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The role of AQP4 and TRPV4 channels in the ischemic brain edema: focusing on glial cells

Role AQP4 a TRPV4 kanálů v edému vyvolaném ischemickým poškozením mozku: zaměření na gliové buňky

Bakalářská práce

Vedoucí bakalářské práce: Ing. Miroslava Anděrová, CSc.

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Podpis

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Abstract

Cerebral ischemia, also known as stroke, is one of the most common causes of death. It is accompanied by the formation of edema, which can be characterized as an influx of water and osmolytes into the brain, causing volume alterations. We recognize two types of cerebral edema – vasogenic, characterized by the disruption of the blood-brain barrier (BBB) and increase of the extracellular volume, and cytotoxic, caused by the increase of the volume of cells, mainly glia. The major contributors to the formation of cytotoxic edema are the astrocytes, which, in physiological conditions, are responsible for the maintenance of the BBB and keeping the homeostasis of the brain and spinal cord or central nervous system. The mechanism responsible for the process of volume and osmotic changes are the transmembrane channels, mainly aquaporin 4 (AQP4) and transient receptor potential vanilloid 4 (TRPV4). AQP4 is the main pathway for water influx as well as efflux when the edema subsides. TRPV4 is likely responsible for the maintenance of the osmotic balance of the organism, although its precise role in the formation of the edema has not yet been fully elucidated. The main aim of this thesis was to categorize the types of cerebral ischemia and edema, and to describe the process of cerebral edema formation and the role of glial cells in this process and nervous tissue recovery following ischemia. Another aim of this thesis was to review and summarize the available information about AQP4 and TRPV4 involvement in these processes.

Key words: brain, ion channels, water transport, astrocytes, NG2-glia

Abstrakt

Ischemie mozku, nebo také mozková mrtvice, je jedna z nejčastějších příčin úmrtí. Je doprovázena tvorbou edému, který se dá charakterizovat jako nadměrné proudění vody a osmolytů do mozku, což způsobuje objemové změny. Rozeznáváme dva druhy edému mozku – vazogenní, charakteristický disrupcí hematoencefalické bariéry a zvýšením extracelulárního objemu, a cytotoxický, způsoben zvětšením objemu buněk, hlavně glií. Hlavními přispěvateli ke vzniku cytotoxického edému jsou astrocyty, které jsou ve fyziologických podmínkách odpovědné za udržování hematoencefalické bariéry a homeostázy mozku a míchy, tedy centrálního nervového systému. Mechanismem odpovědným za osmotické změny a změny objemu jsou kanály, hlavně akvaporin 4 (AQP4) a vápník propustný kationtový kanál – vaniloidní receptor podtypu 4 (TRPV4). AQP4 je hlavním transportérem pro vodu do buněk a také z buněk, když edém odeznívá. TRPV4 je pravděpodobně odpovědný za udržování osmotické rovnováhy organismu, ale jeho konkrétní role v tvorbě edému ještě nebyla úplně popsána. Hlavním cílem této práce bylo kategorizovat typy ischemie a edému mozku a popsat proces vzniku edému mozku, roli gliových buněk v tomto procesu a obnovu tkáně nervového systému po ischemii. Dalším z cílů bylo shrnout dostupné informace o účasti AQP4 a TRPV4 v tomto procesu.

Klíčová slova: mozek, iontové kanály, transport vody, astrocyty, NG2-glie

LIST OF ABBREVIATIONS

ALDH1L – aldehyde dehydrogenase 1 family member L1

AQP – aquaporin

AQP4 – aquaporin 4

ATP – adenosine triphosphate

BBB – the blood-brain barrier

CNS – central nervous system

CSF – cerebrospinal fluid

CXCR2 – C-X-C chemokine receptor type 2

EAA – excitatory amino acid

GFAP – glial fibrillary acidic protein

Glu – glutamate

MCAo – middle cerebral artery occlusion

NG2 – neuron-glial antigen 2

NO – nitric oxide

NOS – nitric oxide synthetase

OL – oligodendrocyte

OPC – oligodendrocyte progenitor cell

PNS – peripheral nervous system

RVD – regulatory volume decrease

TRP – transient receptor potential cation channel

TRPA – transient receptor potential cation channel ankyrin

TRPC – transient receptor potential cation channel cannonical

TRPM – transient receptor potential cation channel melastatin

TRPML – transient receptor potential cation channel mucolipin

TRPP – transient receptor potential cation channel polycystin

TRPV – transient receptor potential cation channel vanilloid

TRPV4 – transient receptor potential cation channel vanilloid 4

* - review article

Contents

1 Introduction

The term cerebral ischemia was first used in the 19th century and ever since the research to elucidate the responsible mechanisms has been ongoing. Ischemia, also known as stroke, is caused by an occlusion of one of the arteries, or by the overall lack of blood (and thus oxygen) supply in the brain. This process is followed by an edema formation, induced by the disruption of the biochemical balance of the brain. In this process, many cells, most importantly neurons, are lost, but glia, and most notably astrocytes have an important role in the edema formation (Kalogeris et al., 2012)*.

The ones responsible for both the onset of edema and the fight against it are glial cells, mainly astrocytes (Hansen & Zeuthen, 1981). Although previously, most of the research focused on neurons, as the most important victims of the ischemia, nowadays, glia are getting into the spotlight as the fighters against its consequences.

The molecular mechanisms of the ischemic edema are being elucidated and the cellular channels responsible for osmotic changes in ischemic edema have been found – aquaporin 4 (AQP4) as the main gateway for water in the onset of edema (Manley et al., 2000) and transient receptor potential vanilloid 4 (TRPV4) as the regulator of osmotic balance (W. Liedtke & Friedman, 2003), although its precise role in the ischemic edema is still unclear.

The understanding of the role of glial cells and these channels in ischemic edema is essential for the advance in the treatment of the cerebral ischemia as well as ischemic edema. This thesis serves as an attempt to gather and summarize all the information that are available on this topic.

