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Synovial TRPV1 is upregulated by 17-β-estradiol and involved in allodynia of inflamed temporomandibular joints in female rats

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ABSTRACT

Women with reproductive capability are more likely to suffer from temporomandibular disorders (TMD), with orofacial pain as the most common complaint. In the past, we focused on the role of estradiol in TMD pain through the nervous system. In this study, we explored estradiol's influence on synoviocyte gene expressions involved in the allodynia of the inflamed TMJ. The influence of 17-β-estradiol on NGF and TRPV1 expression in TMJ synovium was determined *in vivo* and *in vitro* and analyzed by Western blot and real-time PCR. Complete Freund's adjuvant (CFA) injection into the TMJ was used to induce TMJ arthritis. Capsazepine served as a TRPV1 antagonist. Head withdrawal threshold was examined using a von Frey Anesthesiometer. We observed that estradiol upregulated the expressions of TRPV1 and NGF in a dose-dependent manner. In the primary cultured synoviocytes, TRPV1 was upregulated by lipopolysaccharide (LPS), estradiol, and NGF, while NGF antibodies fully blocked LPS and estradiol-induced upregulation of TRPV1. Activation of TRPV1 in the primary synoviocytes with capsaicin, a TRPV1 agonist, dose-dependently enhanced COX-2 transcription. Moreover, intra-TMJ injection of TRPV1 antagonist, capsazepine, significantly attenuated allodynia of the inflamed TMJ induced by intra-TMJ injection of CFA in female rats. This article presents a possible local mechanism for estradiol that may be involved in TMJ inflammation or pain in the synovial membrane through the pain-related gene TRPV1. This finding could potentially help clinicians understand the sexual dimorphism of TMD pain.

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Abbreviations: TMD, temporomandibular disorders; NGF, nerve growth factor; TRPV1, transient receptor potential vanilloid 1; TMJ, temporomandibular joints; CFA, complete Freund's adjuvant; HSP 25, heat shock protein 25; LPS, lipopolysaccharides; DMSO, dimethyl sulfoxide; SEM, standard error of mean.

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1. Introduction

Women with reproductive capability are more likely to suffer from temporomandibular disorders (TMD) with orofacial pain as the most common chief complaint.[1] In post-menopausal females, the possibility of developing a TMD pain case is 30% higher in patients receiving estrogen replacement therapy compared to those not exposed; furthermore, the dose response relationship for estrogens is monotonic.[2] Significantly higher serum estradiol levels were also found in the luteal phase of the menstrual cycle in women who have TMD pain compared to the healthy controls.[3] We previously observed that estradiol levels of synovial fluid in TMD patients are also higher than the levels of healthy controls.[4] Sex hormones appear to be a risk factor for TMD. Recently, we also observed that 17-β-estradiol, the major component of estrogen, enhanced the allodynia of temporomandibular joint (TMJ) inflammation, partially by influencing the expression of transient receptor potential vanilloid 1 (TRPV1) and nerve growth factor (NGF) in the hippocampus–this represents a possible central nervous system (CNS) mechanism for estrogen involved in TMJ pain.[5,6] TMD pain is significantly related to synovitis, internal derangement, and osteoarthritis, indicating that joint inflammation could be a major reason for TMD pain.[7–10]

TRPV1, also known as vanilloid receptor 1, is a nonselective cation channel that was originally identified as the capsaicin receptor.[11] TRPV1 is mainly expressed in the peripheral nervous system and plays a key role in the detection of noxious painful stimuli, such as capsaicin, acid, heat and endogenous ligands.[12] The expression and sensitization of TRPV1 is regulated by nerve growth factor (NGF).[13,14] Activation of TRPV1 in sensory neurons induces the release of inflammatory neuropeptides that cause neurogenic inflammation and pain.[15] TRPV1 activation can also increase COX-2 expression, which is an indicator of inflammation.[16]

TRPV1 is not only expressed in the nerves and vessels distributed in the synovium, but also in the synovial lining cells in both rat and human TMJs.[17,18] However, its biological role in the TMJ remains unexplored. In this article, we explored whether estradiol can induce TRPV1 expression in the TMJ synovium and whether synovial TRPV1 is involved in the allodynia of the inflamed TMJ.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats (180–200 g) were used (Vital River Laboratory Animal Technology CO., LTD, Beijing, China). The experiment was approved by the Animal Use and Care Committee of Peking University and was consistent with the Ethical Guidelines of the International Association for the Study of Pain. The rats were randomly divided into 5 groups (6 rats per group); a group of sham-ovariectomized rats, and 4 groups of ovariectomized rats that received estradiol at doses 0, 20, 80, or 200 μg.

