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ORIGINAL ARTICLE

# 17β-Estradiol regulates the gene expression of voltage-gated sodium channels: role of estrogen receptor α and estrogen receptor β

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**Abstract** Estradiol (E2) plays a key role in pain modulation, and the biological effects of E2 are transduced by binding estrogen receptors (ERs). Voltage-gated sodium (Nav) channels are responsible for the generation and propagation of action potentials in the membranes of most neurons and excitable cells. Adult dorsal root ganglion (DRG) neurons can express the ERs (ERα and ERβ), and Nav channels (TTX-S: Nav1.1, Nav1.6, and Nav1.7; and TTX-R: Nav1.8, and Nav1.9). Although E2 modulates Nav channel currents, little is known about the molecular mechanisms involved. In this study, we investigate the mRNA expressions of Nav channel subtypes mediated differentially by the ERs in the DRGs of wild-type (WT) and estrogen receptor knockout (αERKO and βERKO) mice. By means of quantitative real-time PCR, we found that the expressions of Nav1.1, Nav1.7, Nav1.8, and Nav1.9 subtypes were elevated in αERKO and βERKO mice, whereas Nav1.6 mRNA decreased in αERKO, but not in βERKO mice. The mRNA expressions of Nav subtypes were increased in E2-treated WT ovariectomized animals. We also found that E2-regulation of Nav1.1 and Nav1.9 mRNA expressions is dependent on ERα, ERβ, and another ER, whereas E2-regulation of Nav1.8 appears to be in an ERβ-dependent manner.

**Keywords** Estradiol · Estrogen receptor · Knock out · Voltage-gated sodium channels

## Introduction

Estradiol (E2) is known to influence multiple functions in brain tissue [1], including neuronal development, plasticity and survival, neurotransmitter and neuropeptide synthesis, and pain perception. There is mounting evidence that E2 acts on the central and peripheral nervous systems to affect pain. However, the effects are complex, and the mechanisms are not well understood [2].

The effects of E2 are classically mediated by two estrogen receptors (ERs), ERα and ERβ, which belong to the superfamily of ligand-activated transcription factors. Both ERα and ERβ mRNAs and proteins are expressed in dorsal root ganglion (DRG) neurons. Studies have shown that ERα is expressed only in the small, nociceptive neurons in the DRG, while ERβ mRNA is expressed in all DRG neurons [3].

Voltage-gated sodium (Nav) channels are responsible for the generation and propagation of action potentials in the membranes of most neurons and excitable cells. The Nav channel is composed of a combination of pore-forming α subunits and auxiliary β subunits. Until now, nine α subunits (Nav1.1–Nav1.9) of the Nav channels have been functionally characterized and have also been separated by their sensitivity to tetrodotoxin (TTX) into the TTX-sensitive (TTX-S) Nav channels (Nav 1.1, Nav1.2, Nav1.3, Nav1.4, Nav1.6, and Nav1.7) and the TTX-resistant (TTX-R) Nav channels (Nav1.5, Nav1.8, and Nav1.9) [4]. Each

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Nav channel subunit has a particular tissue localization, consistent with a distinct role for each Nav channel subunit in mammalian physiology. The TTX-S sodium channels Nav1.1, Nav1.6, and Nav1.7, and the TTX-R sodium channels Nav1.8 and Nav1.9 are expressed in the adult DRG neurons [5].

Previous studies have demonstrated that estradiol (E2) may affect Nav channels currents using patch clamp technique. For example, acute estradiol application increases the inward $Na^+$ currents in rat hypothalamic ventromedial nucleus [6] and in MDA-MB-231 cells by a non-genomic mechanism [7]. E2 could also inhibit cation flow through the TTX-S $Na^+$ channel in cultured mouse neuroblastoma cells [8] and attenuate the transient and persistent sodium current in GnRH neurons by chronic, in vivo estradiol treatment [9]. Our previous studies have found that bisphenol A (BPA), an estrogenic compound, inhibited TTX-S and TTX-R $Na^+$ currents in mouse DRG neurons via PKC- and PKA-dependent signaling pathway [10].

However, in spite of the compelling evidence for a physiological regulation of estradiol in Nav channels functions, there have been few studies on the physiological role of the ERs. Therefore, the present study was designed to investigate if the mRNA expression of Nav channels subtypes was mediated differentially by the ERs.

## Materials and methods

All the experiments were conducted in accordance with the NIH Guide for the care and use of laboratory animals, and the study was approved by the local IACUC. All efforts were made to minimize animal suffering and to reduce the number of animals used for the experiments.

### Animals and treatments

Mice of the C57BL/6 strain, which were heterozygous for genomic deletion of ER types α [11] and β [12] were purchased from the Jackson Laboratory (B6.129P2-*Esr1*$^{tm1Ksk}$/J; B6.129P2-*Esr2*$^{tm1Unc}$/J). Inbreeding and appropriate backbreeding of the heterozygotes yielded homozygote knockout ERα (αERKO) and ERβ mice (βERKO). The ERs gene were analyzed following the genotyping protocol of the Jackson Laboratory (BarHarbor, ME). Wild-type (WT) littermates were used as controls. Mice were kept under environmentally controlled conditions (ambient temperature, 22°C; humidity, 40%) on a 12-h light/dark cycle with food and water ad libitum.

