improve therapy. Of course, many factors will determine to what extent a specific drug will alter transporter function. These include inhibitor affinity, its ability to reach the transporter in the target tissue at a sufficient concentration to influence transport, inhibitor reversibility, and so on.

Consider, for example, our recent experiments with the chemotherapeutic, taxol (36). Animal studies suggest that of the few relatively specific p-glycoprotein inhibitors, PSC833 is the most effective in increasing brain drug levels (34, 35). Malignant brain tumors (e.g., higher-grade gliomas) are rarely cured by surgery or radiotherapy, and chemotherapy is of limited value, as long as the blood-brain barrier remains intact. Also, the brain is a sanctuary for metastases in patients with cancer who otherwise respond to cytostatic drugs (37). Taxol and its derivatives are active against various tumors and have been used to treat malignant glioma and brain metastases (38). However, for brain tumors, the therapeutic benefit of taxol has been low and variable, primarily because of limited entry into the CNS. Using a combined *in vitro*/ *in vivo* approach, we identified the mechanism that limits taxol access to the CNS and used that knowledge to validate in an animal model a strategy designed to increase the drug’s effectiveness against brain tumors (36). We first showed in isolated brain capillaries that luminal accumulation of taxol is concentrative, specific, and blocked by the specific p-glycoprotein inhibitor PSC833. We then demonstrated that PSC833 pretreatment not only increased brain levels of taxol (iv dosing), but that it also produced a dramatic therapeutic effect on a paclitaxel-sensitive transplanted human glioblastoma. Although taxol by itself did not affect the volume of intracerebrally implanted U-118 MG tumors, combined PSC833-taxol therapy decreased tumor volume by 90% (animals dosed twice over a 5-week period; Ref. 36). In contrast, neither taxol nor PSC833-taxol therapy affected the volume of implanted U-87 MG tumors, derived from a cell line that is not taxol-sensitive. Neither glioblastoma cell line exhibited a multidrug-resistance phenotype. These findings suggest that coadministration of PSC833 or other p-glycoprotein inhibitors can be of clinical benefit for
chemotherapy of brain tumors sensitive to cytostatics, which are substrates of p-glycoprotein. Whether this turns out to be a general strategy to increase brain permeation of p-glycoprotein substrates remains to be determined.

Rapid Modulation of Transport. For CNS therapy, it would be clearly advantageous to be able to modulate p-glycoprotein function over the short term, while retaining protection over the longer term. One strategy that might accomplish this would be to transiently decrease specific efflux to the blood through rapid regulation of transporter function. Unfortunately, we currently know little about the regulation of p-glycoprotein at the blood-brain barrier, and what we do know concerns mechanisms that work over hours to days rather than minutes (39–40). However, regulatory mechanisms have been described in other barrier and excretory tissues that signal rapid changes in p-glycoprotein function. For example, in hepatocytes, endocytic insertion and retrieval of p-glycoprotein from the canalicular membrane occurs in response to cyclic AMP and taurocholate, which cause insertion, and to cholestasis, which causes retrieval (41). In renal proximal tubules, our recent work has shown that the polypeptide hormone endothelin-1 (ET-1), signaling through an ET_B receptor, nitric oxide synthase (NOS), guanylyl cyclase, protein kinase G, and protein kinase C (PKC) rapidly reduces transport mediated by p-glycoprotein and MRP2 (Fig. 4; Refs. 42, 43).

We have examined the possibility that ET-1 also regulates p-glycoprotein at the blood-brain barrier (44). In intact rat brain capillaries, subnanomolar to nanomolar concentrations of ET-1 rapidly and reversibly decreased p-glycoprotein-mediated transport (luminal accumulation of a fluorescent cyclosporine analog. NBD-CSA; Fig. 4B). With 1–100 nM ET-1, transport was reduced to the same level as found with the p-glycoprotein-specific inhibitor PSC833, suggesting near-complete loss of function. Decreased luminal accumulation caused by ET-1 was not accompanied by increased capillary permeability (e.g., due to opening of tight junctions, as measured using a kinetic dye efflux assay; Ref. 44). In contrast, substantially increased junctional permeability was found with mannitol, sucrose, and 1-O-pentylglycerol, compounds known to open tight junctions rapidly (45–47).