2.2. Estradiol administration

The rats received bilateral ovariectomies or sham ovariectomies and recovered for 1 week. 17-β-estradiol (Huamei Huli Biochem CO., LTD, Beijing, China) was dissolved in corn oil. The ovariectomized rats were dosed with 17-β-estradiol by subcutaneous injections at doses of 0, 20, 80 or 200 μg per rat, in a volume of 200 μl every morning for 12 days. The ovariectomized rats receiving the above doses of estradiol could generate different plasma levels of estradiol.[5] The sham-ovariectomized rats were also dosed with subcutaneous injections of identical amounts of corn oil. The rats were sacrificed with an overdose of sodium pentobarbital (100 mg/kg body weight).

2.3. Western blot analysis

The synovial membrane of the TMJ, which is composed of a synovial lining layer and a connective sublining layer,[19] was dissected from the bilateral TMJs. The membrane was then homogenized in an ice-cold lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin) and centrifuged at 13,000 × *g* for 20 min at 4 °C. The protein concentrations of the supernatants were determined using the BCA assay (Pierce, Rockford, IL, USA) and the supernatants were resolved by 8% polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membrane was blocked in 5% nonfat dry milk in TBS-T buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.05% Tween-20) for 1 hr at room temperature and probed with anti-TRPV1 antibodies (SC-12498, P-19; Santa Cruz Biotechnology, Santa Cruz, CA) at 1:200, or anti-NGF antibodies (Santa Cruz Biotechnology) at 1:500 overnight at 4 °C. The membrane was washed extensively with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. After extensive washing with TBS-T, the membrane was visualized using the ECL kit (Applygen Technologies Inc., Beijing, China). For the internal control, the blots were stripped and reprobed with anti-β-actin polyclonal antibodies (Santa Cruz Biotechnology) at 1:1,000.

2.4. Image analysis

Digital western images were taken with a general electric scanner (Hewlett-Packard Development Company, L.P., USA). Numerical value of TRPV1 and NGF productions were performed with the commonly used image analysis software ImageJ.[20]

2.5. Isolation of TMJ synovial cells

TMJ synovial cells were isolated from intact female Sprague Dawley rats as previously described.[19] Briefly, the inner surface of the TMJ capsule was dissected, washed with phosphate-buffered saline (PBS) three times to remove blood, and maintained at 4 °C in PBS with penicillin (1000 U/ml) and streptomycin (1000 μg/ml) for 10 min. The synovial tissue was cut into small pieces (1–2 mm^3) and digested with 1 mg/ml collagenase IA (Sigma, St. Louis, MO) at 37 °C for 60 min. The cells in the digested solution were collected and washed twice with PBS and cultured in DMEM (Gibco, Gland Island, NY) supplemented

with 10% heat-inactivated fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.6. Immunocytochemistry of cultured primary cells

The cells were cultured on cover slips and fixed in 4% paraformaldehyde in PBS for 10 min. After rinsing with PBS, the cells were treated with 3% H_2O_2 for 10 min to inactivate endogenous peroxidases, and blocked with goat serum for 30 min to reduce non-specific background staining. The cells were then incubated with anti-heat shock protein 25 (HSP 25) polyclonal antibody (Wuhan Boster Biological Technology Ltd., Wuhan, China) at 1:50, or anti-laminin polyclonal antibody (Wuhan Boster Biological Technology Ltd., Wuhan, China) at 1:100. The cells were washed three times with PBS and incubated with horseradish peroxidase-conjugated secondary antibodies for 30 min at room temperature. After thoroughly washing with PBS, the cells were visualized using 3,3′-diaminobenzidine (Zhong Shan Golden Bridge Biological Technology CO., LTD, Beijing, China) for 1 min.

2.7. Treatment of synoviocytes with reagents

Identified synoviocytes between passages 3 and 5 were used. The cells were treated alone or in combination with the following reagents for 24 h: vehicle (0.1% ethanol), 17-β-estradiol (1 nM, dissolved in ethanol), lipopolysaccharides (LPS, 1 mg/ml), NGF (20 ng/ml), and rabbit anti-NGF serum (0.5 μg/ml) as indicated in Fig. 2C. The antiserum was added into the media 30 min before the other reagents. To examine whether the activation of synovial TRPV1 could affect COX-2 expression, the cells were treated with either TRPV1 agonist capsaicin (Sigma, St. Louis, MO, USA) dissolved in adimethyl sulfoxide (DMSO) vehicle or only the vehicle for 24 h.

