Oxytocin receptor: Expression in the trigeminal nociceptive system and potential role in the treatment of headache disorders

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Abstract
Aims: Our studies investigated the location of oxytocin receptors in the peripheral trigeminal sensory system and determined their role in trigeminal pain.
Methods: Oxytocin receptor expression and co-localization with calcitonin gene-related peptide was investigated in rat trigeminal ganglion using immunohistochemistry. Enzyme-linked immunosorbent assay was used to determine the effects of facial electrocutaneous stimulation and adjuvant-induced inflammation of the temporomandibular joint on oxytocin receptor expression in the trigeminal ganglion. Finally, the effects of oxytocin on capsaicin-induced calcitonin gene-related peptide release from dural nociceptors were investigated using isolated rat dura mater.
Results: Oxytocin receptor immunoreactivity was present in rat trigeminal neurons. The vast majority of oxytocin receptor immunoreactive neurons co-expressed calcitonin gene-related peptide. Both electrocutaneous stimulation and adjuvant-induced inflammation led to a rapid upregulation of oxytocin receptor protein expression in trigeminal ganglion neurons. Oxytocin significantly and dose-dependently decreased capsaicin-induced calcitonin gene-related peptide release from dural nociceptors.
Conclusion: Oxytocin receptor expression in calcitonin gene-related peptide containing trigeminal ganglion neurons, and the blockade of calcitonin gene-related peptide release from trigeminal dural afferents suggests that activation of these receptors may provide therapeutic benefit in patients with migraine and other primary headache disorders.

Keywords
Oxytocin, pain, headache, trigeminal, CGRP, inflammation

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Introduction
The nine amino acid peptide oxytocin, first isolated in 1927 (1), is primarily recognized as a hormone involved in the induction of uterine contractions (2), lactation (3), and, more recently, for its role in social behavior (4). In fact, oxytocin infusion has been used for inducing labor since the 1950s (5–8). Additionally, oxytocin was also approved in the USA for nasal application to stimulate milk ejection and for prevention of mastitis from 1960–1997 (9).
In addition to its involvement in uterine contraction, lactation and psychosocial processes, oxytocin has also been implicated as an endogenous modulator of pain. Endogenous oxytocin has been measured in human dorsal root and trigeminal ganglion (11), and terminals of hypothalamic neurons containing specific oxytocin neurophysin carrier proteins have been immunohistochemically localized to the dorsal horn of human spinal trigeminal nucleus (12). Similarly, Swanson and McKellar (13) identified oxytocin receptors within regions of the spinal cord involved in nociceptive transmission in monkeys. Several other groups (14–17) found similar spinal cord distribution of oxytocin receptors in the rat. There is evidence from animal studies for the analgesic effects of oxytocin (18–22) mostly after intrathecal or intracerebroventricular injection. In addition, accumulating evidence suggests that breastfeeding, with the accompanying elevated concentrations of oxytocin, may have a protective effect on migraine recurrence in the postpartum period (23–25). Although the above studies suggest that trigeminal oxytocin receptors may play a role in trigeminal pain perception, there are only limited data on oxytocin receptor expression and their role in the trigeminal ganglion and nucleus, since most studies focused on expression in reproductive tissues, such as mammary gland or endometrium. Clearly, however, expression of oxytocin receptors in these tissues is labile and rapid expression changes have been observed, which are driven, at least in part, by the presence of inflammatory cytokines (26,27).

The focus of the current studies is to investigate whether: (1) oxytocin receptors are expressed on trigeminal primary afferent neurons; (2) oxytocin receptors are co-expressed on calcitonin gene-related peptide (CGRP) positive neurons, presumptively involved in nociception; (3) oxytocin receptor expression on these neurons is enhanced under painful inflammation induced either by injection of complete Freund's adjuvant (CFA) into the temporomandibular joint (TMJ) or through prolonged electrocutaneous stimulation of the cheek; (4) oxytocin can prevent capsaicin-induced CGRP release from trigeminal afferent neurons that innervate the dura mater.

Methods

Animals

Male Sprague–Dawley rats (225–275 g, Harlan Laboratories, CA, USA) were used for the experiments. Rats were maintained two per cage in a controlled environment (temperature: 21.5 ± 4.5°C; relative humidity: 35–55%) under a standard 12 h light/12 h dark lighting cycle. Cage changes occurred twice per week, using standard bedding. All procedures were approved by the Stanford University IACUC.

Oxytocin receptor expression/co-expression with CGRP

Naïve rats (N = 6) were sacrificed using CO2 inhalation and perfused with phosphate-buffered saline followed by 4% paraformaldehyde. Trigeminal ganglia were removed and stored at 4°C overnight in 4% paraformaldehyde containing 20% sucrose. Ganglia were then embedded in paraffin and mounted on a chuck for cryosectioning. Sagittal sections (5 μm thick) were mounted on glass slides, followed by paraffin removal using sequential washes in xylene and ethanol, and then hydrated in deionized water. Standard procedures for antigen retrieval employed heating the dehydrated tissue for 30 min at 95°C in citrate buffer. Sections were rinsed in phosphate-buffered saline supplemented with 0.05% Tween-20 for two washes of 2 min. Sections were blocked in TBST buffer (TRIS-balanced salts containing 0.05% Tween-20) supplemented with 5% goat serum. Primary antibodies: oxytocin receptor, goat polyclonal anti-oxytocin receptor (N-19; Santa Cruz Biotechnology, catalog # sc-810, antibody dilution 1:50; blocking peptide sc-8013p), CGRP, sheep polyclonal anti-CGRP (Abcam, catalog # ab22560, dilution 1:200; rat CGRP peptide, Abcam, catalog # ab47101). Secondary antibodies: rhodamine (TRITC)-conjugated rabbit anti-goat IgG (Abcam catalog # ab6738, dilution 1:200); fluorescein thioisocyanate (FITC)-conjugated rabbit anti-sheep IgG (Abcam catalog # ab6743, dilution 1:500). Separate images of the two fluorophores marking each antigen were obtained using a Nikon PCM-2000 laser scanning confocal microscope at standardized gain settings to minimize overlap of the fluorescence intensity. CGRP-positive cells were selected for measurement only if the image for oxytocin showed a sharp and clear nuclear profile, indicating a mid-diameter cross-section. Such cells were compared with neighboring unstained (i.e. CGRP-negative) cells of similar diameter.

