

The Inhibitory Neuronal Circuit of Spinal Cord in Neuropathic Pain

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ABSTRACT

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Aim of review: Inhibitory interneurons, including GABAergic neurons and glycine neurons, regulate nociceptive information and non-nociceptive information in spinal dorsal horn. Emerging evidence showed that disinhibition of inhibitory interneurons of neuronal circuit in spinal dorsal horn is a pivotal mechanism of neuropathic pain after nerve injury.

Method: In this view, we summarized the recent researches of the structure of inhibitory neurons in spinal dorsal horn and disinhibition of inhibitory interneurons after nerve injury and discussed the primary mechanism.

Recent findings: Much progress has been made with the construction of inhibitory neuronal network in spinal dorsal horn and the dysfunction of inhibitory interneurons in these networks since inhibitory interneurons in spinal dorsal horn firstly integrate nociceptive information and non-nociceptive information from primary afferent fiber and separate non-nociceptive stimuli from nociceptive information. Disinhibition of inhibitory interneurons underlies hyperalgesia and allodynia after nerve injury.

Summary: Loss of inhibitory function of neurons in inhibitory network in the dorsal horn contributes to hyperalgesia and allodynia. The findings of these inhibitory networks provide a new evidence for preventing and curing neuropathic pain.

Neuropathic pain is a challenge for clinicians because the treatment of neuropathic pain is still unsatisfactory. Therefore, increasing understanding of the mechanisms that underlie neuropathic pain will be beneficial for the discovery of new molecular therapy targets. The gate control theory of pain is one of important mechanisms of pain. The theory is the idea that physical pain is modulated by interaction between different neurons. According to the postulate of Melzack and Wall (1), the nerve fibers project to the substantia gelatinosa (SG) of the dorsal horn and the first central transmission cells of the spinal cord. Inhibitory interneurons in the SG act as the gate and determine

which signals should reach the T cells and then go further through the spinothalamic tract to reach the brain. Thus, spinal inhibitory interneurons play an important role in maintaining normal pain. Spinal dorsal horn is the first site of integration of nociceptive and non-nociceptive information within the central nervous system (CNS). Under normal physiological conditions, spinal inhibitory interneurons, including gamma-aminobutyric acid (GABA) neurons and glycine neurons, form axo-axonic contacts with the termination of primary afferent fiber (PAF), exerting presynaptic inhibition control over sensory transmission. Meanwhile, these inhibitory interneurons generate postsynaptic inhibi-

tion, causing hyperpolarization of postsynaptic neurons and decreasing the excitation of excitatory neurons (2). In spinal dorsal horn, especially in lamina I-III, 30% of inhibitory interneurons are organized into intralaminar and trans-laminar neuronal circuit, attenuating the nociceptive inputs to dorsal horn neurons or separating non-nociceptive inputs from nociceptive inputs (3,4). Inhibitory effect of spinal interneurons on PAF and excitatory interneurons is beneficial for maintaining normal pain and isolation pain from other sensory, preventing allodynia and hyperalgesia.

Neuropathic pain is characterized by allodynia and hyperalgesia. Disinhibition of spinal inhibitory neuronal circuit is one of the mechanisms of neuropathic pain (5). Several possible mechanisms have been proposed for the disinhibition of inhibitory network in spinal dorsal horn after nerve injury, including loss of spinal interneurons, reduction of transmitter release, diminished activity of these cells and decreased effectiveness of GABA and glycine (6). However, details of actual circuitries within spinal dorsal horn mostly remain unclear. Similarly, mechanism of the disinhibition of inhibitory network is controversy or obscure. In this view, we focus on dysfunction of spinal inhibitory neuronal circuit.

Structure of Inhibitory Neuronal Circuit in Spinal Dorsal Horn

Spinal dorsal horn receives and integrates peripheral nociceptive and non-nociceptive information. The information is processed by complex circuits involving primary afferent fiber axons, excitatory and inhibitory interneurons, and transmitted to projection neurons for relay to several brain areas. Primary afferent fibers (PAF) innervate peripheral tissues and organs and terminate spinal cord horn. PAF respond to nociceptive and non-nociceptive stimuli from peripheral tissues and organs and transmit nociceptive and non-nociceptive information to interneurons in spinal dorsal horn. According to their diameter and whether or not they are myelinated, PAF are classified into the following categories: the larger myelinated ($A\beta$), fine myelinated ($A\delta$) and unmyelinated (C) fibres. Myelinated δ or unmyelinated C fibers terminate in lamina I and II

