Estrogens Exacerbate Nociceptive Pain via Up-Regulation of TRPV1 and ANO1 in Trigeminal Primary Neurons of Female Rats

Kazuaki Yamagata, Mitsutaka Sugimura, Miki Yoshida, Shinichi Sekine, Akiyo Kawano, Aiko Oyamaguchi, Hiroharu Maegawa, and Hitoshi Niwa

Department of Dental Anesthesiology (K.Y., M.Y., A.K., A.O., H.M., H.N.), Osaka University Graduate School of Dentistry, Suita City, Osaka, 565-0871 Japan; Department of Dental Anesthesiology (M.S.), Field of Oral Maxillofacial Rehabilitation, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima City, Kagoshima, Japan; and Division of Special Care Dentistry (S.S.), Osaka University Graduate School of Dentistry, Suita City, Osaka, Japan

Several trigeminal pain disorders show sex differences, and high levels of estrogens may underlie these differences. The interaction between transient receptor potential vanilloid 1 (TRPV1) and anoctamin 1 (ANO1) plays an important role in peripheral nociception. However, whether TRPV1 and ANO1 are involved in estrogen-modulated trigeminal pain sensitivity is unclear. In this study, we examined estradiol (E2) modulation of nociception through behavioral and immunohistochemical experiments after application of capsaicin (Cap), a selective TRPV1 agonist, onto the ocular surface in ovariectomized rats treated with high-dose E2 (HE) or low-dose E2 (LE) for 2 days. In addition, we used real-time PCR to study the effects of E2 on the expression levels of TRPV1 and ANO1 mRNA in trigeminal ganglia. In the behavioral experiment, the HE group showed significant potentiation of Cap-evoked nocifensive behavior compared with the LE group. Immunohistochemistry showed that Cap evoked a significantly greater number of cells that were immunoreactive for c-Fos, a marker of nociceptive activation, in the trigeminal subnucleus caudalis/upper cervical cord in the HE group than in the LE group. The number of c-Fos-immunoreactive cells in the ventral trigeminal interpolaris/caudalis were similar in the 2 groups. Real-time PCR showed that the levels of TRPV1 and ANO1 mRNA in the HE group were significantly higher than levels in the LE group. Thus, high levels of estrogens may be a risk factor for Cap-evoked nociceptive pain, and estrogen-dependent increases in TRPV1 and ANO1 are likely involved in modulating the nociceptive response in the trigeminal area. (Endocrinology 157: 4309–4317, 2016)

In certain pain disorders, women experience more severe levels of pain, more frequent pain, and a longer duration of pain than men (1–3). Several trigeminal pain disorders, including temporomandibular disorders, trigeminal neuralgia, and burning tongue, show similar sex differences (4–6). Furthermore, several of these disorders are exacerbated in the presence of high levels of the typical female hormone estrogens (7). Therefore, high levels of estrogens may be a risk factor for trigeminal pain disorders in women.

Estrogens affect cells through genomic and non-genomic pathways (8). In the former pathway, estrogens bind to estrogen receptors (ERs), which are located in the cytoplasm and nucleus, and form an estrogen-ER complex. This complex binds to estrogen-response element sequences in the promoter region of some genes and modulates the levels of associated mRNAs and proteins. ERs are expressed in the rat trigeminal ganglia (TG), and their activation alters the expression of certain mRNAs and

Abbreviations: ANO1, anoctamin 1; Cap, capsaicin; E2, 17β-estradiol; ER, estrogen receptor; HE, high-dose E2; IR, immunoreactive; LE, low-dose E2; OVX, ovariectomized; TG, trigeminal ganglia; TRPV1, transient receptor potential vanilloid 1.
proteins in sensory neurons (8, 9). These observations suggest that estrogens may modulate pain sensitivity by affecting signaling pathways in the TG that are involved in peripheral nociception.