Induction of inflammation/chronic pain

In addition to naïve controls (N = 3), which were anesthetized but not treated, trigeminal ganglia were also harvested from rats 24 h after either: (1) CFA injection (N = 3) into the TMJ; or (2) electrocutaneous shock to the face (N = 15, three per time point) as described below.

CFA injection into the TMJ. In order to produce a robust inflammation of trigeminally innervated tissue, the TMJ of some rats were injected with CFA. Rats were
placed in an anesthesia chamber and anesthetized with 2.5% isoflurane. Fifty μL of CFA (DIFCO; Sigma Aldrich, St. Louis, MO, USA) was injected (1 mL syringe with a 26 G × 5/8-inch needle) into the left TMJ in order to produce robust and prolonged orofacial inflammation. To do this, prior to injection, the rat’s mouth was propped open to palpate the target area of the TMJ. In this position, an oval-shaped groove located in the center of the cheek and above the mandible can be distinctly felt. With the syringe positioned at a 30° angle from the rat’s cheek, the tip of the needle was inserted just under the articular disc (approximately 1.5 mm in diameter and 1.0 mm deep). After CFA injection, rats were returned to their home cages.

**Electrocutaneous stimulation.** In order to produce a time-controlled inflammation of trigeminally innervated tissue, some rats were administered electrocutaneous stimulation of the cheek. Rats were lightly anesthetized with 2.5% isoflurane prior to electrocutaneous stimulation. While anesthetized, the left cheek was depilated (NAIR® hair removal cream; Church & Dwight Co., Ewing, NJ, USA). Once hair was removed, the cheek was thoroughly cleaned with 70% ethanol and the rat was returned to the nose cone supplying isoflurane (1.5%). Rats were placed on a heated pad to maintain body temperature during noxious electrical stimulation. Once positioned, the exposed area of cheek was covered with an EEG conductive paste (NuPrep™; Weaver and Company, Aurora, CO, USA) and needle electrodes were inserted into the skin overlaying the left masseter muscle approximately 1 cm apart. Electrical pulses (10 ms duration, 1 Hz, 0.4 mA) were delivered continuously for 2 h using an isostimulator (Model A320; World Precision Instruments, Sarasota, FL, USA).

Twenty-four hours after CFA injection or 2, 4, 6, 8, or 24 h after termination of electrocutaneous stimulation, rats were euthanized by CO2 inhalation. Trigeminal ganglia were harvested within 15 min of death, snap-frozen in liquid nitrogen, transferred to pre-weighed Eppendorf-style micro-centrifuge tubes, and stored at −80°C.

**Tissue processing.** The weight of frozen ganglia was recorded prior to processing. Individual frozen ganglia were transferred onto an aluminium block chilled on a bed of dry-ice, broken in small 1–2 mm pieces using a razor blade and transferred into a chilled Dounce tissue grinder (1 mL volume) with 1 mL of hypotonic buffer added. Tissue was disrupted by 20–30 passes of the glass pestle with the tissue suspension then transferred to a 1.5 mL Eppendorf-style micro-centrifuge tube. Following snap-freezing in liquid nitrogen and thawing on wet ice, a membrane pellet was isolated by centrifugation (20,000 × g for 40 min at 4°C). After removing the supernatant, the pellet was re-suspended in 1 mL hypotonic buffer and again spun (20,000 × g for 40 min at 4°C). Following removal of the supernatant, the membrane pellet was disrupted by addition of lysis buffer (5 μL per mg tissue or a minimum of 150 μL, whichever was greater). Lysis buffer was composed of standard diluent solution, provided with the enzyme-linked immunosorbent assay (ELISA) kit (see below), supplemented with 1.25% (w/v) CHAPS (#220201; EMD Millipore) and protease inhibitor (Protease Inhibitor Cocktail 3, Calbiochem, 1:200 dilution). The lysis solution was transferred into a chilled Dounce tissue grinder (1 mL volume) and the membranes disrupted by 10–15 passes of the tight-fitting pestle. The tubes were stored on wet ice for 1 h with occasional vortexing. Solubilized membrane protein, including oxytocin receptor, was separated from insoluble material by a final spin (20,000 × g for 40 min at 4°C).

**Measurement of oxytocin receptor protein levels.** Oxytocin receptor protein was measured using a commercial ELISA kit (USCN Life Science, Inc., Wuhan, People’s Republic China) according to manufacturer’s protocol. Assay standard was diluted in lysis buffer to produce a standard curve from which oxytocin receptor content was extrapolated following correction for sample dilution. Briefly, the oxytocin receptor content in the ELISA wells was multiplied by the ratio of the total lysate volume divided by the lysate volume used in the ELISA (obtaining units of pg/mL). This value, in turn, was multiplied by the total volume of lysate (in mL) to obtain the oxytocin receptor content of trigeminal ganglia (in units of pg). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test to compare oxytocin receptor concentrations in the trigeminal ganglia of naïve animals to those in inflamed animals (i.e. CFA and shock, respectively). All analyses were conducted at the 0.05 level of statistical significance and used two-tailed tests.

