



# Two-pore Domain Potassium Channels in Astrocytes

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Two-pore domain potassium ( $K_{2p}$ ) channels have a distinct structure and channel properties, and are involved in a background  $K^+$  current. The 15 members of the  $K_{2p}$  channels are identified and classified into six subfamilies on the basis of their sequence similarities. The activity of the channels is dynamically regulated by various physical, chemical, and biological effectors. The channels are expressed in a wide variety of tissues in mammals in an isoform specific manner, and play various roles in many physiological and pathophysiological conditions. To function as channels, the  $K_{2p}$  channels form dimers, and some isoforms form heterodimers that provide diversity in channel properties. In the brain, TWIK1, TREK1, TREK2, TRAAK, TASK1, and TASK3 are predominantly expressed in various regions, including the cerebral cortex, dentate gyrus, CA1-CA3, and granular layer of the cerebellum. TWIK1, TREK1, and TASK1 are highly expressed in astrocytes, where they play specific cellular roles. Astrocytes keep leak  $K^+$  conductance, called the passive conductance, which mainly involves TWIK1-TREK1 heterodimeric channel. TWIK1 and TREK1 also mediate glutamate release from astrocytes in an exocytosis-independent manner. The expression of TREK1 and TREK2 in astrocytes increases under ischemic conditions, that enhance neuroprotection from ischemia. Accumulated evidence has indicated that astrocytes, together with neurons, are involved in brain function, with the  $K_{2p}$  channels playing critical role in these astrocytes.

**Key words:**  $K_{2p}$  channel, astrocyte, passive conductance, glutamate release

## INTRODUCTION

Potassium ( $K^+$ ) channels are membrane proteins that specifically transport  $K^+$  across the membrane and play important roles in cell volume regulation, hormone secretion, heart-beat, synaptic transmission and muscle contraction. The  $K^+$  channels are a superfamily of diverse members involved in  $K^+$  currents in various types of tissues. On the basis of structure, the  $K^+$  channels are categorized into voltage-gated  $K^+$  channel ( $K_v$ ), inward rectifying

$K^+$  channel ( $K_{ir}$ ) and two-pore domain  $K^+$  channel ( $K_{2p}$ ) families [1-3].

The  $K_{2p}$  channels were cloned by data mining from a genetic database, with 15 members currently identified. They induce leak background currents in heterologous systems as well as in endogenous tissues [4-17]. Loss-of-function studies using knockout animals have reported that the channels are expressed in various tissues and are involved in urine production, anesthesia, pain perception, depression, and neuroprotection [18]. Most studies on the  $K_{2p}$  channels performed in nervous system have focused on neurons in the central and peripheral nervous systems, which have been thoroughly discussed in excellent reviews [19-22]. Although astrocytes are the most dominant types of cells in the brain and some  $K_{2p}$  channels are highly expressed in these types of cells, their role in astrocytes have not been well studied.

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Accumulated evidence has recently suggested that astrocytic K<sub>2p</sub> channels play critical roles in brain function. The purpose of this review is therefore to provide an overview of the function of astrocytic K<sub>2p</sub> channels, and to discuss the direction of further studies of these channels in astrocytes.

## THE K<sub>2p</sub> CHANNEL FAMILY

Background K<sup>+</sup> currents have been known since the early period of electrophysiology, but it took more than 50 years to identify the molecular components. Tandem of pore domains in a weak inward rectifying K<sup>+</sup> channel 1 (TWIK1) was identified as an unconventional K<sup>+</sup> channel, which showed very different electrophysiological properties from classical K<sup>+</sup> channels such as K<sub>v</sub> and K<sub>ir</sub> channels. This channel uniquely contains two pore (P) domains, whereas the K<sub>v</sub> and K<sub>ir</sub> channels contain only one P domain [23]. Since TWIK1 was cloned, 14 additional K<sub>2p</sub> channels, sharing a highly conserved molecular architecture, have been identified in mammals. Based on the sequence similarity and functional characteristics, the 15 K<sub>2p</sub> channels are categorized into six subfamilies, including TWIK, TREK (TWIK-related K<sup>+</sup> channel), TASK (TWIK-related acid-sensitive K<sup>+</sup> channel), TALK (TWIK-related alkaline pH-activated K<sup>+</sup> channel), THIK (tandem pore domain halothane-inhibited K<sup>+</sup> channel) and TRESK (TWIK-related spinal cord K<sup>+</sup> channel) families [19, 24, 25].

