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Methylphenidate and dopamine interact at the crayfish neuromuscular junction to modulate EPSP amplitude

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ABSTRACT

Our research explores the underlying neuronal mechanisms and effects of the neurotransmitter dopamine alone and in conjunction with methylphenidate, as well as methylphenidate by itself on the neuron's excitatory postsynaptic potential at the crayfish neuromuscular junction. This topic examines the amplitude of the excitatory postsynaptic potential in four situations to uncover methylphenidate's ability to "switch" dopamine's effect from excitatory to inhibitory: when the neuromuscular junction is exposed to dopamine alone, dopamine then methylphenidate, methylphenidate then dopamine, and methylphenidate alone. Our results show that the amplitude of the excitatory postsynaptic potential (EPSP) at the crayfish neuromuscular junction increases when dopamine alone is present, and decreases when dopamine is added before methylphenidate, methylphenidate is added before dopamine, and methylphenidate is used alone. Dopamine, typically being an excitatory neurotransmitter, must then interact with methylphenidate in some way so that dopamine's effects are swapped to produce an inhibitory response. Several studies, including our own, suggests that dopamine receptors are modulated by the presence of both dopamine and methylphenidate at the neuromuscular junction.

INTRODUCTION

Pine et al. (2010) states that dopaminergic activity in humans is a probable cause in mental conditions such as ADHD, Schizophrenia, and Parkinson's. The literature surrounding the mechanics of dopamine is sparse based on our own searches for relevant primary literature; a search of primary literature on ProQuest through Neurosciences Abstracts, MEDLINE and PsycINFO with the keywords "dopamine," and "EPSP," returns only 177 results, and only 2 of them were studied at the neuromuscular junction. However, this is not because people do not care about the mechanics of dopamine, but instead, nobody knows where to start. We seek to provide the building blocks needed to make testable deductions.

Although the complete understanding of dopamine is distant as observed by searching of primary literature on ProQuest, if everyone is able to deduce at least something from our experiment, we may be able to better understand, and thus treat, ADHD, Schizophrenia, Parkinson's disease, and more issues related to the function of dopamine. Furthermore, it is highly unlikely that the mechanics of dopamine deal only with itself as observed in research done by Dougan et al. (1987) and Seeman et al. (2002). In Dougan et al. (1987) dextroamphetamine was used to "switch" the excitatory effects of dopamine to be inhibitory on a clam ventricle. Our research will test dopamine with another chemical identified to interact with dopamine in a similar way; methylphenidate. Methylphenidate is the active ingredient in the most common ADHD drug Ritalin and an analog to dextroamphetamine. It is also used in the treatment of various other psychological disorders. Methylphenidate acts at the synaptic cleft by inhibiting dopamine and norepinephrine transport proteins, preventing reuptake for both of these neurotransmitters. Our study will allow us to better understand the different combinations of dopamine and methylphenidate and how it affects EPSPs at the neuromuscular junction to attempt to discover the mechanism behind such an interesting interaction. We hypothesize that we will see similar results to Dougan et al. (1987) in which dopamine's effect was switched from being excitatory to inhibitory with the addition of dextroamphetamine, or in our case, methylphenidate.

METHODS

Dissection

A crayfish was placed in an ice bath until movement stopped, which indicated the crayfish's nervous system had been depressed to the point where it could no longer feel pain. The tail was then cut off and the abdominal extensor muscle was exposed by scraping away excess tissue with the thumb. The tail was then placed in a dissection dish and pinned down with needles to await the

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introduction of the various solutions and subsequent intracellular recording.

Solution Preparation

Standard crayfish solution (5.4 mM KCl, 200.7 mM NACl, 12.3 mM $MgCl_26H_2O$, 5 mM sodium hepes buffer, and 6.5 mM $CaCl_22H_2O$) was prepared for the baseline solution in addition to acting as the base for the remaining solutions. A 26 µM dopamine solution (26 mM, 100 µl dopamine solution prepared from pure dopamine powder to 100 mL of standard crayfish saline solution) and a 135 µM methylphenidate solution (135 mM, 100 µl methylphenidate solution prepared from pure methylphenidate powder to 100 mL of standard crayfish saline solution) was prepared from chemicals ordered from Sigma-Aldrich.

Microelectrode Preparation

Two types of electrodes were used in our investigation: a microelectrode for intracellular recording, and a suction electrode for the isolation and stimulation of the nerve. Both electrodes were prepared with a PUL-1 microelectrode puller with the recording microelectrode being filled with 3 M KCl and attached to the the recording assembly, while the intended suction electrode was filed down to a tip large enough to isolate a nerve with sandpaper and attached to the stimulating apparatus.

Intracellular Recording

An ADinstruments Powerlab 4/26 was used to connect the assembly to a computer with the application LabChart7 and the microelectrode was used to record the resting membrane potential of the crayfish extensor muscle while the suction electrode stimulated the nerve while connected to a Grass SD9 Stimulator. The resistance of these electrodes were checked constantly to ensure the resistance stayed above 5 Mega Ohms. For every data set consisting of 200 data points, a minimum of 2 different nerves and muscle groups were tested.

