Estrous Cycle Induces Peripheral Sensitization in Trigeminal Ganglion Neurons: An Animal Model of Menstrual Migraine

Wachirapong Saleeon MSc candidate*, Ukkrit Jansri MSc**,
Anan Srikiatkhachorn MD*, Saknan Bongsebandhu-phubhakdi PhD*

** Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Background: *Many women experience menstrual migraines that develop into recurrent migraine attacks during menstruation. In the human menstrual cycle, the estrogen level fluctuates according to changes in the follicular and luteal phases. The rat estrous cycle is used as an animal model to study the effects of estrogen fluctuation.*
Objective: *To investigate whether the estrous cycle is involved in migraine development by comparing the neuronal excitability of trigeminal ganglion (TG) neurons in each stage of the estrous cycle.*
Material and Method: *Female rats were divided into four experimental groups based on examinations of the cytologies of vaginal smears, and serum analyses of estrogen levels following each stage of the estrous cycle. The rats in each stage of the estrous cycle were anesthetized and their trigeminal ganglia were removed. The collections of trigeminal ganglia were cultured for two to three hours, after which whole-cell patch clamp experiments were recorded to estimate the electrophysiological properties of the TG neurons.*
Results: *There were many vaginal epithelial cells and high estrogen levels in the proestrus and estrus stages of the estrous cycle. Electrophysiological studies revealed that the TG neurons in the proestrus and estrus stages exhibited significantly lower thresholds of stimulation, and significant increase in total spikes compared to the TG neurons that were collected in the diestrus stage.*
Conclusion: *Our results revealed that high estrogen levels in the proestrus and estrus stages altered the thresholds, rheobases, and total spikes of the TG neurons. High estrogen levels in the estrous cycle induced an increase in neuronal excitability and the peripheral sensitization of TG neurons. These findings may provide an explanation for the correlation of estrogen fluctuations during the menstrual cycle with the pathogenesis of menstrual migraines.*

Keywords: *Menstrual migraine, Peripheral sensitization, Estrous cycle, Trigeminal ganglion (TG) neurons, Whole-cell patch clamp recording*

J Med Assoc Thai 2016; 99 (2): 206-12
Full text. e-Journal: *http://www.jmatonline.com*

Migraine is a neurological disorder that is accompanied by migraine attacks that include recurrent painful headaches. Migraine attacks is highly prevalent in females[1,2], and many experience migraine attacks associated to their menstrual cycle, or menstrual migraines. The gonadal hormones, progesterone, and estrogen fluctuate in each stage of the menstrual cycle. These fluctuations in gonadal hormones have been suggested to cause menstrual migraines. Consistent with the hormonal changes in the menstrual cycle[3], the estrous cycle comprises of recurring physiological changes induced by gonadal hormones in mammalian therian females. The estrous cycle of rats can thus be used as an animal model to exhibit the enhancing effects of estrogen on peripheral pain modulation.

The estrous cycle is divided into four stages: diestrus, proestrus, estrus, and met estrus. Estrogen fluctuations are synchronized with the development of the stages of the estrous cycle. The systemic estrogen level is constant, at a baseline level, during the diestrus stage and increases dramatically to its peak level in the proestrus stage. Next, the estrogen level slightly decreases but remains at a considerably high level in the estrus stage. Finally, the estrogen level returns to the baseline level during the metestrus stage and remains steady until the proestrus stage of the next

Correspondence to:
Bongsebandhu-phubhakdi S, Department of Physiology, Faculty of Medicine, Chulalongkorn University, 1873 Rama IV Road, Pathumwan, Bangkok 10330, Thailand.
Phone: +66-83-9860713, Fax: +66-2-2527854
E-mail: saknan@live.jp

estrous cycle. Previous studies have demonstrated that high levels of estrogen can augment pain perception in the trigeminal nociceptive pathway, which is related to the induction of migraine attacks[4]. Estrogen may intensify the activation of trigeminal ganglion (TG) neurons via estrogen receptors, and this process results in peripheral sensitization[5,6]. Additionally, electrophysiological studies have shown that the chronic administration of estrogen increases the total numbers of action potentials in TG neurons.

