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Effects of β-estradiol on cold-sensitive receptor channel TRPM8 in ovariectomized rats

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Abstract: Transient receptor potential cation channel subfamily M member 8 (TRPM8) is associated with sensitivity to cold sensation in mammals. A previous study demonstrated that TRPM8 was overexpressed in the skin of ovariectomized (OVX) rats due to the loss of estrogen. In the present study, we investigated whether estrogen replacement restricts overexpression of the TRPM8 channel in the skin of OVX rats. We divided 15 Sprague Dawley rats into three groups: a non-operated group (NON-OPE), an ovariectomy group (OVX), and a group subjected to estrogen replacement during 4 weeks beginning 7 days after ovariectomy (OVX + E2). Five weeks later, TRPM8 channel mRNA and protein in lumbar skin were quantified by real-time RT-PCR, protein ELISA, and immunohistochemistry. The OVX + E2 group exhibited a trend for decreased expression of the TRPM8 channel in the lumbar skin in comparison with the OVX group, whereas ELISA data and immunohistochemistry data and immunohistochemistry graphs relating to TRPM8 protein did not show any obvious differences between the OVX group and the OVX + E2 group. Estrogen replacement may restrict the overexpression of TRPM8 in the dermis of OVX rats.

Key words: cold-sensitive TRPM8 channel receptor, estrogen, estrogen replacement, oversensitivity to cold, post-menopause

Introduction

Vasomotor symptoms, such as hot flashes, sweating, shortness of breath, and pronounced sensitivity to cold, are the most common manifestations experienced by postmenopausal women [1, 2, 11, 29, 30]. These symptoms often impact their quality of life. Hormone replacement therapy, particularly estrogen supplementation, is the most widely-accepted and effective intervention for these quality of life symptoms [1, 2, 11, 29].

Among vasomotor symptoms, heightened sensitivity to cold is commonly observed below the normal temperature regime of innocuous cold [30]. Mechanisms of oversensitiveness to cold have not yet been completely resolved. In recent studies, certain transient receptor potential (TRP) cation channel subfamily members have been found to be associated with cold sensitivity [4, 5, 7, 13, 17, 18, 20–23, 26, 32, 33]. The 28 characterized mammalian TRP channels can be subdivided into six main subfamilies: TRPC (canonical), TRPV (vanilloid),

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Table 1. Sequences of primers used for real-time RT-PCR assays

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
Trpm8	GCAGTGGTACATGAACGGAGT	TGAAGAGTGAAGCCGGAATAC
Gapdh	AAATCCCATCACCATCTTCCA	AATGAGCCCCAGCCTTCTC

TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin) [22]. Among them, TRPA1 and TRPM8 are each activated by cold stimuli [5, 7, 22, 32]. Dorsal root and trigeminal ganglia have cold-sensitive neurons with TRPA1 or TRPM8 [5, 7, 18, 22, 32]. TRPA1 and TRPM8 are expressed in the skin and by cutaneous sensory neurons mediated by dorsal root ganglia [7, 17, 18]. While TRPA1 is activated by noxious cold (<17°C), TRPM8 is additionally activated by innocuous cold (<30°C) [7, 9, 11, 12]. In other words, TRPM8 is associated with sensitivity to moderate coldness (8–28°C) [22]. The TRPM 8 channel may be overexpressed in the dermis of postmenopausal women who exhibit symptoms of oversensitivity to cold.

Noguchi *et al.* [23] reported that TRPM8 channel protein and mRNA in the skin of ovariectomized (OVX) rats were overexpressed and that cold stress induced detrusor overactivity [13, 23] in such rats accompanied by a lack of estradiol. They demonstrated that α1-adrenergic receptor blockade inhibited cold stress induced detrusor overactivity. They concluded that cold stress induced detrusor over-activity associated with diminished estrogen is associated with TRPM8 channel overexpression in skin and is mediated via adrenergic nerve pathways. This suggests that oversensitivity to cold in menopausal women, whose blood estradiol levels are low, may be related to overexpression of the TRPM8 channel in skin.

In the present study, we administrated β-estradiol to OVX rats to investigate whether dermal overexpression of TRPM8 was restricted by estrogen replacement.

Materials and Methods

Animals

A total of 15 Sprague Dawley rats at postnatal week 30 were used in the experiments.

