Gender aspects of CGRP in migraine

Alejandro Labastida-Ramírez1,*, Eloísa Rubio-Beltrán1,*, Carlos M Villalón2 and Antoinette MaassenVanDenBrink1

Abstract

Background: Migraine is two to three times more prevalent in women than in men, but the mechanisms involved in this gender disparity are still poorly understood. In this respect, calcitonin gene-related peptide (CGRP) plays a key role in migraine pathophysiology and, more recently, the functional interactions between ovarian steroid hormones, CGRP and the trigeminovascular system have been recognized and studied in more detail.

Aims: To provide an overview of CGRP studies that have addressed gender differences utilizing animal and human migraine preclinical research models to highlight how the female trigeminovascular system responds differently in the presence of varying ovarian steroid hormones.

Conclusions: Gender differences are evident in migraine. Several studies indicate that fluctuations of ovarian steroid hormone (mainly estrogen) levels modulate CGRP in the trigeminovascular system during different reproductive milestones. Such interactions need to be considered when conducting future animal and human experiments, since these differences may contribute to the development of gender-specific therapies.

Keywords

CGRP, estrogen, migraine, ovarian steroid hormones, trigeminovascular system

Introduction

Migraine is a complex neurovascular disorder associated with dysfunction of the nociceptive trigeminovascular system (1). Migraine is two to three times more prevalent in women than in men (2). Additionally, women have more frequent and intense headaches, a higher risk of chronification, greater disability and an increased risk of cardiovascular events, including cardiovascular mortality (2,3). Although the precise mechanisms underlying this unique gender-dependent prevalence are not fully understood, they seem to be related to the hormonal fluctuations of ovarian steroid hormones, namely estrogens and progesterone.

Migraine prevalence before puberty is similar between sexes. A notably increased incidence arises in women after menarche, where hormonal fluctuations influence migraine occurrence during different reproductive milestones, such as menstruation, pregnancy, the postpartum state and breastfeeding, perimenopause and menopause (4–6). Furthermore, migraine frequency is affected by the use of oral contraceptive pills, hormone replacement therapy, selective estrogen receptor modulators (i.e. tamoxifen) and medical oophorectomy (7–9). Interestingly, rates of migraine similar to those in females were reported in male-to-female transsexuals under estrogen therapy to induce female sex characteristics (10). This relationship between fluctuations of ovarian steroid hormones and migraine incidence was suggested almost half a century ago by Somerville (11). He stated that in biologically-predisposed women, migraine attacks are triggered by a decline in plasma estrogen concentrations in the late luteal (premenstrual) phase of the menstrual cycle (4,11). On the contrary, phases of rising estrogen levels appear to protect against migraine (12), whereas

1Division of Vascular Medicine and Pharmacology, Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands
2Departamento de Farmacobiología, Cinvestav-I.P. N. (Unidad Sur), Ciudad de México, México
*ALR and ERB contributed equally to this work

Corresponding author:
Antoinette MaassenVanDenBrink, Erasmus MC, PO Box 2040, Rotterdam, 3000 CA Netherlands.
Email: A.vanharen-maassenvandenbrink@erasmusmc.nl
progesterone withdrawal does not seem to precipitate migraine attacks (13). Additionally, migraine prevalence has been associated with menstrual disorders (e.g. menorrhagia, dysmenorrhea), as well as endometriosis and polycystic ovary syndrome (14–16). Thus, migraine incidence is influenced by (the dys)function of the hypothalamo-pituitary-ovarian system.

In the last years, the calcitonin gene-related peptide (CGRP) pathway has been discovered to play a key role in migraine pathophysiology. Revolutionary experiments revealed that during an acute migraine attack CGRP is released in significant amounts in the extracerebral circulation (17), that these elevated levels of CGRP are normalized after triptan treatment (18), and that infusions of CGRP in migraine patients provoke migraine attacks (19). In addition, all clinical trials with anti-CGRP drugs have been positive (20), consolidating the evidence for the role of CGRP in migraine.

Therefore, it is important to consider that fluctuations in ovarian steroid hormones levels may influence CGRP homeostasis. Indeed, the presence of sex hormone receptors in the trigeminovascular system suggests that trigeminal neurons are sensitive to variations in the levels of these hormones (21). However, the exact functional interactions between ovarian steroid hormones and CGRP, which could increase migraine prevalence in women, are not fully understood.

On this basis, the present review will consider the current knowledge on the effects of a changing hormonal environment on the modulation of the CGRP signaling pathway in the trigeminovascular system, and will address some sex-dependent considerations for future CGRP studies.

