CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
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Project Number
S1815

Project Title
Sites of Ethanol Action in P2X4 Receptors

Abstract

Objectives/Goals
The site(s) of ethanol action in P2XRs are unknown. Through site-directed mutagenesis we have been able to identify an important amino acid within the transmembrane (TM1) domain that alters ethanol sensitivity of P2X4Rs. The residue at position 46 within the TM1 domain of P2X4Rs was mutated to amino acids with different physical-chemical properties. The wildtype (WT) and mutant receptors were then expressed in Xenopus laevis oocytes and tested for changes in ethanol sensitivity (200 mM) using two-electrode voltage clamp electrophysiology. Mutating tryptophan at position 46 to alanine (W46A) in P2X4Rs reversed the action of ethanol (inhibition to potentiation). Exchanging W46 residue with other aromatic residues did not significantly alter ethanol sensitivity whereas replacing W46 with aliphatic residues significantly reduced the action of ethanol. Taken together, these findings suggest that physical-chemical properties of the residue at position 46 in the TM1 domain may play an important role in ethanol action of P2X4Rs.

Methods/Materials
To conduct the ethanol sensitivity experiments, currents were generated by applying the P2X4R agonist (ATP at EC10 concentration) for 20 seconds. Upon observing a stable response with the EC10 and allowing a 5 minute washout time, a concentration of ethanol was co-applied with ATP for 20 seconds.

Results
Since the substitution at position 46 (W46A) completely eliminated the effects of ethanol, we extended our study by investigating the role of physical-chemical properties of the residue at this position on ethanol sensitivities.
The results suggest that physical-chemical properties of the residue at position 46 in the TM1 domain may play an important role in ethanol sensitivity of P2X4Rs.

Conclusions/Discussion
The ability to eliminate the effect of ethanol by mutating W46 to alanine indicates that position 46 plays an important role as a target for ethanol action in P2X4Rs.
Identification of specific residues (e.g., W46A) in which the mutation abolishes the action of ethanol without significantly altering receptor function can be used in future studies to investigate the role of P2X4Rs in ethanol-induced behaviors. A better understanding of the sites of ethanol action may help us to unravel the complexity of ethanol sensitivity of native P2XRs in the CNS.

Summary Statement
I am attempting to identify the positions in the TM1 domain of the P2X4 receptor where the effects of ethanol are present.

Help Received
Mentor, Letisha Wyatt, guided me and taught me the proper techniques required for the Two-Electrode Voltage Clamp. Lab equipment and supervision was provided by the University of Southern California's School of Pharmacy. Student of the Bravo STAR II program.