The Role of Ion Channels Expressed in Cerebral Endothelial Cells in the Functional Integrity of the Blood-Brain Barrier (Review)

DOI: 10.17691/stm2016.8.4.29
Received January 19, 2016

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All anatomical elements contributing to the blood-brain barrier (BBB) play a crucial role in maintaining the permeability and CNS homeostasis under physiological/pathological conditions. These elements are endothelial cells, pericytes, astroglia, and neurons that are known as a neurovascular unit (NVU). Being the integral system, NVU contributes to the regulation of neuroplasticity, neurogenesis, intercellular communications and permeability of BBB. Brain capillary endothelial cells (BCEC) are the very important part of NVU. In this review, we discuss the critical role of BCEC ion channels in BBB structural and functional integrity. In last decades, much attention has been paid to the expression of tight junctions and adherence junctions in BCEC whereas less number of studies was focused on the expression and functioning of ion channels in BCEC, however, there is growing evidence supporting their important role in the regulation of NVU/BBB functions. In general, electrophysiological properties of BCEC depend on the expression of various ion channels whose activity, presumably, coordinates intercellular communication within the NVU. Particularly, we focus on BCEC ion channels-dependent mechanisms of NVU functioning, arteriole smooth muscle cells dynamic modulation, and changes in the regional cerebral blood flow. We put special attention on ligand-gated ion channels, store-operated calcium channels, TRP ion channels, calcium-activated, voltage-gated potassium channels in BCEC. Understanding the role of ion channel signaling in the control of cerebral blood flow will helps to reveal the potential therapeutic targets to recover the NVU/BBB functional integrity in different pathological conditions (ischemia, neuroinflammation, neurodegeneration) both *in vivo* and *in vitro* BBB models.

Key words: neurovascular unit; brain endothelial cells; ion channels; blood-brain barrier.

Neurovascular unit in physiological/pathological conditions: the role of endothelial cells. Blood-brain barrier (BBB) plays an important role in the regulation of pivotal brain functions due to ability of brain endothelial cells to provide selective transport of metabolites, xenobiotics, neurotransmitters and hormones, to regulate water exchange, and to take part in the regulation of neurogenesis. All the above-mentioned events take place within the neurovascular unit (NVU) consisting of endothelial cells, pericytes, astroglia, and neurons [1, 2]. Close interactions between the cells of the NVU are based on the coordinated expression and activity of receptors, transporters and channels contributing to establishment and maintenance of the BBB structural and functional integrity in physiological conditions, or to its impairment in central nervous system disorders such as hypertension [3], stroke [4], Alzheimer's disease [5], diabetes mellitus [6] and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [7].

Brain capillary endothelial cells (BCEC) play a key role in BBB properties [8]. It is now accepted that the cerebral endothelium forms the anatomic basis of the BBB in higher animals [9] and that the capillaries make up the primary part of the BBB [10]. BCEC are different from the endothelial cells of other tissues. They mainly have a small height and numerous tight junctions [11], small number of caveolae at the luminal surface of the cell, and large number of mitochondria [12]. The brain capillaries are tightly integrated within the neural parenchyma. Anatomically it is represented as a tight connection of BCEC with other cells of NVU, especially with pericytes and astrocytes. Astroglial endfeet forms a

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fine lamellae closely apposed to the outer surface of the capillary endothelium. Pericytes have a close physical association with the endothelium covering up to 32% of the capillaries. Neurons can regulate the function of blood vessels in response to metabolic requirements by inducing expression of enzymes unique for endothelial cells [13]. Also, endothelial cells and astrocytic processes are directly innervated by noradrenergic, serotonergic, cholinergic, and GABA-ergic neurons, among others [1]. In addition, astroglial cells secrete huge number of molecules affecting transport and homeostatic functions of BCEC, angiogenesis and endothelial repair [14, 15]. Being the integral system, NVU contributes to the regulation of neuroplasticity, neurogenesis, and intercellular communications.

In the evolution, BCEC replaced astroglial cells in their function as main regulators of transport processes between the brain and other tissues. It is generally accepted that in mammals, BCEC regulate selective transport and metabolism of substances from blood to brain as well as in the opposite direction from the parenchyma back to the systemic circulation [16]. As a result, BCEC precisely regulates CNS homeostasis under physiological/pathological conditions by protecting the brain from the fluctuations in plasma constituents [17–19]. Therefore, impairment of NVU functioning is clearly seen in all the types of brain pathology including ischemic brain damage, neurodevelopmental disorders, neuroinflammation and neurodegeneration [20–22].