**CGRP and migraine**

CGRP, a neuropeptide with pleiotropic effects, is involved in different biological processes such as neuromodulation, cardiovascular regulation, inflammation, metabolic function, and aging (16). Two homolog isoforms have been identified, namely, α-CGRP and β-CGRP. α-CGRP is a 37-amino acid neuropeptide widely distributed throughout the nervous system and predominantly expressed in sensory Aβ- and C-fibers. It is produced by transcription and alternative RNA splicing of the primary transcript of the calcitonin gene (23–25). β-CGRP is encoded by a second calcitonin gene, differing by only a single amino acid in rats and by three residues in humans (25,26), and predominantly expressed in the enteric nervous system. Considering that the majority of studies focus on α-CGRP, this review will be limited to this isoform, with a focus on the trigeminovascular system because of its relevance in migraine.

Migraine pathophysiology has long been related with activation and sensitization of the trigeminovascular system, a functional pathway consisting of pseudounipolar neurons peripherally innervating the cranial vasculature and the dura mater, whose cell bodies are located in the ipsilateral trigeminal ganglion (27,28), and centrally projecting axons to the trigeminal nucleus caudalis and the upper two cervical divisions, known as the trigeminocervical complex (29). CGRP is highly expressed throughout the trigeminovascular system (30), it is synthetized in neuronal cell bodies and co-stored with tachykinins (especially substance P) in large granular secretory vesicles (31). During the headache phase of a migraine attack, CGRP is released in the perivascular space, producing neurogenic vasodilation and nociceptive transmission (17,32).

Different experimental migraine models have focused on the various components of the trigeminovascular system. These models indirectly assess peripheral nociceptors in the dura mater and cranial arteries (i.e. neurogenic dilation of pain-sensitive dural blood vessels) or directly study nociceptive trigeminal fibers. The next sections will review animal and human experimental studies focusing on CGRP in the trigeminovascular system, where sex-related differences have been addressed.

**Sex hormone effects in animal CGRP studies**

Migraine is a female-predominant disorder. Surprisingly, the majority of preclinical studies have used male animals to limit experimental variations resulting from natural fluctuations or effects per se of ovarian steroid hormones (33). A recent study revealed that in naïve rat medulla and trigeminal ganglion, there were sex differences in the mRNA levels of CGRP and the three components of its receptor, namely, calcitonin-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1), and receptor component protein (RCP, 34), with higher CGRP levels in the medulla and lower expression of RAMP1, CLR and RCP-encoding mRNA in female tissues compared to that of males. Even considering that no estrous cycle staging or hormone measurements were conducted, this suggests that fluctuating gonadal hormones may regulate CGRP receptor synthesis, expression, or release in the trigeminovascular system.

The cranial vasculature and dura mater are well-documented targets for gonadal steroids, where the expression of estrogen, progesterone and testosterone receptors has been previously demonstrated (see Table 1). These gonadal hormones have diverse and complex physiological functions in the cranial circulation; besides, there are sex differences regarding their
function, such as opposing effects of estrogen and testosterone on vascular tone (35), but their modulation on the CGRP system remains largely unknown. A functional study examined the effects of 17βestradiol pretreatment on the response to CGRP in isolated basilar artery segments of female rats. The basilar artery did not exhibit any variation in the CGRP vascular responses after chronic estrogen (36). Although the basilar artery is not primarily involved in migraine pathophysiology, it was used instead of dural arteries because the small size of the latter is a limitation for in vitro studies. In contrast, estrogen fluctuations during the normal estrous cycle modified dural mast cell density, while, in ovariectomized rats, estrogen pretreatment promoted an increase in dural mast cell density (37), suggesting that gonadal steroids can modify CGRP function.

Furthermore, androgen and progesterone receptors are expressed in the different components of the trigeminovascular system (see Table 1), and a G protein-coupled estrogen receptor (GPER). All estrogen receptors are expressed in different parts of the trigeminovascular system (see Table 1), such as the rodent trigeminal ganglion (41,42) and dura mater (43). Although it is believed that estrogens regulate excitability and sensitization of the trigeminal CGRP pathway, experimental data are scarce and inconsistent. In the female mouse trigeminal ganglion (41) and rat trigeminal nucleus (38), CGRP mRNA levels and CGRP content were constant throughout the phases of the natural estrous cycle, respectively. In contrast, in the rat trigeminal ganglion, ovariectomy significantly increased the gene expression of CGRP compared to cycling (control) rats, and these increased mRNA levels were lowered following estrogen replacement (42). Similarly, in the central nervous system, a deficiency of estrogens increases CGRP levels within the midbrain periaqueductal gray (44), medial preoptic nucleus of the hypothalamus (45) and lumbar dorsal root ganglion (46), while CGRP expression was downregulated after estrogen treatment in the dorsal root ganglion (46) and trigeminal nucleus caudalis (47). However, it has also been described that estrogen positively enhances the expression of CGRP within the dorsal root ganglion (48–50), rat anterior pituitary (51) and medial preoptic nucleus (52). Thus, experimental data suggest that estrogen modulates differently, and in a complex manner, the density of CGRP receptors in the peripheral and central nervous systems. Further studies are clearly required to determine the exact role of estrogens, and which estrogen receptors are involved, in the modulation of CGRP expression in the different components of the trigeminovascular system (Figure 1). The effects of estradiol on the CGRP-ergic system have also been studied using an epigenetic approach, but the