of dorsal horn (7). These fibers respond to nociceptive stimuli to induce pain, such as mechanical, thermal, or chemical stimuli and convey pain to interneurons and projection neurons in the superficial laminae of the dorsal horn. Myelinated $A\beta$ fiber terminates at interneurons in spinal lamina III/IVM (2). Myelinated $A\beta$ fiber responds to non-nociceptive stimuli, including touch and itch, and transmits non-nociceptive information to interneurons in spinal dorsal horn. Primary afferent axons form synaptic connection with interneurons of spinal dorsal horn. As all primary afferents have an excitatory action on their postsynaptic targets, local inhibitory interneurons play a critical role in gating of nociceptive transmission. In spinal dorsal horn, inhibitory interneurons account for the vast majority of interneurons, including GABA-immunoreactivity and glycine-immunoreactive cells (8). Inhibitory interneurons presynaptically inhibit primary afferents. Inhibitory transmitter released from spinal interneurons, such as GABA and glycine, can cause hyperpolarization of primary afferent fibers, and decrease transmitter release from primary afferent (9). Furthermore, these inhibitory transmitters mediate inhibitory postsynaptic currents in excitatory neurons of spinal neurons, reducing excitation of spinal neurons (6).

Distribution of Inhibitory interneurons is highly distinctive within spinal dorsal horn. 25%, 30% and 40% of interneurons are GABAergic neurons in laminae I, II and III/IV (LI, LII, and LIII/IV) (8). Approximately 33%, 27%, and 64% GABAergic neurons co-express glycine in lamina I, II, and III (3). Electrophysiological studies demonstrate that inhibitory neuronal synapses in the lamina II are GABAergic and in lamina III are pure glycinergic (6, 10). These inhibitory interneurons form local neuronal network, which is the region-specific circuit in character.

Inhibitory interneurons exhibit intra- and trans-laminar neuronal connectivity in the superficial spinal dorsal horn. Inhibitory interneurons mainly connect with inhibitory neurons in intralamina (4). On the basis of morphological and electrophysiological properties, inhibitory interneurons in the superficial dorsal horn are classified four types: islet cells, central cells, radial cells, and vertical cells. Kato et al. (4) found that the averaged inhibitory input zone for the lami-

na II population as a whole elongated to the dorsal and ventral borders of lamina II. Some of inhibitory interneurons, such as islet cells, were restricted in spinal lamina II with co-location of their dendrites and body in the same lamina (11). In addition, islet cells display dense axonal arborizations within lamina II. In addition, apart from intralaminar neuronal connectivity, trans-laminar neuronal connectivity is an important part of Inhibitory neuronal circuit in spinal dorsal horn. The inhibitory input zone extended into lamina I in one dorsally placed vertical cell, and into the outer part of lamina III in one ventrally located radial cell. Furthermore, polysynaptic mechanoreceptive A β afferent end in lamina III-IV and connect inhibitory neurons in lamina I and II through inhibitory inter neurons circuit (12). The pathway of superficial dorsal horn neurons that receive this polysynaptic A-beta input is normally silence with blockade of glycine inhibitory interneurons (7), isolating non-nociceptive stimuli from nociceptive stimuli.

GABAergic Neuronal Circuit in Spinal Dorsal Horn

Nociceptive information was transmitted from PAF to projection neurons in lamina I via dorsally-directed neuronal circuits of the superficial laminae of spinal dorsal horn. These neuronal circuits consist of excitatory and inhibitory interneurons. Generally, spinal inhibitory interneurons release inhibitory neurotransmission GABA or co-expression glycine, limiting the excitability of spinal terminals of PAF and facilitating normal sensory processing and the spatial and temporal discrimination of sensory stimuli (13, 14). In the superficial laminae of spinal dorsal horn, GABAergic neuronal circuit is composed of GABAergic neurons in different connection, morphology, function and spatial organization (Figure 1).The inhibitory circuit regulates information propagated by the excitatory pathways (4).

GABAergic neurons form presynaptic connection with nociceptive primary afferent and modulate the input of nociceptive primary afferent fiber in spinal dorsal cord. Immunohistochemistry studies showed that GABAA receptors located on axon terminals of nociceptive primary afferent fiber, which provide a molecular basis for the presynaptic inhibition (12). Lu and Perl com-