2.8. Quantitative real-time PCR

Total RNA was extracted by TRIzol (Invitrogen, Carlsbad, CA). Reverse transcription was performed using an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) in a 20 μl reaction volume containing 1 μg of total RNA, incubated at 25 °C for 5 min, transcripted at 42 °C for 30 min, and terminated by heating at 85 °C for 5 min. Real-time PCR was performed with Power SYBRGreen PCR Master Mix using a 7500 Real-time PCR system (Applied Biosystems, Foster, CA). The reactions were run in duplicates with 1 μl of cDNA template in a 20 μl reaction volume with the program running for 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 1 min. The amplification specificity was confirmed by melting curve. The mRNA level of the target gene was acquired from the value of the threshold cycle (Ct) as relative level to that of β-actin through the formula $2^{-\Delta Ct}$ (ΔCt = β-actin Ct − gene of interest Ct). The primers were synthesized according to the previous study as follows: rat TRPV1 sense/antisense, 5′-GAC ATG CCA CCC AGC AGG-3′/5′-TCA ATT CCC ACA CAC CTC CC-3′[21]; rat COX-2 sense/antisense, 5′-CTG AGG GGT TAC CAC TTC CA-3′/5′-TGA GCA AGT CCG TGT TCA AG-3′[22]; rat β-actin sense/antisense, 5′-TGA CAG GAT GCA GAA GGA GA-3′/5′-TAG AGC CAC CAA TCC ACA CA-3′.[23] p75NTR, forward 5′-AGCCACGTCAACCTGACTG-3′ and reverse 5′-CCTCGCTCGTCACGTTCAC-3′; TrkA, forward 5′-GGCGATGACGTGTTTCTGC-3′ and reverse 5′-AGGAGACGCT-GACTTGGACA-3′. The resultant PCR products were separated by electrophoresis on a 2% agarose gel and visualised under ultraviolet transillumination.

2.9. Measurement of mechanical allodynia of TMJ

Twelve intact female Sprague–Dawley rats (180–200 g) were randomly and equally divided into two groups, either with or without induction of TMJ inflammation. TRPV1 antagonist capsazepine (Sigma) was dissolved in DMSO. For the group without induction of TMJ inflammation, rats ($n = 6$) were only injected with 25 μl capsazepine (600 ng) into the left TMJs and 25 μl of vehicle (saline/DMSO at 1:1) into the right TMJs. For the group with induction of TMJ inflammation, the rats ($n = 6$) were also injected with 25 μl capsazepine (600 ng) into the left TMJs and 25 μl of vehicle into the right TMJs; 25 μl complete Freund's adjuvant (saline/CFA at 1:1) was then injected into the bilateral TMJs 30 min later to stimulate a strong, persistent immune response and induce TMJ inflammation as reported previously.[5] To ensure induction of TMJ inflammation in the proestrous stage, in which the plasma level of estradiol is highest during the rat estrous cycle,[24,25] CFA was injected into the TMJ in the late metoestrous stage. This stage was determined by obtaining a vaginal smear at 4:00 P.M. daily for two consecutive estrous cycles (Supplemental Fig. 1). Twenty hours after CFA injection, the head withdrawal threshold was assessed by applying the filament of an electronic von Frey Anesthesiometer (IITC Life Science, CA, USA) to the skin before the ear around the TMJ until the head of rat was withdrawn as previously reported (Supplemental Fig. 2).[26] The force was increased continuously as the threshold was measured. Each side was tested five times at an interval of a few seconds. The response threshold was defined as the lowest force of the filaments that produced at least three withdrawal responses in five tests.[26] Head withdrawal threshold for one side of the joint were averaged by number of rats per group and presented as mean ± standard error of mean (SEM).

2.10. Statistical analysis

Data is presented as the mean ± standard error of mean (SEM). Comparisons of head withdrawal threshold from bilateral TMJs were examined with paired t tests for the same group and with analysis of variance (ANOVA) for between groups. The other data were examined with ANOVA. Statistical significance was considered at $p < 0.05$.