| Table 1. Expression of sex hormone receptors in the different components of the trigeminovascular system. Receptor expression for animals is only listed when available in the literature. |
|----------------------------------------|------------------|------------------|------------------|------------------|
|                                       | Cranial blood vessels | Dura mater | Trigeminal ganglion | Trigeminal nerve nuclei |
| **Estrogen receptor α**                | rat (82,83)        | rat (43)     | mouse (41), rat (42,86) | rat (43,86,87) |
|                                       | human (84)         | human (85)   | human ND             | human (88,89)   |
| **Estrogen receptor β**                | rat (83)           | pig (90)     | rat (86)              | rat (86)         |
|                                       | human (84)         | human (85)   | human ND             | human (88,89)   |
| **GPER**                               | rat (91)           | rat (43)     | rat (87)              | rat (43)         |
|                                       | human ND           | human ND     | human ND             | human ND         |
| **Progesterone receptor**              | human (92)         | rat (93)     | mouse (94)            | rat (95)         |
|                                       | human (85)         | human ND     | human ND             | human ND         |
| **Androgen receptor**                  | rat (82)           | rat (96)     | rat (97)              | rat (98)         |
|                                       | human (84)         | human ND     | human ND             | human ND         |
| GPER: G-protein-coupled estrogen receptor 1; ND: not determined in humans.
variation in the methylation of the CGRP-ergic genes in that study was too large to allow definite conclusions on the role of estradiol (53).

Therefore, an alternative approach to assess the functional estrogen-CGRP relationship in the whole trigeminovascular system is with specific in vivo animal migraine models. In this context, Gupta et al. (54) have investigated the modulation exerted by 17β estradiol and progesterone (separately and in combination) on dural vasodilation in ovariectomized rats. Periarterial electrical stimulation of the dural vasculature (i.e. the middle meningeal artery) was applied to release CGRP from the activated trigeminal nerve. The subsequent changes in vessel caliber were measured with intravital microscopy on a closed cranial window. Dural vasodilation was induced by endogenous (released by periarterial electrical stimulation or intravenous capsaicin) or exogenous CGRP. The vasodilatory responses to exogenous CGRP or capsaicin were not affected by ovariectomy or by hormonal replacement. However, estradiol pretreatment significantly enhanced neurogenic sensory vasodilation, suggesting that estrogen modulates prejunctionally the sensory release of CGRP in trigeminal perivascular nerve endings in vivo (54).

Another study performed by Martin et al. (55) explored with an in vivo experimental migraine headache model whether sensitization of the trigeminal sensory system changes during different stages of the rat estrous cycle. In anesthetized rats, an electrode was used to record trigeminal nucleus caudalis neurons activity after cutaneous stimuli and chemical stimulation (with topical capsaicin) of the dura mater. There was an enhanced sensitization of the trigeminal system during the later halves of proestrus and estrus, coinciding with an abrupt decline in ovarian steroid hormones (Figure 2). This finding suggests that sensitization of the trigeminal system is dependent on the varying hormonal milieus throughout the estrous cycle (55).

In summary, animal studies have provided evidence that activation of the CGRP system is dependent upon the different stages of the rat estrous cycle, with specific roles for the different sex hormone receptors. However, there are hormonal differences between the rodent estrous cycle and the human menstrual cycle and, therefore, a direct extrapolation to humans should be done cautiously (55, Figure 2). Evidently, further studies will be required to delineate the exact mechanism(s) involved in this (patho)physiological estrogen-CGRP relationship.

**Gender effects in human CGRP studies**

One of the first clinical studies that discovered a relationship between female sex hormones and CGRP was published in 1986 by Stevenson et al. (56). In this study,
concentrations of immunoreactive plasma CGRP (i-CGRP) were measured in healthy controls, throughout normal pregnancy and in the subacute postpartum period. i-CGRP concentrations were significantly increased throughout pregnancy, with the highest concentrations being found towards term. These increased in CGRP levels decreased during the first days after delivery, when they were similar to the controls (56), interestingly, while migraine in many cases seems to be related to increased CGRP levels, during pregnancy and around the delivery, an inverse relationship between CGRP levels and migraine attack incidence seems to be the case. Most likely, this may be attributed to differences between local cranial CGRP levels that are relevant in migraine, and systemic CGRP levels that are increased as part of the cardiovascular adaptation process during pregnancy. Later on, a key study from Valdemarsson et al. revealed that plasma i-CGRP levels are different between both genders. It is remarkable that, in healthy subjects, i-CGRP levels were significantly higher in females than in males, and that the use of combined contraceptive pills was associated with even higher levels of i-CGRP in plasma (57). Accordingly, in postmenopausal women, decreased estradiol serum levels were positively correlated with decreased plasma i-CGRP concentrations (58), ascribing that the CGRP system could be influenced directly by endogenous or exogenous ovarian steroid hormones.