**Effect of oxytocin on capsaicin-induced CGRP-release from dural nociceptors**

In order to provide evidence for a role of oxytocin in trigeminal pain mechanisms, we examined whether oxytocin could block the release of CGRP. Capsaicin-induced CGRP-release from dural nociceptors was measured using a similar protocol as reported by Reeh et al. (28,29). Rats were injected bilaterally with 50 μL of CFA (DIFCO, Sigma Aldrich) into the vibrissal pads to induce robust inflammation or with vehicle (mineral oil) as a control. Two days later, rats were euthanized by CO2 inhalation, followed by immediate decapitation. Skulls were hemi-sectioned, and the brain
tissue removed in order to expose the dura in two (half) skull chambers. Chambers were filled with artificial cerebrospinal fluid (aCSF) containing NaCl 107.8 mM, KCl 3.5 mM, MgSO4·7H2O 0.69 mM, NaHCO3 26.2 mM, NaH2PO4·2H2O 1.67 mM, Na-gluconate 9.64 mM, glucose 5.55 mM, saccharose 7.6 mM, and (added after saturating with CO2) CaCl2 0.025 mM for 10 min to establish baseline CGRP levels (first round). Then new aCSF with capsaicin 10 μM was filled into the hemi-sectioned skulls for 10 min (second round) to activate dural afferents and evoke CGRP release (N=9). Animals without injections of CFA into the vibrissal pads (N=8) were also tested in an identical manner, to quantify increased CGRP-release after facial inflammation. In additional experiments, oxytocin (1 μM (N=7), 10 μM (N=10), and 100 μM (N=8)) was added to the aCSF in the first and second rounds to test for inhibitory effects on capsaicin-induced CGRP release. After each round, aCSF was pipetted into individual Eppendorf tubes (1.5 mL; Santa Cruz Biotechnology, Dallas, TX, USA) and stored at −80°C pending further analysis. CGRP concentrations in aCSF were assayed by ELISA (SPIBio, Bertin Pharma, Montigny le Bretonneux, France) according to the manufacturer’s protocol. For each individual dura sample, CGRP concentrations in the first exposure to aCSF with or without oxytocin were measured and set to 1. CGRP concentrations after capsaicin exposure were then normalized to the baseline concentration measured in the first exposure, and tested for differences using Student’s t-tests for the effect of CFA on capsaicin-induced CGRP-release and one-way ANOVA followed by Tukey’s post hoc tests for effects of oxytocin on capsaicin-induced CGRP-release in CFA-pretreated rats, respectively. All analyses were conducted at the 0.05 level of statistical significance and used two-tailed tests.

Results

Oxytocin receptor expression on trigeminal ganglion neurons

Oxytocin receptors are widely present on a large subset of trigeminal ganglion neurons as demonstrated by epifluorescent microscopy (Figure 1(b,e,h). Pre-absorption

![Figure 1. Oxytocin receptor co-localizes with calcitonin gene-related peptide (CGRP)-positive neurons in trigeminal ganglion of complete Freund’s adjuvant-treated rats. Immunofluorescence images stained for CGRP are shown in (a), (d) and (g) (red), oxytocin receptor staining is shown in (b), (e) and (h) (green) and co-localization is indicated by yellow in the merged images shown in (c), (f) and (i).](image-url)
of the oxytocin receptor antibody with antigens, provided by the manufacturer, completely inhibited fluorescent localization of labelling in cells (data not shown).

**Oxytocin receptor co-expression with CGRP**

Of the cells positive for CGRP ($N = 174$) within the trigeminal ganglion 80% ($N = 140$) also expressed oxytocin receptors (Figure 1(c,f,i)). Pre-absorption of the CGRP antibody with antigens, provided by the manufacturer, completely inhibited fluorescent localization of labelling in cells (data not shown).

**Overexpression with inflammation and electroticuteaneous stimulation**

Mean baseline expression of oxytocin receptor protein in untreated control trigeminal ganglia was $0.13 \pm 0.02$ pg/mg (mean $\pm$ SEM) of ganglion tissue (Figure 2). Inflammation of the TMJ using CFA injections into the TMJ increased oxytocin receptor levels significantly ($p = 0.003$, one-way ANOVA with Tukey’s post hoc test) to $2.38 \pm 1.45$ pg/mg (mean $\pm$ SEM) compared to controls. Similarly, controlled noxious stimulation of the face of anesthetized rats with 2 h of pulsed electroticuteaneous stimulation significantly ($p = 0.04$, one-way ANOVA with Tukey’s post hoc test) increased oxytocin receptor concentrations compared to controls. This up-regulation was fairly consistent at 2, 4, 6, 8, and 24 h after stimulation ($1.1 \pm 0.2$, $1.3 \pm 0.1$, $2.0 \pm 0.3$, $1.6 \pm 0.4$, and $1.7 \pm 0.3$ pg/mg; mean $\pm$ SEM), indicating that the expression change is rapid and persistent for a considerable time after cessation of the noxious stimulus.