This study aimed to investigate the electrophysiological properties of TG neurons in the various stages of the estrous cycle. The results of this study may reveal the involvement of peripheral sensitization in the path physiology of menstrual migraine.

Material and Method

Female Wistar rat aged six to eight weeks old were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. The animals were adults and exhibited high estrogen levels. The rats used in all experiments were housed in ventilated stainless cages, on a 12-hour dark-light cycle, and were fed ad libitum. All procedures were approved by the Animal Care and Use Committee of the Faculty of Medicine, Chulalongkorn University, Thailand.

The rats were divided into four experimental groups according to the following four stages of the estrous cycle, diestrus, proestrus, estrus, and metestrus. The estrous cycle stage for each female rat was identified using the widely used technique of examining cell histology of vaginal smears. Aplastic dropper filled with 0.9% saline was inserted into the vagina to obtain vaginal smears. The saline was allowed to fill the vagina and then mixed two to three times until the saline became turbid. The turbid fluid was then placed on a glass slide. The samples were fixed with fire or air dried and stained with 1% methylene blue for five minutes. The slides were rinsed in water. The resulting samples were examined under a light microscope. The characterization of each stage of the estrous cycle was performed by discriminating three types of cells in the vaginal smear (i.e., epithelial cells, cornified cells, and leukocytes). The stage of the estrous cycle was confirmed by measuring hormone concentrations in the serum. Blood (400-500 µl) was collected in clot blood tubes from the hearts of the rats prior to anesthesia. The blood samples were centrifuged at 3,200 rpm for 10 minutes, and the serum was then stored at -20°C. The serum was analyzed for the level of estradiol (E2) using chemiluminescent micro particle immunoassay (CMIA) method.

The primary cell culture process was modified from a dissociated primary sensory neuron protocol. The rats were anesthetized and then injected intra-peritoneally with an overdose of sodium pentobarbital prior to decapitation. Both trigeminal ganglia were removed and cultured in a 35-mm culture dish with ice-cold Hank's balanced salt solution (HBSS) with penicillin/streptomycin. Next, the trigeminal ganglia were washed two times in HBSS and cut into small pieces with a blade in 1 ml of HBSS. Collagenase and dispase were filtrated through a 0.22 µm filter and added to the sample, which was immediately incubated at 37°C for 20 minutes. Papain was filtrated through a 0.22 µm filter and added to the sample, which was immediately incubated at 37°C for 20 minutes. Next, the sample was centrifuged at RCF 400 g for two minutes, and the supernatant was removed. The first precipitate was ground in L-15 complete medium using a glass pipette three times and was then centrifuged at RCF 400 g for eight minutes. The second precipitate was collected and washed with F-12 complete medium twice. Finally, 400 µl F-12 completed medium was added into the sample, and the sample was placed in a 35-mm laminin/PDL dish. The sample was incubated in an incubator (37°C, 5% CO_2) for three hours. For further electrophysiological studies, the sample was washed in F12 twice.

The patch-clamp recordings were performed at room temperature (26$\pm$0.5°C) using TG neurons that had been maintained in primary culture for three hours after isolation. The patch-clamp recordings were performed on an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA) and recorded in a computer for data analysis with pClampfit 10.2 software (Axon instruments, Foster City, CA, USA). The output signal was filtered at 2 kHz, and the sampling rate was 5 kHz. The glass pipettes were 1.5 mm in outer diameter and 0.86 mm in inner diameter (Sutter Instruments, Navato, CA, USA). The glass pipettes were pulled with a Flaming/Brown micropipette puller (P-97; Sutter Instruments, Navato, CA, USA) with a resistance of 3-5 MΩ[7,8], and filled with internal solution. The internal solution was prepared with nuclease-free water that contained the following (in mM): 144 potassium gluconate, 3 $MgCl_2$, 10 HEPES, 0.2 EGTA, 2 K_2-ATP and 0.3 Na_3-GTP (pH 7.2 and 285-295 mOsm). The plastic chamber that included the primary-cultured TG neurons was placed on the sample stand of an upright microscope (Olympus BX51WI microscope, Olympus,

