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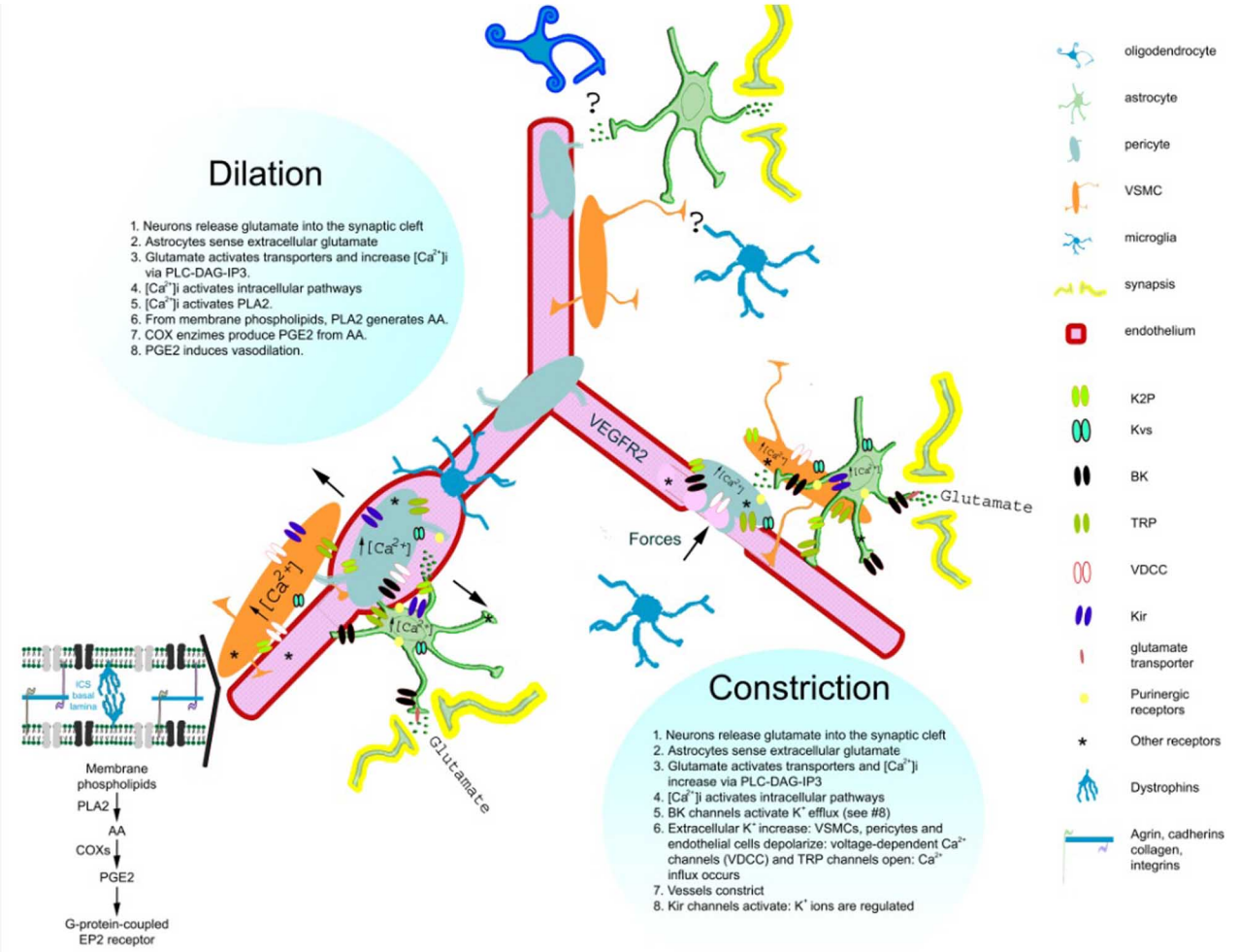

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[CONTENTS]

994 Effects of occupational exposure to ionizing radiation on oxidative stress and inflammatory markers in healthcare workers of a university hospital in Konya, Turkey

Zehra Ardıç, Tahir Kemal Şahin, Mehmet Uyar, Hasan Küçükkendirci, İbrahim Kılınç, Elif Nur Yıldırım Öztürk

1004 The role of ion channels on the physiology of the neurovascular unit and the regulation of cerebral blood flow

Marcelino Montiel-Herrera, Denisse García-Villa, Guillermo López-Cervantes, Daniel Reyes-Haro, J. Abraham Domínguez-Avila, Gustavo A. González-Aguilar

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

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Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

REVIEW ARTICLE

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The role of ion channels on the physiology of the neurovascular unit and the regulation of cerebral blood flow

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List of Abbreviations;

