The emerging role of kainate receptor functional dysregulation in pain

Huili Li1, Junfa Li2, Yun Guan3 and Yun Wang1

Abstract
Pain is a serious clinical challenge, and is associated with a significant reduction in quality of life and high financial costs for affected patients. Research efforts have been made to explore the etiological basis of pain to guide the future treatment of patients suffering from pain conditions. Findings from studies using KA (kainate) receptor agonist, antagonists and receptor knockout mice suggested that KA receptor dysregulation and dysfunction may govern both peripheral and central sensitization in the context of pain. Additional evidence showed that KA receptor dysfunction may disrupt the finely-tuned process of glutamic acid transmission, thereby contributing to the onset of a range of pathological contexts. In the present review, we summarized major findings in recent studies which examined the roles of KA receptor dysregulation in nociceptive transmission and in pain. This timely overview of current knowledge will help to provide a framework for future developing novel therapeutic strategies to manage pain.

Keywords
Pain, kainate receptors, neuron, plasticity, modulation

Date Received: 24 November 2020; Revised 4 January 2021; accepted: 7 January 2021

Introduction
Pain is a normal sensory function that is necessary for survival. It is intended to protect the individual from continued or current injury; however, when the sensation becomes aberrant and develops into a more chronic nature, it transitions into a dysfunctional sensation that handicaps the sufferer, severely affecting quality of life.1 The mechanisms underlying pain have been extensively studied in both humans and animal models. It is well established that glutamatergic transmission is essential to nociceptive transmission.2,3 Glutamate receptors are composed of two major families: ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs).4 While iGluRs form an ion channel pore that becomes activated upon ligand binding, mGluRs do not conduct ion flux, instead they regulate G-proteins to control biochemical processes within cells.

There are three main classes of iGluRs which are structurally and pharmacologically different: NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate (KA) receptors.5,6 NMDA receptors are important for initiating long-term neuronal plasticity such as long-term potentiation,7,8 whereas AMPA receptors control fast excitatory synaptic transmission and plasticity.9,10 Both of these receptors were found to be associated with pain development, and both NMDA and AMPA receptor antagonists were aid in the treatment of pain. Unfortunately, the systemic administration of these drugs in patients has been associated with significant adverse effects including ataxia, loss of motor coordination, memory impairment, and psychotomimetic effects, ataxia.11–13

Unlike NMDA and APMA receptors, KA receptors primarily exert modulatory roles in the peripheral and central nervous system, serving as an important regulator of nociceptive transmission and integration, and thus.
Physiological studies have shown that KA receptors serve as key regulators of synaptic transmission and plasticity. These receptors mediate postsynaptic depolarization and neuronal excitation. In specific excitatory synapse subsets, they can also carry a portion of the synaptic current. KA receptors can also function as modulators of the presynaptic release of neurotransmitters including both glutamate and γ-aminobutyric acid (GABA). In addition, they can facilitate macromolecule and molecular aggregate anchoring, thereby influencing long-term synaptic plasticity in the hippocampus, cortex, and amygdalas.

Owing to a range of regulatory actions, KA receptors can profoundly influence the homeostatic balance between inhibition and excitation in neuronal networks. Accordingly, KA receptor dysfunction and dysregulation may drive the development of pathological conditions, such as pain. The expression of functional KA receptors has been detected via immunohistochemistry and electrophysiology along pain neuraxis including the DRG (Dorsal Root Ganglion), spinal cord dorsal horn, thalamus, and cortex wherein they control nociceptive transmission and pain modulation. The development of research tools including KA receptor agonist, antagonist, and transgenic mice in which these receptors were knocked out helped to unravel important roles of KA receptor dysregulation and dysfunction in peripheral and central sensitization in the context of pain. Intriguingly, besides functioning as ion channels, KA receptors were also found to activate certain G-proteins, thereby may influence long-term changes in synaptic transmission and plasticity, underscoring a potential dual signaling mechanisms for KA receptors to regulate pain. The details of mechanistic understanding for such noncanonical metabotropic signaling, and the factors that determine the roles of KA receptors in the context of pain remain unclear.

