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The Blood-Brain Barrier in Epilepsy

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1. Introduction

The blood-brain barrier is altered in epilepsy. This includes altered expression of transporters and metabolic enzymes as well as barrier leakage that have been linked to antiepileptic drug resistance and seizure genesis, respectively. Here we highlight current understanding of these pathological changes. Three critical components of barrier function - 1) tight junctions, 2) metabolising enzymes and 3) transporter proteins - are introduced and we describe how they are changed in epilepsy and affected by epilepsy treatment. Recent efforts in blood-brain barrier research to overcome drug-resistant epilepsy are also discussed.

2. The blood-brain barrier

The History of Blood-Brain Barrier Discovery. First experiments contributing to the discovery of the blood-brain barrier were performed by Paul Ehrlich in 1885 (**Figure 1**). Ehrlich observed that water-soluble "vital dyes" injected into the blood of rats did not stain the brain (Ehrlich, 1885). In 1900, Lewandowsky made similar observations and coined the term "blood-brain barrier" ("Bluthirnschranke") to explain this phenomenon (Lewandowsky, 1900). Ehrlich's student, Edwin Goldmann, injected the same dyes Ehrlich had used into the subarachnoid space, and found the opposite: intense staining of the brain but no staining of peripheral tissues (Goldmann, 1909; 1913). Goldmann concluded that a barrier had to exist between the brain and the periphery, thus the concept of a vascular barrier was born. In 1923, Spatz postulated that the brain capillary endothelium had to be the structure responsible for barrier function, which initiated a debate that lasted for decades (Spatz, 1933). It was Reese and Karnovsky, and Brightman and Reese who solved the mystery of the blood-brain barrier in the late 1960s. Using electron microscopy, they discovered that tight junctions connect adjacent capillary endothelial cells and seal the intercellular space (Brightman & Reese, 1969; Reese & Karnovsky, 1967). With this, the molecular structure responsible for barrier function was identified and the barrier was localized to the brain capillary endothelium.

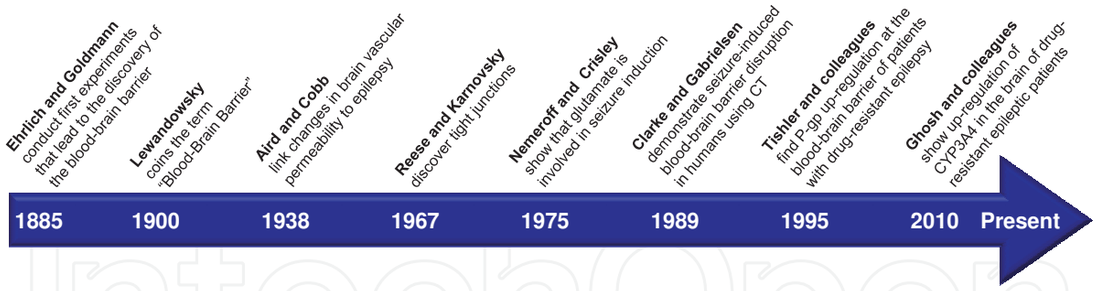

Fig. 1. Evolution of Blood-Brain Barrier Methodology/History

The History of the Blood-Brain Barrier in Epilepsy. In the 1930s, Aird and Cobb discovered that brain uptake of "vital dyes" was increased in epileptic mice. Based on their observation, they suggested that the brain vasculature may be a barrier between the central nervous system (CNS) and the periphery and that altered brain vascular permeability may be a factor contributing to epilepsy (Aird, 1939; Cobb et al., 1938). In the mid 1950s, Bercel used diuretics in patients to increase brain uptake of antiepileptic drugs (AEDs (Bercel, 1955)). Co-administration with diuretics reduced AED doses below toxic levels in ten of ten patients and in seven of these ten patients seizure control was improved (Bercel, 1955). Nemeroff and Crisley made a critical discovery in 1975 when they found that glutamate is involved in seizure induction and increases cerebrovascular permeability in rats (Nemeroff & Crisley, 1975). Further, blood-brain barrier dysfunction was shown to go along with an increase in blood pressure and cerebral vasodilation during seizures (Bolwig et al., 1977; Petito et al., 1977). In 1989, Clarke and Gabrielsen demonstrated seizure-induced blood-brain barrier leakage in humans using computed tomography (Clarke & Gabrielsen, 1989). In 1995, Tishler et al. made the observation that mRNA of *MDR1* (*ABCB1*), the gene encoding the efflux transporter P-glycoprotein (P-gp) is increased at the blood-brain barrier of patients with drug-resistant epilepsy (Tishler et al., 1995). This was a critical finding because P-gp acts as a "gatekeeper" that limits therapeutic drugs from crossing the blood-brain barrier and from entering the brain (Miller et al., 2008). Research in this field initially focused on P-gp, but other transporters such as multidrug resistance proteins (MRPs) and breast cancer resistance protein (BCRP) are also increased in epilepsy animal models or patients (Awasthi et al., 2005; Dombrowski et al., 2001; Sisodiya et al., 2006; Van Vliet et al., 2005).

Today, the role of some of these transporters in epilepsy is still unclear. It has been discussed that P-gp could be involved in seizure generation (Marchi et al., 2004) and that multiple transporters may act in concert to limit brain uptake of a broad range of AEDs (Lazarowski et al., 2007). Recent studies show that AED-metabolizing enzymes such as cytochromes (CYP) 3A4, 2C8, and glutathione sulfotransferase (GST) μ and π are also upregulated in the brain of epileptic patients forming a metabolic barrier that contributes to AED resistance (Ghosh et al., 2010; Shang et al., 2008; Ueda et al., 2007).

2.1 Blood-brain barrier anatomy

Numbers and Facts. The blood-brain barrier is a network of brain capillaries (microvessels). With a diameter of 3-7 μm, brain capillaries are the smallest vessels of the vascular system (**Figure 2A**) (Rodriguez-Baeza et al., 2003). The microvasculature in the human brain is comprised of about 100 billion capillaries forming a highly branched vascular network

(Zlokovic & Apuzzo, 1998). Due to the high capillary density in the brain, capillaries are about 40 µm apart from each other, a distance short enough for small molecules to diffuse within 1 second (Rodriguez-Baeza et al., 2003). This ensures that every neuron (about 100 billion in human brain) is in contact with and perfused by its own capillary, which allows efficient nutrient and oxygen supply. Despite the huge number of 100 billion brain capillaries, the total capillary lumen occupies only about 1% of total brain volume, or about 12-15 ml in an adult human brain of about 1,400 ml (Pardridge, 2003b). Thus, at any given time, about 8-10% (about 10 ml) of total cerebral blood (about 150 ml) is in the lumen of brain capillaries. Not taking the capillary lumen into account, it is estimated that the brain capillary endothelium occupies only about 0.1% of total brain volume (~ 1-1.5 ml) (Pardridge, 2003b). Lastly, the total length of the capillary network is about 600-650 km in an adult human brain with a total surface area of about 20 m^2. This makes the blood-brain barrier the third largest surface area for drug exchange after intestine and lung (Pardridge, 2003a).

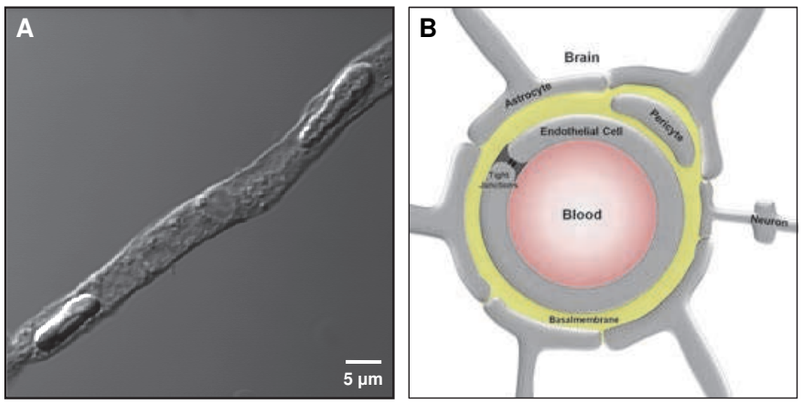

Fig. 2. (A). DIC image of isolated brain capillary. (B) Neurovascular unit.

