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14. ABSTRACT: The funded project examines the impact of estrogen on the analgesic effect of nasally applied oxytocin in a rodent model of headache caused by traumatic brain injury. This report details efforts made during the first year plus of the funded project. The study began during Covid restrictions on laboratory work at Stanford, and so there were (and to some extent still are) delays secondary to the pandemic. However, we were able to achieve IACIC and ACURO protocol approval, recruit a qualified postdoctoral fellow, construct the TBI apparatus, begin demonstration of the allodynic effects of TBI and the analgesic effects of nasal oxytocin in pot-TBI rats. We were also able to begin electrophysiologic testing of trigeminal ganglia (TG) neurons from female rats – a prerequisite of testing of the impact of estrogen pretreatment on the effect of oxytocin as a inhibitor of these pain-sensing neurons. Finally, we used the time during which we could not do laboratory work to publish a paper describing a theory of the pathogenesis of menstrual migraine, which posited that the decrease in estrogen during menses drives decreases in TG oxytocin receptor activity.

15. SUBJECT TERMS
Traumatic brain injury, TBI, Post-traumatic headache, gender, hormones, sex

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1. INTRODUCTION:

The application of nasal application of oxytocin in rats demonstrating chronic head pain following induction of TBI decreases the animal's pain state and improves their performance in a test of depression behaviors. The aim of the experiments is to determine whether an induced increase in estrogen levels will enhance the inhibitory effect of OT on trigeminal neurons and pain behavioral sequelae to TBI in rats. To achieve this, we are first examining the effects of pretreatment of rats with estrogen on the analgesic efficacy of nasal OT in post-TBI rats. We are also aim performing patch-clamp electrophysiological studies of the effects of OT + or - estrogen pretreatment of rats and also. Positive results of this project would likely provide important information as to the optimal approach to the use of nasal oxytocin for alleviation of chronic migraine-like headache and depression secondary to TBI for both male and female warfighters and veterans.

2. KEYWORDS:

Traumatic brain injury, TBI, Post-traumatic headache, gender, hormones, sex

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Groups of 11 male or female rats will be given 4 daily injections of 1 of 3 different doses of estradiol benzoate (EB) or vehicle, following which rats will be subjected to controlled traumatic brain injury. (significant delays resulted from Covid-19 pandemic)

- IACUC and ACURO protocol approval achieved **(04/2021)**
- Recruitment of postdoc **(9/2021)**
- Construction of TBI device **(10/2021)**
- Baseline TBI and facial mechanical pain sensitivity initiated **(02/2022)**
- Subcutaneously injection of estradiol for 4 days/facial sensitivity reassessed **not started**
- Results will be statistically analyzed **not started**

Aim 2: Patch-clamp electrophysiological recordings of effects of oxytocin on trigeminal ganglia neurons from TBI injured rats with or without daily treatment with estradiol.

- Rats euthanized after daily dosing with vehicle of estradiol for 4 days. **not started**
- TG neurons extracted and assessed using current clamp electrophysiology **baseline begun**
- 10 ug/ml oxytocin (OT) will be added to the media and the excitability reassessed **not started**
- Results will be statistically analyzed **not started**

What was accomplished under these goals?

Not surprisingly, the Covid Pandemic significantly slowed progress on this project. Despite this, we did accomplish several components of the study. The early work included IACUC and ACURO protocol approval and onboarding postdoc (letter in appendix). In addition, we constructed the TBI equipment and demonstrated the capacity of induction of post-TBI craniofacial allodynia as well as the analgesic impact of intranasal oxytocin on this allodynia (Fig 1). Similarly, we have demonstrated the capacity to record from freshly excised trigeminal ganglia neurons from female rats (we had always used males in the past) as well as the impact of oxytocin on the excitability of these neurons. (Figure 2).

Fig 1. Post-TBI pain thresholds

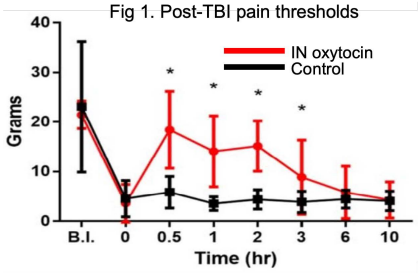
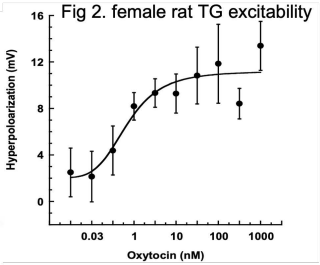


Fig 2. female rat TG excitability



What opportunities for training and professional development has the project provided?

This project provided training opportunities for Dr. Vimala Bharadwaj, a new postdoc in the lab. Dr. Bharadwaj gained greater proficiency in protocol preparation, manuscript preparation, device construction, behavioral testing, and electrophysiology. All of these things will serve her well in her goal to become an academic scientist.

How were the results disseminated to communities of interest?

Literature research performed for this project, combined with our own laboratory work enabled us to prepare and publish a manuscript around a theory of menstrual migraine (PMID: 34125955), which discusses the critical nature of estrogen levels in a related headache disorder. We were able to conduct this research and publication while unable to use the laboratory facilities due to Covid restrictions at Stanford.

What do you plan to do during the next reporting period to accomplish the goals?