2 Cerebral ischemia

Cerebral ischemia is caused by a restriction of the blood flow to the brain that causes a shortage of oxygen, which triggers numerous pathophysiological changes. The brain utilizes a large amount of glucose and oxygen and is dependent on oxidative phosphorylation as a means of energy production. The lack of oxygen after ischemia leads to the oxidative phosphorylation cessation, ATP production is interrupted, the content of ATP is slowly depleted and once it falls under 50%, an efflux of K^+ and uptake of Na^+ and Ca^{2+} occurs (Hansen & Zeuthen, 1981). Following the failure of energy reserves and oxygen depletion, cellular acidosis, triggered by the production and accumulation of metabolic acids caused by anaerobic glycolysis occurs. Osmotically obliged water follows Na^+ and Ca^{2+} into the cells, which causes the formation of edema. The amount of intracellular Ca^{2+} is further increased by the mitochondrial inability to take it up, due to hypoxic conditions. Cellular depolarisation and the release of excitatory amino acids (EAAs), primarily glutamate, by the means of activation of Ca^{2+} channels, ensues (Katsura et al., 1994).

Glutamate initiates a quick surge of nitric oxide (NO), which is synthesized by the Ca^{2+}-dependent enzyme, neuronal nitric-oxide synthase (NOS) that accumulates in the ischemic brain, but soon declines, due to the substrate unavailability; the adverse role of NO in ischemia is the promotion of tissue damage, but its vasodilatory effects might also help increase the post-ischemic blood flow (Iadecola et al., 1994). However, the evidence suggests that inhibition of NOS plays a role in reducing the ischemia - mice with the inducible nitric oxidase knocked out have proven to have reduced ischemic damage (Iadecola et al., 1997).

Another effect caused by the lack of ATP and accumulation of proteolytic enzymes activated by the excessive Ca^{2+} is the degradation of microfilaments and microtubuli, and also phospholipids, which is followed by the accumulation of breakdown products, such as fatty acids, arachidonic acid, diacylglycerides and lysophospholipids (Siesjö, 1984).

An inflammation and invasion of the site of the ischemia by neutrophils and later by macrophages, caused by the expression of adhesive molecules and chemokines also occurs. A major role in this process is played by the interleukin 8 receptor, beta (CXCR2) chemokine receptor and its ligands (Veenstra & Ransohoff, 2012). The aforementioned pathological changes lead to neuronal death by means of necrosis and apoptosis. These changes are summarized in Figure 1.

Two different types of ischemia are recognized – global and focal with significant differences between them.

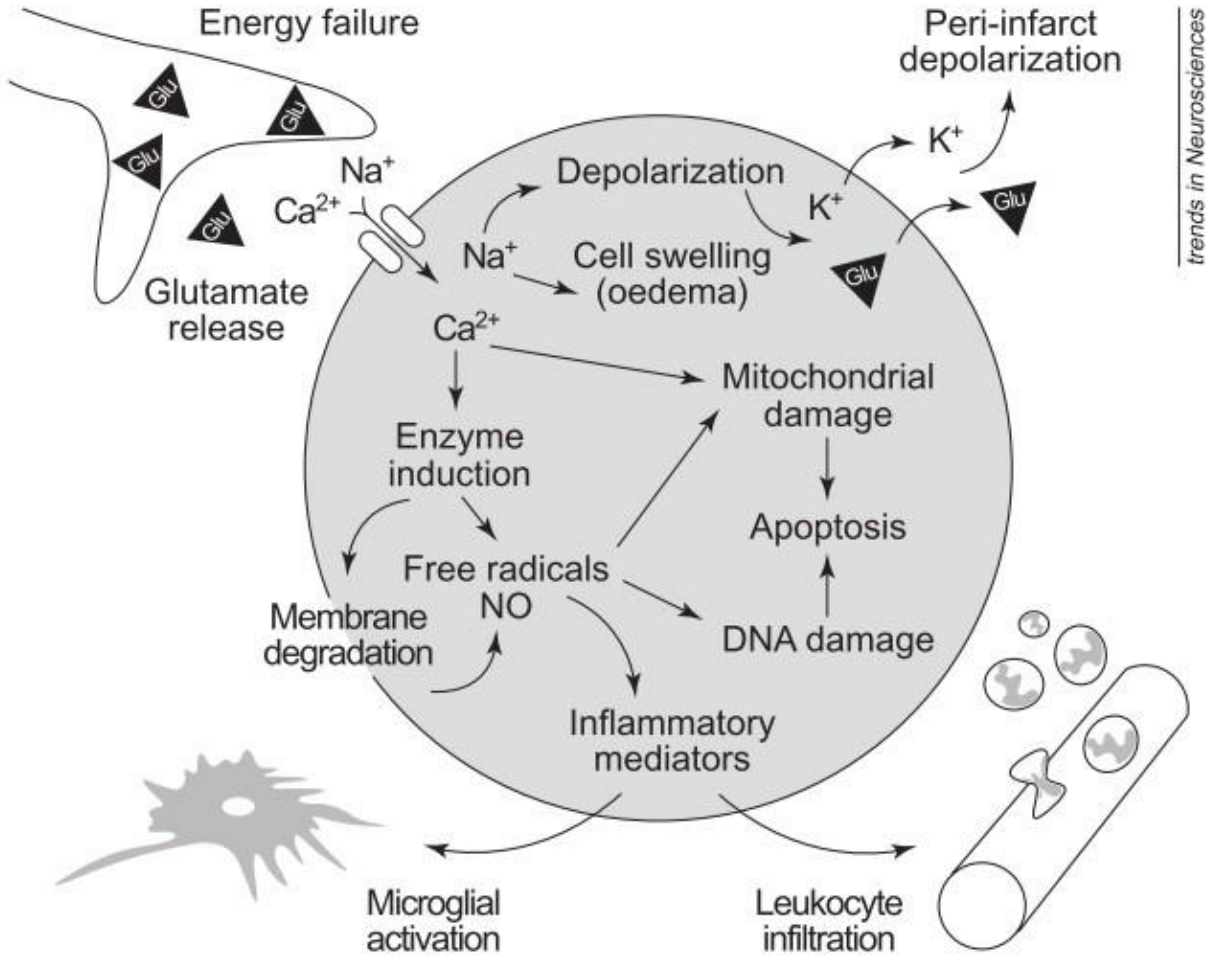

Figure 1: Pathophysiological mechanisms in the ischemic brain. Energy failure leads to depolarization and activation of glutamate receptors, which leads to an influx of Na^+, Ca^{2+}, water, and efflux of K^+. Diffusion of glutamate and K^+ in the extracellular space causes peri-infarct depolarizations. Ca^{2+} triggers the enzyme systems responsible for membrane degradation. Generation of free radicals, which damage mitochondria membranes and DNA, ultimately results in apoptosis. Free radicals cause the activation of microglia and lead to the leukocyte infiltration. Nitric oxide; NO, glutamate; Glu (Dirnagl, Iadecola, & Moskowitz, 1999)*.