Adult female wild-type (WT), αERKO, and βERKO mice (20–25 g body weight) were used. The female mice were ovariectomized (OVX) and supplemented with estradiol. Hence, the group differences can be attributed to the presence or the absence of the receptor rather than to changes in estradiol levels [13]. The animals were ovariectomized under sodium pentobarbital (50 mg/kg i.p.). Surgery was performed according to the method described by Dehpour [14]. The WT, αERKO, and βERKO ovariectomized mice were randomly assigned to experimental groups. Two weeks after OVX, mice received subcutaneous injections of estradiol (100 μg/kg of sesame oil, 48 h before testing) (OVX + E2, $n = 10$). This method (single subcutaneous injection) has been used in previous studies [14–16]. Control groups received vehicle injections (sesame oil) (OVX + oil, $n = 10$).

### Tissue collection, RNA isolation, and quantitative real-time PCR analysis

The DRGs were isolated from acutely dissociated spinal columns of separate groups and stored at −80°C until further processing. DRG tissue ($n = 10$ per group) was homogenized, and total RNA was isolated using the Trizol Reagent (Invitrogen Life Technologies, USA). Dry RNA pellets were dissolved in nuclease-free water, and DNAse-treated to minimize the risk of sample contamination. The method used to assess the integrity of total RNA consists in running an aliquot of the RNA sample on a denaturing agarose gel stained with ethidium bromide. RNA concentration and purity were assessed using $OD_{260}$ and the $OD_{260}/OD_{280}$ ratio and reverse transcribed (RT) to cDNA using PrimeScript™ RT reagent Kit (TaKaRa) with calculated concentrations of 500 ng RNA. Reverse transcription was performed in a 20 μl reaction mixture, at 37°C for 15 min and 85°C for 5 s. Quantitative real-time PCR was performed using 2 μl of sample cDNA on an ABI (Applied Biosystems) 7300 fast real-time PCR system using Eva-Green™ (Biotium, CA, USA) as a detection reagent and the TaKaRa Taq™ Hot Start Version (TaKaRa, Japan) according to the manufacturer's directions [17]. The cycling parameters were as follows: first, 50°C for 2 min followed by DNA polymerase activation step at 95°C for 10 min; and a two-temperature PCR of 40 cycles at 95°C for 15 s (denaturing step) followed by 60°C for 1 min (annealing step); and completed with a dissociation step for melting point analysis with 95°C for 15 s, 60–95°C for 30 s, and 95°C for 15 s. Reaction conditions for each molecule were optimized separately to give the best results for each primer pair. The primer final concentration is 0.2 μmol/l.

The sequences of the primers, according to previous studies [18, 19], are listed in Table 1. To quantify mRNA levels, the cloned DNAs for Nav1.1, Nav1.6, Nav1.7, Nav1.8, Nav1.9, and 18S rRNA genes were used for constructing standard curves to determine the percentage

efficiency [$E = 10(-1/\text{slope}) - 1$] of each amplification, which were made by plotting the cycle threshold versus the log of known concentrations. The cDNAs at series dilutions (1:10) were simultaneously amplified. Those giving 90–100% efficacies were chosen. The standard curves produced high linearity (Pearson correlation coefficient $r^2 = 0.98$ for Nav1.1, 0.98 for Nav1.6, 0.97 for Nav1.7, 0.99 for Nav1.8, 0.98 for Nav1.9, 0.98 for 18S rRNA, and 0.99 for $\beta$-actin). We confirmed PCR specificity by dissociation curve analysis to ensure the presence of only one product. As expected, single peak illustrates that only one product was formed, and the lack of any other peaks illustrates that the primers did not form primer dimers (Fig. 1). The ΔΔCT method normalized the CT (cycle threshold) from each sample for each target gene by subtracting the CT of the reference gene (18S rRNA; ΔCT). The ΔΔCT values were calculated with the ΔCT or the calibrator ΔCT [ΔΔCT = (CT target gene − CT reference gene) − CT of calibrator]. The relative linear quantity of the target gene was calculated using the formula $2^{-\Delta\Delta CT}$ [20]. Similar results were obtained with the $\beta$-actin, which was used as a reference gene (data not shown).

### Statistical analysis

Data were expressed as means ± SEM for all the experiments. Student's *t*-test were used as appropriate to evaluate the statistical significance of differences between two group means, and a one-way ANOVA with a Newman–Keuls post-hoc test was used for multiple groups. All the tests of statistical significance were two-sided and the statistical significance was set at $P < 0.05$.

## Results

### Gene expression of voltage-gated sodium channels in ERKO animals

Quantitative expression profiles of the TTX-S sodium channels (Nav1.1, Nav1.6, and Nav1.7), and the TTX-R sodium channels (Nav1.8 and Nav1.9) mRNAs in αERKO and βERKO animals are shown in Fig. 2a–e. We found that Nav1.1, Nav1.7, Nav1.8, and Nav1.9 mRNAs were elevated in αERKO and βERKO mice compared to WT animals (OVX + oil, $P < 0.05$, $n = 10$), whereas Nav1.6 mRNA (Fig. 2b) significantly (OVX + oil, $P < 0.05$, $n = 10$) decreased in αERKO, but showed no change in βERKO mice (OVX + oil, $P > 0.05$, $n = 10$).