USA). Next, the extracellular superfusion solution was added to the plastic chamber (composition in mM: 1 M CaCl, 1M MgCl, D-gluta and 10% HEPES; adjusted to a pH of 7.40 with 1 M NaOH and an osmolality of 320 mOsm/kg with glucose) at an initial flow rate of 1 ml/min at room temperature. In current-clamp recording mode, the membrane potentials of the TG neurons were manually held at -60 mV and injected with -30 to 70 (10 pA/step) in 11 steps of 500-msec duration. Only the TG neurons with diameters <38 μm were analyzed[9]. The measurements of electrophysiological properties are shown in Fig. 1. Threshold was considered as the depolarizing potential that triggered the first action potential (dash line 1, Fig. 1). Rheobase was the lowest injecting current that produced the first action potential (line 3, Fig. 1).

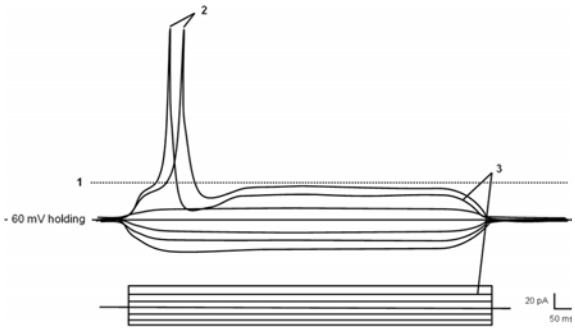

Fig. 1 Electrophysiological parameters; currents in each step are incremented by 10 pA (total 7 currents). 1 = threshold, 2 = spikes (2 total spikes), 3 = rheobase.

Data analysis

All data are presented as the means ± the standard errors of means (SEM). The statistical analyses were performed using Student's t-tests. The $p<0.05$ was accepted as statistically significant.

Results

Morphological studies revealed that the TG neurons in various stages of the estrous cycle exhibited similar structures (Figs. 2A, 2B, 2C, and 2D). TG neurons had a diameter of approximately 24 mm, and contained clear nucleus and nucleolus. In contrast, the cytological studies of vaginal smears demonstrated differences in the distributions of vaginal cells. There were many leukocytes and few epithelial nucleated cells in the diestrus stage (Fig. 2E), and only clusters of nucleated epithelial cells were observed in the proestrus stage (Fig. 2F). The nucleated epithelial cells differentiated to the clusters of cornified epithelial cells in the estrus stage (Fig. 2G). The changes in the cell distributions completed with many leukocytes and few cornified epithelial cells in the metestrus stage (Fig. 2H). The representative structure of nucleated epithelial cells, cornified epithelial cells and leukocyte are shown in Fig. 2I, 2J, and 2K, respectively.