Statistical comparison of treatment groups was carried out using anova followed by Dunnett's post hoc test with 95% confidence interval (CI).

3. Results

3.1. Estradiol induced expressions of TRPV1 and NGF in the TMJ synovial membrane in ovariectomized rats

The expression of TRPV1 in the TMJ synovial membrane (includes neurons, endothelial cells, and synoviocytes) was

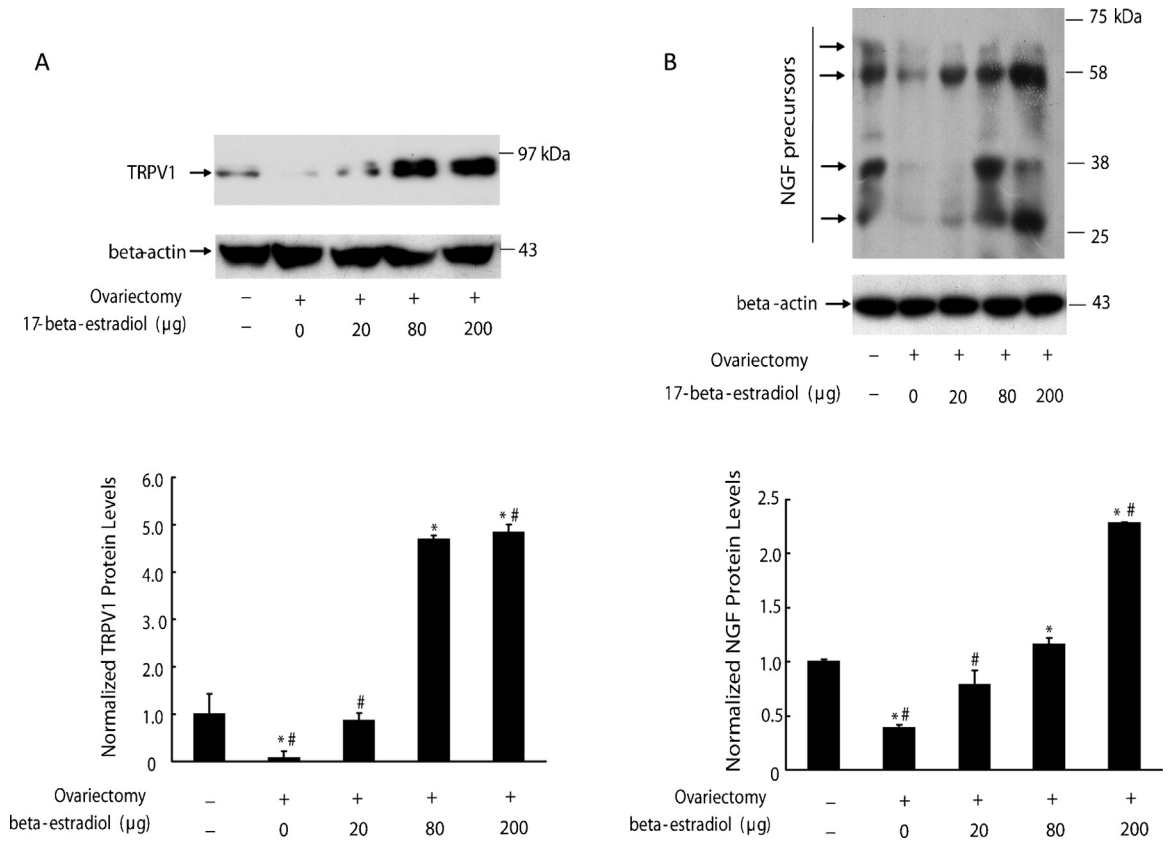

Fig. 1 – Representative immunoblotting of TRPV1 expression (A) and NGF expression (B) in the synovial membrane of the ovariectomized rats receiving 17-β-estradiol. β-actin served as the internal control of equal loading. Results were presented as mean ± SE. $^{*}p < 0.05$ versus control group; $^{\#}p < 0.05$ versus 80 μg group.

lower in the ovariectomized rats receiving no estradiol, compared to the control group. However, estradiol replacement rescued the decrease of TRPV1 expression and further potentiated its expression in a dose-dependent manner (Fig. 1A).