Morphology and Anatomy. Brain capillaries are the next higher level of organisation from endothelial cells that are the smallest anatomical unit of microvessels. Brain capillary endothelial cells are flat, thin, spindle-shaped, polarized cells. Their apical membrane faces the blood (luminal), and their basolateral membrane faces the brain parenchyma (abluminal; (Betz et al., 1980)). It is through the basement membrane that brain capillary endothelial cells are in contact with pericytes, astrocytes, and neurons (**Figure 2B**; (Goldstein & Betz, 1983)). This 4-cell structure is referred to as "*Neurovascular Unit*" and is responsible for maintaining and regulating blood flow, and for controlling barrier function (Begley, 2004).
One fundamental characteristic of endothelial barrier function is a complex, multi-protein structure called a *tight junction*, which is unique in the vascular system (Nagy et al., 1984). Brain capillary endothelial cells also lack intercellular clefts and have low pinocytotic activity, which limits solute exchange between blood and brain. Lastly, to meet the large energy demand of ATP-consuming processes like metabolism and active efflux transport, brain capillary endothelial cells possess a large number of mitochondria (Goldstein & Betz, 1983).

2.2 Blood-brain barrier physiology

Blood-brain barrier functions include CNS protection, and regulation and maintenance of CNS homeostasis. Three components determine barrier function: *1. Tight Junctions, 2.*

Transporters and *3. Metabolising Enzymes*. The following paragraphs describe these components in more detail.

1. Tight Junctions

Tight junctions are cell-cell contacts that seal the intercellular space between adjacent endothelial cells, thereby creating a non-fenestrated endothelium and limiting hydrophilic molecules from paracellular diffusion (Nag, 2003). Tight junctions are multi-protein complexes composed of transmembrane proteins like occludins, claudins, e-cadherins and junctional adhesion molecules as well as adaptor and regulatory proteins (Matter & Balda, 2003a; Vorbrodt & Dobrogowska, 2003). Adaptor proteins include zonula occludens proteins, cingulin, catenin and membrane-associated guanylate kinase inverted proteins that connect junctional transmembrane proteins with cytoskeletal actin filaments (Matter & Balda, 2003b). Regulatory proteins include G proteins, atypical protein kinase C isoforms, and symplektin that are involved in signalling (Matter & Balda, 2003b; Wolburg & Lippoldt, 2002). Together, tight junctions guarantee a tight barrier, and thus, protection of the CNS (Kniesel & Wolburg, 2000). However, under pathological conditions such as epilepsy, tight junctions can be dysfunctional or disrupted, leading to barrier leakage, impaired neuronal function, and brain damage (Huber et al., 2001).

2. Metabolic Enzymes

The concept of a "metabolic barrier" is widely accepted but little information is available on metabolising enzymes at the blood-brain barrier. Early studies focused on phase I enzymes in whole brain tissue but later studies differentiated between different brain cell types. Walther et al. showed that CYP P450 enzymes are located in the inner mitochondrial membrane of neurons and glia from rat, guinea pig, rabbit, and pig brain. This is in contrast to liver, where most CYP isoforms are located at the endoplasmic reticulum (Walther et al., 1986). Consistent with this, Ghersi-Egea et al. found CYP P450 protein expression in mitochondria from rat brain tissue (Ghersi-Egea et al., 1987), and demonstrated CYP activity in various brain regions and isolated human microvessels. They found low 1-naphthol-UDP-glucuronosyltransferase and NADPH-CYP P450 reductase activity, and high GST and epoxide hydrolase activity (Ghersi-Egea et al., 1993). The same group also found Cyp P450 activity in rat brain microvessels (Ghersi-Egea et al., 1994).

Dauchy et al. used isolated microvessels from resected human brain and found mRNA expression of CYP1A1, 1B1, 2B6, 2C8, 2D6, 2E1, 2J2, 2R1, 2S1, and 2U1, and detected CYP1B1 by Western blotting (Dauchy et al., 2008). Immunohistological studies by Rieder et al. confirmed localisation of CYP1B1 in human brain capillaries (Rieder et al., 2000). In a follow up study, Dauchy et al. showed CYP2U1 and CYP2S1 mRNA expression in the human cerebral microvascular endothelial cell line hCMEC/D3 (Dauchy et al., 2009). CYPs with low mRNA expression included CYP2R1, 2B6, 2E1, 1A1, 2D6, 2C18, 1B1, 2J2, 1A2 and 2C8. Except for CYP2C18, all CYP genes found in hCMEC/D3 cells were also detected in isolated human brain microvessels. A novel CYP P450, Cyp4x1, was identified in 2006 by Al-Aznizy et al. in mouse brain (Al-Anizy et al., 2006). Immunohistochemical staining showed strong Cyp4x1 protein expression in neurons, choroid plexus epithelial cells, and brain microvessel endothelial cells. In 2010, mRNA and protein expression of CYP3A4, the most prominent enzyme involved in xenobiotic metabolism in the liver, was found by Ghosh et al. in human brain endothelial cells (Ghosh et al., 2010).

While most blood-brain barrier enzymes have been detected at the mRNA level, protein expression and activity of only few enzymes have been demonstrated. These include

gamma-glutamyl transpeptidase (Beuckmann et al., 1995), alkaline phosphatase (Beuckmann et al., 1995), aromatic L-amino acid decarboxylase (Betz et al., 1980; Matter & Balda, 2003b), the phase I metabolising enzymes CYP1A1 (Filbrandt et al., 2004), CYP1B1 (Filbrandt et al., 2004), CYP3A4 (Ghosh et al., 2010; Ghosh et al., 2011), and Cyp4x1 (Al-Anizy et al., 2006), NADPH-CYP P450 reductase (Chat et al., 1998; Ghersi-Egea et al., 1988; Minn et al., 1991; Ravindranath et al., 1990), epoxide hydrolase (Ghersi-Egea et al., 1988; Minn et al., 1991), and the phase II enzymes, 1-naphthol-UDP-glucuronosyltransferase (Ghersi-Egea et al., 1988) and GSTμ (Shang et al., 2008), and GSTπ (Bauer et al., 2008; Shang et al., 2008).

The presence of these enzymes in the brain microvasculature indicates the existence of a metabolic barrier. However, more studies are needed to better define the role metabolising enzymes play at the blood-brain barrier under physiological and pathophysiological conditions and whether these enzymes can indeed limit AED delivery to the brain.

3. Transporters

The blood-brain barrier is an active, dynamic and selective interface that responds to signals from both the periphery and brain. Key components of barrier function include influx and efflux transporters that are responsible for brain homeostasis, nutrient supply, and protection of the brain from endogenous and exogenous toxins.

Influx transporters that maintain CNS homeostasis and nutrient supply include A-and N-system amino acid transporters (Betz et al., 1980; O'kane & Hawkins, 2003), excitatory amino acid carriers 1, 2, and 3 (O'kane & Hawkins, 2003; O'kane et al., 1999), alanine/serine/cysteine/threonine (ASCT) transporters for neutral amino acids (Boado et al., 2004; Tayarani et al., 1987), glucose transporters GLUT1 and GLUT3/14 (Pardridge, 1991; Simpson et al., 2007), monocarboxylate transporters MCT1 and MCT8 (Braun et al., 2011; Ito et al., 2011; Simpson et al., 2007), and the equilibrative nucleoside transporter ENT1 (Kitano et al., 2002), as well as Na^+-K^+-ATPase (Betz et al., 1980). These transporters belong to the solute carrier (SLC) superfamily. Prominent SLC transporters that have been detected at the blood-brain barrier also include the organic anion transporter Oat3, organic anion transporting polypeptides Oatp1a4, 1b1, 1c1, 2b1, 14, and organic cation transporters OCT1, OCT2 (Ito et al., 2011; Lin et al., 2010). Of these SLCs, Oat3, Oatps, and Octs are involved in drug transport. However, it is currently not known if these SLC transporters can handle AEDs.