All animals were treated in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan) and the rules of the animal experiments committee at Shiga University of Medical Science.

Bilateral ovariectomy

The animals were randomly divided into 2 groups, including 10 with bilateral ovariectomy and 5 with no operation (NON-OPE group). The rats undergoing bilateral ovariectomy were anesthetized with 2% isoflurane. A midline incision was made to expose the lower abdominal cavity. In the OVX + E2 group, 5 rats were administered 5 μg/kg/day β-estradiol by means of subcutaneous injection during 4 weeks beginning on postoperative day 7. We used fat-soluble β estradiol (β-Estradiol≥98%, Sigma-Aldrich, Tokyo, Japan) dissolved in corn oil (Corn oil delivery vehicle®, Sigma-Aldrich, Tokyo, Japan) to 100 μg/ml. The remaining 5 OVX rats were not administered β-estradiol (OVX group). All animals were maintained for 5 weeks on a 12-h alternating light-dark cycle with free access to food and water. Five weeks later, we collected blood from the tail veins of all 15 rats 6 h after the final E2 injection to the OVX + E2 group. Serum estradiol levels were determined at a commercial laboratory (SRL, Tokyo, Japan). Approximately 2 cm^2 lumbar skin portions were harvested with all animals under anesthesia. After sampling blood and lumbar skin, we culled the 15 rats by an intraperitoneal injection of pentobarbital at 100 mg/kg.

Real-time RT-PCR

For real-time RT-PCR, harvested lumbar skin pieces were homogenized in PBS, and total RNA was extracted with an RNeasy MinElute Spin Column® (Qiagen, Hilden, Germany). cDNA was synthesized by total RNA using PrimeScript RT Master Mix® (Takara Bio, Kusatsu, Japan). To determine mRNA expression levels, real-time RT-PCR of the cDNA was performed with a *Trpm8* primer for the TRPM8 channel gene (Table 1, Takara Bio, Kusatsu, Japan) and a *Gapdh* primer for the GAPDH gene (Table 1, Takara Bio, Kusatsu, Japan), which served as an internal amplification control, using a LightCycler 480 SYBR Green 1 Master Kit® (Roche, Tokyo, Japan). The reaction conditions were as follows: 50°C for 2 min and then 95°C for 5 min, followed by 60 cycles at 95°C for 10 s, 55°C for 15 s, and 72°C for 10 s. Gene activity was expressed as the ratio to the internal standard gene

Gapdh.

Protein ELISA

Lumbar skin pieces were homogenized in PBS, and total protein was extracted. TRPM8 protein level was estimated with the use of a TRPM8 ELISA kit (ELISA 121416®, My Bio Source, San Diego, CA, USA). Optical absorbance at 450 nm was measured using a spectrophotometer (NanoDrop 1000®, SCRUM, Tokyo, Japan) and TRPM8 protein levels were calculated from the recorded optical density.

Immunohistochemistry

Lumbar skin portions were fixed in 10%-Formaldehyde Neutral Buffer Solution (Wako Pure Chemical Industries, Moriyama, Japan) overnight at 4°C and then embedded in paraffin. Each sample was cut into 3 μm serial sections. Sections were deparaffinized and rehydrated for immunohistochemistry as follows. Sections were subjected to antigen retrieval by boiling in Immunosaver® (Nisshin EM, Tokyo, Japan) for 45 min. The sections were then treated with 10% goat serum (Nichirei bioscience, Tokyo, Japan) for 30 min at room temperature. After rinsing with PBS, the sections were incubated with an anti-TRPM8 antibody (1:500, rabbit polyclonal, Novus Biologicals, Littleton, CO, USA) and anti-PGP9.5 antibody (1:500, mouse polyclonal, Abcam, Tokyo, Japan) overnight at 4°C. We used PGP9.5 as a pan-neuronal marker. After rinsing with PBS containing 0.03% Tween 20 (PBS-T), the sections were incubated with an Alexa Fluor 594-labelled donkey anti-rabbit IgG secondary antibody, Alexa Fluor 488-labelled donkey anti-mouse IgG secondary antibody, and 1 μg/ml DAPI (Molecular Probes®, Thermo Fisher Scientific, Tokyo, Japan) at room temperature for 1 h. Slides were coated with Fluorescent Mounting Medium (Prolong Diamond®, Thermo Fisher Scientific, Tokyo, Japan) and observed with a confocal laser-scanning microscope (FV-1000D IX-81®, Olympus, Tokyo, Japan).

