Chapter 31
Spinal Synaptic Plasticity in Chronic Pain

Wataru Taniguchi and Terumasa Nakatsuka

Abstract Spinal synaptic plasticity induces pathological chronic pain such as neuropathic pain. However, little is known about the mechanism of spinal synaptic plasticity in chronic pain. Although there are many causes for and various forms of spinal synaptic plasticity, several mechanisms have been revealed in recent years. Spinal synaptic plasticity consists of (1) a change in excitatory synaptic transmission efficiency (e.g., LTP, windup), (2) a change of synaptic network in the dorsal horn (e.g., Aβ-fiber sprouting into lamina II), (3) neurotrophic factors, (4) activation of ion channels (e.g., P2X receptors, TRPV1, TRPA1), and (5) activation of microglia in the spinal cord. In this chapter we review these mechanisms of spinal synaptic plasticity, which induce chronic pain. As we have investigated the activation of P2X receptors, TRPV1, and TRPA1 in the dorsal horn using whole-cell patch-clamp methods, we have discussed these studies in detail.

Keywords Chronic pain • Microglia • Neuropathic pain • Spinal cord • Synaptic plasticity

31.1 Introduction

Nociceptive pain is an important defensive mechanism that warns an individual of imminent damage to the body. However, it only becomes harmful when the alarm is allowed to persist. This pathological pain is a clinical condition termed chronic pain and may be insidious to the life of the individual who is suffering from it. It deviates

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from the role of biophylaxis, and pain itself becomes a target to treat. Although chronic pain is induced by various factors, synaptic plasticity in the spinal cord is recognized as a main cause [1, 2]. Recent studies have shed some light on the mechanisms involved in spinal synaptic plasticity. In this chapter, we introduce several critical molecular mechanisms related to the relationship between spinal synaptic plasticity and chronic pain.

31.2 The Mechanism of Pain Transmission in the Spinal Cord

31.2.1 Excitatory Synaptic Transmission in the Spinal Cord

The dorsal horn neurons in the spinal cord receive nociceptive pain information from primary sensory afferents and relay it to the central nervous system. The impulse (action potential) through Aδ-fibers or C-fibers does not reach dorsal horn neurons directly as an electrical signal, because the dorsal horn neurons in the spinal cord are separated from the central terminals of Aδ-fibers or C-fibers by a synaptic cleft (a distance of approximately 20 nm). The dorsal horn neurons connect C-fibers or Aδ-fibers via chemical synapses, which mediate the excitation of dorsal horn neurons by glutamate released from presynaptic terminals. Glutamate release produces an excitatory postsynaptic potential (EPSP) in dorsal horn neurons [3] (Fig. 31.1). EPSPs evoked by low-frequency action potential discharge of primary afferents are mediated principally by the activation of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors, which are a subtype of glutamate receptors. N-methyl-D-aspartate (NMDA) receptors, another subtype of glutamate receptors, contribute little to the postsynaptic responses to glutamate release under basal conditions because of tonic inhibition of current flow by extracellular Mg^{2+} pore blockade at the resting membrane potential. However, NMDA receptors are important for spinal neuroplasticity, because they contribute to the generation of EPSP and action potentials during chronic pain following activation via kinase-mediated phosphorylation of the receptor (see Sect. 31.2.1). Moreover, nociceptive pain information is modulated by many interneurons and various endogenous neurotransmitters and neuromodulators at the presynapse or the postsynapse in the dorsal horn. For example, when substance P, a neuropeptide, is released from the central terminal of some C-fibers to the synaptic cleft and binds to the neurokinin 1 (NK1) receptor at the cell membrane of dorsal horn neurons, slow EPSP is induced in dorsal horn neurons [4] (Fig. 31.1). This slow EPSP is induced by closing the potassium channel via activation of G proteins in the cell membrane. Similarly, excitatory neuropeptides such as substance P contribute to slow excitatory synaptic transmission through G proteins.

