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Dopaminergic Modulation Of Excitatory Junction Potentials At The Crayfish Neuromuscular Junction

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ABSTRACT

Dopamine (DA), a neurotransmitter found throughout the body, has been found to have modulatory effects on synaptic transmission in both the peripheral and central nervous systems. Modulatory effects take place through dopamine receptor subtypes, which respond in a variety of different ways to the binding of dopamine. This study attempted to first identify the modulatory effects of dopamine at the neuromuscular junction (NMJ) of crayfish, and then quantify the relative contribution of the D2/D3 receptor subtype(s) to overall modulation using sulpiride, a D2/D3-specific antagonist. We placed crayfish preparations in three different solutions: ringer's solution, ringer's solution with dopamine, and ringer's solution with dopamine and sulpiride. While bathed in solution, the preparations were stimulated at increasing voltage levels and the corresponding excitatory junction potential (EJP) amplitudes were recorded. With the addition of dopamine to the ringer's solution, we found a decrease in maximum EJP amplitude and an increase in the voltage level required to stimulate this amplitude. These differences, however, were not statistically significant. The addition of sulpiride to ringer's solution containing dopamine showed a decrease in EJP amplitude and an increase in required stimulatory voltage. These differences were also not statistically significant. Our results may support previous research finding an inhibitory effect of dopamine on synaptic transmission.

INTRODUCTION

Neuromodulation is critically important in organisms demonstrating complex neural processing. In addition to their primary function in synaptic transmission, neurotransmitters have been shown to modulate complex behavioral responses in crustaceans and other invertebrates (Miller *et al* 1985). A number of biogenic amines, such as dopamine (DA), have various modulatory functions throughout the body of many organisms, including regulation of the cardiac neuromuscular junction and pathways involving movement in the central nervous system (Watson *et al* 1985).

Studies examining the modulatory effects of dopamine on synaptic transmission have found varying results. In the neuromuscular junction (NMJ) of various vertebrates and invertebrates, dopamine has been shown to exhibit both excitatory and inhibitory effects at the presynaptic terminal. While research by Watson *et al* (1985) showed enhanced synaptic transmission in the heart of *L. limulus* with increasing levels of dopamine, DA was found to decrease synaptic transmission in the prawn NMJ (Miller *et al* 1985) and larval

CNS-segmental preparations of *D. melanogaster* (Cooper and Neckameyer 1999). Additionally, research by Heinonen (1982) found a 9% hyperpolarization of rat motor nerve terminals in presence of dopamine.

Studies demonstrating inhibitory effects of dopamine have also shown that its effects may be a presynaptic phenomenon. Since decreased synaptic facilitation in the prawn NMJ was noted with increasing concentrations of dopamine, this modulator was proposed to cause an inhibition of calcium (Ca) influx into the presynaptic cell (Miller *et al* 1985). Research by Cooper and Neckameyer (1999) revealed a depression of vesicular release (a presynaptic activity) in *D. melanogaster* with no alteration in the shape of the spontaneous synaptic currents (a postsynaptic activity) in the presence of dopamine.

Dopamine modulation can influence synaptic transmission in various ways through complex interactions between dopamine receptor subtypes. These receptor subtypes can be found in both the presynaptic and postsynaptic terminals. Dopamine receptor subtypes at the presynaptic terminal influence the eventual release of glutamate, and dopamine receptor subtypes at the postsynaptic terminal might cause excitatory or inhibitory responses. By examining the activity of specific DA receptor subtypes during transmission, we can more precisely identify the process through which dopamine modulation occurs.

In our study we first examined the modulatory effect of dopamine in the crayfish NMJ. We then used a specific antagonist to attempt to ascertain the identity of the specific dopamine receptor subtypes involved in this modulation. Characteristics of EJPs, which are evoked upon depolarization of the presynaptic terminal, serve as good indicators of transmission efficacy and cell excitability. Modulation could take two forms: either EJPs would be evoked at a smaller/larger voltage, and/or the amplitudes of the maximum EJP would be larger or smaller. Based on the findings of previous research, we hypothesized that dopamine would depress synaptic transmission in the crayfish NMJ by decreasing maximal EJP amplitude while requiring a larger stimulatory voltage to evoke this amplitude. We found a decrease in maximum EJP amplitude and an increase in the voltage level required to stimulate this amplitude in a preparation bath of dopamine-fortified ringer's solution. These differences, however, were not statistically significant. The addition of the D2/D3 receptor antagonist sulpiride to ringer's solution bath containing dopamine also resulted in a non-significant decrease in EJP amplitude and an increase in required stimulatory voltage.