ET-1 actions on p-glycoprotein-mediated transport were mimicked by the ETB receptor agonist sarafotoxin 6c; both ET-1 and sarafotoxin 6c effects were blocked by an ET_B receptor antagonist but not by an ETA receptor antagonist (44). ET_B receptor was immunolocalized to both the luminal and abluminal surfaces of the capillary endothelium. At present, it is not clear whether activation of the luminal and abluminal receptors are followed by different signaling sequences and thus a different pattern of physiological changes. Sodium nitroprusside (SNP), which generates nitric oxide, also reduced p-glycoprotein-mediated transport, presumably mimicking the effects of NOS activation. Inhibition of PKC blocked the effects of ET-1 and of SNP, while inhibition of NOS blocked the effects of ET-1 but not of PKC activation. The minimal signaling pathway that fits the data is linear, going from the ETB receptor, to NOS, to PKC (Fig. 4A; Ref. 44). This is remarkably similar to the signaling pathway by which ET-1 reduces transport on p-glycoprotein and MRP2 in renal proximal tubule (42, 43, 48–50). These results show, for the first time in brain capillaries, rapid loss of p-glycoprotein transport function followed by rapid and full recovery when the stimulus is removed. This is just the sequence of events needed when treating patients with p-glycoprotein substrates that do not cross the blood-brain barrier. Clearly, use of ET or its agonists in patients is not practical. It remains to be determined whether signaling can be manipulated in such a way as to make this phenomenon useful in the clinic.

Transcriptional Modulation. Regulation of MDR1 gene expression is complex and not completely understood.
Transcription of the p-glycoprotein gene is regulated through the binding of several trans-acting proteins to consensus cis-elements in the promoter region. Consider the gene in humans. Unlike its murine homologue, the hMDR1 promoter lacks a TATA-box and contains an initiator element. For the hMDR1 gene, several promoter elements have been found so far, including a GC-box, a Y-box, a p53 element, an inverse MED1 element, an AP-1 element, and a heat shock element, to name a few. A detailed description can be found in (51, 52). Recently, Geick et al. (53) discovered a complex regulatory cluster of several binding sites for the ligand activated nuclear receptor, pregnane X receptor (PXR), 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 in between the binding motif), one DR3 motif, and one ER6 motif (everted repeat) were identified at about ~8 kilobase pairs. Electrophoretic mobility shift assays further revealed that PXR binds as a heterodimer with the retinoid X receptor α (RXRα) to all DR4 motifs (53). In addition, reporter gene assays confirmed that this cluster of response elements is responsible for PXR-mediated hMDR1 induction.

Available evidence indicates that in a number of barrier and excretory tissues, levels of p-glycoprotein mRNA and protein change as a result of exposure to xenobiotics and mediators of inflammation as well as cellular stress. In the material that follows, each of these factors will be discussed in turn. It should be noted that although the initiating event may be different in each case, all share at least some common signaling elements.

The pregnane X receptor, PXR (NR1I2), was first discovered by Kliewer et al. (54) and Lehmann et al. (55) as a new member of a superfamily of ligand-activated transcription factors, the so-called orphan nuclear receptors. PXR defines a novel steroid signaling pathway, because it is activated by naturally occurring steroids such as pregnenolone and progesterone, and synthetic glucocorticoids and antiglucocorticoids. Importantly, PXR was also found to be activated by a wide range of xenobiotics, including dietary compounds, toxicants, and a large number of commonly prescribed drugs (56, 57). Upon activation by ligands, PXR regulates a number of target genes, mainly in liver and intestine, that are involved in xenobiotic metabolism (phase I and phase II) and efflux. Therefore, PXR is considered to be a “master regulator” of xenobiotic removal (58). Moreover, it is anticipated that PXR, xenobiotic metabolizing enzymes, and efflux transporters are regulated as a core network of defense mechanisms (59, 60). Efflux transporters 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, ABCC3); and p-glycoprotein (ABCB1, MDR1; Refs. 53–55, 61). Importantly, PXR is the only ligand-activated nuclear receptor known to control transcription of MDR1, and thus expression of p-glycoprotein.