An interesting blood-brain barrier transporter is the large neutral amino acid transporter LAT that transports the amino acids valine, leucine, isoleucine, tryptophan, and tyrosine. LAT1 mediates brain uptake of L-DOPA that is used in Parkinson's disease (Del Amo et al., 2008). LAT1 has also been reported to transport the AEDs gabapentin and pregabalin across the blood-brain barrier into the brain (Del Amo et al., 2008; Liu et al., 2008; Su et al., 1995). Whether LATs are affected in epilepsy is unknown.

In total, 21 transporters have been detected at the protein level in brain capillaries and brain capillary endothelial cells from various species by immunohistochemistry, Western blotting, or quantitative LC/MS/MS (Kamiie et al., 2008; Neuwelt et al., 2011). Seven of these transporters belong to the ABC (ATP-binding cassette) transporter family and include P-glycoprotein (P-gp, *MDR1*, *ABCB1*), the multidrug resistance proteins 1, 2, 3, 4, and 5 (MRPs, *ABCC1-5*) and breast cancer resistance protein (BCRP, *ABCG2*). These transporters are ATP-driven and mainly located at the luminal membrane of the brain capillary endothelium (Mrp1 and Mrp4 are also in the abluminal membrane). This "first line of defence" protects the brain from neurotoxicants and limits CNS drugs from entering the brain, and thus, is an obstacle for CNS pharmacotherapy.

Together, transporters ensure proper CNS nutrient supply and mediate efflux of metabolic wastes from the brain, thus, helping maintain CNS homeostasis. The following section describes the role of transporters, metabolic enzymes, and barrier leakage in epilepsy.

3. Blood-brain barrier function in epilepsy

Epilepsy affects more than 60 million people worldwide. The majority of patients respond to treatment with AEDs, but up to 40% of patients are drug-resistant (Kwan & Brodie, 2003; Loscher & Potschka, 2005). Patients with AED resistance suffer from uncontrolled seizures, which elevates their risk of brain damage and mortality (Sperling et al., 1999). These patients experience a low quality of life and, despite advances in pharmacotherapy and neurosurgery, drug-resistant epilepsy remains a major clinical problem (Devinsky, 1999).
Evidence indicates that the blood-brain barrier is altered in patients with epilepsy. Changes in the brain capillary endothelium include upregulation of efflux transporters and metabolic enzymes as well as barrier leakage that have been linked to AED resistance and seizure genesis (Bauer et al., 2008; Ghosh et al., 2010; Marchi et al., 2007). The following section describes the role of transporters, metabolic enzymes, and barrier leakage in epilepsy.

3.1 Transporters in epilepsy

One factor underlying AED resistance is, at least in part, seizure-induced over-expression of drug efflux transporters at the blood-brain barrier (Bauer et al., 2008). Some of these transporters, such as P-gp, Mrp2, and BCRP have been implicated with AED resistance. The first evidence for involvement of efflux transporters in epilepsy goes back to studies by Tishler and co-workers in 1995. These researchers observed increased P-gp mRNA in the brain and protein expression in the capillary endothelium of patients with drug-resistant epilepsy (Tishler et al., 1995). The findings by Tishler et al. were confirmed by other groups (Dombrowski et al., 2001; Lazarowski et al., 1999; Sisodiya et al., 2002) and it was suggested that this phenomenon could prevent AEDs from entering the brain and cause AED resistance. However, studies in cell lines of non-brain endothelial origin showed that some AEDs such as vigabatrin, gabapentin, phenobarbitone, lamotrigine, carbamazepine, and phenytoin are not, or are only weak, P-gp substrates, questioning whether P-gp could be the primary reason for AED resistance (Crowe & Teoh, 2006; Maines et al., 2005; Owen et al., 2001; Weiss et al., 2003). In contrast, Cucullo et al., compared phenytoin permeation in brain capillary endothelial cells from drug-resistant epileptic human brain tissue with that of commercially available human brain microvascular endothelial cells (Cucullo et al., 2007). They demonstrated that phenytoin permeation was 10-fold lower in endothelial cells from AED-resistant patients compared to purchased human endothelial cells. Although this comparison is flawed, inhibiting P-gp increased phenytoin permeation in the AED-resistant cells. Moreover, recent *in vivo* data, including our own studies, demonstrate that P-gp does limit AEDs from entering the brain (Brandt et al., 2006; Liu et al., 2007; Van Vliet et al., 2007). Using a drug-resistant epilepsy rat model, Potschka et al. showed that animals not responding to phenytoin exhibited 2-fold higher P-gp expression levels in brain capillaries compared to animals responding to treatment (Potschka et al., 2004). van Vliet et al. demonstrated that inhibiting P-gp counteracted phenytoin resistance, which reduced seizure occurrence in rats (Van Vliet et al., 2006). Marchi et al. supported these findings showing that patients with high blood-brain barrier P-gp expression had low brain levels of

oxcarbazepine (Marchi et al., 2005). These studies demonstrate that, in drug-resistant epilepsy, certain, but not all AEDs have restricted access to the brain due to increased blood-brain barrier P-gp, and that modulation of P-gp can enhance brain distribution of some AEDs such as phenytoin (Potschka & Loscher, 2001; Van Vliet et al., 2006; Van Vliet et al., 2007).

In addition to P-gp, data indicate that BCRP plays a significant role in drug efflux at the blood-brain barrier. Recent studies show that both transporters, P-gp and BCRP, "team up" and work together to limit chemotherapeutic drugs from permeating across the blood-brain barrier and penetrating into the brain (Chen et al., 2009; De Vries et al., 2007). However, little information is available on the extent to which BCRP contributes to AED resistance and if P-gp and BCRP work in concert in AED efflux from the brain. Some studies found no upregulation of BCRP in human epileptogenic brain tissue and no evidence for BCRP-mediated AED transport *in vitro* (Cerveny et al., 2006; Sisodiya et al., 2003), but other studies reported upregulation of BCRP expression in the microvasculature of epileptogenic brain tumors (Aronica et al., 2005; Vogelgesang et al., 2004) and in chronic epilepsy animal models (Van Vliet et al., 2005). More studies are needed to unequivocally clarify the role of BCRP, especially in conjunction with P-gp, in AED-resistant epilepsy.

Only little information is available on the multidrug resistance proteins (Mrps) in epilepsy. van Vliet et al. used the pilocarpine status epilepticus model in rats and found by immunohistochemistry and Western blotting that Mrp1 and Mrp2 protein expression was upregulated in astrocytes within several limbic structures including the hippocampus (Van Vliet et al., 2005). These findings were confirmed by Hoffmann et al., who also demonstrated Mrp2 upregulation in brain capillaries by immunohistochemistry following pilocarpine-induced status epilepticus (Hoffmann et al., 2006). In control rats, Mrp2 was barely detectable in the brain capillary endothelium, but in status epilepticus rats, Mrp2 staining was evident in brain capillary endothelial cells. MRP2 has also been found to be over-expressed in sclerotic hippocampal tissue of AED-resistant patients with mesial temporal lobe epilepsy (Aronica et al., 2004). In the same patient population, MRP1 expression was upregulated in glial endfoot processes around cerebral blood vessels. Observations of chronic epileptic rats showed that protein levels of Mrp1 and Mrp2 were also upregulated in blood vessels and this over-expression correlated with seizure frequency and reduced brain uptake of phenytoin (Van Vliet et al., 2005). However, phenytoin brain uptake was enhanced by the MRP inhibitor probenecid. While upregulation of mRNA was observed for Mrp1, 5, and 6, increased protein expression was only found for MRP1 and 2 in isolated capillary endothelial cells from patients with drug-resistant epilepsy (Dombrowski et al., 2001; Kubota et al., 2006). A time-course study revealed that 6-24 h after onset of a pilocarpine-induced status epilepticus in rats, mRNA of P-gp, Mrp1, and Mrp5 was decreased in hippocampus, amygdala, and the piriform cotex. This initial decrease in mRNA levels was followed by a 24h period of normal mRNA expression and then increased mRNA levels about 4 days after status epilepticus (Kuteykin-Teplyakov et al., 2009). These findings are in contrast to an earlier study where P-gp mRNA levels in mouse hippocampus were increased by 85% 3-24 h after kainic acid-induced limbic seizures, but returned to control levels after 72 h (Rizzi et al., 2002). Treatment with AEDs for 7 days did not change P-gp mRNA expression (Rizzi et al., 2002). In the same study, the authors also used rats with spontaneous recurrent seizures 3 months after electrically induced status epilepticus. P-gp mRNA levels were increased 1.8- and 5-fold in the hippocampus and entorhinal cortex,

