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Neural Membranes and Transport

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Handbook of Neurochemistry and Molecular Neurobiology

Neural Membranes and Transport

Volume Editor: Maarten E. A. Reith

With 96 Figures and 22 Tables

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Preface

Neural membranes, as plasma membranes of other cells, are composed of lipids, proteins, and carbohydrates. In addition to a structural function for these membrane components, there is a functional role for them as (1) barriers, (2) transporters of ions or other solutes, and (3) receptors or recognition sites. This volume focuses on neural membrane constituents in terms of their functional role. The primary aim of *Neural Membranes and Transport* is to offer a comprehensive picture of the current body of knowledge on neural membranes with an emphasis on their function as barriers and transporters.

The first section of this volume deals with neural membranes and barriers. The main player highlighted here is the blood–brain barrier (BBB), which is covered from many different angles. Tight junctions are discussed, as well as developmental issues, modeling, pathology, astrocytes, and drug transport. There is coverage of P-glycoproteins in the BBB, and of the BBB as an efflux system. The section ends with a discussion of cholesterol, water movement, and endothelial peptide mediators.

The second section covers ion pumps and ion transporters in neural membranes. Sodium/calcium exchange in relation to calcium transport and glutamate excitotoxicity is discussed, as well as copper and zinc transport in the brain.

The final section covers neural membranes and transport of neurotransmitters or other solutes. Various plasma membrane transporters are discussed: GABA and other amino acids, and monoamine transporters. Vesicular transport of GABA, glutamate, acetylcholine, and monoamines is covered, as well as transport of glucose and peptides. Finally, synaptic vesicle recycling and efflux transport are discussed in regard to membrane function.

Each chapter has been put together by experts in the field who have experimentally contributed to advancing knowledge in the area; but coverage goes beyond describing the results of their own research. Rather, these chapters are reviews of the current status of knowledge in each area, aimed at informing the reader about the entire area.

My fascination with neuronal membrane proteins began during undergraduate work as a student in the laboratory of Dr. L.L.M. van Deenen, working on model membrane systems in the form of monolayers and bilayers at Utrecht, the Netherlands. It continued during a predoctoral fellowship at the Center for Neurochemistry in Strasbourg, France with the group of Drs. G. Vincendon, G. Gombos, and I.G. Morgan on purification procedures for plasma membranes from rat brains, during graduate studies on membrane fractionation under the guidance of Drs. D. De Wied, H.S. Jansz, P. Schotman, and W.H. Gispen, and during a postdoctoral fellowship at the Center for Neurochemistry in collaboration with Dr. H. Sershen under the guidance of Dr. A. Lajtha on interactions of nicotine and cocaine with brain membrane components. The latter work laid the foundation for my lasting interest in plasma membrane monoamine transporters. I am honored to be able to contribute to Dr. Lajtha's 3rd edition of the *Handbook of Neurochemistry and Molecular Neurobiology* as editor of this volume. In addition to expressing my gratitude to Dr. Lajtha, I would also like to thank Kristine Immediato for making it possible to edit *Neural Membranes and Transport* while at the same time moving my laboratory from the University of Illinois to New York University. The Internet version of this volume will be updated and will contain future additional chapters to make the subject matter more complete.

Maarten E.A. Reith, PhD
New York, October, 2006

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Table of Contents

Part 1

Neural Membranes and Barriers

1 Tight Junctions in the Blood–Brain Barrier

H. Wolburg · A. Lippoldt · K. Ebnet

Abstract: The blood–brain barrier (BBB) protects the neural microenvironment from changes of the blood composition. It is located in the endothelium, which is both seamless and interconnected by tight junctions. The restrictive paracellular diffusion barrier goes along with an extremely low rate of transcytosis and the expression of a high number of channels and transporters for molecules that cannot enter or leave the brain paracellularly.

Many tight junction molecules have been identified and characterized including claudins, occludin, zonula occludens protein-1 (ZO-1), ZO-2, ZO-3, cingulin, 7H6, junctional adhesion molecule (JAM), and endothelial cell-selective adhesion molecule (ESAM). Signaling pathways involved in tight junction regulation include G-proteins; serine, threonine, and tyrosine kinases; extra- and intracellular calcium levels; cAMP levels; proteases; and cytokines. Most of these pathways modulate the connection of the cytoskeletal elements to the tight junction transmembrane molecules. Additionally, cross talk between components of the tight junctions and the adherens junctions suggests a close functional interdependence of the two cell–cell contact systems.