2.1 Global cerebral ischemia

Global cerebral ischemia is characterized by interruption of the blood flow from systemic conditions. The causes of global ischemia include drowning, carbon monoxide poisoning, severe hypotension, and transient circulatory arrest. It leads to irreversible pathological cellular changes

evolving over days, which typically include delayed neuronal death, while the glial cells tend to proliferate (astrogliosis). The degree of damage caused by global ischemia depends both on the lesions caused by the ischemia itself and those occurring after recirculation (Hossmann, 1982).

2.2 Focal cerebral ischemia

Focal cerebral ischemia (Fig. 2) is caused by a transient or permanent flow restriction in the territory of a cerebral artery, usually caused by an embolic or thrombotic occlusion. The depletion of the energy reserves results in loosing of the membrane potential and depolarization of neurons and glia, which leads to the activation of the Ca^{2+} channels and release of the EAAs in the extracellular space, as described previously (Katsura et al., 1994).

In the focal cerebral ischemia, glutamate excitotoxicity is important as a cause of tissue degradation, by the means of necrosis, but also by triggering processes leading to apoptosis, a delayed cell death. In most cases, it is followed by a pan-necrosis that includes both neurons and glia – astrocytes, oligodendrocytes and also the endothelial cells in the infarct core (Choi, 1990). The core is surrounded by a so-called ischemic penumbra, a border zone of the ischemia with the metabolic activity partially preserved and undisturbed ion homeostasis, but electrically inexcitable. (Astrup et al., 1981) Without treatment, the penumbra will deteriorate, due to peri-infarct depolarizations (Hossmann, 1994).

Figure 2: Permanent focal cerebral ischemia in mouse. Middle cerebral artery occlusion. Tetrazolium chloride staining. The ischemic lesion can be seen in white.

2.3 Ischemic edema of the brain

Ischemic edema is caused by the water that enters cells as an effect of the membrane depolarization and the loss of function of the ion pumps. Many molecular and cellular changes in the blood-brain barrier (BBB) function, regulation, and the cellular volume play a role in edema development. There is also a relationship between the blood flow and the development of edema in the ischemic tissue. During reperfusion, there is a loose relationship between hyperaemia and the extent of cytotoxic edema (Avery et al., 1984). The cause of brain edema is both the cell swelling (cytotoxic edema) and an increase in permeability of the blood vessels (vasogenic edema; Zülch, 1967). The principle of the edema formation was defined by Starling as the identification of the force that drives the substances into the brain and the pore that allows the substance to leave the intercapillary space. In this case, the driving force being the hydrostatic and osmotic forces and the pores that allow the escape of the water being ion channels and reverse pinocytosis and also the shrinkage or loss of tight junctions in the capillary endothelial cells, or loss of the cells themselves by means of necrosis (. Simard et al., 2007)*.

Although these two types are not entirely separable and both occur in brain ischemia, cytotoxic edema is more prominent than vasogenic, due to hypoxia. Vasogenic edema possesses the risk of hemorrhage from damaged vessels (Sage et al., 1984).

2.3.1 Vasogenic edema

Vasogenic edema is mainly caused by the impairment of the BBB, which causes the increased permeability of the vessels and thus a shift of the water to extravascular compartments. In the first days of ischemia, mainly the water, Na^+ (influx) and K^+ (efflux) cross the BBB, while BBB remains impermeable to larger molecules, based on horseradish peroxidase or Evans blue. The vasogenic edema doesn't seem to develop sooner than four hours after the onset of ischemia when the BBB begins to break down and larger molecules invade the extravascular space. Vasogenic edema seems to be more prominent, when infarction is present, than in ischemia alone (Schuier & Hossmann, 1980; Sage et al., 1984).

2.3.2 Cytotoxic edema

The mechanisms responsible for the cellular swelling in cytotoxic edema are the increased Na^+ influx and K^+ efflux, ATP depletion followed by a loss of function of the ion pumps and the uptake of osmotically active solutes (Kraio and Nicholson, 1978; Hansen and Zeuthen, 1981).

These changes cause the uptake of water by the cell along the concentration gradient, which is responsible for the shrinkage of extracellular space caused by this type of edema (Schuier & Hossmann, 1980).

Among the molecules responsible for cytotoxic edema, we recognize the so-called primary drivers and secondary participants. Primary drivers are molecules normally extruded from the cell by the means of active transport and are thus more concentrated extracellularly, such as Na^+. Secondary participants are molecules for which the gradient is created by the primary drivers to maintain the electrical and osmotic neutrality, such as water. In ischemia, the mechanisms that expel the Na^+ outside the cell no longer function, which results in an influx of water into the cell and thus the formation of cytotoxic edema and later necrosis (Simard et al., 2007)*.

It has been shown, that among all the brain cells, the major contributors to the cellular swelling are the astrocytes, by the means of osmosis (Zülch, 1967), but also by the presence of several water and ionic channels, mainly AQP4 channel (Mori et al., 2002), which is discussed in later chapters. Together with other glial cells, astrocytes tend to proliferate after ischemia and contribute to the regulation of the ischemic conditions.

3 Glial cells

At first, neuroglia were thought to be only connective tissue, binding together the elements of the nervous system. They were first described and named by Rudolf Virchow in 1856 and stained by Camillo Golgi in 1885 using the black chrome-silver reaction. For a long time, they were thought to carry out only the supportive function, due to the fact that they do not generate an action potential. The supportive function was highlighted by many, and glia became a term covering many kinds of cells with different origins (both ectodermal macroglia and mesodermal microglia), morphology, properties, and functions, with the unifying property being the maintenance of the homeostasis of the nervous system. Recently, however, numerous important functions of the glial cells, other than being the „glue“ keeping the nervous tissue together, were discovered (Kimelberg, 2004)*.

Other than by their origin, glia can be categorized based on their localisation in the nervous system, those localised in the peripheral nervous system (PNS) being mainly Schwann cells, and

those residing in the central nervous system (CNS) – astrocytes, oligodendrocytes, NG2-glia and microglia (Verkhratsky & Butt, 2013)*.