The K<sub>2p</sub> channels are not activated by a voltage change that induces pore openings of K<sub>v</sub> channels, but mediate an instantaneous current at a wide range of membrane potentials [7, 8, 23, 26]. They show a current-voltage (I-V) relationship similar to the one following the Goldman-Hodgkin-Katz (GHK) equation. They are also time-independent and do not show inactivation kinetics in their current, suggesting that the K<sub>2p</sub> channels have a distinct mechanism of pore gating [7]. However, the K<sub>2p</sub> channels have more or less tendency of rectifying currents. For example, TREK1 and TREK2 carry outward rectifying currents and TWIK1 and TASK3 carry slightly inward currents in heterologous systems that are induced by a specific range of membrane potentials, extracellular K<sup>+</sup> concentrations, or divalent cations, such as magnesium (Mg<sup>2+</sup>) [5, 8, 10, 13, 15, 23]. In addition, a recent study suggested that K<sub>2p</sub> channels have a non-canonical voltage sensing mechanism to gate their pores and show voltage-dependent manners, although this possible process needs to be confirmed in physiological conditions [27]. Each K<sub>2p</sub> channel does not completely follow the electrophysiological properties of an ideal background K<sup>+</sup> channel. However, the 15 isoforms have different voltage dependencies at different ranges of conditions and different expression patterns. It has been suggested that these

characteristics enable the K<sub>2p</sub> channels to function as background channels in excitable and non-excitable cells.

Although the members of the K<sub>2p</sub> family have relatively low sequence homology, they share a very similar molecular architecture. They have 4 transmembrane (TM) domains, 2 P domains, a long extracellular loop between TM1 and P1, and the N- and the C-termini that are oriented into the intracellular space. The K<sub>2p</sub> channels form dimers to make functional K<sup>+</sup> pore, which is composed of pseudo-tetrameric pore units and selectivity filter sequences in each P domain to provide the specificity for K<sup>+</sup>. The 4TM/2P structure of the K<sub>2p</sub> channel is well conserved from *Caenorhabditis elegans* and *Drosophila melanogaster* to mammals [28-30]. In the dimeric structure of TWIK1, the two extracellular loops in each subunit are located close to each other, and two cysteine residues in the extracellular loops form a disulfide bond [12]. In addition, TREK2 and TRAAK have cysteine residues in their external loops, which are involved in intersubunit disulfide bridges in a similar manner to TWIK1 [31-33]. In general, K<sub>2p</sub> channels form homodimers of two of the same isoforms, and the characteristics of the channels are determined by the single isoforms. Recently some K<sub>2p</sub> isoforms were discovered that form heterodimers between two different isoforms, and the heterodimers showed different channel properties from those of the homodimeric channels of each single isoform [34-41]. The heterodimerization of the channels therefore provides diversity in channel properties.

## THE REGULATION OF THE K<sub>2p</sub> CHANNELS

The activity of K<sub>2p</sub> channels are regulated by various modulators, such as physical parameters, chemical signaling and biological signaling. TREK1, TREK2, and TRAAK are mechanosensitive channels, whose activities are enhanced if negative pressure is applied through a patch pipette during an electrophysiological recording [13, 42, 43]. TASK1 and TASK3 are inhibited by extracellular acidification but are insensitive to intracellular pH changes [7, 10]. TREK1 and TRAAK are inhibited by extracellular acidification, while internal acidification activates TREK1 and TREK2 [44-46]. At alkaline pH, TALK1 and TALK2 are inhibited and TRAAK is activated by internal alkalization [47, 48]. TREK1 activity is enhanced by heat between 22~42°C, which is a gradual and reversible process [49]. TREK2 and TRAAK are also controlled by temperature changes [50].