We plan to begin behavioral testing of post-TBI male and female rats following daily injections of estradiol as well as investigations in to the impact of this estrogen treatment on the analgesic effect of intranasal oxytocin. We also will begin electrophysiological recordings of TGs from post-TBI male and female rats to determine the impact of estrogen on the decrease in neuronal excitability induced by oxytocin.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

At this point, the only impactful development that is the result of funding from this grant is our publication of a position paper (attached in the appendix) around our novel theory of the pathogenesis of menstrual migraine. This paper posits that menstrual migraine is caused by a decrease in activity at oxytocin receptors on trigeminal neurons. Oxytocin attenuates the excitability of these pain conveying neurons and so decreases in oxytocin receptor expression and oxytocin levels will disinhibit these neurons allowing migraine triggers to cause headaches. Estrogen both increases oxytocin levels and oxytocin receptor levels. Thus, when estrogen levels drop during menstruation, this increases TG excitability, allowing headaches to be triggered

What was the impact on other disciplines?

The above described paper has the potential to affect how physicians treat menstrual migraine

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Again, the only result of note so far is the paper we were able to publish as the result of the funding as well as the restrictions placed on us by Covid. This paper has the potential to impact patients with persistent headache, including menstrual migraine. If the results of the laboratory work pan out, this could lead to the clinical implementation of oxytocin treatment for post-TBI headache.

5. CHANGES/PROBLEMS:

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

The cause of the delays, so far, have been related to the Covid Pandemic. These continue to some degree but are, at least for now, mainly mitigated.

Changes that had a significant impact on expenditures

Because of the delays in laboratory work caused by the pandemic, the costs of the project are projected to be beyond the original budget as the personnel costs were continuing despite our inability to perform experiments.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Published:
A new hypothesis linking oxytocin to menstrual migraine.
Bharadwaj VN, Porreca F, Cowan RP, Kori S, Silberstein SD, Yeomans DC.
Headache. 2021 Jul;61(7):1051-1059.
Support of this grant acknowedged

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

David C. Yeomans, PhD
Principle Investigator
ORCID #0000-0002-9389-8539
Person Months: 2
Contribution: Dr. Yeomans has overseen all work performed in this project to date.
Funding support: The work described in this project has no other funding support.

Michael Klukinov, MD
Senior Research Scientist
ORCID # 0000-0002-5229-6777
Person Months: 3
Contribution; Dr. Klukinov has overseen construction of tBI apparatus and initial behavioral experiments. He has also contribute to the publication discussed above
Funding Support: The work described in this project has no other funding support.

Vimala Bharadwaj, PhD
Postdoctoral Fellow
ORCID # 0000-0002-6243-0861
Person Months: 3
Dr. Bharadwaj has contributed to the preparation of the publication described above, the behavioral testing, and the elecrophysiological testing
Funding Support: The work described in this project has no other funding support.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

5R33AT00930503 (NIH - Williams, PI): Use of Repetitive Transcranial Magnetic Stimulation to Augment Hypnotic Analgesia **Grant Concluded**

Focused Ultrasound Surgery Foundation (Yeomans, PI) The Needle-less Nerve Block: Targeted Non-Invasive Analgesia with Ultrasonic Uncaging of Local Anesthetics **Grant Concluded**

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

Appendix 1: ACURO Approval Letter

Appendix 2: Published Paper (Bharadwaj et al., A new hypothesis linking oxytocin to menstrual migraine

DEPARTMENT OF THE ARMY

HEADQUARTERS, U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
810 SCHREIDER STREET
FORT DETRICK, MD 21702-5000
April 12, 2021

Director, Office of Research Protections
Animal Care and Use Review Office (ACURO)

Subject: Approval of Proposal Number PR202508, Award Number W81XWH-21-1-0186 entitled, "Nasal Oxytocin for the Treatment of Post-TBI Chronic Headache: Influence of Estrogen"

David Yeomans
The Leland Stanford Junior University
Stanford, CA, US

Dear David Yeomans:

Reference: (a) DOD Instruction 3216.01, "Use of Animals in DOD Conducted and Supported Research and Training"
(b) US Army Regulation 40-33, "The Care and Use of Laboratory Animals in DOD Programs"

In accordance with the above references, ACURO protocol PR202508.e001 entitled, "Estrogen Oxytocin treatment after mTBI," IACUC protocol number 33945, Protocol Principal Investigator David Yeomans, is approved by ACURO as of 04/09/2021 for the use of rats and will remain so until modification, expiration or cancellation. This protocol was approved by the The Leland Stanford Junior University IACUC on 02/26/2021; IACUC approval expires 02/23/2024.

Required Actions:

A. Submit to ACURO for review and approval prior to implementing:

- IACUC-approved de novo reviews of the protocol
- IACUC-approved significant changes to this protocol (see guidance document)

B. Notify ACURO within 5 business days of any of the following:

- Any noncompliance, suspensions or adverse events (see guidance document)
- Receipt of notification that the institution is under investigation by USDA
- AAALAC, International accreditation status change

For further assistance, please contact ACURO at (301) 619-6694, FAX (301) 619-4165, or via e-mail: usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil.

NOTE: Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grant Officer can authorize expenditure of funds. It is recommended that you contact the appropriate Contract Specialist or Contracting Officer regarding the expenditure of funds for your project.

