Recent advance and possible future in TREK-2: A two-pore potassium channel may involved in the process of NPP, brain ischemia and memory impairment

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Summary TREK-2, a new member of the mechanosensitive tandem-pore K⁺ channel family, share 65% amino acid sequence identity and some similar basic electrophysiological and pharmacological properties with TREK-1. It also has some specific regulatory pathway and tissue distribution contrasted with TREK-1 and TRAAK. TREK-2 distributes extensively in CNS and periphery tissue. It can be regulated by G-protein-coupled receptor (GPCR) and may involve in several of physiological and pathophysiological conditions. The long-chain unsaturated free fatty acids such as arachidonic acid (AA), PHi, pressure and temperature can increase the activity of TREK-2. The purpose of this review is to present the recent study and possible importance of TREK-2 in neuropathic pain, thereby emphasizing TREK-2 as one of the important mechanisms underlying. This information should be very useful and prospective for effective chronic pain therapy and future analgesic drug development. This review also further predicts the role of TREK-2 in brain ischemia, memory and other tissue. The specific location and function of TREK-2 in these tissues need further study.

Background

To date, more than 70 K⁺ channel genes have been identified in mammals and are divided into four subfamilies on the basis of their sequence similarities and channel properties. Four families of potassium channels are distinguished in neurons: voltage-gated (Kv), calcium-activated (Kca), inward rectifier (Kir) and two-pore (K2P) K⁺ channels [1]. The two-potassium channels (K2P) is a collection of ion channels whose importance in physiologic mechanisms is still emerging [2,3]. They appear to be an ancient class of channels; K2P channels are found widely, from single-cell yeast to plants to higher mammals [4–6]. More than 50 genes with the predicted K2P channel structure have been identified in Caenorhabditis elegans, as well as 15 human genes [7,8], Fig. 1 shows a dendrogram depicting the evolutionary relationships within the human K2P channel family. The salient feature of K2P membrane topology is that K2P
channels have four transmembrane domains (TM1-4), two pore-forming domains (P1.P2) [4,9,10], while other K⁺ channels are characterized by having two, six or eight transmembrane segments and share one conserved pore-forming domains [11]. The K2P channels can produce leak current that contribute to set and stabilize the resting potential and thus influences the cell excitability in physiological condition, independent of the time and voltage, which is distinguished from other K⁺ channels. The K2P channels are insensitive to classic K⁺ channel blocker such as tetraethylammoni um, apamin, 4-aminopyridine and glybenclamide. In the 2P/4TM K⁺ channels family, there are six subfamilies according to the difference of channel structure and regulation: TWIK, THIK, TASK, TALK, TREK, TRESK. In this K2P channel, the most deeply studied was the family of TREK, including TREK-1, TREK-2 and TRAAK. The recent research showed that TREK-2 is involved in amount of pathologic process such as acute cerebral ischemia [41]. TREK-2 is greatly possible to associate with nociception according to its electrophysiological properties and tissue distribution. The purpose of this review is to present the recent study and possible future in TREK-2 in neuropathic pain, thereby emphasizing TREK-2 as one of the important mechanisms underlying neuropathic pain. This information should be very useful and prospective for effective chronic pain therapy and future analgesic drug development. This review also further predicts the role of TREK-2 in brain ischemia, memory and other tissue.

The molecular structure and tissue distribution of TREK-2

TREK-2, as a new member of the mechanosensitive tandem-pore K⁺ channel family, is firstly reported by Bang and Lesage. It is a 538-amino acid protein and share 65% amino acid sequence identity with TREK-1, has a short N terminus, an extended extracellular loop between M1 and P1, and a long C terminus, structural features typical of nearly all 4TM K⁺ channels (Fig. 2) [12]. Genomic organization of TREK-2 is very close to the genomic organization of both TRAAK and TREK-1 channel genomic organizations [13]. The literature reported that TREK-1 and TRAAK mRNA expression was predominantly CNS specific in contrast to the closely related TREK-2, which was expressed in both CNS and peripheral tissues [14]. However, subsequent studies showed that there are even more widespread distribution of TREK-1 and TRAAK than that of TREK-2 in peripheral tissue such as in the rat heart, mesenteric and pulmonary arteries, human myometrium [15–17]. The K2P distribution analysis in CNS proved that the TREK-2 probe detected two transcripts of 4 and 7.5 kb, and expression of TREK-2 was primarily restricted to the cerebellar granule cell layer and TREK-1 was highest in the striatum and in parts of the cortex (layer IV) and hippocampus (CA2 pyramidal neurons) [18]. It is reported that TREK-2 channels play a relative low role in composed of the standing outward K⁺ (IKso) in cerebellar granule neurons that modulates the resting membrane and cell excitability [19]. TREK-2 is also found in the magnocellular neurosecretory cells (MNCs), cultured rat brain astrocytes and insulin-secreting INS6 cells [20–22]. Future in situ hybridization and immunohistochemical localization studies is needed to identify in which

