The Behavioral and Resting Membrane Potential Responses to Acute and Chronic Ethanol Exposure

JAMES FAUSTO, LISA HABERL and RASHMI TIWARI
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT
The effects of EtOH on the crayfish Procambarus clarkii were examined behaviorally in vivo and electrophysiologically in vitro. Acute and chronic effects of EtOH exposure were monitored over a 14-day period. We verified the behavioral tolerance response as a result of chronic exposure, as well as a physiological correlate through measurements of resting membrane potential. We found significant effects ($p = 0.0001$) of acute and chronic EtOH exposure on the resting membrane potential. Initial acute exposure showed a drop in membrane potential (accounted for as the efflux of potassium). Chronic exposure resulted in a return to control resting membrane potential suggesting an effect on either the influx of calcium or sodium. Further research is needed to resolve this.

INTRODUCTION
Resting membrane potential (RMP) is an indicator of the relationship between many intra- and extracellular factors and can be used to understand the cell and its interactions with its environment, and ultimately, cell health. The plasma membrane, a selectively permeable structure, is a barrier that helps maintain the delicate balance of ions and proteins. Ion transport is one factor that controls this balance by altering ion gradients. The membrane permits several paths for ion flow such as simple diffusion, facilitated diffusion, and active transport. The culmination of these transport pathways is reflected in the RMP. The study of RMP is then important because it reveals an overarching consensus of ionic activity within the cell, which can be used as a starting point for more detailed studies.

Disruption of ion concentrations can produce harmful consequences in the fragile cell environment. Ethanol is one substance that has been shown to affect biological membranes (Rubin and Rottenburg, 1983), and it is the intention of this study to determine if ethanol causes alterations in ion flows, which can be detected as a change in the RMP. Often, physiological studies using alcohol attempt to correlate behavioral responses to draw conclusions about the holistic effect on the organism. Crayfish present an ideal system of study for these types of experiments because they exhibit many of the same axonal and cellular properties as mammals (Blundon and Bittner, 1992), without the complexities associated with the study of mammalian systems.

Procambarus clarkii, has shown behavioral (Friedman et al., 1988) and physiological (Blunden and Bittner, 1992) responses to both acute and chronic exposure to ethanol. However, no research has specifically studied the correlation between behavioral and RMP responses. Thus, to begin the study of this new correlation, we
studied the effect of EtOH on RMP in a crayfish muscle. We found that acute exposure to EtOH (75 mM) resulted in a decreased behavioral righting ability and a decrease of RMP, while chronic EtOH exposure showed signs of behavioral tolerance as well as physiological tolerance, noted by a return to normal resting potential.

MATERIALS AND METHODS

Treatment

We conducted a three week study investigating acute and chronic effects of alcohol on eight crayfish Procambarus clarkii (two control and three sets of two as treatment). The experimental group was bathed in a 75mM EtOH solution for varying lengths of time. We treated the different sets to one day, seven day, and fourteen day exposure period. To prevent aggression, we separated each crayfish in plastic flower pots. The EtOH bathing solution was aerated constantly and changed every other day to ensure ethanol concentration was maintained.

Behavioral Observations

To assess inebriation, we used the standard righting response (Friedman et al. 1988). The crayfish was placed on its dorsal surface, and the amount of time needed to flip ventrally was recorded within a two minute limit. Each crayfish was tested three times, with a one minute reacclimation period between trials.

Physiological Observations

We dissected out the medial fast extensor muscles from the tail of a crayfish and measured membrane potentials via microelectrodes using procedures outlined in the MacLab HyperCard.
Stacks (Stephens 1996). We recorded five membrane potential readings in each crayfish tail preparation. Data were analyzed using MiniTab to perform an ANOVA.

RESULTS

Behavioral effects of EtOH on crayfish righting response

Acute ethanol exposure within the crayfish resulted in an increased righting response time relative to control crayfish (3.5 secs.) (Fig. 1). After a one-day exposure period the crayfish were unable to right themselves within the 120-second time limit. The seven-day exposure showed the onset of behavioral tolerance, as evident by the decreased righting response time (93.35 secs.). By day fourteen, the crayfish were able to right themselves within 31.15 seconds demonstrating further signs of behavioral tolerance to EtOH. An ANOVA analysis of the righting response times showed these results were highly significant (p = 0.0001).

