A new hypothesis of sex-differences in temporomandibular disorders: Estrogen enhances hyperalgesia of inflamed TMJ through modulating voltage-gated sodium channel 1.7 in trigeminal ganglion?

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Objective: Temporomandibular disorders (TMD) are an assorted set of clinical conditions characterized mainly by pain in the temporomandibular joint (TMJ). TMJ inflammation or synovitis is frequently observed in TMD patients and is the major reason for TMD pain. TMD is prevalent in women of childbearing age, at least twice than in men, implying that estrogen may be involved in TMD pain processing. Estrogen affects a cell mainly through the estrogen receptors (ER). The estrogen–ER complex binds to estrogen response element sequences (ERE) in the promoter region of specific genes and then exerts its regulatory potential. The voltage-gated sodium channel 1.7 (Nav1.7), whose single disruption leads to a complete loss of pain, amplifies weak stimuli in the neurons and acts as the threshold channel for firing action potentials and plays a prominent role in pain perception, including inflammatory pain. Furthermore, our previous study showed that trigeminal ganglionic Nav1.7 was involved in the hyperalgesia of the inflamed TMJ. We propose that estrogen may enhance hyperalgesia of inflamed TMJ through decrease nociceptive threshold of TMJ or inflamed TMJ by modulating both expression and channel threshold of Nav1.7 in trigeminal ganglion.

Introduction

Temporomandibular disorders (TMD) are an assorted set of clinical conditions characterized by pain, joint click and joint dysfunction in the temporomandibular joint (TMJ). TMD are 2–3 times more prevalent in women than in men; pain is the most reason for TMD patients seeking care [1,2]. TMD pain is strongly related to joint inflammation or synovitis and concentrated during the reproductive years, typically with onset of pain after puberty and declining after menopause; the sex-differences in the prevalence, severity, and duration of pain in TMD have been attributed to fluctuations in estrogen [1]. Our previous study also showed that 17β-estradiol enhances allodynia of inflammatory temporomandibular joint (TMJ) through upregulation of hippocampal TRPV1 in ovariectomized rats [3]. Although many clinical and basic researches suggest that estrogen increases susceptibility to TMD pain, some researches have also shown that low estrogen or rapid changes in estrogen concentration result in an increase in TMD pain in women [4], suggesting that the estrogen–TMD pain relationship is complicated and remains to be further investigated.

Estrogen may affect a cell through classical nuclear pathways or by signal transduction cascades initiated at cell surface membrane receptors. In the “classical” or “nuclear” mechanism of estrogen action, estrogen diffuses into the cell and binds to the estrogen receptors (ER), which contain ERα and ERβ [5]. The estrogen–ER complex binds to estrogen response element sequences (ERE) in the promoter region of some gene, and then modulates the levels of associated mRNA and protein. The essential ERE has been determined to have the consensus sequence GGTCAnnnTGACC, a 13-nucleotide segment with 10 nucleotides forming an inverted repeat [5,6]. However, only a handful of the most highly estrogen-responsive genes contain perfect consensus EREs. Many genes have been found to contain sequences that appear to be EREs, but most of these vary from the consensus by one or more nucleotides [6]. In the “non-classical” or “non-nuclear” mechanism of estrogen action, G protein-coupled receptor 30 (GPR30) has been identified...
as a novel estrogen receptor, which can activate downstream second messenger pathways in response to estrogen, such as the MAPK extracellular-signal-regulated kinase (ERK) and so on [7].

TMD pain may reflect hyperactivity in primary afferent neurons innervating the TMJ. Trigeminal ganglion (TG) is the location of the primary afferent neuron cell bodies for sensing and relaying the nociceptive sensations of TMJ [8]. Estrogen can affect nociception of TMJ by targeting the TG, which have been proven as one of the most obviously effects relevant to TMD pain [9-11]. In regard to estrogenic modulation of nociceptive neurons in the area of the TMJ, the retrogradely labeled TMJ neurons from ovariectomized rats and ovariectomized rats receiving chronic estrogen replacement were studied using whole cell patch-clamp techniques 3 days after injecting the TMJ with either saline as control or complete Freund's adjuvant (CFA) to induce inflammation, which then proved that estrogen increased the excitability of rat TMJ afferents and exacerbated the inflammation-induced sensitization of these sensory neurons [9]. Furthermore, E2x, E2R and GPR30, which are the three main estrogen receptors, have been demonstrated to present in trigeminal neurons [12,13].

