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Carboxy PTIO decreases the amplitude of EPSP generated in the crayfish tail extensor muscle despite changes in temperature

MIKI NAKAMURA, RUTH CAMPBELL, and NABILA MIRZA
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

Our objective was to investigate the effect that a NO scavenger, Carboxy PTIO, has on the EPSP (excitatory post-synaptic membrane potential) of the crayfish tail extensor muscle, with changing temperature. We measured the membrane potential of the extensor muscle fibres from a dissected crayfish tail in the absence and presence of NO at specific temperatures ranging from 17º C to 0ºC using intra-cellular recording. Our hypotheses, that EPSP amplitude increases with a decrease in temperature, and decreases in the absence of NO, were supported by our experimental findings. We observed that EPSP amplitude increased as we decreased the temperature and this increase was reduced after saturating the crayfish saline with Carboxy PTIO, demonstrating the effects of simultaneously altering temperature and NO (present vs. absent).

INTRODUCTION

Nitric Oxide (NO) is a highly reactive, membrane permeable molecule that is generated in living organisms by N-methyl-D-aspartate (NMDA) receptor stimulation and is involved in the modulation of impulse transmission (Kuriyama and Okhuma 1995). A review article by Kinya Kuriyama and Seitaro Okhuma concludes that NO enhances synaptic transmission by increasing the amount of neurotransmitter release (Kuriyama and Okhuma 1995) whereas Thomas and Robitaille found that in frogs specifically, excess NO production reduced the amount of neurotransmitter released (Thomas 2001). Regardless of these contradictions, an experiment conducted by Wang, Paton and Kasparov (2007) supported the idea that NO's role in the modulation of impulse transmission is pre-synaptic.

Crayfish are cold-blooded crustaceans which survive in an aqueous environment of temperature ranging from 5°C to 20°C. An experiment conducted on crustaceans by Johnson, Peck and Harris-Warrick in 1995 demonstrated the inversely proportional relationship between temperature and the EPSP amplitude. Therefore we know that since temperature regulates synaptic transmission in crayfish, global warming will play a significant role in determining the survival of crayfish in the future. We also know from the review article by Kinya Kuriyama and Seitaro Okhuma (1995) that NO is likely to be another important regulatory factor of synaptic transmission. Some research experiments analyzed the effects of nitric oxide on synaptic transmission, and others have examined the effects of temperature change on synaptic transmission. However, we were unable to find journal articles on experiments which analyze both variables while at the same time looking for possible interactions between the two. Wang, Paton, and Kasparov (1995) suggested that "it is extremely difficult to deduce how much NO was actually released because on the one hand, NO release is temperature- and time dependent and on the other, NO degradation and loss through diffusion are extremely rapid". Since their research did not address the relationship between nitric oxide and changing temperature, we chose to investigate this.

Our primary goal was to determine whether NO plays a modulatory role at the crayfish muscle, and our secondary goal was to investigate how an NO scavenger, in this case Carboxy PTIO, will alter the EPSP amplitude with variations in temperature. As Kinya Kuriyama and Seitaro Ohkuma's review article (1995) stated that NO enhances synaptic transmission, we expected NO's effect to be similar on synaptic transmission in the crayfish extensor muscle. Also, a journal article by Aonuma H, and Newland PL, (2001) stated that the EPSP in the axon of a crayfish tail extensor muscle increases with decreasing temperature, so we predicted that this increase would be reduced in the presence of a NO scavenger. Our experimental results supported both hypotheses.

MATERIALS AND METHODS

Preparing the crayfish tail prior to the experiment

We made standard crayfish saline of pH 7.4 (see Table 1), using a fraction of the preparation to make ice cubes. The crayfish we used was stored in frozen condition to stop all movement. We snipped off the crayfish tail and dissected it to expose the extensor

muscle. The ventral surface was separated from the dorsal surface by cutting the angle between the ventral surface and the side, and the large mass of muscles remaining in the dorsal part was scooped out to expose the nerves, while taking care to avoid scraping the tail's internal side.

