MINIREVIEW

Kainate Receptor Signaling in Pain Pathways

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ABSTRACT

Receptors and channels that underlie nociceptive signaling constitute potential sites of intervention for treatment of chronic pain states. The kainate receptor family of glutamate-gated ion channels represents one such candidate set of molecules. They have a prominent role in modulation of excitatory signaling between sensory and spinal cord neurons. Kainate receptors are also expressed throughout central pain neuraxis, where their functional contributions to neural integration are less clearly

defined. Pharmacological inhibition or genetic ablation of kainate receptor activity reduces pain behaviors in a number of animal models of chronic pain, and small clinical trials have been conducted using several orthosteric antagonists. This review will cover kainate receptor function and participation in pain signaling as well as the pharmacological studies supporting further consideration as potential targets for therapeutic development.

Introduction

Chronic pain is a widespread clinical problem that degrades quality of life and imposes significant financial burdens due to long-term treatment. The pathophysiological mechanisms that give rise to chronic pain are diverse and in many cases poorly understood. Available treatment strategies have limitations that include relatively weak efficacy (nonsteroidal anti-inflammatory drugs), low bioavailability [ziconitide (Prialt; Jazz Pharmaceuticals, Dublin, Ireland)], or high risk of dependency and side effects (opiates); these drugs meet patients' needs to varying degrees. Potential novel therapeutic targets include receptors and channels underlying the aberrant excitation of nociceptive neuronal pathways implicated in neuropathic and other chronic pain states.

Glutamate is the primary excitatory neurotransmitter throughout the peripheral and central nervous systems, and receptors mediating its actions represent candidate targets that have been explored to varying degrees in preclinical and clinical studies. Glutamate acts on both G protein-coupled metabotropic receptors (mGluRs) as well as three families of ligand-gated ionotropic receptors (iGluRs), the *N*-methyl-D-aspartate

(NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors (KARs) (Traynelis et al., 2010). Both mGluRs and iGluRs have been explored as potential therapeutic targets for pain. NMDA receptor antagonists, in particular, have been tested for efficacy in chronic pain models, but their utility has been restricted due to the number of dose-limiting side effects on memory and motor function (Parsons et al., 1998; Chizh et al., 2001; Childers and Baudy, 2007).

KARs could represent more amenable targets given that they play diverse, predominantly modulatory roles in the central and peripheral nervous systems, including in regions critical to transmission and integration of nociceptive input. Pharmacological antagonists selective for a specific KAR subunit have consistently proven to be analgesic for a variety of pain modalities in preclinical models and clinical trials. We will review here the current understanding of how KARs participate in the pathophysiology of pain and evidence that they represent a promising therapeutic target for further drug development.

Kainate Receptor Signaling

Kainate receptors are a family glutamate-gated ion channels composed of varying combination of five different subunits: GluK1 (GluR5), GluK2 (GluR6), GluK3 (GluR7),

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ABBREVIATIONS: ACC, anterior cingulate cortex; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ATPA, (R,S)-2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid; DRG, dorsal root ganglia; EPSC, excitatory postsynaptic current; iGluR, ligand-gated ionotropic receptor; KAR, kainate receptor; LY293558, (3S,4aR,6R,8aR)-6-{2-[1(2)H-tetrazole-5-yl]ethyl}decahydroisoquinoline-3-carboxylic acid; LY382884, (3S,4aS,6S,8aR)-6-[(4-carboxyphenyl)methyl]-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid; mGluR, G protein-coupled metabotropic receptor; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NMDA, *N*-methyl-D-aspartate; PAG, periaqueductal gray.

GluK4 (KA1), and GluK5 (KA2). They were originally defined pharmacologically as an excitatory conductance most sensitive to activation by the seaweed excitotoxin kainic acid (Lodge, 2009), which distinguished them from other iGluRs, the AMPA, and NMDA receptors. Like all iGluRs, KARs operate as tetrameric cation channels, with molecular and functional diversity generated by distinct pharmacological and biophysical properties of the component subunits as well as RNA editing and alternative splicing of subunit transcripts (Perrais et al., 2010; Traynelis et al., 2010; Contractor et al., 2011). Functional diversity is further expanded by the association of KARs with auxiliary subunits, such as the neuropilin- and toll-like proteins (Copits and Swanson, 2012; Tomita and Castillo, 2012).