**Is there a gender difference in the functionality of CGRP and the trigemino-vascular system in humans?**

In the last years, some non-invasive human experimental migraine models have relied on laser Doppler perfusion imaging to study cutaneous antidromic vasodilation induced by chemical stimulation of primary sensory neurons. In this way, topical application of capsaicin on the forearm depolarizes nociceptive neurons via activation of prejunctional transient receptor potential ion channels of the vanilloid type 1 (TRPV1), leading to release of vasoactive peptides, neurogenic inflammation, and subsequent increase of dermal blood flow (59). In this pharmacodynamic model, the capsaicin-induced vasodilation is mediated by release of CGRP, as this response was inhibited by CGRP receptor antagonists (i.e. CGRP8–37 and telcagepant) and by a humanized monoclonal antibody directed against CGRP (60–62).

Using this experimental model, a recent study explored gender differences on sensory CGRP release in healthy subjects and migraine patients, as well as the influence of varying levels of estrogen and progesterone during the menstrual cycle (63). In healthy males, dermal blood flow responses did not vary over time and were comparable with the responses of male migraineurs. However, in healthy women, fluctuations of ovarian steroid hormones influenced CGRP-dependent dermal blood flow, with the highest reactivity observed around the menstruation period, when estrogen levels are low. This is in accordance with the estrogen withdrawal hypothesis, where perimenstrual low levels of estrogen are associated with a higher prevalence of migraine (4,11, Figure 2), assuming that an increased reactivity relates to an increased vulnerability to a migraine attack. Interestingly, in female migraine patients, dermal blood flow responses were elevated compared to healthy subjects, but these responses were independent of the menstrual cycle. In this study, serum estradiol and progesterone between healthy women and migraine patients did not differ across the hormonal cycle. Therefore, the mechanisms involved in the increased dermal blood flow in female migraine patients after capsaicin could be the result of an increased TRPV1 expression or an increased release of CGRP (63).
An optimization of the previous model was done by studying a dermatome in the human forehead innervated by the frontal branch of the ophthalmic (V1) nerve, where the increase in dermal blood flow is produced by trigeminal nerve activation via topical capsaicin application or electrical stimulation of the forehead skin (64).

Using this new specific model, a recent study explored the effects of the menstrual cycle on trigeminal nerve-induced vasodilatation in healthy reproductive women with a regular menstrual cycle, postmenopausal women, and patients with menstrually related migraine (MRM). Cycle-dependent changes in trigeminovascular reactivity were observed in healthy women with enhanced responses around their menstruation (65). Notably, these findings are similar to those found with pain perception, sensory and vasomotor responses, where trigeminal sensitization was produced after an intradermal injection of capsaicin in the forehead (66). In contrast, the physiological absence of ovarian steroid hormones in postmenopausal women did not induce any trigeminal variation during all measurements. Surprisingly, in MRM patients, low estradiol levels were detected during the luteal phase (days 19–21 of the cycle), while cyclic dermal blood flow differences during the menstrual cycle were also absent. This could be attributed to a differential activity of the TRPV1 channel produced by estrogens (65), as they alter the expression of the TRPV1 receptor in sensory neurons (67); however, the precise mechanisms involved were not explored.

Another non-invasive technique commonly used to study the trigeminal nociceptive system is functional neuroimaging. Within this context, Maleki et al. (68) used high-field magnetic resonance imaging (MRI) in male and female migraineurs to determine interictal alterations in brain structures using a thermal pain stressor. Female migraineurs had stronger activation of the spinal trigeminal nucleus compared with male migraineurs and healthy controls (68). These findings were attributed to differences in sensitivity of the trigeminal system due to hormonal processes, but female participants were studied without assessing the stage of the menstrual cycle (68).

A further remarkable study recently reported a female migraine patient who had a high-field MRI every day for 30 days, while standardized trigeminal nociceptive stimulation was done during the experiment. It was found that the hypothalamus, depending on the state of the migraine cycle, exhibited an altered functional coupling with the spinal trigeminal nucleus, with the greatest functional coupling in the last 24 h preceding the onset of the migraine attack (69). Regrettably, the relationship between the stage of the menstrual cycle and the activation of the trigeminal nucleus was not reported in this patient. Using MRI seems a promising tool for detecting cyclic activation of the trigeminovascular system during the different stages of the menstrual cycle, and to the best of our knowledge, these studies have not yet been performed.