To quantify the immunohistochemistry data, we adopted a subjective visual scoring system of the red staining and compared results to data arising from tissues obtained from non-operated rats. The scoring levels were as follows: 0 = absence of staining, 1 = weak staining intensity (equal to non-operated tissue), 2 = moderate staining, and 3 = strong staining intensity.

Statistical analysis

Results are expressed as the mean ± SEM. Differences between the two groups were estimated by Mann-Whitney U test. Statistical significance for all analyses was set at $P<0.05$. Data were analyzed using GraphPad Prism® (GraphPad Software, La Jolla, CA, USA).

Results

Serum estradiol level

The mean serum estradiol levels of the NON-OPE, OVX, and OVX + E2 groups were 36.00 ± 3.77, 22.00 ± 2.32, and 307.4 ± 31.4 pg/ml, respectively. There were statistically significant differences between the mean serum estradiol levels of the NON-OPE and OVX groups ($P<0.01$, Fig. 1), the OVX and OVX + E2 groups ($P<0.01$, Fig. 1) and the NON-OPE and OVX + E2 groups ($P<0.01$).

Relative expression of TRPM8 mRNA

The mean relative expression levels of TRPM8 channel mRNA in the lumbar skin of the NON-OPE, OVX, and OVX + E2 groups were 551 ± 317, 1,945 ± 1,186, and 15.6 ± 13.7, respectively. There were no statistically significant differences between mean relative expression levels of TRPM8 channel mRNA of the NON-OPE and OVX groups ($P=0.4206>0.05$), the OVX and OVX + E2 groups ($P=0.0873>0.05$), or the NON-OPE and OVX+E2 groups ($P=0.5397>0.05$). However, the OVX + E2 cohort exhibited a trend for decreased expression of TRPM8 channel mRNA in comparison with the OVX group ($P=0.0873$, Fig. 2).

TRPM8 protein level

The mean TRPM8 protein levels in the lumbar skin of the NON-OPE, OVX, and OVX + E2 groups were 1.11 ± 0.07, 1.19 ± 0.07, and 1.06 ± 0.04 ng/ml, respectively. There were no significant differences between the mean TRPM8 protein levels of the NON-OPE and OVX groups ($P=0.5476>0.05$), the OVX and OVX + E2 groups ($P=0.3095>0.05$), or the NON-OPE and OVX + E2 groups ($P=0.4206>0.05$). The OVX + E2 cohort did not show a trend for a decreased TRPM8 protein level in comparison with the OVX group ($P=0.3095$, Fig. 3).

Immunohistochemistry

TRPM8 channels and PGP9.5-positive nerve fibers in the lumbar skin of the NON-OPE, OVX, and OVX + E2

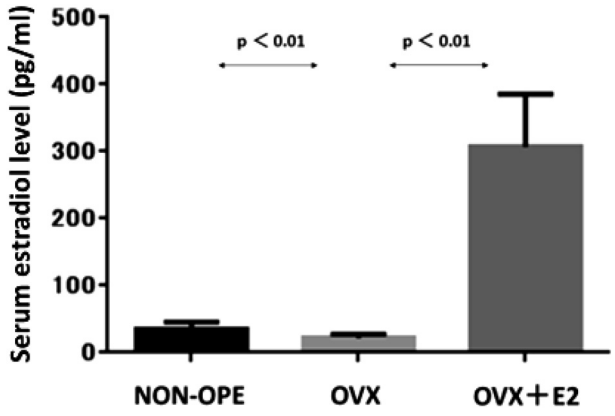

Fig. 1. Serum estradiol level. NON-OPE: non-operated rats. OVX: ovariectomized rats. OVX + E2: ovariectomizd rats treated for 28 days with 17β-estradiol. There was a significant difference among the mean serum estradiol levels of the NON-OPE, OVX, and OVX + E2 groups (P<0.01).

