GABAergic pain modulation

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$GABA_A$ receptors ($GABA_ARs$) are ligand-gated heteropentameric ion channels, most commonly formed by 2α, 2β, and 1γ subunit. They are expressed in spinal cord dorsal horn, both at the pre- and postsynaptic site, controlling the transmission of pain, itch, touch and proprioception.

Keywords: presynaptic inhibition ; GABA ; pain ; spinal cord ; neural circuits ; synaptic transmission

1. GABAergic Inhibition on Primary Afferent Fibers

GABAergic interneurons play a critical role in regulating nociceptive signal strength and separating nociception from touch. Intrathecal application of bicuculline and strychnine (antagonists of $GABA_A$ and glycine receptors, respectively) increases responses evoked by exposure to noxious stimuli [1]. According to the gate theory of pain, proposed by Melzack and Wall [2], stimulation of tactile Aβ fibers activate inhibitory interneurons in the dorsal horn, “closing the gate” to nociceptive transmission. Conditions of disinhibition (either pharmacological or induced by persistent pain) “open the gate”, increasing pain response and causing allodynia (i.e., the perception of an innocuous stimulus as noxious).

Presynaptic GABA receptors located on PAF terminals are involved in gating both tactile and noxious stimuli in the dorsal horn. Indeed, GABA receptors of the A and B type are expressed on both nociceptive and non-nociceptive PAFs, where axo-axonic synapses have been described.

$GABA_A$ receptors ($GABA_ARs$) are ligand-gated heteropentameric ion channels, most commonly formed by 2α, 2β, and 1γ subunit. The composition of $GABA_ARs$ on PAFs is heterogeneous: nociceptive C fibers express the α2, α3, and α5 subunits, while α1, α2, α3, and α5 are present on myelinated A fiber terminals [3][4][5]. The subunit β3 has been shown as the dominant β subunit expressed in dorsal root ganglion (DRG) neurons of both A and C type: In a mouse line where the β3 subunit is selectively knocked out in primary nociceptors, the GABA current in DRG neurons is decreased and the animals exhibit hypersensitivity to noxious heat and mechanical stimulation [6].

1.1. Primary Afferent Depolarization

Presynaptic $GABA_ARs$ expressed on PAF terminals mediate primary afferent depolarization (PAD) in spinal cord dorsal horn. This phenomenon, firstly described in muscle afferents [7], consists of a slow dorsal root depolarization, evoked by the stimulation of an adjacent root. Stimulation of PAFs evokes glutamate release that activates GABAergic interneurons. These neurons, in turn, release GABA binding to $GABA_ARs$ expressed on PAF terminals.

The cellular mechanisms of PAD have been extensively investigated. Primary sensory neurons exhibit a higher intracellular concentration of chloride than most central neurons. This is caused by the high expression of the transporter NKCC1 (transporting Cl^-, Na^+ and K^+ into the cell) and the low, or even undetectable, expression of KCC2 (expelling Cl^- and K^+ out of the cell) [8][9][10][11][12]. Due to the high Cl^- intracellular concentration, the chloride equilibrium potential (E_{Cl}) in DRG neurons is about −30 mV[9][13]. Thus, the activation of $GABA_ARs$ on PAF terminals produces an outward anion flux, leading to membrane depolarization and generation of PAD.

In physiological conditions, PAD exerts an inhibitory effect on glutamate release, through several possible mechanisms. PAF depolarization can lead to the inactivation of voltage-dependent Na^+ and Ca^+ channels, impairing the propagation of action potentials along PAFs and decreasing the calcium influx into the terminals[14][15][16][17][18]. The opening of $GABA_A$ channels could also exert a shunting effect on action potential propagation by decreasing membrane resistivity, as shown by experimental evidence and mathematical simulations [19][20][21][22]. Suprathreshold PAF depolarizations can sometimes trigger action potentials that are conducted antidromically, generating dorsal root reflexes [23][24].