In this chapter, the roles of astrocytes and NG2-glia will be described. Astrocytes possess a significant amount of AQP4 and TRPV4 channels, AQP4s being responsible for both the swelling of astrocytes (Simard & Nedergaard, 2004)* and the regulation of edema, possibly in cooperation with TRPV4 (Jo et al., 2015). TRPV4 might be also responsible for influencing the proliferation of NG2-glia (Ohashi et al., 2018), which play role in replacing the lost astrocytes (Zhu et al., 2007) and oligodendrocytes (Levine et al., 2001).

To mention the remaining CNS glia, oligodendrocytes play a role in the production of myelin that envelops the axons of neurons and in the regulation of axonal functions. Microglia are the main immunocompetent cells of the CNS, supporting the maturation of the nervous tissue (Xiao et al., 2014)*. When activated, during pathological events, they turn into phagocytes (Kreutzberg, 1996).The glia of the CNS can be seen in the illustration below (Fig. 3).

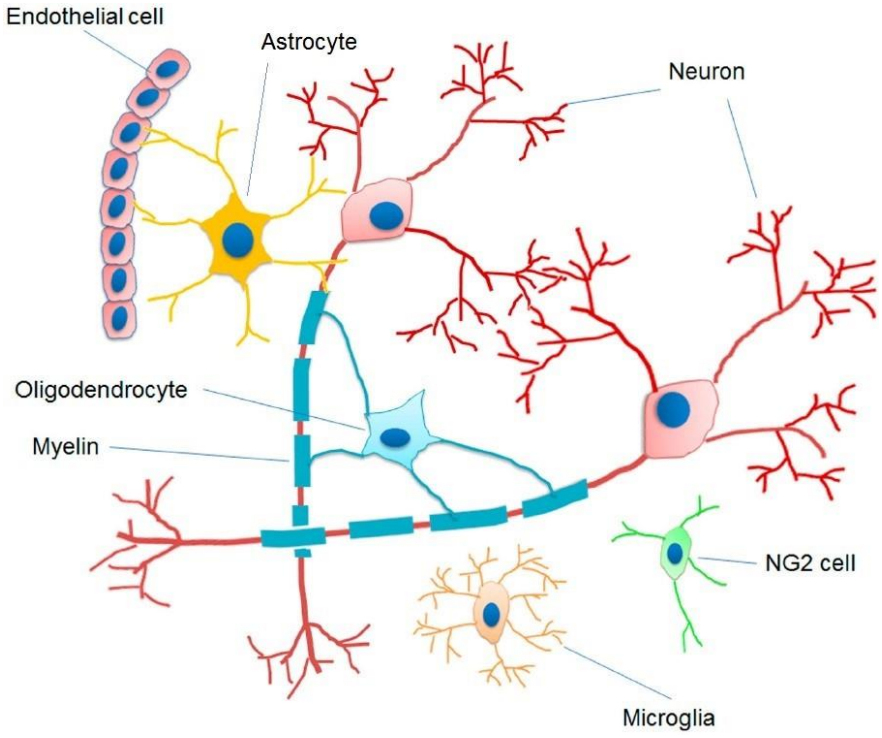

Figure 3: Cells of the CNS and their interactions (modified from Xiao et al., 2014)*.

3.1 Astrocytes

Astrocytes were first stained in 1913 by Santiago Ramón y Cajal using a gold chloride that stains the glial fibrillary acidic protein (GFAP), nowadays used as an astrocytic marker. Cajal was also the first one to use the term astrocyte. The distinction between protoplasmic astrocytes, residing mainly in the gray matter and fibrous astrocytes in white matter was made in the late 19th century, by William Lloyd Andriezen (Kimelberg, 2004)*.

Protoplasmic astrocytes in the gray matter were observed to reside in separate, non-overlapping domains, with their distal tips forming gap junctions. Cortical astrocytes were also observed to enwrap cortical neurons; each astrocyte on average interacts with four neurons (Halassa et al., 2007; Bushong et al., 2018). Being the most numerous cellular population in the brain, astrocytes outnumber neurons 1.4:1. They cover the whole area of the CNS (Sofroniew & Vinters, 2010)*.

Astrocytes are responsible for regulating the cerebral blood flow, forming the BBB, exchanging metabolites with neurons, forming the glial scar, modulating the synaptic activity, releasing neurotransmitters and maintenance of the brain homeostasis. They also express various transporters and receptors, that allow them to control the concentrations of ions, neurotransmitters and metabolites, the levels of water and pH, such as aquaporins (mainly AQP4, localized in their endfeet), for mediating the water fluxes in the nervous system, potassium channels and glutamate transporters, responsible for removing glutamate from the extracellular space (Simard & Nedergaard, 2004)*.

Signaling by Ca^{2+} appears to be the most common astrocytic response towards all kinds of stimuli, regulating the synaptic transmission by the means of Ca^{2+} dependent release of glutamate and thus regulating excessive neuronal activity and probably also regulating the supportive roles of astrocytes (Simard & Nedergaard, 2004)*.

In ischemic conditions, astrocytes are the major contributors to the formation of the ischemic edema, mainly cytotoxic edema. The mechanism by which this happens, is the impairment of the Na^+/K^+ ATPase, due to the depletion of the energy reserves, followed by an efflux of K^+ and uptake of Ca^{2+} and Na^+ into the cell (Hansen & Zeuthen, 1981). The content of Ca^{2+} is further heightened by its release from the endoplasmatic reticulum and mitochondria. The depolarization caused by these changes leads to an uptake of glutamate into the cell, which results in the activation of non-specific cation channels (Kalogeris et al., 2012)*.

The involvement of AQP4 channel in the astrocytic swelling and thus the formation of edema has also been proven. In the AQP4 knockout mice, the cerebral edema was significantly reduced in the model of water intoxication and stroke (Manley et al., 2000). The TRPV4, too, might be involved in the formation of edema (Jie et al., 2015) and act as a partner of the AQP4 in the process of astrocytic swelling (Salman et al., 2017).

The role of the astrocyte in such situation is to combat the swelling by expelling the redundant K^+, Cl^- and the osmotically obliged water from the cell. This process (Fig.4) is called the regulatory volume decrease (RVD; O'Connor & Kimelberg, 1993). Said osmolytes are let out of the cell by the calcium-dependent stretch-activated ion channels, which are activated by the swelling and the amount of intracellular Ca^{2+} present during edema.