Sincerely,

Dawn C. Fitzhugh, VMD, MPH, DACLAM
Colonel, US Army
Director, Animal Care and Use
Review Office

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Cheryle Aird
Dr. Stephen Felt
Alyssa N. Esquivel
David Yeomans
Mr. Jonathan Ryder

Check for updates

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REVIEW ARTICLE

A new hypothesis linking oxytocin to menstrual migraine

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Abstract

Objective: To highlight the emerging understanding of oxytocin (OT) and oxytocin receptors (OTRs) in modulating menstrual-related migraine (MRM).

Background: MRM is highly debilitating and less responsive to therapy, and attacks are of longer duration than nonmenstrually related migraine. A clear understanding of the mechanisms underlying MRM is lacking.

Methods: We present a narrative literature review on the developing understanding of the role of OT and the OTR in MRM. Literature on MRM on PubMed/MEDLINE database including clinical trials and basic science publications was reviewed using specific keywords.

Results: OT is a cyclically released hypothalamic hormone/neurotransmitter that binds to the OTR resulting in inhibition of trigeminal neuronal excitability that can promote migraine pain including that of MRM. Estrogen regulates OT release as well as expression of the OTR. Coincident with menstruation, levels of both estrogen and OT decrease. Additionally, other serum biochemical factors, including magnesium and cholesterol, which positively modulate the affinity of OT for OTRs, both decrease during menstruation. Thus, during menstruation, multiple menstrually associated factors may lead to decreased circulating OT levels, decreased OT affinity for OTR, and decreased expression of the trigeminal OTR. Consistent with the view of migraine as a threshold disorder, these events may collectively result in decreased inhibition promoting lower thresholds for activation of meningeal trigeminal nociceptors and increasing the likelihood of an MRM attack.

Conclusion: Trigeminal OTR may thus be a novel target for the development of MRM therapeutics.

KEYWORDS
estrogen, magnesium, menstrual migraine, oxytocin, oxytocin receptor, therapeutics

INTRODUCTION

Migraine disproportionately affects women.[1] There is little sex difference in migraine prevalence before puberty; yet after puberty, women are two to three times more likely to experience from migraine compared with men.[2] In many women, migraine attacks are associated with the menstrual cycle, a condition termed menstrual-related migraine (MRM). Approximately 42%–70% of female patients

Abbreviations: CGRP, calcitonin gene-related peptide; CNS, central nervous system; IL-6, interleukin-6; Mg^{2+}, magnesium ion; MRM, menstrual-related migraine; mRNA, messenger ribonucleic acid; Na^{+}, sodium ion; OT, oxytocin; OTR, oxytocin receptor; PVN, paraventricular nuclei; TG, trigeminal ganglion; TNC, trigeminal nucleus caudalis.

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report a worsening of migraine headaches during perimenstrual time periods.[3–7] MRM is more severe and incapacitating with higher frequency compared with nonmenstrual migraine.[8,9] Moreover, MRM attacks have a longer duration and are more difficult to treat than nonmenstrual attacks.[2,5,9] Therefore, it is critical to understand the unique underlying pathogenesis peculiar to MRM and to develop therapies to manage this debilitating type of migraine.

The pathogenic relationship between menstruation and migraine has been the subject of much research but remains unclear. The estrogen withdrawal theory postulates that a drop in estrogen is the cause of migraine attacks in vulnerable women.[10] A decrease in estrogen is often strongly linked with factors that also likely contribute to MRM. For example, menstruation has been associated with a decrease in circulating magnesium that temporally coincides with an MRM attack,[11] and ionized magnesium blood levels were found to be significantly lower in MRM attacks compared with non-MRM attacks in MRM patients.[12] Magnesium infusion is abortive in migraine, and magnesium supplementation can be prophylactic for MRM,[13–15] but the mechanism by which these effects occur is not known.

Uncertainties such as these suggest the need for a unitary theory that integrates knowledge of physiological mechanisms underlying MRM more broadly. We propose that the actions of oxytocin (OT) on trigeminal oxytocin receptors (OTRs) may help to unify numerous apparently disparate observations. Clinical[16–18] and preclinical studies strongly support an antinociceptive role of OT in the trigeminal pain system[16,19,20] as well as in the spinal cord.[21,22] OT has been reported to directly inhibit neuronal excitability of cells in the trigeminal ganglion (TG) and to elicit a robust inhibition of nociceptive responses in the trigeminal nucleus caudalis (TNC).[16,20] Therefore, we hypothesize that decreased tonic OT/OTR activity in the perimenstrual period may lower thresholds for activation of trigeminal nociceptive afferents and thus increase the probability of a pain attack. In this review, we suggest that, coincident with menstruation, there are changes in multiple factors that can affect trigeminal OTR activity and thus neuronal excitability, increasing the likelihood of and perhaps dictating a migraine attack.

We first discuss the estrogen withdrawal and magnesium deficiency theories with their respective limitations and propose a theory related to OT for MRM pathogenesis. We also consider the interaction of OT and the OTR, key clinical observations of OT treatment in migraine, and how the mechanism of action of OT in pain modulation may play an important role in promoting MRM. Finally, the therapeutic implications of the proposed OT theory are discussed.