**Effect of oxytocin on capsaicin-induced CGRP-release from dural nociceptors**

CFA injection into the vibrissa of rats, reliably led to swelling and induration of the vibrissal pad indicating significant inflammation. Baseline concentrations of CGRP from the oxytocin treated dura samples were not different from untreated controls (data not shown). CGRP release from dura after CFA pre-treatment was significantly increased when exposed to capsaicin containing aCSF ($11.0 \pm 2.1$ vs. $19.4 \pm 3.8$; mean $\pm$ SEM, $p = 0.04$, Student’s t-test, data not shown) compared to controls not injected with CFA. Adding 10 or 100 μM oxytocin to the aCSF, however, significantly reduced capsaicin-induced CGRP release ($10.0 \pm 1.6$ and $9.5 \pm 0.8$; mean $\pm$ SEM, Figure 3) when compared to CFA-pre-treated control rats ($19.4 \pm 3.8$; mean $\pm$ SEM, $p = 0.026$ and 0.023, respectively, one-way ANOVA with Tukey’s post hoc test, Figure 3). The 1 μM oxytocin dose did not significantly reduce CGRP release in CFA-pretreated rats ($12.7 \pm 3.4$; $p = 0.32$, one-way ANOVA with Tukey’s post hoc test, Figure 3).

**Discussion**

Results of the current experiments demonstrate that oxytocin receptors are expressed in trigeminal ganglion...
neurons; most of which co-express CGRP, which is indicative of their presence on primarily nociceptive trigeminal neurons. Furthermore, oxytocin receptors are up-regulated on trigeminal neurons following inflammatory and/or noxious stimulation. Finally, activation of these receptors by oxytocin can block the release of CGRP, implicating a role in modulating trigeminal pain.

In addition to expression in the uterus, breast, and other non-neural tissue, oxytocin receptors have been localized in human and rodent brain, brainstem, and spinal cord. A particularly high concentration is found in the lower medulla, in which the trigeminal nuclear pain system is located (30,31). A chromatographic study of cadaverous tissue also indicated the presence of oxytocin in human sensory ganglia, including the trigeminal ganglia (11). Furthermore, the paraventricular nucleus of the hypothalamus sends oxytocinergic projection fibers to the spinal cord (32). These projections have been implicated in the modulation of pain signals, particularly after peripheral nerve injury (33). Thus, the localization of oxytocin and oxytocin receptors suggest an anatomical substrate for oxytocinergic modulation of incoming nociceptive neural traffic in the spinal cord. However, to our knowledge the presence of such a substrate was previously unexplored in the trigeminal system.

CGRP is critical in the relay and modulation of nociceptive signals from the periphery to the central nervous system (CNS) (34). Part of this effect is through an action on secondary neurons in the dorsal horn where, by both direct (35) and indirect (36) mechanisms, CGRP can induce sensitization. CGRP is also critically involved in the pathophysiology of migraine headache, and is likely also important in other craniofacial pain states (37). The significant role CGRP has in trigeminal pain disorders is supported by the efficacy of triptan medications (e.g. sumatriptan) in treating migraine headache. Although the pathophysiology of migraine is only partially understood, it is undisputed that the trigeminal ganglion (50) and meningeal afferents further validates the role of CGRP in craniofacial pain. Finally, scalp injections of botulinum toxin (BOTOX®), which is approved for the treatment for chronic migraine has been suggested to act, at least in part, through blockade of CGRP release from trigeminal afferent fibers (41).

Oxytocin produces analgesia when applied to the spinal cord, both in rodents (19,21,22,42,43) and man (44). It has not been clear, however, what the mechanism of this analgesic effect might be. The current results demonstrate that the level of oxytocin receptor expression in the trigeminal system is highly dynamic with craniofacial inflammation increasing the amount of receptor protein by more than ten-fold, 24 h after CFA injection or within 2 h of shock-induced inflammation. Three response elements on the oxytocin receptor gene promoter bind interleukin-6 and are likely responsible for such inflammation-driven upregulation, at least in the CNS (45). These results suggest that inflammation, as occurs with many craniofacial pain states, including temporomandibular disorders, tooth extraction, and, notably, migraine headache, should increase the responsivity of the trigeminal system to oxytocin.

The mechanisms underlying the analgesic effect of oxytocin in the spinal cord likely include presynaptic inhibition of nociceptive primary afferent neurons, possibly through the inhibition of Ca$^{2+}$ influx into spinal terminals (17). Although the current results do not provide conclusive evidence, the presence of oxytocin receptors on CGRP immunoreactive trigeminal ganglion neurons, and the block of evoked CGRP release provides initial support that presynaptic inhibition does, at least, contribute to the analgesic effects observed. In contrast with our findings, Moreno-Lopez et al. (47) found that, in the spinal dorsal root ganglia, oxytocin receptors are primarily found in cell bodies of non-peptidergic C fibers and not in cutaneous nociceptive terminals, mechanoreceptors or peptidergic afferents, possibly indicating a distinction between the spinal and the trigeminal system. Additionally, a recent report demonstrated that oxytocin blocks acid-sensing ion channels in cultured dorsal root ganglia neurons, suggesting a direct effect on nociceptive transduction (48). In addition to these reports of presynaptic effect, patch clamp studies also support a post-synaptic effect of oxytocin. These studies demonstrated that oxytocin can enhance GABAergic and glycinergic inhibitory transmission on spinal substantia gelatinosa neurons (49). Thus, in addition to our demonstration of blockade of CGRP release from trigeminal afferents, there is evidence for numerous mechanisms through which oxytocin could exert an analgesic effect.