Overall, sex-related differences in the trigeminal nociceptive system are evident. Notwithstanding, further experimental research will be required to demonstrate whether the fluctuations of ovarian hormones levels produce: (i) a direct effect on trigeminally innervated blood vessels; (ii) a modulation of prejunctional channels that release CGRP in the dura mater; or (iii) a modulation of trigeminal central pain pathways (Figure 1).

**CGRP receptor antagonism in females**

Because of the unquestionable role of CGRP in migraine pathophysiology, small molecule CGRP receptor antagonists such as olegepant and telcagepant (gepants) were developed and proved to be effective in the acute treatment of migraine headaches (70,71). The safety and efficacy of telcagepant was evaluated for short-term prevention of perimenstrual headaches. When taken perimenstrually for seven days, telcagepant reduced perimenstrual headaches, but it was associated with transaminase elevations in a small number of patients (72). Unfortunately, clinical trials for chronic migraine treatment were terminated because of pharmacokinetic limitations (73) and safety concerns related to hepatotoxicity (74). Notwithstanding the fact that these drugs have been discontinued due to side-effects, CGRP receptor antagonism has been proven effective in migraine treatment and, indeed, other small molecule CGRP receptor antagonists are currently under development. Moreover, at present, there are four monoclonal antibodies (mAb) in clinical development for migraine prophylaxis (75–78), namely, three humanized mAb targeting CGRP (galcanezumab, eptinezumab and fremanezumab) and one fully human mAb targeting the CGRP receptor (erenumab). In migraine patients of both genders, these drugs have shown efficacy, tolerability, and few adverse effects (compared to the gepants) in phase 2 randomized control trials (75–78). However, the exact site and mechanism of action of these novel drugs or the long-term side effects have not been completely elucidated. Until now, there has only been one randomized double-blind, placebo-controlled study, where prolonged CGRP inhibition on cardiovascular parameters was studied in 31 healthy perimenopausal women. Fortunately, no associated hemodynamic changes were found after chronically inhibiting the CGRP pathway with fremanezumab (79), despite the fact that CGRP (receptor) blockade might theoretically have a larger impact on cardiovascular safety in females than in males (80).
Thus, additional randomized control trials with the other mAb should confirm this in women in the reproductive age range.

In summary, CGRP (receptor) blockade is an effective therapeutic option for treating migraine. Clearly, further clinical trials will be required to elucidate whether these novel drugs are safe in individuals with cardiovascular risk factors, if there are any consequences of chronic CGRP inhibition in young reproductive women with a normal menstrual cycle, and whether efficacy depends on the phase of the menstrual cycle.

Future considerations and conclusions

Gender differences are evident in migraine. Animal research is starting to shift from including only male animals to also considering female animals in studies, but there is still much to be done. Consequently, gender-related differences in the CGRP system are emerging. Numerous animal and human studies have shown that cyclic fluctuations of ovarian hormones (mainly estrogen) modulate CGRP in the peripheral and central trigeminovascular system; this is especially relevant now that novel antibodies directed against CGRP or its receptor are currently in clinical trials. Future studies should focus on how fluctuations of gonadal hormones influence migraine pathophysiology in both genders, especially the estrogen-CGRP relationship that seems a key factor in the higher prevalence of migraine in women. Hopefully, these sex-related differences may contribute to the development of gender-specific therapies.

Article highlights

- Sex hormone receptors are expressed in the different components of the trigeminovascular system.
- Fluctuations of estrogen levels modulate CGRP receptor signaling in the trigeminovascular system.
- The estrogen-CGRP relationship seems to be a key factor involved in the higher prevalence of migraine in women.

Acknowledgement

Figure 2 was modified from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic License. http://smart.servier.com/.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Antoinette MaassenVanDenBrink was supported by the Netherlands Organization for Scientific Research (Vidi grant 917.113.349), whereas Carlos M. Villalón, Eloísa Rubio-Beltrán and Alejandro Labastida-Ramírez were supported by Consejo Nacional de Ciencia y Tecnología (CONACyT; Grant No. 219707 to CMV and fellowships No. 409865 to ERB and 410778 to ALR; Mexico City).

