IN VITRO IMPACT OF OXYTOCIN ON HUMAN SENSORY NEURONS

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Background

It has long been suggested that oxytocin (OT) could be used as a non-opioid analgesic. Underlying this potential is the finding that, when applied to rodent sensory neurons, OT produces clear inhibition of excitability. However, the potency of OT analgesic effects is dependent, in part, on the presence of a pre-existing inflammatory injury. This phenomenon, in turn, appears to be driven by response elements on the oxytocin receptor (OTR) promotor for the inflammatory cytokine IL-6. In support of this, we have found that inflammatory injury drives a rapid 5-10 fold increase in rat trigeminal ganglia (TG) OTR (Figure 1), enables oxytocin induced decrease in TG neuronal excitability as shown by increase in resting membrane potential (Figure 2, RMP) as well as craniofacial analgesia after intranasal OT.

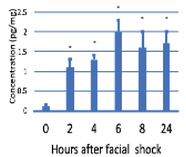


Figure 1. Increase in TG OTR protein after inflammation induced by facial shock[1]

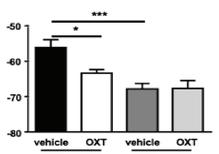


Figure 2. Potentiation of OT induced TG hyperpolarization by pre-existing inflammatory injury in rats[2]

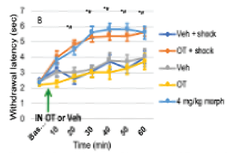


Figure 3. Potentiation of intranasal OT craniofacial analgesia by inflammatory injury[3]

In addition, magnesium ions bind to a pocket in the oxytocin receptor (Figure 4) which increases the affinity of OT for its receptor. Thus, the addition of Mg2+ to an oxytocin formulation enables substantially greater OT potency in terms of hyperpolarization of rat trigeminal neurons (Figure 5) and craniofacial analgesia after nasal application (Figure 6).

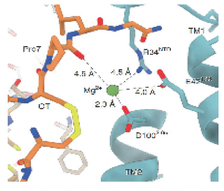


Figure 4. Cryo-EM derived model of OT binding to OT receptor, showing Mg2+ binding site.[4]

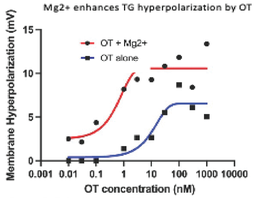


Figure 5. Potentiation of OT induced hyperpolarization of rat TG neurons by Mg2+[5]

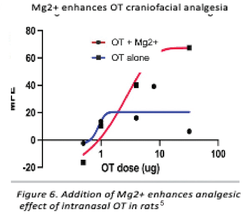


Figure 6. Addition of Mg2+ enhances analgesic effect of intranasal OT in rats[5]

Purpose of the Study

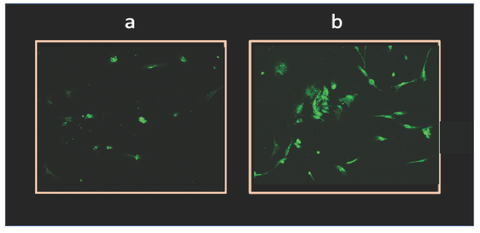
All of the data presented n the background has been generated in rats. Up to now, no direct evidence of inhibition of HUMAN sensory neurons by OT has been established. Thus, the purpose of this study was to examine 1: whether there are OTR on human sensory neurons and whether the expression of these OTR are enhanced by inflammation; 2: whether OT will inhibit the excitability of human sensory neurons and whether this effect is enabled by inflammation; 3) whether the Mg2+ concentration levels will modulate the inhibitory effect of OT on human sensory neurons.