### 17β-Estradiol regulation of sodium channel subtypes in DRG

By using quantitative real-time PCR analysis, we measured Nav1.1, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 mRNA levels in DRG of ovariectomized oil (OVX + oil) and E2-treated (OVX + E2) WT mice. As illustrated in Fig. 3, the mRNA expressions of Nav1.1, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 were significantly increased in DRG in E2-treated WT animals (OVX + E2, $P < 0.05$, $n = 10$).

To elucidate the roles of ERs in the E2 regulation of the mRNA expression of sodium channel genes, we treated ovariectomized WT, αERKO, and βERKO females with E2 and measured the mRNA expressions of Nav1.1, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 subtypes in the DRG (Fig. 3). E2 treatment increased the mRNA expression of Nav1.1 and Nav1.9 in WT ($P < 0.05$, $n = 10$), but decreased its expression in both αERKO and βERKO animals (Fig. 3a, e). The E2-induced increases in Nav1.6 and Nav1.7 mRNA expressions, which were observed in WT ($P < 0.05$, $n = 10$), were also observed in αERKO ($P < 0.05$, $n = 10$) and βERKO ($P < 0.05$, $n = 10$) animals (Fig. 3b, c). Also, E2 treatment up-regulated Nav1.8 mRNA expressions in WT and αERKO ($P < 0.05$, $n = 10$), but this increase was absent in βERKO animals (Fig. 3d).

## Discussion

In the present study, by means of quantitative real-time PCR, we found that the expressions of Nav1.1, Nav1.7, Nav1.8, and Nav1.9 subtypes were elevated in αERKO and βERKO mice, whereas Nav1.6 mRNA decreased in αERKO, but not in βERKO mice. The mRNA expressions

**Table 1** Sequence of primers

| Gene name | Forward primer (5′–3′) | Reverse primer (5′–3′) | Size (bp) |
|---|---|---|---|
| Nav1.1 | CAAAAAAGCCACAAAAGCCT | TTAGCTCCGCAAGAAACATC | 326 |
| Nav1.6 | AGAAGAAGTACTACAACGCC | AGTAGTGTCTCAAGGCAAAC | 285 |
| Nav1.7 | CAGCAAAGAGAGACGGAACC | CCCTCAGTGTCCGTAGAGATT | 536 |
| Nav1.8 | AATCAGAGCGAGGAGAAGACG | CTAGTGAGCTAAGGATCGCAGA | 196 |
| Nav1.9 | AGCCCAACGAAGTGAAGAAA | TCTCCAAGCCAGAAACCAAG | 183 |
| 18 s rRNA | CTTAGTTGGTGGAGCGATTTG | GCTGAACGCCACTTGTCC | 127 |
| β-Actin | AGGCCAACCGTGAAAAGATG | AGAGCATAGCCCTCGTAGATGG | 175 |