References

1. Stankewitz A, Aderjan D, Eippert F, et al. Trigeminal nociceptive transmission in migraineurs predicts migraine attacks. J Neurosci 2011; 31: 1937–1943.
2. Buse DC, Loder EW, Gorman JA, et al. Sex differences in the prevalence, symptoms, and associated features of migraine, probable migraine and other severe headache: Results of the American Migraine Prevalence and Prevention (AMPP) study. Headache 2013; 53: 1278–1299.
3. Kurth T, Winter AC, Eliassen AH, et al. Migraine and risk of cardiovascular disease in women: Prospective cohort study. BMJ 2016; 353: i2610.
4. Pavlovic JM, Allshouse AA, Santoro NF, et al. Sex hormones in women with and without migraine: Evidence of migraine-specific hormone profiles. Neurology 2016; 87: 49–56.
5. Martin VT, Pavlovic J, Fanning KM, et al. Perimenopause and menopause are associated with high frequency headache in women with migraine: Results of the American Migraine Prevalence and Prevention study. Headache 2016; 56: 292–305.
6. Kvisvik EV, Stovner LJ, Helde G, et al. Headache and migraine during pregnancy and puerperium: The MIGRA-study. J Headache Pain 2011; 12: 443–451.
7. Shuster LT, Faubion SS, Sood R, et al. Hormonal manipulation strategies in the management of menstrual migraine and other hormonally related headaches. Curr Neurol Neurosci Rep 2011; 11: 131–138.
8. Martin V, Werner S, Mandell K, et al. Medical oophorectomy with and without estrogen add-back therapy in the prevention of migraine headache. Headache 2003; 43: 309–321.
9. Smitherman TA and Kolivas ED. Resolution of menstrually related migraine following aggressive treatment for breast cancer. Headache 2010; 50: 485–488.
10. Pringsheim T and Gooren L. Migraine prevalence in male to female transsexuals on hormone therapy. Neurology 2004; 63: 593–594.
11. Somerville BW. The role of estradiol withdrawal in the etiology of menstrual migraine. Neurology 1972; 22: 355–365.
12. MacGregor EA, Frith A, Ellis J, et al. Incidence of migraine relative to menstrual cycle phases of rising and falling estrogen. Neurology 2006; 67: 2154–2158.

13. Somerville BW. The influence of progesterone and estradiol upon migraine. Headache 1972; 12: 93–102.

14. Spierings EL and Padamsee A. Menstrual-cycle and menstruation disorders in episodic vs chronic migraine: An exploratory study. Pain Med 2015; 16: 1426–1432.

15. Tietjen GE, Conway A, Utley C, et al. Migraine is associated with menorrhagia and endometriosis. Headache 2006; 46: 422–428.

16. Glintborg D, Hass Rubin K, Nybo M, et al. Morbidity and medical prescriptions in a nationwide Danish population of patients diagnosed with polycystic ovary syndrome. Eur J Endocrinol 2015; 172: 627–638.

17. Goadsby PJ, Edvinsson L and Ekman R. Vasoactive peptides release in the extracerebral circulation of humans during migraine headache. Ann Neurol 1990; 28: 183–187.

18. Goadsby PJ and Edvinsson L. The trigeminovascular system and migraine: Studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. Ann Neurol 1993; 33: 48–56.

19. Lassen LH, Haderslev PA, Jacobsen VB, et al. CGRP and CGRP receptors in migraine. Headache 2002; 42: 54–61.

20. Edvinsson L. The trigeminovascular pathway: Role of CGRP and CGRP receptors in migraine. Headache 2017; 57: S47–S55.

21. Gupta S, McCarson KE, Welch KM, et al. Mechanisms of pain modulation by sex hormones in migraine. Headache 2011; 51: 891–904.

22. Russell FA, King R, Smillie SJ, et al. Calcitonin gene-related peptide: Physiology and pathophysiology. Physiol Rev 2014; 94: 1099–1142.

23. Amara SG, Jonas V, Rosenfeld MG, et al. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 1982; 298: 240–244.

24. Rosenfeld MG, Mermod JJ, Amara SG, et al. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 1983; 304: 129–135.

25. Amara SG, Arriza JL, Leff SE, et al. Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide. Science 1985; 229: 1094–1097.

26. Steenbergh PH, Hoppener JW, Zandberg J, et al. A second human calcitonin/CGRP gene. FEBS Lett 1985; 183: 403–407.

27. Mayberg M, Langer RS, Zervas NT, et al. Perivascular meningeal projections from cat trigeminal ganglia: Possible pathway for vascular headaches in man. Science 1981; 213: 228–230.

28. Norregaard TV and Moskowitz MA. Substance P and the sensory innervation of intracranial and extracranial feline cephalic arteries. Implications for vascular pain mechanisms in man. Brain 1985; 108: 517–533.

29. Goadsby PJ and Hoskin KL. The distribution of trigeminovascular afferents in the nonhuman primate brain Macaca nemestrina: A c-fos immunocytochemical study. J Anatomy 1997; 190: 367–375.

