

Hormones and Migraine

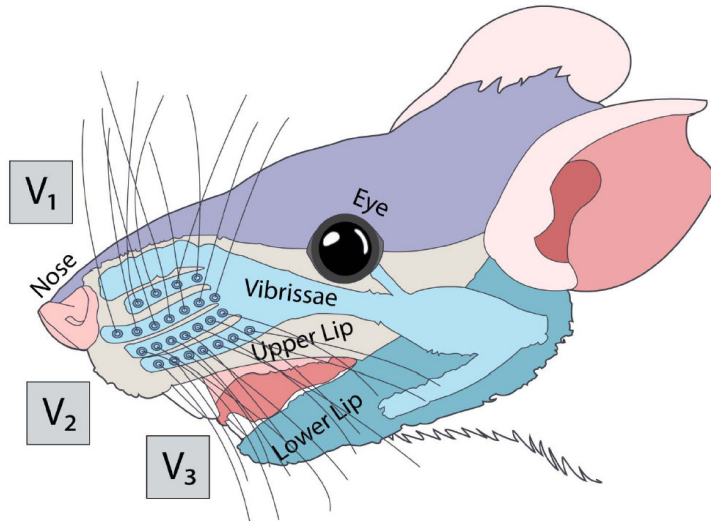
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Migraine is much more common in women than in men,¹ and painful episodes have been linked with the hormonal fluctuations of the menstrual cycle, puberty, pregnancy, and menopause. Evidence that estrogen influences hormonally-related headache was initially provided by Somerville, who not only showed that preventing estrogen withdrawal in the late luteal phase of the cycle prevents migraine, but also that estrogen in oral contraceptives and hormone replacement therapy can trigger migraine.^{2,3} Although estrogen receptors are present throughout the trigeminal vascular system, our studies have focused on their role in the trigeminal ganglion.

In our first study,⁴ we investigated the effect of physiological levels (10⁻⁹ M) estrogen on female rat trigeminal ganglia in vitro. Immunocytochemical analysis demonstrated the presence of estrogen receptor-alpha in a predominantly cytoplasmic location and in neurites. Microarray analysis demonstrated that estrogen treatment regulates several genes with potential relevance to menstrual migraine. The genes that were upregulated included synapsin-2, endothelin receptor type B, activity and neurotransmitter-induced early gene 7 (ania-7), phosphoserine aminotransferase, major histocompatibility complex class 1b (MHC-1b), and extracellular-signal regulated kinase 1 (ERK-1). Down-regulated genes included the *Arabidopsis thaliana* indole-3-acetic acid (IAA) amidohydrolase IL-R1, bradykinin B2 receptor, N-tropomodulin, chemokine (C-C motif) ligand 20 (CCL20), gamma-aminobutyric acid (GABA) transporter protein, fetal intestinal lactase-phlorizin hydrolase, carcinoembryonic antigen-related protein, zinc finger protein 36, epsin 1, and cysteine string protein. Protein activity assays demonstrated that exposure of the cultured neurons to estrogen leads to activation of ERK, which has been linked to inflammatory pain. Immunocytochemistry demonstrated that activated ERK was present in neurons containing peripherin, a marker of nociceptive neurons. Several of the genes in the present study may provide potential targets for understanding the association of estrogen with migraine and other hormone-related orofacial pain.⁴

A number of investigators have shown increases in ERK activation in sensory ganglia following peripheral injury and neuropathic pain.⁵⁻⁷ Furthermore, since blocking ERK reduces pain, ERK activation appears to be involved in the maintenance of pain.^{5,8-10} To determine whether ERK activation plays a role in hormone-related trigeminal pain disorders, we used an established model of inflammatory trigeminal pain, injection of Complete Freund's Adjuvant (CFA) into the masseter muscle.¹¹ We measured withdrawal responses to stimulation of the masseter (V3, primary allodynia) and whisker

pad (V2, secondary allodynia) using graded monofilaments. We study the trigeminal ganglion because it is the primary sensory structure for the head and face; the cell bodies of first order neurons sit in the ganglion and project to the face, dura, and TMJ and back to the spinal trigeminal nucleus.¹²



Adapted from Leiser and Moxon. *J Neurophysiol.* 2006;95:3129–3145.

