The changing role of GABA\(_\alpha\) receptors in the regulation of pain transmission

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Received 14th October © Cell Science 2008

The GABA\(_\alpha\) receptor is a ligand-gated chloride (Cl\(^{-}\)) channel, and the balance between its excitatory and inhibitory properties depends largely on the Cl\(^{-}\) gradient across the cell membrane. Cl\(^{-}\) influx through GABA\(_\alpha\) receptors is normally involved in synaptic inhibition along the pain transmission pathway. However, under certain painful conditions, altered function of cation-Cl\(^{-}\) cotransporters can increase the intracellular Cl\(^{-}\) concentration. As a result, GABA\(_\alpha\) receptor activation could excite pain-sensing neurons by gating Cl\(^{-}\) efflux. We provide a review of the physiological role of GABA and GABA\(_\alpha\) receptors in the control of normal pain transmission at the primary afferent terminal and spinal cord level. We also discuss new findings showing that altered functions of cation-Cl\(^{-}\) cotransporters and GABA\(_\alpha\) receptors contribute to the loss of GABAergic inhibitory tone and increased excitability of pain-sensing neurons in inflammatory and neuropathic pain. Thus, GABA\(_\alpha\) receptors and cation-Cl\(^{-}\) cotransporters represent new targets for pain treatment.

Despite intense research, chronic pain, defined as pain persisting longer than the natural temporary healing period, remains a major clinical problem. Cancer pain, arthritis pain, neurogenic pain, diabetic neuropathic pain, back pain, and postherpetic neuralgia (herpes zoster) all exemplify suffering that continues today without adequate relief. Even for those conditions with relief, medications, such as opioid drugs, frequently come with serious side effects such as sedation, addiction, or constipation, which diminish the quality of life. Thus, research is much needed to identify new drug targets to better treat pain. Here we reviewed the physiological role of GABA\(_\alpha\) receptors in the control of normal pain transmission at the primary afferent terminal and spinal cord level. We also discussed the latest studies about the contribution of cation-Cl\(^{-}\) cotransporters and GABA\(_\alpha\) receptor plasticity to the loss of GABAergic inhibitory tone and increased excitability of pain-sensing neurons in inflammatory and neuropathic pain.

GABA\(_\alpha\) Receptors

Typically, \(\gamma\)-aminobutyric acid (GABA), the naturally occurring amino acid present in the central nervous system, functions as an inhibitory neurotransmitter. GABA produces its actions through three major subtypes of receptors, which include GABA\(_A\), GABA\(_B\), and GABA\(_C\). GABA\(_A\) and GABA\(_C\) receptors are ionotropic receptors, also known as ligand-gated ion channels; whereas GABA\(_B\) is a metabotropic receptor, also known as a G protein-coupled receptor. GABA\(_A\) functions in regulating the flow of Cl\(^{-}\) ions into the target neurons. GABA\(_C\) receptors are similar to GABA\(_A\) receptors but are mostly concentrated within the retina of the eye. GABA\(_A\) and GABA\(_C\) receptors produce fast synaptic inhibition. By contrast, GABA\(_A\) receptors produce slow, prolonged inhibitory signals and functions in stopping the release of neurotransmitters in the central and peripheral nervous systems. Therefore, GABA\(_A\) receptors work faster and, by restraining the central nervous system, control pain messages from reaching the brain.

