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Review article

Estrogen-dependent regulation of transient receptor potential vanilloid 1 (TRPV1) and P2X purinoceptor 3 (P2X3): Implication in burning mouth syndrome

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Abstract Sex differences in the nervous system have gained recent academic interest. While the prominent differences are observed in mood and anxiety disorders, growing number of evidences also suggest sex difference in pain perception. This review focuses on estrogen as the key molecule underlying such difference, because estrogen plays many functions in the nervous system, including modulation of transient receptor potential vanilloid 1 (TRPV1) and P2X purinoceptor 3 (P2X3), two important nociceptive receptors. Estrogen was shown in various studies to up-regulate TRPV1 expression through two distinct pathways, resulting in pro-nociceptive effect. However, estrogen alleviated pain in other studies, by down-regulating nerve growth factor (NGF)–activated pathways and TRPV1. Estrogen may also attenuate nociception by inhibiting P2X3 receptors and ATP-signaling. Understanding the mechanism underlying the pro- and anti-nociceptive effect of estrogen might be crucial to understand pathophysiology of the burning mouth syndrome (BMS), a common chronic orofacial pain disorder in menopausal women. The involvement of TRPV1 is strongly suspected because of burning sensation. Reduced estrogen level of the BMS patient might have caused increased activity of P2X3 receptors. Interestingly, the increased expression of TRPV1 and P2X3 in oral mucosa of BMS patients was reported. The combinational impact of differential modulation of TRPV1/P2X3 during menopause might be an important contributing factor of etiology of BMS. Understanding the estrogen-dependent regulation of nociceptive receptors may provide a valuable insight toward the peripheral mechanism of sex-difference in pain perception.

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Introduction

Sex differences in pain perception have gained growing academic interest. Several clinical reports suggested that women are more sensitive to pain than men, leading to a high percentage of women among patients diagnosed with a pain syndrome.[1–5] Moreover, common chronic pain disorders, such as neuropathic pain, fibromyalgia, osteoarthritis, or migraine, are generally more prevalent in women.[6–8] A large cohort study measuring pressure, mechanical, and thermal pain sensitivity observed greater orofacial pain sensitivity in women.[9] Despite these reports, the mechanism underlying the sex difference in pain perception remains unclear.[1–9]

Pain arises from the activation of specialized primary afferent neurons called nociceptors.[10] The nociceptive information is transmitted to the central nervous system and ultimately perceived as pain.[11] Nociceptors detect noxious stimuli through specific receptors, which have been the subject of extensive research for the past several decades. The two most studied families of nociceptive receptors are transient receptor potential (TRP) receptors and purinergic receptors.

The transient receptor potential vanilloid 1 (TRPV1), a representative member of the TRP family, is a Ca^{2+}-permeable ion channel activated by multiple sensory stimuli, such as heat (>43 °C), protons (pH < 6.0), and vanilloids.[12–14] Interestingly, TRPV1 activation is modulated by inflammatory mediators, such as prostaglandins, extracellular ATP, bradykinin, glutamate, and the nerve growth factor (NGF), suggesting its central role as a pain mediator.[15–18] Compelling amounts of evidence demonstrate the contribution of TRPV1 in the mechanism underlying chronic pain.[19] According to immunohistochemical studies in various peripheral neuropathy models, subsets of sensory neurons within the dorsal root ganglion (DRG) and trigeminal ganglia (TG) express high TRPV1 levels.[18,20,21] While many studies have demonstrated the robust expression and functional contribution of TRPV1 in chronic pain induced by inflammation or nerve injuries of these ganglia, recent findings showing TRPV1 expression in the central nervous system were perplexing and interesting.[14,22]

P2X3 is a purinergic receptor with a well-characterized role in nociception. It is expressed in small-to medium-sized DRG or TG neurons[23,24] and activated by extracellular ATP released from damaged tissue. P2X3 activation generates electrical impulses that are transmitted to the brain and perceived as pain. In particular, the fast desensitization and slow recovery of P2X3 make it a central element in chronic pain conditions.[25]

There are several clinical and animal studies that have suggested that steroid hormones, including sex hormones, influence neuronal survival, neurogenesis, receptor expression, neurotransmitter synthesis, and neuronal excitability.[26–28] In particular, estrogen mainly exerts excitatory actions in both a rapid and delayed mode[26] and play a key role in altering neural function through genomic and non-genomic pathways. Estrogen may play an essential role in chronic pain pathologies.[29–31] Thus, estrogen might alter the functional expression of nociceptive receptors, thereby contributing to the sex difference in pain perception. This review summarizes the current understanding of potential estrogen effects regarding TRPV1/P2X3 receptor function to elaborate the sex difference in nociception mechanisms. Furthermore, we discuss an orofacial pain disorder with extreme prevalence in middle-aged females called burning mouth syndrome (BMS) as a possible result of such difference.