METHOD

We reviewed the literature on MRM on PubMed/MEDLINE database including clinical trials and basic science publications. Inclusion criteria were based on key search items, "pathophysiology of menstrual migraine," "pathogenesis of menstrual migraine," "therapeutics and menstrual migraine," "estrogen withdrawal," "magnesium and migraine," "oxytocin," "oxytocin receptors," "oxytocin and intranasal delivery," "oxytocin and migraine," "blood serum levels and menstrual cycle," and "blood serum levels and migraine." The authors reviewed the citations from review papers involving MRM and OTRs. The final reference list was generated based on relevance to the topics covered in this review.

ESTROGEN WITHDRAWAL THEORY

The estrogen withdrawal theory for MRM, first described over 45 years ago, proposed that the decline in estrogen levels during the late luteal phase could trigger a migraine attack.[10] In this report, women who had MRM and were given estradiol treatment had a delay in their migraine attacks until the level of estradiol dropped once more to pretreatment levels.[10] Studies from the same group suggested that a period of estrogen "priming" with several days of high estrogen level exposure is a necessary precursor for MRM that resulted from estrogen "withdrawal."[23,24] Other clinical studies[25–28] have supported the conclusion that decreased levels of estrogen increase susceptibility for MRM attacks.[12]

The reason fluctuations in estrogen can promote MRM remains uncertain. Estrogen withdrawal has been hypothesized to lead to multiple pronociceptive sequelae including the sensitization of the trigeminal system, modulation of neurotransmitter systems, increased synthesis of neuropeptides, and altered reactivity of microglia.[13,29] Nonetheless, periods of high estrogen appear to increase neuronal excitability and decrease the threshold for spreading depression[30] and sometimes are associated with increased incidence of migraine with aura.[23] Furthermore, estrogen has been used as a therapy to prevent migraine attacks.[24] These paradoxical observations suggest that the effect of estrogen on migraine is complex and multifaceted.[30] One possibility is that the rapid decrease, rather than absolute estrogen levels, is the critical factor contributing to MRM. Additionally, the fluctuation of other factors over the menstrual cycle may also play important roles in MRM pathogenesis. Herein, we suggest that a key hormonal factor in the pathogenesis of MRM attack is OT/OTR activity, which is modulated by estrogen level but is also dependent on other factors that vary over the menstrual cycle.

MAGNESIUM DEFICIENCY

Magnesium (Mg^{2+}) is an essential metallic cation that plays an important role in numerous cellular functions including the maintenance of neuronal transmembrane electric potentials. Mg^{2+} serum concentration has been identified to be an independent risk factor in migraine, and patients have their lowest serum levels of Mg^{2+} during migraine attacks.[31] In fact, the odds of acute migraine headache attack is increased 35-fold when serum levels of Mg^{2+} drop below normal levels.[31] Critically, menstruation has been associated with a decrease

in circulating Mg^{2+}[11] with the timing of this decrease coinciding with MRM attack. In MRM patients, intracellular Mg^{2+} levels in cells isolated from blood samples were significantly reduced compared with controls, and the levels were reported to exhibit an inverse relationship with duration and intensity of migraine attacks.[32] The incidence of Mg^{2+} deficiency was reported to be 45% during menstrual attacks and 14% during menstruation without migraine.[12] The possible contribution of Mg^{2+} in migraine is supported by reductions in MRM attack frequency with oral magnesium supplementation[12,32] as well as by the efficacy of magnesium infusion in terminating *status migrainosus* attacks.[33] Taken together, these clinical studies provide presumptive evidence that deficiency of Mg^{2+} plays a role in MRM.

Mg^{2+} deficiency may contribute to migraine attacks in multiple ways including regulation of calcium ion influx, serotonin receptor activity, platelet aggregation, cerebrovascular tone, and release of nitric oxide and inflammatory mediators such as neuropeptides, substance P, and cytokines.[14,34] These broad neurovascular, neuropeptide, and transmitter actions of Mg^{2+} clearly overlap with known migraine pathogenesis.[14,34,35] Nevertheless, a direct link between Mg^{2+} deficiency and MRM is yet to be demonstrated. Herein, we suggest that Mg^{2+} deficiency is a critical factor in modulating OT/OTR signaling that leads to an increased likelihood of MRM.

OXYTOCIN THEORY

Understanding migraine as a threshold disorder[36] suggests that reduction in inhibitory tone may increase the likelihood of future attacks. OT is a nonapeptide synthesized in the supraoptic and paraventricular nuclei (PVN) and accessory magnocellular nuclei of the hypothalamus.[37,38] The axon terminals of these cells promote the secretion of OT into the bloodstream from the posterior pituitary.[39] OT cells in the PVN also project throughout the central nervous system (CNS) including the amygdala, the striatum, and the superficial and deep lamina of the dorsal horn.[40–42] OT binds to OTRs that are widely localized in the different brain (neuronal and glial cells) and spinal cord regions as well as on peripheral tissues such as the uterus and breast.[43,44] Of key importance for the OT/MRM theory is the inhibitory effect on pain and nociception.[43,45–47] The robust analgesic effect of OT/OTR binding appears to occur via a potassium channel/nitric oxide/K_{ATP} pathway.[45]

