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Corresponding Author: Eunji Cheong

Authors: Go Eun Ha[1], Eunji Cheong[1,*]

Institution: [1]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul 03722, Republic of Korea,

BMB Reports Perspective

Title

Calcium-activated chloride channels as a new target to control the spiking pattern of neurons

Go Eun Ha & Eunji Cheong*

Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul 03722, Republic of Korea

*Corresponding author. E-mail: eunjicheong@yonsei.ac.kr

Abstract (200자 이내)

Neuronal firing patterns and frequencies determine the nature of encoded information in the neural circuits. Here we discuss the molecular identity and cellular mechanisms of spike-frequency adaptation in central nervous system (CNS). Spike-frequency adaptation in thalamocortical (TC) and CA1 hippocampal neurons is mediated by the Ca^{2+}-activated Cl^- channel (CACC) anoctamin-2 (ANO2). Knockdown of ANO2 in these neurons results in significantly reduced spike-frequency adaptation along with increased number of spikes. No previous study has described the finding that CACCs mediate afterhyperpolarization currents, which result in the modulation of neuronal spike patterns in the central nervous system. Therefore, our study proposes a novel role for ANO2 in spike-frequency adaptation and transmission of information in the brain.

Keywords: Anoctamin-2, Calcium-activated chloride channel, Spike-frequency adaptation, Afterhyperpolarization, Thalamocortical neuron

Abbreviations: TC, Thalamocortical; CACC, Calcium-activated chloride channel; ANO2, Anoctamin-2; AHP, Afterhyperpolarization

Perspective to: Go Eun Ha (2016) The Ca2+-activated chloride channel anoctamin-2 mediates spike-frequency adaptation and regulates sensory transmission in thalamocortical neurons. Nature Communications, 2016 Dec 19;7:13791. doi: 10.1038/ncomms13791

본문 (1000word 이내)

Neurons transmit information through the frequency and patterns of action potentials elicited in response to a stimulus or the physiological conditions of an animal. Neuronal firing patterns are crucial for determining the nature of encoded information throughout the brain circuit. The firing patterns of neuron could be transformed according to external stimuli and intrinsic properties, such as expression levels of various ion channels embedded in plasma membrane of cells.

Neurons with a prolonged stimulus initially express a high frequency of firing patterns, followed by gradually declined frequency. This reduction in the firing frequency of the spike response, described as spike-frequency adaptation, has been observed in various types of neurons in the central nervous system. Spike adaptation often follows the extended period of excitation of neurons which generally accompanies the increase of intracellular calcium concentration via voltage-dependent calcium channels. Therefore, the spike adaptation has been ascribed to the slow-type afterhyperpolarization (AHP) mediated by calcium-activated potassium channels. Slow AHP currents can be further categorized into medium AHP (mAHP) and very slow AHP (sAHP) currents (mIAHP and sIAHP), the decay kinetics of which are approximately hundreds of milliseconds and over seconds, respectively. Of these two types of currents, mAHP is known to be mediated by small conductance (SK) or large conductance (BK) Ca2+-activated K+ channels in many types of neurons.

Calcium-activated chloride channels (CACCs), a family of anionic transmembrane ion channels, are widely expressed in different tissues. They have been involved in various physiological conditions, such as contraction of smooth muscle, control of blood pressure, control of cardiac excitability, signal transduction in olfactory and sensory neurons, and cell proliferation. The activation of CACCs could induce depolarization or hyperpolarization of cells according to intracellular chloride

concentration ($[Cl^-]_{in}$). Peripheral neurons have the higher level of $[Cl^-]_{in}$ by the action of sodium-potassium-chloride cotransporter, NKCC2. Therefore, the activation of CACCs triggers the outward chloride flow, and thus results in the depolarization of membrane potential. Anoctamin-2 (ANO2), one of CACCs, expressed in presynaptic end of olfactory epithelium and retinal photoreceptors mediates transepithelial Cl^- secretion and presynaptic Ca^{2+}-activated depolarization of the membrane potential via efflux of Cl^-.