respectively. Thus, changes in P-gp mRNA levels occur after both acute and chronic epileptic activity. The same authors (Rizzi et al., 2002) also used microdialysis and demonstrated that AED brain levels were significantly reduced. While a direct connection between blood-brain barrier P-gp levels and AED brain levels was not shown, it was concluded that seizure-induced changes in P-gp could contribute to AED resistance in epilepsy. Note that none of these studies provided data on transporter protein expression or activity.

3.1.1 Transporter inhibition

The discovery that drug efflux transporters are upregulated at the blood-brain barrier in AED-resistant patients suggested that transporter inhibition could overcome AED resistance in epilepsy. This notion was encouraged by studies that showed enhanced brain uptake of AEDs when co-administered with transporter inhibitors. Using verapamil and probenecid, Potschka et al. used microdialysis and demonstrated in healthy rats that P-gp and Mrp limit carbamazepine brain uptake (Potschka & Loscher, 2001). A follow-up study showed that administration of the metabolic inhibitor sodium cyanide and the P-gp inhibitors verapamil and PSC833 into the frontal cortex significantly increased extracellular fluid concentrations of phenytoin. This indicated that P-gp limits phenytoin distribution into the brain under physiological conditions (Potschka & Loscher, 2001). Similar observations were made with phenobarbital, lamotrigine, and felbamate (Potschka et al., 2002). Verapamil has also been used in case studies with AED-resistant patients (Iannetti et al., 2005; Summers et al., 2004). For example, the status epilepticus in an 11-year old boy who was first unresponsive to conventional AEDs disappeared after administration of verapamil i.v. (Iannetti et al., 2005). However, this anticonvulsive response could have been due to verapamil directly blocking neuronal calcium channels instead of inhibiting P-gp at the blood-brain barrier.

In 2005, tariquidar (XR9576), a non-competitive P-gp inhibitor was first used to block P-gp function. (Martin et al., 1999; Mistry et al., 2001). Tariquidar has a good oral bioavailability, long duration of action and low potential for toxic side effects, all of which make this a favourable P-gp inhibitor. For example, van Vliet et al. demonstrated that inhibiting P-gp with tariquidar significantly reduced seizure duration, frequency and severity, which improved phenytoin efficacy in a rat model for temporal lobe epilepsy. This suggested that combination of AEDs with a transporter inhibitor may be a promising therapeutic strategy for AED-resistant patients (Van Vliet et al., 2006). The same researchers also found that P-gp over-expression in the temporal hippocampus and parahippocampal cortex of chronic epileptic rats reduced phenytoin levels by about 30% in these brain regions. Treating animals with tariquidar significantly increased phenytoin brain levels in regions with over-expressed P-gp (Van Vliet et al., 2007). Another group found that tariquidar restored the anticonvulsive activity of phenobarbital in drug-resistant rats (Brandt et al., 2006). These animal studies demonstrate that transporter inhibition increases AED blood and brain levels and improves seizure control.

Encouraged by animal studies and case reports that suggested transporter inhibition can be used to overcome AED resistance in epilepsy, clinical trials employing P-gp inhibitors were initiated. Currently, two trials using carvediol and verapamil to inhibit P-gp in AED refractory patients are ongoing (www.clinicaltrials.gov, #NCT00524134, #NCT01126307). However, while both carvedilol and verapamil are FDA-approved and readily available, neither drug is a highly specific nor potent P-gp inhibitor (Arboix et al., 1997; Takara et al.,

2004). In addition, to effectively inhibit over-expressed P-gp, high inhibitor plasma concentrations will be needed, which carries the risk of drug-drug interactions and toxic side effects (Pennock et al., 1991). In a recent study, add-on treatment with verapamil to improve seizure control in dogs with phenobarbital-resistant epilepsy had to be discontinued due to detrimental effects (Jambroszyk et al., 2011). Thus, since potent and specific inhibitors that can be safely given to patients are currently not available, transporter inhibition does not seem to be a viable treatment option in drug-resistant epilepsy at this point in time.

3.1.2 Modulation of transporter regulation

Targeting signalling pathways that regulate drug efflux transporters is another strategy to overcome transporter-mediated AED resistance. The advantages of this approach are three-fold. First, modulating transporter regulation to increase AED brain delivery may allow fine tuning of the transporter. For example, modulating the molecular switches of a transporter may allow turning it off for a short, controlled period of time to deliver drugs into the brain, after which it can be turned on again. Second, preventing or blocking seizure-induced upregulation of transporters may normalise transporter expression and functional activity, and thus, prevent or block development of transporter-mediated AED resistance. Third, since transporter upregulation in epilepsy has been linked to increased seizure occurrence, prevention of transporter upregulation holds the promise of better seizure control. Thus, mapping the signalling pathways involved in efflux transporter upregulation at the blood-brain barrier in epilepsy can help identify new targets that may potentially be used to overcome transporter-mediated AED resistance and improve seizure treatment.

Several signalling pathways have been identified that regulate P-gp, BCRP, and Mrp2 at the blood-brain barrier. For BCRP, the most recent signalling mechanisms include Nrf2, NfkB, COX-2, Pim-1 kinase, and the nuclear receptors CAR and AhR (Kalalinia et al., 2011; Singh et al., 2010; Tan et al., 2010; Wang et al., 2010). Of those, CAR and AhR have been shown to upregulate BCRP at the blood-brain barrier (Tan et al., 2010; Wang et al., 2010), which is the opposite of what one would want to improve AED delivery into the brain. Whether targeting any of the other pathways could be used as a therapeutic strategy in AED-resistant epilepsy is unknown and remains to be determined. Mrp2 is also regulated through nuclear receptors (PXR, FXR, CAR; (Bauer et al., 2008; Kast et al., 2002)), but the signalling that upregulates Mrp2 in epilepsy is unknown.

Most information on transporter regulation is available for P-gp, where signalling pathways have been shown to be present in various tissues (liver, kidney, intestine; (Ho & Piquette-Miller, 2006; Nawa et al., 2010; Thevenod et al., 2000). They also involve various signalling molecules: inflammatory mediators including TNF-α, ET-1, IL1-β, IL-6, NO; COX-2 (Dixit et al., 2005; Goralski et al., 2003; Nawa et al., 2010; Patel et al., 2002; Poller et al., 2010; Sukhai et al., 2001; Von Wedel-Parlow et al., 2009), nuclear receptors PXR, CAR, AhR, and GR (Bauer et al., 2004; Bauer et al., 2007; Geick et al., 2001; Narang et al., 2008; Wang et al., 2011; Wang et al., 2010), protein kinase C (Bauer et al., 2007; Chambers et al., 1990a; Chambers et al., 1990b; Hartz et al., 2004; Miller et al., 1998; Rigor et al., 2010), and NFkB (Bauer et al., 2007; Bentires-Alj et al., 2003; Kim et al., 2011; Liu et al., 2008; Thevenod et al., 2000; Yu et al., 2008). These pathways have been found in several diseases including Alzheimer's disease, HIV, and diabetes (Hartz et al., 2010; Hayashi et al., 2006; Nawa et al., 2010).