The expression of synovial NGF was very similar to the expression pattern of synovial TRPV1. However, synovial NGF was detected mainly in the form of precursors NGF (ProNGF) (60, 38, and 27 kDa) (Fig. 1B), a method consistent with a previous report.[27]

3.2. *Characterization of synoviocytes*

To further confirm the results *in vivo*, we cultured primary synovial cells. Cells isolated from the TMJ synovial membrane were characterized by immunocytochemistry. We digested the isolated synovial tissue with collagen IA as described previously.[19] Consistent with the previous study,[19] the majority of the cells demonstrated positive staining for HSP25 (a homologue of human heat shock protein HSP27) and laminin in the cytoplasm (Fig. 2A and B), suggesting that the cultured primary synovial cells were mostly fibroblast-like synoviocytes (type B cells).[19,28] NGF receptor (NGF-R) (TrkA and p75) were expressed in synoviocytes (Fig. 2C), in line with the previous study,[29,30] NGF and NGF-R were unregulated in synoviocytes of patients with rheumatoid arthritis or spondyloarthritis.

3.3. *Induction of TRPV1 transcription by estradiol depended on NGF in synoviocytes*

We treated synoviocytes with estradiol and LPS to verify that estradiol and inflammatory cytokines could enhance synovial TRPV1 expression through an NGF pathway. As shown in Fig. 2D, TRPV1 transcription in the synoviocytes was induced by treatment with estradiol or LPS ($p < 0.05$), but was more significantly induced by NGF ($p < 0.001$). When treated with a combination of estradiol and LPS, TRPV1 transcription was further increased compared to the group treated with estradiol or LPS alone ($p < 0.05$). However, pretreatment with anti-NGF antiserum totally blocked the induction of TRPV1 produced by the combined treatment of estradiol and LPS ($p < 0.05$).

3.4. *Activation of TRPV1 by capsaicin enhanced COX-2 transcription in synoviocytes*

To explore the possible function of synovial TRPV1 in allodynia of the inflamed TMJ, the synoviocytes isolated from the TMJ were treated with TRPV1 agonist capsaicin. As shown in Fig. 3,