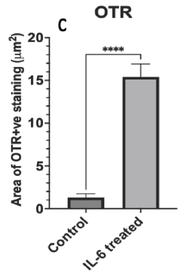
Methods

Fresh human dorsal root ganglia (DRG) neurons were obtained from cadaveric donors (Anabios), dissociated and plated on coverslips, Coverslips were then incubated for 18-22 hours in a solution containing buffer or IL6 Treat with IL-6 (5 X 10-7 M). Study 1: after rinsing, coverslips were incubated in primary oxytocin receptor antibody: goat polyclonal anti-oxytocin receptor (N-19; Santa Cruz Biotechnology, catalog # sc-810, antibody dilution 1:50; blocking peptide sc-8013p), followed by TRITC-conjugated rabbit anti-goat IgG (Abcam catalog # ab6738, dilution 1:200) as a secondary antibody. Confocal microscopy was used to identify and quantify OT receptor expression on DRG neurons. Study 2: 8 control and 4 IL-6 treated DRG neurons were recorded for 3 indices of excitability: RMP, rheobase, and number of action potentials following current injection. Study 3: The effect of OT on the same measures of DRG excitability were assessed in the presence of either 0.5 or 1.75 mM Mg2+ was determined in 10 neurons.

Results: Study 1

Figure 7a and b demonstrate for the first time, that OTR are widely distributed on human DRG neurons. In addition, Figure 7b demonstrates that, following IL-6 incubation, there is a dramatic increase in OTR expression. This increase is semi-quantified in figure 7c, which shows a significant (****p<0.0001) increase in area of OTR staining following IL-6 incubation.





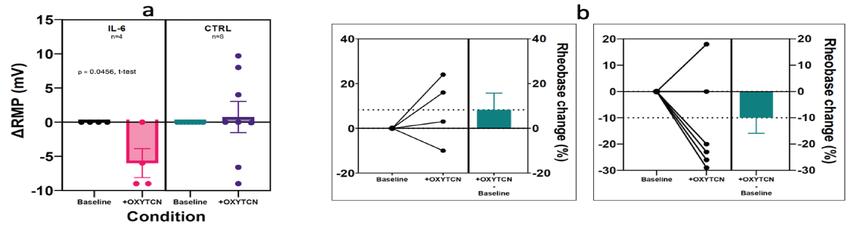
Summary and Conclusions

- Oxytocin receptors are present on HUMAN sensory neurons
- The inflammatory cytokine IL-6 drives OTR expression increase in these neurons
- IL-6 also enables OT decreases in excitability of human DRG neurons
- 1.75, but not 0.5 mM Mg2+ increases the potency of OT in terms of inhibiting the excitability of human DRG neurons

The results of these studies represent the direct evidence for oxytocin receptors on human sensory neurons and the first demonstration of inhibition of human sensory neurons by oxytocin. These results also demonstrate that, as in rodents, the presence of pre-existing inflammation and a "higher than physiological" concentration of Mg2+ are essential to OT efficacy and potency.

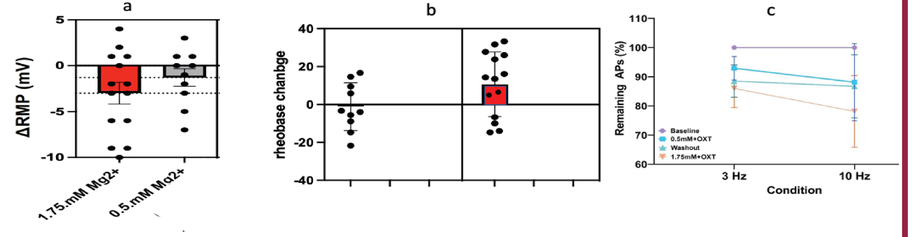
Results: Study 2

Figure 9 demonstrates the impact of IL-6 incubation on the efficacy of OT in decreasing human DRG neuronal excitability. a. IL-6 incubation, significantly (p = 0.0456) increased RMP when compared to control media. b. OT increased rheobase after iL-6 treatment, but control media treatment had no clear effect. c. OT after IL-6 pretreatment decreased following action potentials with a 10Hz stimulus, whereas control media did not.



Results: Study 3

Figure 10 demonstrates the impact of different Mg2+ media concentrations on the efficacy of OT in decreasing human DRG neuronal excitability. All cells were pretreated with IL-6. a. OT in a 1.75 mM Mg2+, but not 0.5 mM Mg2+ media significantly (p = 0.3356) increased RMP when compared to control media. b. OT increased also rheobase in 1.75 mM Mg2+, but this difference did not reach significance (p = 0.055). c. OT IN the presence of 1.75 mM Mg2+ decreased following action potentials normalized to baseline. This effect was particularly evident for higher frequency (10 Hz) when compared to lower frequency (3 Hz) stimulation.



Acknowledgements and References

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