One pathway that involves glutamate signalling through the NMDA receptor (NMDAR) followed by cyclooxygenase -2 (COX-2) and prostaglandin E receptor 1 (EP1) activation seems to be critical for seizure-induced upregulation of P-gp (**Figure 3**, (Bankstahl et al., 2008; Bauer et al., 2008; Pekcec et al., 2009; Zhu & Liu, 2004)). During seizures, neurons release high amounts of the excitatory neurotransmitter glutamate, which can reach interstitial brain concentrations of 10-100 µM for a short period of time (Ronne-Engstrom et al., 1992; Ueda & Tsuru, 1995). Zhu and Liu were the first to connect glutamate with P-gp upregulation at the blood-brain barrier. They found that glutamate increased P-gp expression and activity in rat brain microvessel endothelial cells and suggested that activation of the NMDAR plays a critical role in glutamate-mediated P-gp upregulation (Zhu & Liu, 2004). Consistent with this, Bauer et al. demonstrated that exposing isolated rat and mouse brain capillaries to glutamate increased P-gp expression and activity (Bauer et al., 2008). It was shown that glutamate signalling through NMDAR and COX-2 upregulates blood-brain barrier P-gp, and that COX inhibition prevented P-gp upregulation suggesting that AED brain uptake can be enhanced by COX inhibition. Bankstahl et al. confirmed glutamate involvement in seizure-induced P-gp over-expression and that blocking NMDAR prevents P-gp upregulation and neuronal damage *in vivo* (Bankstahl et al., 2008). Another study showed that pre-treatment with celecoxib, a specific COX-2 inhibitor, prevented seizure-induced P-gp upregulation in rat brain capillaries (Zibell et al., 2009), and yet another study demonstrated that pre-treatment with celecoxib for 6 days followed by administration of phenobarbital for 16 days reduced the frequency of spontaneous recurrent seizures and restored the anticonvulsant effect of phenobarbital in AED-resistant epileptic rats (Schlichtiger et al., 2010). van Vliet et al. evaluated the use of the COX-2 inhibitors SC-58236 and NS-398 in rats with recurrent spontaneous seizures. They found that 2-week treatment with these COX-2 inhibitors prevented P-gp upregulation and enhanced phenytoin brain uptake in chronic epileptic rats (Van Vliet et al., 2010). While these studies are promising, COX-2 inhibitors bear the risk of severe cardio- and cerebrovascular side effects (Mukherjee, 2001; Stollberger & Finsterer, 2003). In addition, it has been demonstrated that COX-2 inhibition can lead to increased seizure frequency and mortality in epileptic rats (Holtman et al., 2010). Thus, although COX-2 inhibition may reduce AED resistance in animal models, it may not be a valid target in the clinic over the long term.

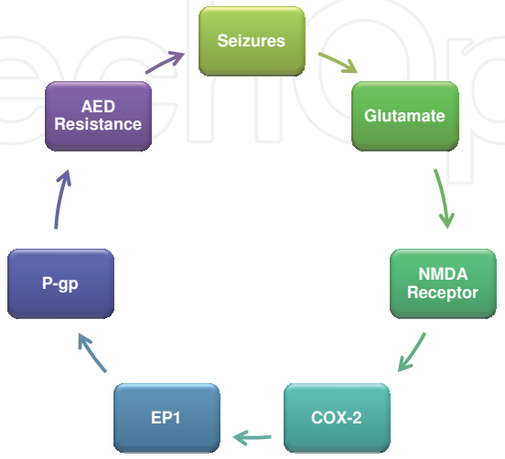

Fig. 3. Glutamate – NMDAR1 – COX-2 – EP1 Signaling Pathway

Another signalling protein involved in glutamate-mediated P-gp upregulation at the blood-brain barrier is the EP1 receptor. EP1 is activated by prostaglandin E2, the main product of COX-2, and was tested as potential target to prevent transporter upregulation in epilepsy. Pekcec et al. found that EP1 is a key signalling protein in the pathway that drives P-gp upregulation during seizures (Pekcec et al., 2009). Studies using the EP1 inhibitors SC-51089 and misoprostol showed that SC-51089 decreased seizure severity in rats when given prior to electrical kindling, but it also prolonged seizure duration at higher doses, whereas misoprostol decreased duration of motor seizure activity (Fischborn et al., 2010).
Together, these studies show that glutamate released during seizures mediates P-gp upregulation through NMDAR, COX-2, and EP1 and that these signalling proteins could potentially be used as therapeutic targets to reduce AED-resistance. Whether this pathway also signals upregulation of other blood-brain barrier proteins is unknown at this time.

3.2 The metabolic blood-brain barrier

Xenobiotic metabolism is a 3-phase process during which low polar molecules (e.g., drugs) are enzymatically converted to polar molecules that are then excreted from the body mostly through bile, faeces, or urine. Most chemicals are pharmacologically or toxicologically inactivated during metabolism, only some are transformed into active metabolites. The liver is recognized as the main site of biotransformation, but extrahepatic tissues such as the kidney, lung, intestine, skin, and brain also contribute to drug metabolism.
The processes involved in the biotransformation of drugs are classified into phase I (functionalisation) and phase II (conjugation) reactions that are followed by phase III excretion of the metabolite (**Figure 4**). Substrates of phase I enzymes are in general lipophilic and undergo functionalisation reactions such as monooxygenation, dealkylation, reduction, aromatisation, or hydrolysis. The modified molecules are substrates for phase II enzymes, which conjugate the functional group with a polar compound, such as an amino acid, sulphate, glutathione, or a sugar (Minn et al., 1991). In the last phase III step, functionalised and conjugated xenobiotics are excreted from cells by efflux transporters.

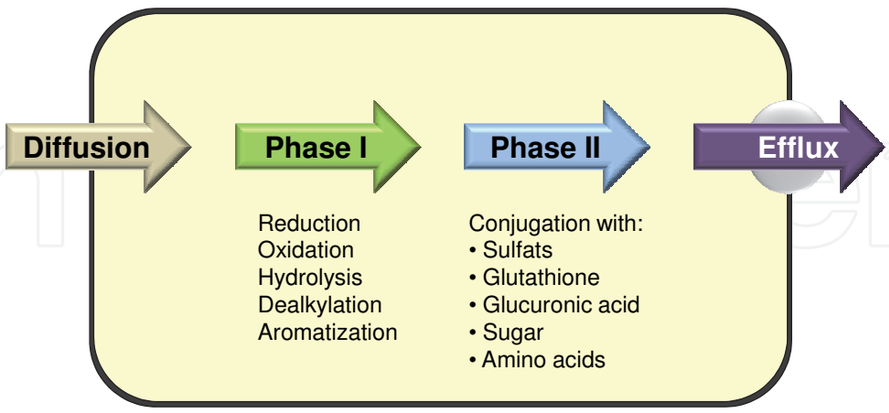

Fig. 4. Schematic of 3-phase drug metabolism and excretion

Most CNS drugs, including AEDs, have to cross the blood-brain barrier and penetrate into the brain parenchyma to reach their target sites. En passage across the barrier and within the brain, drugs can undergo inactivation and elimination comparable to hepatic drug

metabolism. In the brain, the following phase I enzymes have been identified: monoamine oxidases, CYP P450, NADPH-CYP P450 reductase, and epoxide hydrolases (Chat et al., 1998; Dutheil et al., 2010; Ghersi-Egea et al., 1998; Ghersi-Egea et al., 1988; Ghersi-Egea et al., 1993; Minn et al., 1991; Ravindranath et al., 1990). Phase II enzymes identified in the brain include UDP-glucuronosyltransferase (UGT), phenol sulfotransferase (PST), and GST (Dutheil et al., 2010; Ghersi-Egea et al., 1998; Ghersi-Egea et al., 1988; Ghersi-Egea et al., 1993; Minn et al., 1991). Several phase I and II enzymes have been found in the brain capillary endothelium, where they possibly form a metabolic barrier for drugs en route into the brain (Dutheil et al., 2010; Ghersi-Egea et al., 1998; Ghersi-Egea et al., 1993; Minn et al., 1991; Stamatovic et al., 2008). Several reports on metabolism-coupled efflux transport (phase III) suggest that biotransformation of drugs and efflux of the metabolites are part of barrier function. Together, the rodent and human brain, including brain microvessels forming the blood-brain barrier, express enzymes and transporters that are part of the detoxification pathways that affect metabolism of therapeutic drugs. In addition, AED elimination by coupling of two important biological processes – metabolism and efflux transport - could contribute to AED resistance and intractable epilepsy.