Drugs that potentiate GABA\(_A\) receptor activity are widely used clinically as anti-epileptics, anxiolytics, and sedatives. GABA\(_A\) is made up of five subunit proteins arranged in a circle to form a channel and can be constructed from a diverse family of GABA\(_A\) receptor subunits. Each of these five subunit proteins exists as an amino acid string.
running into and out of the cell membrane four times. This channel remains closed until GABA binds to the recognition site. To date, seven subunit families and 16 subunit subtypes [$\alpha$(1–6), $\beta$(1–3), $\gamma$(1–3), $\delta$(1), $\epsilon$(1), $\pi$(1), and $\theta$(1)] have been found in mammalian species. Most native GABA$_A$ receptors contain two $\alpha$, two $\beta$, and one $\gamma$ subunit (Chang et al., 1996; Tretter et al., 1997). The differences in subunits affect GABA$_A$ receptor properties such as receptor opening and conductance. In dorsal root ganglion neurons, the GABA$_A$ receptor primarily contains $\alpha$2 and $\beta$2/3 subunits (Maddox et al., 2004). In the spinal dorsal horn, $\alpha$2 and $\alpha$3 are the most abundant diazepam-sensitive GABA$_A$ receptor $\alpha$ subunits (Knabl et al., 2008).

**Pain Transmission Pathway**

The body detects pain through sensory receptors that are present in the skin and other tissues and organs. The primary afferent (sensory) nerves then transmit these signals from the periphery to the brain where pain is registered and interpreted. The spinal cord lies between the periphery and brain as a relay station of pain signals. In the study of pain mechanisms, the major areas of focus associate with the primary afferent nerves, primary afferent neurons (including dorsal root ganglion and trigeminal ganglion neurons), and spinal dorsal horn. Because these sites participate in transferring pain signals into the spinal cord and toward the brain for interpretation, a closer examination at the pain transmission pathway will make this concept more clear.

**Figure 1.** Diagram shows the pain transmission pathway and its modulation by GABA$_A$ receptors at the spinal level. An impulse travels from the body, down the primary afferent nerves and dorsal root ganglion, and into the dorsal horn within the spinal cord. It is within the spinal cord that dorsal horn neurons release GABA, which travels to bind to GABA$_A$ receptors at the primary afferent terminals or other dorsal horn neurons. The pain signal then transmits out of the spinal cord and projects to the higher brain centers for interpretation.

The spinal cord, when viewed as a cross-section, can be divided laterally into the dorsal and ventral halves - dorsal meaning back and ventral meaning front. The dorsal half controls the communication of sensory impulses into the central nervous system. In contrast, the ventral half controls the communication of efferent motor impulses from the central nervous system back out to the body, concerning mostly with motor movement. The dorsal root ganglion, a
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nodule containing the cell bodies of sensory nerves, passes sensory information to neurons in the spinal cord through the dorsal root fibers. At the end of the dorsal root is the dorsal horn within the spine, which receives the sensory information from the primary afferent nerves and sends this information to the higher-order neurons in the spinal cord and brain (Figure 1).

To fully understand the role of GABA\(_A\) receptors in pain modulation, one must comprehend the process in which the pain information is transmitted and regulated under normal conditions. This process begins when the sensory nerve terminal (nociceptor) is stimulated by painful stimuli. The stimulus initiates a change in the membrane potential on the nociceptor, and this signal transmits onward through the spreading of this changing membrane potential. This traveling wave of electrical excitation is known as an action potential and is set off by a sudden depolarizing of the cell membrane, meaning the membrane potential shifts to become less negative. The depolarizing occurs when a stimulus reaches a needed threshold and opens voltage-gated Na\(^+\) channels, allowing Na\(^+\) entrance to the cell. Like a traveling wave, the action potential continues outward from the original site of depolarization until it reaches the end of the primary afferent nerve terminal.

Once the impulse reaches the central terminal of primary afferent nerves, it must be transferred to the second-order neurons in the spinal dorsal horn across a space known as the synaptic cleft. In other words, the impulse must be transferred from the transmitting, or presynaptic neurons, across the synaptic cleft to the receiving, or postsynaptic neurons. For this to occur, the electrical signal transforms into a chemical signal in the form of excitatory neurotransmitters, such as glutamate. When the action potential reaches the axon terminal, voltage-gated Na\(^+\) channels temporarily open, causing the depolarization of the nerve terminal membrane and the opening of voltage-gated Ca\(^{2+}\) channels. Consequently, glutamate releases from the nerve terminal into the synaptic cleft through exocytosis and quickly travels across the synapse and binds to postsynaptic glutamate receptors on the postsynaptic neuron. As a result, the membrane of the postsynaptic neuron is depolarized, often resulting in firing an action potential. In this case, the glutamate receptors work to convert the chemical signals back into electrical signals in order to continue the information transfer. It is important to note that neurotransmitters, such as glutamate and GABA, at a synapse along the pain pathway create responses that can be either excitatory or inhibitory, thus, either augmenting pain or attenuating pain signals. It is the characteristics of the different receptors on which the neurotransmitters bind that determine the difference in excitation and inhibition of the pain pathway.