The post-transcriptional regulation of KA receptors

The alternative splicing of the GluK1-3 subunits gives rise to additional KA receptor isoforms, whereas GluK4 and GluK5 were not thought to undergo alternative splicing. Figure 1. NTD and CTD regions of GluK1-3 subunits are the primary sites of alternative RNA splicing. For example, the GluK1 extracellular NTD can be alternatively spliced to give rise to the GluK1-1 and GluK1-2 variants, while the CTD exhibits four such variants (GluK1a, GluK1b, GluK1c, and GluK1d). GluK2 and GluK3 similarly exhibit CTD splice variants (GluK2a/GluK2b/GluK2c and GluK3a/GluK3b, respectively). These splice variants are associated with significant changes in KA receptor exit from the
ER (Endoplasmic Reticulum) and surface accumulation, thereby receptor function and neuronal excitability. Because these variants also enable altered interactions between proteins, they may tune KA receptor function in a site-specific manner.45–47

A significant amount of research has been conducted to evaluate how splice variants of growth factors and ion channels affect pain.48 For example, three TRPV1 (Transient Receptor Potential Vanilloid-1) splice variants were found to in the DRG or trigeminal ganglia, and may play a role in nociceptive processing. Splicing affects the N-terminal domain in these splice variants, resulting in a loss of activation by capsaicin and other activators such as protons, resiniferatoxin, or temperature, thus yield a dominant negative channel.49 The expression of different splice variants of voltage-gated calcium channel, particularly Cav2.2, is also enriched in nociceptors. The presence of variants also increases sensitivity to neuronal inhibition through opioid and GABA receptors.50 NMDA receptor splice variants (including NR1-1b, NR1-3b, and NR1-4b) colocalize with NK1 (Neurokinin-1) receptors in projection neurons of the spinal cord dorsal horn, indicating that they may play a role in spinal nociceptive processing.51,52 Yet, formalin-induced nociception was not affected by alternative NR1 splicing.53 In addition, no change in pain behavior or anxiety was observed in mice after knocking in a mutant mGluR7 splice variant (mGluR7a) which lacks the PDZ ((PSD-95, Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1))) domain, indicating that function of this isoform is not required for normal nociceptive processing.54 However, relatively little is known regarding the roles of KA receptor splice variants in pain. Research is needed to establish whether KA receptor splice variants may play a role in the development of pain.

The RNA editing of GluK1 and GluK2 subunit further broadens the KA receptor functional repertoire. GluK1 can undergo editing at the channel pore-forming P-loop (the ‘Q/R’ site), and GluK2 can be edited at the I/V and Y/C sites in the M1 transmembrane domain wherein an isoleucine (ATT) is replaced with a valine (ITT) and a tyrosine (TAC) is replaced with a cysteine (TIC), Figure 1.55,56 These modifications result in amino acid substitutions at critical sites within the subunits, thereby altering amino acid substitutions at critical sites within these receptor subunits.
Because GluK2 subunit Q/R editing suppresses the ability of calcium to pass through KA receptor channels, an increase of GluK2 (Q) variants will increase calcium influx in spinal cord neurons and enhance channel conductance after inflammation. The resultant rises in intracellular calcium level in turn trigger kinase and receptor phosphorylation, thereby bolstering neuronal excitability.62 RNA editing has also been shown to influence neuropathic pain in an L5 spinal nerve transection (SNT) model. SNT markedly decreased the Q/R editing of GluK2 mediated by adenosine deaminase acting on RNA (ADAR, Adenosine Deaminase Acting on RNA) enzyme in the injured DRG neurons. Furthermore, targeting of these ADAR enzymes was sufficient to achieve pain inhibition in SNT model.22 Overall, these studies provided important evidence that post-transcriptional modifications of KA receptors significantly altered receptor functionality, highlighting a warrant of future studies of these modifications as targets for pain treatment.

The post-translational regulation of KA receptors

KA receptors also undergo post-translational modifications such as phosphorylation, which is the most common form of post-translational modification that can alter protein activity, localization, and interactions with other proteins.63,64 A number of residues within KA receptor subunits were shown to be phosphorylated, including S846, S856, S859, S868 S880, S886, S892, and T976.32 Of these, protein kinase C (PKC)-mediated S846, S868, S880, and S886 phosphorylation has been shown to directly impact receptor function. For example, Dildy-Mayfield and Harris first demonstrated that PKC phosphorylated recombinant GluK2, thereby reducing kainite-evoked currents.65 In contrast, Cho et al. showed that activation of PKC by mGluR5 enhanced GluK1-containing KA receptor-mediated excitatory postsynaptic potential (EPSP) in perirhinal cortical neurons.66 KA receptor activation further stimulated PKC-induced GluK1-2b S880 and/or S886 phosphorylation, leading to the internalization of these subunits.67 Evidence also showed that PKC-mediated S868 phosphorylation was associated with endocytosed GluK2 recycling back to the plasma membrane, suggesting that the phosphorylation may regulate the membrane localization of this subunit in a context-dependent manner.68 Such a bidirectional signaling may serve as an important feedback mechanism to prevent KA receptor-mediated neuronal overactivation.67