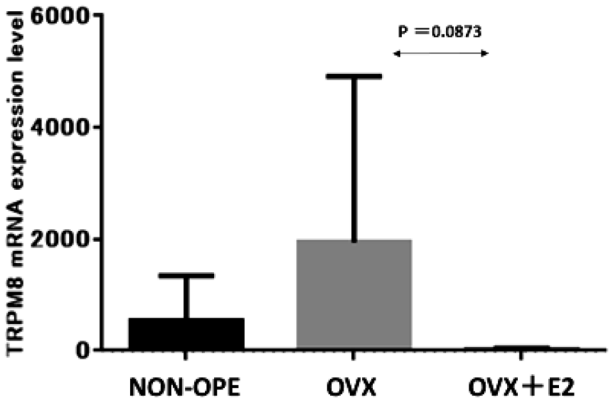

Fig. 2. Relative expression levels of TRPM8 mRNA. NON-OPE: non-operated rats. OVX: ovariectomized rats. OVX + E2: ovariectomized rats treated for 28 days with 17β-estradiol. The OVX + E2 group showed a trend for decreased expression of TRPM8 channel mRNA in lumbar skin in comparison with the OVX group, although the difference between the two groups did not reach statistical significance (P=0.0873>0.05).

groups were visualized by immunohistochemistry.

The mean scores for the NON-OPE, OVX, and OVX + E2 groups were 1.4 ± 0.22, 2.2 ± 0.44, and 1.4 ± 0.22, respectively. There were no statistically significant differences between the mean scores of the OVX and OVX + E2 groups (P=0.2857>0.05). Considering the results of this subjective visual assessment, we were unable to conclude that the expression of TRPM8 in the OVX + E2 rats was weaker than that in the OVX rats. However, our qualitative assessment indicated a tendency for a slight reduction of TRPM8 expression in the OVX + E2 group compared with the OVX group (Figs. 4B and C), whereas the expression of TRPM8 in the OVX group was greater than in the NON-OPE group (Figs. 4A and B).

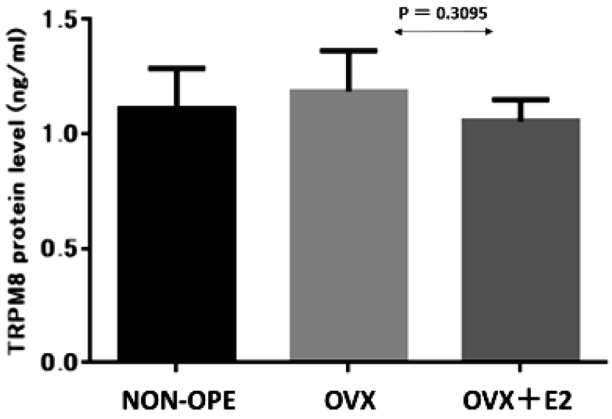

Fig. 3. TRPM8 protein levels. NON-OPE: non-operated rats. OVX: ovariectomized rats. OVX + E2: ovariectomized rats treated for 28 days with 17β-estradiol. There was no statistical difference between mean TRPM8 protein levels in lumbar skin of the OVX and OVX + E2 groups (P=0.3095>0.05).

Discussion

In our study, OVX + E2 rats exhibited a trend for diminished expression of the TRPM8 channel in the lumbar dermis compared with OVX-only animals. This suggests that estrogen replacement restricted the overexpression of TRPM8 in the skin of OVX rats.

Past research involving cold stress-induced detrusor overactivity revealed that the loss of estrogen due to ovariectomy increased expression of TRPM8 channel mRNA and cognate protein in lumbar skin [23]. Other recent studies also showed that OVX rats or mice with a long-term deficiency of estrogen exhibited greater sensitivity to thermal and mechanical stimuli, while estrogen replacement relieved such hyperalgesia [19, 25, 27]. This suggests that the TRPM8 channel may be overexpressed in dermal layers of postmenopausal women with low estrogen levels, who have symptoms of hypersensitivity to cold, and that estrogen replacement may improve the overexpression of TRPM8 channel mRNA.