Control of Pain Transmission by GABA\(_A\) Receptors

GABA-containing (GABAergic) neurons are present throughout the spinal dorsal horn, and many of these neurons make synaptic contact with primary afferent nerve terminals (Barber et al., 1978). When the pain signal reaches the spinal dorsal horn, it can promote GABA release from GABAergic neurons. Pain transmission in the spinal dorsal horn is under tonic inhibitory control mediated largely by GABA (Yoshimura & Nishi, 1995; Cronin et al., 2004; Pan & Pan, 2004). For instance, spinal administration of bicuculline, a GABA\(_A\) receptor antagonist, results in hypersensitivity of dorsal horn neurons and increased pain sensitivity (Sivilotti & Woolf, 1994; Sorkin et al., 1998). Also, the loss of GABAergic neurons (disinhibition) in the spinal cord may have important consequences for the development of neuropathic pain (Castro-Lopes et al., 1993; Moore et al., 2002).

Increased GABA release can diffuse to adjacent primary afferent terminals and dorsal horn neurons to serve as a gate control mechanism to dampen the flow of pain signals (Melzack and Wall, 1965). For example, through activation of presynaptic GABA\(_B\) receptors, it can reduce excitatory neurotransmitter release from primary afferent terminals (Price et al., 1984; Li et al., 2002). GABA can also directly inhibit certain dorsal horn neurons through postsynaptic GABA\(_B\) receptors and opening of G protein-coupled inward rectifying K\(^+\) channels (Marker et al., 2006).

Importantly, increased synaptic GABA release can activate GABA\(_A\) receptors to inhibit normal pain transmission and can do so both presynaptically via depolarization of primary afferent terminals and postsynaptically via hyperpolarization of postsynaptic neurons. The net effect of GABA\(_A\) receptor activation on neurons depends on whether or not the intracellular Cl\(^-\) concentration is maintained below or above the electrochemical equilibrium by cation-Cl\(^-\) cotransporters in the plasma membrane. These cation-Cl\(^-\) cotransporters are responsible for maintaining Cl\(^-\) homeostasis and controlling anion flow through GABA\(_A\) receptors, creating the reversal potential. The reversal
Pain modulation by GABA receptors potential determines whether the GABA\(\alpha\) receptor will function in an excitatory or inhibitory manner, depending on the preexisting membrane potential (Figure 2).

![Figure 2](image.png)

**Figure 2.** The intracellular Cl\(^-\) concentrations, maintained by NKCC1 or KCC2, allow cells to control their excitability levels, as they strive to achieve a near equal balance with their extracellular environment. Thus, the direction of Cl\(^-\) movement follows the electrochemical gradients after activation of GABA\(\alpha\) receptors. A, when the cell begins with a high intracellular Cl\(^-\) concentration, the Cl\(^-\) ions will flow out of the cell through the GABA\(\alpha\) receptors. The cell now has a more positive membrane potential than before and is depolarized. B, if the cell begins with a low intercellular Cl\(^-\) concentration, the Cl\(^-\) ions will flow into the cell through the GABA\(\alpha\) receptors. This ion movement causes the cell to achieve a more negative membrane potential, and the cell is now said to be hyperpolarized. RMP, resting membrane potential.