In addition to PKC, cAMP-dependent protein kinase (PKA) can directly modulate KA receptor functionality. For example, PKA-mediated GluK2 S856 and S868 phosphorylation was shown to potentiate kainite-evoked currents through recombinant KA receptors.69–72 In parallel with the actions of PKA and PKC, CaMKII-induced GluK5 S859, S892, and T976 phosphorylation uncouples these KA receptors from postsynaptic density 95 (PSD-95), improving the overall lateral mobility of these receptors by freeing them from synaptic incorporation, Figure 2.71 Overall, KA receptor phosphorylation is a key regulator of the trafficking of these receptors to synapses, and thus affect synaptic plasticity with respect to both integration and transmission. Consequently, KA receptor phosphorylation plays an important role in long term synaptic plasticity. For example, postsynaptic KA receptors at thalamocortical synapses were rapidly downregulated during the induction of long term potentiation via a mechanism that requires PKC. In perirhinal cortex layer 2/3 pyramidal neurons, a form of long term depression was associated with decreased KA receptor activation, characterized by a rapid reduction in KA receptor-mediated synaptic transmission. Interestingly, this long term depression also requires PKC.73 Recent work suggested a role of GluK2 phosphorylation in regulating KA receptor channel opening and subsequent pro-apoptotic signaling in the context of brain ischemia, suggesting it may play a role in the context of ischemic stroke.74 AMPA/KA receptor-mediated PKA and PKC activation is also required for pain sensation.72 Given that KA receptors can further drive PKC-mediated phosphorylation of KA receptors, this post-translational regulatory mechanism may contribute to thermal stimulus-evoked allodynia. Future research needs to establish whether this mechanism may be targeted to regulate neuronal hyperexcitability in the context of pain.

The regulation of KA receptor activity by interacting proteins

KA receptors do not function in a vacuum. Instead, they participate in a large macromolecular complex at the plasma membrane surface that contains trafficking chaperones, molecular scaffolds, and signaling enzymes capable of shaping the nature of the downstream responses.75 KA receptor-interacting proteins include BTB-Kelch or PDZ/CUB domain-containing proteins.76–78 The major interactions of auxiliary proteins with KA receptors and their relevance in the context of pain are summarized below, Figure 3.

PDZ domain proteins

PSD-95 interacts with KA receptors through the PDZ domain-mediated interactions,79 interacting with PDZ
domains within the CTD of GluK1, GluK2, and GluK5.\textsuperscript{80} Interactions with PSD-95 enable GluK2 homomeric receptors and GluK2/GluK5 heteromeric receptors to recover more rapidly following receptor desensitization.\textsuperscript{81} Such PSD-95 interactions drive KA receptor clustering, as evidenced by the reduction in KA receptors at MF-CA3 synapses in mice lacking PSD-95 expression.\textsuperscript{82} GluK5 and PSD-95 interaction are also necessary for long-term depression at MF-CA3 synapses.\textsuperscript{71}

\textbf{Figure 2.} Post-translational modifications (phosphorylation) of KA receptors. A number of residues within KA receptor subunits were shown to be phosphorylated, including S846/S868/S880/S886 (PKC), S856/S868 (PKA), S859/S892/T976 (CaMKII). All of these phosphorylation is shown to directly impact the kainate-evoked currents, and is also illustrated to be related to the endocytosis of KA receptors, leading to recycling of KA receptors to the membrane or degradation. Moreover, phosphorylation uncouples KA receptors from postsynaptic density 95 (PSD-95), improving the overall lateral mobility of these receptors by freeing them from synaptic incorporation. LBD ligand binding domain, GLU glutamate, N N-terminal domain, C C-terminal domain, P phosphorylation, EC extracellular, IC intracellular.