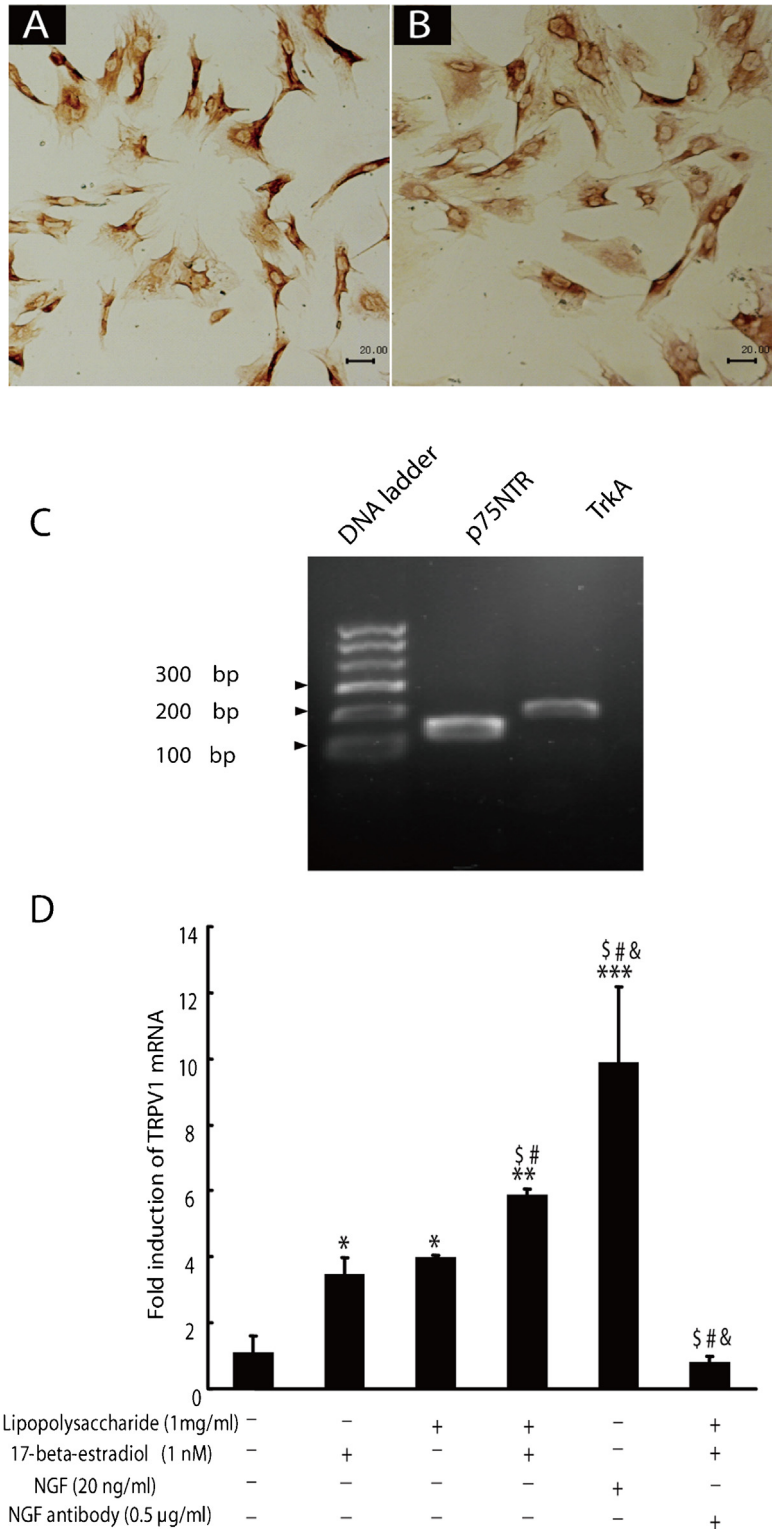

Fig. 2 – The cells were isolated from the TMJ synovial membrane and showed positive staining for heat shock protein 25 (A) and laminin (B) in the cytoplasm. (C) The cultured synovial cells were treated with the indicated reagents for 24 h and TRPV1 expression was evaluated by real-time PCR. $^{*}p < 0.05$ vs. control group; $^{**}p < 0.01$ vs. control group; $^{***}p < 0.001$ vs. control group; $^{\$}p < 0.05$ vs. estradiol group; $^{\#}p < 0.05$ vs. LPS group ($p < 0.05$); $^{\&}p < 0.01$ vs. LPS + estradiol group ($n = 3$, ANOVA).

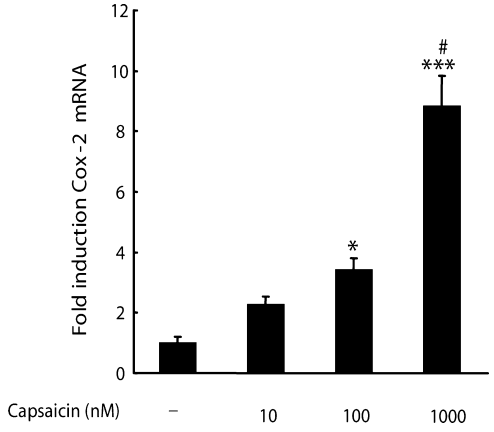

Fig. 3 – The cells were treated with TRPV1 agonist capsaicin for 24 h and COX-2 expression was evaluated with real-time PCR. $^{*}p < 0.05$ vs. control group; $^{***}p < 0.001$ vs. control group. $^{\#}p < 0.05$ vs. other 2 groups ($n = 3$, ANOVA).

the expression of synovial COX-2 mRNA was induced by capsaicin in a dose-dependent manner.

3.5. *TRPV1 antagonist capsazepine pretreatment attenuated allodynia of inflamed TMJ in female rats*

To further explore the role of synovial TRPV1 *in vivo*, TRPV1 antagonist (capsazepine) pretreatment was applied to the TMJ before the induction of TMJ arthritis. As shown in Fig. 4, the group without induction of TMJ inflammation had no difference between the baseline head withdrawal threshold of TMJ on the side with the intra-TMJ capsazepine injection and that of TMJ on the contralateral side with the vehicle injection ($p > 0.05$, $n = 6$, paired t test). In the group with induced TMJ inflammation, the head withdrawal threshold on the side with prior intra-TMJ injection of the vehicle decreased 26% compared to the group without TMJ inflammation ($p < 0.05$, $n = 6$, ANOVA). However, the TMJ inflammation-induced decrease of head withdrawal threshold was partially reserved in the contralateral side with the intra-TMJ pretreatment injection of capsazepine ($p < 0.05$, $n = 6$, paired t test).