***NVU**, Neurovascular unit; **VEGFR2**, Vascular Endothelial Growth Factor Receptor 2; **[Ca^{2+}]i**, Intracellular Calcium Concentration; **PLC**, Phospholipase C; **DAG**, Diacylglycerol; **IP3**, Phosphatidylinositol 3-phosphate; **VSMCs**, Vascular Smooth Muscle Cells; **TRP**, Transient Receptor Potential; **PLA2**, Phospholipase A2; **AA**, Arachidonic Acid; **COXs**, Cyclooxygenases; **PGE2**, Prostaglandin E2; **EP2**, Prostaglandin E2 Receptor; **ICS**, Intercellular Space; **BK**, Big Conductance Potassium Channels; **Kir**, Inward Rectifier K^+ channels; **K-ATP**, Channels Sensitive to Intracellular ATP; **Kv**, Delayed Rectifier K^+ channels; **K2P**, Two-pore Domain K^+ channel; **GIRK**, G-protein Inward Rectifier K^+ Channel; **20-HETE**, 20-Hydroxyeicosatetraenoic Acid; **AMPA/KA**, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic/kainic acids; **ROS**, Reactive Oxygen Species; **PKA**, Protein Kinase A; **PKC**, Protein Kinase C; **AQP4**, Aquaporin-4; **TEA**, Tetraethylammonium.*

Abstract

The neurovascular unit, composed of neurons, brain endothelial cells, and glial cells, regulates cerebral blood flow. Physical and chemical signals govern the physiology of the neurovascular unit within this cell network. This manuscript will briefly discuss recent evidence about the contribution of this cell network and its plethora of ion channels, to the leading cellular mechanisms involved in the physiology of the neurovascular unit.

Keywords: Ion Channels, Astrocyte End-feet, Neurovascular, Endothelial Cell, Vascular Smooth Muscle Cell, Capillaries.

Introduction

The neurovascular unit and cerebral blood flow

Brain cells need an adequate supply of nutrients (e.g., oxygen and glucose) and mechanisms for the clearance of waste metabolites. On a daily basis, these processes are carried out by the cerebral blood flow regulated by the neurovascular unit (NVU), which consists of physical and chemical communication between neurons, brain endothelial cells, pericytes, microglia, and astrocytes (Figure 1). The correct functioning of the NVU is essential for normal brain activity throughout the brain's developmental stages, including the mature brain (Mishra 2017).

Blood supply to brain cells is carried out mainly at the capillary level. Brain cell activity controls this, resulting in a dynamic process that regulates cerebral blood flow (Gordon et al. 2008; Harraz et al. 2018; Reeson et al. 2018). For instance, intense neuronal activity demands a higher metabolic rate, which favors an increased cerebral blood flow to provide enough oxygen and glucose (Gordon et al. 2008; Rosenegger et al. 2015). This process is known as functional hyperemia. Taking into account that metabolic rates in the brain can influence the cerebral blood flow through blood vessels, which is proportional to the vessel radius to the fourth power, a small (~ 10%) change in blood flow will significantly increase vessel diameter, and thus the cerebral blood flow (> 40%), during neuronal activity (Gordon et al. 2008). Since small changes in vessel diameter significantly modify blood microcirculation in the brain, endothelial cells must therefore sense chemical signals and the physical forces generated by blood on the inner layer of the capillary wall and adjust accordingly, in order to maintain adequate blood flow. Dysfunction of hyperemia or obstruction of blood supply is associated with vascular-related neuropathologies (McConnell et al. 2017; Abbott et al. 2018; Reeson et al. 2018), for example, disruption of blood supply produces cerebral edema or brain inflammation (Abbott et al. 2018).

The vascular network in the brain is more susceptible to obstruction of capillaries at its superficial layers and lower-order branches. Considering that the size of erythrocytes is ~7 μm in diameter, these cells can alter their cellular volume to flow even via narrower capillaries that are prone to clog or disrupt pericytes, and endothelial cells must signal each other within the NVU to overcome these events. For instance, about 1-7% of the capillaries in mice brains are transiently obstructed every day in those regions, and a few of them (~ 0.12%) remain obstructed for more than 20 min (Reeson et al. 2018). This triggers the activation of cellular pruning mechanisms and vascular endothelial growth factor receptor 2 (VEGFR2) signaling to regain blood flow and, thus, maintain an adequate supply of oxygen and nutrients and clearance of waste products (Reeson et al. 2018). Interestingly, ~ 30% of the obstructed capillaries prune after 3 weeks, and ~ 70% regain flow via the signaling pathway of VEGFR2 (Reeson et al. 2018). Restored blood flow is accompanied by a transient increase in blood flow velocity and flux in the pruning capillaries and their capillary neighbors. These capillary responses to blood flow indicate that NVU cells must be competently active for proper brain functioning (Stobart et al. 2018).