The K<sub>2p</sub> channels are also differentially modulated by lipid molecules. TREK1, TREK2, and TRAAK are activated by arachidonic acid (AA) and other polyunsaturated fatty acids, such as docosahexaenoic acid and linoleic acid [5, 13, 26, 43, 51]. TREK1

and TRAAK are also activated by lysophospholipids, including lysophosphatidylcholine and lysophosphatidylinositol [51, 52]. In contrast, TRESK is inhibited by AA and docosahexaenoic acid [17]. The  $K^+$  currents mediated by TREK1 and TREK2 are dramatically increased by volatile anesthetics, including chloroform, halothane, isoflurane, and diethyl ether but TRAAK is insensitive to these anesthetics [13, 53]. TASK1 activity is enhanced by halothane and isoflurane but is insensitive or decreased by chloroform and diethyl ether, respectively [53, 54].

The  $K_{2p}$  channels are regulated by various types of G-protein coupled receptors (GPCRs). TREK1 activity is inhibited by the activation of  $G\alpha_q$ -coupled receptors, such as the thyrotropin-releasing hormone receptor 1 (TRHR1) and the Orexin receptor (Orx1R), but is enhanced by the  $G\alpha_i$ -coupled receptors, including the metabotropic glutamate receptor 4 (mGluR4). A series of studies using pharmacological inhibitors and the mutants of TREK1 reported that the phosphorylation status of two serine residues in the C-terminal domain (S300 and S333) is critical in TREK1 regulation by GPCRs, and is dependent on protein kinase C (PKC) and protein kinase A (PKA) [55-57]. Recently, TREK1-mediated fast glutamate release from astrocytes has been reported to be triggered by TREK1 interaction with  $G\beta\gamma$  subunits [38, 58]. The TREK2 is also inhibited by the activation of  $G\alpha_s$ - and  $G\alpha_q$ -coupled receptors, such as 5HT4sR, mGluR1, and the M3 muscarinic receptor [13, 59]. The  $G\alpha_i$ -coupled receptors, such as the mGluR2,  $\alpha_{2A}$  adrenergic receptor and the GABA<sub>B</sub> receptor increase the current mediated by the TREK2 channel [13, 60, 61]. TASK1 and TASK3 are inhibited by activation of  $G\alpha_q$ -coupled receptors, such as group I mGluRs, TRHR1, M3 muscarinic receptor, and angiotensin II (AT<sub>1a</sub>) receptors [37, 62-64].

The binding partners of the  $K_{2p}$  channels have been identified using yeast two-hybrid screening and immunoprecipitation combined with mass spectrophotometry.  $\beta$ -COP, 14-3-3 $\beta$ , and p11 are known to interact with TASK1 and regulate the trafficking of the channel. The  $\beta$ -COP, a subunit Coat Protein Complex I (COPI) keeps TASK1 in the endoplasmic reticulum (ER) by interaction with both the N- and C-termini of the channel, but the 14-3-3 $\beta$  induces forward trafficking of the channel to the cytoplasmic membrane [65, 66]. The p11, a subunit of annexin II, which plays an important role in aldosterone secretion, allows TASK1 to release from ER retention by direct interaction with the channel [67]. A-kinase-anchoring protein 150 (AKAP150) and Mtap2, a microtubule-associated protein, were identified as the interacting proteins that bind to the C-terminal domain of TREK1 and TREK2. The AKAP150 increases the channel currents mediated by TREK1 and TREK2 and modulates the sensitivity of the channels to physical parameters and signaling mediated by

$G\alpha_s$ -/ $G\alpha_q$ -coupled receptors. The Mtap2 enhances localizations to the cytoplasmic membrane and the current densities of TREK1 and TREK2 [68, 69]. The  $\beta$ -COP was identified as the interacting protein of TREK1, and increases both the surface expression and the current by binding to the N-terminal domain of the channel [70]. Intracellular  $Ca^{2+}$ -induced activation of the TRESK channel is regulated by interaction with calcineurin and 14-3-3 $\eta$  [71, 72].

## THE EXPRESSION OF $K_{2p}$ CHANNELS IN THE BRAIN

The  $K_{2p}$  channels have been reported to be expressed in many organs including the heart, kidney, lung, liver, pancreas, placenta, testis, spinal cord, and brain [5-8, 13, 23, 26, 73]. In the brain, TWIK1, TREK1, TREK2, TRAAK, TASK1, and TASK3 are preferentially expressed in specific regions in an isoform-dependent manner [22]. In situ hybridization showed that TWIK1 was expressed in the cerebral cortex, the dentate gyrus of the hippocampus, and the granular layer of the cerebellum [74, 75]. TREK1 was detected in almost all areas of the rat brain including the cerebral cortex, basal ganglia, hippocampus, hypothalamus, mesencephalon and rhombencephalon [76]. TREK2 expression is high in the granule cell layer of the cerebellum [5, 75]. TASK1 also shows a heterogeneous distribution in the brain, and its mRNA levels are high in the cerebral cortex, CA1-CA3, dentate gyrus, paraventricular thalamic nuclei, substantia nigra, and cerebellum [7, 63, 75]. In nervous systems, the channels are expressed in many kinds of neurons, including projection neurons, interneurons, motor neurons, and sensory neurons [75-78].