In last decades, much attention has been paid to the expression of tight junctions and adherence junctions in BCEC, and all these data cannot be reviewed appropriately here. Adhesion molecules like claudins 1 to 15 [23], occludins [24], ZO-1 [25], junction adhesion molecule, endothelial cell-selective adhesion molecule, coxsackie- and adenovirus receptor [26], cadherins [27], catenins [28], integrins etc. are crucial for the regulation of permeability of BBB. Wide spectrum of transporter molecules is expressed in BCEC being extremely important for uptake and release of endogenous and exogenous molecules in the brain [29–32]. Less number of studies was focused on the expression and functioning of ion channels in BCEC, however, there is growing evidence supporting their important role in the regulation of NVU/BBB integral functions [33–35]. In this review, we would like to overview briefly the current understandings on the expression pattern and functional activity of ion channels in endothelial cells of brain microvessels.

Electrophysiological properties of brain endothelial cells. Actually, BCEC as other endothelial cells are non-excitable cells. Cultured BCEC have smaller capacitance than common endothelial cells: approximately 20 pF [36] versus 55–60 pF for endothelial cells of blood vessels [37]. The majority of Na^+ enters from the blood via the sodium, potassium, chloride co-transporter NKCC1 [38] and leaves the cells into the brain via the Na^+, K^+-ATPase, the sodium pump [39–42]. Ca^{2+} leaves from the cell via Ca^{2+}/Na^+ exchanger [43]. Also Ca^{2+} store is regulated via ATP-dependent pump in endoplasmic reticulum [44]. Each of these ion transports in/out the cells at a rate that substantially exceeds the net transport rate from the blood to brain. Thus, there must be mechanisms that under normal conditions allow ions to recycle across both sides of membranes. The most likely candidates for these mechanisms are ion channels. Differential regulation of such channels in the two membranes would allow control of secretion rate of Na^+, Ca^{2+}, K^+ and Cl^- and even under some circumstances remove the exceed amount from the brain, such as during the periods of intense neuronal activity or ischemia [36]. Activity of all the ion channels establishes membrane potential of BCEC at the range of −30 and –45 mV [36] which is similar to endothelial cells of blood vessels [45–48], but much more positive than V_m of neurons and astrocytes [49–52]. The electrophysiological properties of BCEC in native environment (within the BBB) were not examined yet, but it is essential point because these characteristics in dissociated or confluent cells are dramatically different. As it was shown previously in umbilical vein endothelial cells, the dissociated cells have almost 10 times higher input resistance and smaller capacitance than confluent cells because of their electrical communication, presumably through intercellular junctions [37]. For this reason, we can suspect that BCEC in NVU just partly have the same electrophysiological properties as cultured and dissociated common endothelial cells. Whether it is true, remains to be elucidated.

Participation of BCEC in BBB permeability goes by two ways — direct and indirect. Direct influence changes is the endothelial cell permeability. It is poorly understood how ion influx/efflux change the permeability in BCEC, but it was shown that endothelial cells membrane depolarization affect the cell stiffness through molecular mechanism connected with cortical actin cytoskeleton [52]. Indirect influence changes is the volume of pericytes in NVU and decreasing the regional blood flow through smooth muscle cells constriction of brain arterioles.

Gliovascular control of the local blood flow is based on the neuronal activity-dependent K^+ accumulation in the extracellular space followed by the activation of adjacent astrocytes and H^+- or lactate-dependent vasodilatation in the activated brain zones. Extracellular K^+ evokes opening of K^+ conductive channels and hyperpolarization of BCEC and pericytes with subsequent facilitation of blood flow [34].

It was shown that there was a positive feedback from capillary to parenchymal arterioles. These processes are highly dependent on the functional activity of ion channels in the cells of vessel wall. The concentrations of ions in these extracellular compartments dictate their equilibrium potentials and, by extension, both ion channel activity and membrane potentials. This, in turn, affects the level of resting potential in smooth muscle cells [34].