Figure 1 The human two-pore-domain K⁺ (K2P) channels. A phylogenetic tree of the K2P channel family is shown, with the different nomenclatures indicated. Human K2P channels are classified into six structural and functional subgroups. The TREK subgroup (indicated in green) comprises TREK-1 (KCNK2), TREK-2 (KCNK10) and TRAAK (KCNK4), which share structural and many functional properties, including activation by membrane stretch and lipids such as arachidonic acid and lysophosphatidylcholine. The TASK subgroup (indicated in pink) comprises channels that are inhibited by extracellular acidosis, whereas the TALK channels (indicated in blue) are activated at alkaline extracellular pH. TREK-1, TREK-2, TASK-1, TASK-2 and TASK-3 are activated (indicated in green on the tree), whereas THIK-1, TWIK-2, TALK-1, and TALK-2 are inhibited (indicated in red on the tree), by volatile general anaesthetics. Several human K2P channels have little or no functional expression (indicated in light grey on the tree). TRAAK is not modulated by volatile general anaesthetics and the sensitivity of TRESK has not yet been reported (indicated in dark grey). Cited from Nicholas et al. [46] (For interpretation of the references in color in this figure legend the reader is referred to the web version of this article).
cells and at what levels TREK-2 is expressed in the CNS and peripheral tissues.

Electrophysiological and pharmacological properties of TREK-2

Previous studies have shown that TREK-2 passes an instantaneous and non-inactivating current in the nA range and is an inwardly rectifying K⁺ channel in symmetrical 150 mM K⁺ [23]. TREK-2 is insensitive to classic potassium blocker such as tetraethylammonium, quinidine when applied extracellularly, stimulated markedly by acidic pH and inhibited mildly by alkaline pH. TREK-2 possesses potential phosphorylation sites for both protein kinase A and C. The effects of activators of protein kinase A and C on TREK-2 is that phosphorylation by protein kinase A caused a significant reduction of TREK-2 current, whereas phosphorylation by protein kinase C had no effect [12]. TREK-2 like TREK-1 and TRAAK, is a mechanosensitive K⁺ channel, and increased channel activity rapidly when applied negative pressure (0 to −80 mmHg) [23]. TREK-2 is activated by long-chain unsaturated free fatty acids such as arachidonic acid (AA), linoleic acid and oleic acid and insensitive to saturated free fatty acids such as stearic acid and palmitic acid even up to high concentration [12,23].

TREK-2 is reversibly activated by intracellular LPA at atmospheric pressure, and thus channel mechanosensitivity is drastically and reversibly altered. A possible mechanism may involve in a membrane effect of LPA [24,25]. Zn²⁺ can enhance the TREK-2 current in a dose-dependent manner [26]. Otherwise, fenamates can markedly stimulate the TREK-1, TREK-2 and TRAAK and diltiazem (1 mM) inhibited TREK-1 and TREK-2, but not TRAAK. So diltiazem may be a specific blocker for TREK-2.

These novel findings could help to further understand channel functions of the mechanogated 2P domain K⁺ channels [27]. TREK-2 is temperature-sensitive and the thresholds for activation were approximately 25 °C. In cerebellar granule and dorsal root ganglion neurones, TREK-1, TREK-2 and TRAAK were generally inactive in the cell-attached state at 24 °C, but became very active at 37 °C. These results show that TREK-2 in temperature-sensitive channels, is active at physiological body temperature, and therefore would contribute to the background K⁺ conductance and regulate cell excitability in response to various physical and chemical stimuli [28]. Application of H₂O₂ in CHO cells specifically increased TREK-2 currents recorded using a nystatin perforated whole cell technique and that MLCK activation is involved in this process [29].