Voltage-gated sodium channels, which contain Nav1.1–Nav1.9 subtypes, mediate the depolarization phase of the action potential and are key regulators of pain conduction [14]. Nav1.7 is of special interest because it has been linked to a spectrum of inherited human pain disorders, whose single disruption leads to a complete loss of pain [15]. Nav1.7, whose principal δ-subunit is encoded by a sodium channel voltage-gated type IX alpha subunit (SCN9A) gene, is highly expressed in the dorsal root ganglion (DRG), TG, sympathetic ganglions, and the endings of pain-sensing nerves (nociceptors) close to areas where the impulse is initiated [16]. Nav1.7 amplifies weak stimuli in the neurons and acts as the threshold channel for firing action potentials [17]. Nav1.7 has an important function in inflammatory pain. The increased tetrodotoxin-sensitive current amplitude following the inflammation of the hind paw is paralleled by an increase in Nav1.7 mRNA and protein in DRG, i.e., upregulation of Nav1.7 expression concurrently accompanies the enhancement of its function of amplifying stimuli in the neurons [18]. The function of Nav1.7 in inflammatory pain signaling is also supported by the knock-down and knockout studies in mice. Nociceptor-specific knockout of Nav1.7 abrogates inflammation-induced mechanical and thermal hyperalgesia [19]; the knock-down of Nav1.7 in primary afferents prevents inflammatory hyperalgesia [20]. A nucleotide polymorphism in Nav1.7 alters pain perception in patients with either knee or hip osteoarthritis [21]. Furthermore, our previous study also showed that trigeminal ganglionic Nav1.7 was involved in hyperalgesia of inflamed TMJ [22].

Hypothesis

TMD has greater prevalence, severity, and duration in women than in men. TMD pain is mostly due to inflammation or synovitis in TMJ. The effects of estrogen on TMD pain can be complicated. Although we have previously demonstrated that 17-estradiol enhanced hyperalgesia of inflammatory TMJ and Nav1.7 in TG was involved in TMJ inflammatory pain, and estrogen enhances the excitability of rat TMJ afferents and exacerbated the inflammation-induced sensitization of these sensory neurons in other's study, whether estrogen can enhance hyperalgesia of inflamed TMJ by modulating Nav1.7 in TG remains to be explored. It is well known that estrogen exerts its regulatory potential on gene expression through different nuclear and non-nuclear mechanisms. A direct nuclear approach is the interaction of estrogen with ERE. Three sequences, GGTCAagTTCGCT, GCTCactcTGAAT and TGCAGataTGACC, which appear to be ERES, are in the promoter region –1269/-1282, –1214/-1227 and –439/-452 of rat Nav1.7 (SCN9A). Similarly, two ERE-like sequences, AGTCAtcT- GAAA and CAACAccTGACC, are located in the promoter region –1823/-1836 and –1602/-1615 of human Nav1.7 (SCN9A). Therefore, it is highly likely that estrogen might regulate Nav1.7 mRNA expression.

Our hypothesis was as follows: estrogen could enhance hyperalgesia of inflammatory TMJ through modulating trigeminal ganglionic Nav1.7 (Fig. 1) by exerting its regulatory potential on Nav1.7 expression through ER–ERE interaction in the promoter region of Nav1.7 and also promoting the function of Nav1.7 (Fig. 2).

Evaluation of the hypothesis

Our hypothesis is consistent with the phenomenon of sex-differences in TMD. TMD is at least twice more prevalent in women than in men. In a laboratory model of TMD pain, healthy women reported more pain than healthy men after glutamate injection into the masseter muscle [23]. Moreover, serum estradiol levels have been reported to be higher in luteal phase women and in men who have TMD compared to healthy controls [24,25]. In addition, our previous study also showed that 17β-estradiol enhanced allodynia of inflammatory temporomandibular joint (TMJ) through upregulation of hippocampal TRPV1 in ovariectomized rats [3]; these findings suggest that estrogen may modulate TMD pain through the central neural system.

