Human trigeminal ganglia possess oxytocin receptors on CGRP positive neurons: expression increased by inflammation

IHC23-DP-033

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Introduction

We have shown that oxytocin, acting at oxytocin receptors (OTR) on trigeminal neurons can inhibit the excitability of those neurons which could provide the basis for both analgesia as well as decreasing the likelihood of a migraine being triggered. Critically, inflammation appears to play a key role in this phenomenon in that it can drive a rapid upregulation of OTR by interacting with 3 elements on the promotor for the OTR gene that are responsive to interleukin-6 (IL-6). Thus, inflammatory states, such as that accompanying chronic migraine, likely plays a key role in relief or prevention of migraine headache by oxytocin.

We have previously demonstrated, in rodent trigeminal ganglia (TG), that most CGRP expressing neurons also express OTR[1] (Figure 1). We have also shown that facial shock induced inflammation induces a rapid, profound and sustained upregulation of these receptors[1] (Figure 2), and, as a consequence, increasing the craniofacial analgesic efficay of nasally applied oxytocin[2] (Figure 3).

In addition, our preliminary results demonstrate the presence of OTR on human *dorsal root ganglia (DRG)* neurons (Figure 4). Consistent with OTR expression being dependent on inflammation, we also found a robust upregulation of the receptor in human DRG after incubation of the tissue in IL-6 for 4 hours *in vitro* (Figure 5). Consistent with this finding, pre-incubation of DRG with IL-6 also significantly increased the inhibitory effect of OT on DRG neuronal excibatilty as demonstrated by increased resting membrane potential in current clamp electrophysilogy experiments (Figure 6).

Purpose

The pupose of this study was to determine whether, and to what extent OTR are present on HUMAN TG as well as whether OTR expressing TG neurons also express CGRP, a a key peptide in the pathogenesis of migraine. In addition, the study examined whether *in vitro* inflammatory treatment of human trigeminal tissue would drive upregulation of OTR on TG neurons. In addition, the study sought to determine whether exposure to inflammation would drive upregulation of the immediate early genetic markers of neuronal activation pERK and CFOS.

Supporting Data

Figure 1. Immunofluouresence micrograph of rat trigeminal ganglia. Red = oxyocin receptors Green = CGRP. Yellow = double-labelled cells.

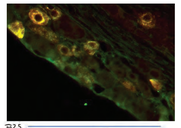


Figure 2. Rapid and sustained up-regulation of oxytocin receptor protein in rat trigeminal ganglia following facial shock induced inflammation.

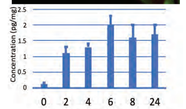


Figure 3. Enablement of nasal oxytocin induced craniofacial analgesia by pre-inflammation

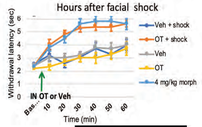


Figure 4. Immunoflourescent micrograph of human DRG showing the expression of OTR immunoreactivity prior to (A) and following (B) in vitro incubation with interleukin-6.

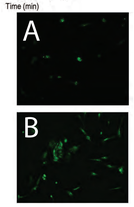


Figure 5. In vitro exposure of human DRG to IL-6 significantly ($p < 0.05$) increases OTR immunoreactivity (n = 5).

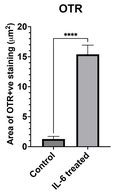
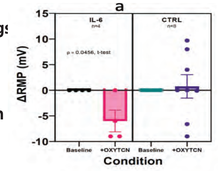


Figure 6. Current clamp recordings of human DRG. In vitro pre-incubation of DRG with IL-6 significantly ($p < 0.05$) increased hyperpolarizing effect of oxytocin (n - 5).



Methods

Human cadeveric trigeminal ganglia (TG) were provided by Anabios (San Diego). Each TG of a pair from a given donor was transected. One half of each TG was injected with 50 ul of either artificial interstitial fluid (aIF) and incubated in aIF as a control or 10ug/mL lipopolysaccharide (LPS) in artificial interstitial fluid (aIF) then incubated with LPS in aIF for 4 hours to induce inflammation.

The ganglia were then immersion fixed in 4% paraformaldehyde overnight. Fixed ganglia were cryo-sectioned (20uM), slide-mounted and incubated in primary antibodiesfor oxytocin receptors, CGRP, and the immediate early gene products c-FOS and p-ERK. Slides were then incubated with appropriate secondary fluorescent antibodies, coverslipped and examined using a laser confocal microscope which allowed semi-quantification of antigens.

Result Summary

This study examined several things: 1. Do human trigeminal ganglia neurons possess oxytocin receptors as do rats? 2. Is there co-expression of oxytocin receptors and CGRP in human TG neurons? 3. Is expression of oxytocin receptors and/or CGRP modulated by *in vitro* inflammation in human trigeminal neurons? 4. Does *in vitro* inflammation drive activation of human trigeminal neurons as indicated by early immediate gene expression?