30. Eftekhar S, Warfvinge K, Blxt FW, et al. Differentiation of nerve fibers storing CGRP and CGRP receptors in the peripheral trigeminovascular system. J Pain 2013; 14: 1289–1303.

31. Gulkemian S, Merighi A, Wharton J, et al. Ultrastructural evidence for the coexistence of calcitonin gene-related peptide and substance P in secretory vesicles of peripheral nerves in the guinea pig. J Neurocytol 1986; 15: 535–542.

32. Zeller J, Poulsen KT, Sutton JE, et al. CGRP function-blocking antibodies inhibit neurogenic vasodilatation without affecting heart rate or arterial blood pressure in the rat. Br J Pharmacol 2008; 155: 1093–1103.

33. Bolay H, Berman NEJ and Akcali D. Sex-related differences in animal models of migraine headache. Headache 2011; 51: 891–904.

34. Stucky NL, Gregory E, Winter MK, et al. Sex differences in behavior and expression of CGRP-related genes in a rodent model of chronic migraine. Headache 2011; 51: 674–692.

35. Krause DN, Duckles SP and Pelligrino DA. Influence of sex steroid hormones on cerebrovascular function. J Appl Physiol 2006; 101: 1252–1261.

36. Gupta S, Mehrrotra S, Villalon C, et al. Effects of female sex hormones on responses to CGRP, acetylcholine, and 5-HT in rat isolated arteries. Headache 2007; 47: 564–575.

37. Boes T and Levy D. Influence of sex, estrous cycle and estrogen on intracranial dural mast cells. Cephalalgia 2012; 32: 924–931.

38. Moussaoui S, Duval P, Lenoir V, et al. CGRP in the trigeminal nucleus, spinal cord and hypothalamus: Effect of gonadal steroids. Neuropeptides 1996; 30: 546–550.

39. Jana B, Palus K, Meller K, et al. Porcine dorsal root ganglia ovarian neurons are affected by long lasting testosterone treatment. Physiol Res 2016; 65: 1019–1030.

40. Gangula PR, Chauhan M, Reed L, et al. 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: 118–123.

41. Purì V, Cui L, Liverman CS, et al. Ovarian steroids regulate neuropeptides in the trigeminal ganglion. Neuropeptides 2005; 39: 409–417.

42. Aggarwal M, Purì V and Purì S. Effects of estrogen on the serotonergic system and calcitonin gene-related peptide in trigeminal ganglia of rats. Ann Neurosci 2012; 19: 151–157.

43. Vermeeren LMM, Gregory E, Winter MK, et al. Exposure to bisphenol A exacerbates migraine-like behaviors in a multibehavior model of rat migraine. Toxicol Sci 2014; 137: 416–427.

44. Wang D, Zhao J, Wang J, et al. Deficiency of female sex hormones augments PGE2 and CGRP levels within midbrain periaqueductal gray. J Neurol Sci 2014; 346: 107–111.

45. Herbison AE and Spratt DP. Sexually dimorphic expression of calcitonin gene-related peptide (CGRP) mRNA in
60. Van der Schueren BJ, Rogiers A, Vanmolkot FH, et al. Immunocytochemical analysis of sex differences in calcitonin gene-related peptide in the rat dorsal root ganglion, with special reference to estrogen and its receptor. *Brain Res* 1998; 791: 35–42.

46. Yang Y, Ozawa H, Lu H, et al. Effect of systemic nitroglycerin on CGRP and 5-HT afferents to rat caudal spinal trigeminal nucleus and its modulation by estrogen. *Eur J Neurosci* 2002; 15: 1803–1809.

59. Caterina MJ, Schumacher MA, Tominaga M, et al. Relationship between sex hormones and rat dural vasodilatation to CGRP, periartrial electrical stimulation and capsaicin. *Peptides* 2003; 24: 1163–1174.

50. Gangula PR, Lanlua P, Wimalawansa S, et al. Regulation of calcitonin gene-related peptide expression in dorsal root ganglia of rats by female sex steroid hormones. *Biol Reprod* 2000; 62: 1033–1039.

51. Gangula PR, Lanlua P, Wimalawansa S, et al. Regulation of calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *Neurology* 2001; 56: 1304–1312.

443
76. Dodick DW, Goadsby PJ, Spierings EL, et al. Safety and efficacy of LY2951742, a monoclonal antibody to calcitonin gene-related peptide, for the prevention of migraine: A phase 2, randomised, double-blind, placebo-controlled study. *Lancet Neurol* 2014; 13: 885–892.

77. Tepper S, Ashina M, Reuter U, et al. Safety and efficacy of erenumab for preventive treatment of chronic migraine: A randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol* 2017; 16: 425–434.