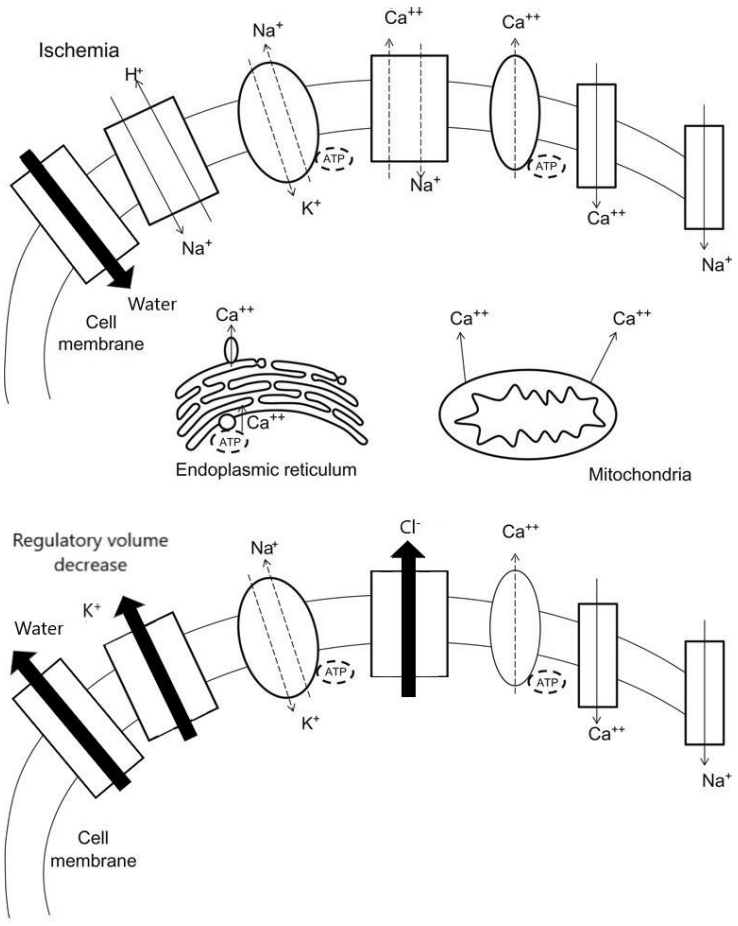

Figure 4: Ionic movements during ischemic edema and following regulatory volume decrease (RVD). Pictured above are the changes in the concentrations of ions during ischemic edema. In the lower picture the efflux of osmolytes, mainly Cl^- and K^+ and passively moving water during RVD is shown. The cell then resumes its physiological shape. (modified from Kalogeris et al., 2012)*.

The progression of RVD is also facilitated by the AQP4 channel, which, on one hand, helps the initial swelling by letting the water into the cell, but on the other promotes the water efflux responsible for cell shrinkage (Mola et al., 2016). The TRPV4 channel likely also participates in the RVD and thus regulation of the cerebral edema (Becker et al., 2005) and the mechanism of cooperation of TRPV4 and AQP4 in this process has been proposed as well (Benfenati et al., 2011).

Other than the role they play in the ischemic edema, astrocytes also serve other functions during ischemia, such as taking up glutamate and producing glutathione, which acts neuroprotectively, and provides neurons with lactate, to serve as a source of energy in times of substrate deprivation. On the other hand, the release of a glutamate precursor, glutamine, may be damaging for surrounding neurons and the heightened concentration of lactate may result in the death of astrocytes because their sensitivity to lactate is higher than that observed in neurons (Dienel & Hertz, 2005).

3.2 NG2-glia

NG2-glia were given their name due to the NG2 proteoglycan, also known as chondroitin sulphate proteoglycan 4 in their membranes (Stallcup & Beasley, 1987). They have also been called oligodendrocyte progenitor cells (OPC), as they have been found to differentiate into oligodendrocytes, (Levine et al., 2001). Despite being only recently discovered, they are nowadays considered to be one of the main glial populations of the CNS, together with astrocytes, oligodendrocytes, and microglia (Zhu et al., 2007). The entire CNS is densely populated with these cells.

When oligodendrocytes are lost in an adult brain, they are not replaced by other surviving oligodendrocytes, but they are replaced by NG2-glia-derived oligodendrocytes and remyelination follows (Fig. 5; Mount & Monje, 2017)*.

NG2-glia have been observed to react to different injuries, by mitosis, morphological changes and the upregulation of NG2. Their numbers tend to increase in zones of demyelination (Dawson et al., 2000) and they are responsive towards various injuries (Levine et al., 2001). Recently, they have been found to give rise not only to oligodendrocytes, but also to protoplasmic astrocytes in gray matter, both *in vitro* and *in vivo* (Zhu et al., 2007).

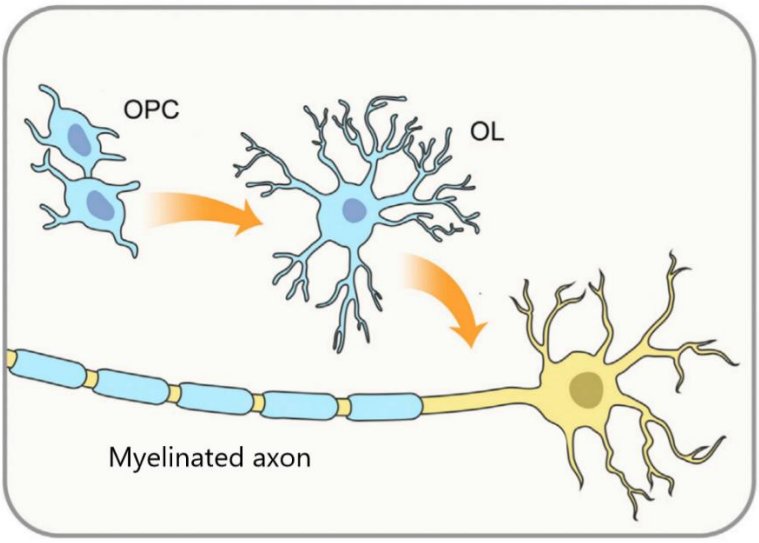

Figure 5: An illustration of the development of oligodendrocyte precursor cell (OPC) into an oligodendrocyte (OL) and the myelination of neuronal axons (modified from Mount & Monje, 2017)*.

Like in other pathologies, they seem to also proliferate during the ischemic injury. Although their number decreases in the core of the infarction, they have been observed to proliferate in the penumbra, with enlarged bodies and hypertrophic processes, taking place of the oligodendrocytes that succumbed to the injury (Tanaka et al., 2001).

