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Thermal Regulation of KCNQ2 Potassium Channels

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Abstract

KCNQ2 (KV7.2) plays a key role in the regulation of neuronal excitability. Although temperature is thought to modulate all ion channels by different magnitudes, temperature dependence of KCNQ channels has not been demonstrated. In the present work, we reported that temperature elevation caused an increased expression of KCNQ2. Notably, the increased protein was retained intracellular. Meanwhile, we found that elevated temperature had a significant effect on the voltage-dependence of KCNQ2 channel activation. These data suggested that temperature can increase the rates of activation of KCNQ2 channel and has a critical role in the trafficking of KCNQ2 subunits to the surface membrane.

Keywords: KCNQ2; ion channels; temperature; regulation.

1. Introduction

Heteromeric KCNQ2 and KCNQ3 channels have been proposed to mediate M-current in neurons [1-2]. Since it is activated at voltages near the threshold for action potential initiation, M-currents are critical determinants of cellular excitability, and contribute functionally to the regulation of neuronal networks, postnatal brain development, and cognitive performance [3-4].

It is widely accepted that temperature critically modulates gating kinetics, expression, and membrane trafficking of ion channels, although the precise effects of temperature vary across channel types [5]. For example, the

voltage-gated sodium channel $Na_V1.2$ exhibits a negative shift in the voltage-dependence of activation (in addition to faster kinetics) in response to elevated temperature [6]. Moreover, a mutation of the inhibitory $GABA_A$ receptor γ2 subunit demonstrates temperature-dependent trafficking deficiencies [7]. However, temperature dependence of KCNQ2 channel has not been investigated. This study examined the impact of temperature elevation on KCNQ2 channel trafficking and function by comparing KCNQ2 channel properties at physiological (37°C) and febrile temperatures (40°C).

2. Material and methods

2.1 DNA Constructs

Full-length cDNAs for human wild-type KCNQ2 gene were cloned by polymerase chain reaction (PCR)-cloned from plasmid pcDNA3.0-KCNQ2, gifts of Dr. Thomas Jentsch, Hamburg University, Hamburg, Germany. The KCNQ2 cDNAs were subcloned into pEGFP-C1 vector (Clontech, Mountain View, CA, USA). All of the inserted sequences were confirmed by DNA sequencing.

2.2 Cell culture and cDNA transfection

HEK 293T cells and mouse neuroblastoma cells (N2a) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated at 37°C in 5% CO_2. At 70-80% confluence, the cells were transfected with the recombinant plasmids using Lipofectamine 2000 (Invitrogen Carlsbad, CA) according to the manufacturer's instructions. Transfected cells were used for experiments 24 or 48 hours after transfections.

2.3 Live cell confocal microscopy and fluorescence quantification

Temperature-controlled confocal microscopy was performed using the CTI Controller 3700 digital with the Tempcontrol 37-2 digital system (Leica Microsystems, Hong Kong, China). The fluorescence intensity values of cells were determined using Leica Confocal Software. Cells were examined with excitation at 488 nm for EGFP and 543 nm for ER. Given that the expression was varied, fluorescence quantification in single cells was averaged. After incubation at 40°C, fluorescence intensities of channels were counted in the same regions using the same image acquisition settings. Co-localization images were analyzed by Image J software (version WCIF, National Institutes of Health), using the plug-in Manders' Coefficient to calculate the Manders' coefficient [8].

2.4 Western Blotting

Forty-eight hours after transfection, HEK 293T cells were transferred to a 40°C incubator for 1 hour. The biotinylation and Western blot analysis were performed as described previously [9] with some modification. The cells were washed 3 times with ice-cold Ca^{2+}/Mg^{2+} PBS and incubated with Sulfo-NHS-biotin (Pierce Biotechnology, Rockford, IL, U.S.A.) for 1 h at 4 °C. Cells were then lysed with RIPA buffer (150 mM NaCl, 20 mM Tris, 1% sodium Deoxycholate, 0.1% SDS, pH 8.0), supplemented with protease Inhibitor mixture (Roche Applied Science, Indianapolis, IN, USA.). One fraction of the extracted supernatant was then incubated with High Capacity Streptavidin Agarose beads (Pierce Biotechnology) for 2 h at 4°C, and the other fraction was prepared as total protein. The total and surface proteins were both separated by electrophoresis on a 8% SDS-PAGE gel and assayed by Western blotting.