Predicted physiological role of TREK-2

TREK-2 and neuropathic pain

The TREK-2 is inhibited by PKA phosphorylation because increasing the intracellular cAMP level led to inhibition of TREK-2 activity at 0 mV [Fig. 3]. The level of TREK-2 activity can be regulated by three different types of G-protein-coupled receptors. The application of Gs-, Gq-coupled receptor, is associated with a decrease of TREK-2 activity, which is associated with activation of phospholipase C. Conversely, activation of the Gi-coupled
leads to a stimulation of TREK-2 activity [Fig. 4] [13]. Activator of Gq-coupled M3 muscarinic receptor, acetylcholine (ACh), induces the inhibition of TREK-2 and occurs primarily via PKC-mediated phosphorylation [30]. TREK-2 regulated by GPCR pathways will probably indicate that TREK-2 activity in neurons is probably fine-tuned by a variety of neurotransmitters. TREK-2 will probably turn out to be an important channel in charge of tuning neuronal excitability in response to a variety of neurotransmitters and hormones and is therefore a natural target of mediators of pain that exert their action via these pathways. Also, TREK-1, which is most homology with TREK-2, involved in polymodal pain perception, is highly expressed in small sensory neurons and extensively colocalized with TRPV1, the capsaicin-activated nonselective ion channel [31]. Recent studies have shown that mRNA transcripts of four major types of K+ channels are identified in dorsal root ganglion [13]; these four K+ channels are TRESK, TREK-1, REK-2, and TRAAK. TREK-2 provide the major background K+ conductance in cell body of small (diameter 10–16)- to medium (diameter 17–25)-sized DRG neurons at 37°C [13,32], is expected to play an active role in the modulation of excitability of DRG neurons and particularly in the regulation of the resting membrane potential if it is active at rest in vivo [33]. After the neuron is injured or constricted, important changes occur in DRG axotomized neurons so that some of quiescent neurons begin to show ongoing discharges that last many days or weeks. This ectopic discharge has great relation with the hyperexcitability of DRG neurons after axotomy. Then, ectopic discharge go into the spinal posterior horn and even transmit to higher central nerve system, which lead to central sensitization to pain. Intense noxious stimuli to nerve and tissue can trigger increased excitability of nociceptive neurons in the spinal cord and cause the release of unsaturated free fatty acid, the change of PH and temperature, and edema leading to the negative mechanical stretch in local position, which can activate the TREK-2 channel [34–36].
Evidence gathered from the electrophysiological properties of TREK-2 and pathological change of neuropathic pain suggest that TREK-2 be activated in the process of neuropathic pain by decreasing the neuronal excitability especially the DRG neurons. It is a paradox contrast with the common idea to the role of potassium channel in NPP. Previous literature suggests that potassium channels play an important role in influencing ectopic discharge of DRG neurons. The hyperexcitability of DRG neurons in chronic pain modal always accompany with reduction of potassium currents. So the exact change of TREK-2 might be upregulated or down-regulated. It is not sure that the TREK-2 change plays a blocked or promoted role in the process of neuropathic pain. To confirm the speculation, the reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis are needed to analyze the mRNA and protein expression of TREK-2 in some neuropathic pain model such as CCI model by Bennett and Xie [37]. The immunohistochemistry and situ hybridization are performed to observe the number of positive neurons and the level of phosphorylation of TREK-2. Also, the TREK-2 channel character in DRG neurons of neuropathic pain model must be studied by whole-cell recording or single-channel recording including the current–voltage curve (I–V curve), unitary conductance, and the ion channel kinetics compared to the normal DRG neurons. Of course, the higher central neuron system needs to be researched such as spinal cord, hippocampus and cerebral cortex. It is prospective to analyze the function of TREK-2 in DRG neurons using the CCI model of TREK-2-/- mice and various techniques mentioned above. At present, pharmacological tool such as the selective agonist for TREK-2 is not yet available and genes of TREKs are highly homologous, making it difficult to test this hypothesis in future experiments. Anyway, the increase of this inwardly rectifying potassium current contributes to the alleviation of abnormal pain sensation in neuropathic pain such as hyperalgia and allodynia. It is possible that the specific activator of TREK-2 becomes an effective drug on chronic pain if the above speculation is sure. To do this, the activator of TREK-2 can be applied to the injury site or directly to the DRG [38], then the ectopic discharge and behavioral signs of mechanical and heat allodynia are evaluated.