In the endothelial cells membrane of blood brain vessels Ca^{2+}, K^+ permeable channels are abundantly expressed. Also Na^+ [53], Cl^- and non-differentiated ion influx without proper characterization what channels it goes through [36] have been reported. Up to date it

Main ion channels expressed in brain capillary endothelial cells and brain arteriolar endothelial cells

Ions	Groups	Channels	Remarks
Ca^{2+}	Ligand-gated	IP_3R	ER membrane glycoprotein complex acting as a selective Ca^{2+} channel activated by inositol trisphosphate (IP_3)
	Store-operated calcium channels	CRAC	Triggered after internal Ca^{2+} store depletion and essential for continuous entry of Ca^{2+} from outside the cell
	TRP (transient receptor potential channels). Non-selective Ca^{2+}-conducting cation channels	TRPV4	TRPV4 are activated under the condition of mechanic stretch resulting in the influx of Ca^{2+}
		TRPC1 TRPC3 TRPC4	Ca^{2+}-conducting cation channels that are activated upon stimulation of receptors coupling to Gαi/Gαo- and Gαq/Gα11-dependent signaling pathways or receptor tyrosine kinases
		TRPM2 TRPM4	Activation leads to intracellular Ca^{2+} overload and endothelial dysfunction
K^+	Ca^{2+}-activated (inhibition in response to rising intracellular Ca^{2+})	SK	Small conductance calcium-activated potassium channels
		IK	Intermediate conductance potassium channels very sensitive to concentration of Ca^{2+} inside the cell
	Inwardly rectifying	$K_{ir}2$	A channel that "inwardly-rectifying" is one that passes current more easily in the inward direction than in the outward direction
	Voltage-gated	K_V1	Participate in setting membrane potential and mediate K^+ secretion into the brain interstitial fluid
Cl^-	Ion channels with poorly understood function mechanisms	Cl^- transport from blood into the brain through the co-operation of multiple cotransporters, pumps, exchangers, and channels	Takes part in formation of resting membrane potential. Cl^- could penetrate through amyloid-β non-selective channels, thus leading to cell swelling

was clearly shown the presence of selective Ca^{2+} IP_3R and CRAC [54] and several nonselective TRP channels in plasma membrane of endothelial cells [34]. These channels are TRPV4 [34], TRPC4 [55], TRPM2 [56] and TRPM4 [57]. Various K^+ channels, such as SK, IK and $K_{ir}2$ [34] play an important role in the regulation of BBB permeability.

Table summarizes the most important features of ion channels expressed in brain endothelial cells within the NVU/BBB.

Ligand-gated Ca^{2+} channels. Many types of ion channels respond to chemical substances (ligands) and not to membrane potential. The most important part of these ligand-gated ion channels is expressed on the surface of neurons and is activated by neurotransmitters. These channels are essential for synaptic transmission and other forms of cell-to-cell signaling phenomena. Other ligand-gated channels are sensitive to chemical signals derived from the cytoplasm of the cells [58]. These channels have ligand-binding domains on their intracellular surfaces that interact with second messengers such as Ca^{2+} or intracellular metabolites such as IP_3. As an example, IP_3 binds with its specific receptor IP_3R. IP_3R is a membrane glycoprotein complex acting as a Ca^{2+} channel and release Ca^{2+} from intracellular stores [59].

In endothelial cells of small peripheral arteries, Ca^{2+} release through IP_3Rs is well-established as an important signaling step leading to regulating vasolidation or vasoconstriction. Ca^{2+} release through IP_3Rs has a key role in communication of the smooth muscle cells with the parenchymal endothelium. In particular, brief, high-amplitude, IP_3R-mediated Ca^{2+} signals localized specifically to microdomains at myoendothelial projections (Ca^{2+} pulsars) activate local IK channels, causing K^+ efflux and hyperpolarization in endothelial cells [60].

Store-operated calcium channels in brain endothelial cells. Although the mechanisms by which extracellular Ca^{2+} ions enter the endothelium remain poorly understood, it is unlikely that voltage-dependent Ca^{2+} channels contribute to Ca^{2+} influx in endotheliocytes. More reliable that local Ca^{2+} entry goes through other channels which are present in non-excitable cells. In electrically non-excitable cells, the major Ca^{2+} entry pathway is the store-operated one, in which the release from intracellular Ca^{2+} stores activates Ca^{2+} influx (store-operated Ca^{2+} entry, or capacitative Ca^{2+} entry) [61]. Release of Ca^{2+} from internal store through IP_3R is necessary to trigger the store-operated Ca^{2+} entry (SOCE) also in BCEC. When the Ca^{2+} stores are exhausted, Ca^{2+} entry from the outside the cell is induced being necessary for maintaining the increased Ca^{2+} level and continuous Ca^{2+} influx in non-excitable cells such as BCEC. The SOCE goes through Ca^{2+} release-activated Ca^{2+} (CRAC) channels [61]. When stromal interaction molecules (STIM) on the endoplasmic reticulum membrane sense store depletion, they translocate to regions close to the plasma membrane and then form functional complexes with Orai molecules, the pore-forming subunits of CRAC channels on the cell membrane. Orai channels and STIM proteins are both required for CRAC channel

activity [62]. In general, CRAC channels are essential for continuous entry of Ca^{2+} from outside the cell. SOCE via CRAC is required for sustained Ca^{2+} influx across the cell membrane [63]. This sustained Ca^{2+} elevation is essential for cell proliferation/death in BCEC [64–66].