Table 1. Composition of standard crayfish saline.

Component	Concentration (mM)
KCL	5.4
NaCL	250
MgCl·6H2O	2.6
NaI+CO3	2.3
Dextrose	2.0
CaCl2·2H2O	13.5

Setting up the apparatus for intra-cellular recording

We heated a thin glass capillary in the middle and pulled it apart from both ends with a large force using an electrode puller, to produce two micro-pippettes of very fine tips which were filled with 3M KCL solution and placed in holders. One of these electrodes and a silver pellet that served as the reference electrode were connected to the micromanipulator and computer, such that any voltage difference detected between the tip of the micro-electrode and the reference electrode would appear on the computer screen. Dipping the electrode into the standard crayfish solution we had prepared, we checked for the resistance of the electrode tip and used it only if the resistance was between 5MΩ and 20MΩ. We used a suction electrode to stimulate the nerve beyond threshold, an amplifier, a microscope and a computer program, Scope, to graphically display the membrane potentials and EPSPs.

Proceeding with the experiment

The dissected crayfish tail, pinned to the dish and immersed in the saline, was observed under the microscope to find a nerve which was stimulated by being sucked up in a suction micro-electrode. The EPSP produced along the axon was measured by probing an extensor muscle with the KCL filled micro-electrodes (see intra-cellular recording above). The difference between the EPSP's peak voltage and the resting membrane potential gave us the EPSP amplitude. We started by measuring the amplitude of EPSP generated at 17°C, then repeated our measurement procedure by reducing the temperature gradually, recording the corresponding EPSP amplitude for each temperature. Temperature of the crayfish tail's environment was modulated by gradually replacing portions of the saline solution with ice-cubes made of the same solution and measured using an electric temperature probe. For each temperature value, EPSP amplitude was measured for more than one nerve and the average value was calculated.

To investigate the effect on the EPSP amplitude in absence of nitric oxide, we saturated the crayfish saline with 20µl Carboxy PTIO solution (made by diluting 50µl of the scavenger in 100ml of water), which removed any NO produced in the extensor muscle on stimulation, and repeated the entire procedure described above.

RESULTS

According to Kinya Kuriyama and Seitaro Ohkuma's review article (1995), NO enhances synaptic transmission, and according to the journal article by Aonuma H, and Newland PL, (2001), EPSP amplitude increases with decreasing temperature. Based on this information, we investigated the relationship between the amplitude of the EPSP generated in the crayfish tail extensor muscle with changing temperature in the presence and absence of NO.

We immersed the dissected crayfish tail in standard crayfish saline (pH 7.4) and stimulated a nerve while observing it under the microscope. We used intra-cellular recording with KCL filled micro-electrodes to record the EPSPs, measuring their amplitude using the Scope program. The temperature of the crayfish tail's environment was modulated by altering the ratio of the saline ice-cubes to solution. Our temperature values ranged from 0ºC to 17ºC.

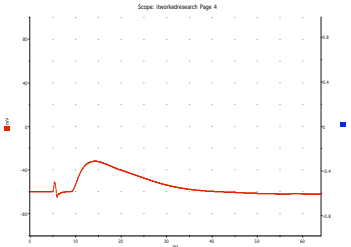

Figure 1a. EPSP produced at 17ºC in the absence of Carboxy PTIO.

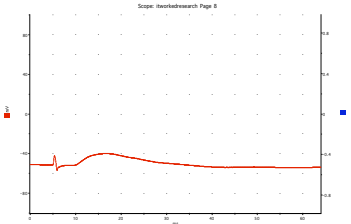

Figure 1b. EPSP produced at 17ºC in the presence of Carboxy PTIO. It can be seen that the peak voltage, and hence the EPSP amplitude, decreased in the absence of NO.

Figures 1a and 1b are examples of the graphical displays of the voltage changes that we obtained using the Scope program. The starting point of each curve

represents the resting membrane potential, the vertical axis shows the voltage and the horizontal axis, the time.