3.2.1 Metabolism of AEDs and the metabolic barrier in epilepsy

Phase I. CYP P450 enzymes are responsible for most phase I metabolic reactions and have the greatest impact on the biotransformation of therapeutic drugs. CYPs form a large and functionally diverse superfamily of enzymes that are found throughout various species ranging from bacteria to humans. In humans, the majority of CYPs are expressed along the inner plasma membranes of mitochondria and the endoplasmic reticulum. Although a distinct group of CYPs (CYP11A1, 11B1, 11B2, 17A1, 21A2) is involved in steroid hormone synthesis in humans (Hrycay & Bandiera, 2009), most CYP enzymes contribute primarily to the elimination of endogenous and exogenous substrates through oxidation to enhance their excretion from the body. By-in-large, CYP-mediated metabolism occurs in the liver and contributes to the "first pass effect" of orally administered drugs. In the liver, several CYPs exist as allelic or genetic variants and such CYP polymorphisms have been shown to influence the plasma concentration of some AEDs. CYPs are also expressed in the kidney and renal excretion is an important elimination route, particularly for most novel AEDs.

With regard to epilepsy it is noteworthy that many AEDs are metabolised by CYPs (**Table 1**). CYP2C9 and CYP2C19 are the two major enzymes involved in AED metabolism including diazepam, phenobarbital, phenytoin, and valproic acid (**Table 1**; (Klotz, 2007)). Several studies demonstrated differences in the biotransformation of these AEDs depending on the underlying CYP genotype. For example: phenytoin metabolism depends on the allelic composition of the gene encoding for CYP2C19 and CYP2C9. Several mutated alleles of these genes are known. Thus, based on genotype, poor phenytoin metabolisers can be distinguished from phenytoin hypermetabolisers and their frequency distributions vary between different ethnic populations (Klotz, 2007).

Regarding CYPs in the brain, *in vitro* and *in vivo* functional expression of CYPs has been detected in various CNS cell types from different species. CYP expression in distinct CNS cell populations is variable but can be as high as in the liver (Bhagwat et al., 2000). Importantly, it has been shown that CYPs are functionally active at the blood-brain barrier (Dutheil et al., 2010).

DRUG	ENZYME/PATHWAY
Carbamazepine	CYP3A4, mEH1 for carbamazepinee-10, 11-epoxide
Ethosuxemid	CYP3A4?, 10-20% renal
Phenobarbital	CYP2C19, hydroxylation, glucuronidation, 25% renal
Phenytoin	CYP2C9, CYP2C19
Valpoate	CYP2C9, glucuronidation, oxidation
Diazepam	CYP2C19, CYP3A4
Felbamate	40-60% renal, hydroxylation, glucuronidation
Gabapentin	Renal
Lamotrogine	Glucuronidation, 8% renal
Levetirazepam	66% renal, hydrolyse
Oxcabazepine	Reduction to active metabolite that is glucuronidated
Pregabalin	98% renal
Tiagabine	CYP3A4, 25% renal
Topiramate	60-80% renal, oxidation, hydrolysis, glucuronidation
Vigabatrin	60-80% renal
Zonizamide	CYP3A4, N-acetylation, glucuronidation, 30% renal

Table 1. Antiepileptic drugs and their route of biotransfomation (modified from Klotz, 2007)

In the human brain, 20 CYP isoforms have been identified so far: CYP1A1, 1A2, 1B1, 2B6, 2C8, 2D6, 2E1, 3A4, 3A5, 8A1, 11A1, 11B1, 11B2, 17A1, 19A1, 21A2, 26A1, 26B1, 27B1, and 46A1 (Dutheil et al., 2010). The exact expression pattern within the CNS depends on the particular CYP and greatly varies between different brain cells. In a recent study, CYP mRNA expression levels were measured in a human brain microvessel cell line and in human microvessels isolated from surgically removed brain tissue from epileptic patients or patients with brain tumours (Dauchy et al., 2008). The authors found mRNA expression of CYP2U1, CYP2S1, CYP2R1, CYP2B6, CYP2E1, CYP1A1, CYP2D6, CYP1B1, CYP2J2, CYP1A2, and CYP2C8. In another study, Gosh et al. used commercially available human microvascular cerebral endothelial cells and found mRNA expression of CYP1A1, 1B1, 2A6, 2B6 2C, 2C9, 2E1, 2J2, 3A4, 4A11, 11b, CYP3A5, 4B1, C1, 21A, and 51A1 (Ghosh et al., 2010). The cells that were used for this study originated from surgically resected brain specimens of drug-resistant epileptic patients, brain specimens resected from aneurism domes, commercially available human microvascular cerebral endothelial cells (used as control), and from human umbilical vein endothelial cells (used as control). mRNA expression from 11 of the 16 CYPs was increased in endothelial cells from epileptic tissue compared to microvascular cerebral endothelial cells. Although this comparison may be flawed due to the different nature of these cells, mRNA expression in endothelial cells from drug-resistant epileptic patients was not different compared to brain specimens resected from aneurism domes without seizures (Ghosh et al., 2010). This result argues against a seizure effect on the regulation of blood-brain barrier CYPs. However, it is clear from these findings that more detailed and accurate studies with appropriate controls are needed.

Overall, expression profiles, tissue and cellular distribution, and relative expression of CYP enzymes seem to depend on study design and the models used. Therefore, additional research is needed to clarify the importance of individual CYPs at the blood-brain barrier under both physiological and epileptic conditions.

CYP Regulation in the CNS

Carbamazepine induces CYP3A4 protein and mRNA expression in human brain endothelial cells and hepatocytes (Ghosh et al., 2010; Luo et al., 2002). But carbamazepine is metabolized by CYP3A4 (, **Table 1**), and high expression of CYP3A4 protein was found in endothelial cells isolated from surgically resected epileptic brain tissue (Ghosh et al., 2011). Neuronal CYP3A4 expression has also been demonstrated in brain sections by immunostaining from patients with temporal lobe epilepsy, tuberous sclerosis, or cavernous angioma, all of whom had intractable epilepsy. In these samples, CYP3A4 was rarely co-localised with the astrocytic marker GFAP (Ghosh et al., 2011). Carbamazepine given to cells that derived from resected epileptic brain tissue underwent metabolism at an extent similar to what was observed in hepatocytes. Thus, increased CYP3A4 expression and metabolic function could be characteristic for endothelial cells in epilepsy, which could contribute to AED resistance.

As mentioned before, AEDs such as carbamazepine act as strong inducers of hepatic and blood-brain barrier CYP expression, thereby influencing the pharmacokinetics of other drugs. Other AEDs have also been reported to increase CYP expression at the blood-brain barrier and exposure of primary rat brain astrocytic cultures to phenytoin increased Cyp2c29 levels (Volk et al., 1995). Moreover, phenytoin was metabolised by the microsomal fraction of astrocyte cultures and chronic treatment of mice with phenytoin resulted in increased levels of phenytoin metabolites in the brain (Volk et al., 1988). These findings support the idea of dynamic CYP regulation at the blood-brain barrier by AED exposure. In general, regulation of CYPs at the blood-brain barrier could be independent from that in the liver. Support for this comes from studies in alcoholics. Levels of CYP2D6 protein were elevated in the brains of alcoholics compared to non-alcoholics (Dutheil et al., 2010; Miksys & Tyndale, 2004). Particularly high CYP2D6 levels were detected in the putamen, globus pallidus, and substantia nigra, but interestingly, CYP2D6 was not elevated in the liver (Dutheil et al., 2010). Nuclear receptors that act as transcription factors control regulation of CYPs and ABC transporters in the CNS. In the human brain, several nuclear receptors have been detected that could control CYP regulation, including AhR, PXR, FXR, CAR, LXRß, RXRα and β, PPAR-α, -δ, and –γ (Dutheil et al., 2009; Nishimura et al., 2004). For example, Dauchy et al. showed that the AhR agonist TCDD increased mRNA expression of CYP1A1 and CYP1B1 in the human cell line hCMEC/D3 (Dauchy et al., 2008). Thus, it is possible that nuclear receptors could be involved in the regulation of CYPs at the blood-brain barrier in epilepsy (Dauchy et al., 2008; Dauchy et al., 2009; Ghosh et al., 2010). This idea is supported by the fact that metabolites of CYP2J2, which is expressed in brain endothelial cells in epilepsy, activate the nuclear receptors PPAR-α (*NR1C1*) and PPAR-γ (*NR1C3*). Future studies are required to address CYP regulation at the blood-brain barrier in health, disease (e.g., epilepsy), and during pharmacotherapy (e.g., AED treatment).