When compared with the number of neurons in the brain, the glia comprise a much larger percentage of the total number of cells in the brain, and astrocytes are one of the dominant cell types of glia [79, 80]. Recently, many studies have reported that  $K_{2p}$  channels are also expressed in various types of astrocytes. TASK1 was detected in the astrocytes of the rat hippocampus by immunohistochemistry [81, 82]. A transcriptome analysis of astrocytes purified from mouse forebrains and a single-cell RT-PCR study with hippocampal astrocytes suggested that TWIK1 and TREK1 were expressed in astrocytes [83, 84].

## THE FUNCTIONS OF THE $K_{2p}$ CHANNELS IN ASTROCYTES

### *Passive conductance*

The electrophysiological properties of astrocytes change during the maturation processes. Mature astrocytes typically show a very negative membrane potential ( $V_M$ ) and a linear I-V relationship. These features are thought to enable astrocytes homeostatic functions in the brain, such as extracellular  $K^+$  buffering and the

uptake of released neurotransmitters from presynaptic neurons [85-88]. This linear I-V relationship, called passive conductance, results from leak K<sup>+</sup> membrane conductance and is not affected by time and voltage. The molecular identity of the passive conductance is not associated with K<sub>v</sub> channels, because is very similar to the linear I-V relationship described by the GHK equation [88-91].

Recent studies have suggested that TWIK1 and TREK1 predominantly mediate the astrocytic passive conductance in the hippocampus [38, 92]. The passive conductance is significantly reduced by quinine that inhibits both TWIK1 and TREK1 channels, while not being affected by Ba<sup>2+</sup>, a blocker of classical K<sup>+</sup> channels [92]. The gene silencing of one or both of TWIK1 and TREK1 by knockdown using shRNA markedly reduced the passive conductance [38]. In addition, TWIK1 and TREK1 form a heterodimeric channel involving an intermolecular disulfide bridge between two cysteine residues in each extracellular loop. The two K<sub>2p</sub> channels mutually regulate the surface expression of each other by heterodimerization in hippocampal astrocytes [32, 33, 38]. The inhibition of channel activity by inhibitors or gene silencing reduces most but not all of the passive conductance. TWIK1 and TREK1 are major isoforms of the K<sub>2p</sub> channels in hippocampal astrocytes and are responsible for at least 80 % of the entire passive conductance (Fig. 1, blue box) [38, 92]. The expression profiles of the K<sub>2p</sub> channels are dependent on the region of the brain, so the passive conductance may be controlled by different K<sub>2p</sub> channels in different regions, suggesting that other K<sup>+</sup> channels expressed in astrocytes, such as TREK2 or pH-sensitive K<sub>2p</sub> channels such as TASKs may partially contribute to the passive conductance [84, 93, 94].

### Glutamate release

In the brain, glutamate, one of the most important neurotransmitters, is released from excitatory presynaptic neurons and mediates synaptic transmission among neurons. Glutamate is also released from astrocytes and functions as a gliotransmitter, that modulates synaptic transmission and finely tunes synaptic plasticity [95]. There is controversy concerning the release mechanism of glutamate from astrocytes, while the release mechanism from neurons is well understood. The controversy regarding glutamate release from astrocytes involves whether it is mediated by vesicular exocytosis or by channels or transporters [96, 97].