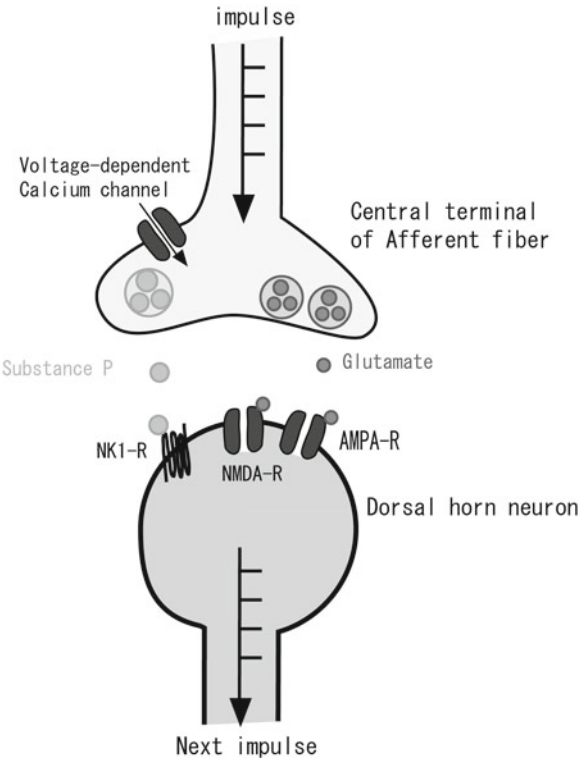

Fig. 31.1 Excitatory synaptic transmission in the dorsal horn. When an impulse is transmitted to central terminals of afferent fibers, excitatory neurotransmitters are released. Glutamate and substance P act at each receptor on the dorsal horn neuron and induce an impulse

Activation of ion channels such as the TRP channel and P2X (ATP receptor) in presynaptic terminals enhances glutamate release, which in turn enhances fast EPSP [5–9] (see Sects. 31.3.4 and 31.3.5).

31.2.2 Inhibitory Synaptic Transmission in the Spinal Cord

Although dorsal horn neuron action potential discharge is driven by glutamatergic EPSPs, the activity of these neurons is powerfully suppressed by inhibitory inputs in both the pre- and postsynapse. The inhibitory effect is mainly produced by γ-aminobutyric acid (GABA) and/or glycine postsynaptically, which are released from the terminals of inhibitory interneurons (Fig. 31.2). These interneurons induce fast inhibitory postsynaptic potentials (IPSPs) on dorsal horn neurons to mediate the activation of $GABA_A$ and glycine receptors. $GABA_A$ and glycine receptors are ligand-gated Cl^- channels. Channel opening inhibits action potentials by

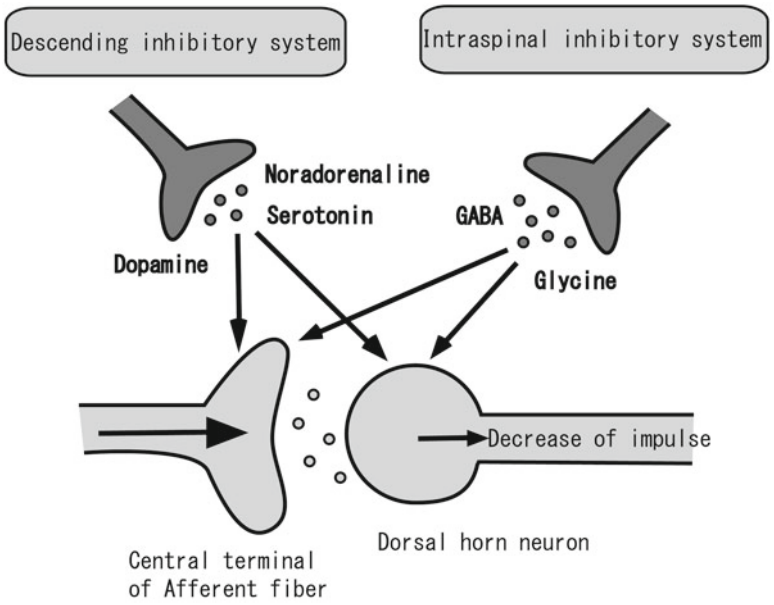

Fig. 31.2 The endogenous inhibitory system. The endogenous inhibitory system consists of the descending inhibitory system and the intraspinal inhibitory system

hyperpolarizing the cell membrane. Activation of G-protein-coupled receptors produces postsynaptic inhibition of excitatory synaptic transmission to hyperpolarize cell membranes by activating K^+ channels. This type of channel includes receptors such as the $GABA_B$ receptor, adenosine receptor, and opioid μ-receptor. Moreover, some endogenous substances suppress the release of glutamate from primary afferent terminals, therefore inhibiting discharge of dorsal horn neurons presynaptically. This presynaptic inhibition involves many of the same chemical mediators that cause postsynaptic inhibition, with receptors localized on the presynaptic terminals of primary afferents. There are not only networks within the spinal cord but also inhibitory projections to the dorsal horn from the brainstem, which is known as the descending inhibitory system. Serotonin [10] from the raphe nucleus, noradrenaline [10, 11] from the locus ceruleus, and dopamine [12, 13] from the hypothalamus A11 act on 5-HT1A receptors, α2 receptors, and D2-like receptors in dorsal horn neurons pre- and postsynaptically, respectively.

