Disclosures
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- Trigemina - Founder
- SiteOne Therapeutics – Founder
- ADYNXX – SAB chair
- Nalu Medical – SAB chair
- Cytonics – SAB chair
- Circuit Therapeutics – SAB chair
- Endo Pharmaceuticals - Consultant
- Orexigen Therapeutics – Consultant
- Rio Grande Neuroscience - Consultant

Learning objectives
• Oxytocin chemical anatomy
• Involvement of endogenous oxytocin in migraine prevalence
• Effect of oxytocin on trigeminal neurons
• Effect of oxytocin in animal pain models
• Distribution of oxytocin after nasal application
• Importance of inflammation in analgesic efficacy of oxytocin in rodents and migraineurs
• Interaction between oxytocin and CGRP in vitro and in vivo
• Effect of addition of magnesium on efficacy of oxytocin
Oxytocin is a 9 amino acid polypeptide hormone/neurotransmitter which is made in the hypothalamus and secreted both into the systemic circulation and into certain CNS sites.

Circumstantial evidence that increases in oxytocin can lead to decreases in migraine.

Breast feeding releases oxytocin and prevents migraines.
Intercourse and orgasm release oxytocin and relieve migraines

Orgasm activates the pituitary release of oxytocin

During orgasm, plasma oxytocin increases 42% in women, 73% in men

Carmichael et al., 1987

During orgasm, plasma oxytocin increases 42% in women, 73% in men

Carmichael et al., 1987

Intercourse and orgasm can alleviate migraine headache

<table>
<thead>
<tr>
<th>Degree of relief</th>
<th>No. (%) of women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete relief</td>
<td>10 (17%)</td>
</tr>
<tr>
<td>Moderate relief</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Temporary relief (&lt;60 min)</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>Relief only for mild headaches</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Any relief</td>
<td>22 (39%)</td>
</tr>
<tr>
<td>No relief</td>
<td>10 (17%)</td>
</tr>
<tr>
<td>Worse</td>
<td>3 (5%)</td>
</tr>
</tbody>
</table>

Huynh et al., 2013

Pregnancy increases oxytocin and decreases migraine

Pregnancy increases oxytocin and decreases migraine

Kuwabara et al., 1987

Adapted from Hoshiyama, 2012

Case studies of alleviation of migraine by IV oxytocin

Case 1
A 37-year-old patient had a migraine during delivery. Following delivery, “her only complaint was continued headache rated 8/10 immediately after delivery of the placenta, oxytocin 20 units was administered in 500 mL of Lactated Ringers Solution, USP over 30 minutes. By the completion of the infusion, the patient experienced complete relief of her headache.”

Phillips et al., 2006

Case 2
“A ten-year-old Caucasian male presented with an acute migraine headache of eight hours duration...His pain intensity rating was 8/10...A peripheral intravenous line was started and 500 mL normal saline with 50 units of oxytocin was administered over 30 minutes. Twenty minutes after beginning the infusion his pain intensity decreased to 2/10.”

Phillips et al., 2006
Oxytocin is released by hugging, massage, sex, looking at your dog, and shooting your gun.

Oxytocin and trigeminal pain

Oxytocin immunoreactivity on trigeminal ganglia neurons
Oxytocin inhibits injured trigeminal ganglia neurons in vitro

Nasal application of oxytocin

Oxytocin is preferentially transported throughout the trigeminal system after nasal delivery