In contrast with the previous studies on ANO2 in olfactory epithelium and retina, we found that ANO2 in thalamocortical (TC) neurons mediates spike-frequency adaptation by generating a hyperpolarizing current. TC neurons responding to a long depolarizing current input displayed the prolongation of inter-spike intervals which is Ca^{2+}-dependent. Knockdown of ANO2 in TC neurons reduced this spike frequency adaptation and significantly decreased mAHP currents. Many factors contribute to make different roles of CACC between peripheral and TC neurons: First, the reversal potential of Cl^- in TC neurons with intact $[Cl^-]_{in}$ is about -70 mV. This potential approximates the resting membrane potential of TC neurons and is also lower than the threshold required to activate voltage-gated Na^+ channels. The endogenous $[Cl^-]_{in}$ corrected with the relative permeability of 0.2 based on a 15 mM $[HCO_3^-]_{in}$ typical of central nervous system neurons was 5.4 mM. This low $[Cl^-]_{in}$ in TC neurons agrees well with previous reports demonstrating that the K^+/Cl^- co-transporter (KCC2) is highly expressed in TC neurons and actively results in Cl^- efflux leading to hyperpolarization of E_{GABA}. Secondly, ANO2 generates an outwardly rectifying current, while ANO1 manifests a linear I-V curve. Therefore, ANO2 would generate relatively large outward currents in neurons with an endogenous ionic content at the depolarized membrane. Moreover, ANO2 activation is relatively less sensitive to Ca^{2+} compared to ANO1, which provides a unique role for ANO2 in controlling the excitability of TC neurons. When TC neurons generate spikes at a low frequency, the membrane potential of TC neurons remains close to the reversal potential of Cl^- with low levels of Ca^{2+} influx. Therefore, ANO2 does not conduct a substantial current, and TC neurons generate spikes at regular intervals. When TC neurons generate a barrage of spikes at high frequencies, the membrane of the TC neurons is depolarized, which is accompanied by high levels of Ca^{2+} influx near the soma. Hyperpolarization of the membrane potential then occurs via the ANO2 current, which elongates the inter-spike intervals. Therefore, ANO2 has functions to generate spike-frequency adaptation, which results in interrupting

excessive firing in TC neurons. The endogenous Cl^- reversal indicate that ANO2 mediates AHP current conduction in TC neurons, which might be assisted by the outwardly rectifying characteristic of ANO2 channels. This phenotype was also observed with knockdown of ANO2 in CA1 hippocampal neurons, which provides evidence that Ca^{2+}-activated Cl^- conductance via ANO2 channels hyperpolarizes the membrane potential in these CNS neurons.

Thalamus-specific ANO2 knockdown significantly increased visceral pain responses, reflecting the level of sensory information transmission from the thalamus to the cortex. The role of ANO2-mediated spike adaptation, which can be considered a type of self-inhibition in TC neurons, was emphasized on the basis of a considerable increase in pain responses in mice with thalamic-restricted ANO2 knockdown. Interestingly, ANO2 currents restrict excessive spike generation but do not interfere with information transmission by TC neurons up to a certain level of spike-frequency. Spike frequency adaption in neurons has been suggested as a crucial contributor to stimulus encoding by neurons. Specifically, spike adaptation may enable neurons to respond more sensitively to coinciding inputs or have a major contribution to network synchronization, which suggests that ANO2-mediated spike-frequency adaptation in TC neurons may facilitate synchronized TC activity.

Question remains if the spike adaptation mediated by CACC could be a general way to restrict the spike generation in the central nervous system. The role of ANO2-mediated spike adaptation in TC and CA1 neurons, which can be considered as a type of self-inhibition in neurons. $[Cl^-]_{in}$ in neurons could play a crucial role in determining if CACC in neurons elicit the hyperpolarization or depolarization of membrane potential. Many neurons in CNS have been known to have relatively low $[Cl^-]_{in}$ when they are matured. In CNS neurons, the action of potassium-chloride cotransporter KCC2 leads to the lower level of $[Cl^-]_{in}$, and the activation of CACCs induces the inward chloride flow, outward chloride current, to hyperpolarize neurons. Therefore, in CNS, the influx of chloride ion through CACCs could have the possibility of the modulation of spike-frequency adaptation by shunting effect.

