Chapter 19

Other Molecules Involved in Pain Transmission

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The aim of this chapter is to bring together data on a number of other molecules that have not been covered in detail in the preceding chapters, but also to include data from other pain states on molecules that may play similar important roles in headache.

EXCITATORY AMINO ACIDS

Glutamate is the major excitatory neurotransmitter, found throughout the mammalian central nervous system (CNS), where it contributes to synaptic plasticity, learning, and memory, as well as pain and sensory transmission as well as neurodegenerative disease states (16). Glutamate acts on a wide range of receptors and these are subdivided into two main groups, ionotropic and metabotropic receptors. The ionotropic receptor (iGluR) group can be further divided into subcategories (16).

α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) receptors
Kainate receptors
N-methyl-D-aspartate (NMDA) receptors

Ionotropic glutamate receptors contain cation-specific ion channels (16). However, the metabotropic receptors (mGluRs) are G-protein linked receptors, coupled to GTP-binding proteins and therefore modulate intracellular cell signaling via certain messenger systems (16). There is an entire family of mGluR G-protein linked receptors ($mGluR_{1-8}$) that exert both inhibitory and excitatory actions on both pre- and postsynaptic sites (16). Interestingly, mGluR2/3 and 5 receptor subunits are expressed widely in the superficial dorsal horn and thus serve to modulate sensory transmission at the spinal level.

The focus of this section is the ionotropic glutamate receptors, because these have been studied in significantly more detail than the metabotropic receptors. The three main iGluR receptors are named according to their agonist specificities, although it is important to note that although kainate and AMPA receptors (non-NMDA receptors) are structurally and functionally different, their respective agonists share the same affinity for the other receptor. Molecular and expression cloning have identified the cDNA's encoding subunits within each of the three ionotropic receptor (16).

AMPA: GluR 1-4, similar size, 68 to 73% sequence homology.
Kainate: GluR5-7, similar size, 75 to 80% sequence homology. KA1 and KA2 are larger than GluR5-7. KA1 and KA2 share 70% sequence homology.
NMDA: NR1 (eight splice variants reported) and $NR2_{A-D,}$ low sequence homology (<18%), 40 to 50% between NR2 subunits. NR3A may be a modulatory subunit, important in development of synaptic elements (7,16).

The AMPA receptor is a major glutamate receptor, mediating fast excitatory transmission throughout the mammalian CNS (16). AMPA receptors are permeable to both Na^+ and K^+ ions, and relatively impermeable to Ca^{2+} ions. However, AMPA receptors, which express anything but the GluR2 subunits (i.e., GluR1, 3, and 4) are indeed permeable to Ca^{2+} ions. Studies have shown that AMPA receptors are located throughout the CNS, particularly the hippocampus and superficial cerebral cortex (16). Despite important roles in pain transmission, the AMPA receptors mediate much CNS excitatory transmission including tactile events and so are unlikely to be a feasible target.

The kainate receptor is widely distributed, although very little is known regarding its function compared to the AMPA receptor. Kainate receptor subunits (iGluR5-7) are distributed within lamina I-III of the spinal cord (35). DRG neurons are also believed to contain functional kainate receptors, strongly implicating expression of kainate receptors on primary afferent neurons in the superficial dorsal

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horn (13). Indeed, it is believed that presynaptic kainate receptors serve to regulate sensory, particularly nociceptive, transmission in the superficial dorsal horn (13), and so may be a target for controlling afferent activity.

The NMDA receptor mediates excitatory neurotransmission in the entire nervous system (26). However the role of the NMDA receptor is extremely diverse and is implicated in neuronal plasticity, gene expression, as well as neuronal growth and survival within the CNS (26). The NMDA receptor has unique properties compared to the AMPA and kainate receptors. NMDA receptors are blocked by Mg^{2+} in a voltage-dependent manner, so at normal resting membrane potentials the NMDA receptor does not allow the passage of ions through its pore and so is nonfunctional (16). Interestingly, NR1-NR2A and NR1-NR2B subunits appear to be blocked by Mg^{2+} ions more efficiently than the other heteromeric complexes, which form the NMDA receptor (17).

NMDA receptor subunits form heteromeric receptor complexes consisting of four subunits (tetrameric) with large current response (16,26). It is therefore widely believed that NR1 NMDA receptor subunits are co-expressed with NR2 subunits, forming functional NMDA receptors. Combination of the NR1 subunit with different NR2 subunits (A–D) form a variety of functional NMDA receptor subtypes but with varying properties; it is thus clear that NR2 subunits are primarily modulatory subunits and differ somewhat in their functionality (16).