The Effects of EtOH Exposure on Resting Membrane Potentials

Having established a behavioral response to EtOH exposure, we proceeded to study the effect of EtOH on RMP. Resting membrane potential showed significant effects to both acute and chronic ethanol exposure in the crayfish (ANOVA, p = 0.0001) (Fig. 2). Control crayfish exhibited a RMP of -57.56 mV. In response to acute ethanol exposure (1 day) the RMP decreased to -78.80 mV, which suggests changes in ion activity. A compensatory increase in RMP was noted at the 7-day exposure (-49.91 mV). This apparent overshoot in RMP was later reduced to -59.49 mV at the 14-day exposure, suggesting a possible development of physiological tolerance within membrane to ethanol.

DISCUSSION

Behavioral Effects of EtOH on Crayfish Righting Responses

Previous studies have shown...
that acute and chronic exposure to EtOH results in predictable behavioral responses within crayfish. The acute exposure causes a depressed ability to respond to stimuli, while chronic exposure results in the eventual appearance of a behavioral tolerance noted by a near return to original responses that occurred prior to EtOH exposure (Friedman et al. 1988; Blundon and Bittner 1992). Our results replicate this trend (Figure 2).

The 24 hour exposure to EtOH produced an intoxication that completely prevented the crayfish from being able to right themselves (even though Figure 2 shows a 2 minute period of righting, this value actually represents the maximum time the crayfish were given to right themselves). Friedman et al. (1988) found a similar result in which they note that 24-hour exposure to EtOH (75-150 mM) produced crayfish intoxication measured by significant increases in righting response times.

Further study of Figure 2 shows possible signs of tolerance in the crayfish as evident by the declining time needed for the crayfish to right themselves as EtOH exposure increased. Again, Friedman et al. (1988) noted a similar response saying that "[a]fter two weeks of chronic exposure to 75 mM EtOH, crayfish showed behavioral tolerance as noted by a decrease in righting time...". Interestingly, Blundon and Bittner draw a parallel between the crayfish model and mammalian systems when they make note of H. J. Little’s (1991) finding that mice are unable to right themselves after EtOH blood levels reach 65 mM (1992).

Basically, these results reiterate prior studies findings and thus show strong support for the previously noted behavioral responses seen in crayfish to acute and chronic EtOH exposures.

The Effects of EtOH Exposure on Resting Membrane Potentials

Ions cross the membrane through selective channels and produce a charge imbalance, measured as membrane potential. For example, potassium ions move down their concentration gradient from inside to outside, leaving behind unbalanced negative charges (hence, the negative resting potential). Similarly, sodium selective channels open to permit sodium ions to enter the cell, producing a different imbalance where positive charges accumulate to exceed negative charges (seen in the upstroke of the action potential) (Zigmond et al. 1999).

It has recently been demonstrated that EtOH has a specific effect on membrane proteins without affecting membrane fluidity (Tapia et al. 1998). The effect on the resting membrane potential, therefore, can be correlated specifically with changes in ion channels. If the ion channels are affected by the exposure to ethanol, the resting membrane potential will reflect this change. As seen in our data, acute exposure results in a slight decrease in the resting membrane potential. Since this altered resting membrane potential has a value close to the equilibrium potential of potassium, it is possible that EtOH has increased the permeability of the cell membrane to potassium.

During chronic exposure (longer than 7 days) the resting membrane potential increases. This might suggest that the membrane is more permeable to either sodium or calcium. Sodium is a smaller ion and its equilibrium potential is less positive than calcium. Moreover, the calcium channel must be extremely selective if it is to allow calcium influx rather than general cations. This observation is based on the knowledge that calcium ions are vastly outnumbered by other cations in the medium outside the channel.
Furthermore, calcium is known to play an integral role in the release of neurotransmitters, which regulate homeostasis or toxicity within a given cell. Therefore, one would expect that the selectivity of the calcium channel would be maintained over the sodium channel.

Alternatively, other evidence suggests that calcium leaks into the cell, increasing the resting membrane potential; a study conducted by Rutten and Moore (1991) further supports this assertion. They demonstrated that low doses of ethanol and calcium ionophores have similar effects on changes in the resting membrane potential. Additionally, they concluded that the hyperpolarizations seen as a result of EtOH exposure were mediated through changes in the level of intracellular calcium. Clearly, calcium could be an important player in mediating EtOH response at the cellular level.

Based on our findings, we see the need to determine exactly which channels are affected by acute and chronic exposure to EtOH. By selectively blocking ion channels, it would be possible to determine which channels are imperative in mediating EtOH interaction. A variety of channel blockers could be easily employed in further studies: TTX to block sodium channels, TEA to block potassium channels, and magnesium to block calcium channels.

REFERENCES


Stephens, P.J. 1996. Teaching Physiology with MacLab and Mac: the Crayfish superficial flexor muscle. HyperCard 2.1