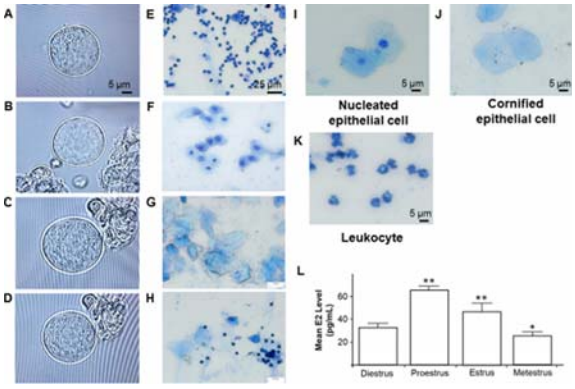

Fig. 2 Evaluation of the stages in the estrous cycle of female rats. (A-D) Representative morphologies of primary cultured TG neurons at each stage of the estrous cycle. (A) Diestrus stage, (B) proestrus stage, (C) estrus stage, and (D) metestrus stage. (E-H) Representative cytologies of the vaginal cells in each stage of the estrous cycle. (E) Diestrus stage, (F) proestrus stage, (G) estrus stage, and (H) metestrus stage. (I-K) Representative vaginal cell types. (I) Nucleated epithelial cells, (J) cornified epithelial cells, and (K) leukocytes. (L) The concentrations of estrogen at each stage of the estrous cycle (n = 8 per stage). * $p<0.05$, ** $p<0.005$ compared with the diestrus stage.

We estimated the estrogen levels of the sera from each stage of the estrous cycle (Fig. 2L; n = 8 in each stage). We found that the estrogen levels were stable at the baseline level in the diestrus stage (32.00±1.00 pg/ml) and increased to the highest level in the proestrus stage (63.43±2.99 pg/ml; $p<0.005$ compared with the diestrus stage). In the estrus stage, the estrogen level had decreased slightly from the highest level but remained significantly higher than in the diestrus stage (45.71±2.98 pg/ml; $p<0.005$ compared with the diestrus stage). In the metestrus stage, the estrogen level significantly decreased to below the baseline level (24.29±3.90 pg/ml; $p<0.05$ compared with the diestrus stage).

Next, we applied 500-msec step pulses and examined the electrophysiological properties of the TG neurons with whole-cell patch clamp techniques (n = 15 in each stage). The TG neurons in each stage of the

estrous cycle exhibited similar RMP values that were not significantly different from those of the diestrus stage (Table 1). Interestingly, the TG neurons in each stage of the estrous cycle exhibited different neuronal excitabilities (Fig. 3). The threshold of the TG neurons in the diestrus stage was -29.31±0.69 mV, whereas the threshold of the TG neurons in the high-estrogen conditions were significantly lower than those in the diestrus stage (proestrus stage: -43.85±0.04 mV; $p<0.005$ compared with the diestrus stage and estrus stage -39.99±0.71 mV; $p<0.005$ compared with the diestrus stage). However, the threshold of the TG neurons in the metestrus stage was not significantly different from that in the diestrus stage (-27.57±2.18 mV; $p = 0.50$). The rheobase values in each stage of the estrous cycle paralleled the thresholds of the TG neurons. The rheobase of the TG neurons in the diestrus stage was 45.33±2.45 pA. Although the rheobases in the proestrus and estrus stages were significantly lower than in the diestrus stage (16.00±0.11 pA, 19.33±1.62 pA; $p<0.005$, $p<0.005$, respectively), the rheobase in the metestrus stage was not significantly different from the rheobase in the diestrus stage (45.33±4.89; $p = 1.00$). Additionally, the summations of peripheral sensitization, as indicated by the total spikes of the TG neurons varied according to the stage of the estrous cycle. The total spikes over the 11 stimulation steps were 6.13±0.78. Peripheral sensitizations were observed as enhancements in the total numbers of spikes in the proestrus and estrus stages compared with the diestrus stage (111.00±7.43, 81.80±8.14; $p<0.005$, $p<0.005$, respectively); however, there was no change in the total number of spikes in the metestrus stage compared with the diestrus stage (6.53±0.91; $p = 0.51$).

Discussion

In the present study, we showed that the stages of the estrous cycle could be determined via cytological examinations of the vaginal epithelium cells that are influenced by the level of estrogen. The high systemic estrogen level conditions of the proestrus and estrus stages increases the numbers of nucleated epithelium cells and cornified epithelium cells. On the other hand, there were many leukocytes in the diestrus and metestrus stages during which estrogen is stable at the baseline level. The change of vaginal cytology in our study is consistent with previous studies[10,11]. The estrogen level had no effect on the morphologies of the TG neurons, but the electrophysiological properties of the TG neurons were altered according to the fluctuations in the estrogen levels across the estrous cycle. The TG neurons exhibited increased excitability in the proestrus and estrus stages, during which the estrogen levels were high. These alterations in TG neuron excitability may be related to peripheral sensitization in the trigeminal nociceptive system.

