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RESEARCH

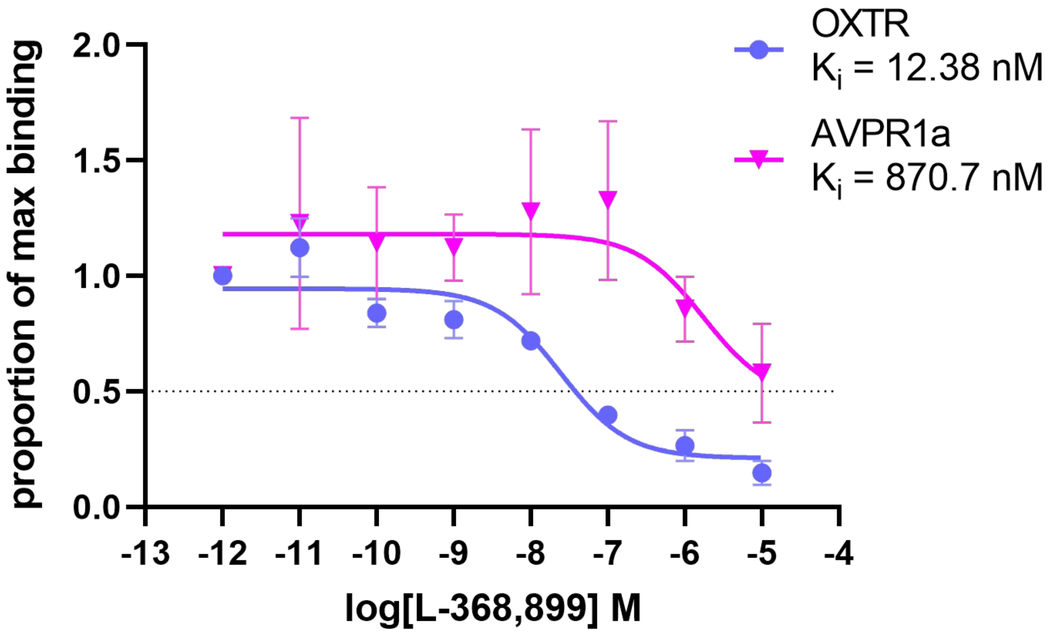
Pharmacological Characterization of the Oxytocin Receptor Antagonist L-368,899 for Coyote Receptors

Introduction

- Oxytocin is a hormone that mediates multiple social behaviors, social recognition, maternal behaviors, and pair-bonding.
- Coyotes display monogamy. In other mammalian models, oxytocin has been shown to influence this behavior.
- Oxytocin and vasopressin's structural similarities result in binding promiscuity -- both molecules bind to both receptors.
- We determined the binding affinity of the oxytocin receptor antagonist, L-368,899 using competitive binding autoradiography.
- **If this antagonist effectively blocks the coyote oxytocin receptor without effectively blocking the vasopressin 1a receptor, it can be used in live coyotes to study oxytocin-dependent social behaviors.**

Methods

- 6 Frozen coyote brains were blocked into slabs and stored at -80C until sectioning.
- Brain sections were sliced using a cryostat at 20 micron thickness, and mounted on microscope slides.
- Competitive binding autoradiography was performed with increasing concentrations of the oxytocin receptor (OXTR) antagonist L-368,899 in competition with a consistent concentration of the commercially available radioligands: 1) OXTR radioligand ^{125}I-ornithine vasotocin analog (^{125}I-OVTA) and 2) vasopressin 1a receptor (AVPR1a) radioligand ^{125}I-linear vasopressin antagonist (^{125}I-LVA).
- After quantifying the binding densities, we generated a competition curve, which calculated binding affinity (K_i).