The general structure of the NVU is composed of the network established by the interactions between neurons, astrocyte processes called "end-feet" that contact blood vessels, and peri-synaptic astrocyte projections (PAP) that contact synapses, microglia, pericytes, and vascular cells (endothelial and vascular smooth muscle cells that have contractile properties) (Filosa 2015; Orellana 2016; McConnell et al. 2017; Stobart et al. 2018). The role of oligodendroglia in the NVU has yet to be explored. An example of the functional coupling in the NVU is given for the parenchymal arterioles that consist of blood vessels that interact with vascular smooth muscle cells (VSMCs) in close contact with astrocytes. The direct contact between astrocytic end-feet and VSMCs has not been demonstrated. The NVU sends different signals to the neighboring neurons and glial cells, including physical forces (mechanical changes produced by an increase in vessel diameter) and chemical transmitters. In addition, in the deep layers of the brain, the NVU is composed of endothelial cells that are anatomically adjacent to pericytes. The NVU is also influenced by neuronal activity, which signals astrocytes and promotes downstream communication with pericytes and endothelial cells. This occurs through gap junctions and peg-and-socket contacts formed between these two cell types; ATP and phosphatidylinositol 4,5-bisphosphate released by endothelial cells, as well as hydrostatic pressure sensed by the vessels, are also involved (Puro 2007; Hall et al. 2014; Hashitani and Lang 2016; McConnell et al. 2017; Harraz et al. 2018; Stobart et al. 2018) (Figure 1).