MATERIALS AND METHODS

Experiments were conducted on four crayfish (Carolina Biological Supply) using the neuromuscular junction (NMJ) preparation described by Stephens (1996). Excitatory junction potentials (EJPs) were evoked by stimulating the presynaptic motor nerve with the Grass SD9 Stimulating electrode. EJP amplitudes were recorded postsynaptically by KCl-filled electrodes placed in the medial tail extensor muscle. The information entering the recording electrode was fed to the MacScope program on our Macintosh Quadra 610. To measure characteristics of cell excitability, maximum EJPs and their corresponding stimulus intensities were found for multiple cells within each preparation. Crayfish were first bathed in a ringer's solution, followed by a dopamine (DA) + ringer's solution. Dopamine

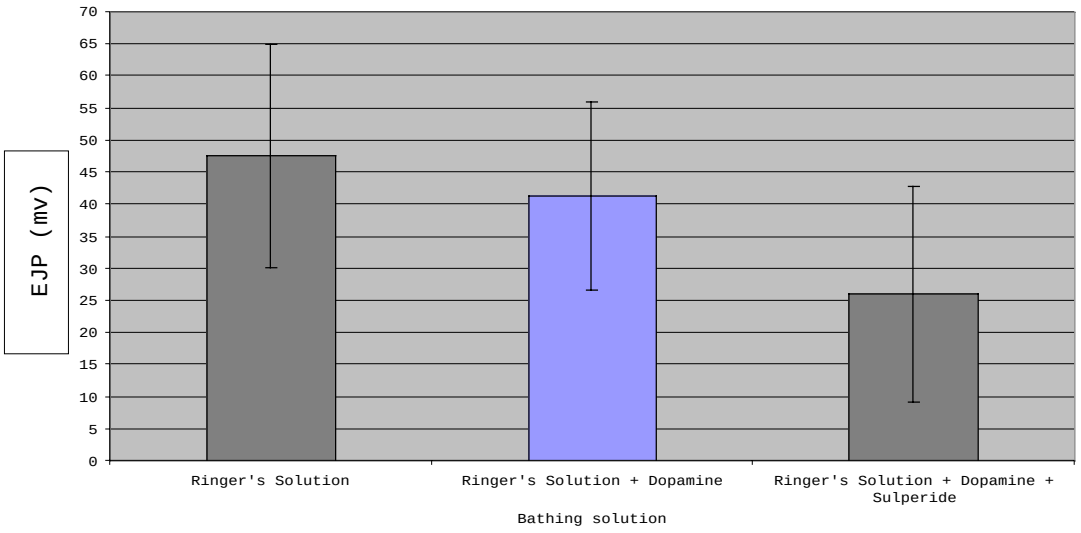
(Sigma Chemical Company) was diluted to 10^{-7} M in ringer's solution, a concentration used previously to modulate EJPs in the prawn neuromuscular junction by Miller *et al* (1982). Voltages required to evoke a maximal EJP and the resulting maximum EJP amplitudes were recorded for preparations bathed in both ringer's solution and ringer's solution + dopamine to reveal any enhancing or inhibiting properties of dopamine in the neuromuscular junction. To negate any order effects, some of the EJP amplitudes were first recorded from crayfish bathed in DA + ringer's solution, followed by recording of EJP amplitudes in dopamine-free ringer's solution.