The BBB endothelial cells are situated on top of a basal lamina, which contains various molecules of the extracellular matrix. Pericytes and astrocytes directly contact this basal lamina; however, little is known about the signaling pathways between these cell types and the endothelium, which possibly are mediated by components of the basal lamina. To analyze the interplay between astrocytes, pericytes, the extracellular matrix, and the endothelial cells is a big challenge for understanding the BBB in health and disease.

1 Introduction

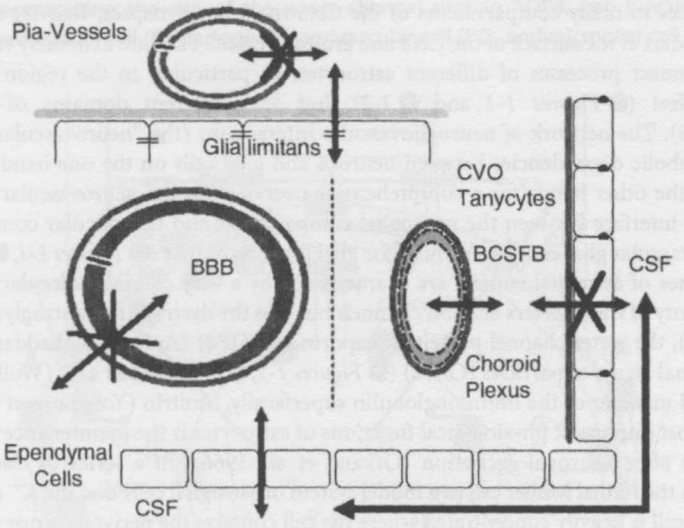
The original finding of Ehrlich (1885) that an infused dye did not stain the brain tissue, together with the complementary observation of his pupil Ernst Goldmann that the very same dye if applied into the cerebrospinal fluid did stain the brain tissue, has lead to the concept of a biological barrier between blood and brain. Due to the free access of the dye from brain ventricle to brain tissue, it was concluded that there is no cerebrospinal fluid–brain barrier. However, the staining of circumventricular organs (CVO) and the choroid plexus, when a dye was applied into the general circulation (Goldmann-I-experiment), and the avoidance of staining of these organs, when a dye was applied into the cerebrospinal fluid (Goldmann-II-experiment), suggested the existence of a barrier between the cerebrospinal fluid and the blood. The cellular basis of these barriers was unclear for decades. Today, we know that in most vertebrates the barrier is located within the endothelium (endothelial blood–brain barrier (BBB); only in elasmobranchs, the BBB is located in astrocytes) and in the epithelial choroid plexus cells and the tanycytes of the CVO (glial blood–cerebrospinal fluid barrier (BCSFB) (◉ *Figure 1-1*).

The structure restricting the paracellular permeability is the tight junction, and this structure implies the necessity of transporters for substances that must overcome the barrier for providing the brain with energy-rich substrates. The BBB is only one part of a huge regulatory neurogliovascular machinery, which controls the blood flow and the delivery of oxygen and substrates to the brain according to continuously changing local requirements. The endothelial BBB is regulated by many more factors than all other endothelial cells outside the central nervous system (CNS). The reason for that is the enormous complexity of the consciousness producing brain and the complete dependency from blood perfusion. This situation makes it very difficult to analyze the network of causal relationships between the different components of the neurogliovascular complex. This brief overview tries to follow some lines of evidence that astroglial cells, together with the extracellular matrix between glial endfeet and the endothelium, manage the barrier properties of the BBB, which is primarily established by tight junctions under the control of the brain microenvironment.

2 The Astrocytes Inducers of the Blood–Brain Barrier

It is now generally accepted that the astrocytes play a decisive role in the maintenance if not induction of the BBB (Janzer and Raff, 1987; Raub, 1996; Abbott, 2002; Brillault et al., 2002; Engelhardt, 2003; Wolburg and

Figure 1-1
Scheme of the principle of barriers in the brain. The BBB is located within the brain microvessels. The barrier between the blood and the cerebrospinal fluid, the BCSFB, is located within the choroid plexus epithelial cells and the tanycytes of the CVO. The ependymal cells as well as the astrocytic endfeet at the glia limitans do not represent a physiological barrier