2.5 Electrophysiology

The whole-cell configuration of the patch-clamp technique was used to obtain voltage-clamp recordings at 37°C and 40°C. Reported temperature was measured using a TA-29 Thermistor (Thermometrics) placed within 500 microns of recorded cells.

Cells were bathed with mammalian Ringer's solution: 2 mM KCl, 150 mM NaCl, 10 mM HEPES, 2 mM $MgCl_2$, 10 mM d-glucose, pH 7.2 (adjusted with NaOH). The pipette solution contained: 140 mM KCl, 5 mM EGTA, 5 mM NaCl, 1 mM Na_2ATP, and 10 mM HEPES (pH 7.2 adjusted with KOH). Cells were held at −80 mV and depolarized in 10-mV steps. Tail currents were recorded at −30 mV and analyzed to generate conductance–voltage plots. Voltage-

clamp recordings were obtained using a Multiclamp 700A amplifier and digitized using a Digidata 1322A (Molecular Devices) for acquisition to a computer. Voltage clamp protocols were generated and data was acquired using Clampex (PClamp 9.0, Molecular Devices). Data were analyzed off-line using Clampfit (Molecular Devices) and Igor Pro Carbon (Wavemetrics). For analyses of voltage-dependence, tail currents at +30 mV were normalized to the amplitude of the tail current subsequent to a voltage pulse to -120 mV in a temperature of 40°C. Boltzmann fits were generated in Igor Pro using the equation $f(x) = 1/(1 + \exp((x-Vhalf)/k))$.

2.6 Data analysis and statistics

Numerical data were expressed as mean ± SEM. Data were analyzed by SPSS 13.0 software using ANOVA or Student's unpaired *t* test to compare the differences between the groups, and the statistical significance was identified by $p < 0.05$.

