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Hydrogen Sulfide Decreases the Negative Presynaptic Effects of Homocysteine on the Crayfish Neuromuscular Junction.

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ABSTRACT

We examined how homocysteine increases oxidative stress in the crayfish neuromuscular junction, and if hydrogen sulfide is able to reverse these changes. We hypothesized that hydrogen sulfide would reduce the negative effect of homocysteine on the crayfish neuromuscular junction by decreasing the overall oxidative stress. This experiment was conducted by using intracellular recording and paired-pulse stimulation in order to test the synaptic response of the crayfish after incubating the crayfish tail in the Ringer's solution, homocysteine, or hydrogen sulfide with homocysteine for two hours. We found that hydrogen sulfide reduced the negative effects of homocysteine, causing the presynaptic response to be similar to that of the control experiment.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects approximately two out of every 100,000 people with 6,000 new cases diagnosed in the United States every year (ALS Association, 2017). The disease causes neurons to die, leading to ineffective communication from muscles to the brain. Loss of muscle control and movement and eventual loss of the respiratory ability occur over time.

Increased concentrations of homocysteine (HCY) have been found in patients with neurodegenerative disorders, such as ALS or Alzheimer's Disease. Although ALS is a potentially fatal disease, several studies suggest that hydrogen sulfide (H2S) has the ability to reduce the oxidative stress that ALS causes in cells and protect neuronal cells from neurotoxicity of HCY. H2S, an antioxidant gas, has been shown to protect neurons from oxidative stress, an increase in reactive oxygen species (ROS) which causes damage to all parts of a cell (Tang et al., 2010). HCY induces ROS formation and stimulates neurotoxicity (Ho, Ortiz, & Rogers, 2001) while hydrogen sulfide reduces ROS formation and prevents the death of neurons caused by oxidative stress (Kimura & Kimura, 2004). Therefore if either one is inhibited, the other effects will be greater (Bukharaeva et al., 2015; Tang et al., 2010 and 2011).

One previous study focused on the effects of HCY and H2S on PC12 cells, a clonal rat cell line used for studying neurons, to examine how hydrogen sulfide-based therapies could be a possible treatment for neurodegenerative disorders, specifically Alzheimer's Disease (Tang et al., 2010). Alzheimer's Disease and ALS are similar, as they are both associated with increased levels of HCY and reduced levels of H2S in the brain.

Our study will concentrate on ALS as opposed to Alzheimer's disease since they are very similar neurodegenerative disorders and we mainly focused on the neuromuscular joint (NMJ) of crayfish since there exists similarities between the NMJ and glutamatergic synapses in the brains of mammals. We will observe the change in presynaptic response caused by HCY and H2S by using paired-pulse stimulation, since it shows paired pulse facilitation which is a form of short-term synaptic plasticity, instead of checking cell viability, apoptosis, and ROS formation. Exogenous HCY and H2S will be applied, as opposed to previously used sodium hydrosulfide, a hydrogen sulfide donor (Tang et al., 2010), and will incubate for two hours. As with previous studies, we aim to further support hydrogen sulfide as a promising therapy and treatment for not just Alzheimer's Disease , but specifically ALS and other neurodegenerative disorders, in slowing the progression of the disease and therefore prolonging life of those affected. We hypothesized that homocysteine would reduce the presynaptic response and H2S would reduce the negative effect of HCY on the crayfish neuromuscular junction, causing the H2S and HCY treatment presynaptic response to be similar to that of the Ringer's solution.

MATERIALS AND METHODS

We conducted three experiments in total. One served as a baseline, one allowed us to examine the effects of homocysteine, and one allowed us to examine the relationship between HCY and H_2S. All experiments were conducted on the crayfish neuromuscular junction

(NMJ). We used intracellular recording and paired-pulse stimulation in order to test the synaptic response of the crayfish. For our control, the crayfish was in normal ringer's saline solution. For our first treatment we incubated a crayfish in homocysteine for two hours and tested the synaptic response. We compared this data to the control (Ringer's solution) to see the effect of homocysteine on the crayfish. For our second treatment, we placed the crayfish in homocysteine for two hours, then added hydrogen sulfide and tested the synaptic response. We compared this data to our homocysteine and control data to see if any changes occurred.

Organism. The live crayfish was submerged in an ice bath to desensitize it. We cut the tail off while the remainder of the crayfish was placed back in the ice bath. We made two parallel cuts along the dorsal side of the shell and removed the underside to expose the muscles and scrape off the muscle using the thumb to expose the superficial extensor muscle. The prepared tail was placed ventral side up and pinned to a dissecting dish filled with Ringer's solution, HCY, or HCY with H2S (Wyttenbach, Johnson, & Hoy, 1999).

Solutions. The synaptic response of the crayfish was measured while submerged in Ringer's solution (5.4mM KCL, 196mM NaCl, 2.6mM $MgCl_2$ $6H_20$,10 μM Hepes buffer, 13.5mM $CaCl_2$ $2H_20$) for two hours for the control. For the treatments, the crayfish was submerged in HCY (500 μM) and HCY (500μM) with H_2S for two hours.