Key clinical observation of OT in migraine

OT levels are lowest at days −2 to +3 of the menstrual cycle, the same time period associated with low estrogen levels and in which MRM attacks occur.[48,49] There is substantial correlative and direct evidence showing that OT can modulate migraine headache.[18,50–52] For instance, over the course of pregnancy, the levels of circulating OT increase,[50] and over the same period, the frequency of headaches decreases.[51] Likewise, women who breastfeed versus bottle-feed their babies have higher OT,[52] and migraine recurrence rates are lower in the breastfeeding group.[51] Furthermore, intranasal OT was shown to relieve headaches in patients in a dose-dependent manner.[18] A recent 40-patient pilot study showed analgesic efficacy of intranasal OT in chronic migraine headache patients but not in low-frequency episodic migraine.[16] Although the study did not meet its primary endpoint of significant pain reduction versus placebo at 2 h postdosing, a significant difference in pain relief was observed by 4 h after dosing in chronic migraine patients.[16] Interestingly, post hoc findings demonstrated that patients who had taken nonsteroidal anti-inflammatory drugs within 24 h were less likely to show OT analgesic efficacy.[16] One plausible theory for this observation is that inflammation can traffic the OTR to the membrane[16,53] allowing for increased efficacy of intranasal OT. A follow-on open-label study in patients with chronic and high-frequency episodic migraine showed that intranasal OT significantly reduced both the pain and the frequency of headache.[16] Although this study suffered from a very high placebo rate of response (74%), intranasal OT induced a striking decrease in frequency from baseline with an average of 14.1 headache days to an average of 5.9 headache days during treatment.[16] In total, these clinical studies provide presumptive evidence showing that OT can attenuate migraine pain in patients.

MECHANISM OF ACTION OF OXYTOCIN IN PAIN MODULATION

Activation of the trigeminovascular system is essential for migraine pain. Thus, the excitability of this system contributes to the probability of a migraine attack.[35,54] OT, binding to trigeminal OTR, decreases the excitability of these trigeminal neurons in rodents[46] and so could contribute to decreased probability of a migraine attack. In humans and animals, OT and OTR expression have been demonstrated in the TG and the brain.[19,20,40,46,55] Immunoreactivity of OT and OTR protein expression was significantly localized to calcitonin gene-related peptide (CGRP) positive and myelinated Aδ sensory neurons and fibers in the TG as well as in trigeminal satellite glial cells.[20,56] The CNS OTR has been demonstrated in areas known to be critical to migraine pathogenesis.[56] Distribution of [^{125}I]-OT using tissue counts and autoradiography after intranasal administration to rodents showed strong localization of OT label in the TG and in all three branches of the trigeminal nerve as well as in brain regions known to be rich in OTR,[20] including areas of the CNS associated with migraine, including the pons, medulla (particularly the dorsal horn of *nucleus caudalis*), hippocampus, thalamus, and midbrain.[16] Taken together, OTR distribution and distribution of OT following nasal application provide a substrate for OT actions on both peripheral and central mechanisms of migraine.

OT, acting at OTR, can have analgesic effects through multiple possible mechanisms (see Dussor et al.[57] for a review). There is strong evidence for direct inhibitory effects of OT on primary afferent nociceptors.[20,57,58] OT can act at OTR to hyperpolarize nociceptors and desensitize signaling at OTR particularly after inflammatory injury.[22,46] Relevant to migraine, activation of OTR by OT blocks

CGRP release from dural afferents in animals following induction of inflammation.[20] Furthermore, a vast majority of OTR immunoreactive neurons in the rat co-express CGRP following inflammation.[25] Interestingly, TG from noninflamed rats does not show inhibition of CGRP release by OT nor show CGRP/OTR colocalization, a finding that is consistent with the known upregulation of OTR and CGRP in the TG by inflammation.[20,59] The nociceptive response to the release of inflammatory cytokines is also thought to be a key component of migraine pathophysiology.[60] The same rapid upregulation of OTR is critical in uterine contraction and is thought to be driven primarily by interleukin-6 (IL-6), for which there are three response elements on the human OTR gene.[61,62]

OT can also influence pain through central actions within the TNC. Single-cell responses in the TNC of rats following noxious facial electrical shock were reduced by more than half by 10 min and by more than 90% by 45 min after intranasal OT administration.[16] Moreover, in a migraine rodent model of nitroglycerin infusion, increased c-Fos expression in TNC neurons was significantly reduced after intranasal OT treatment.[16] Additionally, topical spinal microinjection of OT dose dependently inhibited peripherally evoked activity (V1, ophthalmic) nociceptive transmission in the TNC[47] that was blocked by pretreatment with the selective OTR antagonist.[47] Taken together, these in vivo preclinical studies demonstrate that activation of central TNC neurons is reduced by exogenously applied OT, suggesting the role of OT in pain modulation by influences at the level of the TG and/or the TNC and point to a potential therapeutic direction. Other additional mechanisms by which OT/OTR may produce analgesic actions include postsynaptic effects of OT released from PVN projections to the spinal/trigeminal dorsal horn to inhibit nociception[57,63] and potential activation and desensitization of the transient receptor potential cation channel subfamily V member 1 channel.[57,64]

MODULATION OF OT/OTR ACTIVITY IN MIGRAINE

Several endogenous factors can, therefore, modulate trigeminal pain including endogenous OT levels, Mg^{2+}, estrogen, sodium ion (Na^{+}), cholesterol, and IL-6. Increased likelihood of a MRM attack can result from modulation of (a) endogenous OT levels, (b) OT affinity for the OTR, and (c) the expression of OTR; all of these occur during menstruation. Figure 1 is a concatenation of serial levels of OT, estrogen, Mg^{2+}, Na^{+}, estrogen, cholesterol, and IL-6 over the menstrual cycle that are extracted from published sources.[11,65–68]