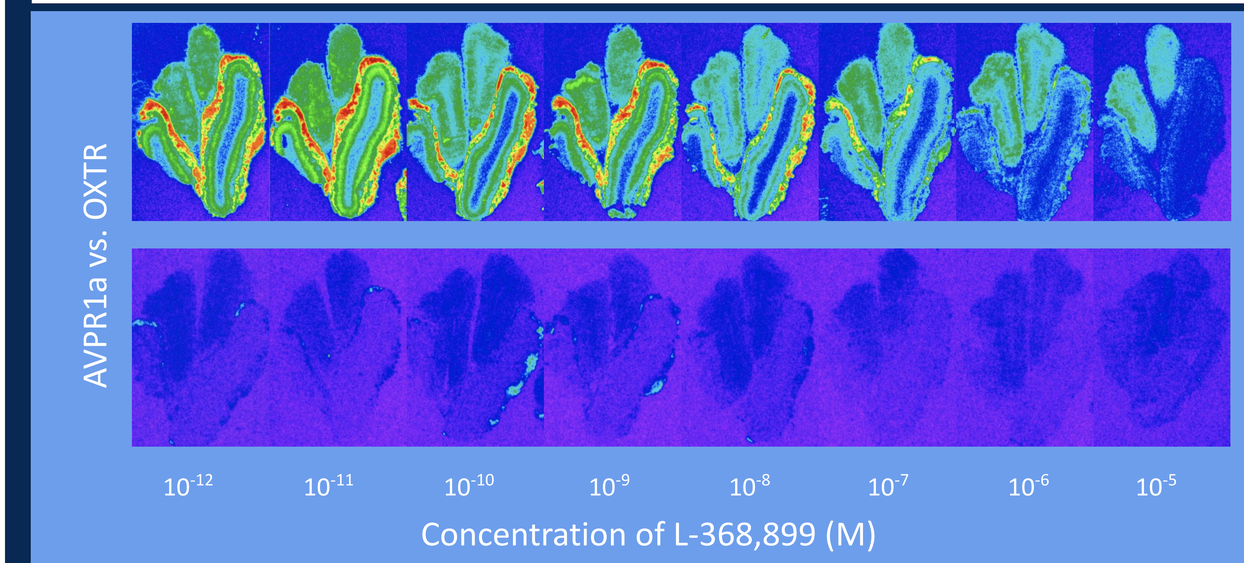


Figure 1 (top)- Binding selectivity competition curves.
Figure 2 (bottom)- Radioligand binding with increasing concentrations of antagonist L-368,899.

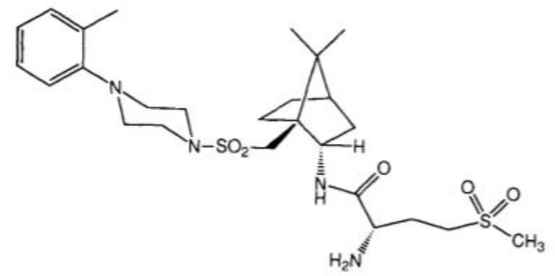


Figure 3- L-368,889 chemical structure.

Results

- Slides exposed to OXTR radioligand exhibited the expected decline in binding as the concentration of L-368,899 increased.
- Slides exposed to AVPR1a radioligand did exhibit some binding density reduction with increased L-368,899, but these slides did not develop as we expected; many were too faint for quantification.
- Based on K_i values, OXTR 12.38 nM and AVPR1a 870.7 nM, **L-368,899 showed a 70x greater binding affinity for oxytocin.**
- ½ of max binding was identified at ~ $10^{-7.5}$ for OXTR. At this same concentration, the antagonist was incapable of competing off the radioligand binding to AVPR1a.

Conclusion

- Increasing concentrations of the antagonist, L-368,899 increasingly inhibits binding of OXTR and AVPR1a radioligands. **L-368,899 is more selective for oxytocin receptors than vasopressin 1a receptors.**
- Lower concentrations of L-368,899 out-compete binding of the oxytocin radioligand, effectively blocking these receptors while leaving vasopressin 1a receptor binding undisturbed.
- We believe variability in AVPR1a selectivity is due to an error in our experimental design. Replication of our experiment is needed to have full confidence in L-368,899's selectivity for future *in vivo* experiments.

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