Transient receptor potential ion channels. Local Ca^{2+} entry goes through nonselective TRP channels, and it has been shown to be important in endothelial cells [67]. TRP channels are nonselective cation channels of six families: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin) [68, 69]. The first type of TRP channel was identified in the Drosophila eye. Now TRP channels are found widely distributed in many organs and tissues of mammals, including the nervous system [70, 71]. The TRP channels function as tetramers and conduct different cations (Na^+, K^+, and Ca^{2+}) when responding to local changes in their environment such as temperature, mechanical pressure, ion changes, pH changes and many other physical and chemical factors. Opening of TRP channels depolarizes the cell membrane and leads to intracellular Na^+ and Ca^{2+} accumulation [71]. Therefore, TRP channels are destined to regulate membrane potential and manipulate intracellular Na^+, K^+ and Ca^{2+} contents in both excitable and non-excitable cells. Activation of several TRP channel members have been shown in ischemic/anoxic conditions and implicated in the cytotoxicity or secondary injury.

In the cerebral circulation, TRPV4 channels have been identified in the endothelium of the isolated middle cerebral (pial) arteries, where they contribute to Ca^{2+} entry in response to the stimulation of purinergic Gq protein-coupled receptors with uridine 5'triphosphate [72]. Similarly, Hamel and co-workers [73] recently confirmed the presence of functional TRPV4 channels in the endothelium of pressurized posterior cerebral (pial) arteries, showing that these channels link muscarinic receptor stimulation (with acetylcholine) to IK and SK channel activation. Also Ca^{2+} entry though TRPV4 evoked by synthetic agonists primarily activates IK channels leading to vasodilation [67].

Ca^{2+} signals in the endothelial cells lead to the release of a similar complement of vasoactive substances as in the smooth muscle cells. Thus, TRPV4 are activated under the condition of mechanical stretch resulting in the influx of Ca^{2+} and up-regulation of endothelial nitric oxide synthase (eNOS) expression and vasodilatation [74] via opening the tight junctions [75]. In general, IP_3Rs and TRPV4 play central roles in Ca^{2+} signaling in the endothelial cells.

Another type of TRP channels that exist in plasma membrane of endotheliocytes are TRPC channels. The mammalian TRPC4 protein is a member of the transient receptor potential canonical subfamily of TRP [76] that are activated upon stimulation of receptors coupling to $G_{\alpha i}/G_{\alpha o}$- and $G_{\alpha q}/G_{\alpha 11}$-dependent signaling pathways or receptor tyrosine kinases [77, 78]. TRPC4 is expressed in a broad range of tissues, including neurons [76, 79], endothelial cells [80, 81], and intestinal smooth muscle cells [82].

Endothelial TRPC channels such as TRPC1 and TRPC4 play an important role in the management of endothelial permeability through endothelium-dependent smooth muscle relaxation [81].