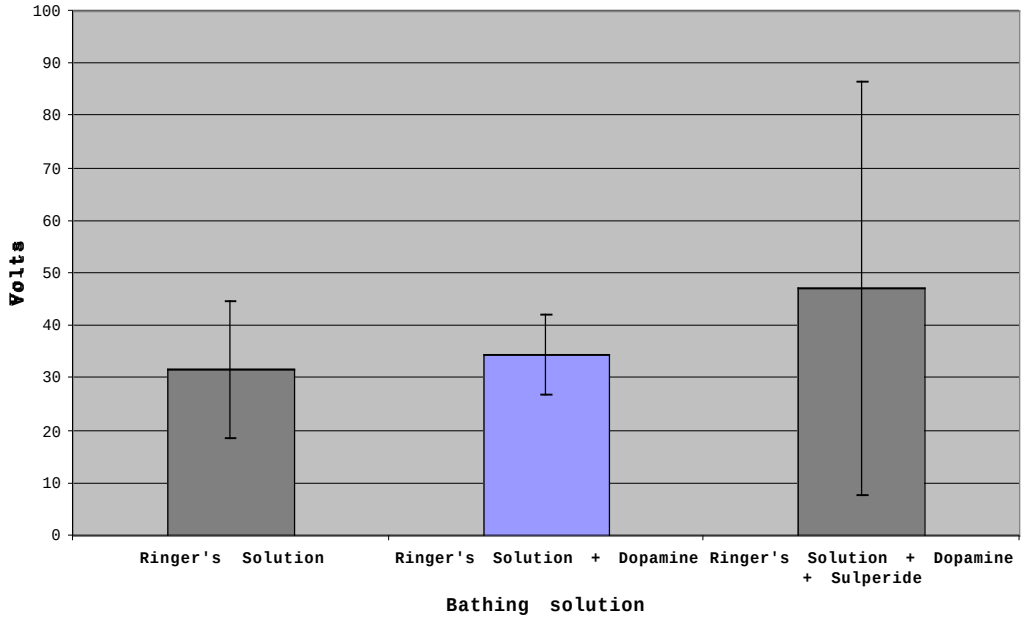
After testing the neuromodulatory effects of dopamine at the NMJ, we attempted to identify the contribution, if any, of the D2/D3 receptor subtypes in dopaminergic modulation. Sulpiride (Sigma Co.), a dopamine receptor subtype antagonist, was diluted with the 10^{-7} M DA + ringer's solution to 10^{-7} M. Upon recording EJP amplitudes and stimulus voltages in preparations containing dopamine and dopamine-free solutions, we recorded EJP amplitudes in a preparation bathed in a solution of dopamine and sulpiride.

Statistical testing was performed using a 2-sample t-test. We found P values for the difference in voltage required to evoke a maximal EJP for preparations bathed in: (1) ringer's solution vs. ringer's + 10^{-7} M DA, (2) ringer's vs. ringer's + 10^{-7} M DA + sulpiride and (3) ringer's + 10^{-7} M DA vs. ringer's + 10^{-7} M DA+ 10^{-7} M sulpiride. We performed the same test in order to compare the maximum EJP amplitudes measured in each of the three solutions. P values less than 0.05 were considered to indicate a significant difference between solution baths.

RESULTS

We attempted to discern any differences in cell excitability in the presence of dopamine by observing maximal EJP amplitudes and their required stimulatory voltages. Mean required voltage for stimulation of the maximum EJP in the medial tail extensor muscle was 31.7($\pm$8.7V) volts for crayfish bathed in ringer's solution (Figure 1). The mean EJP amplitude associated with this





stimulation was 47.7(±11.6mV) (Figure 2). Upon bathing the crayfish in a dopamine solution and applying a stimulus to the motor nerve, we observed a higher mean required voltage for stimulation of maximum EJP in the medial tail extensor muscle (34.5(±4.82) volts) (Figure 1). A t-test revealed, however, that this difference was not significant (P=0.58) suggesting that dopamine does not have a substantial modulatory effect at the crayfish NMJ. A lower mean maximum EJP amplitude observed in the ringer's + dopamine bathing solution (41.4(±9.2mV)) was also non-significant (P=0.41) (Figure 2).

Although the observed differences in EJP amplitude and required stimulatory voltages were not statistically signifcant, we suspected they might demonstrate at trend reflecting DA modulation. Thus we used a D2/D3 antagonist, sulpiride, in an attempt to induce a reversal of the observed trend. After adding sulpiride to the ringer's + DA bathing solution, we observed a mean required stimulus for maximum EJPs (47.2(±46)V) that was higher in voltage than the stimuli recorded for crayfish in ringer's solution and in ringer's + DA (Figure 1). Mean maximum amplitude was also lower in this bathing solution than in the previous two (28.1(±14.2mV)) (Figure 2). Differences of sulpiride-containing solution from ringer's and ringer's + DA solutions in voltage (P=0.57 and P=0.64, respectively) and in amplitude (P=0.071 and P=0.18, respectively) were non-significant, suggesting that sulpiride does not substantially restore (by blocking DA effects) or alter cell excitability characteristics.