Recent work has shown that the interaction between transient receptor potential vanilloid 1 (TRPV1) and anoctamin 1 (ANO1) is a significant pain-enhancing mechanism in peripheral nociception (10). TRPV1 is a polymodal sensor that is activated by heat (43°C), acid, or capsaicin (Cap) (11–13). TRPV1 is up-regulated and activated in the mouse TG after peripheral nerve injury, resulting in thermal hyperalgesia (14). The expression of TRPV1 is abnormally high in patients with burning mouth syndrome (15). Thus, TRPV1 plays an important role in the signaling pathways involved in peripheral nociception. ANO1, which is also known as the transmembrane protein 16A component of Ca2+ option. ANO1, which is also known as the transmembrane protein 16A component of Ca2+ option. ANO1, which is also known as the transmembrane protein 16A, is a significant pain-enhancing protein in sensory neurons (8, 9). These observations suggest that estrogens may modulate pain sensitivity by affecting signaling pathways in the TG that are involved in peripheral nociception.

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Thus, similar to TRPV1, ANO1 plays an important role in the signaling pathways involved in peripheral nociception. However, whether TRPV1 and ANO1 are involved in estrogen-modulated trigeminal pain sensitivity is unclear.

In the present study, we examined estradiol (E2) modulation of orofacial nociception after Cap application onto the ocular surface. The ocular surface is suitable for nociception studies in the trigeminal system, because the nociceptive receptors on the ocular surface have a large representation in the TG via the ophthalmic branch of the trigeminal nerve, and the ocular surface receives the densest nociceptive innervation of the body (18). Thus, to determine whether the estrogen status modulates orofacial nociception, we performed behavioral and immunohistochemical experiments after Cap application onto the ocular surface in ovariectomized (OVX) rats treated with E2 for a short period. In addition, we used real-time PCR (RT-PCR) to study the effects of estrogens on the expression levels of TRPV1 and ANO1 mRNA in TG.

Materials and Methods

Experimental procedures

The study protocols for animal experiments were approved by the Committee of Research Facilities for Laboratory Animal Science, Osaka University Graduate School of Dentistry. The protocols conformed to the established guidelines set by the National Institutes of Health guide for the care and use of laboratory animals (PHS Law 99–138; revised, 2002).

General and endocrine procedures

Age-matched, adult OVX female Sprague-Dawley rats weighing 250–320 g (SLC) were used. Within 21 days after OVX, rats were administered a daily injection of either a low-dose E2 (LE) (4 μg, sc injection) or a high-dose E2 (HE) (40 μg, sc) (Sigma) dissolved in 200-μL sesame oil for 2 days before the experiment. The LE and HE replacement regimens were selected to mimic the plasma levels of E2 in diestrus and proestrus, respectively (19, 20). The doses and regimen of E2 replacement used here have been shown to restore the changes in pituitary hormone secretion and body weight gain that occur after OVX (21, 22). The estrogen status of OVX rats was determined on the day of the experiment by vaginal smear cytology obtained with gentle lavage. Vaginal smears from the LE rats contained mostly leukocytes or a combination of nucleated and squamous epithelial cells and leukocytes, whereas smears from the HE rats primarily consisted of large nucleated epithelial cells or a combination of nucleated and squamous epithelial cells (23). Data were collected in a blinded manner.

Behavior (eye wipe test)

Rats were allowed to acclimate to manual handling daily for 7 days in the behavior laboratory to minimize stress associated with restraint. The experiments were performed by the same well-trained investigator. After a 1-hour acclimation period to the behavior laboratory, Cap (10 μL of a 1mM solution) or vehicle (10 μL) was pipetted directly onto the left ocular surface of the rat that was lightly restrained by hand, and the subsequent nocifensive behavior was monitored in the free-moving condition. This behavior was defined as either holding the eye completely shut or grooming the eye area with either the forepaws or hindpaws. For all experiments, the total number of seconds of this behavior was measured for 5 minutes after the administration of Cap or vehicle, by visual inspection in a blinded manner.