Phase II & III. Metabolism-driven efflux transport, i.e., coupling of phase II and III, has been demonstrated in the liver. Analogous findings from studies at the blood-CSF-barrier show coupling of metabolism and efflux transport also in the CNS. Using cultured rat choroid plexus epithelial cells *in vitro*, Strazielle and Ghersi-Egea demonstrated the presence of a metabolism-driven efflux mechanism for 1-naphthol, a cytotoxic, lipophilic model compound (Strazielle & Ghersi-Egea, 1999). The authors showed that UGT metabolised 1-naphthol *in situ* into a glucurono-conjugate (phase II) that was excreted by an efflux transporter (phase III). In this regard, MRPs have been implicated in cellular export of various glutathione, glucuronide, and sulfate conjugates compounds, and several other

endogenous and xenobiotic compounds (Gerk & Vore, 2002; Jedlitschky et al., 1996; Loe et al., 1996; Oude Elferink & Jansen, 1994). Although Mrp involvement has not directly been shown in Strazielle and Ghersi-Egea's study, Mrp-mediated efflux of the 1-naphthol glucurono-conjugate seems likely as the export was sensitive to the Mrp inhibitor probenecid. Thus, this study demonstrated the biological relevance of metabolism-driven efflux in the brain (Strazielle & Ghersi-Egea, 1999). However, in the human brain, UGT-mediated metabolism of 1-naphthol is less prominent compared to rat brain, and therefore, species differences should be considered (Ghersi-Egea et al., 1993). Activity of another phase II enzyme, GST, seems to be more relevant for human metabolism (Ghersi-Egea et al., 1993). Metabolism-driven transport was shown for GSTπ and Mrp1 by Leslie et al., who demonstrated plasma membrane co-localisation of Mrp1 and GSTπ in H69AR cells and found that functional GSTπ was required for Mrp1-mediated transport (Leslie et al., 2004). mRNA and protein expression of the GST isoform π has been demonstrated in isolated rat and mouse brain capillaries, where GSTπ is predominantly localized in the cytoplasm and the luminal plasma membrane of brain capillary endothelial cells, and to a large extent, co-localises with Mrp2 in the membrane (Bauer et al., 2008). Consistent with regulation by the nuclear receptor PXR, GSTπ protein expression increased in membranes from rat brain capillaries exposed to PCN or dexamethasone, and in capillary membranes from rats dosed with PCN. Immunoblotting of the capillary membrane fraction from hPXR transgenic mice dosed with rifampin further showed enhanced GSTπ expression. GSTπ and Mrp2 upregulation occurred in parallel, suggesting coordinated regulation of phase-II metabolism and phase-III efflux, i.e. Mrp2-mediated transport (Bauer et al., 2008). While these studies provide first insight into the regulation of both GSTπ and Mrp2 in brain capillaries, direct proof of metabolism-coupled efflux transport of chemicals at the blood-brain barrier remains to be shown. Thus, metabolism-coupled excretion of CNS drugs, such as AEDs, by efflux transporters seems likely, but requires further studies. Such studies would have to take into consideration the specific conditions at the human blood-brain barrier. For example, human cerebral microvessels show absence of glucuronidation, low NADPH CYP reductase activity, high GST activity, and pronounced epoxide hydrolase activity (Ghersi-Egea et al., 1993). Understanding metabolising enzymes, specifically at the human blood-brain barrier with respect to their physiological function, regulation in health and disease, and interplay with efflux transporters will allow assessing their impact on drug delivery to the brain, particularly in epilepsy.

3.3 Blood-brain barrier leakage in epilepsy

Seizures are accompanied by impaired blood-brain barrier integrity. This has been observed before, during and after seizures in both experimentally induced seizures in animals as well as in epileptic patients (Cornford & Oldendorf, 1986; Horowitz et al., 1992; Mihaly & Bozoky, 1984; Nitsch & Klatzo, 1983; Padou et al., 1995). As a consequence, impaired blood-brain barrier integrity causes transient barrier leakage, which allows entry of blood borne molecules into the brain (Ndode-Ekane et al., 2010; Seiffert et al., 2004; Sokrab et al., 1989; Van Vliet et al., 2007). It has been shown that seizure duration correlates with reduced barrier function (Cornford & Oldendorf, 1986), and it has been demonstrated that increased blood-brain barrier permeability in epilepsy is limited to anatomically specific brain regions (Bradbury, 1979; Cornford et al., 1998; Nitsch & Klatzo, 1983; Oztas & Sandalci, 1984). Interestingly, brain regions with increased barrier permeability are often anatomically

congruent with the brain regions that are implicated in the development and propagation of seizures. Consistent with this observation, extravasation of blood components into the brain correlates with increased excitability, occurrence of seizures, and epilepsy progression (Friedman et al., 2009; Marchi et al., 2007; Ndode-Ekane et al., 2010; Oby & Janigro, 2006; Seiffert et al., 2004; Tomkins et al., 2008; Van Vliet et al., 2007).

Cause of Blood-Brain Barrier Leakage by Seizures. Studies conducted over the last 3 decades indicate that possibly 3 mechanisms could be involved in causing blood-brain barrier leakage in epilepsy: blood pressure, pinocytosis, and seizure-induced inflammation.

Blood Pressure

The first studies conducted in the 1970s showed that arterial blood pressure is involved in seizure-associated blood-brain barrier leakage (Nitsch & Klatzo, 1983). Several studies unequivocally demonstrated that hypertension has detrimental effects on blood-brain barrier integrity and contributes to barrier leakage (Cornford & Oldendorf, 1986; Lee & Olszewski, 1961; Petito et al., 1977; Westergaard, 1980). Johansson summarized three factors responsible for increased blood-brain barrier permeability: (1) maximal arterial blood pressure, (2) duration of maximal arterial blood pressure, and (3) total increase in blood pressure (Johansson, 1981). The detailed mechanism through which increased blood pressure contributes to barrier leakage is unclear, but a working hypothesis postulates the following (Petito et al., 1977): Neuronal hyperactivity (seizures) leads to increased metabolism, and to an increased nutrient and oxygen demand in the involved brain regions. In turn, cerebral blood flow rises and large cerebral arteries dilate, which leads to increased blood pressure in brain capillaries, small arteries and veins (Ndode-Ekane et al., 2010), and triggers barrier leakage. Consistent with this, extravasation of blood albumin into the brain was found specifically in regions with more EEG spiking activity in humans (Cornford et al., 1998). However, the duration of increased blood pressure is critical and determines the severity of barrier leakage. Thus, more severe seizures that are followed by prolonged blood pressure elevations result in a higher increase in barrier permeability (Oztas & Kaya, 1991; Oztas & Sandalci, 1984). This is also supported by studies where both increased arterial blood pressure and subsequently induced barrier leakage were prevented by cervical cordotomy (Schaefer et al., 1975; Westergaard et al., 1978).