Lippoldt, 2002; Lee et al., 2003; Begley and Brightman, 2003). This concept came up along with experiments showing that astrocytes placed adjacent to endothelial cells in vitro or in vivo supported the development of barrier properties in the endothelial cells. Among the barrier properties tested in these experiments were transendothelial electrical resistance, paracellular permeability of electron-dense tracers, and the expression of barrier-related molecules. Although in vitro models of the BBB have the principal disadvantage of not being able to simulate the whole of the microenvironmental complexity of the brain, they frequently were successful in investigating regulatory mechanisms concerning glio–vascular interactions (Arthur et al., 1987; Méresse et al., 1989; Rubin et al., 1991; Tontsch and Bauer, 1991; Abbott et al., 1992; Wolburg et al., 1994; Stanness et al., 1999; Franke et al., 2000; Gaillard et al., 2001; Cucullo et al., 2002; Nitz et al., 2003; Parkinson et al., 2003). Hamm et al. (2004) demonstrated an increased transendothelial permeability for horseradish peroxidase (HRP) after discontinued coculture with astrocytes, but this change in permeability was not paralleled by a change in tight junction protein expression. The authors concluded that loss of localization of tight junction associated proteins from the BBB tight junctions might be a relatively late event, which is not yet observed in the comparatively short-term in vitro experiments. In vivo, a reversible disruption of tight junctions has been observed after transitory loss of astrocytes as induced by a single dose of 3-chloropropanediol (Willis et al., 2004). Loss of astroglia-derived glial fibrillary acidic protein (GFAP) went along with the fragmentation of occludin immunoreactivity. This observation was in line with experiments performed in GFAP-deficient mice in which an impaired BBB in vivo (Liedtke et al., 1996) or the failure of astrocytes from GFAP-deficient mice to induce BBB properties in aortic endothelial cells in vitro have been described (Pekny et al., 1998). Unfortunately, although close examination of the spatial relationship of glial cells and the expression pattern of GFAP made it tempting to speculate that cellular interactions between neurons, GFAP-expressing glial cells, and endothelial cells supported the establishment of barrier properties in endothelial cells (Gerhardt et al., 1999a), the mechanism of how GFAP-expressing glial cells perform this task is still completely unknown.

In an approach to identify factors that are involved in the induction of BBB properties, the glial cell line–derived neurotrophic factor (GDNF) was found to be successful in BBB induction (Igarashi et al., 1999; Utsumi et al., 2000; Yagi et al., 2000). The *src*-suppressed C-kinase substrate (SSeCKS) in astrocytes

has been reported to be responsible for the decreased expression of the angiogenic permeability factor vascular endothelial growth factor (VEGF) and the increased release of the antipermeability factor angiopoietin-1 (Ang-1). SSeCKS overexpression increased the expression of tight junction molecules and decreased the paracellular permeability in endothelial cells (Lee et al., 2003).

An interesting correlation exists between astroglial differentiation and BBB maturation. The astroglial cells form processes to many compartments of the CNS including synapses, Ranvier nodes, and neural–mesenchymal borders at the surface of the CNS and around vessels. They are extremely rich in gap junctions that not only connect processes of different astrocytes, in particular, in the region of superficial and perivascular endfeet (● *Figures 1-1* and ● *1-2*), but also different domains of the identical cell (Wolff et al., 1998). The network of neurogliovascular interactions (the "neurovascular unit") is involved in manyfold metabolic dependencies between neurons and glial cells on the one hand and glial cells and vascular cells on the other hand (for a comprehensive overview on the neurovascular unit, see Iadecola, 2004). The direct interface between the neuroglial compartment and the vascular compartment is established by the perivascular glial endfeet, forming the glial limiting border (● *Figures 1-1*, ● *1-2*, and ● *1-4a*).

The membranes of astroglial endfeet are characterized by a very special molecular architecture. They not only carry plenty of transporters and ion channels but also the dystrophin–dystroglycan complex (Blake and Kröger, 2000), the water channel protein aquaporin-4 (AQP4) (Amiry-Moghaddam et al., 2004), the so-called orthogonal arrays of particles (OAPs) (● *Figures 1-2*, ● *1-3b*, and ● *1-4*) (Wolburg, 1995a), and a recently identified member of the immunoglobulin superfamily, limitrin (Yonezawa et al., 2003).