Local or circulating OT levels

OT levels vary over the menstrual cycle and are lowest at days −2 to +3 of the cycle[67]—the same time period during which MRM attacks occur. What precipitates this drop? During the menstrual cycle, estrogen drops approximately at the same time as OT drops.[65,67] Estrogen can stimulate both OT synthesis[69] and its release in the systemic circulation[70,71] and CNS structures.[72,73] Thus, the drop in OT during menstruation may be driven, at least in part, by dropping levels of estrogen. Figure 2A illustrates this diagrammatically: decreases in estrogen levels may result in lower OT levels and decreasing OTR activity at trigeminal and other migraine-associated

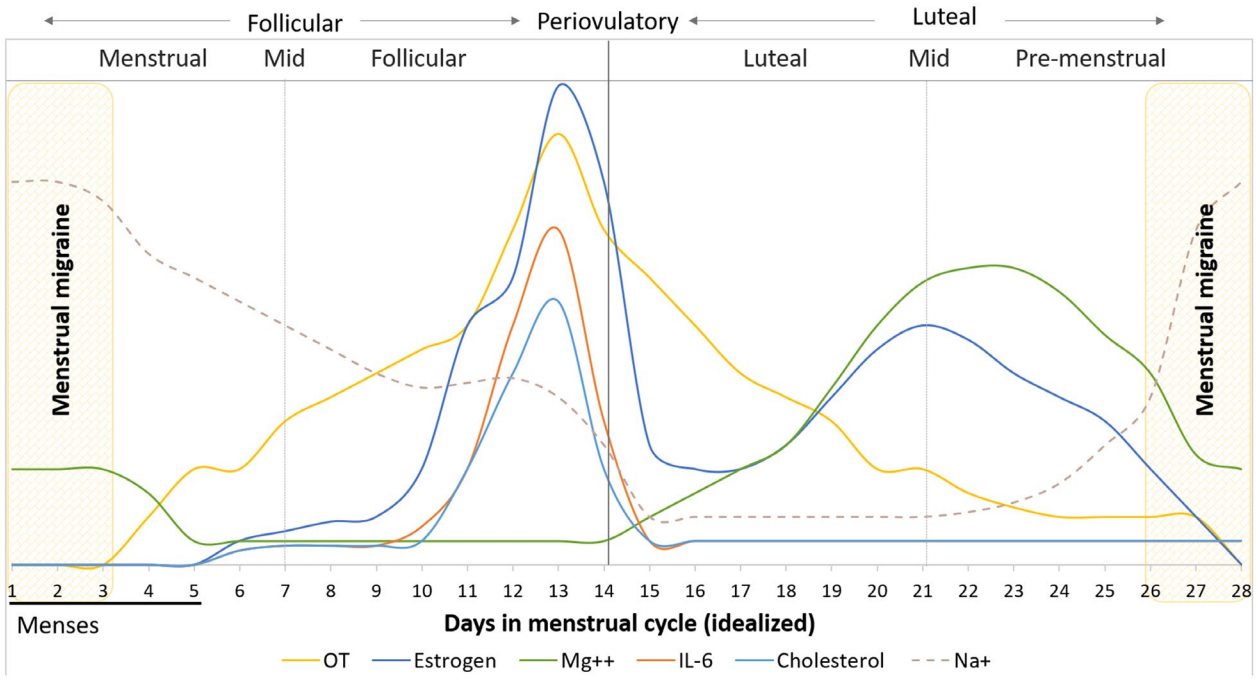

FIGURE 1 Composite illustration extracted from multiple literature sources showing measured and calculated fluctuation of the serum levels of OT, estrogen, Mg^{2+}, IL-6, cholesterol, and Na^{+} level during normal menstrual cycle[11,65–68]

neurons. Thus, the drop in estrogen during the menstrual cycle can increase the likelihood of a painful MRM attack. Future studies are needed to co-serially sample the levels of OT and estrogen in MRM patients. A strong positive correlation, particularly if the order of change could be established, would lend credence to the idea that estrogen levels can modulate OT levels and thus trigeminal OTR tone and nociceptive activity.

Modulation of OT affinity for OTR

The affinity of OT for its receptor is highly variable depending on the biochemical milieu in which the receptor exists (see Figure 2B). OTR affinity is positively allosterically modulated by Mg^{2+} (up to 1500-fold).[74] The local level of Mg^{2+} may, therefore, be involved in the regulation of OT-mediated signaling functions.[74,75] The OTR exists in both a high- and a low-affinity conformation; Mg^{2+} interacts with the ligand-binding site of OTR to stabilize the conformation favoring high affinity and specific binding of agonists[74,75] and can, in addition, increase the binding capacity of OTR.[76] Mg^{2+} levels are normally their highest during the mid-luteal phase of menstruation, falling just before the menstrual period (Figure 1).[11,77] Thus, elevated levels of Mg^{2+} can increase the affinity of available OT at OTR in TG and TNC neurons. However, women who experience MRM show lower levels of Mg^{2+} overall and an additional drop in Mg^{2+} during migraine attacks.[12,78] A drop in Mg^{2+}, which is associated with migraine in general[31] and MRM[12,32] in particular, should decrease the activity of endogenous OT. Consequently, the tonic inhibition of trigeminal and other OTR+neurons promote an increase in the likelihood of MRM attack (see Figure 2B). Thus, the low level of Mg^{2+} in women with MRM may be a precipitating factor in inducing their headaches. As mentioned above, magnesium supplementation is effective in decreasing the frequency of MRM attacks, supporting the idea that levels of this electrolyte are critical in the pathogenesis of MRM.[32]

