# Glutamate and the Presynaptic Control of Spinal Sensory Transmission

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Sensory neurons, in particular the small- and medium-diameter cells that sense painful stimuli, express both ionotropic and metabotropic glutamate receptors, which may regulate transmission between sensory neurons and their targets in the spinal cord dorsal horn. Although the roles that these receptors play in normal physiology are not completely understood, recent work has provided strong evidence for their ability to modulate transmitter release from primary afferent terminals. NEUROSCIENTIST 8(2):89–92, 2002

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Sensory information enters the spinal cord along the axons of primary sensory neurons whose cell bodies are located in dorsal root ganglia (DRG). Each DRG cell gives rise to a single axon that bifurcates, sending one branch out to peripheral sensory endings and the other into the cord via the dorsal roots. The afferent axons extend several segments anterior and posterior from their point of entry and send collateral branches into specific layers of the dorsal horn. Virtually all DRG cells are thought to use glutamate as their primary transmitter. In addition, many DRG cells express peptide cotransmitters including calcitonin gene-related peptide (CGRP), substance P, and somatostatin. Anatomical studies have demonstrated that significant subsets of DRG cells express mRNA or immunoreactivity for ionotropic and metabotropic glutamate receptors (Coggeshall and Carlton 1997). Moreover, physiological recordings have confirmed the presence of functional glutamate receptors on the cell bodies of DRG cells, and in some cases on isolated sensory axon preparations. Until recently, there was relatively little known about the function of glutamate receptors expressed by sensory neurons, although it has long been speculated that they might serve as presynaptic regulators of primary afferent transmission in the spinal cord (e.g., Agrawal and Evans 1986). In this update, we review the evidence for presynaptic glutamate receptors and their role in regulating spinal sensory transmission.

## Kainate Receptors

Kainate receptors are present on the cell bodies and axons of a subset of small-diameter sensory neurons (Davies and others 1979; Agrawal and Evans 1986; Huettner 1990; Hwang and others 2001). Cells bearing the surface carbohydrates recognized by the LA4 and LD2 antibodies account for all of the cells expressing kainate receptors (Lee and others 2001). These neurons terminate in layer II of the dorsal horn and are believed to carry primarily nociceptive sensory information (Dodd and Jessell 1985). Several papers (Sommer and others 1992; Swanson and others 1998) have noted the strong similarities in physiological properties between recombinant receptors formed by homomeric expression of the GluR5 subunit and those of native kainate receptors expressed by DRG cells. Consistent with those observations, GluR5 is highly expressed by small-diameter DRG cells (Bettler and others 1990; Sato and others 1993), although expression of other kainate receptor subunits has also been demonstrated (Partin and others 1993; Petralia and others 1994).

In addition to recent EM immunocytochemistry demonstrating kainate receptor expression on the central terminals of primary afferent fibers (Hwang and others 2001), studies of synaptic transmission indicate that presynaptic kainate receptors regulate glutamate release from sensory nerve fibers. Kerchner, Wilding, and others (2001) recorded synaptic transmission from DRG cells to spinal dorsal horn neurons in dissociated co-cultures and in acute spinal cord slices. In the context of selective AMPA receptor blockade with (RS)-4-(4-aminophenyl)-1,2-dihydro-1-methyl-2-propylcarbamoyl-6,7 methylenedioxyphthalazine (SYM2206), NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) were recorded either in $Mg^{2+}$-free medium or by clamping the postsynaptic cell at +40 mV. Activation of kainate receptors with low to moderate doses of kainate (0.1 to 10 µM), or with the more selective agonist (RS)-2-amino-3-(3-hydroxy-5-tertbutylisoxazol-4-yl) propanoic acid (ATPA) (2 µM), caused a 60% to 80% reduction in the strength of transmission. ATPA was found to be selective for kainate receptors expressed by DRG neurons (Kerchner, Wilding, and others 2001; Wilding and Huettner 2001), providing compelling evidence for a presynaptic action by this agonist.

More recently, a second form of regulation by presynaptic kainate

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receptors has been described in cultures of rat dorsal horn (Kerchner, Wang, and others 2001). In this case, application of kainate in the presence of TTX was shown to elicit an eight- to tenfold increase in spontaneous release of transmitter from dorsal horn inhibitory interneurons. This effect of kainate required extracellular $Na^+$ and $Ca^{2+}$ and was blocked by inhibitors of N and P/Q type voltage-gated $Ca^{2+}$ channels, suggesting a mechanism that involved depolarization of terminals and activation of presynaptic $Ca^{2+}$ channels. In contrast to the strong enhancement of miniature inhibitory postsynaptic currents (mIPSCs), kainate suppressed action potential-evoked transmitter release. Interestingly, this effect was prevented by coapplication of a $GABA_B$ receptor antagonist, suggesting that the increase in spontaneous action potential-independent release events induced by kainate was sufficient to cause activation of presynaptic $GABA_B$ receptors, thereby inhibiting subsequent evoked release. An important functional implication for presynaptic kainate receptor-mediated regulation of inhibitory transmitter release in the spinal cord is that it provides a mechanism for excitatory inputs to interact with the spinal local inhibitory neuronal network.