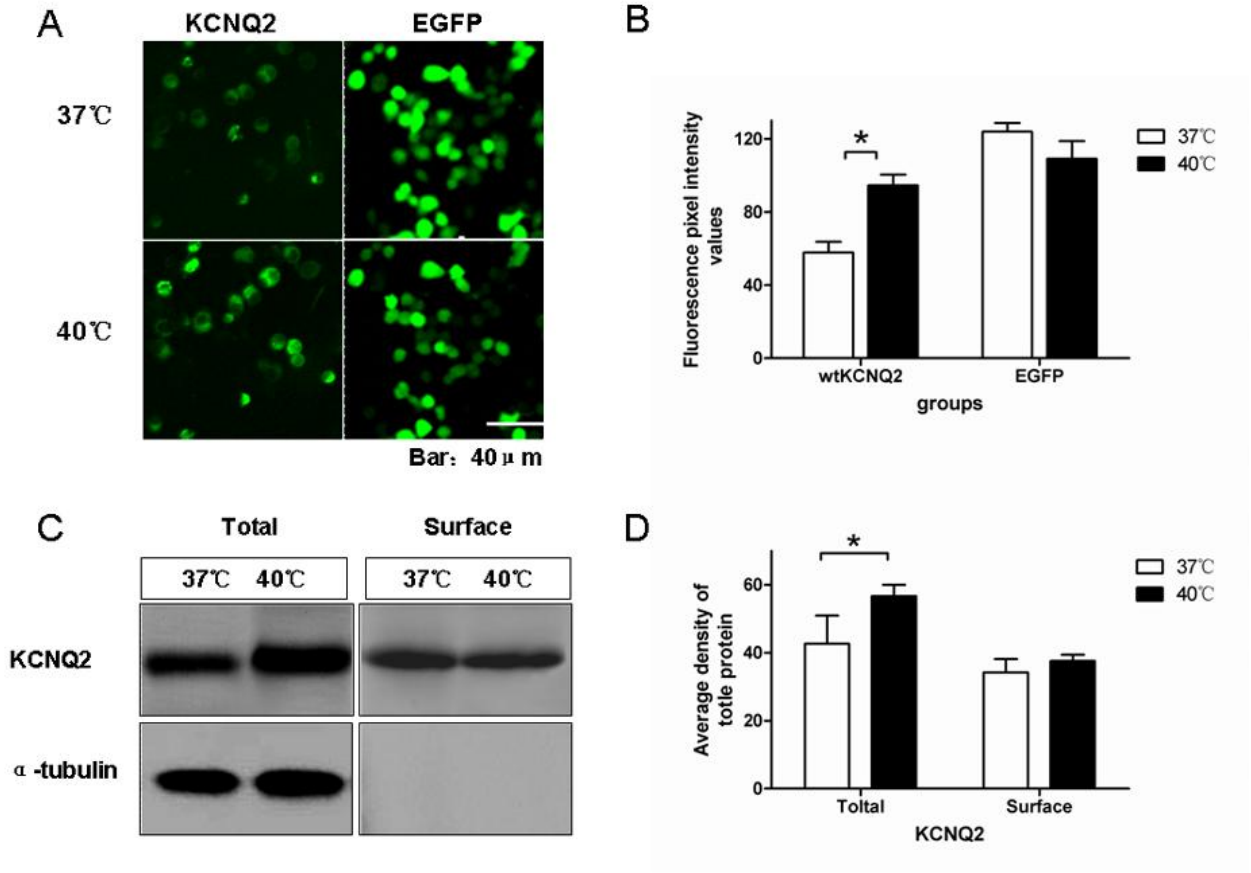

Figure 1. Elevated temperature increased total expression but not surface expression of KCNQ2.
(A) Confocal images of N2a cells transfected with fluorescence-tagged KCNQ2 subunits and EGFP at 37 and 40°C. (B) Fluorescence intensity values of KCNQ2 channel were enhanced after a 30 min incubation at 40°C (* $p < 0.001$, Student's unpaired *t* test). In all groups, data represented the mean ± SEM (n = 25–30 cells from 5 transfections; Student's unpaired *t* test). (C) Western-blot analysis of total and surface expressed KCNQ2 proteins incubated at 37 or 40°C for 1 h. Equal amounts of protein were immunoblotted with mouse monoclonal anti-KCNQ2 ((Millipore, Danvers, MA, USA)). The lower image in this panel shows the same blot stripped and reprobed with anti-α-tubulin antibodies as control. (D) Quantification of Western blots of total and surface protein incubated at different temperatures. Equal quantity of protein was immunoblotted with mouse monoclonal anti-GFP. In all groups, data represent the mean ± SEM (n=5; * $p<0.01$; two-tailed unpaired Student's t test).

3. Results

3.1 Elevated temperature increased total expression but not surface expression of KCNQ2.

To investigate the impact of temperature elevation on the expression of KCNQ2 channel, we initially overexpressed KCNQ2 subunits in N2a cells to examine KCNQ2 expression in neural cells. Interestingly, the fluorescence intensities of EGFP-wtKCNQ2 transfected cells were significantly increased after 30 minutes incubation at 40°C as compared to at 37°C ($p<0.001$) (Fig 1 A and B).

To further determine the effect of temperature elevation on the expression of KCNQ2 channels, western blot analyses were performed on N2a cells transfected to express EGFP-tagged wtKCNQ2. Quantification of western blots revealed that after 1 hour of temperature elevation from 37°C to 40°C, the expression of KCNQ2 subunit were significantly increased ($p<0.05$) (Fig 1C,D).

Meanwhile, surface protein of KCNQ2 channels were labeled by biotin and analyzed to determine whether the elevated temperature affects the surface expression of KCNQ2 channels. There was no significant change in the surface expression of KCNQ2 channels after incubation at 40°C for 1 h as compared to that at 37°C (Fig1C and D).