There are two important types of cation-Cl\(^-\) cotransporters that regulate intracellular Cl\(^-\) concentrations in neurons: Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter-1 (NKCC1) and K\(^+\)-Cl\(^-\) cotransporter-2 (KCC2; Figure 2). NKCC1 is driven by Na\(^+\) and K\(^+\) gradients and typically raises intracellular Cl\(^-\) concentrations. NKCC1 is expressed at early developmental stages and is responsible for the high intracellular Cl\(^-\) concentrations in dorsal root ganglion and trigeminal ganglion neurons (Kanaka et al., 2001; Toyoda et al., 2005). It has been shown that the depolarizing GABA\(\alpha\) responses are dependent on NKCC1 expression because depolarizing GABA\(\alpha\) responses in dorsal root ganglion neurons are reduced in NKCC1-knockout mice (Sung et al., 2000). On the other hand, KCC2 normally lowers intracellular Cl\(^-\) concentrations below its electrochemical equilibrium potential. KCC2 is a key player in the developmental switch from GABA-mediated excitation to inhibition in the brain and spinal cord neurons (Ganguly et al., 2001; Hubner et al., 2001).

Primary afferent depolarization, mediated by GABA\(\alpha\) receptors and GABA released from spinal dorsal horn neurons, determines presynaptic inhibition, a key mechanism in somatosensory processing. The depolarization is due to Cl\(^-\) efflux through GABA\(\alpha\) channels, and the outward Cl\(^-\) gradient is generated by NKCC1. NKCC1 is responsible for intracellular Cl\(^-\) accumulation in dorsal root ganglion neurons and via regulation of intracellular Cl\(^-\), it participates in the modulation of sensory neurotransmission. In the case of presynaptic inhibition, GABA\(\alpha\) receptors function by
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Reducing the release of excitatory neurotransmitters from primary afferent terminals. Primary afferent neurons usually maintain a high intracellular Cl⁻ concentration compared to the extracellular concentration, causing the Cl⁻ ions to naturally exit the neuron through GABAA receptors. This outward flux of Cl⁻ ions brings the cell to a more positive and balanced state creating a depolarized state (Figure 2). To reestablish the Cl⁻ gradient, the NKCC1 activates cellular intake of Cl⁻, Na⁺, and K⁺ ions. The net effect of the GABAA receptor activation is a more positive, or less negative, depolarized state. Primary afferent depolarization jolts the magnitude of incoming action potentials and decreases excitatory neurotransmitter (such as glutamate) release, creating presynaptic inhibition in the spinal cord. Since depolarized GABAA currents reduce incoming action potentials, the quantity of glutamate released from the primary afferent neurons in the dorsal horn is reduced, thus inhibiting pain transmission (Rudomin & Schmidt, 1999; Willis, 1999).

In the case of postsynaptic inhibition, GABAA receptors function by controlling the Cl⁻ movement across the cell membrane via hyperpolarization of postsynaptic membranes. The dorsal horn neurons starts with a low concentration of Cl⁻ ions compared to the ion concentration outside the neuron, which is maintained by KCC2. Thus, the Cl⁻ ions will follow their electrochemical gradient and enter into the neuron through GABAA receptors, raising the intracellular Cl⁻ concentration (Figure 2). Since the neuron now has a more negative and less positive charge, it is said to be hyperpolarized. The hyperpolarizing makes the dorsal horn neuron membrane difficult to depolarize and therefore difficult to generate an action potential (Yoshimura & Nishi, 1995; Pan & Pan, 2004). As a result, pain transmission is inhibited.

**Plasticity of GABAA Receptors and Cl⁻ Transporters in Pain**

Although GABA is best known as the main inhibitory neurotransmitter in the adult central nervous system, it can actually excite neurons during embryonic development. In the immature brain and spinal cord, GABA produces an excitatory action via GABAA receptors because of a high intracellular Cl⁻ concentration (Ben-Ari et al., 1989; Reichling et al., 1994; Serafini et al., 1995). However, in adult neurons, a Cl⁻-extruding mechanism, mediated by KCC2, becomes operative and Cl⁻ is efficiently pumped out from the intracellular milieu. During postnatal development, the switch of GABA-mediated synaptic transmission from excitation to inhibition coincides with the upregulation of expression of KCC2. One consequence of this upregulation is that more Cl⁻ ions are pumped out of the cell, lowering the resting intracellular Cl⁻ concentration. As a result, there is a negative shift in the reversal potential for Cl⁻ ions, and GABA’s action becomes inhibitory. The initial depolarizing GABA-mediated potentials are critical for activation of voltage-dependent Ca²⁺ channels, setting off a signaling cascade that culminates in the developmental upregulation of KCC2 gene expression (Ganguly et al., 2001).