In the process of NG2-glia proliferation, the role of TRPV4 has been proposed. Ohashi et al. claimed that the NG2-glia proliferation is influenced by a Ca^{2+} influx via the TRPV4 (Ohashi et al., 2018).

4 Specific membrane channels

All the living cells are enveloped in a cytoplasmatic membrane, a semipermeable, lipid bilayer that protects the cell from the outside environment. Among other things, it contains channels – highly specific protein structures, that span the membrane and allow the entrance of substances, that would not otherwise be able to cross the membrane, such as various ions, or water, that is able to cross the membrane on its own, but not in a sufficient amount (Roux & Schulten, 2004; Lodish et al., 2000*).

The movements of various substances in and out of the cell in edemic conditions were discussed in the previous chapter, this one is going to focus on two specific types of channels –

aquaporins, (mainly the AQP4) allowing for a rapid movement of water through the membrane, and TRPV4 channel, and their role in the ischemic edema.

4.1 Aquaporin 4

The aquaporins are small, approximately 30 kDa transmembrane proteins, that allow water to cross the cytoplasmatic membrane in a controlled manner, They contain six helical segments that span the entire membrane and two smaller ones that do not. They usually form tetramers with each monomer having a channel (Neely et al., 1999).

They were first discovered in 1992 in the red blood cells of *Xenopus laevis* (Preston et al., 1992). They can be divided into two groups: those permeable only by water: AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8, with AQP6 and AQP8 being parts of this group mainly due to their amino acid sequence, AQP6 being permeable by anions and AQP8 by urea. Members of the second group, aquaglyceroporins, are permeable also by glycerol and other small molecules. This group includes AQP3, AQP7, AQP9, and AQP10. The permeability of these various aquaporins is given by their size (aquaglyceroporins being able to widen to let in other molecules than water), and charge (King et al., 2004)*.

In the brain, two types of aquaporins can be found – AQP1 in the choroid plexus, where it helps the secretion of cerebrospinal fluid (Nielsen et al., 1993) and AQP4 in the ependymocytes and astrocytes, mainly in their processes, that are in contact with the blood vessels (Fig. 6; Nielsen et al., 1997).

Aquaporin 4 was first named the „mercurial-insensitive water channel“, then later renamed (Frigeri et al., 1995). Other than brain, it has been found, to a smaller extent, also in other tissues, such as epithelia in the kidney collecting duct, airways, stomach, and colon (Ma et al., 1997).

Under physiological conditions, AQP4 is responsible for the regulation of the volume of the extracellular space, potassium buffering, circulation of the cerebrospinal fluid, resorption of the interstitial fluid, clearance of the waste, migration of cells, osmosensation, and Ca^{2+} signaling. It is needed for the functioning of the retina, inner ear and olfactory system (Nagelhus & Ottersen, 2013)*.

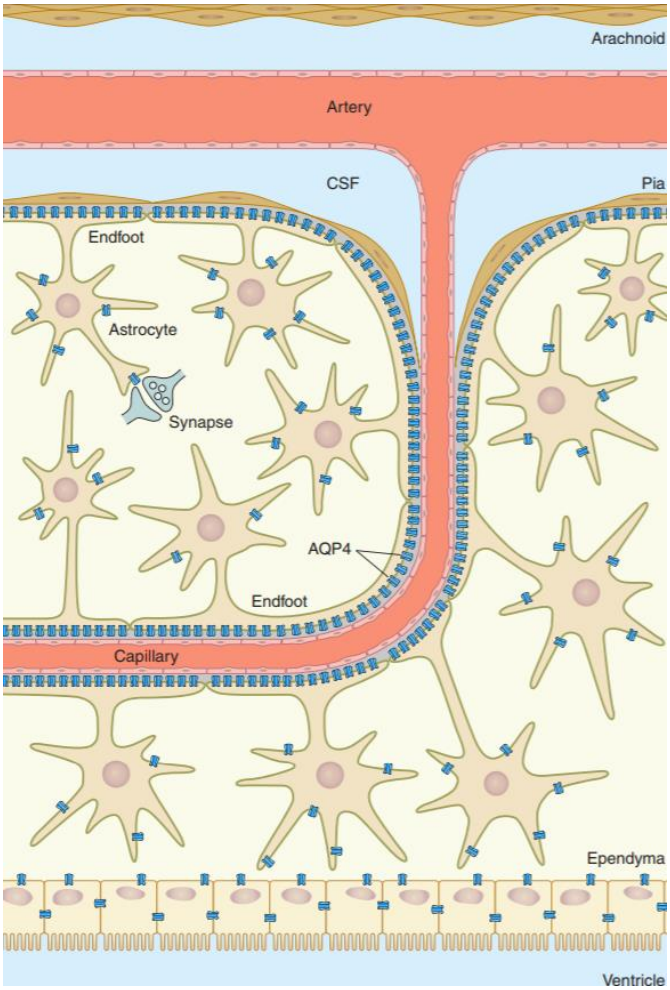

Figure 6: Aquaporin 4 **(**AQP4; blue) in ependymocytes and astrocytic processes interacting with the blood vessels. cerebrospinal fluid, CSF (Nagelhus & Ottersen, 2013)*.

It exists in two isoforms, depending on the initiation of the translation – whether it is on methionine1 (for M1 isoform) or methionine23 (for M23). These two isoforms form tetramers (both homo and hetero) in the membrane (Neely et al., 1999). In rats, a third isoform has been observed, the Mz, with translation initiation 126 bp upstream of that of M1. Mz is, however, absent in both human and mice (Rossi et al., 2011). When it comes to the differences between these three isoforms, a different water permeability has been observed, M1 being the most permeable and M23 the least (Fenton et al., 2010). The permeability also seems to be dependent on the cellular membrane structure and composition (Tong et al., 2012).

The AQP4 tetramers form higher structures, known as “square arrays” (Fig. 7.) in the astrocytic endfeet. Another difference between the M1 and M23 isoform seems to be in their willingness to form these structures – M23 forming large arrays, while M1 tetramers remain as

singlets. Together, they form arrays of an intermediate size. Thus, we can say that these isoforms have an opposite effect on the organization of AQP4 in the membrane (Furman et al., 2003).