bined electrophysiological method with morphologic study to delineate neuronal circuits in spinal dorsal horn (15, 16). In spinal lamina II, GABAergic neurons present four classes: islet, central, vertical and radial cells. Islet cells and central cells were located in lamina Ili, IIo and near the border of laminae Ili and IIo. Their dendritic trees spread in the rostrocaudal direction (17). Somata of vertical neurons located in lamina IIo and pass ventrally through laminae II-IV. The ventrally-directed dendritic arbor allows them to integrate inputs from spinal intralaminar and trans-laminar neurons (4). Radial cells extend in several directions in lamina II. All islet cells are inhibitory neurons with immunocytochemical features of GABAergic cells (17). Central cells include both excitatory and inhibitory neurons. Most vertical and radial cells are mainly excitatory interneurons (18). Furthermore, these cells also are inhibitory neurons (11, 19). But Yasaka T et al. (20) observed that radial cells were excitatory interneurons. Ganley RP et al. found that islet neurons received monosynaptic excitatory inputs exclusively from C- afferents and primary-afferent-evoked GABAergic inhibitory inputs only from A δ -fibres in lamina III (19). However, lamina III GABAergic inhibitory neurons is unlikely responsible for mechanical allodynia (21). Central cells receive excitatory inputs from C- afferents and primary- afferent-evoked inhibitory inputs mediated by both A δ - and C- fibres. The excitatory inputs to radial cells were mediated by both A δ - and C- fibres (17). In addition, the lamina II central, and the radial cells, receive an intralaminar-derived inhibitory input from islet cells. Primary-afferent-evoked inhibitory input to islet, central, and vertical cells was exclusively GABAergic. In spinal lamina I-II, a common synaptic connection consisted of presynaptic GABAergic islet cell and a postsynaptic central cell. Central cells in lamina Ili exhibit monosynaptic inhibitory linkages to vertical cells in lamina IIo. Meanwhile, the monosynaptic connections exist between vertical cells in lamina IIo and projection neurons in lamina I (13). Therefore, the inhibitory input to lamina I neurons derives predominantly from vertical cells inhibited by islet cell (17). Furthermore, lamina I projection neurons receive excitatory input from C fibers. A transgenic mouse,

whose lamina II GABAergic neurones were labeled with green fluorescent (GFP) controlled by the mouse prion promoter. The PrP-GFP neurones have characteristics of the tonic central cell category. The tonic central cell exhibited monosynaptic inhibitory linkages to a nearby islet cell and vertical cells or from a nearby islet cell (22) (Figure 1).

Diversity of GABAergic interneurons also display by coexistence with non-overlapping chemical marker in spinal lamina I-III, including neuropeptide Y (NPY), galanin, neuronal nitric oxide synthase (nNOS) or parvalbumin (23). Subpopulations of GABAergic neurons in laminae I-III form a distinct inhibitory neuronal circuit. NPY- expression GABA boutons account for 15% in laminae I-II and 5% in lamina III (24). Axons that contain NPY and GABA preferentially innervate large projection neurons with neurokinin 1 receptor in lamina III and protein kinase C γ (PKC γ) immunoreactive interneurons in lamina II inner (24). Only 6% NPY-expressing GABAergic neurons present presynaptic connection with innervate giant lamina I projection cells that lack the NK1r. The percentage of nNOS immunoreactive is 17%, 19% and 6% of the GABAergic neurons in laminae I, II and III, respectively. Furthermore, lamina I projection cells that lack the NK1r received synapses from axons of NOS- expression GABAergic neurons (25). Meanwhile, 2-4% of GABAergic neurons that contain nNOS are presynaptic to PKC γ -immunoreactive neurons in laminae II and III (26). In addition, GABAergic axons that contain galanin locate in lamina I and the outer half of lamina II (IIo). The body of galanin cells mainly locate in laminae I and IIo, and little in the inner half of lamina II (IIi) and lamina III. Only 6% of GABAergic in laminae I-IIo and ~1% of those in IIi-III contain galanin-immunoreactive (23). Galanin receptor 1 (GAL1) are expressed in DH neurons of lamina I-III and in the deeper DH. Galanin receptor 2 (GAL2) mostly distribute in ventral horns and in area X of spinal cord (27). Galanin produced a biphasic dose-dependent effect on spinal nociceptive excitability. In pain processing, the activation of postsynaptic GAL1 extent anti-nociceptive actions with decrease membrane excitability of dorsal horn neurons at high doses, while the activation of pre-