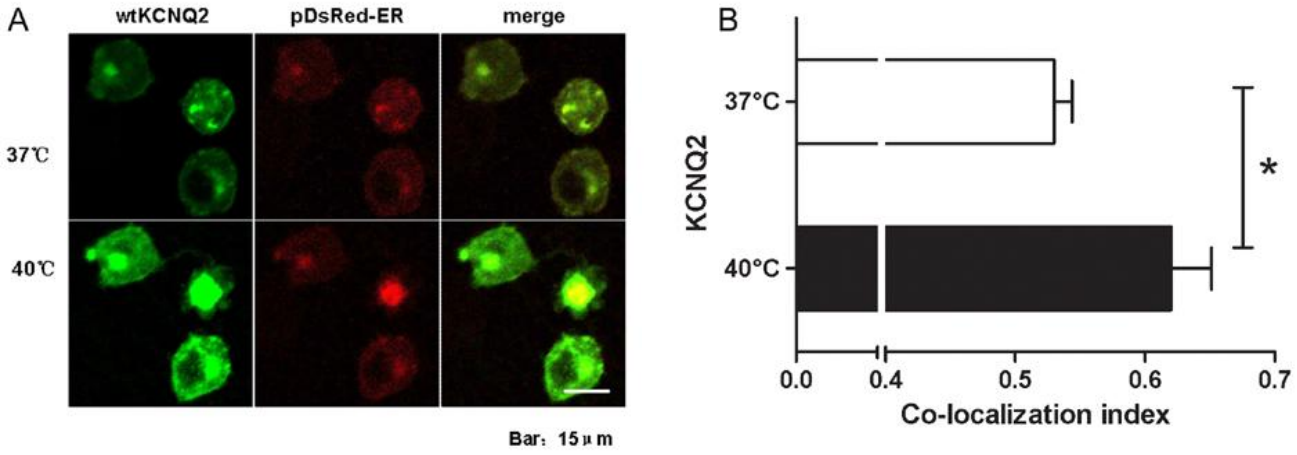

Figure 2. Elevated temperature increased intracellular retention of wtKCNQ2
(A) Confocal microscopy images of N2a cells cotransfected with EGFP-tagged KCNQ2 subunits, and with a DsRed-tagged ER marker after a 30 min incubation at 40°C. (B) Averaged Manders' coefficient of colocalization of cells expressing the different constructs at different temperature ($n>10$). * indicates statistically significant differences between 37°C and 40°C (unpaired Student's t test; $p<0.05$)

3.2 KCNQ2 intracellular localization increased after temperature elevation

To identify the intracellular location of KCNQ2 channels, KCNQ2 subunits were coexpressed with an endoplasmic reticulum (ER) marker, pDsRed-ER [10-11]. Co-localization studies were based on spatial fluorescence overlap of EGFP-labeled KCNQ2 channels (green) and DsRed-labeled ER marker (red) (overlap is pseudocolored yellow, Fig 2A). After a 30 minute incubation at 40°C, the majority of transiently expressed KCNQ2 channels were distributed intracellularly and retained in the ER as shown in Figure 2A, and B.

3.3 Voltage-dependent KCNQ2 activation showed negative shift at elevated temperature

To characterize the impact of temperature elevation on electrophysiological properties of the KCNQ2 channels, we transfected cDNAs encoding EGFP tagged-wtKCNQ2 into HEK 293T cells and recorded whole-cell currents at 37

℃ and 40℃. Fig.3A showed that the current densities (maximum current densities at +30 mV) at 37°C and 40°C (195 ± 73 pA/pF, n = 6 and 151 ± 40 pA/pF, n = 4, respectively) were not significantly different (p = 0.27).

In contrast, elevated temperature had a significant effect on the voltage-dependence of KCNQ2 channel activation, as illustrated by the current activation curves shown in Figure.3B. At high temperature, homomeric wtKCNQ2 channels exhibited a significant leftward shift in the half-maximum activation potential ($V_{1/2}$) of the conductance–voltage curve.

