

MODULATION OF SENSORY INPUT TO THE SPINAL CORD BY PRESYNAPTIC IONOTROPIC GLUTAMATE RECEPTORS

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The centrally directed process of dorsal root ganglion (DRG) neurons conveys peripheral stimuli to the spinal cord and dorsal column nuclei. From these stations, other central neurons, in thalamus and/or brainstem, are interposed before the peripheral event finally reaches the somatosensory cortex. The perception of quality and intensity of the stimulation is not linearly related to the physical properties of the stimulus itself as a result of modulatory mechanisms present at all levels of the chain of propagation of the peripherally-generated signal.

The unique functional characteristics of synaptic endings at different levels of the somatosensory path contribute to the modulation of the release of their neurotransmitter and, thus, to their synaptic efficacy. The release of neurotransmitter by terminals of the central process of DRG neurons is modulated by a variety of presynaptic mechanisms. As an example of these mechanisms, DRG neurons express and transport receptors that are inserted in the membrane of the central axon terminal and are, therefore, referred to as presynaptic receptors. These include ligand-gated ionotropic and metabotropic receptors. Receptor ligands include the main inhibitory neurotransmitter in the CNS, γ -aminobutyric acid (GABA). Receptors for GABA can be the ionotropic subtype A, GABA_A (3) and the metabotropic subtype B, GABA_B (76). Presynaptic GABA receptors on central terminals of DRG neurons are believed to play an important role in one of the best studied forms of modulation of peripheral input to the spinal cord, presynaptic inhibition. This form of inhibition is mediated by axo-axonic contacts by GABAergic interneurons, and was reported to affect large myelinated, low-threshold mechanosensory (A α) fibers (2, 69). Terminals of these interneurons activate GABA_A receptors at the synaptic endings of DRG afferents thereby opening Cl⁻ channels (69). The equilibrium potential for Cl⁻ in DRG central terminals is above resting potential, due to the expression in DRG neurons of a Cl⁻ co-transporter which uses the energy from the Na⁺ gradient generated by the Na⁺-K⁺-ATPase pump to increase the intracellular concentration of Cl⁻. Thus, the opening of Cl⁻ channels in DRG terminals results in an efflux of Cl⁻ ions which depolarizes, rather than hyperpolarizes, the axon terminal membrane (4, 81). Depolarization of the synaptic ending can inhibit release of neurotransmitter by

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probably depending on the concentration of the agonist, Ca^{2+} influx at Ca^{2+} -permeable receptors, and other factors not yet adequately explored (21, 27, 34).

Although all AMPA receptor subunits may be synthesized by DRG neurons (20, 43, 47, 71, 75), evidence for central transport has been provided mainly for the GluR3 and GluR4 subunits (47), suggesting a selective expression of Ca^{2+} -permeable homomeric or heteromeric complexes of presynaptic AMPA receptors in central afferents. While presynaptic GluR4 is primarily expressed in terminals in superficial laminae (I and II) of the dorsal horn, presynaptic GluR3 is expressed primarily in deeper laminae which receive low-threshold mechanosensory input (47). In the only study thus far focused on the effects of activation of AMPA receptors in central terminals of DRG neurons in rats, excitatory postsynaptic currents in dorsal horn neurons of spinal cord slices were suppressed, in the presence of a cocktail of pharmacological agents ensuring that the decrease in glutamate release was mediated presynaptically (43).

Central transport of NR1 from DRG neurons was first reported in C fibers by Liu et al (45). Since glutamate is released by terminals of central processes of DRG neurons, presynaptic NMDA receptors, like kainate and AMPA receptors in the same terminals, were interpreted as autoreceptors. More recent work has demonstrated expression of presynaptic NR1 in a large proportion of terminals of A fibers as well (48). Different subtypes of the NR2 subunit may colocalize with NR1 in different types of DRG neurons and their central terminals (13, 49, 52) to form heteromeric complexes which require NR1 and one subtype of NR2 to be functional (54). Presynaptic NMDA receptors may facilitate release of substance P from C fibers (46, 50, 51). In contrast, activation of the same receptors inhibits glutamate release from central terminals of DRG neurons (10).

As the presence of PIGR on central terminals of DRG neurons has become validated by the evidence summarized above, several questions arise. Among these, three are most prominent: 1) What is the source of glutamate that activates PIGR? 2) What is the mechanism by which activation of PIGR may modulate release of glutamate? 3) What is the role of PIGR in the context of modulation of sensory input to the spinal cord?

1) Glutamate which activates PIGR can spill over from the synaptic cleft at glutamatergic synapses. Theoretical and experimental studies have determined the diffusion rate of glutamate in synaptic complexes in hippocampus and cerebellum (9, 27, 29). This rate is compatible with activation of PIGR by spilled over glutamate from the same, or immediately adjacent, terminal. However, this possibility has not been tested experimentally for central terminals of DRG neurons. Many factors besides diffusion rate, may influence the efficacy of spilled over glutamate in activating PIGR, including the distance of the receptors from the synaptic cleft, the affinity of the receptors, the density of glutamate transporter molecules, the rate of desensitization, and other presynaptic mechanisms that may variably determine synaptic strength in different synapses (8).