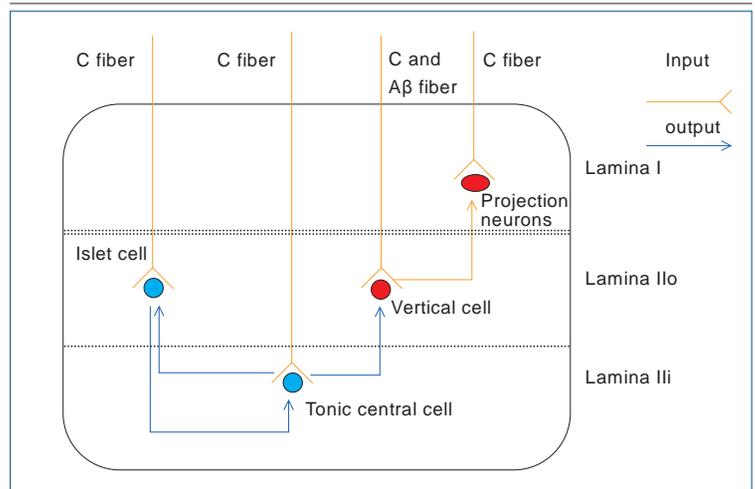


Figure 1. Schematic Illustration of GABAergic Inhibitory Neuronal Networks in the Superficial Dorsal Cord.

Inhibitory and excitatory neurons are depicted in yellow or red, respectively. Islet cell and tonic central cell are GABAergic interneurons. Vertical cell and projection neuron are excitatory neurons. The inhibitory input to lamina I neurons derives predominantly from islet cell inhibition of vertical cell via inhibitory tonic cell. Nerve injury induces disinhibition of GABAergic neuronal circuit, resulting in inhibitory/excitatory imbalance and hyperalgesia.

synaptic GAL2 media pro-nociceptive action at low doses (28, 29). Spinal parvalbumin(PV) positive cells mainly distribute in lamina I-III. 82.9% PV cells were detected in lamina III and 14.3% PV cells in lamina II inner, a few cells in lamina I and II outer (30). Most of PV cells are islet or central cell-like morphology. The dendrites of PV-expressing cells form postsynaptic connection with myelinated afferents and receive direct inputs from these fibers in lamina II inner and III. In addition, the axon terminals of PV cells expressed VGAT, which form pre-synaptic input with myelinated fibers and modulate sensory information from myelinated afferents. PV cells that extent synaptic connects with PKC γ excitatory neurons inhibit the excitation of PKC γ neurons in lamina II inner. Furthermore, PKC γ neurons directly receive A β -fiber input, but, under normal situation, PKC γ neurons is inhibited by inhibitory interneurons and blocked from non-nociceptive pathway to nociceptive pathway for preventing touch inputs from activating pain circuit (31, 32). As ablating of PV cells cause the disinhibition of PKC γ neurons, inducing tactile allodynia (33). Thus, PV cells are the gate-keeper of touch-evoked pain after nerve injury.

Disinhibition of Spinal GABAergic Interneurons and Neuro-pathic Pain

Neuropathic pain is characterized by spontaneous pain, allodynia and hyperalgesia. Disinhibition of spinal inhibitory interneurons is an important mechanism of neuropathic pain. Electrophysiologic studies showed that loss of inhibitory neurons in spinal dorsal horn inhibitory, including GABAergic neurons and glycine neurons, reduced inhibitory tone in CCI and SNL-treated rats (31, 34, 35). Furthermore, knockout of inhibitory neurons or blockade of these neurons receptors induce allodynia and hyperalgesia (32, 36). But ablating of apoptotic GABAergic interneurons or transplantation of GABAergic neural progenitors attenuates neuropathic pain in rats (32, 37, 38). These data suggested that inhibitory interneurons play a key role in neuropathic pain processing. However, the mechanism of disinhibition of inhibitory interneurons is not unclear.

Several possible mechanisms have been proposed for the disinhibition of inhibitory interneurons in spinal dorsal cord after nerve injury. These mechanisms include loss of spinal interneurons, reduction of transmitter release, diminished activity of these cells and decreased effectiveness of inhibitory interneurons (38).

Loss of GABAergic interneurons of spinal dorsal horn was observed in neuropathic pain model (34, 35). Loss of the inhibitory interneurons might be due to the activation of apoptotic pathway (34). Blockade of apoptotic pathway activation prevented neuronal apoptosis and the decrease in spinal inhibition in lamina II, and neuropathic pain-like behavior (34). Furthermore, transplant of GABAergic neuron precursors enhanced spinal cord GABAergic inhibition and reverses the mechanical and heat hypersensitivity, which provided indirect evidence that the reduction of GABAergic interneurons of spinal dorsal horn might contribute to injury-induced neuropathic pain (39). However, Polgar et al. (8) reported that no loss of GABAergic neurons were found in the chronic constriction injury model of neuropathic pain. It is difficult to explain the discrepancy. Mounting studies suggested that astrocyte, as a GABAergic cell, expresses glutamic acid decarboxylase (GAD) and synthe-

sizes GABA and releases GABA (40). Furthermore, astrocytes express GABAA and GABAB receptors (41). With expression of GABA transport, astrocytes involve in GABA uptake to regulate extracellular concentrations of GABA (42). In spare nerve injury model of neuropathic pain, GABA uptake increase with upregulation of the GABA transport 1 (GAT-1) in activated spinal astrocytes (42). In ischemic injury, activated astrocytes up-regulate the expression of GAD and GABA (43). These data suggested that neurons are not the only cells that synthesize and uptake GABA in the central nervous. Thus, it is necessary to identify cell-type-specific GABA expression, if loss of GABAergic neurons is regarded as one of mechanisms of disinhibition of inhibitory interneurons in neuropathic pain.