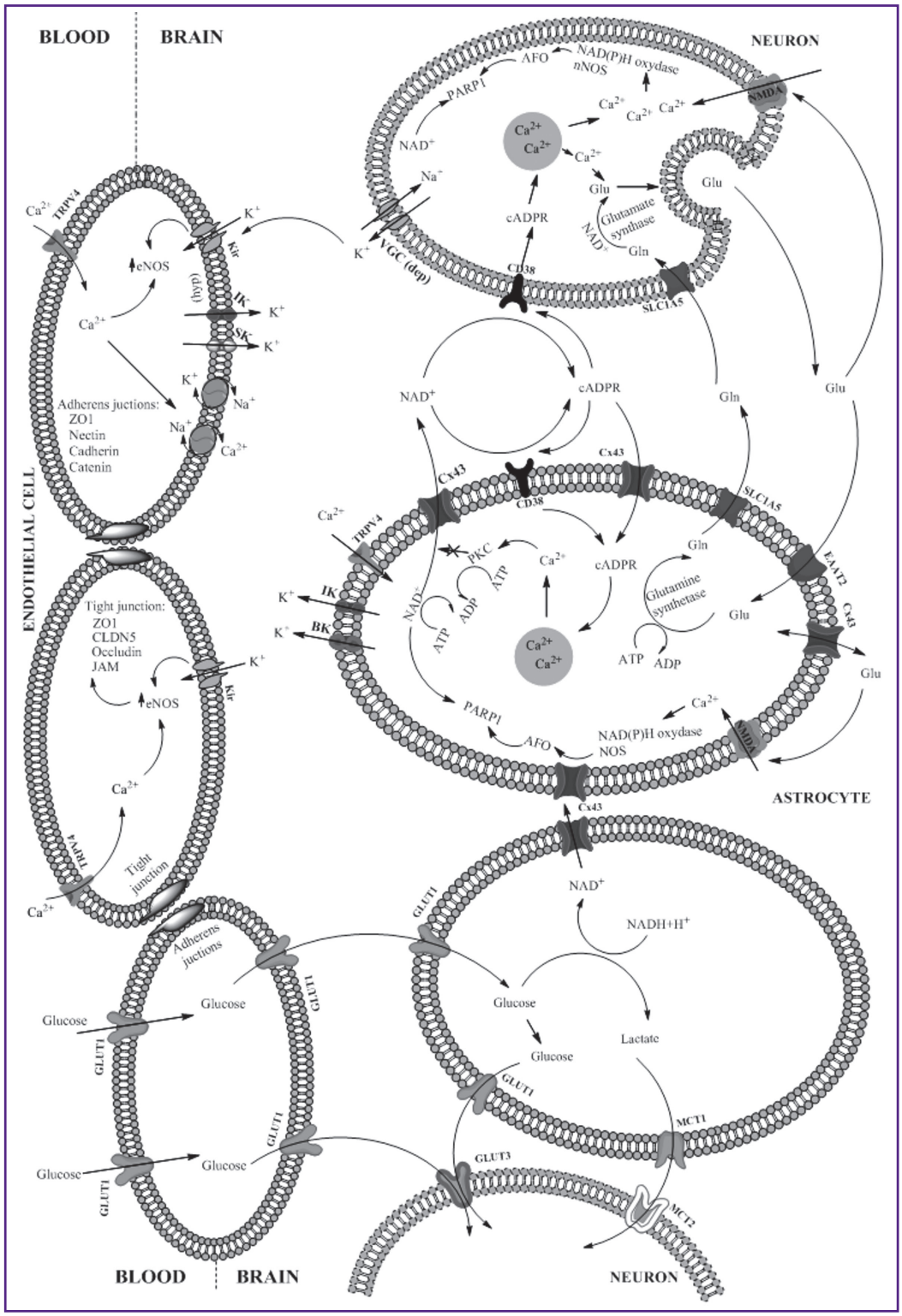
On the other hand, Ca^{2+} entry through the TRPM channels has the negative influence. Prolonged opening of TRPM2 channels in endothelial cells leads to intracellular Ca^{2+} overload and endothelial dysfunction in Alzheimer's disease [56]. The same mechanism underlies TRPM4 activation. The role of TRPM4 in brain pathology is under the investigation, and it was shown that activation of these channels leads to oncotic cell death during the ischemic stroke [83].

Calcium-activated potassium channels. Calcium-activated potassium channels are potassium channels gated by calcium and structurally or phylogenetically relate to calcium-gated channels. They contain two different subunits, α and β. The α subunits have six or seven transmembrane segments, similar to the potassium voltage-gated channels but with an additional N-terminal transmembrane helix. The β subunit is a regulatory subunit of the channel [84].

Ca^{2+} signals are tightly connected with the function of K^+ channels in endothelial cells. Increasing the Ca^{2+} concentration inside the cell activates local IK and SK channels of endothelial cells which take part in regulation of regional blood flow by the modulation of vessels diameter. Activation of these channels results in K^+ efflux and hyperpolarization [60]. Then, hyperpolarization can be transmitted via gap junctions directly to the underlying smooth muscle cells [34]. IK and SK channels are also involved in the development of endoplasmic reticulum (ER) stress. ER is one of the major organelles that contribute to the regulation of Ca^{2+} concentration. In addition, ER plays a central role in the folding of secreted proteins. Multiple stimuli and pathological conditions disturb ER homeostasis and result in ER stress leading to BCEC death [85]. ER stress-mediated suppression of IK and SK channels leads to endothelial dysfunction and prolonged vasoconstriction [86].