Following exposure to estrogen, cells are activated by the process of protein expression. Estrogen binds to the estrogen receptors (ER) ER-α and ER-β, which function using both genomic and non-genomic mechanisms[12]. High systemic estrogen levels activate the ER receptors in vaginal cells, which results in the proliferation of nucleated epithelium cells and cornified epithelium cells. Estrogen receptors are also expressed in TG neurons[13]. Our results revealed that the high estrogen levels of the proestrus and estrus stages reduced the stimulation thresholds and

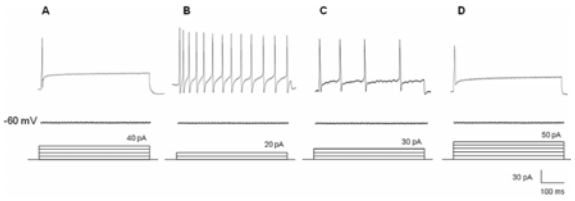

Fig. 3 Neuronal excitabilities of the TG neurons at each stage of the estrous cycle. A) In the diestrus stage, a rheobase of 40 pA induced the first response. B) In the proestrus stage, a rheobase of 20 pA induced the first response. C) In the estrus stage, a rheobase of 30 pA induced the first response. D) In the metestrus stage, a rheobase of 50 pA induced the first response.

Table 1. Electrophysiological properties of the TG neurons in each stage of the estrous cycle.

	Diestrus (n = 15)	Proestrus (n = 15)	Estrus (n = 15)	Metestrus (n = 15)
RMP (mV)	-48.93±0.68	-47.90±1.83	-48.35±1.03	-44.32±1.53
Threshold (mV)	-29.31±0.69	-43.85±0.04*	-39.99±0.71*	-27.57±2.18*
Rheobase (pA)	45.33±2.45	16.00±0.11*	19.33±1.62*	45.33±4.89
Total spikes	6.13±0.78	111.00±7.43*	81.80±8.14*	6.53±0.91

The values are presented as the means ± the SEMs. *$p< 0.05$ compared with the diestrus stage

rheobases required to evoke action potentials in TG neurons. The thresholds and rheobases reflect the neuronal excitability of TG neurons, which are modulated by various neurotransmitters and neuropeptides. It has been demonstrated that the administration of estrogen decreases trigeminal pain thresholds in female rats and increases the excitability of TG neurons[14]. Estrogen increases peripheral sensitization in the trigeminal system by enhancing bradykin in signaling in TG neurons[15,16]. In addition, estrogen also augments endothelial nitric oxide synthase (eNOS) levels, and these levels directly modify peripheral sensitization[17].

As estrogen increases calciton in gene-related peptide (CGRP) and serotonin (5-HT) levels, both known to have important roles in pain perception in the trigeminal system, estrogen is thought to be a modulator factor in menstrual migraines[4,18]. Other studies have reported enhanced effects of estrogen in CGRP synthesis due to nerve growth factor (NGF)-mediated mechanisms and activation of the transient receptor potential cation channel V1 (TRPV1) in the dorsal root ganglia[19]. The chronic administration of estrogen increases CGRP levels in dorsal root ganglia[20]. Estrogen also modulates nociceptive responses through its effects on other neuropeptides, such as galan in and neuropeptide Y[21]. Thus, our study suggests it is highly possible that fluctuation of estrogen levels during the estrus cycle induces the alteration of neurotransmitters, resulting in the peripheral sensitization.