The mechanisms of oversensitivity to cold in postmenopausal women are not fully understood. Estradiol dilates blood vessels and increases blood flow by relax-

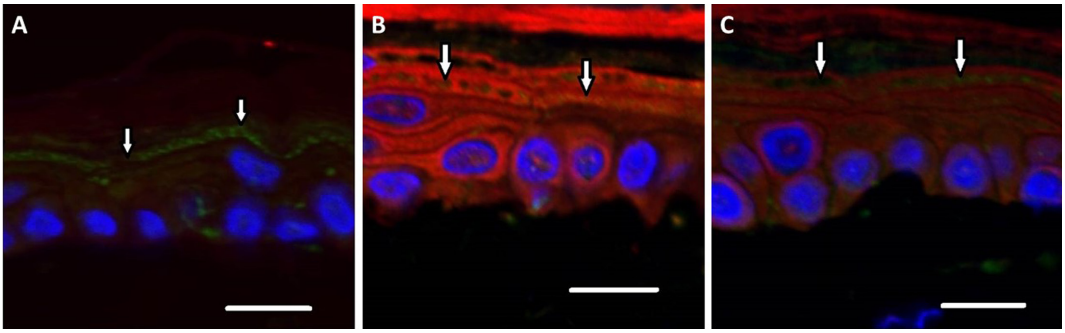

Fig. 4. Immunohistochemistry. A. NON-OPE: non-operated rats. B. OVX: ovariectomized rats. C. OVX + E2: ovariectomized rats treated for 28 days with 17β-estradiol. TRPM8 channels and PGP9.5-positive nerve fibers in lumbar skin of NON-OPE, OVX, and OVX + E2 rats were visualized by immunohistochemistry. TRPM8 channels were stained red, and PGP9.5-positive nerve fibers were stained green. Cell nuclei are shown in blue. PGP9.5-positive nerve fibers were scattered in a line at the external side of the epidermal tissues (arrows). Scale bar, 10 μm. Expression of TRPM8 in the OVX rat was greater than in the NON-OPE rat (A, B), whereas expression of TRPM8 in the OVX + E2 rat was lesser than in the OVX rat (B, C).

ing vascular smooth muscles [16, 24, 30]. It has also been found that estradiol promotes the generation of nitric oxide (NO), which is an endothelium-derived relaxing factor (EDRF), by endothelial nitric oxide synthase (eNOS) [16, 24]. Therefore, it has been proposed that decreased blood flow in peripheral tissues due to reduction in estradiol levels results in cold sensation in postmenopausal women [30].

On the other hand, two recent studies in mice revealed that TRPM8 and TRPA1 play important roles in cold-induced vascular response [3, 28]. Johnson *et al.* [14] reported that TRPM8 channels are present in rat arterial vascular smooth muscle and are involved with vasoconstriction and vasodilation. Ding *et al.* [8] reported that Icilin, a TRPM8/TRPA1 agonist, produces a dose-related hyperthermic response mediated by NO production in rats. Furthermore, Pan *et al.* [25] reported that, in rats, TRPM8/TRPA1 promotes oxaliplatin-induced-vasodilation, which is associated with NO metabolites, and this vasodilation is attenuated by 17β-estradiol. Together with these reports, TRPM8 and estradiol seem to be related with mechanisms of thermosensation, involving vascular regulation mediated by NO production. However, the mechanistic basis remains unclear. Further studies are clearly required in this area.

The relationship between TRPM8 and estrogen has also remains unknown. Chodon *et al.* [4] reported the correlation between TRPM8 expression and estrogen receptor (ER) status in breast cancer cells. They found that TRPM8 overexpression was observed in 77.8% of the ERα-positive tumors and in 37.5% of the ERα-negative tumors (P<0.05). This provided evidence of an ER-mediated increase in TRPM8 mRNA expression. Considering their research, estrogen may have increased TRPM8 gene expression in breast cancer cells. The relationship between TRPM8 and estrogen in normal (non-tumor) cells may be different from that in tumor cells.

There are some limitations to our study. First, the number of rats (n=15) was small. This was one reason why we could not gain statistical significance. A study using a larger number of animals is now necessary, although the principles of the 3Rs should be respected from the viewpoint of animal welfare.

Second, in the process of measuring TRPM8 protein levels with the ELISA approach adopted, we intended to extract TRPM8 proteins only from the epidermis; however TPPM8 protein from subcutaneous tissues may nevertheless have been admixed with those from the epidermis. Therefore, we may not have measured exact TRPM8 protein levels in the epidermis of rats. We should have used a microdissection technique. However, admixture could not explain the discrepancy between mRNA expression (a trend of reduced expression) and protein expression (no change), because TRPM8 mRNA, as well as TRPM8 protein, were included in the mixed tissue.