78. Dodick DW, Goadsby PJ, Silberstein SD, et al. Safety and efficacy of ALD403, an antibody to calcitonin gene-related peptide, for the prevention of frequent episodic migraine: A randomised, double-blind, placebo-controlled, exploratory phase 2 trial. *Lancet Neurol* 2014; 13: 1100–1107.

79. Bigal ME, Walter S, Bronson M, et al. Cardiovascular and hemodynamic parameters in women following prolonged CGRP inhibition using LBR-101, a monoclonal antibody against CGRP. *Cephalalgia* 2014; 34: 968–976.

80. MaassenVanDenBrink A, Meijer J, Villalón CM, et al. Wiping out CGRP: Potential cardiovascular risks. *Trends Pharmacol Sci* 2016; 37: 779–788.

81. Chai NC, Peterlin BL and Calhoun AH. Migraine and estrogen. *Curr Opin Neurol* 2014; 27: 315–324.

82. Gonzales RJ, Ansar S, Duckles SP, et al. Androgenic/estrogenic balance in the male rat cerebral circulation: Metabolic enzymes and sex steroid receptors. *J Cereb Blood Flow Metab* 2007; 27: 1841–1852.

83. Kemper MF, Zhao Y, Duckles SP, et al. Endogenous ovarian hormones affect mitochondrial efficiency in cerebral endothelium via distinct regulation of PGC-1 isoforms. *J Cereb Blood Flow Metab* 2013; 33: 122–128.

84. Zuloaga KL, O’Connor DT, Handa RJ, et al. Estrogen receptor beta dependent attenuation of cytokine-induced cyclooxygenase-2 by androgens in human brain vascular smooth muscle cells and rat mesenteric arteries. *Steroids* 2012; 77: 835–844.

85. Giuffre R, Palma E, Liccardo G, et al. Sex steroid hormones in the pathogenesis of chronic subdural haematoma. *Neurochirurgia* 1992; 35: 103–107.

86. Bereiter DA, Cioffi JL and Bereiter DF. Oestrogen receptor-immunoreactive neurons in the trigeminal sensory system of male and cycling female rats. *Arch Oral Biol* 2005; 50: 971–979.

87. Liverman CS, Brown JW, Sandhir R, et al. Role of the oestrogen receptors GPR30 and ERalpha in peripheral sensitization: Relevance to trigeminal pain disorders in women. *Cephalalgia* 2009; 29: 729–741.

88. Alimi-Allrath T, Ricken A and Bechmann I. Expression of estrogen receptors alpha and beta in the trigeminal mesencephalic nucleus of adult women and men. *Ann Anat* 2014; 196: 416–422.

89. Fenzi F and Rizzuto N. Estrogen receptors localization in the spinal trigeminal nucleus: An immunohistochemical study in humans. *Eur J Pain* 2011; 15: 1002–1007.

90. Glinskii OV, Abraha TW, Turk JR, et al. Microvascular network remodeling in dura mater of ovariectomized pigs: Role for angiopoietin-1 in estrogen-dependent control of vascular stability. *Am J Physiol Heart Circ Physiol* 2007; 293: H1131–H1137.

91. Murata T, Dietrich HH, Xiang C, et al. Protein-coupled estrogen receptor agonist improves cerebral microvascular function after hypoxia/reoxygenation injury in male and female rats. *Stroke* 2013; 44: 779–785.

92. Khalid H, Shibata S, Kishikawa M, et al. Immunohistochemical analysis of progesterone receptor and ki-67 labeling index in astrocytic tumors. *Cancer* 1997; 80: 2133–2140.

93. Meffre D, Delespierre B, Gouézou M, et al. The membrane-associated progesterone-binding protein 25-Dx is expressed in brain regions involved in water homeostasis and is up-regulated after traumatic brain injury. *J Neurochem* 2005; 93: 1314–1326.

94. Manteniottis S, Lehmann R, Flegel C, et al. Comprehensive RNA-Seq expression analysis of sensory ganglia with a focus on ion channels and GPCRs in trigeminal ganglia. *PLOS One* 2013; 8: e79523.

95. Haywood SA, Simonian SX, van der Beek EM, et al. Fluctuating estrogen and progesterone receptor expression in brainstem norepinephrine neurons through the rat estrous cycle. *Endocrinology* 1999; 140: 3255–3263.

96. Lin IC, Slump AE, Hwang C, et al. Immunolocalization of androgen receptor in the developing craniofacial skeleton. *J Craniofac Surg* 2004; 15: 922–927.

97. Lee KS, Zhang Y, Asgar J, et al. Androgen receptor transcriptionally regulates mu-opioid receptor expression in rat trigeminal ganglia. *Neuroscience* 2016; 331: 52–61.

98. Simerly RB, Chang C, Muramatsu M, et al. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. *J Comp Neurol* 1990; 294: 76–95.