Pinocytosis

It was hypothesized that pinocytosis is involved in transport across the capillary endothelium, thus, affecting barrier permeability (Palade, 1961). The pinocytosis rate at the blood-brain barrier is low, which contributes to a tight barrier endothelium. However, Petito et al. observed that seizure-induced blood-brain barrier leakage correlates with increased micropinocytosis (Petito et al., 1977). In an elegant study using intravenous HRP injections in adult male rats with seizures, the authors made two important findings: (1) Brain capillary vesicles from animals that suffered seizures did contain HRP compared to vesicles from control animals that did not contain HRP; (2) the number of HRP-containing vesicles was higher directly after seizures (within 30 sec of last seizure (Petito et al., 1977)). From these observations the authors concluded that an increased micropinocytosis rate during and shortly after seizures increases blood-brain barrier permeability and counteracts barrier function. Nitsch and Hubauer confirmed these studies and showed in kainic acid-injected rats that blood-brain barrier opening was due to increased transendothelial pinocytosis, while tight junctions stayed intact (Nitsch & Hubauer, 1986).

Seizure-induced Inflammation

Another factor causing barrier leakage in epilepsy is seizure-induced inflammation that could be enhanced by extravasation of blood-borne components into the brain. It has been shown that blood-brain barrier permeability is increased by inflammatory mediators, including histamine, substance P, endothelin-1, bradykinin, VEGF, TGFß, IL1ß, TNFα, INFγ, PGE2, PGF2a, chemokines, free radicals, and other factors such as metalloproteinases, thrombin, amyloid-ß, intracellular calcium, and leukocytes that directly interact with endothelial cells (Stamatovic et al., 2008). Only limited information is available on how these factors alter the blood-brain barrier but some, e.g., IL1ß and chemokines, seem to exclusively affect paracellular permeability (Stamatovic et al., 2008). It is currently unknown if secretion of these factors is a direct consequence of epileptic seizures. In addition, depending on epilepsy aetiopathology, the composition of the inflammatory "cocktail" and the contribution of individual inflammatory mediators to blood-brain barrier damage could vary significantly. The effect of inflammation on barrier permeability is context-dependent, complex and not well understood. It likely depends on the model, dose, time and location of the inflammatory mediators involved.

One hypothesis that could explain some of the phenomena observed at the blood-brain barrier in epilepsy is that seizure-released glutamate activates signalling reducing barrier integrity and increasing permeability. Glutamate release occurs during seizures at sites in the brain with excessive neuronal activity. On the one hand, high glutamate levels are cytotoxic, which contributes to brain damage. On the other hand, subtoxic glutamate levels trigger molecular processes such as local release and activation of matrix-degrading enzymes that breach integrity (Michaluk & Kaczmarek, 2007; Nishijima et al., 2010). It is possible that glutamate-initiated signalling and inflammatory mediators cause barrier leakage and breakdown in epilepsy. Such events would allow extravasation of blood-borne compounds. Whether this scenario is part of seizures remains to be shown.

Consequences of Blood-Brain Barrier Leakage in Epilepsy. Epilepsy is often a consequence of a prior brain insult (e.g., traumatic brain injury, stroke) and seizures are a symptom of an underlying brain disorder (e.g., brain tumour, Alzheimer's disease, brain inflammation; (Marchi et al., 2006; Salazar et al., 1985; Tomkins et al., 2011). Although the factors that are involved in the development of epilepsy remain unclear, impaired barrier function is common after an initial brain insult and likely contributes to epilepsy pathology. This particular topic has recently attracted major interest in the epileptology field.

It has been shown in patients that brain injury, post-ischemic or vascular inflammation often cause seizures and barrier leakage (Stamatovic et al., 2008; Tomkins et al., 2011; Tomkins et al., 2008). The blood-brain barrier is also impaired in epileptic patients and in seizure animal models, and consequently, it has been postulated that barrier leakage is involved in epilepsy aetiology (Friedman et al., 2009; Marchi et al., 2007; Ndode-Ekane et al., 2010; Oby & Janigro, 2006; Seiffert et al., 2004; Tomkins et al., 2011; Tomkins et al., 2008; Van Vliet et al., 2007). In addition, it has been shown that osmotic barrier opening causes seizures (Oby & Janigro, 2006). However, not all implications of barrier opening have been studied. It is known that intra-arterial injection of hyperosmotic mannitol in patients and rodents results in EEG changes and induces seizures (Fieschi et al., 1980). In a recent study, Marchi et al (2007) observed seizures in patients undergoing osmotic barrier opening for delivering chemotherapeutics to treat brain lymphomas. In 25% of patients, seizure onset occurred

immediately after barrier opening. Using a pig model, the authors demonstrated that seizure occurrence correlated with barrier opening and was neither attributed to the existing brain lymphoma nor to chemotherapy (Marchi et al., 2007).

The molecular and cellular events that are triggered by barrier opening and that result in seizures and neuronal hyperactivity are a matter of research. Rigau et al. (2007) demonstrated loss of functional tight junctions and immunoglobulin leakage into the brain in surgically resected hippopcampal tissue from AED-resistant epilepsy patients (Rigau et al., 2007). Additional evidence from rodents and resected epileptogenic human brain tissue shows that extravasation of albumin into the brain triggers epileptogenesis (Friedman et al., 2009; Ivens et al., 2010). It was shown that astrocytes incorporated extravasated albumin, which induced proepileptogenic transformations, including reduced expression of potassium and aquaporin channels and gap junction proteins, impairment of astrocytic glutamate metabolism, and increased release of pro-inflammatory mediators (Friedman et al., 2009). All these changes had detrimental effects on seizure threshold and susceptibility (Friedman et al., 2009). One could postulate that seizures or other factors that induce barrier leakage trigger albumin extravasation with subsequent astrocytic transformation eventually causing seizures. Such a scenario implies a pernicious feedback loop where seizures drive barrier leakage leading to more seizures. Although this hypothesis is a matter of discussion, many epileptologists are convinced that 'seizures beget seizures' and that epilepsy has a progressive nature (Hauser & Lee, 2002). In this regard, alterations of the blood-brain barrier and extravasation of blood-borne compounds could be a critical part of epilepsy pathology that could potentially be a target for new therapies.

4. Conclusions

Research over the last century demonstrated a key role of the blood-brain barrier in the development of epilepsy and AED resistance. Despite the advances that have been made, what we currently know about AED resistance is mostly limited to descriptive observations rather than understanding of the mechanisms underlying the disease. We know that the blood-brain barrier is altered in epilepsy including changes in transporters, metabolic enzymes, and tight junctions. We also know that transporters, enzymes and tight junctions are affected by and/or contribute to epilepsy pathology. Yet, whether each of these molecular players is part of a cause-effect jigsaw puzzle and how each of the pieces fit together is unclear. Thus, AED resistance in epilepsy remains an unsolved clinical problem.

To solve this problem future studies will have to address the mechanism of AED resistance at the molecular level taking all aspects into account in a "big picture approach" rather than focusing on one single piece of the puzzle. The stimuli of blood-brain barrier transporter, enzyme and tight junction regulation in epilepsy will have to be identified and the detailed chain of signalling events will have to be unravelled. Such information will provide novel targets and therapeutic strategies that hold the promise to advance this research field and eventually improve treatment of patients with AED-resistant epilepsy.

5. Acknowledgments

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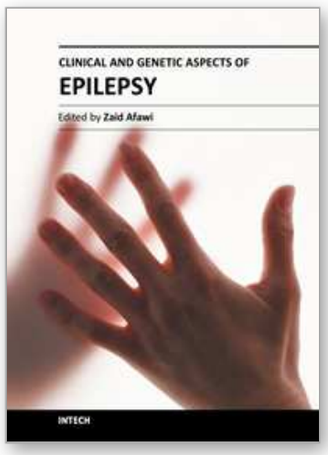

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This book on Epilepsy was conceived and produced as a source of information on wide range of issues in epilepsy. We hope that it will help health care providers in daily practices and increase their understanding on diagnosis and treatment of epilepsies. The book was designed as an update for neuroscientists who are interested in epilepsy, primary care physicians and students in health care professions.

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