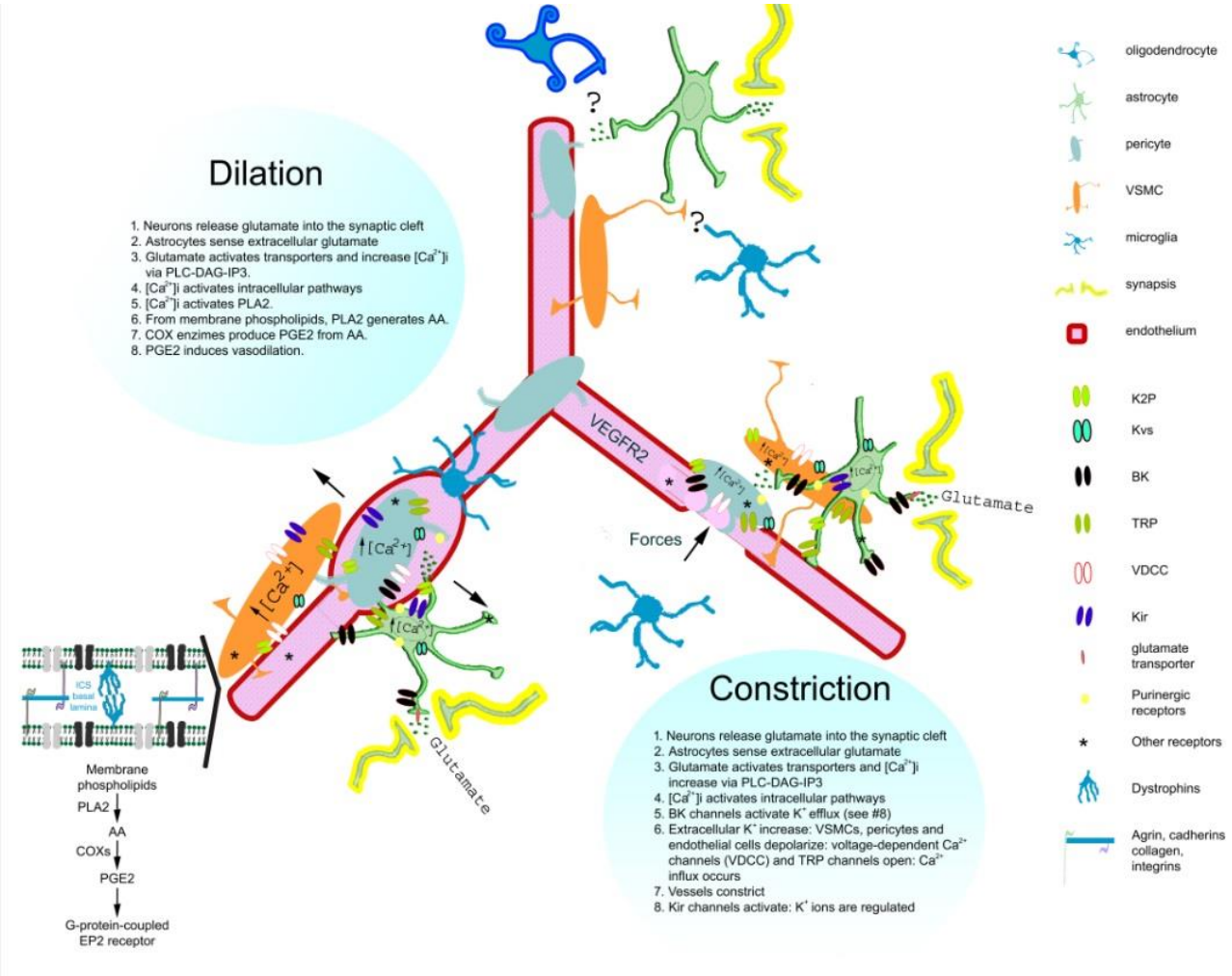

Figure 1. *Schematic representation of the brain neurovascular unit. Both types of vessel responses are shown, those mediated by synaptic glutamatergic transmission and by calcium-astrocyte, which influence vascular smooth muscle cells and endothelial cells. Well-known metabolic pathways of membrane phospholipids facing intracellular space of neurons, astrocytes and vascular cells that influence vessel responses are also depicted. However, the roles of intercellular membrane phospholipids and proteins facing each other between all cells of the neurovascular unit are poorly understood. [Ca^{2+}]i, intracellular calcium concentration; PLC, phospholipase C; DAG, diacylglycerol; IP3, phosphatidylinositol 3-phosphate; VSMCs, vascular smooth muscle cells; TRP, transient receptor potential; K2P, Kvs, BK and Kir, types of potassium channels; VEGFR2, vascular endothelial growth factor receptor 2; PLA2, phospholipase A2; AA, arachidonic acid; COXs, cyclooxygenases; PGE2, prostaglandin E2; EP2, prostaglandin E2 receptor; ICS, intercellular space.*

Another function of the NVU is regulating the transfer of substances from the blood through the cell membrane of endothelial cells (Harraz et al. 2018). Endothelial cells are equipped with several membrane transporters, ion channels, gap junctions, and transmitter receptors that enable them to detect and transmit long-distance intercellular signals (Hashitani and Lang 2016; Harraz et al. 2018). Notably, they can detect, transmit and summate electrical signals along with the microcirculation network of capillaries of the brain (Figure 1) (Jackson 2005; Harraz et al. 2018).

Another critical player in the NVU is the pericyte, which envelops around 37% of brain capillaries and close contact with endothelial cells. Pericytes express big conductance potassium channels (BK), inward rectifier K^+ channels (Kir), Kir channels sensitive to intracellular ATP (K-ATP), delayed rectifier K^+ channels (Kv), and voltage-dependent calcium channels (Mishra 2017) (Figure 1) and respond to ATP, dopamine, angiotensin II, endothelin-1, noradrenaline, 20-hydroxyeicosatetraenoic acid (20-HETE), adenosine and prostaglandin E2 (PGE2). The signaling pathways of all of these chemical messengers are

directly associated with NVU activity. In addition, neurotransmitters are also associated with the NVU, for example, angiotensin II and endothelin-1 induce retinal vasoconstriction, and histamine, bradykinin, and cholinergic and β-adrenergic agonists produce retinal vasodilation (Puro 2007). Even with this established knowledge, the contribution of pericytes and endothelial cells to blood flow regulation is controversial, since a single microinjection of 500 μM KCl did not produce significant changes in vessel diameters, apparently to pericytes lack contractile properties (Hill et al. 2015). It is essential to consider that capillary filtration and reabsorption are highly influenced by the hydrostatic pressure exerted on vessels, which can determine the final response given by the NVU (Hashitani and Lang 2016). Altogether, specific anatomical arrangement of cells, hydrostatic forces, and cellular communication skills strongly suggest an active and fast pericyte signaling, which contributes to maintaining a constant supply of nutrients and oxygen to the NVU under high neuronal activity.

The role of astrocytes in regulating cerebral blood flow

As previously mentioned, astrocytes are important components of the NVU that, with their end-feet, influence synapses through PAP and blood vessels. Astrocytes express a plethora of ion channels, chemical-transmitter receptors, and transporters that allow them to sense and modulate synaptic activity and, hence, influence NVU activity (Sibille et al. 2015; Mishra 2017). For instance, it is known that dilation or constriction depends predominantly on the changes of Ca^{2+} and K^+ concentration in the vascular network (Girouard et al. 2010). Astrocytic K^+ channels are highly involved in regulating extracellular K^+, and allow these cells to respond to changes in extracellular K^+, with changes in intracellular Ca^{2+} concentration. For example, in mouse cortical brain slices, an increment of extracellular K^+ (up to 20 mM) evoked Ca^{2+} increments in the astrocytic end-feet and dilation of arterioles. However, if astrocytic intracellular Ca^{2+} concentration increases to more than 700 nM, the Ca^{2+}-dependent BK channels are activated and produce vascular constriction (Figure 1). Activation of ligand-gated channels in astrocytes produces changes in their intracellular Ca^{2+}, for example, astrocytes respond to the neurotransmitters released by neurons with variations in their intracellular Ca^{2+} concentration. In the NVU, activating astrocytic metabotropic glutamate receptors 1/5, produces intracellular Ca^{2+} responses mediated by the phospholipase C (PLC) pathway. This increased astrocytic intracellular Ca^{2+} concentration also impacts the activity of Ca^{2+}-dependent ion channels (e.g., Ca^{2+} sensitive-chloride channels), which in turn affect membrane potential (Figure 1). Astrocytes express α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic/kainic acids (AMPA/KA) receptors, whose activation produces astrocytic Ca^{2+} responses (Mishra et al. 2016). It was recently demonstrated that neuronal activity *in vivo* produces fast (~100-120 ms) intracellular Ca^{2+} increments in both astrocyte processes and end-feet (Stobart et al. 2018). These signals are involved in releasing chemical transmitters to the extracellular space by vesicle-mediated mechanisms that are Ca^{2+}-dependent (Evanko et al. 2004; Covelo and Araque 2018). Disruption of astrocytic Ca^{2+} signals, for instance, using Ca^{2+} chelators, produces a dysfunction of the NVU, including functional hyperemia (Rosenegger et al. 2015; Mishra et al. 2016). It has been proposed that astrocytes also release transmitters through ion channels (Woo et al. 2012; Hwang et al. 2014; Seifert et al. 2018), but this mechanism remains unclear. It has also been found that astrocyte end-feet interact with vascular cells at all levels, from capillaries to parenchymal arterioles and veins, which, in particular, contribute to maintain the brain's basic metabolic needs (Orellana 2016).