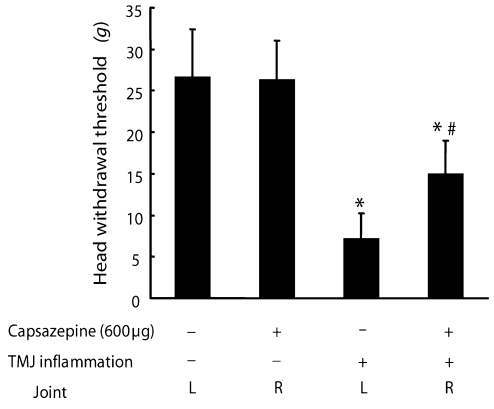

Fig. 4 – Intra-TMJ injection of TRPV1 antagonist capsazepine significantly attenuated mechanical allodynia of the inflamed TMJ. $^{*}p < 0.05$ vs. the group without TMJ inflammation; $^{\#}p < 0.05$ vs. the contralateral side within the same inflammation group. L = left; R = right. $n = 6$, paired t test.

4. Discussion

Pronociceptive or antinociceptive effects of estradiol remain fiercely controversial in the literature.[31] In the present study, we showed that estradiol potentiated expressions of TMJ synovial NGF and TRPV1 in ovariectomized rats. Furthermore, we also showed that intra-TMJ injection of TRPV1 antagonist partially attenuated mechanical allodynia of an inflamed TMJ. These results suggest that estradiol may augment nociception from an inflamed TMJ through the upregulation of TRPV1 expression in the TMJ synovial membrane–this is a possible local mechanism underlying the predominance of females among TMD patients.

4.1. *Rat temporomandibular joint inflammation induced by injection of CFA*

TMD represents a heterogeneous cluster of diseases and joint inflammation could be a major reason for TMD pain.[7–10] Thus far, several methods have been attempted to create animal models of TMD, including disc displacement (DD),[32] mouth opening,[33] drug-inducing,[34,35] and spontaneously occurring methods.[36] Because of the limited availability of special animal species, complicated operations, and slow progression of the disease, the use of spontaneous or surgical-induced methods was limited.[35,37] CFA is a solution of antigen emulsified in mineral oil and consists of inactivated and dried mycobacteria. LPS, a main component of gram-negative bacteria outer membranes, binds specifically to Toll-like receptor 4 (TLR-4) expressed in various immune and non-immune cells and induces a robust immune reaction via induction of proinflammatory cytokines such as TNFα, IL-1β, IL-6, and chemokines, including other inflammatory factors.[38–40] The rat TMJ synovial tissue expressed TLR-4 and it's the target of LPS,[41] in line with the activation of TLR signalling in synovial fibroblasts that leads to secretion of cytokines, chemokines and matrix-degrading enzymes.[42–45] Twenty-four hours after injection of CFA into TMJ, chromodacryorrhea in the eyes and intense redness, swelling over the TMJ region and allodynia of inflammatory temporomandibular Joint were observed in all the CFA-injected groups.[31,46] Histopathologic examination showed that the synovial tissues were hypertrophied, with an increase of synoviocytes and infiltrated leukocytes in the CFA-injected TMJ.[31] Angiogenesis and fibrin-like exudate in the superior joint space were also observed in the CFA-injected TMJ compared with the control joint.[31] The expression of the tumor necrosis TNF-α, IL-1β, IL-6, COX-2, and inducible nitric oxide synthase in the synovial membrane was upregulated, indicating that injection of CFA into the TMJ successfully induced TMJ inflammation.[47] CFA injection is simple and reproducibly induces TMJ inflammation, as shown in previous work by us and others with a focus on the phase of inflammation.[31]