**The possible signal transduction pathway and role in some drugs**

To elucidate the details of role of TREK-2, delineation of the precise signal pathway needs to be established. ERK integrate the signal of PKA and PKC in the superficial of spinal cord and play a substantial role in enhancing the excitability of DRG neuron after CCI [39,40]. However, further details about whether ERK is involved in the cell signal pathway of TREK-2 as a downstream of the PKA and PKC need to be examined in future studies. The inhibition of PKA or/and PKC, ERK may relieve the neuropathic pain through regulatory current of TREK-2. Others, it would be prospective to interfere with the process of NPP by selectively manipulating the TREK-2 gene.

On the other hand, numerous studies demonstrate that the opening of several potassium channels participate in the antinociception induced by some drugs such as opioid analgesic, nonsteroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants, etc [1]. The possible involvement of TREK-2 opening in the antinociception induced by these drugs remains unexplored to date. Research about that would help better understanding the mechanism of these drugs and why or why not these drugs are effective to the NPP.

**TREK-2 and neuroprotection**

In rat ischemia model, TREK-1, TREK-2, and TRAAK showed enhanced expressions both in cortex and hippocampus at mRNA and protein level after the middle cerebral artery occlusion (MCAO) and even higher expression levels of TREK channels 24 h after MCAO surgery in the survival neurons. It is predicted that TREK channels be activated by the pathological changes in the microenvironment, but in a relatively long period, their genes are activated and more functional proteins are expressed. Thus the TREK channels probably provide a protective function during brain ischemia by the increase of the channels activation, which can cause the efflux of K+ from cells, membrane hyperpolarization and decrease the cell excitability [41]. But in chronic cerebral ischemia model, the gene expressions of TREK-1 and TRAAK were increased markedly at 3 days (97% and 87%, respectively) and 30 days (63% and 47%, respectively) in hippocampus after permanent bilateral carotid artery ligation (BCAL). However, TREK-2 gene expression level had no change [42]. Therefore, the protective function of TREK-1, REEK-2 and TRAAK is different in different ischemia state. TREK-2 can be stimulated by application of the inhalational anesthetic chloroform, halothane, and isoflurane at a clinical dose [13]. At present, solid experimental evidence supports neuroprotection by anesthetic agents that the mechanisms appear to involve in suppression of excitatory neurotransmission, and potentiation of.
inhibitory activity, which may contribute to the reduction of excitotoxic injury and neuronal death either by necrosis or apoptosis. Activation of intracellular signaling cascades that lead to altered expression of protective genes may also be involved [43–45]. Some literature show that the TREKS have an important role in the general anaesthetic properties of volatile agents, such as halothane, and provide an explanation for the neuroprotective properties of polyunsaturated fatty acids [46]. TREK-2 is also activated by application of the neuroprotective drug riluzole [13]. Taken together, TREK-2 is probably an important ion channel to involve in the neuroprotection by tuning the level of resting potential, reducing the brain cell excitability and release of stimulative neurotransmitters. The research in this field contributes to explore new direction to the mechanism and the cure of brain ischemia.

TREK-2 and memory impairments

In memory impairments induced in rats by single icv injection of beta-AP25-35 (2 mmol L⁻¹) 5 microL, the mRNA expression levels of TREK-1, TREK-2 TRAAK were increased significantly in the hippocampus by 40.0%, 27.9% and 18.9%, respectively; while no significant change was observed in the cortex [47]. Further studies are needed to reveal the real role and mechanism in the memory impairments. In magnocellular neurosecretory cells (MNCs) of the rat supraoptic nucleus (MNCs) at rest, TREK-2 can be found and may modulate the excitability of MNCs [20]. TREK-2 may involved in several of periphery function speculated by the extensive distributions as well as in the CNS. TREK-2 could be clearly identified in insulin-secreting MIN6 cells, and contributes to the background K⁺ conductance in MIN6 cells, and may regulate depolarization-induced secretion of insulin [22]. TREK-2 is expressed in kidney, pancreas, testis, colon, small intestine and pulmonary arteries. Very faint signals were obtained in liver, heart, prostate, and thymus [12,13,16]. TREK-2 may modulate the cell excitability in these tissues mentioned above and regulate the secretory function of the cell. TREK-2 also probably involved in the stretch regulation of smooth muscle cells in blood vessel and digestive tract.

To summarize, though some evidences have provided that TREK-2 is identified in a lot of tissues and may involve various physiological and pathophysiological states, further studies in TREK-2 are needed to identify the specific location and elucidate the details of the role in these tissue.

References