During high neuronal activity, astrocytes uptake glucose, metabolize it to lactate, and release it to the NVU. Lactate is a vasoactive metabolite that modulates the diameter of brain's microvasculature, in an oxygen-dependent manner (Gordon et al. 2008). Evidence suggests that the oxygen-dependent response is hierarchically significant for the NVU to regulate cerebral blood flow, since this phenomenon proceeds despite astrocytic glutamatergic signaling. Since low and high oxygen pressure produce vasodilation and vasoconstriction, respectively (Figure 1), oxygen levels could modulate astrocytic Ca^{2+} signaling, via phospholipase A2-cyclooxygenase-prostaglandin E2 pathways (Gordon et al. 2008; Rosenegger et al. 2015). Low oxygen-induced astrocytic-mediated vasodilation has also been shown to be enhanced by extracellular adenosine (Gordon et al. 2008), similar to ATP, another important modulator of vessel physiology, which induces vasoconstriction or vasodilation at high or low levels, respectively (Kur and Newman 2014). Other transmitter systems (e.g.,

adrenergic, purinergic, GABAergic) (Figure 1) and cellular mechanisms are known to participate in regulating NVU physiology (Sugiyama, 2014; Otsu et al. 2015; Longden et al. 2016; Stobart et al. 2018).

The present review makes it clear that there is a complex physiology underlying the NVU. To better understand, it is necessary to design carefully controlled experiments, because even small changes in the levels of chemical transmitters may yield opposite effects on vessel function. It is important to pay attention to methodologies used, to study conditions (e.g., temperature), stimulus type/strength, and effects of anesthetics, and other pharmacological procedures, because all these variables could yield misleading results about the specific contribution of every cell type in the regulation of cerebral blood flow (Filosa, 2015; Mishra, 2017; Rungta et al. 2017). To overcome these protocol-related variable results, Masamoto et al. (2015) employed a channelrhodopsin-2 (light-gated cation channel) transgenic mouse model to optogenetically stimulating astrocytes in the intact NVU. They found that the optogenetic activation of astrocytes controls local cerebral blood flow through the modulation of extracellular K^+, affecting the vascular cells. However, Rungta et al. (2017) reported that light itself produces a Ca^{2+} decrease in VSMC arterioles, that leads to vasodilation without the involvement of neurons and astrocytes. This makes it clear that it is imperative to pay close attention when developing accurate methodologies for the study of the NVU; additional studies remain necessary to elucidate the role of every single cell component of the NVU in regulating cerebral blood flow (Hall et al. 2014).

The following section gives an update about the particular contributions of ion channels to specific cell types that assure the correct functioning of the NVU.

The contribution of ion channels to the physiology of the NVU

Arteriole tone involved in the regulation of cerebral blood flow in response to varying neuronal activity requires ion channels. In particular, K^+ channels widely expressed in all vertebrates have been studied at the NVU (Longden et al. 2016; Stobart et al. 2018). Interestingly, neurons, glial cells, and vascular cells express a vast array of K^+ channels involved in many physiological tasks such as K^+ homeostasis and setting the resting membrane potential. There are more than 90 genes that encode K^+ channels (Alexander et al. 2017; Zhang et al. 2018), which can be grouped into four categories: **1)** K^+ channels sensitive to nucleotides, pH, temperature, mechanical forces, and chemical transmitters; **2)** Ca^{2+}-modulated K^+ channels; **3)** Kir channels; **4)** Kv channels. K^+ channels are distributed and assembled on cell membranes and contribute to maintaining the ionic homeostasis of the brain. The following paragraphs provide further details regarding the roles of K^+ channels in K^+ homeostasis and NVU physiology.