Glutamic acid decarboxylase (GAD) is the enzyme responsible for the synthesis of GABA. GAD exists as two major isoforms called GAD65 and GAD67. Both forms display different patterns of spatial distribution in spinal cord. In spinal ventral horn, high level of GAD67 is present in axons boutons, while high level of GAD65 is observed in cluster of boutons. In spinal dorsal cord, GABAergic boutons appear to have relatively high levels of GAD65 and low levels of GAD67 (44). In neuropathic pain status, protein expression and mRNA of GAD reduce in the dorsal horn ipsilateral to the nerve injury in rats (45, 46). Nerve injury induced reduction of GAD65 expression in spinal lamina I and lamina II. The greatest drop of GAD65 occurs in lamina II around 3-4 weeks after nerve injury (46). However, Moore et al. (36) found that there was a significant depletion of GAD65, but not GAD67, in lamina II of the dorsal horn in animal model of neuropathic pain. Furthermore, up-regulation of GAD67 and GAD65 increased GABA level, which contributed to attenuating mechanical allodynia and thermal hyperalgesia following nerve injury (47, 48). Thus, depletion of GAD might be implicated in disinhibition of spinal GABAergic neurons in spinal cord. However, mechanism of down-regulation of GAD was unclear. Some studies revealed that GAD67 in the cytoplasm synthesizes GABA and is responsible for cell metabolism and tonic, whereas GAD65 in axon terminals mediate activity-dependent synthesis and vesicle release of GABA during intense synaptic ac-

tivities (49, 50). In addition, GAD65 is coded by GAD2. GAD2 knockout mice are sensitive to pain (51). Meanwhile, upregulation of GAD2 inhibits pain. Thus, coexistence of decrease of GABA and GAD65 down-regulation might be contribute to disinhibition of GABAergic neurons in neuropathic pain model.

Clearance of GABA maintains the homeostasis of extracellular GABA and normal GABA transmission. GABA transporters are expressed on the plasma membrane in neurons and astrocytes. These transporters, including GAT-1 and GAT-3, can transiently bind extracellular GABA, remove it from synaptic cleft and then translocate it from the cytoplasm back into the extracellular space (52). Under normal conditions, a feedback mechanism involved in the regulation of extracellular GABA concentration at the synapse (52). GABA transporters mainly control the concentration of extracellular GABA. High level of extracellular GABA time-dependently up-regulate GAT-1, following by the increase of GABA uptake (53). In addition, some intracellular signaling cascades alter the expression of GABA transporters in neurons. For instance, PKC activation and tyrosine kinase inhibition decrease the expression of GABA transporters and GABA uptake. Depolarizing events up-regulate GABA transporters and enhance the recycling rate GABA transporters (54). In neuropathic pain model, up-regulation of GAT in astrocytes contributed to disinhibition of spinal dorsal horn (55). Blocking of spinal GAT-1 ameliorates mechanical allodynia and thermal hyperalgesia (42, 56). Plasticity of GABA transporters shapes inhibitory synaptic transmission and then influence inhibitory GABAergic tone.

Normal GABA neurotransmission is dependent on precise regulation of the level of intracellular chloride. Intracellular chloride level is maintained by the balance between the inward $\text{Na}^+ - \text{K}^+ - \text{Cl}^-$ cotransporter isoform 1 (NKCC1) and the outward $\text{K}^+ - \text{Cl}^-$ cotransporter isoform 2 (KCC2) in cells (57). As KCC2 mediates Cl^- extrusion, the down-regulation of KCC2 increases intracellular Cl^- , following by activation of cation channels and excitation of neurons (57). In addition, enhancement of KCC2 restores spinal inhibition and reserves neuropathic pain (58). KCC2 knockdown impairs glycinergic synapse

maturation in cultured spinal cord neurons (59). Taken together, decrease of KCC2 destroyed chloride homeostasis and affected the inhibition of inhibitory neurons.