Additionally, our results also revealed that the total numbers of spikes during the proestrus and estrus stages increased. These findings indicate that estrogen may modulate the activation of several voltage-gated ion channels. Estrogen has been found to increase the activity of calcium-dependent potassium channels and induce augmented depolarization in dorsal root ganglion cells[22,23]. Estrogen increases the activation of mitogen-activated protein (MAP) kinase and extracellular signal-regulated kinase (ERK), which results in the phosphorylation of voltage-gated sodium channels and voltage-gated potassium channels[6]. These effects are involved in the regulation of neuronal excitability[24]. Thus, the fluctuations of estrogen levels during the estrous cycle are correlated with neuronal excitability and peripheral sensitization in the trigeminal system. Our findings indicate possible adverse effect of estrous cycle causing peripheral sensitization of TG neurons, which may be the fundamental mechanism underlying menstrual migraine in humans.

Conclusion

Our results revealed that the fluctuations in estrogen levels during the various stages of the estrous cycle are related to the cytology of vaginal epithelial cells; however, the estrous cycle did not affect the morphologies of TG neurons. Interestingly, the high estrogen levels of the proestrus and estrus stages in duce peripheral sensitization by lowering the thresholds and rheobases of stimulation and by increasing the total spikes numbers in the TG neurons. We conclude that the estrous cycle induces peripheral sensitization, in an animal model of menstrual migraine.

What is already known on this topic?

Several previous studies indicated that different four stages of estrous cycle correlate to the alteration of trigeminal nociceptive system. In proestrus and estrus stages which estrogen levels are higher than other stages, female rats have lower thresholds of response to trigeminal stimuli.

What this study adds?

The present study revealed that female rats develop peripheral sensitization of trigeminal nociceptive system proestrus and estrus stages. The higher levels of estrogen in proestrus and estrus stages may induce peripheral sensitization by lowering thresholds and rheobases of stimulation and by increasing total spikes numbers in the trigeminal neurons. Thus, the estrous cycle induces peripheral sensitization in an animal model of menstrual migraine.

Acknowledgement

This research was supported by the Research Unit and Center of Excellence for Neuroscience of Headache, "Integrated Innovation Academic Centre: IIAC", 2012; the Chulalongkorn University Centenary Academic Development Project, the National Research University Project, the Office of Higher Education Commission (WCU-008-HR-57), Ananda mahidol Foundation, the Government Research Budget 2014-2019 and the Ratchadapiseksompoj Fund from the Faculty of Medicine, Chulalongkorn University.

Potential conflicts of interest

None.

References

1. Stewart WF, Wood C, Reed ML, Roy J, Lipton RB. Cumulative lifetime migraine incidence in women and men. Cephalalgia 2008; 28: 1170-8.