c-Fos immunohistochemistry

After a bolus injection of pentobarbital (50 mg/kg, ip injection), 10 μL of 1mM or 10mM Cap or vehicle was pipetted directly onto the left eye of the rat. Two hours later, rats were perfused through the heart with 0.02M PBS followed by 4% paraformaldehyde in 0.1M PBS. Then, the lower brainstem and upper cervical spinal cord segment were removed for c-Fos immunohistochemistry and postfixed in cold fixative for 3 hours, followed by immersion in cold 30% sucrose in 0.1M PBS. Serial frozen transverse sections (30-μm thickness) were cut on a vibratome through the caudal medulla and first segment of the spinal cord (C1) and collected in 0.1% phosphate buffer (pH 7.4). Every tenth section was processed for c-Fos immunohistochemistry as follows. Sections were incubated in 1% normal goat serum (Vectastain; Vector Laboratories) for 20 minutes at 4°C,
followed by incubation in rabbit polyclonal anti-Fos antibody (1:7000; Santa Cruz Biotechnology) for 12 hours at 4°C. Subsequently, the sections were washed and incubated in biotinylated goat antirabbit antibody (1:500) for 2 hours at 37°C, followed by washing and incubation in avidin-biotin-peroxidase complex (ABC solution, Vectastain; Vector Laboratories) for 1 hour at 25°C. c-Fos-immunoreactive (IR) neuronal nuclei were visualized by incubation in 0.05% 3,3′-diaminobenzidine-tetra HCl (Sigma), 0.1% nickel ammonium sulfate, and 0.01% peroxide in 0.05M Tris buffer (pH 7.2). After rinsing in phosphate buffer, sections were mounted on slides, air-dried, and cover-slipped with mounting medium. Specific staining with the above protocol was confirmed by omission of the primary antiserum on some sections.

The obex was used as a landmark for counting and analysis of c-Fos-IR cells on the rostral side from +1.0 to −6.0 mm. The average number of c-Fos-IR cells after Cap stimulation of the eye was compared in 3 areas: the boundary area between the trigeminal subnucleus interpolaris (Vi) and the trigeminal spinal nucleus caudalis (Vc) (Vi/Vc, from +0.5 to −1.0 mm), the area of the middle trigeminal spinal nucleus caudalis (mid-Vc, from −1.5 to −2.5 mm), and the boundary area between the Vc and C1 (Vc/C1, from −3.0 to −5.0 mm). The superficial layer (laminae I/II) and magnocellular region (laminae III/V) of the mid-Vc and Vc/C1 were analyzed separately.

Quantitative real-time PCR (relative quantification analysis)

Rats were deeply anesthetized with pentobarbital (50 mg/kg ip) and decapitated, and the TG was removed immediately. Total RNA was extracted from the tissues using an RNeasy mini plus kit (QIAGEN) according to the manufacturer’s protocol. Total RNA was reverse transcribed to cDNA with Prime Script reverse transcriptase Master Mix (Takara) at 37°C for 15 minutes, followed by 85°C for 5 seconds. Real-time PCR was performed to quantify the mRNA expression of TRPV1 and ANO1 with the KAPA SYBR FAST qRT-PCR kit (NIPPON Genetics) using Rotorgene 6000 (QIAGEN). cDNA samples were incubated at 95°C for 2 minutes, followed by 40 cycles of amplification at 95°C for 10 seconds and 64°C for 30 seconds using the following specific primers:

- β-actin, (forward) 5′-TGTTAACACTGGGACGAC-3′ and (reverse) 5′-GGTGTGAGGTCTCAAACAT-3′;
- TRPV1, (forward) 5′-GACATGCCACCCAGCAGG-3′ and (reverse) 5′-GACATTCCACACACCTCC-3′; and
- ANO1, (forward) 5′-CCATCGAGCAGAACATCGG-3′ and (reverse) 5′-GGGCTGTGGGACTGTGGTTA-3′.