KARs depolarize neuronal membranes upon activation by glutamate or other agonists, as is typical for iGluRs, but also are unusual in that they display a second mode of signaling mediated by nonionotropic, G protein-coupled activation of protein kinases. How the receptors couple to these metabotropic pathways remains poorly understood. In the central nervous system, KARs use these dual modes of signaling, ionotropic and metabotropic, to modulate neural circuits via diverse mechanisms that include postsynaptic depolarization at a subset of synapses, presynaptic modulation of both glutamate and GABA release, and direct alteration of neuronal excitability through actions on voltage-gated ion channels that regulate action potential firing (Contractor et al., 2011). Several recent reviews discuss molecular and biophysical aspects of KAR function in detail (Paternain et al., 1995; Traynelis et al., 2010; Contractor et al., 2011); here we focus primarily on their association with nociceptive signaling and on KAR-based pharmacological intervention for alleviation of pain.

Peripheral and Central Pain Pathways

To discuss of the role of KARs in pain, it will be of use to describe briefly the relevant signaling pathways and some underlying mechanisms as they are currently understood. Pain is a complex perceptual phenomenon that culminates from a series of signaling events that occurs at multiple levels of the peripheral and central nervous systems (Carrasquillo and Gereau, 2008; Basbaum et al., 2009). Acute pain signals are initiated by the peripheral stimulation of the nerve endings of high threshold A δ and C-fiber nociceptors (often as a result of injury), propagated as action potentials through the cell soma residing in the dorsal root ganglia (DRG) to the dorsal horn of the spinal cord, and then sent through second-order neurons in the thalamus to the cortex for higher-order processing. Descending control of nociceptive signaling originates in the somatosensory cortex and travels through the midbrain to the spinal cord. Among other important functions, this pathway serves to produce an analgesic effect through both stimulation of endogenous opioid receptors and modulation of inhibitory GABAergic tone on nociceptive dorsal horn neurons. This sensory transduction pathway, under normal conditions, allows nociceptors to respond to noxious stimuli, which are then perceived as a painful threat (Carrasquillo and Gereau, 2008; Basbaum et al., 2009). Perception of stimuli as painful serves the purpose of preventing us from further injuring ourselves and protection of the injured tissue to facilitate the healing process.

Modulation of signal transduction in peripheral and central nociceptive pathways will therefore alter perception of pain.

Persistent or recurrent pain is associated with a variety of disorders that can have divergent pathophysiological bases and is a preeminent unmet health issue in modern society. The underlying pathologic manifestation of chronic pain can be the result of injury (e.g., pain from back injury), damage to the nervous system (neuropathic pain), or dysfunction of the pain transduction pathway despite the lack of a peripheral stimulus (e.g., fibromyalgia). Regardless of the cause, chronic pain can be manifested spontaneously (without external stimuli) and result in hyperalgesia and allodynia after an injury has healed and therefore often serves no useful purpose to an organism. Persistent pain arises from pathologic increases in excitability, or sensitization, of one or more peripheral or central components of pain transduction pathways (Carrasquillo and Gereau, 2008; Costigan et al., 2009; Woolf, 2011). Peripheral sensitization results from a reduction in firing threshold and an increase in responsiveness of the peripheral nociceptors, which can result initially from local exposure to neurogenic inflammatory factors such as calcitonin gene related peptide, substance P, ATP, and serotonin and later a welter of noxious chemicals known as an "inflammatory soup" (Basbaum et al., 2009). Central sensitization occurs as nociceptive neurons of the dorsal horn of the spinal cord become persistently hyperexcitable, which can manifest in several forms as wind-up of dorsal horn neurons, long-term potentiation of excitatory synaptic strength, or conditioning-driven sensitization (Seal et al., 2009; Pfau et al., 2011; Woolf, 2011). Mechanistically, central sensitization can be driven by pre- and postsynaptic changes as well as increases in postsynaptic membrane excitability (Latreoliere and Woolf, 2009). Long-term alterations in neuronal excitability is not limited to the spinal cord, and indeed a major component of persistent pain is now thought to arise from adaptive changes in structure and function of a number of central brain regions both directly and secondarily implicated in higher processing of pain-related sensory information (Woolf, 2011).