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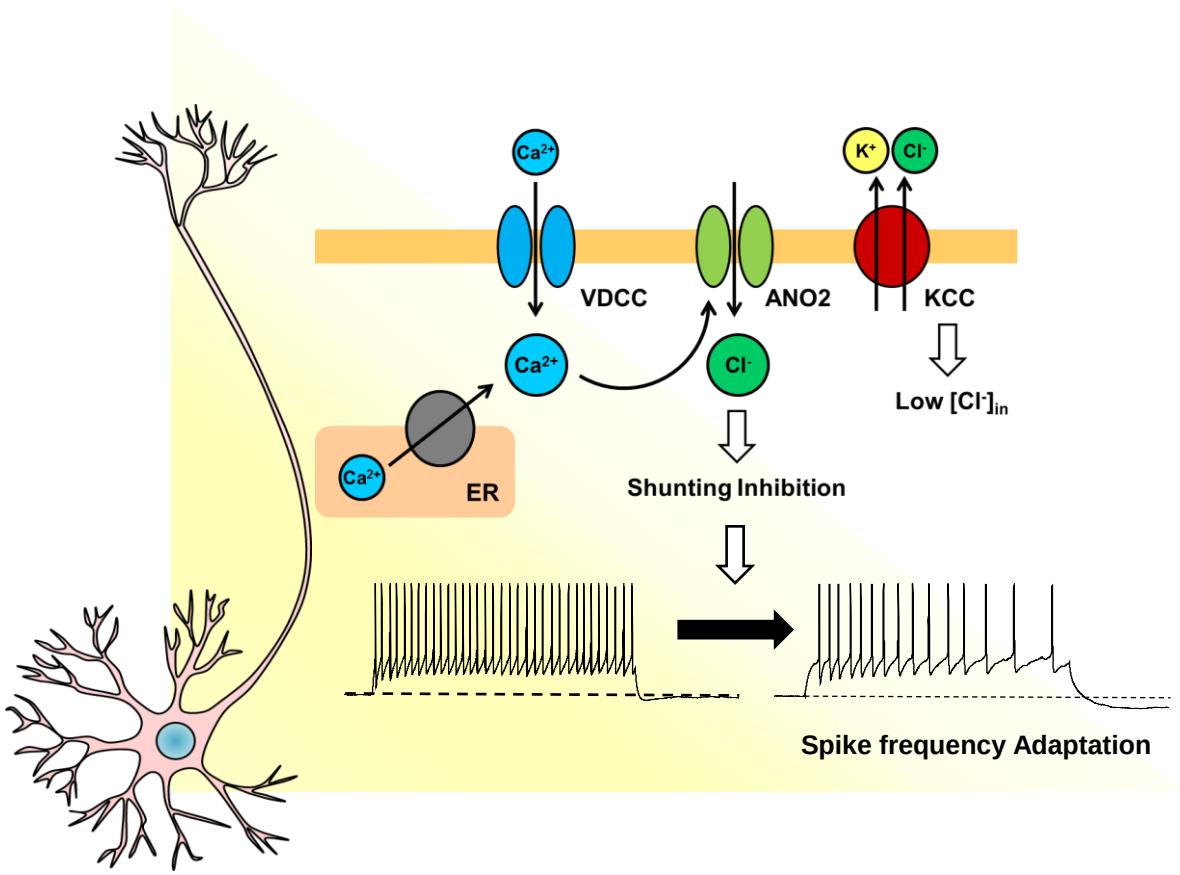
Ca2+
K+
Cl-
VDCC
ANO2
KCC
Ca2+
Cl-
Low [Cl-]in
Ca2+
ER
Shunting Inhibition
Spike frequency Adaptation