Spinal Glycinergic Neural Circuit in Spinal Dorsal Horn

Spinal glycinergic neurons are largely present in lamina III of the dorsal horn and ventral horn, whereas less in lamina I-II (60). Glycine immunoreactive was observed in the large bouton in laminae I-III of the spinal dorsal horn, such as in symmetrical axodendritic, axosomatic or axoaxonic synapses, where the postsynaptic boutons frequently resembled the terminals of myelinated primary afferents (60, 61). Dendrites of glycinergic neuron in laminae II and III were postsynaptic to the central axons of type II glomeruli, which suggests that glycinergic neurons receive a major monosynaptic input from myelinated primary afferents (61). Furthermore, pharmacogenetic activation of dorsal horn glycinergic neurons mitigates neuropathic hyperalgesia (60). These data suggested that glycinergic neurons are play a key role in somatosensory processing involving low threshold myelinated cutaneous primary afferents.

Glycine binds glycine receptor (GlyR) in the postsynaptic membrane of neurons and then open the GlyR integral anion channel, resulting in the influx of Cl^- and the hyperpolarization of the postsynaptic cell, thereby inhibiting neuronal fire (62). Glycine receptors composed of α and β subunit. α and β subunit combine to form subtypes of GlyRs, including homomeric GlyRs, $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 4$, and heteromeric GlyRs $\alpha 1\beta$, $\alpha 2\beta$, $\alpha 3\beta$ and $\alpha 4\beta$ (63). GlyRs $\alpha 3$ is expressed nociceptive neurons in lamina I and II with gephyrin, which anchors and then clusters GlyRs at postsynaptic site in rat spinal cord (64). GlyRs $\alpha 3$ mediate glycinergic inhibitory neurotransmission in nociceptive sensory neuronal circuits in laminae of spinal dorsal horn. Blockade of GlyRs $\alpha 3$ suppress neuropathic pain (65). Furthermore, Glycine transporter (GlyT) modulate glycinergic neurotransmission by clearing synaptically released glycine or supplying glycine to the neurons (66). Thus, GlyT modify pain signal transmission in the spinal cord. These are two glycine transporters, including gly-

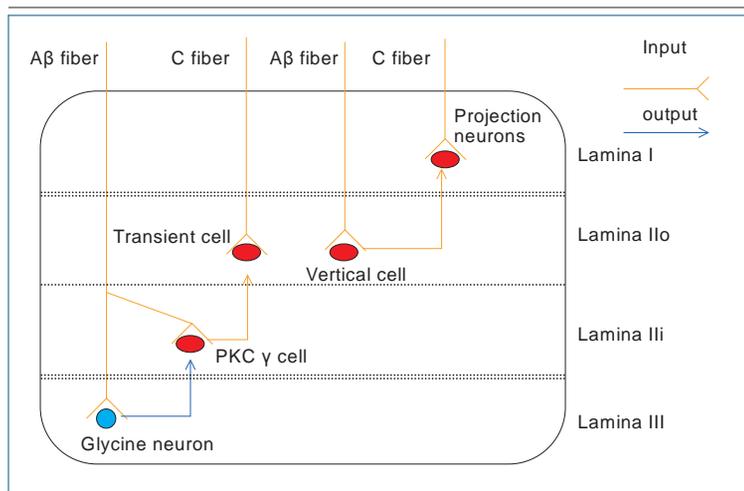


Figure 2. A Feed-Forward Spinal Cord Glycinergic Neural Circuit.

Inhibitory and excitatory neurons are depicted in yellow or red, respectively. Glycinergic neurons act as “gate control” units for preventing the interaction between innocuous and nociceptive signals. Glycinergic neurons control excitatory linkage from PKC γ + cell to TC cell in normal physiological conditions. Nerve injury results in disinhibition of PKC γ + neurons and allows innocuous stimuli to activate the nociceptive pathway.

cine transporter 1 (GlyT1) and GlyT2 (66). GlyT2 is the only marker of glycinergic neurons in CNS, except in the cerebellum (65). Few immunostained GlyT2 present in lamina I-II. But densely stronger immunoreactive GlyT2 were observed in lamina III and ventral horn (31, 32). As the distribution of GlyT2- immunoreactive boutons matches that of glycine axons in spinal cord, the distribution of GlyT2- immunoreaction are presumed to be glycinergic neurons. According to the distribution of these marks associated with glycine synapse, it is suggested that glycine neuron might involve in regulation of non-nociceptive information transmitted from spinal cord to brain.