Figure 7 demonstrates that, as in rats, human cadeveric trigeminal neurons express oxytocin receptors and that, most of these neurons co-express CGRP. Figure 8 demonstrates that, in human cadeveric trigeminal cell bodies, exposure to lipopolysaccharide in vitro dramatically increases the expression of both oxytocin receptors and CGRP. Finally, in figures 9 and 10, we found that exposure of human trigeminal ganglia to LPS also activated trigeminal neurons in that some cells expressed the immediate early genes cFos and p-ERK.

References

[1]Tzabazis, A., et al., Oxytocin receptor: Expression in the trigeminal nociceptive system and potential role in the treatment of headache disorders. Cephalalgia, 2016. 36(10): p. 943-50.

[2]Tzabazis, A., et al., Oxytocin and Migraine Headache. Headache, 2017. 57 Suppl 2: p. 64-75

Results

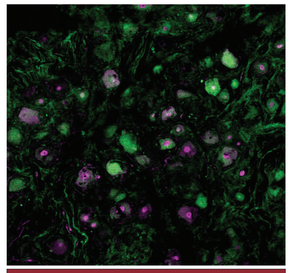


Figure 7. Immunoflourescence micrograph of human TG. Green represents OTR immunoreactivity; Purple represents CGRP immunoreactivity. Note numerous cell exprssing both

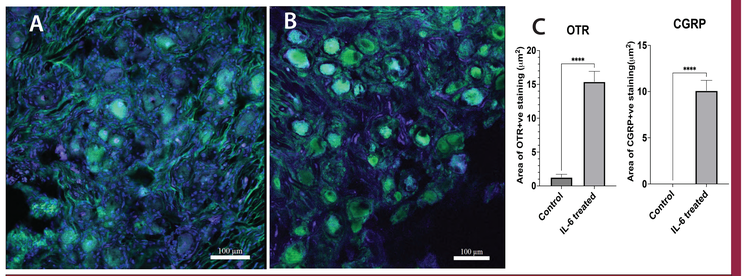


Figure 8. Immunoflourescence micrographs of human TG after exposure to artifical interstitial fluid (A) or lipopolysaccaride in aIF (B). In vitro exposure of TG tissue for 4 hours induced a significant increase in immunofluorescence of both oxytocin receptors and CGRP (C).

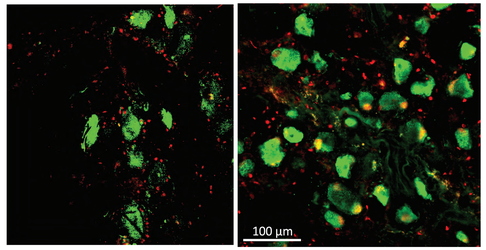


Figure 9. Immunoflourescence micrographs of cFos immunoreactivity in human TG TG after exposure to artifical interstitial fluid (A) or lipopolysaccaride in aIF (B). LPS exposure induced clear activation of cFos early immediate gene product, likely in satellite cells. Green represents OTR+ neurons.

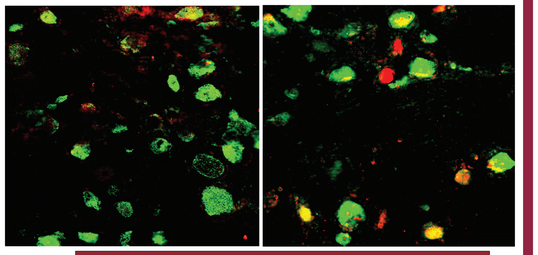


Figure 10. Immunoflourescence micrographs of pERK immunoreactivity in human OTR + TG neurons after exposure to artifical interstitial fluid (A) or lipopolysaccaride in aIF (B). LPS exposure induced clear activation of pERK early immediate gene product in neurons. Green cells are OTR + neurons.

Conclusions and Support

Oxytocin has been shown to inhibit the firing of sensory ganglia neurons and, when applied intranasally, to produce a craniofacial analgesic effect in rats as well as a headache abortive effect in chronic migraineurs. These effects appear to be mediated, in part, but the blockade of CGRP release from these neurons[2]. Data presented here demonstrates, for the first time, that oxytocin receptors are present on human trigeminal neurons and that there is a strong co-expression of CGRP in these neurons. Furthermore, in the presence of inflammation, which is persistently present in chronic migraine, there is a robust increase in the expression of both oxytocin receptors and CGRP in human trigeminal neurons as occurs in rat ganglia. Furthermore, these cadeveric neurons demonstrate viability in that they show clear activation in the presence of inflammation. Thus, the presence of inflammation plays a critical role in the analgesic efficacy of oxytocin and may may be a predictive biomarker of that efficacy.

Support provided by CDMRP W81XWH-21-1-0186 from DOD and a grant from Tonix Pharmaceuticals