Estrogen treatment in the presence of inflammation increased withdrawal response to stimulation of both masseter and whisker pad compared with inflammation alone, indicating an additive effect of inflammation and estrogen on both primary and secondary allodynia.¹¹ We examined ERK activation in trigeminal ganglia from each treatment group using western blot and immunohistochemistry. Both masseter inflammation and estrogen treatment increased ERK activation, and combined treatment had an additive effect. Both masseter inflammation and estrogen increased the percentage of pERK immunoreactive neurons in divisions 1 and 2 (V1/2), and combined treatment increased pERK immunoreactivity in V1/2 compared with inflammation alone. We stereotactically administered ERK antagonist U0126, or inactive control U0124, to the trigeminal ganglion of CFA+E2-treated rats. U0126 decreased withdrawal responses to mechanical stimulation of the whisker pad compared with U0124-treated rats. Because the secondary allodynia in V2 after inflammation in V3 was reduced by antagonizing ERK activation in the periphery, these data suggest a peripheral component to secondary allodynia mediated through ERK activation.¹¹

Since estrogen increases facial allodynia through its actions on activation of ERK in trigeminal ganglion neurons, our goal in subsequent work was to determine which estrogen receptor is required for behavioral sensitization.¹³ Immunohistochemical studies demonstrated the presence of estrogen receptor alpha (ERalpha) in nuclei of larger neurons and cytoplasm of smaller neurons, and the novel estrogen receptor G-protein coupled receptor 30 (GPR30) in small diameter neurons that also contained

peripherin, a marker of unmyelinated C-fibers. Specific agonists for ERalpha (PPT) and GPR30 (G-1), but not ERbeta (DPN), activated ERK in trigeminal ganglion neurons *in vitro*. Both G-1 and PPT treatment increased allodynia after CFA injections into the masseter of ovariectomized Sprague-Dawley rats. Treatment with estrogen increased expression of ERalpha but not GPR30, while masseter inflammation increased GPR30 but not ERalpha. Differential modulation of these ERK-coupled receptors by estrogen and inflammation may play a role in painful episodes of temporomandibular disorder and migraine.¹³

These data indicate that GPR30 and ERa are expressed in trigeminal nociceptors and show that ERa and GPR30 specific ligands activate ERK *in vitro* and sensitize trigeminal neurons *in vivo*. They also suggest that GPR30 is upregulated by inflammation, while ERa is upregulated by estrogen.

References

1. Lipton RB, Stewart WF, Diamond S, Diamond ML, Reed M. Prevalence and burden of migraine in the United States: data from the American Migraine Study II. *Headache*. 2001;41:646–657.
2. Somerville BW. Plasma estradiol level linked to migraine during menstrual period. *JAMA*. 1972;221:845–846.
3. Somerville BW. The role of estradiol withdrawal in the etiology of menstrual migraine. *Neurology*. 1972;22:355–365.
4. Puri V, Puri S, Svojanovsky SR, et al. Effects of oestrogen on trigeminal ganglia in culture: implications for hormonal effects on migraine. *Cephalalgia*. 2006;26:33–42.
5. Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. *Nat Neurosci*. 1999;2:1114–1119.
6. Doya H, Ohtori S, Takahashi K, et al. Extracellular signal-regulated kinase mitogen-activated protein kinase activation in the dorsal root ganglion (DRG) and spinal cord after DRG injury in rats. *Spine (Phila Pa 1976)*. 2005;30:2252–2256.
7. Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. *Pain*. 2005;114:149–159.
8. Ji RR, Befort K, Brenner GJ, Woolf CJ. ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. *J Neurosci*. 2002;22:478–485.
9. Obata K, Noguchi K. MAPK activation in nociceptive neurons and pain hypersensitivity. *Life Sci*. 2004;74:2643–2653.
10. Seino D, Tokunaga A, Tachibana T, et al. The role of ERK signaling and the P2X receptor on mechanical pain evoked by movement of inflamed knee joint. *Pain*. 2006;123:193–203.
11. Liverman CS, Brown JW, Sandhir R, Klein RM, McCarson K, Berman NEJ. Oestrogen increases nociception through ERK activation in the trigeminal ganglion: evidence for a peripheral mechanism of allodynia. *Cephalalgia*. 2009;29:520–531.
12. Leiser SC, Moxon KA. Relationship between physiological response type (RA and SA) and vibrissal receptive field of neurons within the rat trigeminal ganglion. *J Neurophysiol*. 2006;95:3129–3145.
13. Liverman CS, Brown JW, Sandhir R, McCarson KE, Berman NEJ. Role of the oestrogen receptors GPR30 and ERalpha in peripheral sensitization: relevance to trigeminal pain disorders in women. *Cephalalgia*. 2009;29:729–741.