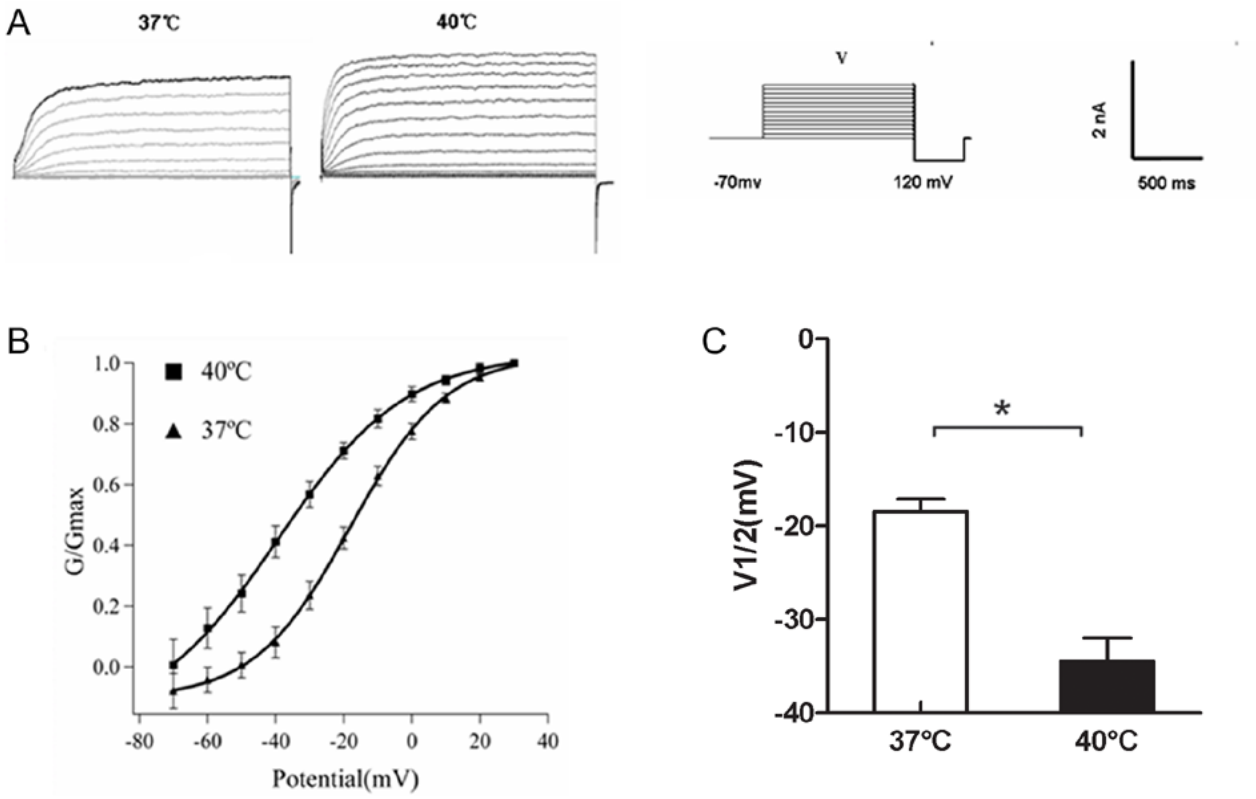

Figure 3. Modulation of homomeric KCNQ2 current by elevated temperature
(A) Whole-cell KCNQ2 currents from a HEK 293T cell in bathing solutions of differing temperature were elicited by depolarizing voltage steps (1.5 s duration) from a holding potential of -70 mV to +30 mV.(B) Whole-cell KCNQ2 current activation curves in bathing solutions at 40°C and 37°C. Curves are Boltzmann distributions fitted to the mean data, where $V_{1/2}$ is the voltage of half-maximal activation. (C) $V_{1/2}$ values with standard error of the mean (SEM). *Indicates statistically significant differences between 37°C and 40°C (unpaired Student's t test; $p < 0.05$).

4. Discussion

Studies have revealed that elevation of brain temperature alters many neuronal functions, including the function of several temperature-sensitive ion channels [12] that contribute to shaping neuronal firing and synchronized neuronal activity. In this study, our data indicated that elevated temperature alters expression and gating of KCNQ2 channels.

It has been shown that in the immature CNS, KCNQ channels play an important role in inhibitory neurotransmission system[14]. Notably, we found that when the temperature was elevated, the intracellular localization of KNCQ2 subunits was increased, suggesting that temperature affects the trafficking of KCNQ2 channels. However, the increased expression did not show a concomitant increase of membrane-associated channels. Indeed, channel trafficking

is a complex process that involves subunit assembly, ER exit, transport to the membrane and maintenance therein, endocytosis, and degradation [13]. Thus, only under the appropriate conditions, a large reservoir of intracellular channels can properly traffic to the plasma membrane, and temperature could act at any of multiple stages in this complex process to cause intracellular retention of KCNQ2 channels. Further studies need to clarify the mechanism of the increased steady-state channel expression in response to 30 min of elevated temperature: is it increased synthesis, decreased degradation or a combination of the two?

We also observed a negative shift in the voltage-dependent activation of KCNQ2 at febrile temperature, although KCNQ2 macroscopic current density did not change upon rapid temperature elevation. The result would suggest an enhancement of KCNQ channel open probability at high temperature. In fact, during the early stages of development, the function of KCNQ channels may be very important for neuronal excitability[14], and increasing the function of the KCNQ channel may a protective mechanism in children with fever. Given the important impact of KCNQ2 channels on inhibition of the immature CNS, KCNQ2 channel dysfunction in a background of febrile temperatures could increase the susceptibility to seizures, which proved KCNQ2 could be an important target the therapy of other heat-triggered dysfunction of CNS, such as seizure and epilepsy.