Two-pore domain K^+ channel (K2P)

The two-pore domain K^+ channel (K2P) family comprises 15 members (including TASK1, TREK1, TREK2, TWIK1) that contribute to the constitutive leak of K^+. These K2P channels regulate the resting membrane potential and excitability of neurons (Seifert et al. 2018). Its activity is modulated by physical and chemical stimuli, including temperature, shear stress, pH, polyunsaturated fatty acids, hormones, and neurotransmitters (Cho et al. 2017). For example, it is known that heterodimers composed of TWIK1/TREK1 channels are coupled to G protein (Gαi) signaling (known as G-protein inward rectifier K^+ channel; GIRK) and contribute to vascular vasodilation/vasoconstriction responses generated in response to neuronal activity (Cho et al. 2017). In astrocytes, K2P channels contribute with a remnant passive K^+ current, although it should be mentioned that knockout animal models of TWIK1 and TREK1 do not change the current patterns of astrocytes *in situ* (Seifert et al. 2018). Moreover, in Müller glial cells, TASK1 channels are sensitive to pH and bupivacaine, which are involved in swelling cellular mechanisms. These swelling cellular mechanisms may target changes in vessel diameters and contribute to signaling the NVU (Figure 1). However, further studies are still needed to understand the functional role of all members of the K2P family in the physiology of the NVU.

Big conductance potassium (BK) channels (Ca^{2+}-modulated K^+ channel)

BK channels are expressed on almost every excitable cell, and are composed of α and β subunits, with the α subunit having a voltage sensor in its S4 region. The Ca^{2+} sensitivity of BK channels is modified by local intracellular Ca^{2+} and the phosphorylation state of several intracellular enzymes, and are therefore targets of several

intracellular stimuli (Vivas et al. 2017). In general, the activation of BK channels depends on both voltage membrane and intracellular Ca^{2+} concentration. In the absence of intracellular Ca^{2+}, BK channels are activated over 100 mV, while their Ca^{2+} affinity ranges between 1-10 mM (Vivas et al. 2017). BK channels form clusters coupled to low voltage-activated Ca^{2+} channels (Cav1.3 or Cav2.2), in a cooperative association that ensures rapid repolarization after neurons fire action potentials (Vivas et al. 2017). In this context, a possible scenario emerges, where cells of the NVU might express multi-channel complexes of BK channels (composed of BKα and β subunits). There is evidence that, in the VSMC, intracellular Ca^{2+} increments originated by extracellular Ca^{2+} entry through voltage-activated Ca^{2+} channels activate BK channels, producing hyperpolarization of membranes that exert a relaxation effect (Figure 1) (Zhu et al. 2018). BK channels composed by the α subunit, with a cysteine-rich 59-aminoacid insert between RCK1 and RCK2, and are called "stress-axis regulated exon"; they are particularly relevant for NVU physiology. These BKα channels have an enhanced sensitivity to voltage and Ca^{2+} (Zhu et al. 2018), which could regulate the relaxation of cell membrane potentials. On the other hand, dysfunction of the β1-subunit of BK channels is associated with diabetes, hypertension, and other vascular pathologies. BKβ1 subunit knockout mice have BK channels with a significantly reduced Ca^{2+} sensitivity (Zhu et al. 2018). It is also noteworthy that the BKγ1 subunit also affects voltage and Ca^{2+} sensitivity on these channels. BK channels contain leucine-rich domains encoded by LRRC26, LRRC52, LRRC55, and LRRC38 genes. The literature indicates that LRRC26 is expressed by arterial smooth muscle cells, while knockout models of this protein have reduced voltage and Ca^{2+} sensitivities, and decreases the frequency and amplitude of BK currents (Zhu et al. 2018).

BK channels are modulated by diverse signaling pathways, such as those activated by angiotensin II, reactive oxygen species (ROS), protein kinase A and C (PKA and PKC, respectively), and other molecules like nitric oxide (NO), fatty acids (Ω-3 docosahexaenoic acid) and their metabolites. Moreover, some diseases are associated with a dysfunction of vascular BK channel activity; for instance, diabetes mellitus is associated with a downregulation of β1-subunit, with no effects on BKα subunits (Zhu et al. 2018). This can be partially attributed to the fact that diabetes increases ROS (superoxide anion and hydrogen peroxide among others), which in turn influence the channels' activity. For example, it has been described that ROS molecules produce changes in BK currents in vascular cells and that genetic disruption of the β1-subunit impairs vascular relaxation and modifies Ca^{2+} and voltage sensitivities, thereby compromising BK channel function and vascular tone (Zhu et al. 2018). Interestingly, unpublished studies performed in our laboratory suggest that streptozotocin-induced hyperglycemia in rats does not alter the expression of β1-subunits in the frontal lobe (unpublished results). In spite of these references, few studies are available on the subject of ROS and their effect on BK channel function; further information is needed to understand the pathophysiology of ROS and chronic hyperglycemia on the physiology of the NVU.