| Broad Distribution of 125I-Oxytocin in Trigeminal Ganglia After Nasal Application |
|---------------------------------|----------|----------|----------|----------|
|                                | Maxillary | Mandibular | Ophthalmic | Other Tissues |
|                                | Branch   | Branch   | Branch   | Tissue   |
|                                | Ganglion | Ganglion | Ganglion | Muscle   |
|                                | TRIGEMINAL | NERVE | NERVE | Liver   |
|                                | NUCLEUS | BULBS | Cortex | Kidney   |
|                                | Brain   | Brain  | Caudate | Lung     |
|                                | SPINAL  | Cord  | Thalamus | Heart    |
|                                | Cord    | Cord   | Midbrain | Cord     |
|                                | OTHER   | TISSUES | Cerebellum | Cord     |
| TRIGEMINAL  | NERVE | NUCLEUS | Cortex | BLOOD |
|                | Maxillary | 471 ±117 | 34 ±10 | 63 ±4 |
|                | Mandibular | 676 ±235 | 39 ±12 | 23 ±4 |
|                | Ophthalmic | 423 ±143 | 15 ±6 | 20 ±8 |
|                | Other Tissues | 50 ±5 | 26 ±10 | 26 ±10 |
|                | Muscle | 16 ±3 | 34 ±9 | 34 ±9 |
|                | Liver | 16 ±2 | 5 ±1 | 5 ±1 |
|                | Kidney | 50 ±5 | 15 ±6 | 15 ±6 |
|                | Lung | 25 ±4 | 23 ±4 | 23 ±4 |
|                | Heart | 23 ±4 | 26 ±10 | 26 ±10 |

Basis of Oxytocin: Inhibition of Trigeminal Pain Sensing Neurons

Nasal application of oxytocin

 Autoradiograms of trigeminal ganglia

Trigeminal nociceptive system: Immunofluorescence
Intranasal Analgesia: Oxytocin Inhibits Brainstem Pain Responses

Nasal oxytocin inhibits pain responses of trigeminal nucleus neurons in vivo.

Nasal oxytocin decreases nitroglycerin-induced c-fos expression in the trigeminal nucleus.

Intranasal, but not intravenous oxytocin produces antagonist-reversible analgesia after TMJ inflammation in rats.

Nitroglycerin can induce migraines in humans.

C-fos expression change in the trigeminal nucleus before (a) and after (b) nitroglycerin infusion, and (c) after nasal oxytocin treatment.
Nasal delivery of oxytocin to the trigeminal system of humans

Pilot clinical study: effect of nasal oxytocin in episodic migraine

Percent of patients showing improvement at 2 hrs

<table>
<thead>
<tr>
<th></th>
<th>IV Oxytocin</th>
<th>IV Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain reduction</td>
<td>33%</td>
<td>26%</td>
</tr>
<tr>
<td>Photophobia</td>
<td>21%</td>
<td>9%</td>
</tr>
<tr>
<td>Phonophobia</td>
<td>16%</td>
<td>2%</td>
</tr>
<tr>
<td>Nausea</td>
<td>27%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Rating at 24 hrs

<table>
<thead>
<tr>
<th></th>
<th>IV Oxytocin</th>
<th>IV Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>14%</td>
<td>2%</td>
</tr>
<tr>
<td>Good</td>
<td>14%</td>
<td>7%</td>
</tr>
<tr>
<td>Fair</td>
<td>11%</td>
<td>17%</td>
</tr>
<tr>
<td>Poor</td>
<td>41%</td>
<td>14%</td>
</tr>
</tbody>
</table>

Inflammation and oxytocin analgesic efficacy
Oxytocin gene promoter has multiple response elements for Inflammatory cytokine IL-6

hOTR gene Promoter
Nucleotide Sequence (with cleavage Sites)
Consensus Response Elements
Transcription start site
NF-IL6 -181
NF-IL6 -777
NF-IL6 -816

IL-6 response elements
Schmid et al., 2001

IL-6 response elements

Inflammation increases expression of oxytocin receptors on trigeminal ganglia neurons 5-10 fold

Nasal oxytocin reduces trigeminal thermal nociception only after painful inflammation
Nasal, but not IV oxytocin is analgesic for head pain after brain injury

Nasal oxytocin is as effective as multiple doses of carbamazepine in a rat model of trigeminal neuralgia

Pilot clinical study: Nasal oxytocin reduces pain in chronic migraines. Blocking IL-6 with NSAIDs decreases efficacy
Oxytocin and CGRP: mechanism to reduce migraine frequency