Kainate Receptors in Pain-Associated Pathways

KARS in Dorsal Root Ganglia Sensory Neurons. KARs are expressed throughout the peripheral and central nervous system, including in those pathways comprising the pain neuraxis. These receptors were first implicated in pain-related physiologic pathways well before the cloning of cDNA encoding the first subunit, GluK1, in 1990 (Bettler et al., 1990). Dorsal roots from sensory neurons were found to depolarize in response to relatively low concentrations of kainic acid (Davies et al., 1979; Agrawal and Evans, 1986), and, in subsequent voltage clamp recordings, iGluR currents detectable in a subpopulation of DRG neurons were clearly attributable to KARs (Huettner, 1990). Indeed, neonatal DRG neurons comprise the only neuronal cell type in the central and peripheral nervous systems in which glutamate-evoked iGluR currents arise completely (or nearly so) from KARs (Huettner, 1990; Sommer et al., 1992; Wong and Mayer, 1993; Mülle et al., 2000; Lee et al., 2001), and in that respect these neurons were particularly useful in the characterization of the receptor currents before pharmacological isolation was made possible with the development of selective AMPA receptor antagonists (Paternain et al., 1995). KAR-mediated

depolarizations were found in dorsal roots classified as C-fibers, which together with the observation that KAR currents arose from DRG neurons with soma of small to medium diameter (Agrawal and Evans, 1986; Huettner, 1990), suggested that these receptors were positioned to impact nociceptive signaling.

Further delineation of the subpopulation of DRG neurons expressing functional KARs strengthened the association with nociceptive signaling. DRGs contain a highly heterogeneous population of neurons, which have been categorized based on criteria that include expression of immunohistochemical markers and sensitivity to activation by specific types of stimuli (Dodd and Jessell, 1986; Carr and Nagy, 1993; Julius and Basbaum, 2001; Hjerling-Leffler et al., 2007; Teichert et al., 2012). Agonist application elicited KAR currents from 50–65% of small to medium diameter (<30 μm) acutely isolated neonatal rat DRG neurons (Huettner, 1990; Lee et al., 2001). KAR-containing neurons also overlapped nearly completely with neurons expressing the glycoconjugate recognized by monoclonal antibody LA4 (Lee et al., 2001), which colabels isolectin B4 positive C-fiber nociceptors that project predominantly to the inner layer of lamina II in the spinal cord (Nagy and Hunt, 1982; Stucky and Lewin, 1999; Gerke and Plenderleith, 2001; Fang et al., 2006). KAR subunit immunoreactivity is found in this afferent termination zone (Petralia et al., 1994; Hwang et al., 2001). Very little coexpression of KARs with substance P was observed, whereas the majority (62%) of neurons with KAR currents also expressed the heat-sensing channel TRPV1 (Lee et al., 2001). These data support the interpretation that KARs detectable in isolated DRG neurons are expressed predominantly by C-fiber, somatostatin-positive nociceptors (Dodd and Jessell, 1985), some of which act as thermal sensors.

The physiologic characteristics and pharmacological profile of DRG KAR currents appear remarkably similar to those gated by recombinant homomeric GluK1 receptors reconstituted in heterologous expression systems (Huettner, 1990; Herb et al., 1992; Sommer et al., 1992; Swanson and Heinemann, 1998). Consistent with this interpretation, GluK1 mRNA is most prominently expressed by DRG and trigeminal neurons, although GluK5 mRNA can also be detected at modest levels (Partin et al., 1993; Sato et al., 1993; Sahara et al., 1997). Isolated DRG neurons derived from mice with a targeted deletion of the GluK1 subunit lack KAR-mediated currents in large part (Mulle et al., 2000; Kerchner et al., 2002) (Fig. 1A). Curiously, deletion of the GluK2 subunit altered current kinetics but not density in isolated DRG neurons (Kerchner et al., 2002), which was unexpected given the relative paucity of mRNA for this subunit in the ganglia (Partin et al., 1993).

GluK1 was also established as the key subunit comprising DRG KARs in pharmacological studies with relatively selective agonists and antagonists. In general, development of discriminatory pharmacological tools has been problematic in the KAR field, but the GluK1 subunit represents a notable exception (Jane et al., 2009). Selective agonists such as ATPA and 5-iodowillardiine activate GluK1-containing and DRG neuronal KARs with similar potencies and gating properties (Wong et al., 1994; Clarke et al., 1997; Swanson et al., 1998; Kerchner et al., 2001b; Wilding and Huettner, 2001). As well, competitive antagonists that inhibited DRG KAR responses all exhibited varying degrees of GluK1 selectivity (e.g., the

Fig. 1. KARs in the pain neuraxis. (A) Dorsal root ganglia neurons express KARs that contain GluK1 as a critical component subunit. Whole cell voltage-clamp recordings were made from acutely isolated dorsal root ganglia neurons from wild-type and GluK1^{-/-} (GluK5) knockout mice. Domoate application to small- to medium-diameter neurons elicited a KAR current in ~60% of wild-type neurons whereas neurons from knockout animals were devoid of detectable responses. Adapted from Mulle et al. (2000). (B) Superficial dorsal horn neurons contain post-synaptic KARs at high-threshold inputs. Voltage-clamp recordings were made from spinal neurons in slice preparations during stimulation of the dorsal entry zone. KAR synaptic currents were only observed when the stimulation strength was high. Adapted from Li et al. (1999). (C) Presynaptic KARs regulate inhibitory transmission between thalamic reticular and relay neurons. ATPA, a selective GluK1 agonist, reduced IPSP amplitudes reversibly in intracellular recordings from thalamic relay neurons in slice preparations. Adapted from Binns et al. (2003).