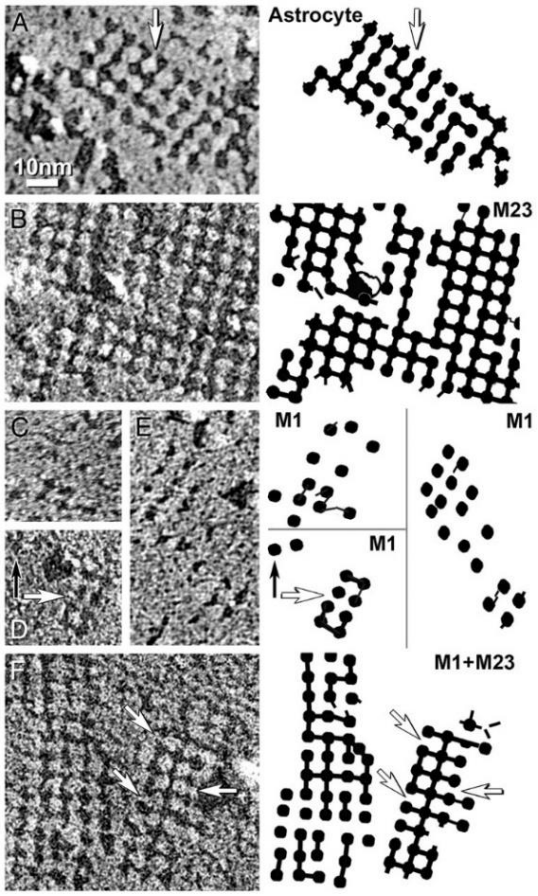

Figure 7.: Square arrays in an astrocyte; M23 isoform, on its own, forms largest arrays with more than 90 % of the individual pits (corresponding to individual tetramers) connected by furrows, M1 tetramers tend to remain in the form of singlets, M1 and M23 form arrays of an intermediate size with about half the furrows as in M23 (Furman et al., 2003).

Mz, on their own, are very much like the M1, when it comes to forming the arrays – they diffuse and tend not to form the arrays. But when co-expressed with M23, they form heterotetramers and arrays together, same as M1 with M23 (Rossi et al., 2011).

4.1.1 AQP4 in ischemic edema

An increase of AQP4 immunoreactivity was proven in hyponatremic mice (Vajda et al., 2000) and the role of AQP4 in hypoosmotic conditions was confirmed using AQP4 knockout mice, which showed reduced brain water content and a decrease of swelling of pericapillar astrocytic processes in hypoosmotic edema. In addition, these mice show a better recovery than their wild-

type counterparts (Manley et al., 2000). These effects were caused by a delayed K^+ clearance in these mice (Binder et al., 2006). Moreover, hypoosmocity and also hypothermia have been shown to increase the expression of AQP4 in the membranes of astrocytes. The hypothermia-mediated expression has been shown to require the activity of TRPV4 (Salman et al., 2017).

It has been shown that knocking out dystrophin, a protein associated with many cytoskeletal proteins in muscle and brain, resulted in AQP4 absence in astrocytic endfeet (Vajda et al., 2002), as did alpha-syntrophin knockouts. Alpha-syntrophin is one of the proteins associated with dystrophin. These studies suggest that alpha-syntrophin could be the anchor for AQP4 in astrocytic endfeet. The expression of endothelial AQP4 in alpha-syntrophin knockouts was not altered (Amiry-Moghaddam et al., 2004).

The dystrophin and alpha-syntrophin knockouts both show a delayed onset of hypoosmotic edema and reduced mortality, compared to wild-type mice, same as in AQP4 knockouts (Vajda et al., 2002; Amiry-Moghaddam et al., 2004).

The delayed and diminished edema in alpha-syntrophin knockouts was further observed by Anderova et al. and Dmytrenko et al. in hyponatremic conditions, after middle cerebral artery occlusion (MCAo), water intoxication, hypoosmotic stress, and terminal ischemia. This seems to be associated with K^+ transport and suggests an alteration in the K^+ transport in the alpha-syntrophin knockouts. In the models stimulating vasogenic edema, on the other hand, the clearance of the intraparenchymal fluid was slower in alpha-syntrophin knockouts and thus the edema was more severe (Dmytrenko et al., 2013; Anderova et al., 2014).

The role of astrocytic AQP4 in the formation of edema has been further confirmed by a glial-conditional AQP4 knockout (Haj-Yasein et al., 2011) as well as its role in the recovery after focal cerebral ischemia – in a model of MCAo followed by reperfusion, the volume of the infarction was significantly smaller in AQP4 knockout mice (Yao et al., 2015).

The smaller lesion in AQP4 knockout mice after ischemia was further confirmed by Hirt et al. using the model of MCAo. Lower mortality and faster recovery have also been observed. These findings suggest reduced neuroinflammation and neuronal death in AQP4 knockout mice (Hirt et al., 2017).

The downregulation of AQP4 has been proven as an efficient way to both combat the effects of cytotoxic edema and the lesion in ischemia. The vasogenic edema, on the other hand, is worsened in AQP4 knockout mice. In vasogenic edema, water and solutes are driven into extracellular space

by hydrostatic forces, and the models of BBB-disrupting diseases, such as brain tumors and abscesses have shown that the removal of the fluid from extracellular space was significantly slower in AQP4 knockouts (Papadopoulos & Verkman, 2013)*.

4.2 TRPV4

The TRP (transient receptor potential) channel family can be divided into six subfamilies: TRPV (vanilloid), TRPA (ankyrin), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin), and TRPP (polycystin) (Shibasaki, 2016)*.

The one that plays a role in the formation of edema is the TRPV, namely its subtype, TRPV4. It was first described as a channel activated by swelling in hypotonic conditions and initially named vanilloid receptor-related osmotically activated channel (Liedtke et al., 2000). It is a Ca^{2+} permeable, nonselective cation channel. It is a polymodal, ionotropic receptor activated by various stimuli, such as heat, acidic pH, pressure or hypotonicity. It is responsible for maintenance of the concentration of Ca^{2+} and osmotic homeostasis and can be found in a wide variety of cells. In CNS it can be found in astrocytes or microglia, in endothelia of cerebral arteries, where it regulates the influx of Ca^{2+}, in endothelia of the choroid plexus TRPV4 regulates the permeability of the BBB, in hippocampal neurons, it also regulates the activity of Na^{+} channels (White et al., 2016).