To examine the potential effects of fatigue on the muscle, t-tests were performed to compare data from crayfish that were bathed in ringer's solution prior to ringer's + DA vs. from crayfish that were bathed in ringer's + DA prior to a ringer's solution bath. Results from t-tests revealed no significant difference between these two different orders.

DISCUSSION

In contrast to previous research (Miller *et al* 1985, Cooper and Neckameyer 1999), we found no significant modulatory effect of dopamine on synaptic transmission at the neuromuscular junction. Specifically, EJP amplitudes and required stimulatory voltages were not significantly different between crayfish preparations bathed in control (ringer's) and DA solutions. Because we observed no modulatory effects of dopamine at the NMJ, we observed no significant countermodulatory effects of sulpiride antagonist on synaptic transmission.

Although our preparations bathed in dopamine did show a reduced average maximum EJP amplitude and a greater required voltage for maximum EJPs, these changes were not significantly different from control values. These results may indicate that dopamine has no modulatory effect on the crayfish neuromuscular junction; however, the non-significant difference between response in control and DA-containing preparations could be due to other factors. First, a greater number of trials may have yielded a more significant difference. Resting membrane potentials, voltages required to evoke a maximal EJP, and maximum EJP amplitudes varied considerably across cells within a single preparation, and our average values of these latter two parameters may have been influenced by the response properties of a particular group of cells.

While the differences in maximum EJP amplitudes between the three solution baths were not significant, the decrease in maximum EJP amplitudes between preparations bathed in ringer's solution vs. preparations in ringer's + DA + sulpiride was very close to being significant (as displayed by the p-value). We observed a larger decrease in cell excitability (max EJP amplitudes and their required stimulatory voltages) in the presence of sulpiride + DA compared with DA alone. This result is opposite to what we hypothesized earlier. It is possible that during the sulpiride trials (which were performed last), the DA from the previous dopamine bath and the DA in the sulpiride bath had enough time initiate its modulatory effects. This may explain the larger decrease, though not statistically significant, in the excitability characteristics of the NMJ in the sulpiride solution. It is also possible that sulpiride may have decreased the NMJ's excitability independently of blocking dopaminergic activity. We believe, however, that decreased excitability was most likely due to the fatiguing of the extensor muscle. The sulpiride trials were performed after testing the preparation for 2 to 3 hours, possibly causing the lower EJP amplitudes and higher stimulatory voltages for the sulpiride bath in comparison to the ringer's solution.

These effects of fatigue may have been a factor throughout the experiment, leading to an increased difficulty in stimulating cells to fire and yielding decreased EJP amplitudes over time. Future research should attempt to eliminate potential variation in synaptic transmission due to muscle fatigue by changing the crayfish preparation more often. It would also be advantageous to run trial baths in a number of different orders (e.g. by bathing the preparation in the sulpiride-containing bath first, then in the standard ringer's solution bath, and then in the DA + ringer's solution bath).

Though most research has found that dopamine decreases synaptic transmission in the neuromuscular junction, more recent research in this field has shown that the presence of dopamine in the neuromuscular

junction may alter activity by a number of different mechanisms. These varied mechanisms can result in different types of modulation by single type of neurotransmitter. Harris-Warrick *et al*'s (1998) research with the crustacean pyloric neural network revealed that DA can simultaneously increase and decrease the excitability of a particular neuron. This may happen via an alteration of the cell's baseline physiological properties or a modulation of ionic currents necessary for signal transmission. In addition, dopamine's modulatory effects may not be confined to the presynaptic terminal. Harris-Warrick *et al*'s results emphasize that dopamine can potentially modulate both terminals, sometimes with opposing results. These findings could potentially explain the variation, and therefore non-significance, we found for our amplitude and voltage data in different solutions.

In conclusion, our findings suggest that although DA may alter synaptic activity, it does not change the excitability characteristics of neurons significantly. In addition, sulpiride, the D2/D3 selective antagonist, may have an independent inhibitory effect on synaptic transmission not related to the blocking of any dopaminergic activity. As the mechanisms underlying the activity of dopamine and dopamine receptor subtypes in the nervous system are better understood, pharmaceutical companies will be able to create selective antagonists and agonists that more successfully block or mimic the effects of dopamine. These agents could then be used to treat a variety of disorders resulting from dopamine level abnormalities.

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