decahydroisoquinolines LY293558, LY382884, and LY466165 and the UBP series of willardiine derivatives) (Bleakman et al., 1996; O'Neill et al., 1998; Dolman et al., 2005; Weiss et al., 2006; Dargan et al., 2009), as did a very weak partial agonist, MSVIII-19, used as a functional antagonist (Qiu et al., 2011). A strong consensus therefore exists as to the critical molecular components and pharmacological selectivity of the KARs expressed by nociceptive neurons in the DRG.

Kainate receptors expressed by DRG neurons serve at least two functions: peripheral chemosensing and presynaptic modulation of glutamate release from afferent terminals in

the dorsal horn of the spinal cord. Similar to other iGluRs, KARs can be found in sensory nerve endings, where they presumably sample the extracellular environment and respond to physiologic or pathologic stimuli to initiate action potential firing (Carlton et al., 1995; Coggeshall and Carlton, 1998; Du et al., 2006; Miller et al., 2011). Accordingly, peripheral application of KAR agonists elicits nociceptive signaling by sensory neurons and consequent pain behaviors (Ault and Hildebrand, 1993; Du et al., 2006). Experimentally induced inflammation in the rodent paw enhanced KAR subunit immunoreactivity and agonist-induced responses to thermal and mechanical stimulation (Carlton and Coggeshall, 1999; Du et al., 2006), which was proposed to occur as a result of upregulation of receptor numbers in the KAR-expressing subpopulation of sensory neurons (Du et al., 2006).

KARs localized to the presynaptic sensory neuron terminal in the dorsal horn regulate release of vesicular glutamate (Kerchner et al., 2001b), as do both AMPA and NMDA receptors (Liu et al., 1994; Lee et al., 2002). Excitatory postsynaptic currents (EPSCs) recorded from dorsal horn neurons that were evoked by stimulation of DRG neurons in either cocultures or intact slice preparations were strongly reduced by activation of KARs with either kainic acid or the GluK1-selective agonist ATPA (Kerchner et al., 2001b, 2002; Lee et al., 2002). This presynaptic negative feedback modulation has been proposed to occur either via stimulation of primary afferent depolarization (Lee et al., 2002) or alternatively through a G protein-mediated metabotropic signaling pathway that diminishes calcium channel activity (Rozas et al., 2003). Whether suppression of excitatory afferent transmission accurately reflects the physiologic action of KARs when synaptically released glutamate constitutes the stimulus is less clear, and indeed modulation of synaptic strength was observed to be biphasic and facilitatory upon application of a very low concentration of kainic acid (Youn and Randic, 2004). A biphasic effect on EPSC amplitudes also occurs upon activation of presynaptic hippocampal mossy fiber KARs with exogenous agonist (Contractor et al., 2000; Kamiya and Ozawa, 2000; Schmitz et al., 2000). Physiologic activation of that particular population of hippocampal presynaptic KARs clearly enhances synaptic strength (Contractor et al., 2001). It remains unknown how synaptically released glutamate alters the modulatory function of DRG KARs. Presynaptic suppression of EPSC amplitudes by kainic acid was absent when DRG neurons from GluK1^{-/-} mice were co-cultured with wild-type dorsal horn neurons (Kerchner et al., 2002), supporting the interpretation that the GluK1 subunit is a key constituent of the presynaptic KAR complement. Knockout studies also have implicated GluK2-containing receptors in presynaptic modulation, but these conclusions have been difficult to explore further given the absence of GluK2-selective agonists. In sum, a variety of approaches clearly implicate KARs in regulation of primary afferent transmission from nociceptive neurons to dorsal neurons in the superficial spinal cord, the initial site of synaptic integration in nociceptive signaling pathways.

