Comparing L-368,899 and ALS-III-61 as human-selective oxytocin receptor antagonists

Blake Nielson and Matthew Webb, *Utah State University* | Dr. Sara Freeman, *Utah State University*

UtahStateUniversity
DEPARTMENT OF BIOLOGY

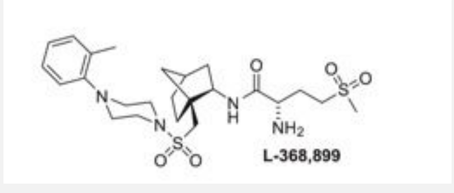
Introduction

- Oxytocin and vasopressin are two hormones that act in the brain to modulate social functions in both humans and animals.

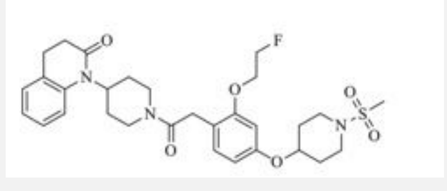
-Oxytocin acts in the brain by binding to the oxytocin receptor (OXTR), a G-protein coupled receptor. Oxytocin also has an affinity for vasopressin 1a receptors (AVPR1a), since oxytocin and vasopressin have similar chemical structures.

-Therefore, it is important to develop and validate selective drugs to target each of these receptors for research purposes.

-Compound L-368,899 (or the "Merck compound") is a commercially-made drug used as an oxytocin antagonist, but there is limited evidence for its effectiveness.



-ALS-III-61 (or the "Smith compound") is a novel oxytocin antagonist that was provided by a collaborator, who has changed careers and is no longer synthesizing it .

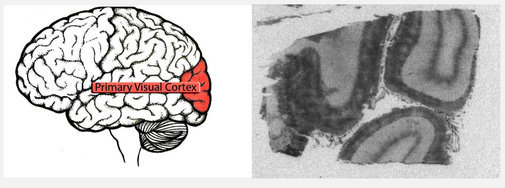


Methods

-Fresh frozen, postmortem human brain specimens (provided by the NIH NeuroBioBank) were sliced using a cryostat at -15°C at 20 µm sections and mounted to microscope slides.

-The human substantia nigra is known to contain a high amount of OXTR and was used as our source for OXTR: .

-The human primary visual cortex is known to contain a high amount of AVPR1a and was used for AVPR1a: .



-We used competitive binding autoradiography; increasing concentrations of each competitor ligand (either the Merck or Smith compounds) were incubated on the tissue in the presence of a constant concentration of one of the commercially available radioligands: ^{125}I-ornithine vasotocin analog for OXTR and ^{125}I-linear vasopressin antagonist for AVPR1a. We then measured radioligand displacement.

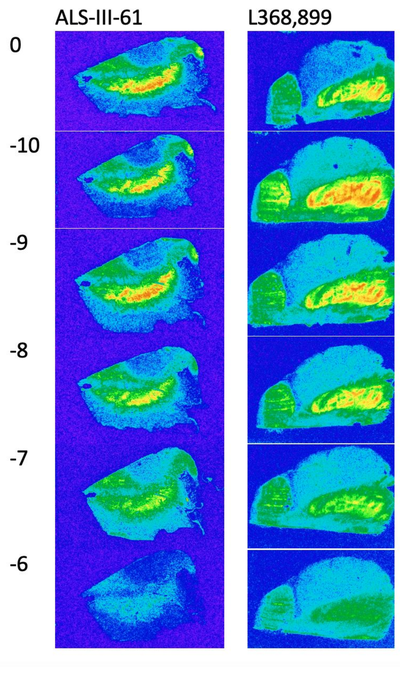


Figure 1 (left) – Human brain sections of the substantia nigra showing the ability of competitor ligands ALS-III-61 and L-368,899 at increasing concentrations to displace the OXTR radioligand.

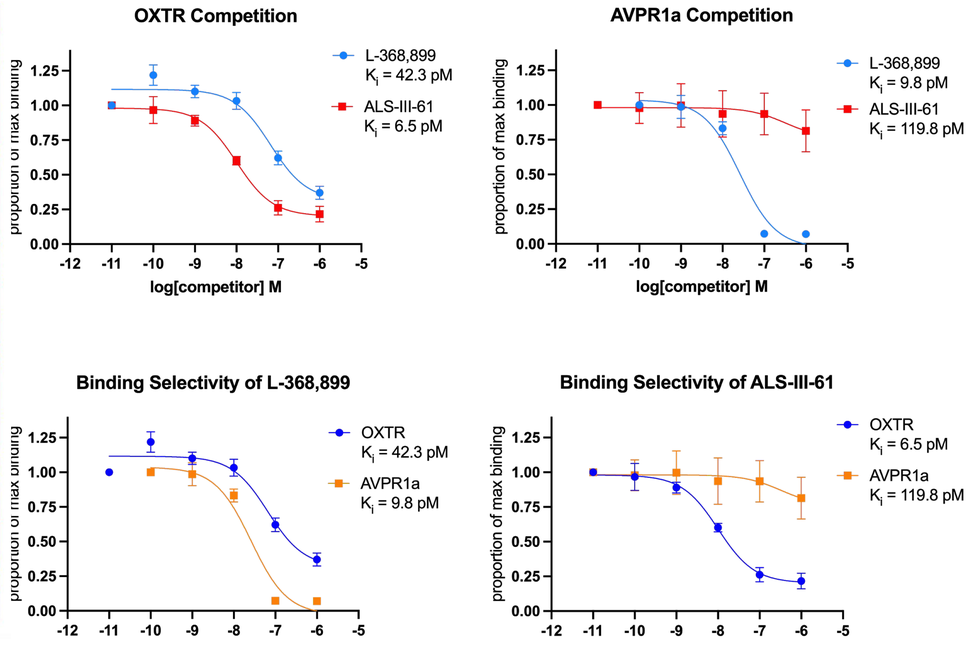


Figure 2 (right) – Competitive binding curves comparing both antagonists for OXTR and for AVPR1a (top); and binding selectivity curves for each antagonist for both receptors (bottom).

Results

ALS-III-61 has 6.5x higher binding affinity for OXTR than L-368,899 has for OXTR.

L-368,899 binds with 4.3x higher affinity to AVPR1a than to OXTR.

ALS-III-61 binds with 18.4x higher affinity to OXTR than to AVPR1a.

Conclusions

Because our data shows that L-368,899 has a 4.3x higher affinity to AVPR1a than to OXTR, **we do not recommend using L-368,899 as an antagonist for OXTR-related experiments.** We recommend the continued use of ALS-III-61 for such experiments due to its higher selectivity to OXTR.

Due to some unexpected assay results, we wish to repeat this experiment to confirm our findings.

References:
1.Aaron L. Smith, Sara M. Freeman, Ronald J. Voll, et al. Carbon-11 N-methyl alkylation of L-368,899 and in vivo PET imaging investigations for neural oxytocin receptors Bioorganic & Medicinal Chemistry Letters 23 (2013) 902–906

2. Aaron L. Smith, Sara M. Freeman, Jeffery S. Stehouwer, et al. Synthesis and evaluation of C-11, F-18 and I-125 small molecule radioligands for detecting oxytocin receptor. Bioorganic & Medicinal Chemistry Volume 20, Issue 8, 15 April 2012, Pages 2721-2738.