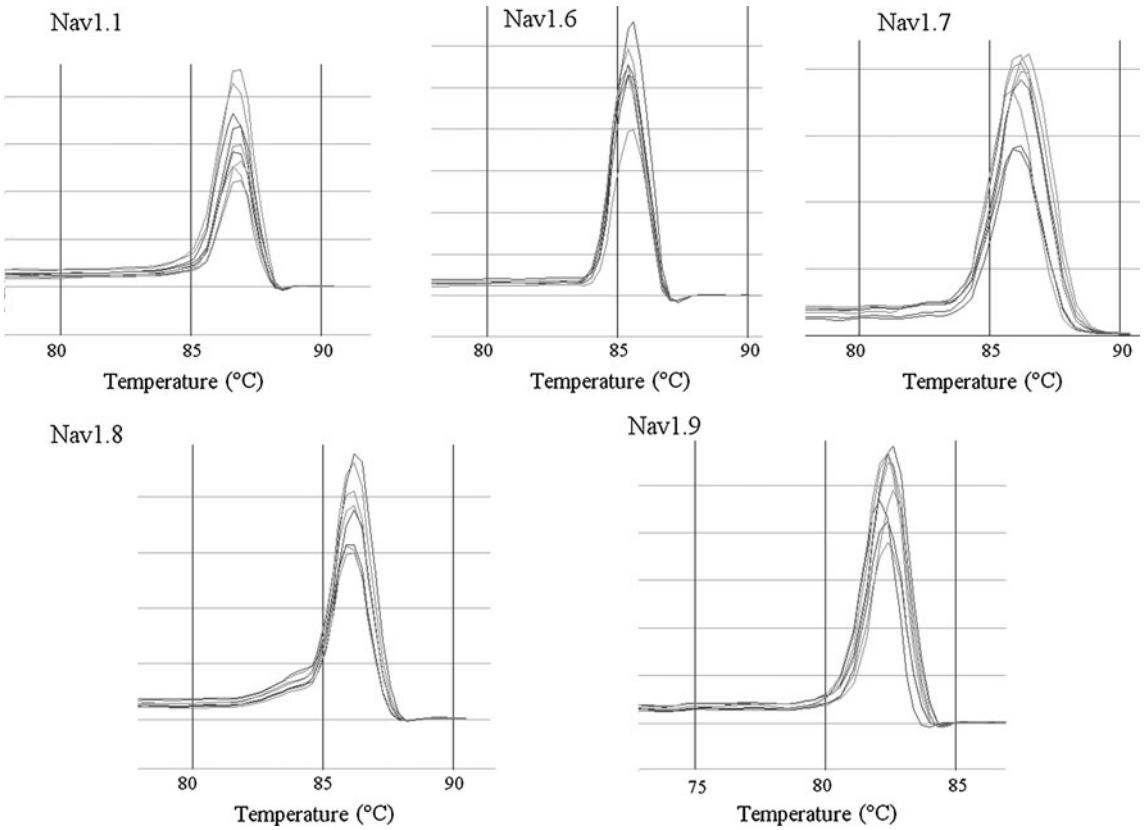


**Fig. 1** Representative dissociation curves, showing single-product melting at 86.5°C for Nav1.1, 85.4°C for Nav1.6, 86.2°C for Nav1.7, 86.4°C for Nav1.8, and 82.4°C for Nav1.9. The dissociation curve analysis revealed a single peak for each PCR product

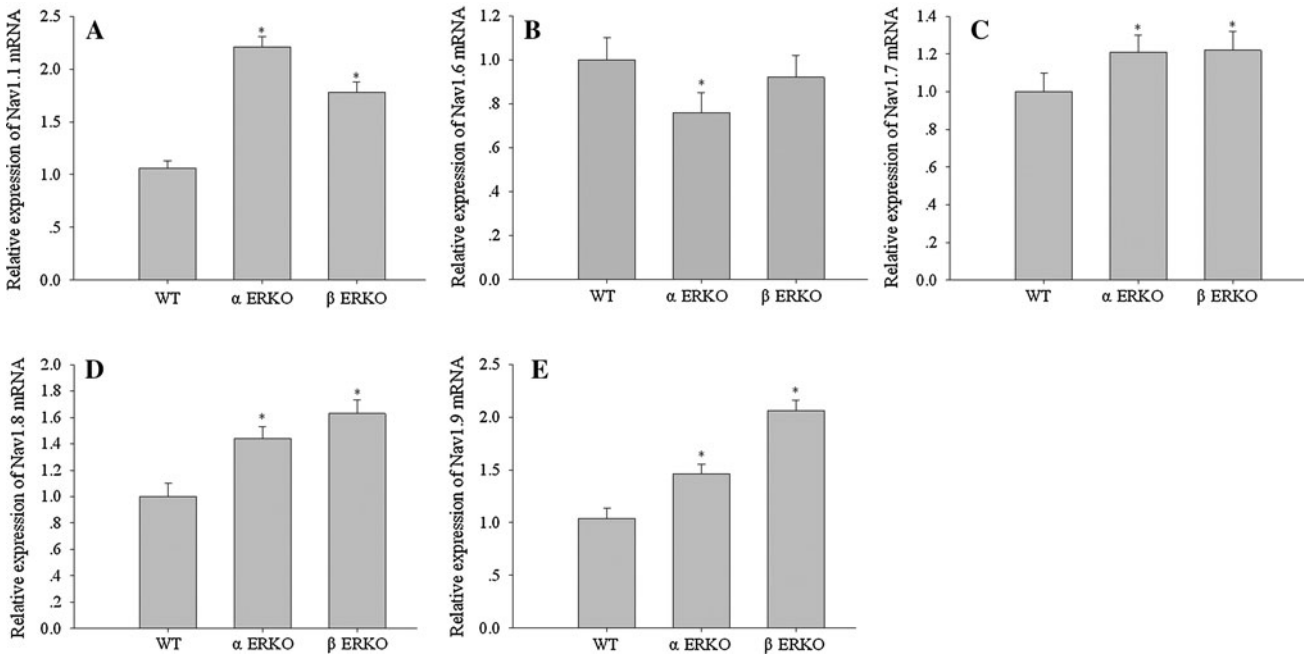


**Fig. 2** Quantitative real-time PCR analysis of the expressions of Nav1.1 (**a**), Nav1.6 (**b**), Nav1.7 (**c**), Nav1.8 (**d**), and Nav1.9 (**e**) mRNAs in the DRGs of female wild type (WT) and estrogen receptor knockout ($\alpha$ERKO and $\beta$ERKO) mice (OVX + oil). The relative quantity of target mRNA was calculated by the $\Delta\Delta$CT method [$\Delta\Delta CT = (CT\ target\ gene - CT\ reference\ gene) - CT\ of\ calibrator$], where the calibrator was the mean $\Delta$CT of the WT group. Data are expressed as means ± SEM. ($^*P < 0.05$, $n = 10$, compared to the WT group)