Although the pathophysiology of migraine is only partially understood, it is undisputed that the trigeminal ganglion (50) and meningeal afferents (51) play a key role. However, there are only limited data suggesting an inhibitory effect of oxytocin on migraine. Hoshiyama et al. (23) found significantly lower migraine recurrence rates when comparing breastfeeding an inhibitory effect of oxytocin on migraine. Hoshiyama et al. (23) found significantly lower migraine recurrence rates when comparing breastfeeding (i.e. presumably higher oxytocin levels) to bottle-feeding mothers for at least 6 months post-partum. These findings were corroborated by another study by Sances et al. (24), in which 1 month after delivery of their baby, 100% of bottle-feeding mothers had recurrence of migraine compared to 43% of mothers who were breastfeeding. Similarly, Serva et al. (25) found that breastfeeding was protective for migraine.
recurrence. In addition, there is a single case series of two patients suffering from an acute migraine headache who had experienced relief after intravenous infusion of oxytocin (52).

The results presented here provide presumptive evidence that oxytocin could act at trigeminal receptors, in a state of upregulation due to inflammation, to block the release of CGRP from nociceptive primary afferent neurons. This effect may occur in concert with other pre- and post-synaptic mechanisms, thereby reducing the intensity and frequency of migraine headaches and potentially of other trigeminal system associated pain conditions.

**Key findings**
- Oxytocin receptors were present on the vast majority of calcitonin-gene-related-peptide (CGRP)-positive trigeminal ganglia neurons
- Oxytocin receptors were upregulated in the trigeminal ganglion after noxious stimulation of the face
- Exogenous oxytocin blocked capsaicin-induced CGRP release from trigeminal dural afferents

**Declaration of conflicting interests**
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Oxytocin and Pain
A Systematic Review and Synthesis of Findings
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Objectives: A review of the literature was conducted to assess the association between oxytocin (OT) and pain.

Methods: PsychInfo, PubMed, and Medline (EBSCO) research databases were searched for peer-reviewed articles written between 1950 and 2012. Of a total of 1166 articles returned, 50 (9 human, 33 animal, and 8 spinal cord samples) met full inclusion criteria and were included in the review.

Results: OT had a reliable effect as defined by increasing pain tolerance in 29 of 33 animal studies reviewed. This effect persisted across central and peripheral modes of administration and type of noxious stimulus used (eg, heat, electric). The results suggest that OT acts as an analgesic for acute pain in animals. Preliminary research with humans offers consistent evidence to suggest that OT decreases pain sensitivity, though the reliability and stability of such effects cannot yet be determined. Although the findings are encouraging, there is a need for methodologically rigorous work in humans where OT is administered centrally.

Discussion: Further research seems to be warranted as the existence of biologically and psychologically plausible mechanisms linking OT and pain have been well supported using animal models with limited but encouraging human research. Implications and recommendations are discussed. Findings from this research may inform therapeutic methods for the management of pain.

Key Words: oxytocin, pain, noiception, analgesia, review

Research examining the role of oxytocin (OT) in pain sensitivity has increased slowly but steadily in recent years. The methodologies, samples, and noxious stimuli used to study this subject have been diverse. Although the majority of research has concluded that OT decreases sensitivity to noxious stimuli, contradictory findings have been reported. Given the important implications for clinical practice, the strength of evidence for an analgesic effect of OT was assessed by reviewing and synthesizing research that investigated the relationship between OT and pain sensitivity.

OT is a neuropeptide that is produced in the supraoptic and paraventricular nuclei of the hypothalamus and released into circulation by way of magnocellular neurons extending down to the posterior pituitary.1 OT released into the bloodstream from the posterior pituitary is presumed to comprise the majority of the peripherally released OT and is believed to be the primary source reflected in blood and saliva.2 Additional OT is released from paraventricular neurons that project throughout the CNS to the amygdala, the striatum, the raphe nuclei, and the superficial and deep lamina of the dorsal horn.3–6 Thus, one stream of OT is released centrally and confined largely within the CNS, whereas the other is released peripherally. The central route represents a biologically plausible descending mechanism for pain control.

The dorsal horn represents a site at which pain signals are modulated in a dynamic manner. A high concentration of nociceptors terminate in the superficial and deep dorsal horn; Aδ-afferents terminate in the superficial lamina I and the deeper lamina V, whereas C-fibers terminate superficially in lamina I and II.6 A well-documented direct hypothalamo-spinal projection, which originates from the paraventricular nucleus (PVN), also terminates in the dorsal horn (lamina I, II, and IV).7 This projection contains several neuropeptides, the most abundant being OT.8 Further, a subset of dorsal horn neurons in lamina I and II (≈35%) also contain OT receptors that influence glutamate and GABA cellular signaling.9 The effects of OT on GABAergic interneurons seems to be more prominent than the effects on glutamine interneurons and result in pre-synaptic inhibition of Aδ-fiber and C-fiber signals at nociceptive-specific and wide dynamic range (WDR) neurons.10

The presence of a descending oxytocinergic pathway along with an abundance of OT receptors in an area with a high concentration of nociceptive fibers suggests the involvement of OT in pain signaling. Given the expanding but limited research base examining OT-pain relationships, the present review presents a summary of the total literature evaluating these associations in both animals and humans.

METHODS

Literature Search
PsychInfo, PubMed, and Medline (EBSCO) research databases were reviewed between October 2011 and February 2012. Searches included the term oxytocin combined with 1 of 3 pain classifiers (ie, pain, noiception, or analgesia). Smart searches using an “and” classifier were used to locate search terms anywhere in the abstract, title, or text of articles published between 1950 and 2012. The first author (J.A.R.) performed the literature search and the second author (A.A.-C.) duplicated the search to ensure accuracy. To ensure no relevant articles were missed, references of included manuscripts were checked for relevant contributions to the review.

Review Process
Two review authors assessed the studies for eligibility. Articles that included OT and a pain classifier in the title,
abstract, text, or as a keyword were selected for additional review. Trials were not blind assessed because author name, institution, and source of publication were known.