One of the most important physiological functions of astrocytes is the maintenance of the extracellular K^+ concentration after neuronal excitation (Orkand et al., 1966). In a series of classical experiments, Newman found in the retinal Müller cell as a model system of astroglial cells that the K^+ conductivity across the surface of the cell is heavily concentrated where the cell contacts the perivascular or the superficial glial limiting membrane (as reviewed in Newman and Reichenbach, 1996; Kofuji and Connors, 2003). The principle of spatial buffering (in the brain) or siphoning (in the retina) means that K^+ efflux takes place spatially apart from the site of K^+ influx effectively stabilizing the extracellular K^+ concentration. Later on, the inwardly rectifying $K^{\pm}$ channel, Kir4.1, was identified as one important member of K^+ channels, which

■ Figure 1-2

Schematic view of the endothelial BBB. The processes of one endothelial cell (E) are interconnected by tight junctions (TJ). The endothelial cells are underlined by a basal lamina (BL), in which pericytes are embedded and at which astroglial endfeet (A) are attached. These glial endfeet are interconnected by gap junctions (GJ), and their membranes facing the perivascular basal lamina carry numerous OAPs, which were now identified as the water channel protein aquaporin-4

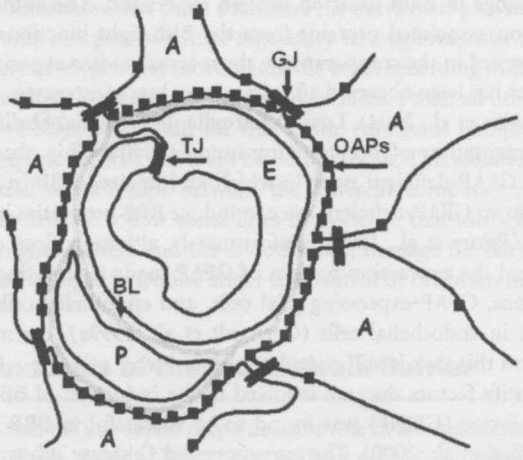
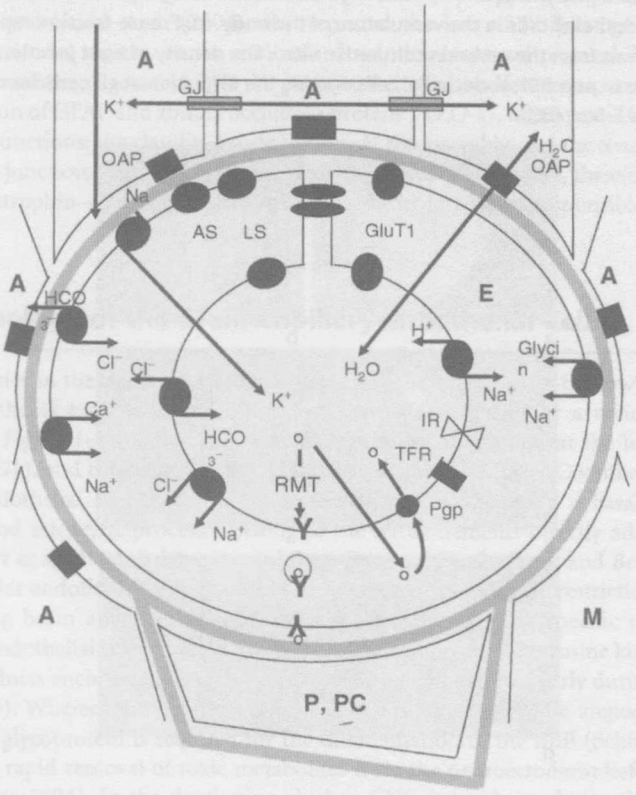


Figure 1-3

Schematic view of the distribution of some transporters and receptors at the BBB. A-system of amino acid transport as present in the abluminal membrane (AS), L-system of the amino acid transport (LS) as present in the both the luminal and abluminal endothelial membrane. Receptor-mediated transport (RMT), transferrin receptor (TFR), Insulin receptor (IR), P-glycoprotein (Pgp), orthogonal arrays of particles in the glial endfoot membrane (OAP), representing the site of the water channel protein AQP4, gap junctions (GJ) between astroglial cells (A), endothelial cell (E), pericyte (P), perivascular cell (PC), and microglial cell (M)