The observation that small vesicular organelles containing vesicular glutamate transporters were found in astrocytes and astrocyte-dependent synaptic potentiation was prevented by introduction of the active light-chain of tetanus neurotoxin (TeNT) into astrocytes suggested that astrocytic glutamate release is

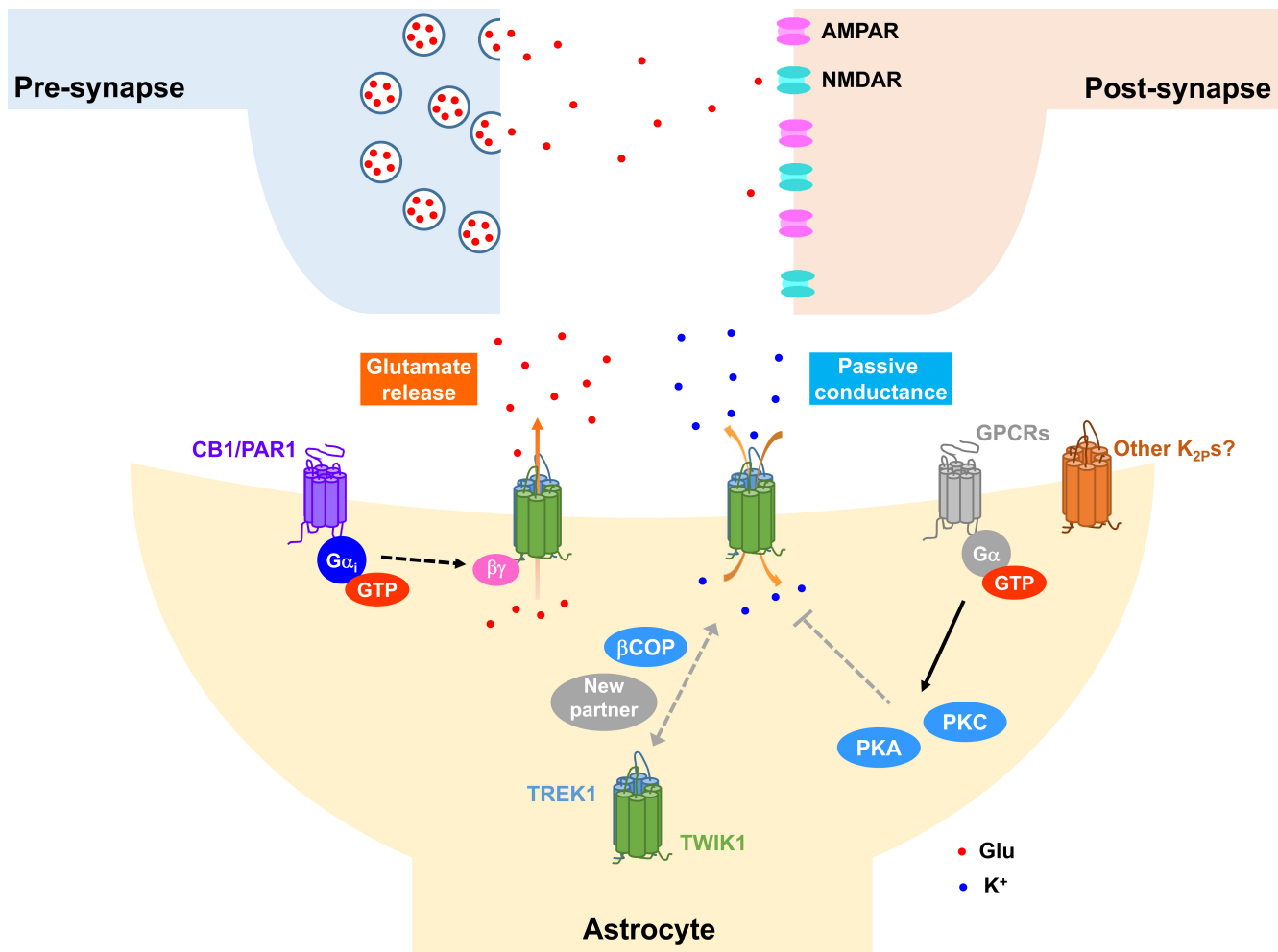
mediated by vesicular exocytosis [98, 99]. However, these studies did not directly show that the glutamate release from astrocytes by astrocytic stimulation. In addition, another study reported that ATP- or hypotonicity-induced astrocytic glutamate release was not affected by TeNT [100]. Studies using pharmacological inhibitors reported that some anionic channels were involved in glutamate release from astrocytes without exocytotic machinery. Anionic channel blockers, such as 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid, 5-nitro-2-(3-phenylpropylamino)-benzoic acid, flufenamic acid, and gossypol drastically inhibited astrocytic glutamate release [100, 101].