We have begun to examine transcriptional regulation of p-glycoprotein at the blood brain barrier. In earlier published reports, PXR expression was not detected in whole brain homogenates (54, 62, 63). However, capillaries comprise less than 1% of brain volume. If PXR expression were restricted primarily to those structures, mRNA levels in whole brain might be below the detection limits of the technique used (Northern blotting). Using reverse transcription–polymerase chain reaction (RT-PCR), we detected PXR mRNA in isolated rat brain capillaries; immunostaining confirmed receptor expression in the endothelial cells (64). Exposing isolated rat brain capillaries to two PXR ligands, pregnenolone 16α-carbonitrile (PCN) and dexamethasone, increased mRNA (via quantitative RT-PCR) and protein (via Western blotting and quantitative immunostaining) levels for p-glycoprotein as well as p-glycoprotein–mediated transport in the capillaries (56). Moreover, dosing rats with PCN and dexamethasone increased p-glycoprotein expression in plasma membranes from liver and brain capillaries and up-regulated specific transport in the capillaries (Fig. 5). Our initial experiments also show parallel up-regulation of MRP2 (64) as well as of phase II drug metabolizing enzymes (Bauer et al., unpublished data), suggesting...
coordinate regulation of drug metabolism and efflux at the blood-brain barrier.

These in vitro and in vivo dosing experiments provide the first evidence for PXR expression in brain and for regulation by nuclear receptors of xenobiotic transporters at the blood-brain barrier. They disclose one mechanism by which the activity of p-glycoprotein, a primary gatekeeper of the blood-brain barrier, is modulated and argue for selective tightening of the blood-brain barrier in patients exposed to the wide range of xenobiotics that are PXR ligands. They also suggest a mechanistic basis for earlier experiments in which p-glycoprotein expression was found to be increased in brain capillary endothelial cells from patients with epilepsy (65) and in primary cultures of rat brain capillary endothelial cells exposed to puromycin, rifampicin, and dexamethasone, all PXR ligands (40, 66).

Because p-glycoprotein is responsible for the efflux of neurotoxic chemicals and metabolites from the CNS, increased pump expression in response to PXR ligands should result in enhanced neuroprotection. However, because many p-glycoprotein substrates are used to treat CNS disorders, increased pump expression also implies reduced access of those drugs to sites of action within the CNS. Three practical aspects require further comment. First, many p-glycoprotein substrates are also PXR ligands. Thus, it is possible that PXR activation up-regulates p-glycoprotein expression, and thus drug resistance, when certain substrates are given chronically. Second, PXR action is known to be affected by multiple corepressors and coactivators that modify PXR’s actions within the cell nucleus. However, at present, there is no practical way to intervene at the level of PXR-gene interactions. Third, it is not clear to what extent p-glycoprotein expression can be down-regulated by removing PXR ligands from the diet. We would expect the effects of diet modification to be greatest on transporter expression in tissues of the gastrointestinal tract, but there may be a benefit to the blood-brain barrier of patients scheduled to undergo pharmacotherapy for CNS disorders.