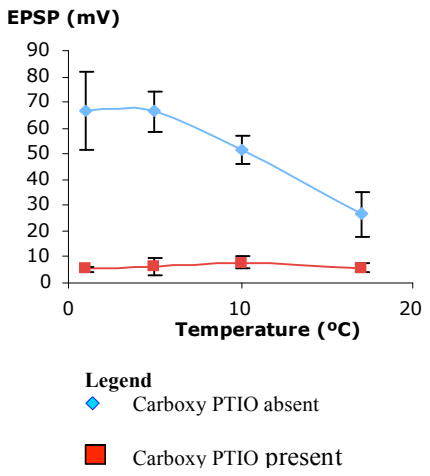

Figure 2. Relationship between temperature and EPSP amplitude. Each dot represents the mean EPSP at different temperatures (°C). The line joining the red dots shows the trend in EPSP amplitudes at specific temperatures in presence of Carboxy PTIO and the line connecting the blue series of dots represents the EPSPs measured at the same temperatures in the absence of the scavenger. The temperatures at which measurements were made both in the absence and presence of Carboxy PTIO were about 1°C, 5°C, 10°C and 17°C. The EPSP amplitudes in the presence of Carboxy PTIO and in the absence of Carboxy PTIO were compared for every temperature. From the t-test applied for the comparison of EPSP amplitudes at each temperature, we found that the p-value was always below 0.05, showing that the differences between the EPSP values at each temperature were almost entirely due to the presence or absence of NO, and were not due to chance.

It can be seen in Figure 2 that in both cases, either in the presence or in the absence of the scavenger, as temperature decreases, there is a general upward trend in the lines connecting each set of dots, which demonstrates that EPSP amplitude increases as temperature falls. At any temperature within the given range, 0ºC to 17ºC, the EPSP amplitude significantly decreased in the presence of Carboxy PTIO, which proves that NO greatly enhances synaptic transmission. For instance, at 10ºC, in the presence of Carboxy PTIO (and thereby the absence of NO), the EPSP amplitude decreased by 85% and similarly at 5ºC, there was a decrease of 90%. Our results contradict the finding by Aonuma and Newland (2001) that the EPSP amplitude increases to a greater extent when temperature is lowered below 5ºC; in the presence of NO, the EPSP amplitude did not change when the temperature was lowered below 5ºC.

DISCUSSION

Our results demonstrate that above 5ºC, the EPSP amplitude increases with decreasing temperature, regardless of NO's presence in the crayfish tail's environment, as we expected. A possible reason for this may be because NO degradation is temperature dependent, as Wang, Paton, and Kasparov (1995) suggested (see "Introduction," pg. 1, cl. 2); NO degradation rate increases with increase in temperature. Our prediction that NO would reduce the increase in EPSP amplitude with reduction in temperature was also proved correct, as can be seen from Figure 2, and this agrees with Kinya Kuriyama and Seitaro Ohkuma's findings in 1995 that NO enhances synaptic transmission. The amplitudes of the EPSPs generated in the absence of NO remained almost constant at all the temperatures that we considered, and was relatively much lower than of those generated in presence of NO at the same temperatures.

Our findings support the conclusion of Aunoma H, and Newland PL (2001) to a certain extent. They found that the EPSP amplitude increases more rapidly at temperatures below 5ºC, while we found that below 5ºC, the EPSP amplitude remained constant. However, our results are limited to a minimum temperature of 0ºC, and we may have possibly found different results had we lowered the temperature to below 0ºC. Another factor which may account for this decrease is time, as EPSP amplitude decreases with time.

Further research may be done to find out the impact of different NO scavengers and NO inhibitors on the EPSP amplitude, including a comparison between the effect of a NO inhibitor and a NO scavenger on the EPSP amplitude. Our measurements were made from the extensor muscle only, and nitric oxide may affect the EPSP amplitude in the flexor muscle differently. A comparative study can be done by repeating the experiment for the flexor muscle to find out a more specific role of nitric oxide in synaptic transmission.

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