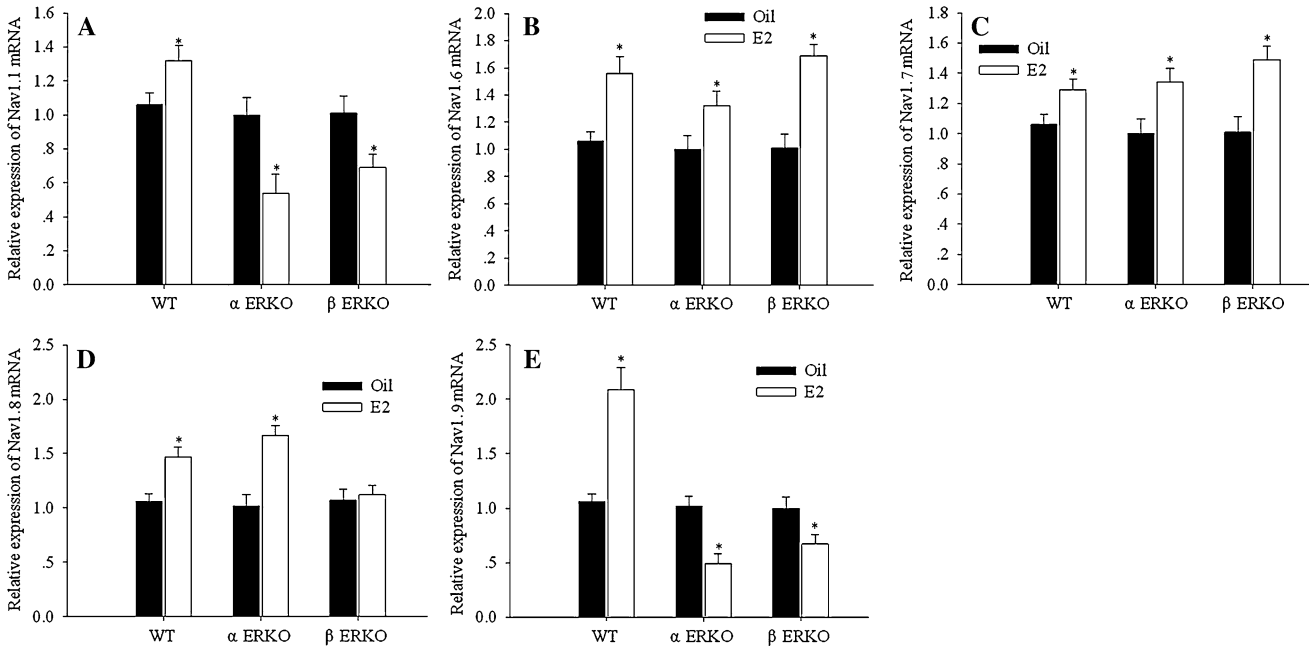


**Fig. 3** Quantitative real-time PCR analysis of the expression of Nav1.1 (**a**), Nav1.6 (**b**), Nav1.7 (**c**), Nav1.8 (**d**), and Nav1.9 (**e**) in the DRG from ovariectomized WT, αERKO, and βERKO C57BL/6 mice after oil-(OVX + oil) and E2-treatment (OVX + E2) ($n = 10$ per group). The relative quantity of target mRNA was calculated by the ΔΔCT method [ΔΔCT = (CT target gene − CT reference gene) − CT of calibrator], where the calibrator was the mean ΔCT of the oil-treated animals. Each data point represents means ± SEM. *$P < 0.05$ versus the same genotype oil-treated animals (OVX + oil)

of Nav subtypes were increased in E2-treated WT ovariectomized animals.

The existing literature from both human and animal studies as to whether estrogen displays pro- or anti-nociceptive roles is contradictory. In the human population, a number of chronic pain conditions, such as migraine, temporomandibular joint pain, and interstitial cystitis, are more prevalent in women than in men [2, 21], suggesting that estrogen is involved. However, when estrogen levels are constantly elevated as in pregnancy, pain sensitivity is known to decrease [2]. In laboratory tests of animal model, acute estrogen administration after ovariectomy enhances pain responses and sensitivity [22]. However, the absence of estrogen has often been shown to result in an increase in pain sensitivity. Some studies have also shown an anti-nociceptive role for estrogen in pain sensitivity [23].

Given the diverse actions of estrogens described above, it is not surprising that pain modulation by estrogens via ERs is complex. Previous studies have found that the sex difference in basal mechanical pain threshold and inflammatory hypersensitivity are eliminated in mice lacking either the ERα or ERβ [24]. However, the action of ERs on nociception modulation is unclear. Estradiol acting primarily through activation of ERβ may have a developmental role that affects spinal cord structures that are important for transmission of nociceptive information [25]. Marchand and co-workers [26] showed the involvement of both ERα and ERβ in pain modulation. The female βERKO mice showed reduced response in the formalin test compared to WT controls [13], suggesting a pronociceptive role of ERβ, and the pronociceptive nature of ERβ was confirmed using specific ERβ-selective agonist (DPN) injections in ovariectomized WT mice [26]. The female αERKO mice presented a small increase in nociceptive behaviors during phase 1 of the formalin test and the injection of ERα-selective agonist (PPT) in ovariectomized WT mice induced a decrease of pain behaviors during phase 1, interphase, and late phase 2, suggesting an anti-nociceptive effect of ERα [26]. Furthermore, the selective activation of ERβ by ERb-131, a non-steroidal ERβ ligand, appears sufficient to provide beneficial effects in animal models of symptoms associated with neuropathic pain [27]. These studies suggest that ERs may be a fruitful target for developing estrogenic medications for certain types of pain.