Interestingly, studies in rat brains have revealed that the NMDA receptor subunits are expressed in distinct areas of the CNS in varying quantities. NMDA receptors are largely distributed in the hippocampal CA1 region, as well as other areas of the brain (predominantly the forebrain). The NR1 subunit, consistent with these findings, is also found abundantly throughout the entire CNS. However, the regional distributions of the NR2 subunits are relatively distinct and include spinal cord (16,35).

Immunocytochemical studies in the rat have revealed that, within the dorsal horn of the spinal cord NMDAR2B, subunits are found predominantly in lamina I-III, as are NMDAR1 subunits (35). Other reports suggest that NR2D subunits can also be located in the superficial dorsal horn. Interestingly, NMDAR2A and NMDRAR2C subunits were not found in the dorsal horn, suggesting these receptors play a minimal role in nociceptive transmission at the spinal cord level (35). These points are pertinent to the issue of attempting to control the actions of a ubiquitous transmitter. The available drugs that act on the NMDA receptor are presently ketamine, which is effective in patients with difficult pains yet with undesirable side effects, and drugs such as dextromethorphan, which lack potency and have failed in some trials with facial pains. Subtypes of the receptor may allow selective actions on those receptors that are implicated more in pain than global forebrain function (5).

It is clear that both glutamate and the amino acid glycine are required to activate the NMDA receptor channel, and such findings suggest glycine is a co-agonist in the activation and function of NMDA receptor activity (34). Furthermore, the NMDA receptor structure also contains modulatory sites for polyamines, protons, redox agents, and Zn^{2+} suggesting that other agents may affect the activity of NMDA receptors, alongside glycine (26).

NMDA receptors, as mentioned, have a variety of distinct properties. One of the most intriguing properties is the wind-up effect consistently attributed to NMDA receptor channels, following repetitive, high-threshold stimuli of nociceptive neurons (8,11). *Wind-up* describes the sharp increase in response following repetitive constant stimuli and this effect is thought to underlie the mechanisms for acute pain transmission and central sensitization in the CNS (8,11). Central sensitization frequently arises following tissue injury or neuronal insult. Such damage often evokes increased excitability of dorsal horn neurons, increased receptive field sizes, and heightened response properties in nociceptive and sensory nerve fibers (8,11,25,27).

In electrophysiologic studies, wind-up can be evoked following repetitive electrical stimuli administered at C-fiber thresholds (8,11). Repetitive noxious stimuli prompts peptide released from presynaptic C-fiber afferents within the superficial laminae and this, in turn, results in the depolarization required to remove the voltage-dependent Mg^{2+} block of NMDA receptors. The recruitment of NMDA receptors in the neuronal response to repetitive noxious stimuli is also enhanced by nitric oxide production (8,11,25). Wind-up is thus the result of progressive increases in neuronal response properties, which are no longer proportional to the original evoked stimulus and which decay after minutes if the afferent barrage subsides. Electrophysiologic studies whereby the wind-up response is significantly blocked by NMDA receptor antagonists confirm such a role of NMDA receptors in the wind-up response and alterations after peripheral (27). Similar studies in the trigeminal complex have revealed that wind-up, which depends on NMDA receptors, can be observed and that *c*-fos (33) labeling of nociceptive neurons in nucleus caudalis can be modulated by ionotropic and metabotropic glutamate receptors (20). There is further convincing evidence that sagittal sinus activation activates NMDA-dependent mechanisms in nucleus caudalis (6).

Following pathology or trauma, changes in the normal transmission of sensory information can result in various abnormal pain-related behaviors. Longer term changes than wind-up can be induced in spinal neurons. Also attributed to the NMDA receptor is long-term potentiation (LTP) and long-term depression (LTD), whereby NMDA receptors underlie a persistent change in synaptic strength and functionality lasting for many hours. Bliss and Lømo (4) used brief high-frequency trains of electrical stimuli to

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increase efficiency of synaptic transmission at hippocampal synapses. This phenomenon was named LTP; it was also shown that synaptic transmission could also be depressed for long time periods (LTD). Since then LTP and LTD have been proposed to represent synaptic models for storage of information throughout the CNS.