Upregulation of TRPV1 by estrogen

Recent experimental data revealed that estrogen regulates TRPV1 and P2X3 activity and expression.[22,32] Upregulation of those nociceptive receptors by estrogen can provide important insights into the neuronal mechanisms of pain and sex differences. To date, many studies have suggested that estrogen has a pro-nociceptive effect on TRPV1, while it has the opposite effect on P2X3.[28,32–37] According to the studies related to TRPV1, estrogen upregulates TRPV1 through two different pathways: a classical genomic pathway and a non-classical pathway.

Estrogen is a lipid-soluble hormone that activates the classic nuclear estrogen receptors (ERα and ERβ), affecting the transcription of target genes and resulting in the classical long-term effect.[38] The first attempt to demonstrate the relationship between TRPV1 and estrogen showed that ERα and ERβ knockout mice had significantly lower TRPV1 expression levels than wild-type mice.[32] Another group revealed that 17β-estradiol, the most potent estrogen, binds to the estrogen receptor located in the cytoplasm of DRG neurons. Then, the estrogen receptor translocates to the nucleus, where it binds to the estrogen response elements located in the promoter region of the TRPV1 gene.[28] This results in the transcription of the TRPV1 gene, increasing its expression in the DRG and promoting pain. Besides the DRG, immunohistochemistry experiments showed that high estrogen doses increased TRPV1 mRNA expression in the TG.[33]

A behavioral test using ovariectomized rats supported a similar pro-nociceptive estrogen effect. It showed that ovariectomy completely reversed the enhanced sensitivity to the capsaicin-induced nocifensive response.[34] Besides, ovariectomized rats treated with high estrogen doses for 2 days showed greater capsaicin-evoked nocifensive behavior than ovariectomized rats treated with low estrogen doses.[33] In addition, observing rats at different estrous cycle phases has helped to prove the hormonal effect.

Consistent with the previous studies, rats in the proestrus phase (where estrogen levels rise) display a higher capsaicin-induced response than rats in other phases.[34]

By contrast, TRPV1 can be upregulated through a non-classical pathway involving the membrane-bound G-protein-coupled receptor 30 (GPR30). GPR30 is an important non-genomic receptor modulating pain perception expressed in neurons of the primary sensory ganglia.[39,40] In female rats, ovariectomy downregulates GPR30 expression in DRG neurons; estrogen replacement can help recover GPR30 expression.[41] Moreover, estrogen and GPR30 agonists injected into rats induced mechanical hyperalgesia, whereas GPR30 antagonists inhibited it.[42,43] A recent study suggested a protein kinase C epsilon (PKCε)-dependent mechanism, independent from classical estrogen receptors, to explain the GPR30-mediated pain pathway. It observed that estrogen acts through GPR30, triggering an intracellular signaling pathway that activates PKCε, which phosphorylates TRPV1.[44] Rats with TRPV1 phosphorylated by PKCε in their DRG were hypersensitive to capsaicin, heat, and acid.[45,46] This indicates that estrogen is a downstream signaling factor of PKCε-induced TRPV1 sensitization.

Downregulation of TRPV1 and P2X3 by estrogen

In contrast to the above findings, accumulating evidence suggests that estrogen alleviates pain. For example, prolonged elevation of estrogen levels during pregnancy elevates the pain threshold.[47] Another study showed that female rats tested in diestrus, proestrus, or estrus endured a hot plate significantly longer than others.[48] Recent clinical researches suggest that, besides the endogenous ones, exogenous hormones can lower pain in postmenopausal women. Although this so-called estrogen replacement therapy still has an unclear, non-generalizable mechanism, it has been widely used for postmenopausal diseases, such as osteoporosis, pelvic pain, or temporomandibular joint pain.[30] Animal studies also support estrogen replacement therapy inhibiting pain by an estrogen receptor-mediated process.[49,50]

With regard to TRPV1, downregulation of NGF by estrogen was reported as a mechanism responsible for its pain reduction effects. A recent time-course study showed that NGF could sensitize TRPV1, cause the acute-to-chronic transition, and maintain pain.[51] A major effect of NGF was to translocate TRPV1 to the cell surface membrane through tropomyosin-related kinase A (TrkA) signaling.[52,53] Estrogen significantly reduced NGF mRNA and protein expression levels in rat chondrocytes.[54] This is similar to previous studies showing that estrogen reduced NGF levels in rat hippocampus and sympathetic neurons.[55,56] Moreover, long-term estrogen replacement decreased TrkA mRNA levels in the DRG, which can alter NGF response.[57]