To exist in a high-affinity state, OTR requires at least two factors, namely a divalent metal cation such as Mg^{2+} and a high-cholesterol environment.[76] In fact, depending on the presence of

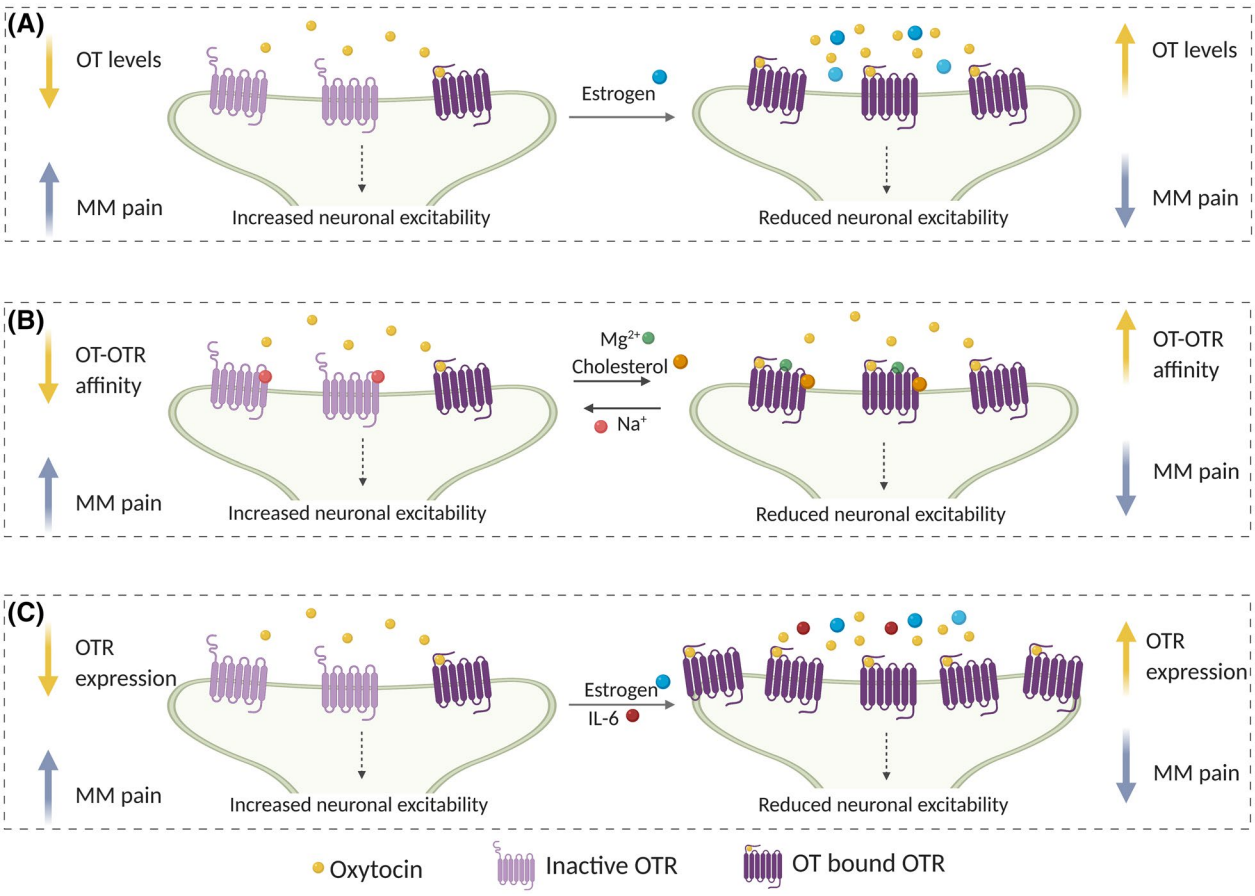

FIGURE 2 (A) Estrogen leads to increased OT levels thereby reducing excitability of the trigeminal pain system. The drop in OT during menstruation may be driven, at least in part, by the drop in estrogen. (B) Na^+ may lead to decrease in OTR-OT affinity and thereby increase excitability of the trigeminal pain system. Mg^{2+} and cholesterol increase in OTR-OT affinity and thereby reduce excitability of the trigeminal pain system. High levels of Na^+ and low levels of Mg^{2+} and cholesterol can contribute to the probability of onset of MRM attack by modulating OTR affinity for OT in the trigeminal pain pathway. (C) IL-6 and estrogen both increase OTR expression. Decreased IL-6 and estrogen levels during menstruation could decrease OTR expression, reducing the inhibitory effect of OT on trigeminal excitability and thus increasing the likelihood of painful MRM attacks. Created with BioRender.com

these factors, the receptor can reversibly change its conformation from low affinity ($K_d \sim 100$ nM) to high affinity ($K_d \sim 1$ nM) and vice versa[76] for OT. In the presence of Mg, cholesterol binding to its specific domain in the OTR causes a conformational change that both increases the affinity of OT for the OTR and stabilizes the receptor against proteolytical degradation.[79] Cholesterol (total cholesterol and low-density lipoprotein) levels are highest during the follicular phase and decline during the luteal phase reaching the lowest levels just before menstruation[68,80] corresponding to times associated with MRM attacks (see Figure 2B). Although some studies show elevated overall cholesterol levels in some migraine patients,[81–83] levels of cholesterol and OT have not been co-serially investigated in MRM patients.

