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Short communication

A non-toxic dose of cobalt chloride blocks hair cells of the zebrafish lateral line

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ABSTRACT

Experiments on the flow-sensitive lateral line system of fishes have provided important insights into the function and sensory transduction of vertebrate hair cells. A common experimental approach has been to pharmacologically block lateral line hair cells and measure how behavior changes. Cobalt chloride ($CoCl_2$) blocks the lateral line by inhibiting calcium movement through the membrane channels of hair cells, but high concentrations can be toxic, making it unclear whether changes in behavior are due to a blocked lateral line or poor health. Here, we identify a non-toxic treatment of cobalt that completely blocks lateral line hair cells. We exposed 5-day post fertilization zebrafish larvae to $CoCl_2$ concentrations ranging from 1 to 20 mM for 15 min and measured 1) the spiking rate of the afferent neurons contacting hair cells and 2) the larvae's health and long-term survival. Our results show that a 15-min exposure to 5 mM $CoCl_2$ abolishes both spontaneous and evoked afferent firing. This treatment does not change swimming behavior, and results in >85% survival after 5 days. Weaker treatments of $CoCl_2$ did not eliminate afferent activity, while stronger treatments caused close to 50% mortality. Our work provides a guideline for future zebrafish investigations where physiological confirmation of a blocked lateral line system is required.

1. Introduction

The flow-sensitive lateral line system of fishes is composed of a series of mechanoreceptor clusters distributed across the surface of the body. Each cluster, called a neuromast, consists of hair cells that detect information about the surrounding fluid (e.g., pressure and velocity). Experiments which block the hair cells of the lateral line have revealed the crucial role of the lateral line in capturing prey (Coombs and Conley, 1997), evading predators (Stewart et al., 2013), and navigating and orienting to current (Windsor et al., 2008; Montgomery et al., 1997). Blocking occurs when the afferent neurons contacting hair cells stop firing, which is typically achieved by exposing fish to aminoglycoside antibiotics (Harris et al., 2003; Ton and Parng, 2005; Owens et al., 2009; Van Trump et al., 2010), such as neomycin and gentamycin, or divalent cations such as cobalt (Karlsen and Sand, 1987). Aminoglycoside antibiotics are ototoxic and block the lateral line by triggering hair cell death (Huth et al., 2011). Recovery of function involves the regeneration of the affected hair cells (McHenry et al., 2009).

Divalent cations like cobalt can block the lateral line by competitively inhibiting calcium from entering the hair cells through voltage-gated channels of the cell membrane (Karlsen and Sand, 1987), which halts calcium-dependent synaptic vesicle fusion and neurotransmitter release to stop communication with downstream afferent neurons. In high concentrations, cobalt can also trigger cell death after entering the hair cells of the lateral line (Becker, 2013) or the mammalian cochlea (Li et al., 2015; Lee et al., 2016). The mechanisms for the death of hair cells and associated neurons from high concentrations of cobalt exposure are poorly understood. In rats, we know that cobalt has the potential to become toxic by acting as an inhibitor of zinc-dependent enzymes (Hartwig, 2001). Other deleterious effects may be caused by its activity as a calcium channel antagonist, which promotes the inhibition of calcium entry and calcium signaling during critical cellular activities (Barnes and Hille, 1989; Akbar et al., 2011). Prolonged or high-dose treatment with cobalt chloride switches cell metabolism from aerobic respiration to anaerobic glycolysis (Regazzetti et al., 2009). This induction of oxidative stress can provoke cell death through the increase in expression of the highly toxic superoxide anion in hair cells and of caspase-3 (Jomova and Valko, 2011; Lee et al., 2013; Huk et al., 2004).

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Treatments of both antibiotics and divalent cations can be toxic to fish at high doses (Kaus, 1987; Janssen, 2000). Therefore, the main goal of this paper is to identify a dose of cobalt that blocks hair cells of the lateral line without compromising the overall health of the animal. This is important because interpretations of behavioral changes after treatment should be attributed to the loss of lateral line function, and not a systemic response to a toxic over dose. Here, we provide a recommendation for safely blocking lateral line hair cells with cobalt chloride ($CoCl_2$) by measuring how cobalt affects 1) the spiking activity of afferent neurons in response to direct stimulation of connected neuromasts and 2) the long-term behavior and survival of fish. Zebrafish larvae were chosen for this study because they provide a particularly strong system in which to investigate the neural circuitry of a tractable hair cell system *in vivo*, and are a widely-used model system in the fields of development, genetics, and neuroscience.

2. Material and methods

2.1. Measuring the effect of cobalt on survival

We evaluated how $CoCl_2$ concentration affected the survival of zebrafish larvae. 15 groups of 20 healthy, 5 days post fertilization larvae ($N = 300$) were randomly assigned one of five treatments (control, 1, 5, 10, and 20 mM $CoCl_2$ in Hank's solution). The larvae were exposed to their respective treatments for 15 min and the number of surviving larvae was recorded at 5, 10, and 15 min during treatment. The larvae were then transferred to dishes containing Hank's solution and their survival was monitored for 5 days.