A discrepancy between mRNA and protein is often observed and is frequently due not only to technical difficulties but also to translational control in the literature [6, 10, 31, 34]. In the OVX group, the translation of TRPM8 protein might have been suppressed by some factors, although the transcription of TRPM8 mRNA was

overexpressed. On the other hand, the translation level of TRPM8 protein in the OVX + E2 group might have been stabilized in spite of an extremely low level of TRPM8 mRNA. Further research is needed to determine the mechanisms through which TRPM8 is translationally regulated at the gene level.

Third, the serum estradiol level of the OVX + E2 rats was unexpectedly high. We decided on a low dose of 5 μg/kg/day fat-soluble β-estradiol with reference to previous reports [9, 12, 15]. However, the serum estradiol level of OVX + E2 rats in our study after 28 days of subcutaneous injection of fat-soluble β-estradiol was approximately one-third higher than the predicted value of 100 pg/kg/day. Compared with water soluble β-estradiol, fat-soluble β-estradiol may have been sequestered in the bodies of the rats.

In conclusion, our research showed that β-estradiol restricted the expression of TRPM8 channel mRNA in the OVX rat dermis, while the expression of TRPM8 channel protein was not remarkably changed by β-estradiol. Although statistical significance was not established, trends suggest that estrogen replacement may improve oversensitivity to cold in postmenopausal women.

Conclusions

β-estradiol may restrict overexpression of TRPM8 in the skin of OVX rats.

Conflicts of Interests

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

1. Alexander, J.L., Burger, H., Dennerstein, L., Woods, N.F., Davis, S.R., Kotz, K., Van Winkle, J., Richardson, G., Ratka, A., and Kessel, B. 2007. Treatment of vasomotor symptoms in the menopausal transition and postmenopausally: psychiatric comorbidity. *Expert Rev. Neurother.* 7:(Suppl): S115–S137 . [Medline] [CrossRef]
2. AACE Menopause Guidelines Revision Task Force. 2006. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of menopause. *Endocr. Pract.* 12: 315–337. [Medline] [CrossRef]
3. Aubdool, A.A., Graepel, R., Kodji, X., Alawi, K.M., Bodkin, J.V., Srivastava, S., Gentry, C., Heads, R., Grant, A.D., Fernandes, E.S., Bevan, S., and Brain, S.D. 2014. TRPA1 is essential for the vascular response to environmental cold exposure. *Nat. Commun.* 5: 5732 . [Medline] [CrossRef]
4. Chodon, D., Guilbert, A., Dhennin-Duthille, I., Gautier, M., Telliez, M.S., Sevestre, H., and Ouadid-Ahidouch, H. 2010. Estrogen regulation of TRPM8 expression in breast cancer cells. *BMC Cancer* 10: 212 . [Medline] [CrossRef]
5. Colburn, R.W., Lubin, M.L., Stone, D.J. Jr., Wang, Y., Lawrence, D., D'Andrea, M.R., Brandt, M.R., Liu, Y., Flores, C.M., and Qin, N. 2007. Attenuated cold sensitivity in TRPM8 null mice. *Neuron* 54: 379–386 . [Medline] [CrossRef]
6. Dengler, M.A., Weilbacher, A., Gutekunst, M., Staiger, A.M., Vöhringer, M.C., Horn, H., Ott, G., Aulitzky, W.E., and van der Kuip, H. 2014. Discrepant NOXA (PMAIP1) transcript and NOXA protein levels: a potential Achilles' heel in mantle cell lymphoma. *Cell Death Dis.* 5: e1013 . [Medline] [CrossRef]
7. Dhaka, A., Murray, A.N., Mathur, J., Earley, T.J., Petrus, M.J., and Patapoutian, A. 2007. TRPM8 is required for cold sensation in mice. *Neuron* 54: 371–378 . [Medline] [CrossRef]
8. Ding, Z., Gomez, T., Werkheiser, J.L., Cowan, A., and Rawls, S.M. 2008. Icilin induces a hyperthermia in rats that is dependent on nitric oxide production and NMDA receptor activation. *Eur. J. Pharmacol.* 578: 201–208 . [Medline] [CrossRef]
9. Fedotova, J. 2014. Effects of SCH-23390 in combination with a low dose of 17β-estradiol on anxiety-like behavior in ovariectomized rats. *BioMed Res. Int.* 2014: 847821 . [Medline] [CrossRef]
10. El Hajj, P., Gilot, D., Migault, M., Theunis, A., van Kempen, L.C., Salés, F., Fayyad-Kazan, H., Badran, B., Larsimont, D., Awada, A., Bachelot, L., Galibert, M.D., Ghanem, G., and Journe, F. 2015. SNPs at miR-155 binding sites of TYRP1 explain discrepancy between mRNA and protein and refine TYRP1 prognostic value in melanoma. *Br. J. Cancer* 113: 91–98 . [Medline] [CrossRef]
11. Hall, G. and Phillips, T.J. 2005. Estrogen and skin: the effects of estrogen, menopause, and hormone replacement therapy on the skin. *J. Am. Acad. Dermatol.* 53: 555–568, quiz 569–572 . [Medline] [CrossRef]
12. Hao, F., Gu, Y., Tan, X., Deng, Y., Wu, Z.T., Xu, M.J., and