The NKCC1 proteins accumulate Cl⁻ intracellularly in dorsal root ganglion neurons, and it is the primary mechanism that sets the reversal potential for Cl⁻ conductance through GABAA receptors (Sung et al., 2000). Unlike most neurons in the brain and spinal cord, NKCC1 is responsible for the increase in intracellular Cl⁻ in dorsal root ganglion neurons and maintain depolarizing responses to GABAA receptor agonists throughout postnatal development. In certain pain conditions, GABAA responses could be enhanced such that the normal small depolarization of primary afferent fibers by GABA is augmented to the point that it directly induces firing activity. Injection of capsaicin, a pain-producing substance, into the colon causes a rapid increase in phosphorylated NKCC1 proteins in the spinal cord (Galan & Cervero, 2005). It has been demonstrated that NKCC1-knockout mice display reduced responses to noxious heat and reduced touch-evoked pain (Laird et al., 2004). These findings suggest that NKCC1 plays an important role in the modulation of inflammatory pain.

It has been shown that peripheral nerve damage reduces the expression of KCC2 in the spinal dorsal horn (Coull et al., 2003). The reduction in the transmembrane Cl⁻ gradient could cause normally inhibitory synaptic responses to be excitatory. As a result, these changes contribute to increased excitability of dorsal horn neurons leading to neuropathic pain. In the spinal cord of diabetic rats, KCC2 protein levels are also reduced, while levels of NKCC1 and the GABAA receptor are not changed (Jolivalt et al., 2008). Increased GABA levels, along with the decrease in KCC2 expression, may underlie the abnormal neuronal activity detected in the spinal cord of diabetic rats (Morgado et al., 2008). Reduction in KCC2 expression can lead to increases in intracellular Cl⁻ concentration and, in this condition, GABA binding to GABAA receptors induces membrane depolarization, provoking neuronal excitaiton.
Pain modulation by GABA receptors rather than inhibition. Thus, a loss of GABA-mediated inhibitory tone within the spinal cord may result in neuronal hyperexcitability in diabetic neuropathic pain (Morgado et al., 2008).

The expression level of KCC2 proteins in the spinal cord is also reduced in a rat model of inflammatory pain (Zhang et al., 2008). Therefore, inflammation induces downregulation of KCC2 in the spinal dorsal horn, which may in turn facilitate the development and/or maintenance of inflammatory pain. These data support the notion that loss of GABAergic inhibition in the spinal cord is a general feature of inflammatory and neuropathic pain conditions.

Targeting GABAA Receptors and Cl⁻ Transporters for Pain Control

Persistent pain could be produced from loss of GABAergic inhibition of spinal dorsal horn neurons. This loss can be compensated with facilitation of GABAergic neurotransmission through modulation of GABAA receptors. In normal rats, administration of GABAA receptor antagonists to the spinal cord causes thermal hyperalgesia and tactile allodynia, the condition in which ordinarily, nonpainful stimuli evoke pain. In rats subjected to peripheral nerve injury, spinally administered GABAA receptor agonists reverse thermal hyperalgesia and tactile allodynia (Malan et al., 2002). Thus, GABAergic manipulation at the spinal level after peripheral nerve injury may represent an alternative treatment for neuropathic pain.