A wide variety of ion channels, playing important roles in the regulation of cell excitability, have been shown to be modulated by estrogens in DRG neurons. Lee et al. [28] found that 17β-estradiol inhibited high-voltage-activated $Ca^{2+}$ channel currents in rat DRG neurons via activation of pertussis toxin-sensitive G-protein(s) and non-genomic pathways. After the colon was inflamed, Fan et al. [29] showed that $P2X_3$ receptor (adenosine triphosphate receptor subunit) mRNA in the ovariectomized rats was significantly decreased in DRG, and estrogen significantly increased $P2X_3$ mRNA expression compared with that in

ovariectomized rats. Furthermore, E2 increased *N*-methyl-D-aspartic acid receptor (NMDAR) currents in adult male and female DRG neurons [30]. Estrogen promotes DRG nociceptor axon sprouting by up-regulation of the angiotensin II receptor type 2 ($AT_2$) mRNAs and proteins in cultured neonatal and intact adult DRG neurons [31].

The Nav channels are critical for the electrical excitability of sensory neurons and play a key role in pain sensation by controlling afferent impulse discharge. In this study, by means of quantitative real-time PCR, we found that the mRNA expressions of Nav1.1, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 were increased in E2-treated WT ovariectomized mice (WT, OVX + E2).

A number of channels are likely modulated by ERs in DRG or other neurons. For example, in DRG neurons from $\beta$ERKO mice, E2 attenuated the ATP-induced $[Ca^{2+}]_i$ flux as it did in WT mice, but in $\alpha$ERKO mice, E2 failed to inhibit the ATP-induced $[Ca^{2+}]_i$ increase. These results show that the rapid attenuation of ATP-induced $[Ca^{2+}]_i$ signaling is mediated by ER$\alpha$ [32]. Using ER$\alpha$- and ER$\beta$-deficient C57BL/6 mice, Bosch et al. [20] found that E2-induced increase in the mRNA expression of T-type calcium channel subunit Cav3.1 in the hypothalamus and pituitary was dependent on ER$\alpha$, whereas the E2 effect on Cav3.2 was dependent on both ER$\alpha$ and ER$\beta$. In this study, quantitative real-time PCR analysis showed that the gene expression levels of TTX-S (Nav1.1 and Nav1.7) and TTX-R (Nav1.8 and Nav1.9) sodium channel subtypes were elevated in DRGs of $\alpha$ERKO and $\beta$ERKO mice, whereas Nav1.6 mRNA decreased in $\alpha$ERKOs but showed no changes in $\beta$ERKO mice (OVX + oil). These results support our hypothesis that both ER$\alpha$ and ER$\beta$ play important roles in the regulation and/or functional integrity of Nav channels.

We also found that the E2 treatment increased the mRNA expressions of Nav1.1 and Nav1.9 in WT, but decreased the same in both $\alpha$ERKO and $\beta$ERKO animals. Recently, G protein-coupled receptor 30 (GPR30), a novel integral membrane ER was identified [33]. Previous studies have reported that GPR30 mRNA was expressed in the DRG [34, 35], and estrogen could act at GPR30 in nociceptors to produce a PKC$\varepsilon$-dependent mechanical hyperalgesia [34]. Therefore, the decreased mRNA expressions of Nav1.1 and Nav1.9 in $\alpha$ERKO and $\beta$ERKO group may indicate that E2 is acting on another receptor (e.g., GPR30). E2-induced increases in Nav1.6 and Nav1.7 mRNA expressions, which were observed in WT were also observed in $\alpha$ERKO and $\beta$ERKO animals. Also, E2 treatment up-regulated Nav1.8 mRNA expression in WT and $\alpha$ERKO, but this increase was lost in $\beta$ERKO animals. This would suggest that, in the DRG, E2-regulation of Nav1.8 may be through an ER$\beta$-dependent mechanism.

Our present findings fit well with previous studies that estradiol may modulate Nav channel currents. Wang et al. [9] found that both the transient and persistent sodium current are attenuated in GnRH neurons by chronic, in vivo estradiol treatment, which could lead to a downregulation of cell excitability. E2 could also inhibit cation flow through TTX-S $Na^+$ channel in cultured mouse neuroblastoma cells [8]. However, Fraser et al. [7] found that external application of E2 increased the current amplitude of voltage-gated $Na^+$ channels (VGSCs) in MDA-MB-231 cells. In addition, acute estradiol application increases inward $Na^+$ currents in rat hypothalamic ventromedial nucleus [6]. Moller et al. [36] examined the effects of E2 on the major cardiac currents, showed that addition of estradiol, only a minor current reduction of the sodium channel 5A (SCN5A)-mediated sodium inward current was observed.

Although studies on mRNA of Nav channels are limited, there is overwhelming physiological evidence for the expressions of Nav channel currents in the DRG. Previous studies support the idea that Nav channels are critical for neuronal excitability. Nav channels mediate a rapid and transient increase in $Na^+$ permeability in response to changes in membrane potential, thereby contributing to the generation and conduction of action potentials that serve as sensory signals from the periphery to the spinal cord through the primary afferent neurons [5]. Hence, the sodium channels in sensory neurons are implicated in the development of neuropathic pain.

In summary, the present study shows that E2 regulates the Nav channel subtypes' mRNA expressions in the DRG. E2-induced regulation of Nav1.1 and Nav1.9 mRNA expression is dependent on ER$\alpha$, ER$\beta$, and another ER (e.g., GPR30), whereas E2 treatment leads to increase the mRNA expression of Nav1.8 in an ER$\beta$-dependent manner. Therefore, the robust E2 regulation of the Nav channels mRNA expression could be an important mechanism by which E2 affects the excitability of DRG neurons and modulates the pain. However, further investigation will be required to determine the mechanism.