Wang, W.Z. 2016. Estrogen replacement reduces oxidative stress in rostral ventrolateral medulla of ovariectomized rats. *Oxid. Med. Cell. Longev*. 2016: Article ID 2158971, 8pages.

13. Imamura, T., Ishizuka, O., Sudha, G.S., Lei, Z., Hosoda, T., Noguchi, W., Yamagishi, T., Yokoyama, H., Kurizaki, Y., and Nishizawa, O. 2013. A galenical produced from Ba-Wei-Die-Huang-Wan (THC-002) provides resistance to the cold stress-induced detrusor overactivity in conscious rats. *Neurourol. Urodyn.* 32: 486–492 . [Medline] [CrossRef]
14. Johnson, C.D., Melanaphy, D., Purse, A., Stokesberry, S.A., Dickson, P., and Zholos, A.V. 2009. Transient receptor potential melastatin 8 channel involvement in the regulation of vascular tone. *Am. J. Physiol. Heart Circ. Physiol.* 296: H1868–H1877 . [Medline] [CrossRef]
15. Kim, I.D., Ahn, K.H., Lee, S., Hong, S.C., Kim, S.H., and Kim, T. 2013. Effect of ovariectomy, 17-β estradiol, and progesterone on histology and estrogen receptors of bladder in female partial bladder outlet obstruction rat model. *J. Obstet. Gynaecol. Res.* 39: 1259–1267 . [Medline] [CrossRef]
16. Kypreos, K.E., Zafirovic, S., Petropoulou, P.I., Bjelogrlic, P., Resanovic, I., Traish, A., and Isenovic, E.R. 2014. Regulation of endothelial nitric oxide synthase and high-density lipoprotein quality by estradiol in cardiovascular pathology. *J. Cardiovasc. Pharmacol. Ther.* 19: 256–268 . [Medline] [CrossRef]
17. Li, M.C., Yang, G., Zhou, X.D., Tselluyko, S., and Perelman, J.M. 2014. The pathophysiological mechanisms underlying mucus hypersecretion induced by cold temperatures in cigarette smoke-exposed rats. *Int. J. Mol. Med.* 33: 83–90. [Medline]
18. Lippoldt, E.K., Elmes, R.R., McCoy, D.D., Knowlton, W.M., and McKemy, D.D. 2013. Artemin, a glial cell line-derived neurotrophic factor family member, induces TRPM8-dependent cold pain. *J. Neurosci.* 33: 12543–12552 . [Medline] [CrossRef]
19. Ma, B., Yu, L.H., Fan, J., Cong, B., He, P., Ni, X., and Burnstock, G. 2011. Estrogen modulation of peripheral pain signal transduction: involvement of P2X(3) receptors. *Purinergic Signal.* 7: 73–83 . [Medline] [CrossRef]
20. Madrid, R., Donovan-Rodríguez, T., Meseguer, V., Acosta, M.C., Belmonte, C., and Viana, F. 2006. Contribution of TRPM8 channels to cold transduction in primary sensory neurons and peripheral nerve terminals. *J. Neurosci.* 26: 12512–12525 . [Medline] [CrossRef]
21. McKemy, D.D., Neuhausser, W.M., and Julius, D. 2002. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416: 52–58 . [Medline][Medline] [CrossRef]
22. Nilius, B., Owsianik, G., Voets, T., and Peters, J.A. 2007. Transient receptor potential cation channels in disease. *Physiol. Rev.* 87: 165–217 . [Medline] [CrossRef]
