Chronic Exposure to RoundUp® Ready to Use Weed and Grass Killer Increases the Resting Membrane Potential of Crayfish Lateral Extensor Muscle Cells

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ABSTRACT
Agricultural runoff is a significant source of pollution of aquatic environments. This is of substantial concern to areas dependent on commercial farming, specifically the rural area of Grinnell, Iowa. It has previously been determined that chemical toxicity results in an increase in resting membrane potential in affected mussel muscle cells. Our research investigated the possibility that chemical toxicity can be measured in this manner in the lateral extensor muscles of crayfish, an animal native to the Grinnell area. We hypothesized that increasing concentrations of RoundUp® Ready to Use Weed and Grass Killer, an herbicide commonly used in farming, would result in an increase in resting membrane potential.

We exposed crayfish to environments containing various concentrations of RoundUp® (0.1%, 0.5%, and 1.0%) for 70h and recorded the resting membrane potential of the right lateral extensor muscle. For each concentration we compared results against resting membrane potentials collected from control subjects raised in Grinnell tap water. Our results showed that environmental concentrations of 1.0% of RoundUp® caused significant increases in resting membrane potential compared to control subjects raised in Grinnell tap water.

INTRODUCTION
Agricultural runoff often results in contamination of both animal habitats and human drinking resources, creating a potential threat for both populations. Pesticide residuals have been found in groundwater in many communities, especially in rural areas (Eitzer and Chevalier 1999). The large agricultural population of central Iowa makes this of specific concern for the inhabitants of Grinnell, Iowa, the residence of Grinnell College. While human drinking water is carefully processed before consumption, freshwater contamination is still a threat for the inhabitants in the streams and ponds that receive agricultural runoff. In addition to the chemical presence in water, pesticide residues have also been found in bottom sediments of such aquatic systems (Kreuger et. al 1999). This provides an
additional source from which the inhabitants may come into contact with chemical contaminants.

This contact with contaminants threatens the health of the animals through alterations to cellular components. According to Borseth et. al (1992) one component that agricultural pollutants affect is the sodium gradient of mussel muscle cells. The alteration of this gradient, in turn, modifies important physiological processes such as the generation of action potentials, the accumulation of amino acids, and the cellular extrusion of calcium (Borseth et. al 1992). The induced increase in intracellular sodium concentration results in an increase in the membrane potential of the cell. From this finding, Borseth et. al (1992) concluded that increases in membrane potential could be a useful indication of chemical toxicity.

The toxicity of various types of pesticides has been researched in many areas of the world, but no information is known on the effects of pesticides commonly used in the Grinnell area. RoundUp® Ready to Use Weed and Grass Killer is one of the most popular herbicides among central Iowa farmers and therefore serves as an ideal agent for our investigation. As previously mentioned, chemical runoff is a recognized problem for aquatic wildlife in agricultural areas. The crayfish is an organism commonly found in Iowa freshwater streams, residing not only in the open water but also in the sediments of streams. Thus any pesticides present in either of these substrates may affect the organism. We hypothesize that exposure to increasing concentrations of RoundUp® will be increasingly toxic to crayfish subjects and result in a measurable increase in resting membrane potential of the medial extensor muscles.

MATERIALS AND METHODS

Crayfish. All crayfish (Carolina Biological Supply, Greensborough, NC) were kept in aquariums of either Grinnell tap water (control) or tap water with the respective concentration of herbicide (experimental) for 70h and chilled on ice before data collection for 15 minutes to achieve appropriate anesthesia. Two experimental crayfish were tested from each of the three RoundUp® #7008 contaminated solutions: 0.1%, 0.5%, and 1.0%. Two control crayfish were also observed in conjunction with each experimental group to account for any variations between shipments of crayfish specimens.

Dissection and membrane potential recording.

The subjects were prepared and membrane potential recordings were taken from the third segment of the right lateral extensor muscle as outlined by Stephens (1996) on the Macintosh Scope. During dissection and recordings the specimens were bathed in crayfish saline. Ten resting membrane potential readings were recorded in succession from different locations within the muscle segment on each subject; the most negative reading observed during each insertion of the microelectrode served as the reported data point.

Data analysis.