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**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. T.A. Roepke, O.K. Ronnekleiv, M.J. Kelly, Physiological consequences of membrane-initiated estrogen signaling in the brain. Front. Biosci. **16**, 1560–1573 (2011)
2. R.M. Craft, Modulation of pain by estrogens. Pain **132**(Suppl 1), S3–S12 (2007)
3. N. Taleghany, S. Sarajari, L.L. DonCarlos, L. Gollapudi, M.M. Oblinger, Differential expression of estrogen receptor alpha and

beta in rat dorsal root ganglion neurons. J. Neurosci. Res. **57**(5), 603–615 (1999)
4. H. Tu, L. Zhang, T.P. Tran, R.L. Muelleman, Y.L. Li, Reduced expression and activation of voltage-gated sodium channels contributes to blunted baroreflex sensitivity in heart failure rats. J. Neurosci. Res. **88**(15), 3337–3349 (2010)
5. S.D. Dib-Hajj, T.R. Cummins, J.A. Black, S.G. Waxman, Sodium channels in normal and pathological pain. Annu. Rev. Neurosci. **33**, 325–347 (2010)
6. L.M. Kow, N. Devidze, S. Pataky, I. Shibuya, D.W. Pfaff, Acute estradiol application increases inward and decreases outward whole-cell currents of neurons in rat hypothalamic ventromedial nucleus. Brain Res. **1116**(1), 1–11 (2006)
7. S.P. Fraser, I. Ozerlat-Gunduz, R. Onkal, J.K.J. Diss, D.S. Latchman, M.B.A. Djamgoz, Estrogen and non-genomic upregulation of voltage-gated $Na^+$ channel activity in MDA-MB-231 human breast cancer cells: role in adhesion. J. Cell. Physiol. **224**(2), 527–539 (2010). doi:10.1002/jcp.22154
8. M. Barann, M. Gothert, M. Bruss, H. Bonisch, Inhibition by steroids of [14C]-guanidinium flux through the voltage-gated sodium channel and the cation channel of the 5-HT3 receptor of N1E-115 neuroblastoma cells. Naunyn-Schmiedeberg's Arch. Pharmacol. **360**(3), 234–241 (1999)
9. Y. Wang, M. Garro, M.C. Kuehl-Kovarik, Estradiol attenuates multiple tetrodotoxin-sensitive sodium currents in isolated gonadotropin-releasing hormone neurons. Brain Res. **1345**, 137–145 (2010)
10. Q. Wang, J. Cao, Q. Zhu, C. Luan, X. Chen, X. Yi, H. Ding, J. Chen, J. Cheng, H. Xiao, Inhibition of voltage-gated sodium channels by bisphenol A in mouse dorsal root ganglion neurons. Brain Res. **1378**, 1–8 (2011)
11. D.B. Lubahn, J.S. Moyer, T.S. Golding, J.F. Couse, K.S. Korach, O. Smithies, Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc. Natl. Acad. Sci. USA **90**(23), 11162–11166 (1993)
12. J.H. Krege, J.B. Hodgin, J.F. Couse, E. Enmark, M. Warner, J.F. Mahler, M. Sar, K.S. Korach, J.A. Gustafsson, O. Smithies, Generation and reproductive phenotypes of mice lacking estrogen receptor beta. Proc. Natl. Acad. Sci. USA **95**(26), 15677–15682 (1998)
13. M.F. Spooner, P. Robichaud, J.C. Carrier, S. Marchand, Endogenous pain modulation during the formalin test in estrogen receptor beta knockout mice. Neuroscience **150**(3), 675–680 (2007)
14. D. Kalbasi Anaraki, S. Sianati, M. Sadeghi, M. Ghasemi, M.J. Paydar, S. Ejtemaei Mehr, A.R. Dehpour, Modulation by female sex hormones of the cannabinoid-induced catalepsy and analgesia in ovariectomized mice. Eur. J. Pharmacol. **586**(1–3), 189–196 (2008)
15. K.P. Lawson, S. Nag, A.D. Thompson, S.S. Mokha, Sex-specificity and estrogen-dependence of kappa opioid receptor-mediated antinociception and antihyperalgesia. Pain **151**(3), 806–815 (2010)
16. L.A. Berglund, H. Derendorf, J.W. Simpkins, Desensitization of brain opiate receptor mechanisms by gonadal-steroid treatments that stimulate luteinizing-hormone secretion. Endocrinology **122**(6), 2718–2726 (1988)
17. Q. Wang, L. Lu, X. Gao, C. Wang, J. Wang, J. Cheng, R. Gao, H. Xiao, Effects of raloxifene on voltage-dependent T-type $Ca^{2+}$ channels in mouse spermatogenic cells. Pharmacology **87**(1–2), 70–80 (2011)
18. J. Zhao, M.C. Lee, A. Momin, C.M. Cendan, S.T. Shepherd, M.D. Baker, C. Asante, L. Bee, A. Bethry, J.R. Perkins, M.A. Nassar, B. Abrahamsen, A. Dickenson, B.S. Cobb, M. Merkenschlager, J.N. Wood, Small RNAs control sodium channel expression, nociceptor excitability, and pain thresholds. J. Neurosci. **30**(32), 10860–10871 (2010)