2. Lipton RB, Bigal ME, Diamond M, Freitag F, Reed ML, Stewart WF. Migraine prevalence, disease burden, and the need for preventive therapy. Neurology 2007; 68: 343-9.
3. Emanuele MA, Wezeman F, Emanuele NV. Alcohol's effects on female reproductive function. Alcohol Res Health 2002; 26: 274-81.
4. Bolay H, Berman NE, Akcali D. Sex-related differences in animal models of migraine headache. Headache 2011; 51: 891-904.
5. Flake NM, Bonebreak DB, Gold MS. Estrogen and inflammation increase the excitability of rat temporomandibular joint afferent neurons. J Neurophysiol 2005; 93: 1585-97.
6. Puri V, Puri S, Svojanovsky SR, Mathur S, Macgregor RR, Klein RM, et al. Effects of oestrogen on trigeminal ganglia in culture: implications for hormonal effects on migraine. Cephalalgia 2006; 26: 33-42.
7. Hullugundi SK, Ansuini A, Ferrari MD, van den Maagdenberg AM, Nistri A. A hyperexcitability phenotype in mouse trigeminal sensory neurons expressing the R192Q Cacna1a missense mutation of familial hemiplegic migraine type-1. Neuroscience 2014; 266: 244-54.
8. Hullugundi SK, Ferrari MD, van den Maagdenberg AM, Nistri A. The mechanism of functional up-regulation of P2X3 receptors of trigeminal sensory neurons in a genetic mouse model of familial hemiplegic migraine type 1 (FHM-1). PLoS One 2013; 8: e60677.
9. Junsre U, Bongsebandhu-phubhakdi S. ASICs alteration by pH change in trigeminal ganglion neurons. J Physiol Biomed Sci 2014; 27: 20-5.
10. McLean AC, Valenzuela N, Fai S, Bennett SA. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. J Vis Exp 2012; e4389.
11. Byers SL, Wiles MV, Dunn SL, Taft RA. Mouse estrous cycle identification tool and images. PLoS One 2012; 7: e35538.
12. Martin VT. Ovarian hormones and pain response: a review of clinical and basic science studies. Gend Med 2009; 6 (Suppl 2): 168-92.
13. Papka RE, Storey-Workley M, Shughrue PJ, Merchenthaler I, Collins JJ, Usip S, et al. Estrogen receptor-alpha and beta- immunoreactivity and mRNA in neurons of sensory and autonomic ganglia and spinal cord. Cell Tissue Res 2001; 304: 193-214.
14. Martin VT, Lee J, Behbehani MM. Sensitization of the trigeminal sensory system during different stages of the rat estrous cycle: implications for menstrual migraine. Headache 2007; 47: 552-63.
15. Rowan MP, Berg KA, Roberts JL, Hargreaves KM, Clarke WP. Activation of estrogen receptor alpha enhances bradykinin signaling in peripheral sensory neurons of female rats. J Pharmacol Exp Ther 2014; 349: 526-32.
16. Rowan MP, Berg KA, Milam SB, Jeske NA, Roberts JL, Hargreaves KM, et al. 17beta-estradiol rapidly enhances bradykinin signaling in primary sensory neurons in vitro and in vivo. J Pharmacol Exp Ther 2010; 335: 190-6.
17. Brandes JL. The influence of estrogen on migraine: a systematic review. JAMA 2006; 295: 1824-30.
18. Averbeck B, Izydorczyk I, Kress M. Inflammatory mediators release calcitonin gene-related peptide from dorsal root ganglion neurons of the rat. Neuroscience 2000; 98: 135-40.
19. Gangula PR, Lanlua P, Wimalawansa S, Supowit S, DiPette D, Yallampalli C. Regulation of calcitonin gene-related peptide expression in dorsal root ganglia of rats by female sex steroid hormones. Biol Reprod 2000; 62: 1033-9.
20. Gangula PR, Chauhan M, Reed L, Yallampalli C. Age-related changes in dorsal root ganglia, circulating and vascular calcitonin gene-related peptide (CGRP) concentrations in female rats: effect of female sex steroid hormones. Neurosci Lett 2009; 454: 118-23.
21. Puri V, Cui L, Liverman CS, Roby KF, Klein RM, Welch KM, et al. Ovarian steroids regulate neuropeptides in the trigeminal ganglion. Neuropeptides 2005; 39: 409-17.
22. Scholz A, Gruss M, Vogel W. Properties and functions of calcium-activated K+ channels in small neurones of rat dorsal root ganglion studied in a thin slice preparation. J Physiol 1998; 513 (Pt 1): 55-69.
23. Valverde MA, Rojas P, Amigo J, Cosmelli D, Orio P, Bahamonde MI, et al. Acute activation of Maxi-K channels (hSlo) by estradiol binding to the beta subunit. Science 1999; 285: 1929-31.
24. Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. Nat Neurosci 1999; 2: 1114-9.