\textbf{Figure 3.} Schematic of domain organization of PSD95, GRIP, PICK1, and Netos with KA receptors. PDZ domain proteins (PSD95, GRIP and PICK1) are shown to interact with KA receptors through the PDZ domain-mediated interactions, which are necessary for the appropriate regulation of KA receptor-mediated synaptic functionality. Interactions with PSD95 enable KA receptors to recover more rapidly following receptor desensitization. Such interactions also drive KA receptor clustering, and KA receptors-PSD95 complex can additionally interact with mixed-lineage kinases 2 and 3 (MLK2 and MLK3), which drives JNK kinase activation. GRIP regulates KA receptor anchoring at synapses, and that the PICK1-targeted phosphorylation of KA receptors by PKC stabilizes GRIP binding. Netos (NETO1/2) interact with KA receptors via their PDZ-ligand domains and CUB domains, thereby forming stable complexes with KA receptors. Netos interaction with KA receptors typically slow KA receptor deactivation kinetics, and such interactions also represents a regulator of KA receptor trafficking. EC extracellular, IC intracellular.
Owing to these regulatory functions of KA receptors, the interactions between KA receptors and PSD-95 were also suggested to be involved in a range of pathological contexts. For example, a PDZ inhibitor peptide was shown to protect against the neuron apoptotic death due to ischemia/reperfusion, suggesting that disrupting GluK2-PSD-95 interactions may represent an effective approach for neuroprotection. The GluK2-PSD-95 complex can additionally interact with mixed-lineage kinases 2 and 3 (MLK2 and MLK3), which bind to the PSD-95 Src homology 3 (SH3) domain. The resultant interaction then drives JNK kinase activation, which is known to be important to chronic inflammatory pain and neuropathic pain. Interactions between KA receptors and PSD-95 may thus regulate pain at multiple steps by both directly impacting the functionality of these receptors and by influencing downstream signaling pathways.

A number of other PDZ domain-containing proteins may also interact with KA receptors to influence their functionality. For example, Hirbec et al. showed that GluK1-2b, GluK1-2c, and GluK2 bind to the PDZ domain-containing proteins PICK1 (protein interacting with CUB domains, CUB domain-containing proteins PICK1), GRIP (glutamate receptor-interacting protein 1), and syntenin via their CTDs. GRIP and PICK1 are necessary for the appropriate regulation of KA receptor-mediated synaptic functionality at mossy fiber-CA3 synapses. Disrupting these interactions interferes with synaptic transmission facilitated by these KA receptors. GRIP can also directly bind to kinesin motor proteins, indicating it may control the transport and trafficking of KA receptors. Hiberc et al. also suggested that GRIP regulates KA receptor anchoring at synapses, and that the PICK1-targeted phosphorylation of GluK1-2b S880 and/or S886 by PKC stabilizes GRIP binding, as evidenced by the disruption of KA receptor-mediated currents from inhibiting PICK1 interaction or PKC activity. A lack of appropriate interactions between PDZ domain-containing protein and KA receptors thus interferes KA receptor plasma membrane stability and functionality, which may lead to network instability, neuron hyperexcitability, and pathological changes including pain.

**CUB domain proteins**

Neto1 and Neto2 are neuropilin- and tollloid-like (Neto) proteins that contain CUB (complement subcomponent C1r, C1s/sea urchin embryonic growth factor Uegf/bone morphogenetic protein 1)-domains, and interact with KA receptors. Both Neto1 and Neto2 are auxiliary proteins that interact with scaffolding proteins via their PDZ-ligand domains and CUB domains, thereby forming stable complexes with KA receptors. How these Neto proteins impact the properties of KA receptor channels has been shown previously. In brief, Neto proteins typically slow KA receptor deactivation kinetics, explaining why these receptors exhibited distinct properties in vivo from that in cell lines which are lack of Neto protein expression. These Neto proteins additionally control neuronal network inhibition via regulating somatodendritic and presynaptic KA receptors in somatostatin, cholecystokinin, cannabinoid receptor 1, and parvalbumin-containing interneurons. Yet, the specific roles of Neto proteins in the context of KA receptor trafficking and synaptic incorporation remain to be defined. Early studies suggested that Neto1/2 had minimal impact on GluK2 surface expression in a heterologous system, nor were GluK2/GluK5 abundance impacted in PSD fractions obtained from mice lacking Neto1 expression. In addition, Neto1 and Neto2 co-expression failed to bolster exogenous CA1 pyramidal neuron KA receptor responses, even though these cells typically lack postsynaptic KA receptor EPSCs. These findings suggest that Neto proteins have no impact on GluK2-containing KA receptor synaptic incorporation. However, hippocampal synaptic GluK2 was found to be reduced in Neto1 knockout mice, and similar findings were found in cerebellar PSD fractions from mice lacking Neto2, indicating that Neto proteins may be important regulators of GluK2 synaptic targeting. Neto1 and Neto2 have also been shown to increase the cell surface expression of GluK2 in HEK293 cells, and injecting GluK2 and Neto2 into oocytes also enhanced the surface expression of GluK2. These data suggest that Neto proteins may play a role in controlling KA receptor cell surface localization in at least certain contexts.

