EPG RECORDINGS AS A NEW TOOL FOR TOXICOLOGY IN C. elegans

USING THE SCREENCHIP SYSTEM - BY DR. JANIS C. WEEKS, PH.D

PURPOSE

The NemaMetrix ScreenChip System is a microfluidics platform for recording electropharyngeograms (EPGs) from the nematode worm Caenorhabditis elegans and other species. C. elegans is a well-validated experimental subject for toxicological research\(^1\) with which to investigate the physiological effects of specific toxic substances and genes that influence sensitivity or resistance to these substances. C. elegans also provides a whole-animal system for assaying the toxicity of environmental soil or water samples. The U.S. National Institute of Environmental Health Sciences has highlighted C. elegans as a valuable system for toxicological research\(^2\). Various endpoints have been used to detect toxic effects, including development, reproduction, induction of stress-related genes, lethality and—relevant to the ScreenChip system—pharyngeal pumping. Toxicants that inhibit pumping in C. elegans include heavy metals, insecticides, organophosphate pesticides and cyanobacterial toxins. Here we used EPG recordings to investigate toxic effects of the heavy metal, copper (Cu\(^{2+}\)), which inhibits pharyngeal pumping\(^3\). Exposure to high levels of Cu\(^{2+}\), e.g., from corrosion of copper pipes by acidic water, is likewise toxic to humans and can damage the liver and kidneys\(^4\). We found that microfluidic EPG recordings detected quantitatively the presence of Cu\(^{2+}\) in aqueous samples at concentrations within the range found in contaminated home water supplies.

RESULTS AND DISCUSSIONS

Experiment 1 was performed using 8-channel microfluidic EPG chips with perfusion capability\(^5,6\). Fig. 1A shows pharyngeal pump frequency in control solution (K medium + 10 mM 5HT), followed at \(t = 0\) min by switching the perfusate to the same solution containing a range of Cu\(^{2+}\) concentrations. In control worms (black line), pump frequency remained steady over time, at ~ 4 Hz (pumps/s). In response to Cu\(^{2+}\) exposure, pump frequency decreased in a concentration-dependent manner, with inhibition apparent within 5 min at the higher concentrations. Steady-state pump frequency, defined as the mean frequency between \(t = 30\) and 60 min, was plotted in Fig. 1B to derive an IC\(_{50}\) value (the concentration that
caused a 50% reduction in pump frequency) of 49 mg/L Cu^{2+}. For comparison, a prior study reported an IC_{50} of 3.32 mg/L Cu^{2+} when pumping was counted visually after 24 h exposure on Cu^{2+}-containing agar plates. Thus—not unexpectedly—Cu^{2+}-induced inhibition of pumping depends on both the concentration and duration of exposure.

Experiment 2. In these experiments, the ScreenChip system was used to compare pump frequency in worms exposed to control or 50 mg/L Cu^{2+} solutions (Fig. 2; n = 26-28 worms/group; mean ± S.E.M.). EPG recordings (2 min per worm) were started 30 min after the onset of Cu^{2+} exposure, during the steady-state inhibition of pumping (see Fig. 1A). The Cu^{2+}-exposed group showed a significant reduction in pump frequency of ~68% compared to controls (P < 0.00001; 2-tailed Mann-Whitney Wilcoxon Test).

CONCLUSION

These data demonstrate the use of microfluidic EPG recordings to quantify concentration- and time-dependent effects of Cu^{2+} on pharyngeal pumping. To our knowledge, this is the first demonstration of a rapid, electrophysiological effect of Cu^{2+} on C. elegans. EPG recordings detected [Cu^{2+}] as low as 10 mg/L (Fig. 1), well within the range of copper-contaminated water in the United States, which can exceed 30 mg/L; for comparison, the U.S. Environmental Protection Agency’s upper limit for safe drinking water is 1.3 mg/L Cu^{2+}. We conclude that the ScreenChip system provides rapid and sensitive detection of the toxic heavy metal, Cu^{2+}, in a concentration range suitable for testing environmental samples.

METHODS

Synchronized N2 (wild-type) worms were cultivated at 20 °C to the first day of adulthood on plates containing nematode growth medium (NGM) seeded with E. coli OP50, using standard methods. For microfluidic EPG recordings, reagent-grade Cu^{2+} solutions were prepared in K medium (32 mM KCl, 51 mM NaCl in dH2O) containing 10 mM 5HT to stimulate pumping. EPG recordings were acquired using Spike2 software (Cambridge Electronic Design Ltd.) for 8-channel chips or NemAcquire software for the ScreenChip system. Detailed ScreenChip methods are available elsewhere. Recordings were analyzed using custom software in IGOR Pro; soon to be superseded by the release of NemaMetrix’s NemAnalysis software.

REFERENCES


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