LTP relates to pain; it has also been seen in the dorsal horn of the spinal cord. Here, using field potentials (15) and by recording single deep neurons (30) and by patch clamping superficial neurons (21) clear long-term increases in excitability have been reported in the spinal cord. On this basis, LTP may underlie some forms of afferent-induced hyperalgesia (21).

Repetitive, high-frequency electrical stimulation of the sciatic nerve induces LTP of synaptic transmission from Aδ- and C-fibers (21,30) in vitro and in vivo. Furthermore, strong natural noxious stimuli such as skin burns, contusions, inflammation, and nerve injury have been shown to induce spinal LTP. Simultaneous activation of multiple receptors such as the NMDA receptor, the NK1 receptor for substance P, and mGluRs are required for the induction of spinal LTP. The magnitude and time course of spinal LTP depends on the type and intensity of conditioning stimulation and the activity of descending controls (21,30). LTD of synaptic strength at Aδ-fiber synapses in the superficial spinal dorsal horn has also been demonstrated following burst-like stimulation of Aδ-fibers. However, selective stimulation of the low-threshold Aα/Aβ–fibers does not induce LTP (21,30). Thus, the conditioning stimuli that induce synaptic LTP in the superficial spinal dorsal horn are similar to those that trigger hyperalgesia. Further, spinal LTP and injury-induced hyperalgesia share signal transduction pathways, time course, and pharmacologic profile, which makes use-dependent LTP an attractive model of injury-induced, longer term central sensitization and hyperalgesia.

Understanding these events at spinal and trigeminal levels will lead to a better understanding of central hyperexcitability. However, it is very clear that spinal transmission is not simply a question of local events, but can be modulated from certain supraspinal sites, such as the PAG and rostral ventromedial medulla (RVM) (19,32). Earlier behavioral studies paid attention to the PAG–RVM descending inhibitory influence on the spinal cord, based on reductions in pain behavior following electrical stimulation, or after morphine (2). However, it is now becoming very clear that the same areas can support the opposite function—the contribution to pain of excitatory drives arising from the same brainstem areas, in particular the RVM (22).

Recent reviews cover the body of anatomic and pharmacologic evidence for descending facilitatory functions and their potential contribution to the substrates of central sensitization after injury (22,32). After nerve neuropathy, chemical inactivation of the RVM or selective lesions of certain RVM neurons can reduce the tactile and thermal hyperalgesia that follow the injury (22). Thus, descending facilitatory influences may then underlie some of the neuropathy-induced plasticity observed at the spinal level. One such descending excitatory pathway uses 5HT as a major transmitter, released into the spinal cord from pathways that originate in the RVM, and driving major excitatory effects by activating spinal $5HT_3$ receptors expressed on the nerve terminals of small-diameter afferents (9,36). Blocking spinal $5HT_3$ receptors with the selective antagonist ondansetron or genetic deletion of the receptor confirms the pronociceptive role of this receptor (9,36).

ORIGINS OF THE DESCENDING FACILITATION ARE NK1-EXPRESSING LAMINA I NEURONS

Substance P is located within and released (along with glutamate) from C-fiber afferents in the spinal cord. Substance P acts preferentially on the NK1 receptor, and the other tachykinins, NK A and B act on NK 2 and 3 subtype receptors. It is thought that the majority of substance P receptors are located on post-synaptic neurons in the dorsal horn, particularly nociceptive neurons (31).

Lamina I neurons, which express the NK1 receptor for substance P, are predominantly projection neurons that respond only to noxious stimuli; that is, they send ascending axons to a number of brainstem areas that are important in both sensory and affective aspects of nociceptive processing (31). A key supraspinal target is the parabrachial (PB) area, which in turn projects to brainstem areas such as the PAG and the RVM. There is good evidence that trigeminal neurons activated by noxious stimuli also have ascending PB projections (1). These areas are at the origin of many descending projections that, in turn, project back to the spinal cord, forming complex circuits that allow the brain to control spinal activity. The 5HT3 is the final target for one of these pathways.

NK1-expressing lamina I neurons play an important role in central sensitization, and have been shown to be critical for the allodynia and hyperalgesia seen in animal models of persistent pain (12,18,21). The use of SP-conjugated to the neurotoxin saporin (SP-SAP) allows for the selective ablation of lamina I NK1 receptor-expressing population of neurons. This was first used to show a marked attenuation of pain behavior in rats in various models of inflammatory pain (12). Furthermore, SP-SAP treatment prevented allodynia when given either before or after the development of neuropathic pain (21). More recently, recordings of spinal neurons revealed key marked changes in the responses of deeper dorsal horn neurons. Not only was there reduced central sensitization of deep dorsal horn neurons and diminished wind-up, but the receptive field size and the mechanical and thermal evoked responses of spinal neurons were attenuated. In models of

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persistent pain, reduced responses to chemical (formalin) inflammation were seen (28).