Inflammation is a major component of many CNS diseases. The acute response is a complex immunological reaction that is triggered by a wide variety of stimuli, including infection, trauma, and cell stress. Inflammation results in the release of proinflammatory cytokines and a change in gene expression in affected tissues. The changes observed in liver and intestine are particularly interesting for students of blood-brain barrier function. In both tissues, inflammation, induced experimentally by injection of lipopolysaccharide (LPS, endotoxemia), reduces expression (mRNA and protein) of certain drug metabolizing enzymes and drug efflux transporters, including p-glycoprotein, and MRP2 (67, 68). In hepatocytes, sinusoidal efflux transporters (e.g., MRP3) are up-regulated to protect against intracellular accumulation of toxic bile salts. As one might expect, inflammation also changes the disposition of a number of xenobiotics, and these changes are consistent with the known effects on enzyme and transporter expression. A similar hepatic response is observed with experimental cholestasis, induced by bile duct ligation, which induces a severe inflammatory response in the liver (67). The responsible inflammatory mediators include interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-α (TNF-α), each of which reduces transporter expression (27, 69). The mechanisms responsible for the profound changes in hepatic enzyme and transporter expression in cholestasis are complicated, and multiple factors appear to be involved, including bile salts, proinflammatory cytokines, oxidative stress, retinoids, drugs, and hormones (67, 70). Cytokines do appear to suppress the activation by nuclear receptors of genes coding for luminal drug efflux transporters (e.g., MRP2 and p-glycoprotein) and drug metabolizing enzymes (67, 71). At a minimum, inflammation appears to down-regulate hepatic receptor expression, but this may not be the only underlying mechanism involved.

Consistent with this picture, recent studies show that chronic inflammation reduces p-glycoprotein activity in brain tissue in general, and in brain capillary endothelial cells in particular (72–74). For example, Goralski et al. (72) 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.

In contrast to inflammation, cellular stress (e.g., exposure to heavy metals, reactive oxygen species, and some chemotherapeutics as well as heat stress) up-regulates expression of p-glycoprotein and some MRP isoforms in tumor cells and some epithelial tissues (70, 75, 76). Increases in pump expression, along with increases in expression of heat shock proteins can be seen as a response designed to remove from the cells both the offending agents and the products of their actions. Consistent with this, recent studies showed that oxidative stress increased p-glycoprotein expression in rat brain and rat brain capillary endothelial cells in primary culture. Using a rat brain ischemia model, Samoto et al. (77) observed a loss of p-glycoprotein followed by recovery of expression during the postischemic period. In this regard, anoxia followed by reperfusion is known to generate reactive oxygen species. In stroke, hypoxia followed by reperfusion leads to generation of reactive oxygen species, and these are believed to contribute substantially to subsequent cell death. In brain capillary endothelial cells, exposure to hydrogen peroxide over a period of 1–2 days increased both expression of p-glycoprotein and p-glycoprotein–mediated transport (78). Exposure was followed by activation of intracellular signaling through protein kinases (ERK1/2 and stress-
activated protein kinase C) and transcription factors (e-Jun and Akt, which activate transcription and NF-κB, which depending on circumstances, may activate or inhibit; Ref. 39). Certainly, we would want to know whether brain capillaries in situ respond to oxidative stress in a similar manner. It would also be important to determine whether this response increases chances of survival after stroke or is part of a longer-term pathological process.

**Perspectives**

It has become increasingly clear that the brain capillary endothelium is a dynamic barrier, with the properties of the passive elements (tight junctions) and active/selective elements (expressed transporters) capable of changing dramatically as a result of hormonal signaling, injury, disease, and therapy. Because of the key role that p-glycoprotein plays in determining xenobiotic entry into the brain, understanding how the activity of this efflux transporter is modulated may provide practical strategies for selectively manipulating barrier permeability for a large number of drugs. Although we are making rapid progress in identifying the multiple mechanisms that modulate p-glycoprotein function, much still remains to be discovered. Areas to be investigated further include: (i) practical aspects of the use of specific p-glycoprotein inhibitors (e.g., targeting, optimal dosing protocols, reversibility, and safety), (ii) dietary and pharmacological treatments to modify nuclear receptor actions, and (iii) possible use of inflammatory and stress pathways to lower p-glycoprotein expression at the blood-brain barrier.