A recent study using the sniffer-patch technique, a highly sensitive detection system for glutamate release from astrocytes revealed that TREK1 and Best1, an anion channel, mediated fast and slow glutamate release, respectively [58]. These two types of astrocytic glutamate release were independent of the exocytotic machinery, and the fast release mediated by TREK1 did not require an increase in intracellular Ca<sup>2+</sup>. Furthermore, to facilitate glutamate permeability, TREK1 needed to form a heterodimer with TWIK1, and gene silencing of only one of the two K<sub>2p</sub> isoforms inhibited the glutamate release from astrocytes [38]. Because the astrocytic glutamate release is triggered by the activation of GPCRs, such as PAR1 and CB1, the synaptic transmission can be fine-tuned by input from various synaptic environments (Fig. 1, orange box) [38, 58].

### The protection from ischemia

In the brain, ischemia elicits hypoxia and hypoglycemia that induce neuronal excitotoxicity due to glutamate and cause can severe brain damage resulting in disability and death [102]. Astrocytes play pivotal roles in the protection of neurons from toxicity induced by stress, while also supporting neuronal survival and general physiological functions. Astrocytes retain much hyperpolarized membrane potential and leak K<sup>+</sup> conductance in the resting condition, which provides a major driving force for glutamate uptake and K<sup>+</sup> spatial buffering [103, 104]. Many studies have suggested that K<sub>2p</sub> channels contribute to the ability of astrocytes to protect neurons against ischemia.

TREK1<sup>-/-</sup> mice were more vulnerable to global ischemia following transient bilateral occlusion of common carotid arteries. Furthermore, TREK1<sup>+/+</sup> mice showed an improved survival against ischemia after injection of linoleic acid or lysophosphatidylcholine, that are activators of TREK1, but the survival rate of TREK1<sup>-/-</sup> mice was not affected [105]. TREK1 expression was drastically and gradually increased after cerebral ischemia induced by middle cerebral artery occlusion in a time dependent manner in hippocampal CA1 and cerebral cortex [106, 107]. The



**Fig. 1.** The  $K_{2p}$  channels in astrocytes. The scheme summarizes the known functions and regulatory mechanisms of  $K_{2p}$  channels in astrocytes, and suggests the strategies for future studies. In astrocytes, TWIK1 (green) and TREK1 (blue) are highly expressed and form heterodimeric channels. The TWIK1-TREK1 channel mainly contributes to passive conductance that is a leak  $K^+$  background current in astrocytes (blue box). The heterodimeric channel also mediates glutamate release from astrocytes that does not require exocytotic machinery proteins, such as SNARE components. The release process is triggered by a direct interaction between a TREK1 subunit and the  $G\beta\gamma$  subunits after the activation of  $G\alpha_i$ -protein coupled receptors (orange box).  $\beta$ COP, PKA, and PKC are already known to control TREK1 by protein-protein interaction or by phosphorylation after GPCR activation in non-astrocytic cells. However, the effector proteins are also expressed in astrocytes, so they may regulate the TWIK1-TREK1 channel by similar mechanisms (gray objects and dashed lines). In addition, other novel interacting partners of the  $K_{2p}$  channels that can be novel regulators are expected to be identified in astrocytes in future studies. As suggested by some studies, there is the possibility that other  $K_{2p}$  channels, such as TASK1 may be expressed and could contribute to the function of astrocytes ( $K_{2p}$  channel in orange).

proliferation of cultured cortical astrocytes was increased by exposure to hypoxic condition, but was suppressed by treatment with quinine [107]. The clearance ability of extracellular glutamate by astrocytes was inhibited by blocking of TREK1 with quinine or bupivacaine under hypoxic conditions [108]. In addition, linoleic acid reduced apoptotic cell death, aquaporin 4 expression, and microglial activation induced by cerebral ischemia [106]. TREK2 expression were also increased by hypoxic exposure in cultured cortical astrocytes and hippocampal astrocytes *in vivo*, and the increase was mediated by *de novo* translation of TREK2 protein

[109, 110]. The increase of TREK channels by hypoxic conditions may therefore be a defense mechanism against glutamate toxicity induced by hypoxia.