An amplification plot from a series of dilutions of cDNA from control rats was used to obtain the linear correlation between the threshold cycle and the log amount of template present. The relative ratio of target mRNA for each sample was calculated from its threshold cycle using a software program (LightCycler System Software version 3.3; Roche Molecular Biochemicals) according to the manufacturer’s instructions. Data were normalized to the values for β-actin mRNA.

Statistical analysis

All data are expressed as the mean ± SEM. Two-way ANOVA with post hoc Scheffe’s F test and the unpaired Student’s t test were used for comparative analyses. Differences in the data were considered significant at P < .05.

Results

Behavioral examination (eye wipe test)

After application of vehicle to the eye, the durations of nocifensive behavior in the LE and HE groups were 1.0 ± 0.3 and 1.4 ± 0.2 seconds, respectively; the difference was not statistically significant. After application of 1mM Cap to the eye, the durations were 64.8 ± 15.4 seconds in the LE group and 143.4 ± 21.2 seconds in the HE group. The duration in each Cap group was significantly longer than that for the corresponding vehicle group (ANOVA and Scheffe’s F test, P < .05 in the LE group, P < .01 in the HE group) (Figure 1). In addition, after 1mM Cap application, the duration in the HE group was significantly longer than that in the LE group (ANOVA and Scheffe’s F test, P < .01) (Figure 1).

Immunohistochemical examination (c-Fos immunoreactivity)

Cells that were immunopositive for c-Fos after application of vehicle, 1mM Cap, or 10mM Cap to the left eye were stained black/gray (Figures 2 and 3). On the ipsilateral side after application of 1mM or 10mM Cap, most of the labeled cells were present in the Vi/Vc or Vc/C1 area of the trigeminal spinal nucleus and showed a biphasic distribution (Figure 4A). The numbers of c-Fos-IR cells per section in the Vi/Vc of the LE and HE groups were 13.1 ± 2.1 and 11.5 ± 1.9 after application of vehicle, 15.6 ± 2.5 and 15.6 ± 1.5 after application of 1mM Cap, and 22.3 ± 1.2 and 22.2 ± 1.8 after application of 10mM Cap, respectively (Figure 5). For each Cap dose and vehicle, no

![Figure 1](https://academic.oup.com/endo/article-abstract/157/11/4309/2758405)
statistically significant differences were found between the LE and HE groups. In addition, for the LE and HE groups, the number of cells after application of 10mM Cap was higher than after application of vehicle but was not higher than after application of 1mM Cap (ANOVA and Scheffe's F test, $P < .05$) (Figure 5).

Figure 2. Expression of c-Fos-IR cells in the VJVc. Photomicrographs show the ipsilateral side to vehicle or 1mM or 10mM Cap application. a, LE + vehicle; b, HE + vehicle; c, LE + 1mM Cap; d, HE + 1mM Cap; e, LE + 10mM Cap; f, HE + 10mM Cap.

Figure 3. Expression of c-Fos-IR cells in the Vc/V1. Photomicrographs show the ipsilateral side to vehicle or 1mM or 10mM Cap application. a, LE + vehicle; b, HE + vehicle; c, LE + 1mM Cap; d, HE + 1mM Cap; e, LE + 10mM Cap; f, HE + 10mM Cap.
The numbers of c-Fos-IR cells per section in the superficial layer (laminae I/II) of the Vc/C1 in the LE and HE groups were 0.7/0.4 and 0.6/0.3 after application of vehicle, 9.9/1.1 and 20.9/2.5 after application of 1mM Cap, and 25.8/1.5 and 38.0/3.7 after application of 10mM Cap, respectively (Figure 6). Application of 1mM and 10mM Cap resulted in a significant increase in the number of cells in the HE group compared with the LE group (ANOVA and Scheffe’s F test, P < .05) (Figure 6). Additionally, in the LE group, 10mM Cap induced a greater increase in the number of c-Fos-IR cells compared with the vehicle, and 10mM Cap increased the number compared with the vehicle and 1mM Cap (ANOVA and Scheffe’s F test, P < .01) (Figure 6).