Endothelial cells in the vascular bed release epoxyeicosatrienoic acids (EETs) that exert vasodilation/vasoconstriction effects on adjacent VSMCs, hyperpolarizing their membranes by opening of BK channels, which in turn generate vasodilation (Goto et al. 2018). It is known that prolonged hypertension generates dysregulation of the vascular endothelia, according to experiments performed in BK-deficient mice that developed high blood pressure (Goto et al. 2018). This suggests that BK channels play an essential role in the regulation of systemic blood pressure, although their activity in endothelial cells is not fully understood.

Inward rectifier K^+ (Kir) channels.

Kir channels were first described by Sir B. Katz (1949) as K^+ channels that increase their conductance in hyperpolarizing potentials, and decrease it under depolarization (Hille, 2001). That is, K^+ influx to the cell generates an inward current during hyperpolarization. Kir channels are very diverse in brain cells, for example, K-ATP channels are weakly Kir channels sensible to intracellular ATP. They are composed of Kir6.1 or Kir6.2 subunits with one of four types of auxiliary sulfonylurea receptors that open in the absence of cytoplasmic ATP, which means they hyperpolarize cells that are low on energy. In arterioles, K-ATP channels may participate in the vasodilation response to hypoxia and acidosis (Longden et al. 2016).

Astrocytes express diverse K^+ channels (Montiel-Herrera and García-Colunga 2010) that satisfy their

functional and specific needs (Seifert et al. 2018). In particular, astrocyte-Kir channels dominate K^+ conductance in the adult brain, but other channels like Ca^{2+} channels, Kvs, and passive K2P channels are believed to contribute to specific functions, although they are yet to be discovered. Astrocytes, not neurons, express Kir4.1 channels. Several studies indicate that Kir4.1 maintains glutamate uptake by lowering the astrocytic membrane potential towards its K^+ equilibrium potential and improving K^+ buffering during high periods of neuronal activity (Seifert et al. 2018), although their dysfunction leads to excitotoxicity and cell damage (Xiao et al. 2018). For example, by using si-RNA-mediated Kir4.1 knockdown in cortical astrocytes, glutamate uptake decreases over 30-50% (Milton and Smith 2018). The genetic deletion of Kir4.1 channel generates a strong depolarization and increases astrocyte membrane resistance. These biophysical characteristics of Kir4.1 channels have confirmed that this particular subunit sets the resting membrane potential of astrocytes.

Kir4.1 channels also distribute the lean processes of astrocytic end-feet facing blood vessels and synapses (PAP). This non-uniform distribution suggests that Kir4.1 channels serve specific functions of astrocytes (e.g., rapid uptake, re-distribution of K^+, and setting the resting membrane potential) that have an influence on NVU physiology (Figure 1) (Seifert et al. 2018). Recent studies have shown that Kir4.1 channels and aquaporin-4 (AQP4) might be co-distributed in raft-like membrane domains along with volume-regulated ion channels, whereby their interactions may play essential roles in regulating water homeostasis in the brain and, therefore, in the control of cerebral blood flow and vascular tone (Wolburg et al. 2009).

Astrocytes also express the Kir5.1 subunit, which confers high pH sensitivity when co-expressed with the Kir4.1 subunit in the retrotrapezoid nucleus, thus, these channel subunits contribute to chemoreception-mediated breathing control. Interestingly, the heterologous expression of AQP4 and Kir4.1/5.1 gives volume sensitivity to Kir channels. In this sense, this macromolecular arrangement in astrocytic end-feet along with BK channels would be expected to modulate volume and K^+ homeostasis in the control of cerebral blood flow via the NVU (Seifert et al. 2018). Moreover, focal ischemia reduces Kir currents, Kir4.1 expression, and astrocyte localization (Milton and Smith, 2018). Kir4.1 channel re-distribution in astrocytes is attributed to ensuring neuronal survival following ischemia via the mammalian target of rapamycin complex 1-phosphatidylinositol 3 kinase/protein kinase B pathway.

VSMCs and endothelial cells express Kir channels, Kv channels, K-ATP, and Ca^{2+}-modulated chloride channels (McConnell et al. 2017; Goto et al. 2018). The membrane localization of Kir channels is different in these cells, and evidence shows specific roles related to the vascular bed they comprise. For instance, VSMCs express Kir channels that predominantly distribute in smaller resistance vessels (and appear functionally absent in larger vessels), where they influence the resting membrane potential toward the equilibrium potential for K^+ (Gollasch et al. 2018; Goto et al. 2018). Under basal conditions, VSMCs have a resting membrane potential (-35 to -45 mV) that is more positive than their K^+ equilibrium potential (-103 mV, assuming 3 mM extracellular K^+ and 140 mM intracellular K^+) (Longden et al. 2016). Thus, the activation of K^+ channels should try to hyperpolarize the membrane of VSMCs, influencing rapidly over the vascular diameter. For example, when raising extracellular K^+ from 3 to 8 mM, Kir hyperpolarizes the VSMC membrane around 40 mV, closed by voltage-dependent calcium channels, generating stronger vasodilation (Longden et al. 2016). However, a comprehensive set of data on Kir subunit expression in VSMCs in arterioles and endothelial cells downstream in the cerebral blood flow has yet to be elucidated. Although experimental evidence shows that Kir 2.1 channel activation leads to vasodilation, animal model mutants of Kir2.1 channels show an increase in blood pressure, probably due to the enhanced microvascular resistance (Goto et al. 2018). Moreover, knockout of Kir2.1 subunits, but not Kir2.2, completely ablates the dilatory effect of vessels due to increased extracellular K^+, suggesting a pivotal role for Kir2.1 subunits in the brain's blood supply (Hasan and Jaggar 2018). Harraz et al. (2018) have recently shown that Kir2.1 and TRPV4 channel activities in endothelial cells of the brain govern the balance between depolarizing and hyperpolarizing responses of brain capillaries to modulate the NVU.