KARs in the Spinal Cord. KARs are located at pre- and postsynaptic sites in the dorsal horn of the spinal cord, in both projection pathways as well as local microcircuits, where they recapitulate many of the physiologic roles initially described in hippocampal circuits (Contractor et al., 2011). How these diverse activities participate in spinal neural integration is

less well understood, however. Spinal neurons express KAR mRNAs across many laminae (Tolle et al., 1993; Dai et al., 2002), with GluK5 mRNA particularly dense and GluK4 quite sparse (also see Allen Brain Atlas, <http://mousespinal.brain-map.org>). Pharmacologically isolated KAR-mediated EPSCs (EPSC_{KAR}) were detected in voltage-clamped lamina II neurons upon stimulation of primary sensory afferents (Li et al., 1999) (Fig. 1B) but appear to be absent from C-fiber postsynaptic sites in lamina I (Dahlhaus et al., 2005). Like EPSC_{KAR} found at hippocampal mossy fiber, CA3, and a number of other synapses (Castillo et al., 1997; Vignes and Collingridge, 1997), lamina II spinal EPSC_{KAR} exhibited slow gating kinetics and small amplitudes relative to EPSC_{AMPA} at the same synapses (Li et al., 1999). The extended time course of decay of postsynaptic KAR depolarizations is thought to prolong the temporal window for synaptic integration and enhance neuronal excitability during higher-frequency bursts of stimuli (Frerking and Ohliger-Frerking, 2002; Sachidhanandam et al., 2009), although there is no direct evidence yet that they serve this function in superficial spinal neurons. High stimulation intensities were required to elicit EPSC_{KAR} from superficial spinal neurons, whereas lower intensities of stimulation exclusively evoked EPSC_{AMPA}. These results suggested that AMPA and kainate receptors were unequally segregated at synapses with low- and high-threshold afferents and that postsynaptic KARs were therefore preferentially distributed to synapses contacting Aδ/C-fiber (high-threshold) nociceptive inputs (Li et al., 1999). Moreover, retrograde labeling of spinal ascending processes from the thalamus confirmed the presence of EPSC_{KAR} in principle output neurons in layer II of the dorsal horn. Both GluK1 and GluK2 subunit-containing KARs contribute to whole cell KAR currents in cultured dorsal horn neurons, based on studies from knockout animals (Kerchner et al., 2002), but it remains unclear whether these represent overlapping or separate populations of receptors.

KARs expressed by spinal interneurons regulate local inhibitory tone. Inhibitory and excitatory contacts are in close proximity on dendrites of dorsal horn neurons, and crosstalk between the neurotransmitter systems occurs in part via spillover of glutamate and heterosynaptic activation of KARs located on interneuron terminals (Kerchner et al., 2001a). Presynaptic KARs act as biphasic feedback pathway for GABA and glycine release through a two-step process: (1) glutamate binding to KARs depolarizes presynaptic terminals, triggering calcium entry and enhanced release of inhibitory transmitters, which in turn then (2) bind to G protein-coupled GABA_B autoreceptors that reduce GABA and glycine vesicle release probability. The latter negative feedback is likely to occur preferentially following a strong burst of afferent excitatory input (Kerchner et al., 2001a). Both GluK1- and GluK2-containing KARs appear to contribute to heterosynaptic regulation of inhibitory transmission in the dorsal horn (Kerchner et al., 2002).

Glutamatergic signaling is a central component of sensitization that occurs at the level of the spinal cord in chronic pain states (Szekely et al., 2002). AMPA, NMDA, and mGluRs have been known to be key signal transducers in the sensitization process, whereas KARs have been implicated in one form of sensitization, wind-up following inflammation (Stanfa and Dickenson, 1999), but have not been explored in other mechanisms of central sensitization that occur at the level of the dorsal horn. As modulators of both excitatory and

inhibitory synaptic tone, they are well positioned to shape sensitization. Inhibition of GABA and glycine receptors in the spinal cord increases A-fiber-mediated excitatory transmission in the superficial dorsal horn (Baba et al., 2003) and produces tactile allodynia (Sivilotti and Woolf 1994). A loss of inhibitory tone in the dorsal horn can play an important role in chronic pain conditions (von Hehn et al., 2012). For example, partial peripheral nerve injury results in a reduction of GABA-induced IPSCs in the dorsal horn (Janssen et al., 2011; Moore et al., 2002), which appears to be due to an excitotoxic loss of GABAergic interneurons (Scholz et al., 2005). Given that KARs are engaged as heterosynaptic modulators of inhibitory tone, it is reasonable to postulate that their function might be altered in pathologic states of hyperexcitability, particularly given that KAR antagonists are effective analgesics (see below).