Fewer than 3 c-Fos-IR cells were seen in the magnocellular region (laminae III/V) of the Vc/C1 in both groups after application of vehicle or either dose of Cap.

The numbers of c-Fos-IR cells per section in the superficial layer (laminae I/II) of the Vc/C1 in the LE and HE groups were 1.2 ± 0.2 and 1.0 ± 0.4 after application of vehicle, 6.4 ± 1.1 and 6.2 ± 1.1 after application of 1mM Cap, and 11.6 ± 1.4 and 11.1 ± 1.0 after application of 10mM Cap, respectively; no significant differences between the LE and HE groups were seen. In addition, the number of c-Fos-IR cells produced in the superficial layer of the Vc/C1. Application of 1mM and 10mM Cap resulted in a significant increase in the number of c-Fos-IR cells in the HE group compared with the LE group (a, P < .05 and b, P < .05, respectively). In the LE group, 10mM Cap induced a greater increase in the number of c-Fos-IR cells than 1mM Cap or vehicle (†, P < .01). In the HE group, 1mM Cap significantly increased the numbers of c-Fos-IR cells compared with the vehicle (*, P < .05), and 10mM Cap increased the number compared with the vehicle and 1mM Cap (§, P < .01). Sample size, n = 6 in each group. Statistical analysis was performed using two-way ANOVA followed by Scheffe’s F test. Each value represents the mean ± SEM.
numbers of c-Fos-IR cells after application of 10 mM Cap, but not 1 mM Cap, in the LE or HE groups were higher than after application of vehicle for each group (ANOVA and Scheffe’s F test, \( P < .01 \)) (data not shown). Fewer than 3 c-Fos-IR cells were seen in the magnocellular region (laminae III–V) of the mid-Vc in both groups after application of vehicle or either dose of Cap.

The numbers of c-Fos-IR cells per section on the contralateral side were low and less than the numbers on the ipsilateral side (Figure 4B).

**Molecular biological examination (RT-PCR)**

The levels of mRNA expression of TRPV1 and ANO1 in the HE group were significantly higher than levels in the LE group (unpaired \( t \) test, \( P < .01 \) and \( P < .05 \), respectively) (Figure 7).

**Discussion**

The present study showed that Cap stimulation of the trigeminal area in the HE group increased the duration of pain-related behavior, the number of c-Fos-IR cells in the Vc/C1 area of the trigeminal spinal nucleus, and the levels of TRPV1 and ANO1 mRNAs in the TG. These findings indicate that E2 likely mediated the enhanced Cap-induced pain sensitivity via up-regulation of TRPV1 and ANO1 in trigeminal primary neurons.

**Behavioral examination (eye wipe test)**

The ocular surface, especially the cornea, is rich in Aδ and C fibers, which are intimately involved in nociceptive transmission (18). Therefore, the cornea is a useful target for the study of experimental acute pain, and the eye wipe test has been used as a means to assess pain-related behavior in the HE and LE groups. These replacement regimens after OVX were selected to mimic the proestrous and diestrous phase in the estrous cycle, respectively (19, 20). Application of 1 mM Cap induced nocifensive behavior in the HE and LE groups, resulting in a significantly prolonged duration of the eye wipe behavior compared with each vehicle group. In addition, the duration of the behavior in the HE group was longer than that in the LE group. Therefore, these findings suggest that E2 enhances Cap-induced pain sensitivity in a dose-dependent manner.