Inwardly rectifying potassium channels. The released excessive K^+ may also trigger the opposite effect by activating local K_{ir} channels in the endothelium and/or smooth muscle cells [87]. K_{ir} are the "inwardly-rectifying" channels where K^+ current more easily passes in the inward direction than in the outward direction. This unusual phenomena of inward rectification of K_{ir} channels is the result of channel pore block by spermine and magnesium ions. Such blockage of channel pore stops the outward current from K_{ir}. This block causes currents only in the inward direction [88]. K_{ir} channels take part in membrane potential stabilization. But the more prominent role of K_{ir} in endothelial cells is regulation of eNOS expression [89].

Also it has been shown that membrane hyper-polarization due to the activation of inward rectifier K^+ channel ($K_{ir}2.1$) regulates Ca^{2+} concentration to cause cell death in t-BCEC117 cells derived from BCEC [64]



Interconnection of NVU (neurovascular unit) cells. Endothelial cells separate blood from the internal brain space. They selectively filtrate essential substances for the brain such as glucose, ketone bodies, lactate; coordinate efflux of metabolites and transport of xenobiotics. In addition, endothelial cells are in close metabolic connection with other cells of NVU. Structural and functional integrity of the blood-brain barrier is controlled by tight and adherence junctions whose functioning is compromised in brain pathologies, i.e. in neuroinflammation. Numerous ion channels expressed in the cells of NVU are important for the coordinated activity of NVU, i.e. membrane potential fluctuations through activation of Ca^{2+} and K^+ permeable channels have the dramatic influence on the endothelial permeability

Up-regulation of $K_{ir}2.1$ is responsible for the elevated Ca^{2+} concentrations and cell death in ER stress-affected t-BCEC117 [65].

Voltage-gated potassium channels. Voltage-gated potassium channels are membrane channels selective for K^+ and sensitive to voltage changes in the cell membrane potential. In excitable cells, they play a crucial role in repolarization during action potentials. We should take into the consideration that the expression of voltage-gated K^+ channels in non-excitable cells such as BCEC [36] is very low. But from another point of view, it was clearly shown that K_V channels help setting the resting membrane potential in non-excitable cells [90]. In BCEC, K_V1 channels also participate in setting membrane potential and mediate K^+ secretion into the brain interstitial fluid [36], thus, presumably contributing to the BBB-mediated regulation of K^+ concentrations in the activated brain areas and establishment of the gliovascular control of the local blood flow.

Peculiarities of electrophysiological recordings in brain endothelial cells. Non-excitable cells usually are not the bailiwick of electrophysiologists. But the tight coupling of BCEC with other cells of NVU and critical role in BBB permeability force the researchers to study the electrical properties and ion channels function of these cells. Till today there are the few studies where endothelial cultures derived from the brain cortex have been used for the assessment of cerebral endothelial cells electrophysiology, like rat brain endothelial cells [91, 92] or primary culture of cerebral capillaries from porcine brain [93, 36]. But the patch clamp technique for endothelial cells is not remarkably different from the conventional patch clamp of neurons and other excitable cells. All main configurations like whole-cell and perforated patch are suitable for these experiments that can be performed after the establishment of gigaohm seal formation and membrane rapture [94, 95]. In a case of BCEC, the main difference from patching the neuronal cells is in the protocol of voltage-clamp experiments. Due to high membrane potential of endothelial cells they should be clamped at –40…–50 mV [36].

Figure shows contribution of main ion channels, transporters and other signaling molecules expressed in endothelial and astroglial cells to the intercellular interactions within the NVU/BBB under physiological conditions.

Conclusion. Endothelial cells of the NVU express a wide spectrum of ion channels that engage in extra- and intercellular signaling to control the diameter of parenchymal arterioles and modulate cerebral blood flow through control of smooth muscle cell membrane potential and release of vasoactive factors. Understanding the role of ion channel signaling in the control of cerebral blood flow will helps to reveal the potential therapeutic targets to recover the NVU/BBB functional integrity in different pathological conditions (ischemia [4], neuroinflammation [96–98], neurodegeneration [99, 100]) both *in vivo* and *in vitro* BBB models [2]. In addition, recently observed similarities in the expression pattern of ion channels in brain endothelial cells and adjacent astrocytes within the NVU [34] suggest further progress in deciphering novel molecular mechanisms of BBB functioning.

Acknowledgements. The study is supported by the Russian Science Foundation (Project No.14-25-00054).

Conflicts of Interest. The authors indicated no potential conflicts of interest in this study.

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