BK channel in microglia as a potent therapeutic molecular target for neuropathic pain

Yoshinori Hayashi^{1,*} and Hiroshi Nakanishi^{1,2}

¹Department of Aging Science and Pharmacology, Faculty of Dental Sciences, Kyushu University, Fukuoka 812-8582, Japan

²Japan Science and Technology Agency, Core Research for Evolutional Science and Technology, Tokyo 102-0075, Japan

*Corresponding author. Tel.: +81 92 642 6412; fax: +81 92 642 6415.

E-mail address: yoshinor@dent.kyushu-u.ac.jp (Y. Hayashi),

nakan@dent.kyushu-u.ac.jp (H. Nakanishi).

Abstract

Background

Ketamine, noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, has been used in the treatment of chronic neuropathic pain. However, serious troubles have been still remained in ketamine for the clinical treatment because of the strong adverse side effects during the long-term use. *S*-ketamine, one of the ketamine enantiomers, has a potent analgesic effect and weak side effects compared with *R*-ketamine. To date, analgesic potency of *S*- and *R*-ketamine was not fully account for the binding affinity to NMDA receptors. In the present review, we provide a novel analgesic mechanism of ketamine on chronic pain.

Conclusion

These findings suggest that potent analgesic effects of *S*-ketamine arise from the inhibition of microglial BK channels in addition to neuronal NMDA receptors. Thus, BK channels in microglia are a potential target for the treating of neuropathic pain.

Keywords: neuropathic pain, microglia, *S*-ketamine, large-conductance Ca^{2+} -activated K^{+} channel

1. Introduction

Ketamine is synthesized as an anesthesia and is clinically used for the treatment of chronic pain for almost 20 years. Ketamine, a noncompetitive NMDA receptor antagonist, has several adverse side effects such as psychotomimetic effects [1]. Clinically used ketamine contains an equimolar mixture of a pair of enantiomers because there is one chiral center in its structure. The two enantiomers are classified as either "*S*" or "*R*" and have different actions like thalidomide. *S*-ketamine has a 3-4 times more potent analgesic effect than *R*-ketamine. However, *R*-ketamine is a higher occurrence of psychotomimetic effects than *S*-ketamine [2, 3]. The different potency of the side effect was considered as the binding affinity to sigma receptors [4]. US Food and Drug Administration (FDA) have approved optically pure drugs account for more than 50% of total novel drugs, whereas racemate is lower than 10% of them [5]. Therefore, optically pure *S*-ketamine is an effective therapeutic medicine from the aspect of minimal side effects. Neuropathic pain is a chronic pain state that is usually accompanied with tissue damage or dysfunction in the nervous system.

Neuropathic pain is resistant to currently available analgesic drugs, including

non-steroidal anti-inflammatory drugs or opioids [6]. The development of effective therapeutic strategy to alleviate chronic pain is a big issue for the pain management. Effectiveness of ketamine has already proved in the chronic pain, including postherpetic neuralgia, complex regional pain syndrome (CRPS), cancer pain, orofacial pain, and phantom limb pain [7]. Although the main target of ketamine is NMDA receptors, the different analgesic potency of *S*- and *R*-ketamine was not fully account for the blocking efficacy of neuronal NMDA receptors. The following points can be given as a reason that *S*-ketamine can only blocked neuronal NMDA receptors mediated currents twice as strong as *R*-ketamine in vitro experiments, unlike analgesic potency of ketamine enantiomers. A growing body of evidence indicates that activated microglia in the spinal cord yield several cellular events that directly influence the pathogenesis of pain hypersensitivity [8, 9]. It was demonstrated that LPS-induced microglial activation was significantly suppressed by ketamine *in vitro* study [10]. In addition, activated microglia express NMDA receptors [11, 12], presumably molecular target of ketamine. Therefore, we speculated that the different potency of analgesic effect of ketamine enantiomers might arise from microglia-based mechanism in addition to neuron-based one. In the present review, we summarized novel molecular mechanisms underlying the therapeutic action of *S*-ketamine on neuropathic pain with a specific focus on BK channels.

2. The function of NMDA receptors in spinal microglia

We first analyzed analgesic effects of the ketamine enantiomers on neuropathic pain that was made by lumbar 4 spinal nerve transection (so-called peripheral nerve injury, PNI). Optically pure *S*- and *R*-ketamine were synthesized according to the method of asymmetric synthesis [13] developed by Dr. Ryoji Noyori who received