Inclusion and Exclusion Criteria for the Selection of Articles

For this systematic review, the inclusion and exclusion criteria were met if the article had the following characteristics: (1) written in English; (2) directly assessed the relationship between OT and pain; and (3) the study was empirical, offering quantitative data. Trials using small sample sizes (n ≤ 5) from which firm conclusions could not be based were excluded. Neurosurgical studies removing a significant portion of the brain were excluded because such surgery may have unreliable effects on pain perception and the potential role of OT. Given that central OT concentration has been found to peak within 30 to 60 minutes after exogenous administration, a delay of ≥ 3 hours between OT administration and pain testing was also grounds for exclusion. Articles assessing OT and pain during pregnancy, and labor were excluded due to the consideration that pregnancy is a period associated with the release of a myriad of hormones that may confound regular associations between OT and pain.

Calculation of Effect Size

Wherever possible, relevant statistics (eg, F values, correlations, χ²) were converted into a measure of standardized effect as Cohen d. Effect size calculations were performed using the commercially available software, ClinTools (Melbourne, Australia). Effects were pooled within studies that reported several OT-pain outcome measures. If a study reported results regarding the effects of multiple concentrations of OT on pain then a conservative approach was taken and the median value was used. Within-group estimates of effect were corrected using a formula developed by Morris and DeShon. Correlation (r) values were transformed using a method developed by Friedman. To standardize nonparametric tests, χ² values were computed into r which was then computed into a d.

RESULTS

The literature search resulted in 1166 articles across 3 databases (See Fig. 1 for a description of the review process). After a thorough review of abstracts, 1107 articles did not meet our inclusion criteria (eg, did not directly assess the relationship between OT and pain, made assessment during pregnancy or labor, did not contain enough information to make a firm conclusion). A total of 59 articles assessed the relationship between OT and pain and moved to a full review. Of these articles, 9 did not meet our inclusion criteria or exhibited methodological weaknesses (assessed OT in a cursory manner to another analgesic,15,16 removed a substantial portion of the brain,17,18 delay between OT administration and pain testing [eg, 46d to 4mo],19,20 provided insufficient description of the painful stimulus,21 or reported on a sample of <5 patients). Table 1 orders the studies according to support for the analgesic effects of OT and date of publication. Nine studies examined the relationship between OT and pain in humans (7 in adults and 2 in children), 8 in samples of spinal cord neurons,10,24,30,33–35,39,49 and 33 using animals (27 in rats, 25–29,32,37,38,40,41,43,45,46,48,50–52,55,59–62,64,65,67,70,72 4 in mice,31,42,66,71 and 2 in dogs)24,49

Animal Studies

The method used to assess the influence of OT on pain in animals varied widely. In animal models, injections of OT and/or an OT antagonist were used in 29 studies,25–29,32,37,40–43,45,46,48,50–52,55,59–62,64–67,69,70,72 2 studies measured OT concentrations in blood plasma,36,54 and 2 studies used mice that had the genetic expression for OT removed.31,71 Nociceptive stimuli used to produce pain also varied widely. Studies inflicting acute pain used electrical29–32,46,61 (eg, iontophoresis), mechanical31,32,40,43,51,69 (eg, tail clip or von Frey filament test), cold,43 heat,31,32,37,40,43,52,55,59,60,64–67,71–73 (eg, hotplate, 50°C water), chemical31,42 (eg, formalin test, intraperitoneal acetic acid injection), or some combination of painful stimuli. The relationship between OT and the natural occurrence of chronic pain was only assessed in 1 animal study.

The most reliable evidence indicating that OT increased pain tolerance came from studies using animal models. All but 49–72 of the 33 animal studies showed strong support for the analgesic effects of OT. The average effect reported by these studies was large, Cohen d = 2.28. This effect persisted when OT was injected peripherally, average Cohen d = 1.86,31,32,42,45,55,60,65 or centrally, mean Cohen d = 2.47,25–27,29,37,40,41,43,46,48,50–52,56,61,62,66,67,72 and was also evidenced in blood plasma by higher OT levels in dogs with chronic pain relative to normal controls.54 Analgesic effects were strongest 20 to 30 minutes after exogenous administration and lasted for approximately 1 hour.

Human Studies

In human samples, 4 studies assessed OT using blood plasma,36,47,53,58 2 studies administered OT using injections (1 intrathecal,63 1 intravenous67). 1 study assessed the inhalation of OT vapor,27 and 2 studies administered OT intranasally using a placebo-control double-blind design.48,68 Studies assessing acute pain used electric,68 mechanical,36,56,57 (eg, finger prick), cold,68 (eg, cold pressor), or heat.56 Studies assessing chronic physical conditions resulting in the natural occurrence of pain were primarily conducted on humans.44,47,53,56,58,63

Research assessing the effect of OT on pain in humans was generally consistent, suggesting that OT may decrease pain sensitivity. Four studies assessed endogenous OT concentrations in blood plasma,36,47,53,58 4 studies administered exogenous OT relative to a placebo,44,56,57,68 and 1 study assessed the exogenous administration of OT relative to a placebo as well as endogenous OT concentration in blood plasma.63 Higher endogenous OT concentrations were associated with lower pain sensitivity in 4 of the 5 studies assessing blood plasma OT. Children with chronic abdominal pain (n = 103) exhibited lower blood plasma OT concentration than pain-free control children (n = 113) matched on age and sex.47,58 Adults with chronic (n = 83) and acute (n = 72) low back pain had lower blood plasma OT concentration than controls (n = 65).63 Greater blood plasma OT concentration in pain-free women (n = 48; 25 black, 23 white), was also associated with higher cold pressor and ischemic pain tolerance but not thermal heat pain tolerance.63 Of interest, black women displayed lower pain tolerance across all pain tasks, and exhibited lower plasma OT concentrations suggesting that OT may contribute to ethnic differences in pain tolerance. Only 1 study indicated that there was no difference between blood plasma OT concentrations between women with fibromyalgia (n = 39) and controls (n = 30) matched on age and sex.53 Yet, blood plasma OT concentrations were lower among patients
scoring higher on pain, stress, or depression, highlighting that severity of condition may be important.