Earlier studies have demonstrated the involvement of $GABA_A$Rs in the generation of PAD: The $GABA_A$ antagonist picrotoxin blocks presynaptic inhibition on spinal monosynaptic reflexes, while iontophoretic application of GABA generates depolarization of group I afferent fibers [25][26]. More recently, Witschi et al. have shown that mice lacking the $GABA_A$ α2 subunit specifically in primary nociceptors exhibit a lack of effect of the $GABA_A$ modulator diazepam in potentiating PAD and decreasing inflammatory hyperalgesia, confirming the involvement of presynaptic $GABA_A$Rs in PAD generation and pain inhibition [27]. Interestingly, neither glycine receptors nor gephyrin clusters have been detected on C fibers expressing $GABA_A$Rs, in contrast with inhibitory synapses on postsynaptic neurons [4]. The presence of unclustered $GABA_A$Rs on presynaptic terminals suggests a more diffuse mode of inhibition at these synapses, consistent with the slow kinetics of GABA-mediated PAD. Beside $GABA_A$Rs, also glutamatergic receptors of the AMPA and NMDA type, expressed on PAF central terminals, have been reported to contribute to the generation of PAD in the spinal cord [28].

PAD has been proposed as one of the most powerful mechanisms of sensory control, producing several effects: (1) reduction in the effectiveness of one sensory input over the others and selective control of convergent inputs; (2) generation of surround inhibition, producing localized reactions to sensory stimuli (a small stimulus to the skin produces PAD in the same spinal cord segment, but also in many rostral and caudal segments, both ipsi- and contra-lateral); and (3) increase of the temporal contrast of a somatic sensory input. Accordingly, PAD mediated by $GABA_A$Rs is composed of a phasic and a tonic component (likely mediated by receptors expressing the α5 subunit [29]): The first increases the perception of a sudden stimulus, while the latter represents the ongoing inhibition of slow changes of sensory inputs.

The functional properties of PAD in muscle afferents and LT cutaneous PAFs have been investigated by several studies [16][23][30][31]. We recently demonstrated that presynaptic $GABA_A$Rs are involved in short term synaptic depression during repetitive stimulation of Aβ fibers [32] (Figure 1). We performed electrophysiological experiments on rat spinal cord slices, recording from unidentified laminae III–IV neurons, in voltage-clamp at −70 mV. The dorsal root attached to the slice was electrically stimulated with four pulses at the frequency of 10–20 Hz and intensity of 10–25 μA, recruiting LT A fibers, mainly of the Aβ type. The evoked excitatory postsynaptic currents (EPSCs) showed a strong depression after the first response, which was particularly evident in the second EPSC (Figure 1B, black trace). Application of the $GABA_A$ antagonist gabazine unmasked an additional component in the second EPSC (blue trace): This indicates that GABA, released after the first pulse, acts on presynaptic $GABA_A$Rs, reducing glutamate release from Aβ fibers at the second stimulus. By mainly affecting the second response in a train of stimuli, presynaptic $GABA_A$Rs inhibit glutamate release from PAFs with a high temporal precision, controlling the earliest part of an afferent response to touch

