Commentary: Serotonin Receptor 2B Mediates Mechanical Hyperalgesia by Regulating Transient Receptor Potential Vanilloid 1

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ABSTRACT

Serotonin [5-hydroxytryptamine (5-HT)] is an inflammatory mediator which contributes to inflammatory pain. We previously demonstrated that 5-HT-induced mechanical hyperalgesia is mediated by 5-HT$_{2B}$ but not by other 5-HT receptors. Our recent article provided further evidence how 5-HT$_{2B}$ regulates 5-HT-induced mechanical hyperalgesia, and suggested, that 5-HT$_{2B}$ mediates mechanical hyperalgesia through G$_{q/11}$-phospholipase Cβ (PLCβ)-protein kinase Cε (PKCε) pathway. Interestingly, transient receptor potential vanilloid 1 (TRPV1) also involves in 5-HT$_{2B}$-mediated hyperalgesia. It was the first evidence that 5-HT receptor regulates TRP channel to affect mechanical hyperalgesia. It is a commentary on the recent article that suggests distinct roles of peptidergic (IB$_4$-negative) and non-peptidergic (IB$_4$-positive) nociceptors in regulating 5-HT-induced mechanical hyperalgesia. In IB$_4$-negative neurons, 5-HT$_{2B}$ in response to 5-HT mediates PLCβ-PKCε to regulate TRPV1 function. In IB$_4$-positive neurons, 5-HT$_{2B}$ may control 5-HT$_3$ or other channels to regulate mechanical hyperalgesia.

Serotonin [5-hydroxytryptamine (5-HT)] is known as a neurotransmitter, which is involved in regulating transmission of nociceptive signals in central nerve system. It is also an inflammatory mediator, released from immune cells, platelets and epithelial cells in the peripheral tissues. Mounting evidence has suggested that 5-HT is a pro-inflammatory and pro-nociceptive agent which can cause pain and hyperalgesia through activating various subtypes of 5-HT receptors which are present in primary afferents. We previously demonstrated that 5-HT-induced mechanical hyperalgesia is attributed to 5-HT$_{2B}$ activation. Our recent article provided more detail mechanism that 5-HT$_{2B}$ mediates G$_{q/11}$-phospholipase Cβ (PLCβ)-protein kinase Cε (PKCε) pathway to control mechanical hyperalgesia in both IB$_4$-negative and -positive neurons. Interestingly, transient receptor potential vanilloid 1 (TRPV1) and 5-HT$_3$ are also regulated by 5-HT$_{2B}$ in response to 5-HT mediates PLCβ-PKCε to regulate TRPV1 function. In IB$_4$-positive neurons, 5-HT$_{2B}$ may control 5-HT$_3$ or other channels to regulate mechanical hyperalgesia.

The distinct roles of IB$_4$-negative and IB$_4$-positive neurons in 5-HT-induced mechanical hyperalgesia

Pain transduction from the periphery to central nerve system depends on A6 and C fiber sensory neurons. C-fiber sensory neurons can be divided into two classes depended on their molecular properties. The peptidergic c-fiber expresses the neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP). The non-peptidergic c-fiber binds Isolectin B$_4$ (IB$_4$) and expresses glia...
cell-derived neurotrophic factor receptors (GDNF) and P2X3 receptors. IB4-positive neurons have low action potential (AP) threshold and shorter AP duration than IB4-negative neurons. IB4-negative neurons are more important in transducing information about stimuli. Consistent with that 5-HT injection dramatically enhanced 5-HT-evoked intracellular calcium signals in IB4-negative neurons, but not in IB4-positive neurons. Therefore, IB4-negative neurons are the major neurons responsible for transducing 5-HT stimuli to induce mechanical hyperalgesia. Blocking 5-HT_{2B} PLCβ or PKCε before 5-HT injection inhibited the enhanced calcium signals in IB4-negative neurons. It is correlated with behavioral results that blocking of 5-HT_{2B}-PLCβ-PKCε pathway inhibited mechanical hyperalgesia.

Although IB4-positive neurons did not increases intracellular calcium signals after 5-HT injection, the number of the neurons responding to 5-HT was increased. IB4-positive neurons with higher density of tetrodotoxin (TTX)-resistant Na+ channel and longer AP could lead more efficient calcium influx into the presynaptic terminal, resulting in more transmitter release. IB4-positive neurons mediating more reliable synaptic connection could participate maintenance of hyperalgesia. As expected, blocking 5-HT_{2B} PLCβ or PKCε before 5-HT injection also inhibited the calcium signals in IB4-positive neurons.

In IB4-negative neurons, 5-HT-induced calcium signals were inhibited by removal of extracellular calcium; while only some of IB4-positive neurons were sensitive to calcium-free condition. It indicated that channels allow calcium influx may participate in 5-HT-induced calcium signals. TRPV1 and 5-HT3 are identified to participate in the downstream of 5-HT_{2B}-mediated signaling pathway in IB4-positive and IB4-negative neurons, respectively.