In contrast to the positive modulating effects of Mg^{2+}, sodium has been shown to negatively modulate the affinity of OT for the OTR.[84] Specifically, sodium (Na^+) allosterically modulates the agonist-binding site stabilizing the receptor in a low-affinity receptor state.[84] The concentration of Na^+ in plasma is lowest during the luteal phase[85,86] and peaks at menstruation[11] corresponding to when MRM attacks occur. Moreover, Na^+ concentrations were reported to be significantly higher in the cerebrospinal fluid in patients with migraine than in healthy controls.[87,88] These clinical studies may suggest that peak levels of serum Na^+ during menstruation could negatively modulate the OT affinity for the OTR, leading to increased neuronal excitability in the trigeminal system (see Figure 2B). The levels of Na^+ and OT have not yet been co-serially investigated in MRM patients.

As shown in Figure 2B, magnesium and cholesterol, taken together, positively modulate OT binding to OTR and Na^+ negatively modulates OT-OTR affinity. Thus, during menstruation, the drop in Mg^{2+} and cholesterol, along with the increase in Na^+ should lead to reduced OTR affinity to OT. The reduced OTR affinity to OT should lead to an increase in neuronal excitability in the trigeminal system and the probability of an MRM attack.

OTR expression levels

OTR expression is highly variable and can rapidly change. Similar to the expression in the uterus, trigeminal OTR expression is strongly and rapidly enhanced in the presence of inflammatory mediators.[20,61] Inflammation rapidly upregulates OTR protein expression by more than 10-fold within 2 h postinflammation induction and potently enhances trigeminal antinociception in rats.[16] Circulating IL-6 is a likely candidate to promote changes in OTR expression as there are three response elements in the OTR.[53] Results from both clinical and preclinical studies demonstrate that OT analgesia is substantially enhanced in the presence of IL-6 and other inflammatory mediators.[16,20] IL-6 serum levels are significantly higher in migraine patients during an attack compared with control.[89] Additionally, serum IL-6 levels follow estrogen levels, increase during preovulation and drop to the lowest point during the luteal phase and menstruation[66] (see Figure 2C).

In addition to IL-6, there is an estrogen response element on the OTR promoter, and consequently, estrogen can cause a significant increase in OTR messenger ribonucleic acid (mRNA) in both peripheral and CNS tissues.[43,90,91] The estrogen-induced increase in OTR expression may in part be mediated by de novo synthesis of OTR mRNA or by alterations in the stability of OTR gene transcripts.[43,92] Specifically, preclinical studies show that estrogen treatment induced a fourfold increase in the hypothalamus, a threefold increase in the amygdala, a 1.7-fold increase in the hippocampus, and a threefold increase in the myometrium compared with ovariectomized female rats.[90–92] Additionally, estrogen can lead to increased number and immunostaining of OT fibers in regions of the hypothalamus.[90,91] Thus, in addition to modulating OT levels, serum estrogen can modulate expression levels of OTR. In summary, estrogen and inflammatory mediators including IL-6 can increase OTR expression. During menstruation, a drop in IL-6 and estrogen serum levels may decrease OTR expression collectively promoting an increased likelihood of a MRM attack (see Figure 2C).

We recognize that many other factors can influence OT/OTR tone and thus may influence MRM. It should also be noted that OT and the OTR show structural similarities with vasopressin and its receptors and OT agonists and antagonists can act at vasopressin receptors, especially at higher concentrations.[93,94] As such, a detailed examination of the effect of the same menstrually related factors, including magnesium, cholesterol, and sodium, on vasopressin and its receptor and its effect on OT/OTR tone is warranted.

CONCLUSION: CLINICAL PRACTICE AND THERAPEUTIC IMPLICATIONS

Trigeminal neurons possess OTR and are inhibited by OT. The same neurons also often contain CGRP, and the release of CGRP is inhibited by OT. Blockade of CGRP or CGRP receptors with either monoclonal antibodies or small molecule receptor antagonists is effective in preventing migraine in many patients.[95–97] A recent study using telcagepant, a CGRP receptor antagonist, showed a reduction in on-drug premenstrual headaches.[98] Thus, OT-induced CGRP release inhibition could be a targeted mechanism for MRM therapeutics. Similarly, clinical studies have shown Mg^{2+} to be effective in attenuating MRM attacks.[32] Furthermore, the elevation of both OT levels and OTR expression likely provides the mechanism through which rapid changes in estrogen level can precipitate MRM as well as the mechanism through which MRM can be prevented by pharmacological estrogen.[28] More directly, elevating OT levels within the trigeminal system could be effective in preventing or aborting MRM.[16] Thus, OT, estrogen, and Mg^{2+}, either alone or in combination, should decrease the excitability of the migraine-associated trigeminovascular system that is activated in MRM providing a basis for novel therapeutics for MRM.

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CONFLICT OF INTEREST

David C. Yeomans and Shashidhar Kori are consultants for Tonix Pharmaceuticals. All other authors have no conflict of interest.

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