The data collected were analyzed using Excel 98 to obtain
RESULTS

We investigated the effects of chronic chemical exposure on the resting membrane potential of crayfish muscle cells. We exposed our experimental groups to three different concentrations of RoundUp® herbicide and compared their resting membrane potentials to those of control crayfish raised in Grinnell tapwater. We found that the resting membrane potential of the experimental crayfish increased (became more positive) as the herbicide concentration increased, with a significant difference between the experimental and control groups at 1% (p<0.05; resting membrane potentials: control, -70mV; 1% RoundUp®, -49mV) (Fig. 1). The 0.5% concentration showed no significant difference (resting membrane potentials: control, -52mV; 0.5% RoundUp®, -52mV), while the 0.1% showed a significantly higher resting membrane potential for the control than the experimental groups (p<0.05; resting membrane potentials: control, -44mV; RoundUp®, -60mV).

DISCUSSION

Our research showed that increases in herbicide (glyphosate) concentration (1.0%) produced increases (more positive) in the resting membrane potential of lateral extensor muscle cells of crayfish. According to Borseth et al. (1992), this increase in membrane potential is a result of an increase in intracellular sodium concentration, caused by the presence of toxin in the environment, and is a valid indication of chemical toxicity. Previous studies have shown that herbicides inhibit the action of adenosine triphosphatase (ATP), thus inhibiting the sodium/potassium pump, which is
active in maintaining the sodium gradient across the cell membrane (Delrot et al., 2000). The hydrolysis of ATP to adenosine diphosphate (ADP) and an inorganic phosphate releases the energy needed to fuel the sodium/potassium pump, which moves three sodium ions out of the cell while two potassium ions are allowed to enter the cell (Kandel and Schwartz, 1985). This process creates a sodium concentration gradient with a much higher concentration outside the cell. With the inhibition of ATP, the cell accumulates intracellular sodium, thus eliminating the efficacy of the gradient in important physiological processes such as the generation of action potentials, the accumulation of amino acids, and the cellular extrusion of calcium.

We also observed a significantly higher resting membrane potential for the control group than the experimental group at 0.1%. Since the concentration of herbicide was low enough to not produce an effect on the experimental crayfish, we attribute this difference to the individual subjects from which we collected data. There was no significant difference observed between the control and experimental groups at 0.5%, therefore the concentration of RoundUp® was still below that needed to affect the resting membrane potential.

In addition to the effects that we studied, herbicides have also been observed to inhibit the activity of acetylcholinesterase, which plays an important role in the functioning of the nervous system. As the neurotransmitter acetylcholine is released into the synaptic cleft, acetylcholinesterase acts to hydrolyze the neurotransmitter and thus stop the signal being sent across the synapse. The inhibition of acetylcholinesterase would then allow for excessive amounts of acetylcholine to reach the postsynaptic receptors, resulting in abnormal respiration, feeding, and social interaction (Kandel and Schwartz, 1985).

Although we found significant differences in resting potential between crayfish exposed and not exposed to RoundUp®, more research should be done to relate our findings to the effects of herbicides in the environment. We measured significant increases in resting membrane potential in crayfish exposed to 1.0% concentration of herbicide, but this concentration may not be the minimal amount of herbicide required to observe a significant change in membrane potential. It is also unknown whether this amount is comparable to concentrations found in the environment or if the cellular damage to the organism is equivalent. More research is needed to determine the herbicide concentrations found in the Grinnell area, as well as the minimal amount of toxin required to produce significant effects on the exposed crayfish.

While our research focused on the effects of a specific herbicide, other environmental pollutants result in similar cellular toxicity. One such example is caffeine, which inactivates the sodium/potassium pump, mentioned previously, by blocking the sodium and potassium channels. Caffeine also acts as an antagonist to GABA receptors (Kardos and Blandl, 1994; Mukhopadhyay and Poddar, 1995). This effect is significant with respect to the increasing problem...
of human contamination of natural habitats. Along with our results, this finding indicates the strong need for improvement in not only agricultural runoff prevention, but also in the overall environmental awareness in order to maintain a hospitable environment for all native organisms.

REFERENCES


Stephens, Philip J (1996) Teaching Physiology with MacLab and Mac: theCrayfish superficial flexor muscle. HyperCard 2.1