Delayed rectifier K^+ (Kv) channels

Twelve subfamilies of Kv channel α-subunits (Kv1 to Kv12) that can assemble as homo- or heterotetramers have been identified to date (Hasan and Jaggar 2018). These channels generate a tetraethylammoniun (TEA)-

sensitive delayed rectifier (Kdr) K^+ current, and a sensitive 4-aminopyridine transient (A-type) K^+ current (Reyes-Haro et al. 2005; Montiel-Herrera and García-Colunga 2010).

Rat cerebral arteries express Kv1.2, Kv7.1, Kv7.4, and Kv9.3 channels, while the literature indicates that VSMCs express diverse Kv channel isoforms that may depend on vessels' anatomical localization and needs (Hasan and Jaggar 2018). In mesenteric arteries, Kv1.5 channel activation can lead to vasodilation, but these channels are trafficked by several extra- and intracellular stimuli (e.g., ROS, oxyhemoglobin, serotonin, angiotensin II and hyperglycemia). For example, the activation of angiotensin II receptors promotes Kv1.5 channel trafficking and degradation through G_{q-11} protein-coupled PKC pathways in mesenteric arteries, leading to a decrease in Kv1.5 current density to vasoconstriction. Hence, in brain vascular cells, Kv1.5 channels may also contribute to modulating the vascular tone (Hasan and Jaggar 2018). It has been recently described that diabetes suppresses Kv2.1 channels in arterial VSMCs, through biochemical mechanisms involving transcriptional regulation through AKAP150-Ca^{2+}/calmodulin-dependent phosphatase-transcription factor NFATc3 (Hasan and Jaggar 2018).

The delayed rectifier Kv7 channel family comprises five members, Kv7.1–Kv7.5, encoded by the KCNQ genes (KCNQ1-5), of which neurons express KCNQ2-5 (Gollasch et al. 2018). Kv7.2/Kv7.3 heterotetramers are the channels responsible for “M-currents” in neurons, that is, outwardly rectifying Kv currents that are suppressed by the activation of muscarinic receptors by acetylcholine (Byron and Brueggemann 2018; Gollasch et al. 2018). Vasopressin (100 pM) partially suppresses Kv7.4 and Kv7.4/7.5 currents, suggesting that these currents contribute to signal transduction pathways used by systemic artery cells to modulate vascular contractility.

Many vasoconstrictor molecules affect VSMCs through binding to G_{q-11} protein-coupled receptors, which are linked to PKC activation and its second messenger-related pathways. For instance, endothelin-1 and serotonin via G_{q-11}-coupled receptors and PKC activation suppress Kv7 currents on VSMCs (Byron and Brueggemann, 2018). The pharmacology and activation/inactivation of Kv7 channels are well-reviewed in Gollasch et al. (2018).

The NVU is responsible for ensuring blood flow to the brain. The information available to date indicates that the ion channel network (e.g., voltage-dependent ion channels and agonist-ionotropic and metabotropic receptors) contributes to the signaling translation between membrane potential of NVU cells and their biochemical intracellular Ca^{2+} pathways, to regulate and finely tune diameters of arterioles and capillaries in the healthy brain. From a hierarchical point of view, a vessel response (e.g., dilation or constriction) primarily obeys oxygen concentration and, by extension, the metabolic rate of the brain, in order to satisfy its energy demands, although the physiology of the NVU under pathological conditions is poorly understood. Experimental evidence on models of chronic hyperglycemia, vascular dystrophy, and ischemia (among other diseases) have been used in order to understand plastic changes displayed by ion channels and agonist receptors expressed by NVU cells and their vasoactive signaling. This knowledge could soon be used to target vessel-related therapies. Finally, an NVU-transcriptome and splicing database of glial cells, neurons, and vascular cells (Zhang et al. 2014; Mishra 2017) would be of great help to understand the role of ion channels in regulating brain blood flow during health and disease. The generation of this novel information will help to clarify and understand regulation of ion channels that occurs under different stress condition.

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