KARs in Supraspinal Pain Pathways. Nociceptive dorsal horn neurons project via the spinothalamic tract to the ventral posterolateral nuclei in the thalamus. Inhibition of GluK1-containing KARs is known to attenuate enhanced signaling in the spinothalamic tract following peripheral neuropathy in primates (Palecek et al., 2004). The thalamus as a whole also exhibits a diverse nuclei-specific expression of KARs mRNAs (Wisden and Seeburg, 1993; Bahn et al., 1994; Ibrahim et al., 2000). Thalamic relay neurons receive inhibitory input from reticular neurons, which contain presynaptic GluK1-containing KARs positioned on the inhibitory terminals that reduce GABA release in electrophysiological recordings from the rat ventrobasal thalamus; the receptors therefore were proposed to function as a mechanism for disinhibition of relay neurons (Salt, 2002; Binns et al., 2003) (Fig. 1C). Inhibition of thalamic KARs with a GluK1-selective antagonist degraded excitatory responses in response to sensory input (whisker stimulation) (Binns et al., 2003), which, if analogous mechanisms occur in nociceptive pathways, could be of benefit in cases of spinalthalamic hyperexcitability underlying aberrant pain states. Presynaptic KARs also modulate release of glutamate from cortical inputs to thalamic neurons in an intriguing bidirectional manner: presynaptic GluK1-containing KARs at cortical terminals on relay neurons depress glutamate release; conversely, non-GluK1 receptors facilitate release on terminals contacting reticular neurons (Miyata and Imoto, 2009). Because relay neurons are inhibited by reticular neurons, the two distinct populations of corticothalamic KARs are poised to reduce excitability of relay neurons through combined monosynaptic and disynaptic control of glutamate release. Postsynaptic KARs in ventrobasal thalamic neurons also contribute to temporal integration at excitatory connections with corticothalamic but not lemniscal inputs (Binns et al., 2003; Miyata and Imoto, 2006); analogous studies have not been carried for spinothalamic inputs to ventral posterolateral relay neurons.

Thalamocortical projections associated with nociceptive signaling target a number of cortical structures that include the somatosensory, insular, and anterior cingulate (ACC) cortices, in which KARs play diverse pre- and postsynaptic functions (Huettnner, 2003; Lerma, 2006; Contractor et al., 2011; Koga et al., 2012). In the somatosensory cortex, presynaptic KARs regulate glutamate release at thalamocortical synapses in young rats (Kidd et al., 2002; Jouhannau et al., 2011), which also transiently expressed a postsynaptic KAR that undergoes activity-dependent downregulation early in

development (Kidd and Isaac, 1999). Layer V neurons, on the other hand, express KARs at predominantly extrasynaptic sites and exhibit a very small EPSC_{KA} that nonetheless is maintained past early developmental stages (Eder et al., 2003). The ACC, which is associated with the perception of stimuli as painful and the affording of emotional significance, particularly in chronic pain states (Vogt and Sikes, 2000; Apkarian et al., 2005; Basbaum et al., 2009), also contains both pre- and postsynaptic KARs. Activation of presynaptic GluK1-containing KARs facilitates GABA release from interneuron synapses on layer II/III pyramidal neurons in the ACC and potentially modifies tonic inhibitory currents that impact neuronal excitability (Wu et al., 2007b), similar to what had been described previously in the CA1 region of the hippocampus (Cossart et al., 1998). On the postsynaptic side of the synapses, characteristically slow EPSC_{KA} evoked in layer II/III pyramidal neurons in both the ACC and the insular cortex were dependent upon the presence of both GluK1 and GluK2 subunits (Wu et al., 2005; Koga et al., 2012). How these diverse functions of KARs are altered as a consequence of the structural and circuit adaptations that occur in cortical regions in neuropathic or other chronic pain states (Costigan et al., 2009; Metz et al., 2009; Li et al., 2010) has not been examined but could be relevant to understanding the mechanistic basis for the analgesic effect of KAR antagonists.

KAR actions in descending pain pathways have not been characterized to a great extent with the exception of the midbrain periaqueductal gray (PAG), a brain stem nucleus in a variety of physiologic activities, including descending pain modulation. Tonic inhibition regulates output of PAG neurons, and presynaptic GluK1-containing KARs modulate the release of GABA in cultures of dissociated PAG neurons (Nakamura et al., 2010).

This brief review of KAR actions in pain-related pathways underscore three important points: (1) these receptors are expressed at all levels of the pain neuraxis examined to date, (2) their functional activities are consistent with roles in modulation of circuit excitability, and (3) little is known regarding how KAR function is altered in chronic pain states.