Glycinergic neuronal circuit is a “gate” for the interaction between nociceptive and nociceptive signals of the separation pathway from non-nociceptive pathway. The circuit located at the junction of spinal laminae II/III, in which the Gly inhibitory interneurons and PKC γ + neurons are activated by the same excitatory input from low-threshold A β fibers. Glycine neurons mediate translamina synaptic input to PKC γ interneurons of inner lamina II (6, 18). PKC γ interneurons, which contain gamma isoform of protein kinase C, express glycine receptors GlyR α 3 subunit and receive the inhibitory output of glycine

neurons and the excitatory output of low-threshold A β -fiber (31, 67). As PKC interneurons receive two types of input, excitatory postsynaptic potentials (EPSPs) from A β -fiber and inhibitory postsynaptic potential (IPSPs) from glycine neurons, a biphasic response was evoked in PKC γ + cells after using dorsal root (DR) stimulation (6). Under normal physiologic situation, with the control of the inhibitory input, excitatory input can not elicited action potentials in the PKC γ + neurons. Thus, the neuronal circuit activated by innocuous mechanical stimulation is a silent pathway. But glycine receptor blockade can wakes up a normally silent circuit (31). Furthermore, lamina II transient central cells, as excitatory neurons, are postsynaptic to PKC γ excitatory neurons at the II-III border. Then, Lamina II transient central cells connected vertical cells (Figure2). Finally non-nociceptive information was sent to lamina I projection neurons (59) by which the information is transmitted to brainstem. Therefore, glycinergic neuronal circuit is critical for maintaining of the separation nociceptive pathway from non-nociceptive pathway.

Disinhibition of Spinal Glycine Neuronal Circuit and Allodynia

Disinhibition of spinal glycinergic interneurons contributes to mechanical allodynia. Studies showed that blockade or reduction of glycine receptors and glycinergic neurons ablation or silencing in spinal dorsal horn induce mechanical allodynia (60, 67). Furthermore, enhancing the activation of the glycinergic neurons in spinal cord produced a profound antiallodynia effect in an animal of neuropathic pain. This may indicate that dysfunction of glycinergic neurons is an important mechanism of mechanical allodynia in neuropathic pain.

Blockade of glycine receptors within the spinal cord induces profound tactile allodynia (36). Although blockade of GABA(A) receptor resulted in mechanical allodynia, glycine receptors antagonists, strychnine, inhibit more than 70% of inhibitory postsynaptic currents (IPSCs) evoked by blue light stimuli, whereas the remaining portion was blocked by the GABA(A) receptor antagonist bicuculline in a study of the combination of electrophysiology and opto-genetics (60).

Consequently, non-nociceptive mechanical stimuli unmasking the activation of PKC α -dependent activation of a local excitatory circuits, which are normally blocked local excitatory circuits onto nociceptive output neurons. Then, the information is transmitted to projection neurons for relay to several brain area (6, 31). Glycine inhibitory dysfunction turns non-nociceptive input to nociceptive input. In addition, c-Fos is a marker of neural activity and nociceptive processing. After disinhibition by intracisternal injection of strychnine, c-Fos-positive neurons were detected in the ipsilateral superficial laminae I and II (31). Thus, a feed-forward spinal glycinergic neural circuit gates mechanical allodynia.

The α 3-containing glycine receptors (GlyR α 3) plays an important role in pain sensitization. GlyR α 3 sparsely distribute in the lamina II of the spinal dorsal horn, a region for integrating nociceptive information (63). A selective inhibitor of GlyR α 3, PGE2, induces central and peripheral pain sensitization by suppressing inhibitory glycinergic neurotransmission onto superficial dorsal horn neurons in animal model of inflammation pain (68, 69). Furthermore, Xiong et al. found that cannabidiol (CBD), as an effective drug for patients with neuropathic pain and other types of chronic pain, suppress neuropathic pain by targeting GlyR α 3 (65). The analgesic effect of CBD is absent in mice lacking the GlyR α 3. In GlyR α 3 knockout (Gla3 $^{-/-}$) mice, pain sensitization is a complete lack in inflammation pain(63). However, some studies suggested that Gla3 $^{-/-}$ mice is not pivotal for induction or maintenance of neuropathic pain (70). Thus, the role of α 3 GlyR in neuropathic pain needs to be demonstrated in the future.

Glycine transporter 2 (GLYT2) is necessary to refill synaptic vesicles in inhibitory spinal cord neurons (110). Selective GLYT2 inhibitors enhance glycinergic inhibition in the spinal dorsal horn with regulation of the extracellular concentration of glycine and prolonging the duration of the glycinergic postsynaptic currents in the spinal cord (110- 112). Therefore, GLYT2 inhibitors have spinal anti-disinhibition of glycinergic inhibitory interneurons effect for neuropathic pain models in mice (13). Anti-allodynia action of glycine transporters inhibitor in neuropathic pain suggested that dysfunction of glycinergic neurons

contributes to development of neuropathic pain.