The exogenous administration of OT decreased pain sensitivity in 3 of 5 studies. Reports of finger prick pain were reduced when pain-free participants were infused with the continuous inhalation of OT (n = 16) relative to the continuous inhalation of a placebo (n = 16). Likewise, the continuous intravenous administration of OT led to a dose-response decrease in reports of pain induced by inflating a barostat bag in the descending colon of 26 (11 female) patients with abdominal pain. Similarly, the central administration of OT in doses between 50 and 400 μg/kg relieved pain among patients with acute and chronic low back pain. One of the 5 studies assessing the exogenous administration of OT on pain sensitivity was promising, whereas 1 was inconclusive. The administration of intranasal OT resulted in a significant reduction in abdominal discomfort and a nonsignificant reduction in abdominal pain among women with daily abdominal symptoms and chronic constipation randomized to receive an OT (n = 23) or placebo (n = 26) nasal spray twice each day for 13 weeks. Post hoc calculations indicated that 60 to 120 women would be required to detect an effect of OT on abdominal pain. Neither pain intensity nor threshold was assessed and null findings may be attributable to a limited group of 20 relatively homogenous male participants.

**Spinal Cord Samples**

In spinal cord samples, 5 studies administered electric current to the PVN of the hypothalamus to facilitate the release of OT. Two of these studies also administered OT topically. Two studies cultured spinal cord samples and topically administered OT and OT antagonists. Mechanistic studies using spinal cord samples support an association between OT and pain. Findings from 5 studies using spinal cord samples, and 1 animal study conducted by the same laboratory, indicated that electrical stimulation of the hypothalamus, in particular the PVN, produced OT release, led to an increased concentration of OT in the spinal cord, and acted to inhibit pain at the dorsal root. This inhibition of pain was blocked by the administration of a selective OT antagonist. Findings from the remaining 3 studies showed that PVN stimulation, or topical OT, acted to selectively attenuate pain signals from incoming C-fiber and Aδ-fiber in lamina II. OT activated presynaptic OT receptors at the terminals of glutamatergic interneurons, which activated local GABAergic interneurons and suppressed C-fiber and Aδ-fiber action potential firing by closing K⁺ and Ca²⁺ channels. OT inhibited both wide range dynamic and nociception-specific cells. Further, both PVN stimulation
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and intrathecal OT injection reduced long-term potentiation in the dorsal horn, which is theorized to be the sensitizing mechanism turning acute pain to chronic.74

### Discussion

Research on the effects of OT on pain has spanned nearly 3 decades yet the literature base remains modest, with 33 animal and 9 human studies identified. We conducted a review of the literature on OT and pain from nearly 3 decades yet the literature base remains modest, with 33 animal and 9 human studies identified. We conducted a review of the literature on OT and pain from the application of OT increased pain tolerance and attenuated acute pain in mice and rats. This was observed through exogenous administration of OT and endogenous stimulation of brain centers that facilitate OT release. This effect was consistent across 29 of 33 animal studies, persisted when OT was administered peripherally or centrally, and was blocked by a selective OT antagonist. These findings may be taken as reasonable evidence, given the large magnitude of effect sizes, for the analgesic properties of OT. Given this robust set of findings arising from animal research, the relative dearth of human findings is surprising. Nine studies have examined OT-pain relationships in humans. Five of these studies administered OT exogenously, whereas 5 studies measured plasma OT concentrations (1 study assessed exogenous and endogenous OT). Four of 5 studies reported that the exogenous administration of OT increased pain tolerance and 3 of 4 studies measuring OT concentrations in blood plasma concluded that pain tolerance is higher when OT concentrations are higher.

There are at least 2 potential biologically plausible mechanisms connecting OT and pain. The first involves direct OT projections to the dorsal horn. Stimulation of the PVN or administration of OT activate presynaptic OT receptors located superficially in the dorsal horn (lamina I and II) and subsequently excite inhibitory GABAergic interneurons.10 Activation of GABAergic interneurons, in turn, presynaptically inhibit Aδ-fiber and C-fiber signals at nociceptive-specific and WDR neurons in lamina I and II.30,34,39 This OT-based reduction in nociceptive responding is associated with a reduction in fast transient (Ih) and delayed rectifier (IKDR) K+ currents,32 and an inhibition in the increase in intracellular calcium from membrane depolarization.24 These effects are reversible with a selective OT antagonist.34 Moreover, PVN stimulation reduces the extent to which long-term potentiation facilitates WDR neuronal responses evoked by Aδ-afferent and C-afferent.30,35 It is important to note that this first mechanism may also work at a postsynaptic level by modulating postsynaptic dorsal column projection neuron responses to nociceptive input.39

The second potential mechanism linking OT and pain involves an indirect pathway through the endogenous opioid system. Nine studies reported that the injection of a μ-opioid or κ-opioid receptor antagonist partially blocked the analgesic effects of OT.27,40,42,43,46,48,50,51 Blocking the analgesic effects of OT with an opioid antagonist suggests that OT binds to opioid receptors. Further, OT may stimulate the release of endogenous opioids in the brain. An opioid system located in the PAG activates a series of descending controls that prevents spinal cord transmission regarding injury.75 OT administered into the PAG results in antinociception that can be blocked by the administration of a μ-opioid or κ-opioid antagonist.27,42,51 It is possible
that the indirect effect of OT on endogenous opioids in the brain is mediated by a closely related neuropeptide known as arginine-vasopressin. Arginine-vasopressin is synthesized by a class of PVN neurons, projects to the PAG, and has been observed to influence the endogenous opioid system. Systematically administered OT has been reported to produce nociception in mice by an action that is mediated by the arginine-vasopressin-V1a receptor.