31.3 Spinal Synaptic Plasticity Induces Pathological Chronic Pain

There are two types of plasticity in the nervous system: one is central nervous sensitization, and the other is peripheral sensitization. In particular, central sensitization in the spinal cord has much to do with chronic pain. Although spinal synaptic

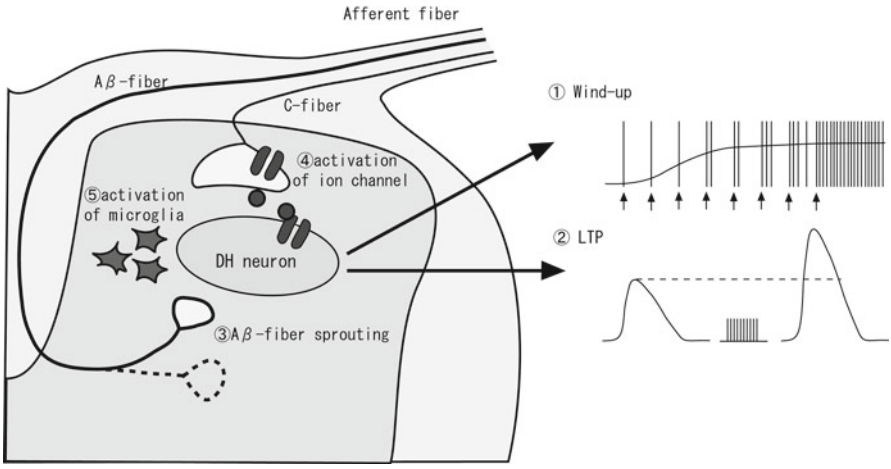

Fig. 31.3 Spinal synaptic plasticity. Spinal synaptic plasticity is induced by various causes. For example, ① windup, ② LTP, ③ Aβ-fiber sprouting into laminaII, ④ activation of ion channels on the central terminal of afferent fibers, and ⑤ activation of microglia

plasticity induces pathological chronic pain, there are many causes and various forms of spinal synaptic plasticity. In recent years, the influential mechanisms involved in spinal synaptic plasticity have become clear. We describe some convincing mechanisms in the following explanation.

31.3.1 Change in Efficiency of Excitatory Synaptic Transmission

The change in efficiency of excitatory synaptic transmission by nervous activation plays an important role in the enhancement of pain, as well as memory and learning in the hippocampus. With respect to electrophysiological phenomena, the enhancement of efficiency in excitatory synaptic transmission occurs by windup and long-term potentiation (LTP). Windup is when noxious peripheral stimulation that is more intense or sustained induces primary afferent nociceptors to discharge at higher frequencies (Fig. 31.3①). LTP refers to high-frequency primary afferent stimulation that induces the potentiation of glutamate receptor-mediated responses at synapses onto dorsal horn neurons (Fig. 31.3②). Windup and LTP are considered to be involved in the release of peptide neuromodulators such as substance P, together with glutamate, from central terminal C-fibers. These peptides act on their G-protein-coupled receptors to produce depolarizing synaptic potentials lasting up to tens of seconds. Moreover, both phenomena are also considered to be related to the activation of NMDA receptors by phosphorylation via kinase, and the subsequent current flow through NMDA receptors leads to a rise in intracellular Ca^{2+} levels.

31.3.2 Alterations to the Synaptic Network in the Dorsal Horn

The synaptic network changes dynamically according to neural activity, for example, growth or regression of axons, the appearance of new synapses, or the disappearance of existing synapses. Alterations to the synaptic network (synaptic morphology change) in the dorsal horn have been suggested to lead to pain in chronic inflammation [14] or peripheral nerve injury. Under normal conditions, noxious information is transmitted through Aδ- and C-fibers to the superficial dorsal horn, especially substantia gelatinosa (SG) neurons (lamina II of Rexed), while innocuous mechanical information is transmitted through Aβ-fibers to the deep dorsal horn (lamina III–IV). In past studies, it was shown that C-fibers were missing in the sciatic nerve amputation pain model, and approximately 10 % of Aβ-fibers started to extend axons (sprouting), with input into lamina II [15, 16] (Fig. 31.3③). Moreover, Aβ-fiber sprouting was observed in the inflammation pain model [17]. Aβ-fiber sprouting in these pain models plays an important role in brain-derived neurotrophic factor (BDNF), which is released from C-fibers into the spinal cord. The mechanism of allodynia has been suggested to occur when innocuous mechanical information is converted to noxious information. This process occurs when Aβ-fibers connect to SG neurons, which project noxious information to the more central nervous system during pathological conditions such as chronic inflammation and neuropathic pain.