4.2. *Estradiol inducing TMJ synovial TRPV1 expression depended on NGF*

Estrogen receptor alpha is expressed in type B synovial cells of the rat TMJ, so the TMJ synovial membrane is an estrogen target tissue.[31] In this investigation, we showed that estradiol could potentiate the expressions of TRPV1 and NGF in the TMJ synovial membrane in ovariectomized rats. Moreover, the expressions of TRPV1 and NGF in the synovial membrane appeared to be dependent on the plasma level of estradiol (Fig. 1), as their expressions were both decreased in the ovariectomized rats without estradiol replacement compared to the control. Our results are in line with studies showing that estradiol enhances TRPV1 and NGF expression in the dorsal root ganglion and hippocampus.[6,48] However, whether the enhancement of TRPV1 expression by estradiol was NGF-mediated remains unknown. TPRV1 is upregulated by NGF both *in vitro* [13] and *in vivo*.[49] Considering that the synovial NGF was also induced by estradiol in this study, we examined whether NGF mediated estradiol-enhanced synovial TRPV1 expression in cultured TMJ synoviocytes. We confirmed that estradiol-induced TRPV1 transcription depended on NGF, as anti-NGF antiserum completely blocked the combined effects of LPS and estradiol on TRPV1 in synovial cells (Fig. 2). The results also suggested that NGF mediated LPS-induced synovial TRPV1 transcription. Moreover, it appeared that estradiol could enhance the effects of LPS on TRPV1 expression. Since LPS promotes the secretion of pro-inflammatory cytokines in many cell types, it may induce synovial TRPV1 expression through inflammatory mediators or cytokines.

4.3. *ProNGF involved in allodynia of inflamed TMJ*

NGF is secreted as a precursor and cleaved by its regulators to generate mature NGF.[50] ProNGF is generally thought to be cleaved to the mature, biologically active NGF prior to secretion. It has been commonly held that NGF performs its effects primarily through TrkA, inducing a cascade of tyrosine kinase-initiated responses, while proNGF binds more strongly to p75NTR. A coimmunoprecipitation study further confirmed that p75NTR bound pro-NGF more than mature NGF.[51] There are increasing reports on this precursor form of neurotrophins upregulated in pathophysiological conditions.[52–54] It is showed that pro-NGF binding to p75NTR is responsible for Inflammatory thermal hypersensitivity.[55,52] In the present study, the upregulation of endogenous pro-NGF induced by estradiol may involve in allodynia of inflamed temporomandibular joints in female rats induced by CFA.

4.4. *Synovial TRPV1 involved in allodynia of inflamed TMJ*

Activation of TRPV1 in the joint afferents may cause a secondary release of neuropeptides.[56] Here, we demonstrated that activation of TRPV1 by capsaicin dose-dependently increased COX-2 expression in TMJ synoviocytes (Fig. 3). COX-2 is the major enzyme in the biosynthesis of prostaglandins, such as PGE2 and PGI2, which are important inflammatory mediators. Injection of PGE2 or PGI2 through the artery close to the knee has been found to sensitize joint afferents in response to mechanical and chemical stimuli.[57,58] In addition, the level of PGE2 in TMJ fluid is significantly and positively correlated to an index of clinical joint pathology or pain during joint movement in the TMJ with inflammatory disorders.[59,60] TRPV1 is constitutively activated in inflamed tissues due to low pH and increased temperature. Therefore, our results regarding capsaicin's effects in synoviocytes suggested that once synovial TRPV1 was activated, it would increase the release of prostaglandins from the synoviocytes and lead to hyperalgesia or allodynia of the inflamed joint. This suggestion was in fact supported by our results demonstrating that intra-articular injection of capsazepine, a competitive TRPV1 antagonist, significantly attenuated mechanical allodynia of the inflamed TMJ (Fig. 4). This also revealed that synovial TRPV1 could be involved in nociception of the inflamed TMJ. The TMJ synoviocytes may receive noxious stimuli and initiate vicious cycles of synovial TRPV1 activation and inflammation, leading to the sensitization of TMJ afferents.

However, targeting synovial TRPV1 alone may not sufficiently block hyperalgesia or allodynia of the inflamed TMJ. Our intra-articular injections of capsazepine did not completely block allodynia of the inflamed TMJ, which is in line with a previous report showing that hyperalgesia is greatly decreased in TRPV1 knockout mice but not completely absent compared to the wild type mice.[61] This indicates that multiple mechanisms may be involved in the complex processes of joint pain. Since pain and inflammation are inherently linked, peripheral application of a TRPV1 antagonist with anti-inflammatory drugs may still have clinical significance.

5. Conclusions

In conclusion, the NGF-TRPV1 signalling pathway in TMJ synoviocytes was induced by estradiol and may be involved in the allodynia of inflamed TMJs. Estradiol may potentiate TMD pain through the induction of synovial TRPV1. Peripheral application of TRPV1 antagonist with anti-inflammatory drugs may have anelgesic effects on TMD pain.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical approval

The experiment was approved by the Animal Use and Care Committee of Peking University and was consistent with the Ethical Guidelines of the International Association for the Study of Pain. The reference number is LA2012-59.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.archoralbio.2015.05.011.

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