Similar to our results, a few recent reports have demonstrated that proestrous female rats exhibit greater Cap-induced nocifensive responses on the dorsal surface of the hindpaw compared with estrous females (27) and that greater thermal or mechanical hyperalgesia is observed in proestrous female rats compared with diestrous females after application of complete Freund’s adjuvant to the hindpaw (28, 29). In contrast to our results, one report showed that the nociceptive thresholds of female rats during diestrus, proestrus, and estrus measured as hindpaw withdrawal latencies from a hot-plate and tail withdrawal latencies from warm water do not significantly differ from each other (30). Taken together, how estrogens modify Cap-induced pain sensitivity may depend on the pain model. Therefore, we should keep in mind that different pain models may exhibit different mechanisms of nociception and may thus be differentially influenced by estrogens.

**Immunohistochemical examination (c-Fos immunoreactivity)**

We used anesthetized rats to examine the c-Fos-IR cells induced purely by Cap stimulation onto the ocular surface. We were concerned about the expression of c-Fos-IR cells induced indirectly by Cap-evoked nociceptive behaviors. Therefore, we maintained rats under deep anesthesia that was sufficient to prevent nociceptive behaviors after Cap application. Pentobarbital reduces the number of c-Fos-IR cells in the Vc after heat stimulation (31). In our study, the c-Fos-IR cells induced by Cap stimulation may have been reduced by deep anesthesia. However, we were still able to evoke strong expression of c-Fos-IR cells by Cap stimulation in the Vc.

Cap application to the ocular surface induced expression of c-Fos in many ipsilateral cells in the Vi/Vc and Vc/C1 areas of the trigeminal spinal nucleus, and the distribution was biphasic (Figure 4A).

Following labeling of the neural circuit with a radioactive tracer, the primary sensory nerve from the ocular surface was shown to project to the superficial layer of the...
Vi/Vc or Vc/C1 area of the trigeminal spinal nucleus (32–35). In addition, a variety of types of nociceptive stimulation to the ocular surface evoke the expression of c-Fos and/or phosphorylation of extracellular signal-regulated kinase in the Vi/Vc and the Vc/C1 (36–38), which is consistent with the results of the present study. However, the previous reports suggest the possibility of some differences in the function of the Vi/Vc and the Vc/C1. For example, Chang et al (39) reported that c-Fos-IR cells induced by Cap application to the ocular surface are Cap concentration dependent in the Vc/C1 but not in the Vi/Vc. In addition, Meng et al (40) reported that the neurons in the Vc/C1, but not those in the Vi/Vc, are strongly involved in nociceptive transmission from the ocular surface because specific nociceptive neurons are abundant in the Vc/C1. On the other hand, many neurons in the Vi/Vc are low-threshold mechanoreceptive neurons that respond to nonnociceptive stimulation (40). The present results also showed that the numbers of c-Fos-IR cells in both the LE and HE groups are Cap concentration dependent in the Vc/C1 area but not in the Vi/Vc (Figures 5 and 6). In addition, vehicle application evoked low numbers of c-Fos-IR cells in the Vc/C1 area, whereas the reaction in the Vi/Vc was similar to that induced by 1mM Cap application (Figures 5 and 6). Our data thus support the argument by Meng et al (40). On the other hand, the Vi/Vc area may be involved in reflex tearing, because tearing is blocked when input to the Vi/Vc area is inhibited, whereas tearing is not blocked by inhibiting the Vc/C1 (41). Therefore, the presence of c-Fos-IR cells in the Vi/Vc may also be explained by neuronal excitation through reflex tearing right after ocular application.

We observed c-Fos-IR cells on the contralateral side to Cap application as well as the ipsilateral side (Figure 4). The number of c-Fos-IR cells observed on the contralateral side was lower than that observed on the ipsilateral side (Figure 4). Previous studies have demonstrated that nocuous stimulation to the ocular surface evokes c-Fos-IR cells on both sides in the Vi/Vc and Vc/C1 area (36, 37, 39), which is consistent with the results of the present study. Nocuous stimulation to the ocular surface is transduced by the ophthalmic branch of the trigeminal nerve and transmitted to the ipsilateral neurons in the Vi/Vc and Vc/C1 area. Thus, the c-Fos-IR cells on the ipsilateral side, but not the contralateral side, are important markers of nociceptive activation to noxious stimulation. A recent study has reported that the release of proinflammatory mediators, such as IL-1β, TNF-α, and substance P, evokes responses on the contralateral side to the inflamed cornea after alkali burn (42). The contralateral reaction observed in this study may be induced by the same mechanism.