31.3.3 Neurotrophic Factors

Neurotrophic factors are supplied to nerve cells by many kinds of cells (e.g., glia cells, dorsal root ganglion (DRG) neurons, Schwann cells, keratinocytes, and fibroblasts) and have various influences on the transmission of noxious information. During inflammation, the production of nerve growth factor (NGF) increases and causes hyperalgesia. NGF interacts with the TrkA receptor at the peripheral terminals of nociceptive fibers and is carried to DRG neurons by a retrograde axial. The NGF-TrkA complex controls gene expression in DRG neurons and promotes the production of neuropeptides, excitatory ion channels, and BDNF. BDNF is transferred to the central terminal of nociceptive fibers, which are projected to the spinal cord, where it acts upon its receptor, TrkB, on dorsal horn neurons. The subsequent activation of TrkB receptors on dorsal horn neurons modulates pain information [18]. Furthermore, BDNF also contributes to the change in the synaptic network in the dorsal horn, as mentioned above, during inflammation and neuropathic pain.

31.3.4 Activation of P2X Receptors

Much attention has been given to ion channels in the central terminals of Aδ- and C-fibers, which enhance nociceptive pain information by increasing glutamate release when these channels are activated by endogenous mediators (Fig. 31.4).

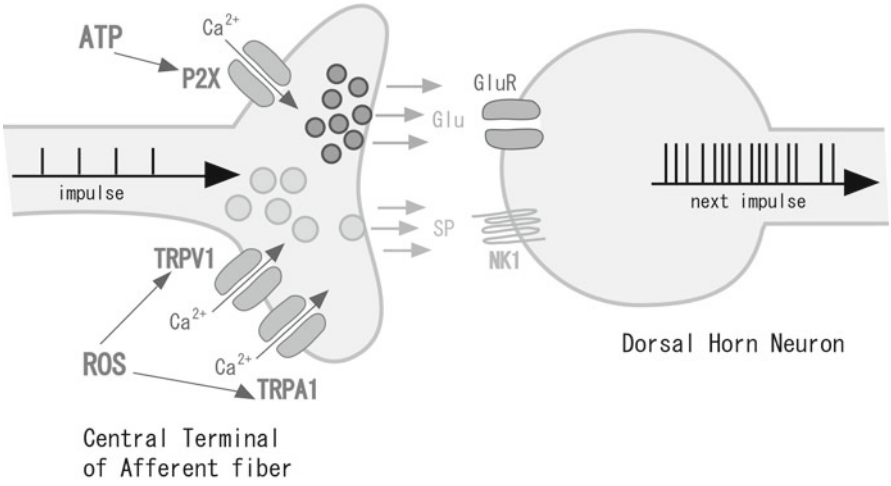

Fig. 31.4 Activation of ion channels. When ion channels in the central terminals of afferent fibers are activated, excitatory neurotransmitters are released. The P2X receptor is activated by ATP. TRPV1 and TRPA1 are activated by ROS and so on

For example, these ion channels include the ATP-dependent P2X receptor, the transient receptor potential (TRP) receptor family, voltage-dependent Na^+ channels, and acid-sensing ion channel 3 (ASIC3). In particular, we focused our attention on the P2X receptor in the dorsal horn. The P2X receptor family consists of at least seven P2X subunits ($P2X_1$–$P2X_7$). Each functional P2X receptor is composed of three or more P2X subunits, forming a pore structure that is permeable to cations including Ca^{2+}. Therefore, the P2X receptor family is a ligand-gated ion channel family. We examined the role of P2X receptors in modulating excitatory synaptic transmission by using patch-clamp recordings from dorsal horn neurons of the rat spinal cord [6, 7, 19, 20]. Distinct subtypes of P2X receptors were located at central terminals of primary afferents that innervate onto lamina II and lamina V neurons. On activation, these P2X receptors enhanced the release of glutamate in various ways. In lamina II neurons, the modulation of glutamate release by presynaptic P2X receptors was mainly transient. In contrast, the P2X receptor-mediated modulation of glutamate release was relatively long lasting in lamina V neurons [20]. Pharmacological studies suggested that lamina II neurons were involved in homomeric $P2X_3$ receptors, while lamina V neurons were involved in non-$P2X_3$ receptors [20]. Differences among P2X-expressing afferent fibers innervating lamina II and lamina V neurons were also seen in terms of capsaicin sensitivity [19]. P2X-expressing afferent central terminals in lamina II were derived from capsaicin-sensitive primary afferents, while those in lamina V were from capsaicin-insensitive primary afferents [19]. Because $P2X_3$-expressing afferent central terminals directly make synapses with lamina II neurons, the inputs from these afferent terminals could forward excitatory synaptic activities. On the contrary, the activities conveyed to lamina V neurons from $P2X_3$-expressing primary afferents were polysynaptic; these inputs together