23. Noguchi, W., Ishizuka, O., Imamura, T., Kurizaki, Y., Yamagishi, T., Yokoyama, H., Lei, Z., Silwal, S.G., Nishizawa, O., and Andersson, K.E. 2013. The relationship between α1-adrenergic receptors and TRPM8 channels in detrusor overactivity induced by cold stress in ovariectomized rats. *J. Urol.* 189: 1975–1981 . [Medline] [CrossRef]
24. Orimo, A., Inoue, S., Ikegami, A., Hosoi, T., Akishita, M., Ouchi, Y., Muramatsu, M., and Orimo, H. 1993. Vascular smooth muscle cells as target for estrogen. *Biochem. Biophys. Res. Commun.* 195: 730–736 . [Medline] [CrossRef]
25. Pan, Y., Chen, F., Huang, S., Cai, Z., Lan, H., Tong, Y., Yu, X., and Zhao, G. 2016. TRPA1 and TRPM8 Receptors May Promote Local Vasodilation that Aggravates Oxaliplatin-Induced Peripheral Neuropathy Amenable to 17β-Estradiol Treatment. *Curr. Neurovasc. Res.* 13: 309–317 . [Medline] [CrossRef]
26. Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M., Earley, T.J., Dragoni, I., McIntyre, P., Bevan, S., and Patapoutian, A. 2002. A TRP channel that senses cold stimuli and menthol. *Cell* 108: 705–715 . [Medline] [CrossRef]
27. Sanoja, R. and Cervero, F. 2005. Estrogen-dependent abdominal hyperalgesia induced by ovariectomy in adult mice: a model of functional abdominal pain. *Pain* 118: 243–253 . [Medline] [CrossRef]
28. So, K., Tei, Y., Zhao, M., Miyake, T., Hiyama, H., Shirakawa, H., Imai, S., Mori, Y., Nakagawa, T., Matsubara, K., and Kaneko, S. 2016. Hypoxia-induced sensitisation of TRPA1 in painful dysesthesia evoked by transient hindlimb ischemia/reperfusion in mice. *Sci. Rep.* 6: 23261 . [Medline] [CrossRef]
29. Sofowora, G.G., Singh, I., He, H.B., Wood, A.J.J., and Stein, C.M. 2005. Effect of acute transdermal estrogen administration on basal, mental stress and cold pressor-induced sympathetic responses in postmenopausal women. *Clin. Auton. Res.* 15: 193–199 . [Medline] [CrossRef]
30. Ushiroyama, T. 2001. Clinical efficiency of HRT on cold sensation. *Jpn. J. Obstet. Gyneco. Ther.* 83: 663–667.
31. Wang, D. 2008. Discrepancy between mRNA and protein abundance: insight from information retrieval process in computers. *Comput. Biol. Chem.* 32: 462–468 . [Medline] [CrossRef]
32. Wang, T., Jing, X., DeBerry, J.J., Schwartz, E.S., Molliver, D.C., Albers, K.M., and Davis, B.M. 2013. Neurturin overexpression in skin enhances expression of TRPM8 in cutaneous sensory neurons and leads to behavioral sensitivity to cool and menthol. *J. Neurosci.* 33: 2060–2070 . [Medline] [CrossRef]
33. Weil, A., Moore, S.E., Waite, N.J., Randall, A., and Gunthorpe, M.J. 2005. Conservation of functional and pharmacological properties in the distantly related temperature sensors TRVP1 and TRPM8. *Mol. Pharmacol.* 68: 518–527. [Medline]
34. Wennemers, M., Bussink, J., van den Beucken, T., Sweep, F.C.G.J., and Span, P.N. 2012. Regulation of TRIB3 mRNA and protein in breast cancer. *PLoS One* 7: e49439 . [Medline] [CrossRef]