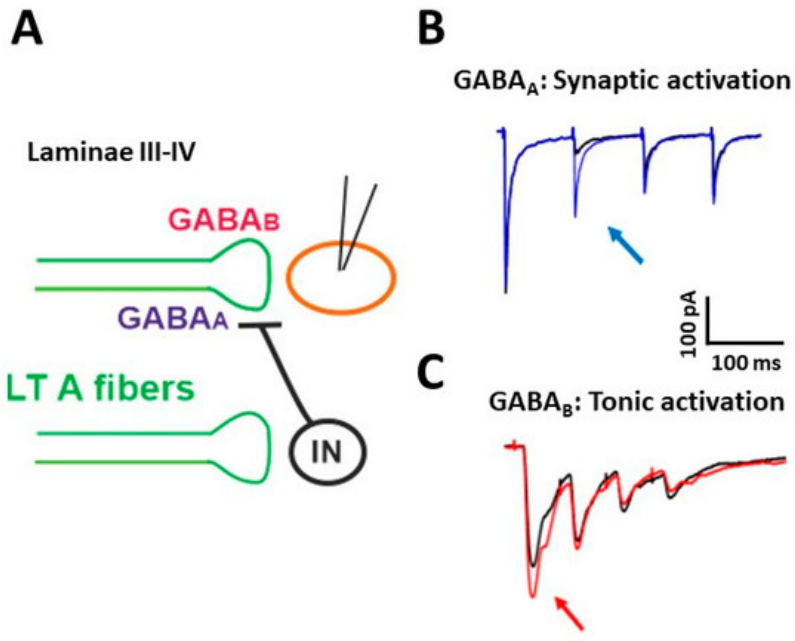

Figure 1. Presynaptic modulation mediated by $GABA_A$ and $GABA_B$ receptors on low threshold (LT) A fibers in deep dorsal horn (laminae III–IV). (**A**) Schematic representation of the circuit activating presynaptic GABA receptors. $GABA_A$ receptors can be recruited by a synaptic mechanism: an inhibitory interneuron (IN) is activated by LT A fibers and releases GABA onto fibers of the same type, causing the inhibition of glutamate release and synaptic depression. $GABA_B$Rs ($GABA_B$ receptors) can tonically inhibit the release of glutamate from LT fibers. (**B**) Representative traces of EPSCs recorded from a lamina III–IV neuron, evoked by stimulating LT fibers with four pulses at 10 Hz. A strong depression of the second response was evident in control (black trace). Application of the $GABA_A$R antagonist gabazine (10 μM) increased the

second EPSC (blue trace, arrow) in 10 out of 17 recorded neurons. (**C**) Representative traces of EPSCs, evoked by stimulating LT A fibers with four pulses at 20 Hz. In the presence of the $GABA_B$ antagonist CGP 55,845 (5 μM), the first EPSC increased in five out of 13 lamina III–IV neurons (red trace, arrow). Modified with permission from [33].

Using a cesium-fluoride intracellular solution, able to block $GABA_A$Rs expressed on the recorded neuron, the effect of gabazine on the second EPSC was not abolished, confirming the involvement of presynaptic $GABA_A$Rs. Application of strychnine was ineffective in increasing the second EPSC, indicating that glycine receptors are not importantly involved in presynaptic modulation on PAF terminals. By recording at −10 mV from laminae III–IV neurons and stimulating at Aβ fiber threshold, we observed inhibitory postsynaptic currents, mediated by both $GABA_A$ and glycine receptors. Thus, $GABA_A$Rs modulate the first synapse between Aβ fibers and dorsal horn neurons in two ways: through a negative feedback mechanism at PAF terminals and by a feed-forward control on postsynaptic neurons.

Differently from LT afferent fibers, functional studies about PAD on HT nociceptive fibers are still limited. By using an ex vivo spinal cord preparation, Fernandes et al. [34] recently reported that noxious C-fiber input to rat lamina I neurons (both projection and local neurons) is presynaptically modulated by Aβ, Aδ, and C fibers. Thus, presynaptic inhibition mediated by these different groups of afferents may control the inflow of nociceptive input to superficial dorsal horn, playing a role in nociceptive discrimination and lateral inhibition.

1.2. $GABA_B$ Receptors as Presynaptic Modulators

In addition to $GABA_A$, activation of $GABA_B$ receptors ($GABA_B$Rs), expressed on nociceptive and non-nociceptive PAF terminals, also contributes to presynaptic inhibition, exerting analgesic and anti-hyperalgesic effects [35]. $GABA_B$Rs are G protein-coupled receptors, expressed as obligate heterodimers of the two subunits $GABA_{B1}$ and $GABA_{B2}$. The $GABA_{B1}$ isoforms 1a and 1b, together with the subunit $GABA_{B2}$, have been found in small and large DRG neurons and in the spinal cord, both at PAF terminals and on dorsal horn neurons [36][37][33]. Endogenous or exogenous activation of $GABA_B$Rs in superficial dorsal horn causes both pre- and postsynaptic effects. Electrophysiological studies performed on rats have shown that presynaptic $GABA_B$Rs inhibit pinch- and touch-evoked synaptic responses in vivo [38] and decrease glutamate and peptide release from A- and C-type PAFs and dorsal horn neurons [37][39][40][41][42]. The inhibitory effect of $GABA_B$Rs on transmitter release is due to the concurrent inhibition of presynaptic calcium channels [43][44] and release machinery downstream of calcium entry into the nerve terminals [45].

By performing electrophysiological experiments similar to those described above, we showed that the block of $GABA_B$Rs increases the first EPSC in a train of four stimuli, recorded from lamina III–IV neurons [33] (Figure 1C, red trace). This suggests that, differently from $GABA_A$Rs, which require the release of GABA through synaptic activation, $GABA_B$Rs are tonically activated, confirming the finding of a previous study performed in lamina II [46].

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