**Participation of TRPV1 in 5-HT signaling transduction in IB4-negative neurons: contribution to induction of mechanical hyperalgesia**

TRPV1 is known as a heat and capsaicin receptor, which is widely expressed in sensory neurons, especially in c-fiber nociceptors. TRPV1 is expressed both in peptidergic and non-peptidergic c-fiber in rat but predominantly in peptidergic c-fiber in mouse by immunohistochemical analysis. Although capsaicin-induced calcium signals were greater in IB4-positive neurons than in IB4-negative neurons before 5-HT injection, capsaicin-evoked calcium signals were significantly enhanced in IB4-negative neurons after 5-HT injection. Therefore, capsaicin-sensitive IB4-negative neurons may play a role in 5-HT-induced mechanical hyperalgesia under regulation of 5-HT_{2B}-PKCε.

As confirmed in animal behavioral studies, administration of a TRPV1 antagonist before 5-HT injection in mice inhibited 5-HT-induced mechanical hyperalgesia. Mice lacking TRPV1 genes also showed the absence of mechanical hyperalgesia after 5-HT injection. Even though TRPV1 participates in thermal hyperalgesia, several lines of evidence have also suggested that the involvement of TRPV1 in capsaicin, acid or CFA-induced mechanical hyperalgesia which indicated the involvement of TRPV1 in mechanical hyperalgesia.

How 5-HT_{2B} receptor may mediate PLC leading AEA formation to activate TRPV1. Arachidonic acid (AA) is the precursor that can be metabolized by various enzymes. The products of lipoxgenase include 12- and 15-HEPETO, and 5-HETE that are also TRPV1 agonists. 5-HT_{2B} receptor activation activate phospholipase A2, leading the neuronal secretion of AA. Therefore, peripheral 5-HT_{2B} activation by 5-HT may relieve phosphatidylinositol 4,5-bisphosphate-dependent channel inhibition and generates endogenous ligands AEA or AA to activate and regulate peripheral TRPV1 function, resulting in mechanical hyperalgesia.

**Participation of 5-HT_{3} in 5-HT-induced calcium signal in IB4-positive neurons: contribution to maintenance of mechanical hyperalgesia**

The calcium signals in IB4-negative neurons were completely dependent on 5-HT_{2B}-PLCβ-PKCε signaling and TRPV1 activation as described above. 5-HT-induced calcium signals in IB4-positive neurons were partially sensitive to removal of extracellular calcium, suggesting that the calcium signals may be from channels in both the plasma membrane and the endothelial reticulum (ER). 5-HT_{3} receptor antagonist (Granisetron) specifically inhibited 5-HT-induced calcium signals in a small set of IB4-positive population, explaining sensitivity of these neurons to removal of extracellular calcium. Thus, there are at least two distinct pathways in IB4-positive neurons in response to 5-HT stimulation. One is 5-HT_{2B}-PLCβ-PKCε pathway and the other is 5-HT_{2B}-PLCβ-PKCε/5-HT_{3} pathway.

5-HT_{3} was found in pain-related regions and is involved in pain processing. In our previous study, mice with pre-injection of 5-HT_{3} antagonist did not inhibit mechanical hyperalgesia but shortened the duration of pain after 5-HT.
injection. Data from the recent article are consistent with the previous study by Lin et al., that 5-HT is not involved in 5-HT-induced mechanical hyperalgesia. However, the shortening of mechanical hyperalgesia suggests that 5-HT may have an influence on modulating the maintenance of hyperalgesia. Stucky and Lewin’s suggested that IB4-positive neurons have a higher action potential (AP) threshold and longer AP duration than IB4-negative neurons. Also, there are some reports showing that IB4-positive neurons can exhibit sustained responses, but not transient or mixed responses to low pH. Therefore, the responses in IB4-positive neurons that are sensitive to graminetron are thought to be responsible for extending the duration of 5-HT-induced mechanical hyperalgesia.

The GPCR-TRP channel axis in 5-HT-induced pain and itch-like sensation

In previous studies, 5-HT can induce pain and itch sensations in mice and humans. A subset of 5-HT-sensitive neurons is also sensitive to histamine and chloroquine, suggesting that these neurons are involved in itch perception. A distinct subgroup of c-fibers that were preferentially excited by pruritc compounds has been reported. 5-HT receptors were reported to respond for 5-HT-induced itch through activating Gq/11-PLC pathway, and PKC activation. 5-HT-induced itch-like sensation could be also involved in itch sensation.

Conclusions

The recent article demonstrated that peptidergic and non-peptidergic nociceptors mediate 5-HT signaling through distinct mechanisms to induce mechanical hyperalgesia. The axis of 5-HT2A-Gq-PLCβ-PKCε-TRPV1 in peptidergic neurons contributes to induction of hyperalgesia, while the axis of 5-HT2A-Gq-PLCβ-PKCε-5-HT3 in non-peptidergic neurons participates in maintenance of hyperalgesia. The GPCR-TRP channel axis used in pain sensation could be also involved in itch sensation.

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