Laboratory experiments also point out that estrogen may attenuate pain by altering nociceptive signaling via P2X3. Prolonged exposure to 17β-estradiol (0.1—1 μM) had an anti-nociceptive effect on DRG neurons, inhibiting ATP-induced intracellular Ca^{2+} influx in the DRG.[58,59] The involvement of P2X3 receptors in the anti-nociceptive effect of estrogen has been recently discussed.[60] P2X3 activation in the rat DRG, which promoted ATP-induced peripheral hyperalgesia, may be reversed by estrogen replacement (30 μg/kg, 0.4 mL/day).[61] Another study also showed that estrogen replacement (30 μg/kg, 0.4 mL/day) reversed the ovariectomy-induced increase in P2X3 mRNA and protein expression levels in the DRG. By contrast, using progesterone on female rats or estrogen on male rats had no effect.[62,63] In the TG, estrogen treatment (30 μg/kg, 0.4 mL/day) also decreased P2X3 mRNA and protein expression levels. The estrogen-induced trigeminal P2X3 mRNA and protein expression decrease correlate with reduced facial mechanical pain. These results suggest that estrogen inhibits P2X3 receptors through a genomic mechanism.

The estrogen effect on nociceptors is most likely concentration-dependent. The inhibitory effect of 17β-estradiol on ATP-mediated responses occurs at 100 nM but not at 10 nM.[63] Applying 30 μg/kg of 17β-estradiol on mice for 6 weeks decreased P2X3 mRNA expression,[36,60,61,63] but applying 0.1 μg/kg for the same duration had no effect.[64] Moreover, different estrogen concentrations have anti-nociceptive effects on TRPV1. A report showed that exposure of DRG neurons to 17β-estradiol (10—100 nM) overnight inhibited TRPV1 activation.[37] Overnight incubation with 1 nM of 17β-estradiol or short (10 min) incubation did not remarkably alter TRPV1 expression.[65] Considering that a 1-day exposure to 100 pM 17β-estradiol rather significantly increased TRPV1 mRNA expression,[65] the anti-nociceptive effects of estrogen on TRPV1 may require a relatively high concentration or long exposure duration.

It is conceivable that estrogen has both pro-nociceptive and anti-nociceptive effects, depending on the nervous system region, the exposure conditions, and the pathway it affects. Moreover, studies on estrogen-related pain mechanisms should be considered according to the estrogen concentration and duration applied to come up with a consensus.

Burning mouth syndrome

Burning mouth syndrome (BMS) is an idiopathic chronic and intractable oral pain condition characterized by lowered sensory and pain thresholds in the absence of detectable oral mucosa changes.[66] Many clinical and laboratory studies have searched the causes of this syndrome, such as psychiatric, local, and systemic factors, yet the underlying mechanism remains unclear. Although its etiology is quite multifactorial and poorly understood, BMS is remarkably more prevalent in women with a sex ratio of 7:1.[67] In particular, most BMS patients are middle-aged or older women during peri- or post-menopause.[68] Estrogen replacement therapy has been suggested for BMS patients, revealing that most patients who responded to this therapy had an increased estrogen receptor expression.[69] This strongly implies that estrogen plays an essential role in BMS. Moreover, BMS is related to a small-fiber idiopathic neuropathy of the trigeminal system affecting oral sensation.[70] This indicates that key ion channels involved in pain perception at the peripheral level, TRPV1 and P2X3, should also be investigated

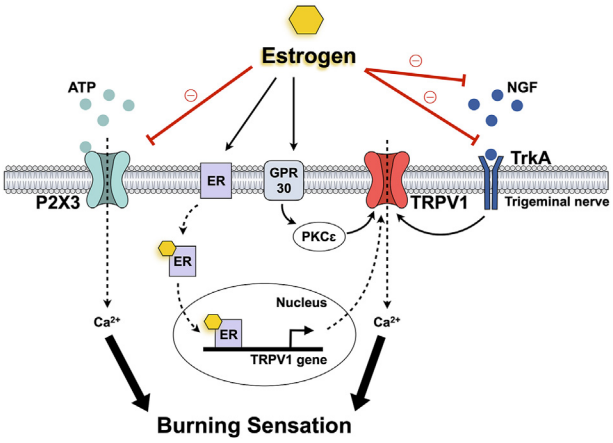

Figure 1 Estrogen regulation on TRPV1 and P2X3 with burning mouth syndrome. Abbreviations: ATP, adenosine triphosphate; ER, estrogen receptor; GPR30; G-protein-coupled receptor 30; NGF, nerve growth factor; P2X3, P2X purinoceptor 3; PKCε, protein kinase C epsilon; trkA, tropomyosin-related kinase A; TRPV1, transient receptor potential vanilloid 1.

together to understand the role of estrogen on these nociceptive ion channels and neuropathic pain pathways of BMS (Fig. 1).