KARs as Targets in Animal Models of Pain and Clinical Studies

The initial characterization of KARs in peripheral nociceptive pathways stimulated interest in exploring how signaling by these receptors might contribute to acute or chronic pain-related behaviors and therefore represent potential targets for therapeutic treatment. Constraints exist on how effectively KARs can be selectively targeted with antagonists, however, and in large part only receptors containing the GluK1 subunit can be inhibited while avoiding antagonism of other types of KARs (or AMPA receptors in many cases) (Jane et al., 2009). As well, all compounds tested to date act at the orthosteric glutamate binding site on KARs as competitive antagonists or strongly desensitizing partial agonists, which tend to be polar molecules with low bioavailability and therefore poor drug candidates. Allosteric modulators like those isolated for AMPA, NMDA, and mGluRs, which could be viable alternatives that achieve both better selectivity and desirable chemical characteristics, comprise a pharmacological domain largely unexplored for KARs; to date very few negative

allosteric modulators have been reported and those have been only partially characterized at the mechanistic level (Valgeirsson et al., 2003; Christensen et al., 2004; Valgeirsson et al., 2004). Application of recent high-throughput screening approaches to discovery of allosteric modulators of NMDA and mGluRs (e.g., Ogden and Traynelis, 2011; Sheffler et al., 2011; Hansen et al., 2012) suggest there are grounds for optimism that similar efforts could produce novel pharmacology for KARs.

Preclinical Models of Pain. KAR antagonists have been tested in a variety of animal models of acute pain as well as persistent pain arising from inflammatory and neuropathic insults, and a limited number of studies in gene-targeted mice have supplemented those pharmacological approaches. Early studies noted that nonselective antagonism of both AMPA and kainate receptors ameliorated inflammatory signaling and pain in rats (e.g., Hunter and Singh, 1994; Jackson et al., 1995; Simmons et al., 1998; Stanfa and Dickenson, 1999). Subsequent use of more selective compounds have generally led to conclusions that inhibition (or genetic ablation) of GluK1-containing receptors produces analgesic effects following induction of either inflammatory or neuropathic persistent pain (Wu et al., 2007a and summarized below) (see chemical structures in Fig. 2). These observations are consistent with the predominance of GluK1 receptors in nociceptive DRG neurons and the established role of this subunit in presynaptic receptors that regulate inhibitory transmission. On the other hand, the participation of KARs predominantly composed of the other principle subunits, GluK2 and GluK3, cannot be excluded yet because there simply are not adequately selective pharmacological tools to test their potential roles in nociception.

Competitive antagonists with selectivity for GluK1 receptors were among the first KAR-targeting compounds to

demonstrate analgesic efficacy. A series of molecules designed on a decahydroisoquinoline scaffold exhibited varying degrees of specificity for receptors containing this subunit (Jane et al., 2009); the most selective, LY382884, attenuated pain-associated behaviors following formalin injection into the paw of rats without ataxic effects that accompany coincident inhibition of AMPA receptors (Simmons et al., 1998) (Fig. 3A). Esterified prodrugs of LY382884 and a less selective decahydroisoquinoline, LY293558, were analgesic when delivered orally in the formalin model as well as inflammatory thermal and mechanical hyperalgesia models of pain (Dominguez et al., 2005; Jones et al., 2006). LY293558 also reduced mechanical hyperalgesia in a rat model of postoperative pain (paw incision) when administered intrathecally (Lee et al., 2006). Interestingly, the antinociceptive efficacy of the decahydroisoquinolines correlated well with their activity on GluK1-containing receptors specifically when introduced centrally into the cisterna magna (Jones et al., 2006),

KAR Competitive Antagonists with Analgesic Activity

KAR Agonists with Analgesic Activity

Fig. 2. Structures of several KAR competitive antagonists and weak partial agonists that attenuated pain behaviors in inflammatory, neuropathic, or other models. For details, see text and references therein.

Fig. 3. KARs contribute to formalin-induced pain behaviors. (A) Intrathecal injection of the GluK1 weak partial agonist (effectively a functional antagonist) reduced pain behaviors following formalin injection into mice in a dose-dependent manner. This figure has been reproduced with permission of the International Association for the Study of Pain (IASP). The figure may not be reproduced for any other purpose without permission. (B) Formalin injection into mouse paw did not elicit pain behavior in the GluK1^{-/-} mouse, whereas GluK2^{-/-} responded to the same degree as wild-type animals. Adapted from Ko et al. (2005).

suggesting that supraspinal KARs comprised an important site of action of the antagonists.