Another approach to increase GABAergic inhibitory tone is the use of drugs known as benzodiazepines, which do not directly bind to the GABAA receptor recognition site. When benzodiazepines bind to their specific site, they allow the structure of GABAA receptors to change and allow GABA to more easily bind to its own recognition site. This creates a higher intake of Cl⁻ ions into the neurons and an increase in inhibitory inputs at the postsynaptic neurons. In support of this possibility, L-838,417, a benzodiazepine-site ligand targeting the α2 and α3 subunits of GABAA receptors, can effectively reduce neuropathic and inflammatory pain while avoiding sedation and tolerance development (Knabl et al., 2008). Further development of this type of drugs may hold great promise as a method to control chronic pain and avoid the side effects of classical GABAA receptor agonists.

A recent study also has examined the role of GABAA receptors at the dorsal root ganglion level in the control of neuropathic pain in rats. When muscimol, a GABAA receptor agonist, is applied at the time of nerve injury, it abolishes neuropathic pain. Furthermore, when applied after neuropathic pain development, muscimol can dose-dependently reduce neuropathic pain symptoms (Naik et al., 2008). When bicuculline is administered alone after nerve injury to the dorsal root ganglion, it exacerbates thermal hyperalgesia, the excessive sensibility to pain (Naik et al., 2008). These data suggest that increasing GABAA receptor activity on the primary afferent neurons can suppress pain transmission.

However, despite the above studies showing the benefit of treating painful conditions by enhancing the GABAergic inhibition and GABAA receptor activity, other studies have shown that enhanced GABAergic transmission and GABAA receptor activity may actually lead to sensitization of spinal dorsal horn neurons and worsening of inflammatory and neuropathic pain. When administered to the spinal cord, muscimol increases, but the GABAA receptor antagonist gabazine decreases, pain sensitivity in normal rats (Anseloni & Gold, 2008). However, after induction of inflammation, the effects of these compounds are reversed: muscimol exacerbates the inflammatory pain, but gabazine reduces inflammatory pain in rats (Anseloni & Gold, 2008). Hence, an inflammation-induced shift in spinal GABAA receptor signaling from inhibition to excitation appears to underlie inflammatory pain.

Furthermore, when GABAA receptor antagonists, picrotoxin and bicuculline, are administered to the spinal cord, they can reverse the sensitization of dorsal horn neurons induced by peripheral injection of painful chemicals (Garcia-Nicas et al., 2006). Similarly, spinally administered bicuculline reduces formalin-evoked paw flinching and also dose-dependently reduces mechanical pain sensitivity in diabetic rats (Jolivalt et al., 2008). These studies suggest that an enhanced GABAergic transmission may lead to hyperexcitability and sensitization of spinal dorsal horn neurons in acute and chronic pain. It is not entirely clear why manipulation of GABAA receptors produces distinct effects. It is possible that drugs used in the above studies may affect various types of target neurons in the dorsal root ganglion, spinal dorsal horn, and different brain regions.
Several studies have also indicated that cation-Cl⁻ cotransporters could be targeted to treat inflammatory and neuropathic pain. For example, downregulation of the KCC2 level in the spinal cord can reduce neuropathic pain in rats (Coull et al., 2003). Also, delivery of the NKCC1 blocker bumetanide to the spinal cord inhibits painful behaviors caused by formalin injection and mechanical allodynia induced by capsaicin injection into the hindpaw in rats (Granados-Soto et al., 2005; Valencia-de Ita et al., 2006; Pitcher et al., 2007). These results suggest that targeting the abnormal changes in cation-Cl⁻ cotransporters could reduce pain by restoring the normal Cl⁻ homeostasis and GABAergic inhibitory tone at the spinal level.

In summary, the changing activity of GABA receptors on the primary afferent terminals and spinal dorsal horn neurons can distinctly influence the role of GABA in pain transmission in normal and painful conditions. By manipulating GABA receptor activity in these locations, pain transmission can be modified for the better control of pain. It is likely that cation-Cl⁻ cotransporters and GABA receptors will receive increased attention in the future as a possible step up the ladder toward a better understanding of pain mechanisms and the development of more effective pain treatments.

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