Another potential source of glutamate for PIGR activation is astroglia. The classical view of astrocytes as passive supporting elements for neurons has been repla-

3) A role for PIGR in the context of modulation of sensory input to the spinal cord is, at present, only a matter of speculation. If it will be confirmed that the predominant effect of PIGR activation is to decrease glutamate release, these receptors may provide a more generalized, and perhaps more powerful, mechanism acting in parallel with GABA-mediated presynaptic inhibition. In fact, PIGR may be the main presynaptic inhibitory mechanism in A δ - and C fibers in spinal cord, which may not be affected by GABA-mediated presynaptic inhibition (see above). Were this the case, PIGR may have a prominent role in the control of nociception.

Expression of PIGR, however, is not limited to central terminals of DRG neurons, nor is it limited to glutamate-releasing spinal neurons. Recent work has shown that about a third of GABA-ergic neurons in superficial laminae of the dorsal horn of the spinal cord express PIGR (Lu *et al.*, in press). If activation of PIGR in these inhibitory neurons results in decrease of GABA release, the net result of PIGR activation in a network of neurons releasing different transmitters in the laminae of termination of A δ - and C fibers can vary and even contribute to central sensitization and hyperalgesia.

SUMMARY

Sensory input from peripheral nerves to the dorsal horn of the spinal cord is mediated by a variety of agents released by the central terminals of dorsal root ganglion (DRG) neurons. These include, but are not limited to, amino acids, especially glutamate, peptides and purines. The unraveling of the mechanisms of synaptic transmission by central terminals of DRG neurons has to take into account various ways in which the message from the periphery can be modulated at the level of the first central synapse. These include postsynaptic and presynaptic mechanisms. Homomeric and heteromeric complexes of receptor subunits for the different transmitters released by DRG neurons and interneurons, clustered at the postsynaptic site of central synapses, can be expressed in different combinations and their rate of insertion into the postsynaptic membrane is activity-regulated. Inhibitory mechanisms are an important part of central modulation, especially via presynaptic inhibition, currently believed to involve GABA released by inhibitory intrinsic neurons.

Recent work has established the occurrence of another way by which sensory input can be modulated, i.e. the expression of presynaptic ionotropic and metabotropic receptors in central terminals of DRG neurons. Microscopic evidence for the expression, in these terminals, of various subunits of ionotropic glutamate receptors documents the selective expression of glutamate receptors in functionally different DRG afferents. Electrophysiological and pharmacological data suggest that activation of presynaptic ionotropic glutamate receptors in central terminals of DRG neurons may result in inhibition of release of glutamate by the same terminals. Glutamate activating presynaptic receptors may spill over from the same or adjacent synapses, or may be released by processes of astroglial cells surrounding synaptic terminals.

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blocking propagation of action potential via inactivation of voltage-gated Na⁺ channels, or by an increase in membrane conductance that "shunts" Na⁺ currents (26, 27, 30). Evidence for presynaptic inhibition of small myelinated (A δ) and unmyelinated (C) fibers is not as robust as for A α fibers, but several investigators have suggested depolarization of terminals of these afferents (14, 18, 28, 32, 66). However, the sparseness of GABAergic axo-axonic contacts over terminals of C fibers (2, 11) and the absence of dorsal root reflexes in C fibers (81) suggest that mechanisms other than classic presynaptic inhibition may be responsible for the modulation of C fiber afferents in spinal cord (69).

Besides GABA receptors, DRG neurons express both ionotropic and metabotropic glutamate receptors (20, 33, 44, 45, 49, 52, 59, 62, 63, 71, 74). Both types of glutamate receptors are transported peripherally (15, 22), and centrally (16, 35, 37, 42, 43, 45, 47, 48). Peripherally transported receptors can be activated by application of glutamate or its agonists to the skin (15, 77, 83). Their discovery has opened the way for a new interpretation of the mechanism for sensitization and for the exploration of novel therapeutic strategies for pain (23, 25). The remainder of this article will focus on presynaptic ionotropic glutamate receptors (PIGR) in terminals of DRG central processes and their functional properties.

Different ionotropic glutamate receptors are identified, and referred to, according to the agonist that activates them: i) kainate, ii) α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and iii) *N*-methyl-D-aspartic acid (NMDA). Receptors in each group can be heteromeric association of different subunits. PIGR in central terminals of DRG neurons were first suggested by selective depolarization of C fibers by kainate (1, 24), and by the microscopic demonstration of NR1 receptors in terminals of primary afferents in the superficial laminae of the dorsal horn (45). In subsequent work on a variety of preparations, presynaptic kainate, AMPA and NMDA receptors have been proposed to modulate the release of glutamate from terminals of central processes of DRG neurons (10, 40, 42, 43, 70).

The expression of PIGR in terminals of central processes of DRG neurons was surveyed by confocal and electron microscopy in the dorsal horn of the adult rats. The results showed which subunits of kainate, AMPA, and NMDA receptors are expressed in terminals of myelinated and/or unmyelinated afferents. The functional types of terminals that express the different subunits were inferred on the basis of their morphology and simultaneous labeling with functional markers.

The KA1 and KA2 subunits of the kainate receptor are expressed in a variety of terminals, including nociceptive afferents to laminae I and II (Lucifora *et al.*, in preparation). Of the three subunits of the low-affinity kainate receptor, GluR5 is also expressed in terminals of A- and C fibers (35, 59, 80, 82), and it is the critical subunit for activation of the heteromeric complex of presynaptic kainate receptors (41). The degree to which high- and low-affinity kainate receptor subunits may be coexpressed in the same presynaptic terminals has not been tested, but GluR5 and KA2 form the predominant heteromeric pair in postsynaptic kainate receptors in the hippocampus (34). Activation of presynaptic kainate receptors on central endings of DRG neurons may result in an increase (42) or a decrease (40) of glutamate release,

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