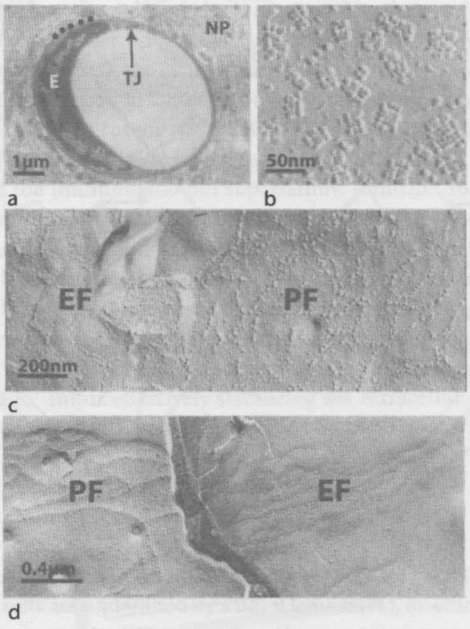


is highly concentrated at glial endfeet membrane domains and responsible for spatial buffering (Kofuji et al., 2002; Kofuji and Newman, 2004). In rodents, the K^+ channel is expressed in astrocytes surrounding synapses and perivascular endfeet around blood vessels (Higashi et al., 2001; Li et al., 2001a, b) (*Figure 1-3*).

Interestingly, the distribution of the Kir4.1 channel protein and the K^+ conductivity is similar to that of the dystrophin–dystroglycan complex and the water channel protein AQP4 (Blake and Kröger, 2000; Amiry-Moghaddam and Ottersen, 2003; Connors et al., 2004; Nagelhus et al., 2004; Warth et al., 2005). In contrast, within the neuropil the parenchymal astroglial membranes do express these molecules to an essentially less extent. The polarization of astrocytes, which is related to the distribution of OAPs and the K^+ conductivity, arises concomitantly with the maturation of the BBB (Wolburg, 1995b; Nico et al., 2001; Brillault et al., 2002; Yonezawa et al., 2003; Nicchia et al., 2004). Regarding the OAPs, it is well-known that they contain at least the water channel protein AQP4 (*Figure 1-4*). Aquaporins mediate water movements between the intracellular, interstitial, vascular, and ventricular compartments, which are under the strict control of osmotic and hydrostatic pressure gradients (Badaut et al., 2002; Papadopoulos et al., 2002). The involvement of AQP4 in the OAP formation was demonstrated by the absence of OAPs in astrocytes of the

Figure 1-4

Electron microscopy of the BBB. (a) Ultrathin section of a mouse brain capillary. The *arrow* points to a tight junction (TJ) interconnecting two processes of an endothelial cell (E). The nucleus of the endothelial cell is at the left-hand side. The points mark the perivascular astroglial membrane, which is shown by means of freeze-fracturing in part b. (b) Freeze-fracture replica of a perivascular astroglial endfoot membrane from the mouse brain, studded with orthogonal arrays of intramembranous particles. (c) Freeze-fracture replica of microvascular endothelial tight junctions from the rat brain. The tight junction particles at the E-face (EF) and the P-face (PF) are roughly equal in density. This BBB-specific high association of tight junctional particles with the P-face is unique among all endothelial cells in the vasculature of the body. (d) Freeze-fracture replica of microvascular endothelial tight junctions from the rat brain cultured in vitro. The density of tight junctional particles at the PF is extremely low such as in non-BBB endothelial cells outside the CNS. Almost all particles of the tight junctions are associated with the E-face (EF)



AQP4-deficient mouse (Verbavatz et al., 1997), by formation of OAPs in chinese hamster ovary cells stably transfected with AQP4 cDNA (Yang et al., 1996), and by the immunogold fracture-labeling technique showing that AQP4 is a component of the arrays (Rash et al., 1998, 2004). Moreover, Nielsen et al. (1997) were able to demonstrate by immunogold immunocytochemistry that the distribution of the AQP4-related immunoreactivity was identical to that of the OAPs. It should be stressed that AQP4 is the only member of the aquaporin family, which is associated with a membrane structure demonstrable by electron microscopy (*Figure 1-4b*).

Under brain tumor conditions, the density of OAPs of astroglioma cells has been demonstrated to decrease (Neuhaus, 1990). However, the AQP4 content as detected by immunocytochemistry was increased (Saadoun et al., 2002) and the localization no more restricted at the perivascular endfeet but redistributed across the whole surface of the cell (Warth et al., 2004). These apparently conflicting findings can only be resolved, if one suggests that under glioma conditions AQP4 exists separated from the OAP in the membrane and is no more restricted to the glial membranes contacting the basal membrane. There was a positive correlation between AQP4 restriction at the endfoot membrane (polarization of the astrocyte) and presence of agrin in the vessel basal lamina (Warth et al., 2004). Importantly, the heparan sulfate proteoglycan agrin, an extracellular matrix component, does not bind to AQP4 but to α-dystroglycan