#### FUTURE $K_{2p}$ CHANNELS STUDIES IN ASTROCYTES

The  $K_{2p}$  channels are expressed in both of neurons and astrocytes. In general, excitable neurons and non-excitable astrocytes have different cellular contents and physiology, so it is expected that the function and regulation of  $K_{2p}$  channels in astrocytes are different

from those in neurons. However, most studies on K<sub>2p</sub> channels in the brain have been focused on neuron, and we do not well understand how the channels function in astrocytes. Our current knowledge provides some clues regarding future studies of K<sub>2p</sub> channels in astrocytes (Fig. 1, grey objects and dashed lines).

A series of knockout animals with deleted genes encoding the K<sub>2p</sub> channels were developed and used to investigate the loss-of-function of the channels. These studies made significant contributions to the current knowledge of K<sub>2p</sub> channels [18]. However, the whole body knockout animals did not simultaneously express the target channels in neurons and astrocytes, so it is not clear whether the effect of gene silencing originated from neurons or astrocytes. To definitively establish astrocyte-specific loss-of-function, the gene-silencing should be performed using astrocyte-specific promoters, such as glial fibrillary acidic protein (GFAP), glutamate-aspartate transporter (GLAST), or glutamate transporter-1 (GLT-1) promoters [111, 112]. Conditional knockout mice where the K<sub>2p</sub> channel expression is removed only in astrocytes will provide a better system to assess the astrocyte-specific function of the channels.

The cellular localization and the activity of the K<sub>2p</sub> channels are regulated by various interacting proteins [65-72]. Although no interacting partner of the K<sub>2p</sub> channels in astrocytes has not been identified yet, there is a strong possibility that the channels can be regulated by interacting proteins in astrocytes. Importantly, some of the known proteins interacting with K<sub>2p</sub> channels in other types of cells are also expressed in astrocytes [113-115], and should be the objects of future studies. In addition, new interacting partners expressed in astrocytes should be identified to learn more about the regulatory mechanisms of the channels.

The K<sub>2p</sub> channel activity is dynamically and diversely regulated by signals via GPCRs [55-57, 59-64]. In particular, PAR1 and CB1 are already known to induce glutamate release from astrocytes by interaction with TREK1 [38, 58]. In astrocytes, various kinds of GPCRs are expressed and contribute to cell-to-cell communication and functional regulation of the cells [116, 117]. These results suggest that there may be many regulatory mechanisms of K<sub>2p</sub> channels involving GPCRs, which can provide new therapeutic targets for the treatment of neurodegenerative and psychiatric diseases.

## CONCLUSIONS

The K<sub>2p</sub> channels have distinct characteristics as K<sup>+</sup> channels that differ from K<sub>v</sub> or K<sub>ir</sub> channels. The K<sub>2p</sub> channels mediate leak K<sup>+</sup> conductance independently of time or voltage and are not characterized by inactivation kinetics. The K<sub>2p</sub> channels therefore

function as background K<sup>+</sup> channels that are evolutionarily conserved from *C. elegans* to mammals. The 15 members of the mammalian K<sub>2p</sub> family are specifically expressed in a wide variety of tissues in an isoform-dependent manner, and are involved in various physiological and pathophysiological phenomena. In astrocytes, TWIK1, TREK1, TREK2, TASK1, and TASK3 are the dominantly expressed isoforms that contribute to the functions of astrocytes. Furthermore, K<sub>2p</sub> channels play critical roles in the induction of passive conductance, in glutamate release, and in neuroprotection from hypoxia. Recently, the K<sub>2p</sub> channels have been reported to be involved in hippocampal epilepsy and cellular volume regulation [118-122]. These findings suggest that the K<sub>2p</sub> channels can be new therapeutic targets for treatment of neurodegenerative and psychiatric diseases in astrocytes.

Because astrocytes are physically and functionally associated with neurons, and the K<sub>2p</sub> channels are expressed in both types of cells, it is difficult to study the K<sub>2p</sub> channels in astrocytes using conventional strategies. To investigate the function of the K<sub>2p</sub> channels in astrocytes, it is therefore necessary to use astrocyte-specific conditional knockout animals, high-throughput screening to find the interaction proteins with the channels in astrocytes, and small molecular modulators. The new approaches should provide novel strategies to better understand the K<sub>2p</sub> channels in astrocytes.

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