with monosynaptic inputs from P2X-expressing and capsaicin-insensitive afferents were shown to converge on lamina V neurons. These results indicate that distinct subtypes of P2X receptors are expressed in central terminals of primary afferents innervating onto superficial and deep dorsal horn neurons and modulate glutamate release in a different manner. Furthermore, P2X receptors were localized at lamina V neurons to mediate postsynaptic sensory transmissions [21]. Using the whole-cell patch-clamp technique, we investigated whether the activation of postsynaptic P2X receptors can modulate synaptic transmission in lamina V neurons. The ATP analogue generated an inward current in lamina V neurons. These pre- and postsynaptic actions may serve to understand P2X receptor-mediated modulation of various sensations.

31.3.5 Activation of TRPV1 and TRPA1 in the Central Terminals of Afferent Fibers

We have also focused on and examined the role of TRP receptors in modulating excitatory synaptic transmission. TRP channels belong to a family of ion channels that are activated by temperature and which are expressed in primary sensory nerve terminals where they provide information about thermal changes in the environment. There are six thermosensitive ion channels in mammals (TRPV1, TRPV2, TRPV3, TRPV4, TRPM8, and TRPA1), all of which belong to the TRP superfamily [22]. These channels are involved in chemical, mechanical, and thermal nociception. TRP channels are drug targets for the relief of pain, including neuropathic pain. In particular, TRPV1 (TRP vanilloid 1) and TRPA1 (TRP ankyrin 1) have been a major focus of research into the mechanisms of inflammatory and neuropathic pain. In addition to their expression at peripheral nerve endings, we revealed that TRPV1 and TRPA1 are expressed at the central terminals of primary afferent fibers and that their activation facilitates excitatory synaptic transmission in SG neurons by increasing glutamate release by whole-cell patch-clamp recording [5, 8, 9]. Therefore, activation of TRPV1 and TRPA1 at the central terminals of primary afferent fibers results in the enhancement of glutamate release (Figs. 31.3④ and 31.4). This enhancement leads to central sensitization and spinal synaptic plasticity. However, it is unclear which endogenous mediators act on TRPV1 or TRPA1 in the spinal cord. Reactive oxygen species (ROS) have been recognized to play an important role in the central neuropathic pain of the spinal cord. Spinal cord injury (SCI) increases levels of highly toxic ROS, which can damage neural, glial, and microvascular elements, resulting in sensory and motor neuron apoptosis. We investigated the effect of ROS on glutamatergic excitatory synaptic transmission in SG neurons [23], using the whole-cell patch-clamp technique. We showed that ROS enhances the spontaneous release of glutamate from presynaptic terminals onto SG neurons through TRPA1 and TRPV1 channel activation [23] (Fig. 31.4). Therefore, excessive activation of these ion channels by ROS may induce central sensitization in the

spinal cord and result in chronic pain such as that following SCI. Thus, ROS may be one of the mediators which activate TRPV1 or TRPA1.