TRPV4 is expressed in several places other than the brain, mainly the kidney, where it acts as an osmoregulator of blood. Its expression has been proven to increase following CNS injuries associated with the cerebral edema and swelling of astrocytes. The activation of TRPV4 leads to an increase in the intracellular concentration of Ca^{2+} that does not happen otherwise.

While the TRPV4 knockout phenotype is of a relatively mild nature in mice and they do not display any obvious undesirable effects of the lack of this channel, in humans, the mutations of TRPV4 are a cause of a wide range of diseases. The TRPV4 channelopathies in humans include for example skeletal dysplasias with symptoms such as short stature, platyspondyly, and defects in ossification, or neuropathies with respiratory, motor and sometimes sensory defects (Nilius & Voets, 2013)*. A recent study also shows, that TRPV4 activation in microglia might play a role in the apoptosis of oligodendrocytes and, thus, in demyelination in neuroinflammatory diseases (Liu et al., 2018).

4.2.1 TRPV4 in ischemic edema

Same as AQP4, the TRPV4 has been observed in the membranes of astrocytic processes, mainly at the interface between the cell and extracerebral liquid spaces, such as blood vessels (Benfenati et al., 2007).

A study on TRPV4 knockout mice has proven the importance of TRPV4 in osmotic regulation. The TRPV4 knockouts were hyperosmolar compared to their wild-type littermates and drank less water, suggesting the TRPV4 could be one of the molecular mechanisms that maintain the osmolar balance and evoke thirst (Liedtke & Friedman, 2003).

Furthermore, a mechanism of hyperthermia activation of TRPV4 in ischemic conditions *in vitro* was proposed, using brain slices treated with oxygen-glucose deprivation, which caused the rise in brain temperature and consequently the activation of the TRPV4 channels, which led to the onset of ischemic edema. This was further confirmed by an *in vivo* experiment using TRPV4 knockout mice (Hoshi et al., 2018). On the other hand, hypothermia inhibits the activity of TRPV4 (Shibasaki et al., 2015).

A different role of TRPV4 in the ischemic edema was proposed by Becker et al. suggesting direct participation of TRPV4 in the RVD using human keratinocyte cells in hypotonic environment (Becker et al., 2005). Another study negated the hypothesis of TRPV4-assisted RVD, but claims to have observed an antiedematous role of TRPV4 after the induction of ischemia (Pivonkova et al., 2018), while yet another study claims the opposite - Jie et al. found, that the blockage of TRPV4 has an antiedematous effect through an inhibition of metalloproteinases in mice after MCAo (Jie et al., 2015).

TRPV4 has also been observed to be involved in pathological Ca^{2+} signaling in astrocytes during ischemia, although the mechanism of its activation in this process is still unclear, the swelling of astrocytic endfeet might be triggering the activation (Butenko et al., 2012).Moreover, the TRPV4-mediated calcium influx has been proposed to play a role in the peri-infarct depolarizations and enhance the accumulation of glutamate in the extracellular space in the model of cerebral ischemia (Rakers et al., 2017).

4.3 AQP4 and TRPV4 cooperation

A complex of TRPV4 and AQP4 in the astrocytes has been found to react to hypoosmotic conditions by the increase of intracellular Ca^{2+} aided by the TRPV4. In this study, the coexpression

of AQP4 and TRPV4 was proven to be essential for the onset of RVD in astrocytes (Benfenati et al., 2011).

The interplay of AQP4 and TRPV4 has further been confirmed by Chmelová et al. and TRPV4 and AQP4 were proposed to slow down the shrinkage of the extracellular space during severe ischemia *in vivo* (Chmelova et al., 2019).

Furthermore, the colocalization of AQP4 and TRPV4 has been observed in Muller astrocytes in the retina. Suppressed transcription of the genes coding for TRPV4, AQP4 and inwardly rectifying potassium (Kir) 4.1 channel was found in TRPV4 and AQP4 knockout mice. The model of regulation of AQP4 expression, volume, and swelling via the concentration of Ca^{2+}, that is regulated by TRPV4 was proposed as the way of cooperation of these two channels (Jo et al., 2015).

Moreover, an involvement of TRPV4 in a hypothermia-induced swelling of astrocytes and increase in the abundance of AQP4 in plasmatic membranes has been found (Salman et al., 2017). On the other hand, another study shows a neuroprotective effect of hypothermia by inhibition of a surge of AQP4 upregulation that closely follows an ischemic injury (Kurisu et al., 2016).

Recently, however, a cooperation between AQP4 and yet another channel of the TRP family, has been discovered. The TRPM4 is not expressed in the astrocytes under physiological conditions, it is only upregulated after CNS injuries, causing a phenomenon termed AQP4 dysregulation, which involves the switch from TRPV4 as a partner for AQP4, to TRPM4 and a shift of AQP4 from the astrocytic endfeet to a widespread expression throughout plasmalemma (Lafrenaye, 2019)*. The physical and functional association of AQP4 with TRPM4 and its heterodimerizing partner, SUR1, results in a heteromultimeric water/ion channel complex, that serves as a fast, transmembrane water transport method and thus causes a significant increase in astrocytic swelling triggered by ischemia-induced Ca^{2+} influx both *in vivo* and *in vitro* (Stokum et al., 2017).

5 Conclusion

In this thesis, the types of ischemic injury and the cerebral edema were categorized. The information on the formation and regulation of the edema were summarized. The role of glial cells, that is of the utmost importance in this process was described. The most involved in this process are the astrocytes, that contribute to both the formation of edema and the RVD afterward. The overall role of astrocytes and NG2 glia in the ischemic injury was presented too – NG2-glia being the ones that replace oligodendrocytes and astrocytes after their death during the ischemia and astrocytes playing many roles even besides regulation of the volume, such as nourishment of the neurons, maintenance of the BBB and control of the blood flow. The available information on the role of AQP4 and TRPV4 in this process were reviewed – the role of AQP4 found in the astrocytic endfeet being that of a pathway of water into the cell, and out of it, when the edema subsides, the reviewed articles mostly agreeing on its role played in this process.

The part played by the TRPV4, however, is still largely unknown, with many of the articles giving contrary opinions on the topic of TRPV4 involvement in the formation of the edema and the RVD. Nevertheless, its role as an osmoregulator is mostly clear, as is its overall involvement in the formation of the edema.

Going forward, the goal is to fully elucidate the role of the TRPV4 and AQP4 channels in the edema and the role of their cooperation in phenomena related to the ischemic edema.

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