วงจรความเป็นสัดเหนี่ยวนำให้เกิด peripheral sensitization ในเซลล์ปมประสาทไทรเจมินัล: แบบจำลองในสัตว์สำหรับโรคไมเกรนในช่วงที่มีรอบเดือน

วชิรพงศ์ สลีอ่อน, อุกกฤษฎ์ จันทรศรี, อนันต์ ศรีเกียรติขจร, ศักนัน พงศ์พันธุ์ผู้ภักดี

ภูมิหลัง: ผู้หญิงส่วนมากมักจะเคยมีประสบการณ์เกี่ยวกับโรคไมเกรนในช่วงที่มีรอบเดือน ซึ่งจะมีอาการปวดหัวซ้ำไปมาเกิดขึ้นในช่วงดังกล่าว ในวงจรรอบเดือนของมนุษย์เพศหญิงจะมีการเปลี่ยนแปลงของระดับฮอร์โมนเอสโตรเจนสอดคล้องกับการเปลี่ยนแปลงระหว่างระยะ follicular phase และ luteal phase มักจะมีการนำวงจรความเป็นสัดของหนูแรทมาศึกษาเป็นแบบจำลองในสัตว์ เพื่อทดสอบผลของการเปลี่ยนแปลงระดับฮอร์โมนเอสโตรเจน

วัตถุประสงค์: เพื่อทดสอบระยะต่างๆ ในวงจรความเป็นสัดจะเกี่ยวข้องกับการเกิดไมเกรนได้จริงหรือไม่ โดยเปรียบเทียบระดับการกระตุ้นของเซลล์ปมประสาทไทรเจมินัลในหลากหลายระยะของวงจรความเป็นสัด

วัสดุและวิธีการ: นำหนูแรทเพศเมียมาแบ่งเป็น 4 กลุ่ม ตามระยะของวงจรความเป็นสัด ตัดสินการแบ่งกลุ่มโดยการทำสเมียร์ช่องคลอดและวิเคราะห์ระดับเอสโตรเจนในซีรัม หนูแรทในแต่ละกลุ่มจะได้รับการฉีดยาสลบ และผ่าตัดนำปมประสาทไทรเจมินัลออกมา จากนั้นจะนำเซลล์ในปมประสาทมาผ่านกระบวนการเพาะเลี้ยง 2-3 ชั่วโมง และนำไปทดลองด้วยวิธี whole-cell patch clamp เพื่อวัดผลคุณสมบัติทางไฟฟ้าสรีรวิทยาของเซลล์ปมประสาทไทรเจมินัล

ผลการศึกษา: ในระยะ proestrus และ estrus พบเซลล์เยื่อบุเป็นจำนวนมากในช่องคลอด ควบคู่กับการเพิ่มระดับของเอสโตรเจน ในการศึกษาทางไฟฟ้าสรีรวิทยาพบว่า เซลล์ปมประสาทไทรเจมินัลในระยะ proestrus และ estrus แสดงค่า threshold สำหรับการกระตุ้นที่ต่ำลง และมีจำนวนการเกิดศักย์ไฟฟ้าทำงานเพิ่มขึ้นเมื่อเทียบกับเซลล์ในระยะ diestrus

สรุป: ผลการทดลองของงานวิจัยนี้บ่งชี้ว่าเอสโตรเจนที่มีระดับสูงขึ้นในระยะ proestrus และ estrus มีผลเปลี่ยนแปลงค่า threshold และ rheobases สำหรับการกระตุ้นรวมถึงการเกิดศักย์ไฟฟ้าทำงานในเซลล์ปมประสาทไทรเจมินัล ซึ่งนำไปสู่การเพิ่มระดับการกระตุ้นและ peripheral sensitization ในเซลล์ปมประสาทไทรเจมินัล การค้นพบนี้น่าจะนำไปสู่ความเชื่อมโยงของการเปลี่ยนแปลงระดับเอสโตรเจนระหว่างช่วงรอบเดือนกับพยาธิกำเนิดของโรคไมเกรนในช่วงที่มีรอบเดือน