Our hypothesis is consistent with the view that estrogen modulates the nociceptive neurons innervating the TMJ, i.e., estrogen may also modulate TMD pain through the peripheral neural system. Acute TMJ inflammation induced by mustard oil injection produced greater fos-like immunoreactivity in proestrous compared to diestrous female rats at the major terminal zone for sensory afferents innervating the TMJ, indicating that TMJ inflammation produces greater neural activation when ovarian hormones are higher [26]. Estradiol also dose-dependently increased trigeminal afferent discharge induced by NMDA injection into the masseter muscle in ovariectomized rats [27]. Moreover, estrogen increases the excitability of rat TMJ afferents and exacerbates the inflammation-induced sensitization of these sensory neurons [9]. These findings strongly suggest that estrogen modulates trigeminal afferent discharge in the area of the TMJ.

Our hypothesis is consistent with the view that Nav1.7 plays an important role in pain perception. Nav1.7 is highly expressed in dorsal root ganglion (DRG), TG, sympathetic ganglions, and the endings of pain-sensing nerves (the nociceptors) close to areas where the impulse is initiated [16,28]. Mutations of Nav1.7 contribute to three human pain syndromes. Gain-of-function mutations cause primary erythromelalgia [29] and paroxysmal extreme pain disorder [30]. Loss-of-function mutations congenital inability to experience pain [15], revealing that its single disruption leads to a complete loss of pain. Moreover, carrageenan induced inflammation of hindpaw could increase Nav1.7 mRNA and protein expressions in DRG, which is paralleled by an increase in tetrodotoxin-sensitive current amplitude, i.e., enhances the function of Nav1.7 [18]. In addition, the nociceptor-specific knock-out of Nav1.7 [19] and the knock-down of Nav1.7 [20] in primary afferents abrogate inflammatory hyperalgesia. Our previous study also found trigeminal ganglionic Nav1.7 was involved in hyperalgesia of inflamed TMJ [22]. These findings strongly suggest that Na1.7 plays an important role in pain.

In conclusion, estrogen can increase TMD pain through modulating activities of the trigeminal neurons innervating the TMJ. Nav1.7 amplifies weak stimuli in the neurons and acts as the threshold channel for firing action potentials and plays an important role in pain perception. Therefore, our hypothesis that estrogen might enhance hyperalgesia of inflammatory TMJ through modulating trigeminal ganglionic Nav1.7 will worth exploring.
Fig. 1. TMJ inflammation induces expressions of cytokines, such as TNFα, IL-1β, and NGF. These cytokines act on the nerve endings, initiate impulse and induce Nav1.7 expression in the trigeminal ganglion (TG). Estrogen may further upregulate trigeminal ganglionic Nav1.7 expressions, which amplifies weak stimuli in the neurons, through estrogen receptors (ERα and ERβ) and then enhances hyperalgesia of inflammatory TMJ.

Fig. 2. Estrogen diffuses into the cell and binds to the estrogen receptor (ER), which contains ERα and ERβ. This estrogen–ER complex binds to estrogen response element sequences (ERE) in the promoter region of Nav1.7, and then modulates mRNA and Nav1.7 expressions.

How might this hypothesis be tested?

1. To test whether there is a sex-difference of TMJ nociception in rats, and if it does, then to further examine whether it is proportionally related to Nav1.7 expression in TG, rats will be divided into male group and female group; head withdrawal threshold, which indicates TMJ nociception [31], and mRNA and protein expressions of Nav1.7 in TG will be measured.

2. To examine whether change in expression of Nav1.7 in TG correlates with estrogen fluctuation in estrous cycle of rats, and to further examine whether change in TMJ nociception is related to change of Nav1.7 expression in TG, female rats will be divided into 4 groups according to the estrous cycle determined by vaginal cytology [32]: preoestrus group (which has highest serum level of oestrogen), diestrus group (which has higher serum level of oestrogen than the rest two groups), estrus group, and metestrus group; head withdrawal threshold and Nav1.7 mRNA and protein expressions in TG of these groups will be measured.