Nobel Prize in Chemistry in 2001. S-ketamine preferentially alleviated mechanical allodynia compared with racemate or *R*-ketamine. To analyze the effect of ketamine on NMDA receptors in spinal microglia, whole-cell patch clamp analyses was examined from visualizing microglia in spinal dorsal horn that was prepared from Iba1-EGFP mice [14] 3 days after PNI. It is well known that NMDA receptors, a glutamate receptor, was blocked by extracellular Mg^{2+} in a voltage-dependent manner and potentiated by the presence of glycine, co-agonist of NMDA receptors [15]. We, therefore, used Mg^{2+} -free extracellular solution added glycine (1 μ M) during patch clamp recordings. Contrary to our expectations, NMDA (1mM)-induced inward currents were observed on neither contralateral nor ipsilateral side of spinal microglia. In contrast, both sides of spinal microglia were elicited the currents in response to ATP. Immunohistochemical analyses revealed that both contralateral and ipsilateral side of spinal microglia express NR1 subunit of NMDA receptors following nerve injury [16]. According to the microdialysis analyses, basal glutamate concentration in the cerebrospinal fluid was found to be no difference in normal (265.7 ± 77.3 ng/ml; $1.80 \pm 0.53\mu$ M) and neuropathic pain (131.8 ± 54.8 ng/ml; $0.90 \pm 0.37\mu$ M) rats [17]. Glutamate concentration in the synaptic cleft is considered to reach 1.1 mM during synaptic glutamate release and decayed with a time constant of 1.2 ms [18], indicating that the NMDA concentration in our experiments was sufficient to evoke NMDA receptors. Thus, NMDA receptors in microglia do not have a functional role in the spinal cord. Microglia are ubiquitously distributed in the brain and spinal cord where regulate the neuronal microenvironment in the physiological situation [19-21]. In response to PNI, microglia in the spinal cord change into activated phenotype that is defined as hypertrophic morphology and an increase in the cell number. Activated microglia

release several bioactive molecules such as cytokines and amino acids [15], which in turn lead to hyperexcitability of spinal dorsal horn [22]. *S*-ketamine preferentially suppressed PNI-induced microglial activation such as morphological change and productivity of cytokines, including interleukin-1 β (IL-1 β). Therefore, *S*-ketamine should affect another therapeutic target on spinal microglia.

3. Molecular target for analgesic effect of ketamine.

Ketamine has several molecular targets to elicit its action. Ketamine suppresses sodium channels like as local anesthesia [23], resulting in the reduction of firing rate in the dorsal root ganglion (DRG) neurons [24]. Agonistic effect of GABA α receptors is another feature of ketamine properties [25]. Recently, hypnotic action of ketamine was found to be mediated by the inhibition of HCN1 channels [26]. It is implicated that ketamine bind to the Ca²⁺-binding site of Ca²⁺-activated K⁺ (K_{Ca}) channels, especially in large conductance K_{Ca} (BK) channels [27, 28]. Besides the anesthetic effects, acute antidepressant effect of subanesthetic dose of ketamine is exerting mammalian target of rapamycin (mTOR)-dependent synapse formation [29]. Among the abundant targets of ketamine, BK channels are well-known molecule to modulate the activation state of microglia [30]. BK channels are one of the K⁺ channels, which are operated by intracellular Ca²⁺ concentration and membrane voltage and ubiquitously expressed in the tissue. In electrically excitable cells such as neurons or muscles, BK channels contribute to repolarization of action potential by the efflux of potassium ion [31]. In other words, BK channels work as a “braking system” in excitable cells. In contrast, BK channels in electrically non-excitable cells such as immune cells or glioma were involved in the proliferation and cellular activation [32, 33]. The function of BK

channels in non-excitabile cells is highlighted [34] as the sensor of cellular activity because BK channels in non-excitabile cells are ready to activate by physiological membrane voltage without experiencing large depolarization. They are working as an “accelerator” of cellular activity in non-excitabile cells. Pharmacological analyses revealed that K^+ currents in spinal microglia after PNI were sensitive to the ChTx, BK channel inhibitor, but not apamin, SK channel (small conductance Ca^{2+} -activated K^+ channel) inhibitor. However, we could not observe any change in the expression level of BK channels in spinal microglia after PNI [16]. The immunoreactivity for SK channels was never detected in the spinal microglia even after cellular activation [35]. These findings well corresponded to our data that current activation in BK channels but not SK channels is a specific event in the activated spinal microglia. Bath application of S-ketamine significantly suppressed BK currents recorded from the ipsilateral side of spinal microglia. As above mentioned, BK channels are operated by membrane depolarization. Resting membrane potential of microglia is very shallow (-37.1 ± 4.29 mV) compared with neuron (around -70 mV). Microglia are characterized by high input resistance and little voltage-gated membrane currents [36, 37], thus they have very low membrane potential. This fact might indicate that microglial BK channels already set on “ready-to-go” state in the physiological situation and activate in response to abnormal signals immediately.

4. Possible involvement of BK channels in the pathology of neuropathic pain.

We further address whether microglial BK channels are the cause of the pathology of neuropathic pain by using NS1619, a BK channel opener. Interestingly, intrathecal administration of NS1619 (low concentration: 20 μ M, 0.072 μ g/10 μ l) in naïve mice

exhibited allodynia-like behavior. In contrast, high concentration of NS1619 (5555 μ M, 20 μ g/10 μ l) has no effects on behavior, consistent with the previous report [38]. This discrepancy might be due to the different sensitivity of neuron and microglia against NS1619. The effective dose of NS1619 on neuronal activity *in vivo* study was $\sim$ 30 μ g/10 μ l [39]. This concentration of NS1619 can suppress the spike generation of DRG neurons [40]. In contrast, both concentrations of NS1619 could activate spinal microglia. We observed depolarizing shift of resting membrane potential (8.22 ± 2.62 mV) in spinal microglia even in the low concentration of NS1619 [41]. The analgesic effect of high concentration of NS1619 might attribute to suppress neuronal activity with masking microglial activation. To further clarify the involvement of microglial BK channels in neuropathic pain, BK channel inhibitor was intrathecally administered in the mice following PNI. ChTx, BK channel inhibitor, significantly suppressed both neuropathic pain and microglial activation in the spinal dorsal horn.