31.3.6 Activated Microglia in the Spinal Cord Induce Neuropathic Pain

Glia cells consist of astrocytes, oligodendrocytes, and microglia. The number of glial cells is several times greater than neurons in the spinal cord. Glial cells are considered to have no electrical activity and mainly play a role in the maintenance and supplementation of neuron function. Microglia were thought to play a role in immunity; however, recent studies revealed that glia cells are also involved in the adjustment of nervous activity. In particular, microglia in the spinal cord contributes to the mechanism of neuropathic pain after peripheral nerve injury [24, 25]. Recently, we showed that the number of activated microglia in the SG correlated to the frequency and amplitude of spontaneous excitatory postsynaptic current (sEPSC) using in vivo patch-clamp methods [26]. Under normal conditions, microglia are in their "resting" state, but following injury, such as peripheral nerve injury, they become "activated" (Fig. 31.3Ⓢ). The activated microglia causes morphologic changes such as cell soma enlargement and shrinkage. Moreover, it was reported that the $P2X_4$ receptor specifically increases on activated microglia in the neuropathic pain model. The $P2X_4$ receptor is one subtype of the P2X receptor family and is found to be expressed in only microglia. When ATP stimulates the $P2X_4$ receptor on activated microglia, mechanical hypersensitivity in the neuropathic pain model is induced [25]. With respect to this mechanism, it becomes clear that BDNF, which is released from activated microglia in the spinal cord, acts on dorsal horn neurons [27]. BDNF involves the reduction of expression of the K^+-$C1^-$ cotransporter KCC2 in SG neurons. KCC2 normally plays a role in the extrusion of Cl^- from cells. As a consequence, there is a rise in intracellular Cl^- in SG neurons, resulting in a depolarizing shift in the anion reversal potential and hyperexcitability by means of dramatically reducing $GABA_A$-ergic and glycinergic inhibition (Fig. 31.5). This is recognized as one mechanism of allodynia. It is also becoming clear that intermediates, such as interferon γ [28] and interferon regulatory factor 8 [29], activate "resting" microglia.

31.4 Conclusion

Pain itself plays an important role as a warning signal; however, chronic pain is known to be pathological and hazardous to the body and goes beyond the warning signals. Chronic pain is therefore refractory because disease-developing mechanisms are complex, limiting the selection of effective treatments. However, in the world of fundamental research, we are on the verge of discovering the mechanism of chronic pain once and for all, which will allow us to improve treatments dramatically.

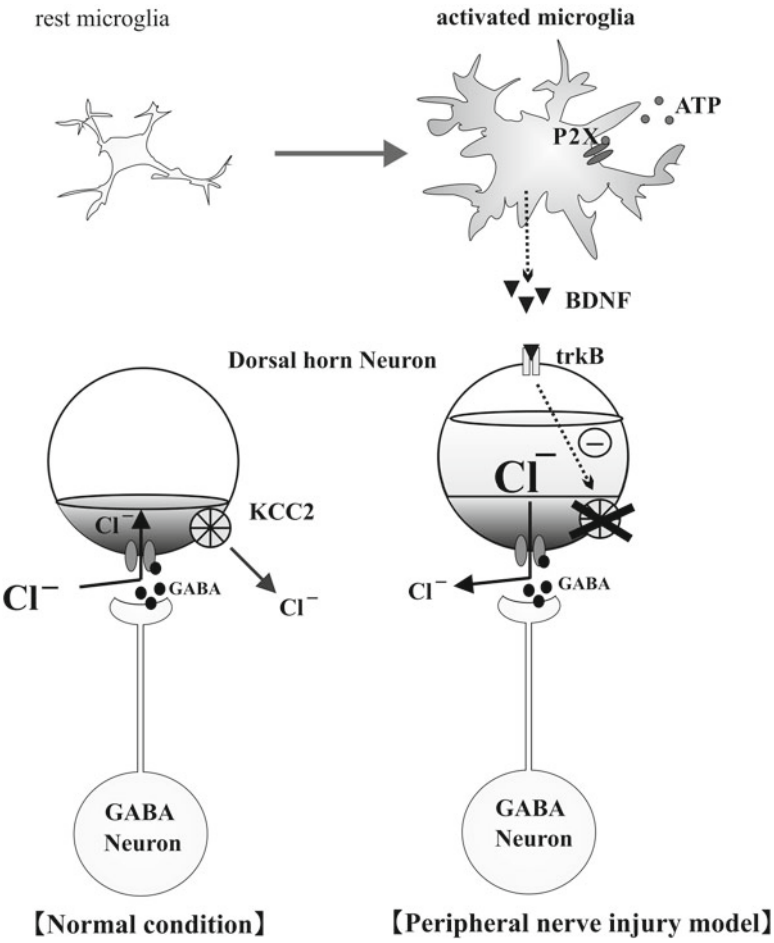

Fig. 31.5 Mechanism of activated microglia-induced allodynia. In the peripheral nerve injury model, resting microglia are activated and release BDNF. BDNF acts on TrkB receptors and causes downregulation of KCC2. Because intracellular Cl^- concentrations are increased, dorsal horn neurons are depolarized when GABA acts on $GABA_A$ receptors

Conflict of Interest The authors declare that they have no conflict of interest.

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