2.2. Measuring how cobalt affects the sensitivity of the lateral line

We next evaluated how the concentrations of $CoCl_2$ used in our survival experiments affected the firing of the afferent neurons connected to neuromasts. We did this by measuring both the spontaneous and evoked spiking rates of afferent neurons with electrophysiology. Each fish was paralyzed (0.1% α-bungarotoxin) and secured to a dish containing 3 mL of extracellular solution (XCL, 134 mM NaCl, 2.9 mM KCl, 1.2 mM $MgCl_2$, 2.1 mM $CaCl_2$, 10 mM glucose, 10 mM HEPES buffer, adjusted to a pH of 7.8 with NaOH). Extracellular, loose patch recordings were made from single afferent neurons in the posterior lateral line ganglion, as previously (Haehnel-Taguchi et al., 2014). During the recording, we deflected the connected neuromast with a 30 Hz sine wave stimulus for 10 s using a glass probe driven by a piezoelectric actuator. The rounded end of the probe (~300 μm diameter) was positioned ~100 μm to the side of the neuromast and was driven back and forth over a distance of 150 μm (Fig. 1*a*). We then removed 1.5 mL of XCL from the dish and carefully perfused a 1.5 mL solution of concentrated $CoCl_2$ in XCL (or XCL alone for control experiments) as appropriate to bring the final concentration to the desired treatment level. After mixing in the $CoCl_2$ solution, the afferent response to the stimulus was immediately measured, and then again at two minute intervals for 20 min. Preceding each stimulation, we also recorded the spontaneous spiking for at least two seconds. Experiments were conducted on 5 larvae for each treatment. All data were analyzed in Matlab.

2.3. Measuring the behavior of zebrafish larvae after cobalt treatment

Our survival experiments and electrophysiological recordings allowed us to identify the optimal treatment of $CoCl_2$. We next sought to determine how long this optimal treatment blocked the lateral line. In our hands, it was not possible to maintain patch recordings of afferent neurons long enough to document the recovery of spikes after cobalt washout. Therefore, we determined the functional recovery of the lateral line by measuring the probability that treated larvae responded to a small fluid jet during the days following exposure. 100 larvae (5 days post fertilization) were exposed to the optimal treatment of $CoCl_2$ and equally divided into 25 petri dishes. Each larva was carefully exposed to fluid jets generated manually with a pipette (McHenry et al., 2010). This stimulus was chosen for its efficacy and to minimize vestibular cues caused by whole-body displacement of the animal. The opening of the pipette was positioned ~10 mm beside a larva and 3 small and sequential jets were aimed at the animal. Dye visualizations before experiments showed that each jet produced a small vortex ring that collided with the animal. Larvae that responded to the jet executed an obvious and rapid escape maneuver. All larvae were exposed to these jets for 14 times over the first 5 h after treatment, and then once a day for 5 days. To further verify that the lateral line mediates the responses to the fluid jet, we measured the ability of 20 larvae (5 dpf) to respond to fluid jets before and after a 1-h treatment with 250 μm neomycin sulfate. This dosage has been demonstrated to reliably ablate lateral line hair cells (McHenry et al., 2009). We found that the probability of response decreased sharply from 0.95 to 0.05 following the neomycin treatment, as expected.

To evaluate how the optimal treatment affected the health of the zebrafish larvae, we measured spontaneous swimming before and after treatment. 50 larvae were placed together in a large petri dish. After 30 min to allow acclimation, a 1 min video of the larvae was digitally recorded to measure the probability of spontaneous swimming. All larvae were then treated with $CoCl_2$ and the probability of spontaneous swimming was measured 7 times over the next 5 h. As a control, we repeated these measurements on an additional set of 50 untreated fish.

3. Results

All animals survived the initial 15-min exposure to all $CoCl_2$ treatment concentrations (0–20 mM $CoCl_2$), but there was mortality in each group after 5 days (Fig. 2*a*). Survivability was optimal at the 1 and 5 mM concentrations, with at least 85% of the larvae surviving. However, for concentrations above 10 mM, only about 50% of larvae survived. All individuals from the control group survived.

Cobalt quickly decreased the sensitivity of the lateral line afferent neurons to neuromast stimulation (Fig. 1*b*). The spiking rate of afferent neurons decreased within minutes for all treatments (Fig. 2*b*), with higher concentrations of $CoCl_2$ causing the spiking rate to decrease more rapidly (decay coefficient, Fig. 2*c*). Fifteen minutes of exposure to 1 mM $CoCl_2$ decreased the evoked spike rate over 40%, while 5 and 10 mM $CoCl_2$ effectively abolished it. The evoked firing rate from the control group did not change over 20 min. We found that the synchronization of the spikes to deflection (i.e., vector strength, Fig. 2*d*) was the lowest for 10 mM $CoCl_2$, and exhibited the highest variation for 1 mM $CoCl_2$.

Exposure to high concentrations of $CoCl_2$ quickly abolished spontaneous spiking in afferent neurons. After only 4 min of exposure to 5 and 10 mM $CoCl_2$, spontaneous activity was, on average, absent from afferent neurons (Fig. 2*e*). For 1 mM $CoCl_2$, we observed wide fluctuations of the spontaneous spike rate, at times increasing considerably above, but never below, control levels. The spontaneous activity of afferent neurons from the control group did not change over time.

Larvae recovered their ability to sense flow one day after $CoCl_2$ treatment. We measured the probability that larvae treated with 5 mM $CoCl_2$ responded to a fluid jet, and found that the probability of a response slowly increased after treatment (regression,