5. Activation mechanism of BK channels in spinal microglia

Sensory A β fibers transmit innocuous information, which innervate into deeper dorsal horn laminae. Under normal condition, GABAergic inhibitory neurons in deeper dorsal horn laminae suppress the activity of nociceptive neurons. After nerve injury, these neurons lose K⁺/Cl⁻ co-transporter KCC₂, thereby altering the response to GABA. Dysregulation of the intracellular chloride homeostasis results in the disinhibition of nociceptive transmission from lamina I neurons [42]. Therefore, patients feel intense pain, even with innocuous tactile stimuli via sensory A β fibers. The core signaling pathway is through P2X₄ receptor on spinal microglia and subsequent release of BDNF which trigger the reduction of KCC₂ expression [43]. Both P2X₄ receptors and BDNF

in spinal microglia were well recognized as good marker for the neuropathic pain. Notably, we found that intrathecal administration of BK channel inhibitor suppressed the expression of P2X4 receptors and BDNF in the spinal microglia after PNI [16]. We finally addressed what molecule is the causative factor to activate BK channels in spinal microglia following PNI. Chemokine, cytokine, and lipid are thought as important mediators of the development of neuropathic pain [44]. Among of them, we focused on lysophosphatidic acid (LPA) which is simple lipid with glycerol, fatty acid, and phosphate in its structure. Intrathecal administration of LPA initiates allodynia-like behavior [45]. Interestingly, microglia respond to LPA through LPA3 receptors that increase intracellular $[Ca^{2+}]_i$ and induce the expression of P2X4 receptors and subsequent BDNF synthesis [46]. Time course of *de novo* LPA production in the spinal dorsal horn after nerve injury was strongly correlated with microglial activation [47]. Therefore, LPA might be a critical mediator to induce neuropathic pain through the microglial activation. Our study demonstrated that S-ketamine could strongly suppress LPA-induced pain behavior and current activation of BK channel in the spinal microglia. LPA also induce IL-1 β in the microglia which was significantly suppressed by ChTx. Plausible mechanism underlying BK channel activation in the spinal microglia following nerve injury was as follows. Nerve injury triggers the intense stimulation of spinal neurons, which induce *de novo* LPA production. The increased expression of P2X4 receptors in LPA-stimulated microglia further mobilize Ca^{2+} from extracellularly. Increased $[Ca^{2+}]_i$ operated BK channels, then induce IL-1 β secretion (Fig. 1). It is still unclear how BK channel regulate the secretion of IL-1 β . A potassium efflux is the core mechanism to activate the NLRP3 inflammasome for IL-1 β processing and secretion [48]. BK channel mediated potassium efflux might be involved in the production of

IL-1 β [49]. BDNF secreted microglia in turn further enhanced BK channel activity [50, 51]. Thus, BK channel inhibitor as well as *S*-ketamine likely to be breaking the vicious cycle of microglial activation following PNI, resulting in the potent analgesic effect.

6. Conclusions

Our study demonstrated that BK channels play an important role in the activation of spinal microglia and development of neuropathic pain. Potent analgesic effects of *S*-ketamine were mediated by blocking of microglial BK channels in addition to neuronal NMDA receptors. Inhibition of BK channels leads to the reduction of P2X4 receptors expression and BDNF synthesis in the spinal microglia after PNI. Our study might facilitate therapeutic strategy based on optically pure drug. Further study needed for the characterization of microglial BK channels to develop microglia specific BK channels inhibitor because of the BK channels diversity. BK channels in microglia might be promising a target with low side effect for the treatment of neuropathic pain.

Ethical approval

This review did not require ethical approval.

Acknowledgments

This work was supported by the Japan Science and Technology Agency, Core Research for Evolutional Science and Technology (H. N.), and Grants-in-Aid for Young Scientific Research (No. 24791979 to Y. H.) from the Ministry of Education, Science, and Culture Japan, and Takeda Science Foundation, Japan (Y. H.).

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Figure legend

Fig.1. Schematic illustration of activation mechanism of spinal microglia through the BK channels following PNI. Nerve injury triggers the production of LPA which accelerate the expression of P2X4 receptors and BDNF synthesis. BK channels are sequentially activated by Ca²⁺ influx through the P2X4 receptors. Microglia releasing molecules such as IL-1 β or BDNF cause hyperexcitability of neuronal networks in the dorsal horn, which develop chronic neuropathic pain. BK channels might facilitate negative spiral of cellular activation in the microglia. S-ketamine and BK channel inhibitor have a crucial role in breaking the vicious cycle of microglial activation, thereby, work as potential analgesic effect.

Figure 1