### Molecular biological examination (RT-PCR)

The interaction between TRPV1 and ANO1 is a significant pain-enhancing mechanism in peripheral nociception (10, 16, 17). However, whether TRPV1 and ANO1 are involved in estrogen-modulated trigeminal pain sensitivity was unclear. Based on the results from the behavioral and immunohistochemical examinations of the present study, we used RT-PCR to examine the impact on the expression levels of TRPV1 and ANO1 mRNA in the TG due to estrogen fluctuation via E2 administration. We observed an increase in the expression levels of these two mRNAs in the HE group (Figure 7). These data suggest that the enhanced nociception in the TG may be due to an E2-dependent increase in TRPV1 and ANO1 expression. These molecules may be important targets for elucidating the mechanism of female pain sensitivity in the peripheral trigeminal area. Although to the best of our knowledge, no report has investigated this phenomenon in the trigeminal area, another study using human tissue and cell culture of embryonic stem cell-derived sensory neurons incubated with E2 showed increases in TRPV1 mRNA and that an ERβ-selective agonist increases the levels of TRPV1 mRNA (43), in agreement with our results. Moreover, the ANO1 expression level in the mouse ovary is remarkably higher in the proestrus and estrous stages during the estrous cycle (44). In addition, the interaction between TRPV1 and ANO1 is an important pain-enhancing mechanism in the peripheral nervous system, as shown electrophysiologically using the whole-cell patch-clamp technique in HEK293T cells expressing mouse TRPV1 and/or mouse ANO1 (10). These reports and our results in the trigeminal peripheral area suggest that the E2-dependent increase in TRPV1 and ANO1 expression levels evoked pain exacerbation. In addition, peripheral excitation is transmitted to secondary neurons, resulting in excitation of the Vc/C1 area.

### Table 1. Antibody Table

| Peptide/Protein Target | Antigen Sequence (if Known) | Name of Antibody | Manufacturer, Catalog Number, and/or Name of Individual Providing the Antibody | Species Raised in; Monoclonal or Polyclonal | Dilution Used |
|------------------------|-----------------------------|-----------------|--------------------------------------------------------------------------------|---------------------------------------------|--------------|
| c-Fos                  | Rabbit                      | c-Fos antibody  | Santa Cruz Biotechnology, sc-52                                               | Rabbit; polyclonal                          | Saline       |
Limitations and prospects

Estrogens mediate their effects through ERs, which consist of three subtypes: ERα, ERβ, and G protein-coupled estrogen receptor 1. The spatial distribution of ERs varies among tissues (8). ERα and ERβ are somewhat highly expressed in the TG, but the expression level of G protein-coupled estrogen receptor 1 is lower. Thus, estrogens likely modulate nociception through the first 2 receptor subtypes (45). Our study did not elucidate whether the E2-dependent increase in TRPV1 and ANO1 mRNA expression levels is mediated through ERα and/or ERβ, and thus, future research is needed to clarify the mechanism.

In summary, the present study demonstrated that estrogen-dependent increases in TRPV1 and ANO1 are likely involved in modulating the nociceptive response in the trigeminal area. Our results contribute to the understanding of trigeminal nociceptive changes during the menopausal stage.

Appendix

See Table 1.

Acknowledgments

Address all correspondence and requests for reprints to: Kazuaki Yamagata, Department of Dental Anesthesiology, Graduate School of Dentistry, Osaka University, 1–8 Yamadaoka, Suita City, Osaka, 565–0871 Japan. E-mail: brassosu@yahoo.co.jp.

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