3. To examine whether estrogen can dose-dependently upregulate Nav1.7 expression in TG and also enhance sensitivity of TMJ nociception, female rats will be divided into 4 groups based on ovariectomy and estrogen replacement to exclude the impact of other possible factors in estrus cycle, such as progesterone: Sham group (which received sham-operation of ovariectomy) and 3 groups of ovariectomized rats treated with 0 μg, 20 μg, and 80 μg of 17β-estradiol as our previous study [3]. Head withdrawal threshold and Nav1.7 mRNA and protein expressions in TG will be measured.

4. To examine whether estrogen can enhance sensitivity of inflamed TMJ nociception through modulating Nav1.7 expression, female rats will be divided into 5 groups: control group, sham-ovariectomized group, and 3 groups of ovariectomized rats treated with 0 μg, 20 μg, and 80 μg of 17β-estradiol. The last four groups will be injected with 50 μl of CFA into the bilateral TMJs to induce TMJ inflammation on the tenth day of estradiol treatment, and the control rats will be injected with vehicle (50 μl saline). Head withdrawal thresholds and Nav1.7 mRNA and protein expressions in TG will be measured. To block the function of Nav1.7 in TG, ovariectomized rats treated with 80 μg of 17β-estradiol were equally divided into 3 groups: control, vehicle group, and Nav1.7 antibody group. The vehicle group and Nav1.7 antibody group will be injected with 50 μl of CFA into the bilateral TMJs to induce TMJ inflammation. The Nav1.7 antibody group will be microinjected with Nav1.7 antibody into the trigeminal ganglion performed as previously described [22]. Then head withdrawal threshold and food intake (another indicator for TMJ nociception [31]) will be measured.

5. To examine whether estrogen exerts their regulatory potential on Nav1.7 expression through ERs, ICI 182,780 (estrogen receptors α and β inhibitor) will be used to observe if there is reversion of inflammation-induced pain behavior and Nav1.7 expressions. Female rats will be divided into 3 groups: control, inflammation without ICI 182,780 treatment, and inflammation with ICI 182,780 treatment. Head withdrawal thresholds, food intake and Nav1.7 mRNA and protein expressions in TG will be measured.

6. To examine whether estrogen regulates Nav1.7 expression through ER-ERE interaction in the promoter region of Nav1.7 (SCN9A), rat Nav1.7 (SCN9A) promoter of 1.5 kb containing the region −1269/−1282 (GGTCAagtTGCtC) and −439/−452 (TGCCAgtaTGACC) will be cloned into luciferase reporter and transfected into nerve growth factor (NGF)-induced and ERα-transfected PC12 cells, which are ERα-null cells and possess neuron property [33]. Luciferase activity will be measured after the cells treated with increasing doses of 17β-estradiol for 24 h.

7. To investigate whether estrogen regulates the channel activity of Nav1.7, patch-clamping experiments will be performed. ERα and β-transfected HEK 293 cells will be co-transfected with SCN9A fused with a red fluorescent protein and the auxiliary sodium channel β1 and β2 subunits fused with green fluorescent protein to form a functional Nav1.7, and only cells clearly expressing SCN9A (red fluorescence) and β1 and β2 subunits (green fluorescence) will be used for electrical activity measurement as reported previously [15]. The cells will be treated with vehicle, 17β-estradiol, IL-1β/17β-estradiol, and IL-1β, respectively, and the amplitude of Na+ current will be measured.
Significance of the hypothesis

To the best of our knowledge, the relationship between Nav1.7 and sex-differences of TMJ pain has never been put forward. We consider that estrogen may enhance hyperalgesia of inflammatory TMJ through both upregulation of trigeminal ganglionic Nav1.7 expression and function. If this hypothesis is proven, it may help better understand the sex-differences of TMD or TMD pain and even help develop new therapy for TMD pain.

Conflict of interest

None declared.

References