Because most of the effects of ablating these NK1 neurons were reproduced by blocking the pronociceptive $5HT_3$ receptor in the spinal cord, these cells must form part of an important ascending pathway to the brainstem (28). Thus, loss of the ascending lamina I–PB pathway must underlie these reduced pain responses, seen both behaviorally and in deep spinal neurons. Importantly, the only neuronal responses that did not depend on the $5HT_3$ receptor were wind-up and LTP, although they were highly sensitive to SP-SAP treatment. Thus, wind-up and LTP are intrinsic spinal phenomena relying on spinal events, but not on descending excitations (28).

The hypothesis that the brain can amplify spinal pain processes through a serotonergic circuit is supported by recent findings that reveal an enhanced efficacy of ondansetron after peripheral nerve injury compared to normal conditions (29). $5HT_3$-mediated descending pathways are therefore not only crucial for the full coding of polymodal peripheral inputs by spinal neurons, but also appear to be enhanced after neuropathy, showing a capacity for change in the spino-bulbo-spinal loop. Thus, supraspinal sites can drive sustained and enhanced facilitatory influences on the spinal cord following neuropathy, in keeping with data on these types of pathways in chronic pain states (3,21,29). $5HT_3$ receptor blockade had a greater effect on the mechanical punctate-evoked responses compared to thermal responses in neuropathic animals (29). This, and the report that spinal transection (severing supraspinal circuits) blocks nerve injury-induced tactile allodynia but not thermal responses (3), may indicate strong descending facilitatory influences on allodynia. Not only could this be a major contributor to the mechanisms that underlie central sensitization in concert with spinal wind-up and LTP, but these findings suggest that allodynia may have central generators. Because the lamina I–PB pathway impacts emotional areas of the brain that alter in states of fear and anxiety, these affective states may contribute to the aberrant processing of afferent inputs. One could speculate that this sort of central neuronal activity may become altered with the general malaise that precedes migraine and then contribute to the resultant pain and allodynia independently of peripheral afferent activity. In conclusion, the behavioral molecular and electrophysiologic evidence for a descending facilitatory pathway originating from the RVM mediated by $5HT_3$ receptors and driven by lamina I neurons is convincing (29). Furthermore, the data suggest that these circuits exert differential supraspinal controls on selective primary afferent input, which could allow the brain to exert submodality-dependent regulation of spinal neuronal responses.

These states of excitation can be controlled by inhibitory controls and three examples are briefly covered. γ-Aminobutyric acid (GABA) is the most well-recognized inhibitory amino acid within the CNS. More specifically, it is evident that GABAergic axons terminate presynaptically on mechanical afferents or their postsynaptic neurons in lamina I and II of cat and monkey spinal cords, thus controlling the transmission of nociceptive mechanical stimuli Electrophysiologic studies whereby Aδ-fiber and noxious mechanical responses are facilitated in deep dorsal horn neurons following bicuculline mediated block of $GABA_A$ receptors, confirm these effects (14,24). Reductions in spinal GABA tone would allow mechanical inputs to exert greater activation of spinal neurons, which may contribute to allodynia like processes.

Galanin is also found within nociceptive afferents and is believed to have both excitatory and inhibitory actions within the spinal cord. However, it has been shown that, following peripheral nerve injury, exogenous galanin exerts primarily inhibitory effects on spinal dorsal horn neuronal response properties (10), making this peptide an interesting target.

Finally, noradrenaline is in other descending pathways that may exert a modulatory effect on pain transmission. Noradrenaline pathways originate predominantly from A5 and A6 clusters in the pons and project to the spinal cord (19). Indeed, inhibitory α_2 receptors are located throughout the superficial and deep dorsal horn layers. Studies largely implicate noradrenaline as a major transmitter in inhibitory descending pathways involved in the control and modulation of pain transmission (19).

Thus, the balance of activity in afferent, spinal, and descending pathways and the interactions between the excitatory and inhibitory systems with a complex pharmacology determines the final sensation. The challenge is to understand how these mechanisms translate into the clinic and then to attempt manipulations to restore normal function.

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