The TRPV1 channel mediates pain perception and may participate in BMS symptoms. BMS patients have shown a low tolerance to noxious heat stimulation, including capsaicin.[71] The burning sensation comes from a small-fiber sensory neuropathy affecting the tongue's epithelial and sub-papillary nerve fibers.[72] The expression of TRPV1 and its regulator NGF was significantly increased in the epithelial fibers of BMS patients, and this expression correlated with their capsaicin pain score.[70] While a high TRPV1 expression in trigeminal afferents may be associated with hypersensitivity in BMS, a high NGF concentration plays a crucial role in enhanced intracellular signaling. Considering that estrogen can reduce NGF/TrkA signals to TRPV1, increased NGF concentrations might increase pain sensitivity in postmenopausal women.

Furthermore, a recent study has found a significant correlation between P2X3 and BMS. It found an increased P2X3 immunoreactivity in the trigeminal sensory system in the lingual mucosa of patients with BMS.[73] P2X3 in the trigeminal sensory system may play a pivotal role, along with TRPV1, in developing and maintaining BMS symptoms. With regard to the anti-nociceptive effect of estrogen, 17β-estradiol inhibits ATP-induced intracellular Ca^{2+} influxes in ganglia neurons.[59] Considering that P2X3 receptors are ATP-dependent Ca^{2+}-permeable channels, estrogen might also regulate P2X3 expression by attenuating ATP-induced Ca^{2+} influxes. In fact, many studies have suggested that estrogen inhibits P2X3. Estrogen decreased P2X3 mRNA and protein expression levels in primary afferent neurons.[35,36] Also, estrogen replacement reversed ovariectomy-induced increased P2X3 expression in rats.[63] These results suggest that estrogen inhibits P2X3-mediated peripheral pain transduction.

Despite extensive research on the role of TRPV1 and P2X3 receptors on pain perception, knowledge about the interaction of these receptors in sensory ganglion activity remains sparse. Previous studies have reported colocalization of TRPV1 and P2X3 on peripheral afferent fibers[23,74] and a high co-expression percentage of these receptors in pain conditions, including BMS.[32] However, those channels certainly respond differently to various inflammatory factors.[75] Understanding the complex peripheral mechanism of BMS would require further examination of the interaction of co-expressed TRPV1 and P2X3 receptors. Moreover, 17β-estradiol modulates both channels. Considering the inconsistent estrogen effect on pain perception, the combined effect of estrogen on those nociceptive receptors together should also be addressed. In conclusion, to explain the sex differences in BMS observed in clinical practice and explore the therapeutic role of estrogen, further studies need to confirm the interaction between TRPV1 and P2X3.

Discussion

In summary, it is evident that estrogen regulates nociceptive receptors, TRPV1 and P2X, through genomic and non-genomic pathways, potentially contributing to the sex difference in pain. Many studies reported the activation of TRPV1 and inhibition of P2X3 by 17β-estradiol in TG and DRG, although a few studies with varying concentration of 17β-estradiol, or with the different pain models reported inconsistent results.

BMS is one of the representative diseases where sex is a strong predisposing factor. The strong prevalence of BMS in post-menopausal woman suggests reduced estrogen as a triggering factor. The estrogen-deficient status of the postmenopausal women might cause the enhanced NGF signaling and TrkA, thereby increasing TRPV1 activity in the peripheral nerves. Decreased estrogen level can also increase pain sensitivity by reducing estrogen-dependent modulation of P2X3 in the sensory neurons. These results suggest that reversing the activity of NGF, TrkA or P2X3 could be a proposing therapeutic strategy of BMS. Studies that test the inhibitors of NGF, TrkA or P2X3 might provide valuable new insights on the etiology or treatment of BMS, especially of peripheral nerve origin.

In conclusion, the interaction between P2X3, TRPV1, and estrogen in sensory neurons may represent a novel mechanism that can explain gender-driven discrepancies in pain perception, and uncovering it could help figure out appropriate therapeutic targets and developing treatments.

Declaration of competing interest

The authors have no conflict of interest relevant to this article.

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