A potential relationship between OT and pain may also be explained through a psychological mechanism whereby OT decreases pain sensitivity by improving mood, decreasing anxiety, and mitigating the stress response. There is a large literature base suggesting that OT improves mood and mitigates stress. OT is negatively associated with depression and anxiety, and the administration of OT has been observed to reduce fear-related activation of the amygdala. There are reports that exogenously administered OT is associated with lower cortisol levels after stressful tasks, such as vigorous physical exercise and couples conflict. In an informative placebo-controlled, double-blind study, the intranasal administration of OT in men resulted in greater calmness, less anxiety, and a trend toward lower cortisol during the Trier Social Stress Test. Prominent improvement in mood and reduction in stress are 2 ways that OT may decrease pain sensitivity.

The majority of research on OT and pain has examined the influence of OT on transient or acute pain with less attention to persistent pain. Seven studies assessed OT-pain relationships using populations experiencing persistent pain. Five of these studies assessed endogenous OT concentration and generally concluded that peripheral OT concentrations were lower among patients with chronic pain. Two studies reported that plasma OT concentrations were lower among children experiencing recurrent abdominal pain. One study observed that plasma OT concentrations were elevated among dogs who had previously experienced spinal cord compression. Another study indicated that plasma OT concentrations did not differ between women with fibromyalgia and pain-free controls. An additional study reported that plasma OT concentrations were lower among patients with low back pain. Findings from the 5 studies measuring endogenous OT concentration in blood plasma are complicated given that blood plasma assays tag molecules other than OT and may not give a reliable representation of concentrations in the CNS. The relationship between centrally and peripherally produced OT is uncertain and depends on the method of measurement. When basal concentration is assessed there seems to be little to no association between peripheral and central OT concentration. Coordination between central and peripheral OT release in response to stimulation is inconsistent, with some studies showing coordination for certain stimuli (eg, birth, suckling, sex) and other findings indicating no such coordination (eg, forced swimming, social defeat). It is unknown whether central and peripheral OT release is coordinated in response to pain.

Peripheral OT concentration seems to reflect central concentration when OT is administered exogenously. Although OT does not cross the blood-brain barrier, the exogenous intranasal administration of OT bypasses the blood-brain barrier and reaches behaviorally relevant brain areas, and this uptake is paralleled by changes in plasma OT in animals and saliva OT in humans. Despite the potential discrepancy between peripheral and central OT concentration, peripheral levels of OT may be clinically relevant given that the peripheral administration of OT was reported to decrease pain sensitivity. Further, 3 studies indicated that the exogenous administration of OT decreased pain sensitivity among individuals with chronic pain. The continuous infusion of 30 mU/min of OT increased tolerance for bladder distention pain among adults with irritable bowel syndrome. OT injected into the cerebrospinal fluid relieved pain among adults with chronic back pain. Finally, the administration of OT over a 13-week period tended to reduce abdominal pain in women reporting chronic constipation.

Additional research is needed to determine the stability and reliability of the OT-pain relationship in humans. Only 9 studies have assessed the potential for this relationship to date, and have involved several significant methodological shortcomings. Three studies did not provide enough data to calculate the magnitude of effects. One study assessed unpleasantness but not pain intensity. Three studies reported that the administration of OT relieves acute or chronic pain, whereas 2 studies found no such effect (although this may be related to an inadequate sample size). Some of the research to date has included both men and women but there has been no attempt to assess for potential sex differences in the OT-pain relationship, despite the very different endogenous manifestation of this hormone between the sexes. The limited nature of the findings suggests that there is a need for more methodologically rigorous work. This may involve the exogenous intranasal administration of OT using a double-blind placebo control design administering multiple doses across several noxious stimuli. Intranasal administration is an effective way for neuropeptides to pass the blood-brain barrier with fewer side effects than a parenteral route. One study observed that intravenous or intramuscular infusion; however, it is less common given that this means of delivery is not approved by the Food and Drug Administration. A placebo-controlled, double-blind design controls for the high placebo effect typically observed in pain trials and the use of several noxious stimuli is important given that effects have been reported for some but not all methods of pain induction.

Overall, the animal studies reviewed illustrate that a consistent and dose-dependent relationship exists between OT and pain perception. The administration of 1 to 10 mg/kg of OT to a Sprague-Dawley rat or a mouse seemed to result in therapeutic effects on pain with greater concentrations offering additional relief. There did not seem to be an upper limit of this effect. Further, biologically and psychologically plausible and evolutionarily adaptive mechanisms exist to account for this effect. Yet, although there is evidence to suggest that OT causes analgesia for acute or chronic pain in humans, additional rigorous trials are needed to determine the stability and reliability of effects. Laboratory studies of OT and pain have important implications for the management of pain in real-life situations. If proven therapeutic in humans, OT may represent a relatively inexpensive form of pharmacotherapy for pain management with little potential for addiction. Findings from this literature also have important implications for other forms of pain management. OT is released during warm interpersonal contact suggesting that the presence of social support and nurturing physical contact may improve outcomes among acute and chronic pain patients.
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