Recording. Microelectrodes used for intracellular recording were made using 1.2 mm glass micropipettes. We heated and pulled apart micropipettes using the electrode puller. The microelectrodes were filled with 3mM KCl for intracellular recording with a range of resistance between 4 and 20 MΩ . Additional microelectrodes were made using 1.2 mm glass micropipettes heated and pulled apart, but we shaved down the tips of these electrodes using emery paper in order to create a small hole to suck up nerves.

Measuring. We measured the percent change in synaptic response by subtracting the first amplitude of the excitatory junction potential (EJP_1) from the second (EJP_2), and dividing it by EJP_1 and multiplying by 100. The formula was ((EJP_2 - EJP_1)/ (EJP_2)) x 100. We found the EJPs by sending a twin pulse and measuring the amplitudes (mV) using an amplifier. We used an unpaired one-tailed t-test with a null hypothesis of .05 to quantify significant difference.

RESULTS

We focused on the presynaptic response of the crayfish neuromuscular joint when exposed to three treatments.. We hypothesized that homocysteine would reduce the presynaptic response and H_2S would reduce the negative effect of HCY on the crayfish neuromuscular junction, causing the H_2S and HCY treatment presynaptic response to be similar to that of the Ringer's solution. We stimulated the crayfish NMJ with twin pulses in order to get two EJPs, which we then used to calculate the percent change between the amplitudes (mV) of EJP_1 and EJP_2. Comparisons of the percent change between the three treatments gauged whether or not the H_2S reduced the negative effects of HCY.

For each treatment, we conducted trials by isolating a nerve and probing the crayfish superficial extensor muscle in the same segment as the nerve. We sent twin pulses to stimulate synaptic responses to get EJPs and continued to do so for each trial on the same nerve since viable nerves were difficult for us to find. We conducted 16 trials for the control, 12 for the HCY, and 19 for the HCY with H_2S. The range for percent change of the treatments were large for each treatment with the control having a range of 28.82%, HCY having a 25.03% range, and the HCY with the H_2S having a range of 39.89%.

As shown in Figure 1, the average percent change in synaptic response for the control group (20.726%) and the H_2S with HCY (21.378%) are within .652% of each other while the HCY alone had a negative percent change (-1.695%), meaning EJP_1 was larger that EJP_2. The negative average of HCY shows no paired pulse facilitation while the control and HCY with H_2S do.

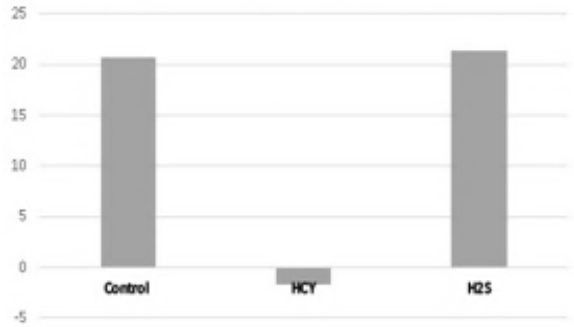

Figure 1: The average percent change in the pre-synaptic response of the crayfish NMJ. The average percent change for the control and the hydrogen sulfide treatments are within 1% of each other while the homocysteine is a negative percent change.

DISCUSSION

The data we collected supports our hypothesis that hydrogen sulfide decreases the negative presynaptic effects of homocysteine on the crayfish neuromuscular junction. HCY caused a negative percent change in synaptic response, meaning there was no paired pulse facilitation so no synaptic plasticity. However, both control and the HCY with H_2S showed synaptic facility since there was paired pulse facilitation. The hydrogen sulfide reduced the negative effect of homocysteine, resulting in similar results for both the baseline and hydrogen sulfide experiments (Figure 1). The average percent change for the control and the hydrogen sulfide treatments are within 1% of each other, while the homocysteine produced a negative percent change.

Our results support the previous research being done on homocysteine and hydrogen sulfide. HCY has been shown to cause neuronal toxicity in other organisms such as PC12 cells, and H_2S has been shown to reduce the negative effect of HCY by reducing ROS formation and preventing the death of neurons caused by oxidative stress (Tang et al., 2010; Kimura & Kimura, 2004). Our results further suggest hydrogen sulfide reduces negative effects of HCY, specifically on synaptic plasticity in our study.

Similar experiments should be pursued in this area in the future, as some error was present in our experiments. Due to the death of one of our crayfish, the crayfish used for our control data did not incubate for two hours prior to our experiment, creating experimental inconsistencies in our experiments. Future research should be pursued to clarify whether this inconsistency. created unreliable results. Future work should also be done on hydrogen sulfide and its beneficial effects to investigate if eventually it could be used as a therapy to inhibit the negative effects homocysteine on neurons, possibly improving and prolonging life for patients with ALS and other neurodegenerative diseases.

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