Ablation or silencing glycinergic neurons of spinal dorsal horn induces mechanical, heat, and cold hyperalgesia. In rats with ablation glycinergic neurons, 70% of IPSCs originated from purely GABAergic neurons was blocked by the glycine receptor antagonist strychnine. The data proved an important role for glycine in inhibitory input to dorsal horn neurons (58). Furthermore, activation of spinal glycinergic neurons effectively alleviates neuropathic pain (60).

Treatment of Disinhibition of Inhibitory Interneurons in Neuropathic Pain

A reduction in the inhibitory tone in the spinal cord involve in neuropathic pain. Restoring the inhibitory tone is a reasonable therapeutic approach for neuropathic pain. The way to restore the inhibitory tone includes activation of spinal GABA receptors, inhibition of glycine transporter 2 and enhancement of chloride extrusion (58, 71-73). Nonselective GABAA receptors agonist, diazepam (DZP), binds GABAA receptors and causes the opening of Cl $^{-}$ channel, leading to hyperpolarization and enhancing synaptic inhibition (72). However, nonselective GABAA receptors agonist displays side effects, including insufficient efficacy after systemic administration dose-limiting sedation, impaired motor coordination and dependence and addiction (73). The present study has shown that addictive properties of BDZs require the α 1-containing GABAA (74). Agonists at the benzodiazepine-binding site of GABAA receptors targeting α 2GABAA Rs have the highest antihyperalgesic efficacy in triple GABAA R point-mutated mice (73). Furthermore, glycine transporter 2 inhibitors, including ALX-393 and Org-25543, produce a profound anti-allodynic effect in mouse neuropathic pain. But acute toxicity of the inhibitors limits their usefulness (75). In addition, chloride extrusion enhancers, including KCC2-dependent Cl $^{-}$ extrusion enhancer and inhibition of carbonic anhydrase, increase chloride extrusion or reduce bicarbonate efflux, restoring inhibition compromised by chloride dysregulation (76). However, although these drugs achieve significant analgesia for nerve injury-induced neuropathic pain, the side effects of these drugs or the inability of

systemic drug administration limit the clinical utility. Therefore, transplanting embryonic GABAergic neuronal precursors in the dorsal horn of the spinal cord was used for injury-induced neuropathic pain (39). Transplanted cells make functional connections with both primary afferent and spinal cord neurons, integrating into the host spinal cord circuitry. Finally, these grafted cells reverse the persistent pain produced by peripheral nerve injury. At present, GABAergic interneuron transplantation is beneficial for improving animal's behaviors in neuropathic pain. But, source of primary MGE-derived cells are limited in a future clinical setting. Meanwhile, efficiency of other cell-derived GABAergic interneurons is low (77). Thus, further study should focus on increase of cell-derived GABAergic interneurons and improvement of efficiency of stem cell-derived GABAergic interneurons.

Conclusion

Dysfunction of spinal inhibitory circuits is an important mechanism of neuropathic pain. Although recent studies have uncovered synaptic connection of spinal inhibitory circuits that involve in allodynia and hyperalgesia, knowledge of neuronal inhibitory circuitry in spinal dorsal horn is still limited. Meanwhile, spinal interneurons present complex subunit and function as well as diverse receptors and ion channels. In addition, Ishibashi H et al. found that inhibitory transmit-

ter GABA at GABA/ mixed synapses, but not glycine, may prevent pathophysiological hyperexcitability (78). However, Rousseau F et al. showed that with the requirements for glycine refilling in two phases of vesicle release elicited by high-frequency trains of stimuli, GlyT2 played a central role in determining inhibitory phenotype and therefore in the physiology and pathology of inhibitory circuits (79). Therefore, the role of GABAergic interneurons and glycinergic neurons in neuropathic pain should be further clarified. Furthermore, acetazolamide, a blockade of carbonic anhydrase, reversed the effects of chloride dysregulation, but it did not reverse the effects of GABA or glycine receptor blockade (76). The differential effects of carbonic anhydrase blockade suggest that not all disinhibitory mechanisms are equivalent. It is prerequisite to establish the pattern of expression of receptors and channels on different neuronal types and to identify the different components of these circuits in order to explore how to sensory information transmit in these neuronal circuits. Apart from primary afferent sensory nerve fibers, fiber tracts descending from noradrenergic and serotonergic fibers of supraspinal areas activated GABAergic and glycinergic interneurons and modulate pain (80). However, the precise mechanisms of the effect of the supraspinal fiber on spinal inhibitory circuits have not been fully elucidated.

The authors declare no conflicts of interest.

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