Given these conflicting findings, exactly how Neto proteins influence the trafficking of GluK2-containing KA receptors remains to be established. The variable model systems used in previous studies may partially cause the discrepancy. In addition, this may also reflect the complex nature of interactions between KA receptors and Neto proteins, which can be impacted by differential subunit expression and cell type-specific interacting protein expression. GluK2 undergoes a range of post-translational modifications such as phosphorylation, ubiquitination, SUMOylation, and palmitoylation, all of which can have a direct or indirect impact on Neto protein activity. For instance, phosphor-deficient mutant Neto2 S409A impeded GluK1 trafficking to synapses, suggesting that Neto2 Ser-409 phosphorylation inhibited synaptic targeting of GluK1. Disrupting Neto protein activity may thus adversely impact KA receptor functionality and the synaptic networks regulated by these receptors.

Several studies have highlighted the impact of such disruptions in pathological contexts. For example, Neto2-knockout mice exhibit decreased...
pentylenetetrazole (PTZ)-induced seizure latency and increased severity of seizures. Furthermore, Sargin D suggested that Neto2-mediated KA receptor modulation is a key driver of fear memory in mice. They found that a lack of Neto2 expression was associated with decreased synaptic KA receptor subunit accumulation at synapses in the brain fear center, driving the development of behavior phenotypes consistent with post-traumatic stress disorder in animals. Vernon and Swanson also found that mice lacking Neto1 and Neto2 expression exhibited normal thresholds for acute thermal and mechanical pain, indicating that KA receptors are not important to acute pain signaling. In contrast, a delayed upregulation of Neto2 following sciatic nerve crush indicated that KA receptors may contribute to the development of chronic neuropathic pain which is Neto-dependent.

In addition to Neto1/2, SEZ6 (Seizure protein 6) is another CUB domain-containing protein that is capable of interacting with KA receptors. Membrane proteome analyses conducted in neurons lacking SEZ6 expression revealed that cell surface GluK2 and GluK3 levels were selectively reduced, and that GluK2 post-ER transport in the secretory pathway had been altered in these neurons. SEZ6 knockout also decreased kainite-evoked currents in CA1 pyramidal neurons in acute hippocampal sections. From a mechanistic perspective, SEZ6 may represent a regulator of KA receptor trafficking. When interactions between these proteins and KA receptors are dysregulated, neuropathies caused by KA receptor-dysfunction may develop. Notably, SEZ6 proteins are widely expressed throughout the brain, and have been implicated in neurodevelopmental and psychiatric disorders. It was shown that a lack of SEZ6 family proteins also impaired motor functions, short-term memory, and cognitive flexibility. Most importantly, recent evidence suggested that SEZ6 is a driver of the onset of inflammatory hyperalgesia. Although there has been no direct evidence supporting the role of SEZ6 interactions with KA receptors in pain, the aforementioned findings strongly suggest the notion that SEZ6 may control KA receptor functionality in this context.

Conclusions

Herein, we provide an updated overview about roles of KA receptors in synaptic transmission and plasticity, through regulating of the receptor trafficking toward and from the synaptic membrane, ion channel gating, and downstream signaling. KA receptor function can be regulated via a host of mechanisms including post-transcriptional RNA editing and alternative splicing, post-translational phosphorylation, and interactions with accessory proteins including PSD-95, NETO, and SEZ6. These regulatory mechanisms have been suggested to play a role in nociceptive signaling, pain transmission and sensitization, highlighting KA receptors as a promising target of pharmacological interventions for pain treatment.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by grants from the National Natural Science Foundation of China (81771181, 81571065); the Beijing Natural Science Foundation (7202053); Beijing Hospitals Authority Youth Program (QML20180105); and Scientific Research Common Program of Beijing Municipal Commission of Education (KM201910025018).

ORCID iDs

Huili Li https://orcid.org/0000-0001-9316-4087
Junfa Li https://orcid.org/0000-0002-1930-9724
Yun Wang https://orcid.org/0000-0003-0695-8861

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