Because kainate receptors are expressed in small DRG cells, such presynaptic regulation may be especially important for nociceptive transmission. In vitro studies of spinal nociceptive reflexes indicate that ATPA can inhibit dorsal root–evoked ventral root potentials (Procter and others 1998). In addition, intrathecal application of the GluR5-selective antagonist, LY382884, was shown to suppress single unit responses evoked by repetitive C-fiber stimulation (Stanfa and Dickenson 1999), suggesting that activation of GluR5 kainate receptors by endogenous glutamate may actually enhance transmission in vivo and contribute to nociception. Involvement of spinal kainate receptors in behavioral nociception was also demonstrated by Li and colleagues (1999), who found that intrathecal injection of the AMPA and kainate receptor antago-

nist CNQX produced greater antinociceptive effects than an antagonist selective for AMPA receptors alone.

The relative contribution of GluR5, GluR6, and other kainate receptor subunits to acute nociception versus chronic pain remains unclear. Behavioral experiments using systemic drug administration show that GluR5-containing kainate receptors may play a role in chronic pain, although the targets of drug action in these experiments may have included receptors in the periphery, the spinal cord, and supraspinal structures where kainate receptors are also expressed (Proctor and others 1998; Simmons and others 1998; Sutton and others 1999). Collectively, these in vivo studies indicate that pharmacological manipulation of kainate receptors, including presynaptic receptors on spinal neurons or primary afferents, has the potential to affect nociceptive transmission, but clearly more work must be done before useful therapeutic strategies can be developed. In addition, further work is needed in adult spinal cord slice preparations to determine whether presynaptic kainate receptors affect glutamate release from nonnociceptive afferent fibers.

## AMPA Receptors

Expression of AMPA receptor subunits by DRG cells has been documented by northern analysis (Partin and others 1993) and immunocytochemistry (Sato and others 1993; Lee 2001), but electrophysiological evidence for these receptors was lacking until recent work demonstrated functional AMPA receptors in a significant fraction of small-diameter neurons cultured from embryonic rat DRGs (Lee and others 1999; Lee 2001). Although responses mediated by AMPA receptors were much more rare in cell bodies isolated from postnatal rats, recordings in acute slices from postnatal animals showed that application of AMPA receptor agonists to the dorsal horn depolarized the terminals of primary afferents and suppressed the evoked release of transmitter elicited by dorsal root stimulation (Lee 2001).

## NMDA Receptors

Evidence for expression of presynaptic NMDA receptors comes from both anatomical and physiological studies. Lovinger and Weight (1988) reported whole-cell currents mediated by NMDA receptors in freshly isolated DRG cells from adult rats. The prevalence of cells responding to NMDA appears to be lower in younger animals (Huettner 1990). Expression of the NR1 subunit of NMDA receptors by both large and small DRG cells has been documented by in situ hybridization (Sato and others 1993) and immunocytochemistry (Liu and others 1994). Transport of NR1 along central and peripheral DRG axons was demonstrated, and NR1 immunoreactivity was detected on approximately 35% of presynaptic terminals in the dorsal horn of the spinal cord (Liu and others 1994). Less is known about DRG cell expression of NR2 subunits, which are required for formation of functional channels.

Intrathecal injection of NMDA in rodents elicits nociceptive behaviors and stimulates the release of substance P from primary afferents (Liu and others 1997); however, it remains to be established whether these effects of NMDA are mediated by a direct action on presynaptic NMDA receptors or by an indirect mechanism involving NMDA receptors on postsynaptic spinal neurons. It also appears possible that release of glutamate and substance P may be regulated differently, as application of NMDA to spinal slices inhibits primary afferent transmission mediated by postsynaptic AMPA/kainate receptors (Bardoni and others 2000; Li and Zhuo, unpublished observations).

## Metabotropic Receptors

Subsets of DRG cells, as well as dorsal horn neurons, express several different G-protein-coupled metabotropic glutamate receptors. Group I receptors (mGluR1 and mGluR5), which mobilize calcium via phospholipase C, are found in small- and medium-diameter DRG cells and on central and peripheral sensory axons (Valerio and others 1997; Hongge and others 1999; Bhave and

others 2001). In addition, group II (mGluR2/3) and group III (mGluR4a, mGluR7) receptors, which down-regulate adenylyl cyclase, are present on small-diameter DRG cells (Ohishi and others 1995; Petralia and others 1994; Hongge and others 1999; Azkue and others 2001). Slice recordings from neurons in superficial layers of the dorsal horn have shown that exposure to agonists selective for group II or group III receptors causes substantial reduction in primary afferent transmission, with minimal direct effect on postsynaptic cells (Gerber and others 2000). Group I receptor agonists also reduce primary afferent transmission (Zhong and others 2000), possibly by a presynaptic reduction in release. In addition, however, group I agonists elicit direct depolarization of postsynaptic spinal neurons (Zhong and others 2000). Blockade with selective antagonists of group I or group II mGluRs prevents the induction of long-term depression at synapses formed by primary afferent A-δ fibers, whereas a group III antagonist had no effect on LTD (Chen and Sandkühler 2000).

## Perspective

The localization of glutamate receptors mainly to small-diameter, nociceptive DRG cells makes them a promising target for analgesic drugs. Activation of presynaptic glutamate receptors by exogenous agonists typically produces inhibition of evoked transmission (Gerber and others 2000; Kerchner, Wilding, and others 2001); however, much less is known about the conditions under which these receptors may be activated by endogenous glutamate. In addition to their role as presynaptic modulators of transmitter release in the dorsal horn, glutamate receptors may also function at sensory nerve endings in the periphery (e.g., Bhave and others 2001). Future studies on the behavioral effects of selective agonists and antagonists, as well as studies with subunit knockout mice (Mulle and others 1998), may help to unravel the physiological functions that presynaptic glutamate receptors play in spinal sensory pathways.

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