CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
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Project Number
S0405

Project Title
Investigating Ethanol's Effects on GABA-A Receptors Expressed in Xenopus laevis Oocytes

Abstract

Objectives/Goals
Alcohol is the most widely abused drug in the United States today and although we know what ethanol can do to the brain, we are barely beginning to understand its molecular mechanisms. Using oocytes (unfertilized frog eggs) from the Xenopus laevis frog, our lab is able to study ligand-gated ion channels that mediate ethanol activity and the intitial binding sites of ethanol. The objective of this experiment was to see if pressure is an ethanol antagonist on GABA-A Receptors.

Methods/Materials
The frogs used are Xenopus laevis frogs, more commonly known as the South African clawed frog. The oocytes are surgically removed from the Xenopus laevis frogs by our lab technician twice every week. The proposed experiment will utilize cDNAs expressed in Xenopus oocytes. Female Xenopus laevis frogs will be used as the donor source of oocytes for electrophysiological assays. This approach minimizes the use of animals by conducting experiments in unfertilized oocytes. The frogs that undergo surgery are allowed to recover for one week after surgery before being reintroduced into their normal tank. The frog that undergoes surgery is only allowed to have surgery 5 times, with each surgery capped at once every 4 months. After the 5 surgeries, the frog is euthanized according to USC biological safety guidelines approved by the State of California. After oocytes are removed from the Xenopus laevis frog, the follicular layer is removed which allows easy injection of the cDNA and is important for accurate two-electrode voltage clamp readings. The oocytes are injected with GABA-A cDNA and left in an incubation medium for 48-72 hours. Afterwards, these oocytes are individually voltage clamped and exposed to different drugs. When finished, the oocytes are safely disposed of according to the University of Southern California's regulations.

Results
Ethanol potentiation under normal conditions shows a 60% effect, while under pressure, ethanol potentiation is around 35%.

Conclusions/Discussion
Pressure does not physiologically modify the GABA-A receptor in any way and can be seen as a direct agent. Pressure is a direct ethanol antagonism on GABA-A receptors.

Summary Statement
The synthesis of GABA-A receptors on Xenopus laevis oocytes allow me to test pressure#s role in ethanol antagonism.

Help Received
I used lab equipment at University of Southern California#s School of Pharmacy under the supervision of Dr. Daryl L. Davies.