Dural CGRP release appears to be critical to migraine

Oxytocin Receptors are co-expressed on CGRP + Trigeminal Neurons
Oxytocin’s Inhibition of trigeminal neurons decreases dural (and likely central) CGRP release

Oxytocin Reduces Capsaicin-Induced CGRP Release from Rat Dura Mater

Oxytocin-induced Reduction of Dural CGRP release is Dose-Dependent

Fold increase in CGRP release

Oxytocin concentration

Laser stimulation of the cheek induces CGRP release-dependent flare

Nasal oxytocin reduces flare response to laser pulse measured by scanning laser doppler after cheek inflammation

Adapted from Russell et al., 2014
Oxytocin blocks release of CGRP and so could directly block both peripheral and central sensitization

Oxytocin blocks trigeminal activity and neuronal release of CGRP, which could block both peripheral and central inflammation and sensitization.

Dural inflammation
Trigeminal neuron activity
Dural CGRP release
Brainstem CGRP release
Oxytocin blocks trigeminal neuron sensitization
Dural inflammation
Trigeminal neuron sensitization
CNS inflammation
Glial activation
Central sensitization
CGRP antibodies bind CGRP released from dural trigeminal neurons

Oxytocin Receptors also co-express with PACAP in trigeminal ganglia neurons

Overlay of OXTR (Green) and PACAP-38 (Red) immunoreactivity

Pilot study: effect of nasal oxytocin on migraine day frequency

SITES
- CHILE: 4 Sites
- AUSTRALIA: 3 Sites
- NEW ZEALAND: 2 Sites

SCREENING
High Frequency Episodic Migraine
- Chronic Migraineurs
- High Frequency Episodic Migraine (15+ headache days/month)
- Must also experience other migraine symptoms (such as nausea, photophobia)

RANDOMIZATION
n=318

TREATMENT
Nasal OT (n=143) Matching Placebo (n=75)

FOLLOW-UP
Telephone Follow-Up at 30, 60 and 90 Days After Exit From Study

• Cholinergic Signatures
• Headache Medications
• High Frequency Episodic Migraine
• Migraineurs (≥30 headache days/month)
• Must also experience other migraine symptoms (such as nausea, photophobia)
Placebo in Australia/New Zealand vs. Chile
Geographical Comparison in Weekly Migraine Headache Days (MHD) Reduction

Normalized Placebo Response Curve
Expected Drug Response Curve, Similar Between Venues
Abnormal, Drug-like Placebo Response Curve

p Values Provided are for Entire Period of Study
Based on Prespecified Primary Endpoint

Placebo IN oxytocin
Placebo IN oxytocin

IP Use Lower in Treatment Arm Compared to Placebo

WEEKLY IP USE BY GROUP

RESULTS

TI-001, a novel, magnesium containing oxytocin formulation
Magnesium ions act as positive allosteric modulators at the oxytocin receptor, increasing the potency of oxytocin in vitro.

Nasal TI-001 analgesia is superior to oxytocin alone.

Nasal TI-001 is more effective than oxytocin for Autism/FXS symptoms, e.g., anxiety.
Summary

• As oxytocin levels increase, migraines decrease
• Oxytocin receptors are present on trigeminal ganglia neurons
• Oxytocin decreases excitability of injured trigeminal ganglia neurons
• Nasally applied oxytocin concentrates in the trigeminal system and inhibits central neuronal responses and behavioral responses to painful stimuli
• Nasal oxytocin has minimal effect in low frequency episodic migraine
• Inflammation drives upregulation and enables efficacy in rat trigeminal pain models
• Nasal oxytocin is analgesic in chronic migraineurs who have not taken NSAIDs
• CGRP is co-expressed in trigeminal ganglia neurons; CGRP release is blocked by oxytocin
• PRN nasal oxytocin decreases migraine frequency in chronic migraineurs, presumably via blocking CGRP release
• Addition of magnesium salt to oxytocin produces synergistic, analgesic effects and results in a proprietary formulation

Thank you!