Partial agonists that effectively desensitize GluK1 receptors also exhibit antinociceptive activity. The high-affinity agonist (2S,4R)-4-methylglutamate (SYM2081) potently activates and desensitizes both GluK1- and GluK2-containing KARs and has been used extensively as a functional antagonist because receptors remain in a nonconducting state in the presence of the compound. SYM2081 has been tested in a number of pain models, including chronic constriction injury, capsaicin, and carrageenan injections, in which it provides analgesic effect on both mechanical and thermal hyperalgesia when given both intrathecally and intraperitoneally (Sutton et al., 1999; Ta et al., 2000; Turner et al., 2003). Similarly, MSVIII-19, a synthetic derivative of a tetrahydrofuran toxin isolated from a marine sponge (Sanders et al., 2005), also acts as a high-affinity inhibitor of GluK1-containing receptors and was analgesic for thermal and mechanical hyperalgesia in inflammatory and chronic constriction models but not in acute or visceral pain models (Qiu et al., 2011).

The pharmacological evidence for participation of GluK1-containing receptors in nociceptive signaling was supported by the observation that gene-targeted mice lacking this subunit exhibited attenuated pain behaviors. Paw licking caused by either capsaicin or formalin injection was greatly reduced to vehicle control levels in GluK1 but not GluK2 knockout mice (Ko et al., 2005) (Fig. 3B). Mechanical allodynia induced by complete Freund's adjuvant was normal in the knockout mice, however, as was acute thermal and mechanical sensitivity. In summary, studies with a variety of pharmacological agents and gene-targeted mice support the hypothesis that GluK1-containing receptors play a key role in enhanced pain sensitivity for several sensory modalities following experimentally induced inflammation or neuropathy.

GluK1-containing receptors are expressed by trigeminal neurons (Sahara et al., 1997), leading to interest in the role of these KARs in migraine. Animal models of migraine use surrogate biochemical measures, such as protein extravasation and c-fos activation in the nucleus caudalis, rather than pain behaviors, to assess efficacy of compounds. Both GluK1-selective decahydroisoquinoline compounds prodrugs as well as the highly selective fluorinated antagonist LY466195 effectively reduced both these measures following stimulation of the trigeminal nerve (Filla et al., 2002; Weiss et al., 2006). Conversely, selective activation of GluK1 receptors with a willardiine analog attenuated calcitonin gene related peptide-induced vasodilation caused by dural stimulation, an experimental paradigm that mimics some of the neurogenic processes thought to occur during migraine (Andreou et al., 2009). The efficacy of topiramate, an anticonvulsant, in prevention of migraines also was recently proposed to arise from antagonistic actions on KAR-driven signaling in the trigeminovascular pathways (Andreou and Goadsby, 2011). Among other pharmacological activities, topiramate appears to selectively reduce signaling through GluK1-containing KARs through mechanisms that are not well understood (Gryder and Rogawski, 2003; Braga et al., 2009).

KARs as Therapeutic Targets

The success of KAR antagonists in a variety of animal models of persistent pain and migraine led to several small

clinical trials with the aim of validating these receptors as therapeutic targets. The decahydroisoquinoline LY293558 was efficacious in alleviation of inflammatory pain caused by capsaicin injection (Sang et al., 1998), postoperative pain (Gilon et al., 2000), and migraine pain and associated conditions (Sang et al., 2004), with minimal side effects and acceptable tolerance. Beneficial effects were observed with LY293558 (also known as tezampanel or NGX424) in a phase II clinical trial focused on alleviation of migraine pain (Murphy et al., 2008). Further studies on LY293558 (also known as tezampanel or NGX424), or its oral prodrug form (NGX426), for treatment of migraine appear to have been suspended in 2009, although positive results from a large phase I trial with NGX426 were reported by the most recent licensee of the molecule, Raptor Pharmaceutical Co. (press release, 11/23/2009).

Conclusions

KARs play integral signaling roles at multiple levels of the pain neuraxis, and abundant preclinical and clinical evidence suggests that pharmacological targeting of GluK1-containing receptors, in particular, ameliorates hyperalgesia and allodynia in a number of persistent pain states. A number of important and challenging questions remain unresolved, however. For example, the efficacy of KAR antagonists in reducing inflammatory or neuropathic pain, but the absence of effect (in most studies) in acute pain, suggests that KAR function is altered following central sensitization in a way that is poorly understood currently. How KARs impact signaling in descending pathways also is unclear. Finally, we do not know if KAR antagonists have their analgesic effects through inhibition of a particular population of receptors—peripheral, spinal, or supraspinal—although the latter is most consistent with results from studies on decahydroisoquinolines. The premature end to clinical studies with LY293558 is unfortunate, given the apparent efficacy of the drug in both migraine and a variety of pain states in patients; in effect this therapeutic approach remains one with a great degree of promise in an area of substantial unmet clinical needs in the human population.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Bhargoo, Swanson.

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