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References

[1] Cooper, E.; Jan, L. M-channels: neurological diseases, neuromodulation, and drug development. Archives of Neurology, 2003,60(4): 496-500. DOI:10.1001/archneur.60.4.496

[2] Wang, H.; Pan, Z.; Shi, W.; Brown, B.; Wymore, R.; Cohen, I.; Dixon, J.; McKinnon, D. KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science, 1998,282(5395): 1890-1893. DOI: 10.1126/science.282.5395.1890

[3] Peters, H.; Hu, H.; Pongs, O.; Storm, J.; Isbrandt, D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. Nature Neuroscience, 2005,8(1): 51-60. Doi:10.1038/nn1375

[4] Brown, D.; Passmore, G. Neural KCNQ (Kv7) channels. British Journal of Pharmacology, 2009,156(8): 1185-1195. Doi:10.1111/j.1476-5381.2009.00111.x

[5] Rodriguez, B.; Sigg, D.; Bezanilla, F. Voltage gating of Shaker K+ channels. The effect of temperature on ionic and gating currents. Journal of General Physiology, 1998,112(2): 223-242. Doi: 10.1085/jgp.112.2.223

[6] Thomas, E.; Hawkins, R.; Richards, K.; Xu, R.; Gazina, E.; Petrou, S. Heat opens axon initial segment sodium channels: a febrile seizure mechanism? Annals of Neurology, 2009,66(2): 219-226. Doi:10.1002/ana.21712

[7] Kang, J.; Shen, W.; Macdonald, R. Why does fever trigger febrile seizures? GABAA receptor gamma2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. Journal of Neuroscience, 2006,26(9): 2590-2597. Doi: 10.1523/JNEUROSCI.4243-05.2006

[8] Gomez-Posada, J.; Etxeberria, A.; Roura-Ferrer, M.; Areso, P.; Masin, M.; Murrell-Lagnado, R.; Villarroel, A. A pore residue of the KCNQ3 potassium M-channel subunit

controls surface expression. Journal of Neuroscience, 2010,30(27): 9316-9323. Doi: 10.1523/JNEUROSCI.0851-10.2010

[9] Su, J.; Cao, X.; Wang, K. A novel degradation signal derived from distal C-terminal frameshift mutations of KCNQ2 protein which cause neonatal epilepsy. Journal of Biological Chemistry, 2011,286(50): 42949-42958. Doi: 10.1074/jbc.M111.287268

[10] Etxeberria, A.; Aivar, P.; Rodriguez-Alfaro, J.; Alaimo, A.; Villace, P.; Gomez-Posada, J.; Areso, P.; Villarroel, A. Calmodulin regulates the trafficking of KCNQ2 potassium channels. FASEB J, 2008,22(4): 1135-1143. Doi: 10.1096/fj.07-9712com

[11] Alaimo, A.; Gomez-Posada, J.; Aivar, P.; Etxeberria, A.; Rodriguez-Alfaro, J.; Areso, P.; Villarroel, A. Calmodulin activation limits the rate of KCNQ2 K+ channel exit from the endoplasmic reticulum. Journal of Biological Chemistry, 2009,284(31): 20668-20675. Doi:10.1074/jbc.M109.019539

[12] Shibasaki, K.; Suzuki, M.; Mizuno, A.; Tominaga, M. Effects of body temperature on neural activity in the hippocampus: regulation of resting membrane potentials by transient receptor potential vanilloid 4. Journal of Neuroscience, 2007,27(7): 1566-1575. Doi: 10.1523/JNEUROSCI.4284-06.2007

[13] Heusser, K.; Schwappach, B. Trafficking of potassium channels. Current Opinion in Neurobiology 2005,15(3): 364-369. DOI:10.1016/j.conb.2005.04.001

[14] Maljevic, S.; Wuttke, T.; Seebohm, G.; Lerche, H. KV7 channelopathies. Pflugers Archiv-European Journal of Physiology, 2010,460(2): 277-288. DOI: 10.1007/s00424-010-0831-3