19. V. Haufe, J.A. Camacho, R. Dumaine, B. Gunther, C. Bollensdorff, G.S. von Banchet, K. Benndorf, T. Zimmer, Expression pattern of neuronal and skeletal muscle voltage-gated $Na^+$ channels in the developing mouse heart. J. Physiol. **564**(Pt 3), 683–696 (2005)
20. M.A. Bosch, J. Hou, Y. Fang, M.J. Kelly, O.K. Ronnekleiv, 17Beta-estradiol regulation of the mRNA expression of T-type calcium channel subunits: role of estrogen receptor alpha and estrogen receptor beta. J. Comp. Neurol. **512**(3), 347–358 (2009)
21. K.J. Berkley, Sex differences in pain. Behav. Brain Sci. **20**(3), 371–380 (1997). Discussion 435–513
22. N.M. Flake, D.B. Bonebreak, M.S. Gold, Estrogen and inflammation increase the excitability of rat temporomandibular joint afferent neurons. J. Neurophysiol. **93**(3), 1585–1597 (2005)
23. S. Sarajari, M.M. Oblinger, Estrogen effects on pain sensitivity and neuropeptide expression in rat sensory neurons. Exp. Neurol. **224**(1), 163–169 (2010)
24. L. Li, X. Fan, M. Warner, X.J. Xu, J.A. Gustafsson, Z. Wiesenfeld-Hallin, Ablation of estrogen receptor alpha or beta eliminates sex differences in mechanical pain threshold in normal and inflamed mice. Pain **143**(1–2), 37–40 (2009)
25. X. Fan, H.J. Kim, M. Warner, J.A. Gustafsson, Estrogen receptor beta is essential for sprouting of nociceptive primary afferents and for morphogenesis and maintenance of the dorsal horn interneurons. Proc. Natl. Acad. Sci. USA **104**(34), 13696–13701 (2007)
26. M.A. Coulombe, M.F. Spooner, I. Gaumond, J.C. Carrier, S. Marchand, Estrogen receptors beta and alpha have specific pro- and anti-nociceptive actions. Neuroscience **184**, 172–182 (2011)
27. F. Piu, C. Cheevers, L. Hyldtoft, L.R. Gardell, A.L. Del Tredici, C.B. Andersen, L.C. Fairbairn, B.W. Lund, M. Gustafsson, H.H. Schiffer, J.E. Donello, R. Olsson, D.W. Gil, M.R. Brann, Broad modulation of neuropathic pain states by a selective estrogen receptor beta agonist. Eur. J. Pharmacol. **590**(1–3), 423–429 (2008)
28. D.Y. Lee, Y.G. Chai, E.B. Lee, K.W. Kim, S.Y. Nah, T.H. Oh, H. Rhim, 17beta-Estradiol inhibits high-voltage-activated calcium channel currents in rat sensory neurons via a non-genomic mechanism. Life Sci. **70**(17), 2047–2059 (2002)
29. J. Fan, L.H. Yu, Y.M. Zhang, X. Ni, B. Ma, G. Burnstock, Estrogen altered visceromotor reflex and P2X(3) mRNA expression in a rat model of colitis. Steroids **74**(12), 956–962 (2009). doi:10.1016/j.steroids.2009.07.002
30. J.A. McRoberts, J. Li, H.S. Ennes, E.A. Mayer, Sex-dependent differences in the activity and modulation of N-methyl-D-aspartic acid receptors in rat dorsal root ganglia neurons. Neuroscience **148**, 1015–1020 (2007). doi:10.1016/j.neuroscience.2007.07.006
31. A. Chakrabarty, A. Blacklock, S. Svojanovsky, P.G. Smith, Estrogen elicits dorsal root ganglion axon sprouting via a renin-angiotensin system. Endocrinology **149**(7), 3452–3460 (2008). doi:10.1210/en.2008-0061
32. V.V. Chaban, P.E. Micevych, Estrogen receptor-alpha mediates estradiol attenuation of ATP-induced $Ca^{2+}$ signaling in mouse dorsal root ganglion neurons. J. Neurosci. Res. **81**(1), 31–37 (2005)
33. C.M. Revankar, D.F. Cimino, L.A. Sklar, J.B. Arterburn, E.R. Prossnitz, A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science (New York, N.Y.) **307**(5715), 1625–1630 (2005)
34. J. Kuhn, O.A. Dina, C. Goswami, V. Suckow, J.D. Levine, T. Hucho, GPR30 estrogen receptor agonists induce mechanical hyperalgesia in the rat. Eur. J. Neurosci. **27**(7), 1700–1709 (2008)
35. S.L. Dun, G.C. Brailoiu, X. Gao, E. Brailoiu, J.B. Arterburn, E.R. Prossnitz, T.I. Oprea, N.J. Dun, Expression of estrogen receptor GPR30 in the rat spinal cord and in autonomic and sensory ganglia. J. Neurosci. Res. **87**(7), 1610–1619 (2009)
36. C. Moller, R. Netzer, Effects of estradiol on cardiac ion channel currents. Eur. J. Pharmacol. **532**(1–2), 44–49 (2006)