Solution Exchange

To swap out one solution for another from the dissection tray, the existing solution was removed via flexible tubing connected to a syringe and discarded safely. Then the new solution was poured into the dish with special effort to “rinse” the remaining old solution off of the crayfish extensor muscles. In every trial, only enough solution was used to cover the top of the tail carapace and the crayfish was exposed to each solution for 10 minutes before any intracellular recording took place.

RESULTS

The data below was collected to showcase the effects of methylphenidate and dopamine added alone and in concert (in different orders) by comparing the experimental data sets of EPSPs at the crayfish neuromuscular junction to a control group. The EPSP amplitudes are recorded through intracellular recording. The amplitude is calculated from measuring the difference between the peak of the EPSP and the start of the EPSP before the stimulus artifact. We gathered 200 EPSPs for each of the combinations of chemicals. We specifically gathered 200 EPSPs because we believed that more data points will help prove the consistency of our data as well as aid us in our overall accuracy. We finalized our data collection by converting the values into % changes to account for the fact that we used a different crayfish for each combination of chemicals. After all of the data was organized as such, we created a data table representing the values and a bar graph for each of the experiments so that a clear comparison may be observed. We used the ANOVA test to acquire the p-values of our data.

As seen in fig. 1, the results of each experiment had a significant effect on the average amplitude of the EPSP under all conditions. Dopamine showed the largest amplitude of the four experimental conditions, while methylphenidate showed the smallest. When compared to the baseline in terms of percentage, as in fig. 2, methylphenidate showed the greatest change from the baseline.

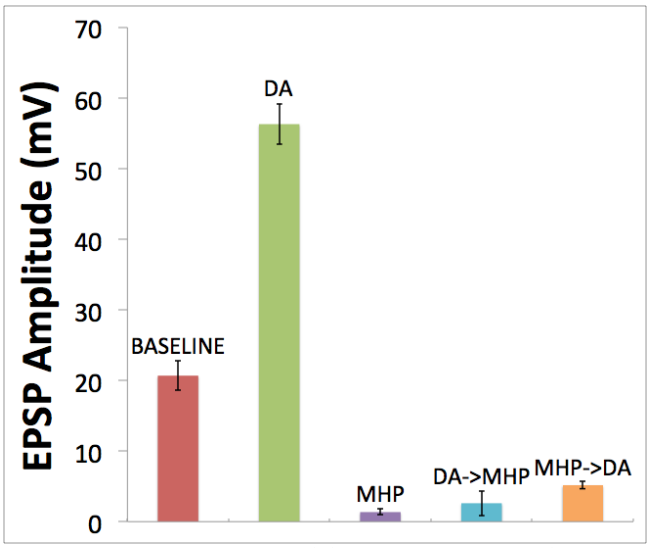

Fig 1. The various experiments and their EPSP’s average peak in millivolts. The error bars show the standard deviation for each sample set. Alone, dopamine (DA) was excitatory, but when paired with methylphenidate (MHP) in any order, it was inhibitory. Methylphenidate alone was also inhibitory. N = 200

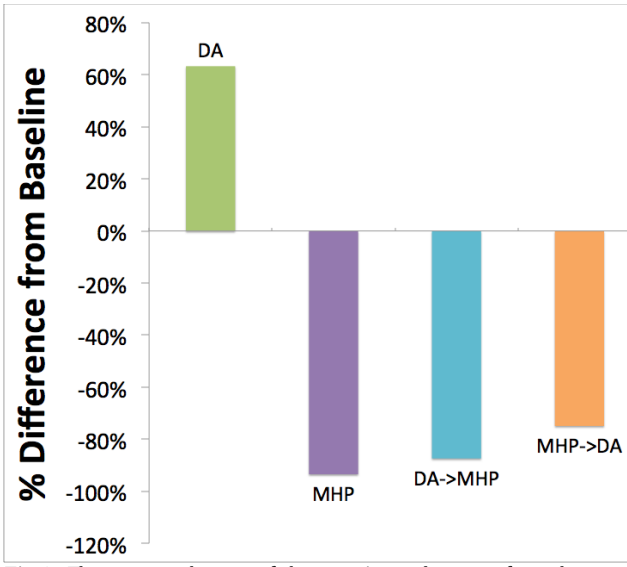

Fig 2. The percent changes of the experimental groups from the baseline in Fig. 1. Standard deviation for all samples sets are <1%. Ultimately, only dopamine (DA) caused an excitatory response, while both methylphenidate (MHP) and any combination of methylphenidate and dopamine was inhibitory to varying degrees. The most effective inhibitor was methylphenidate by itself. N = 200

DISCUSSION

Our research showed that dopamine was excitatory, but in any combination with methylphenidate, the EPSP was inhibited. Methylphenidate alone was also inhibitory. Additionally, the combinations of methylphenidate and dopamine were shown to be less inhibitory than methylphenidate alone. Considering dopamine alone produced an exceptionally strong excitatory reaction while dopamine after methylphenidate produced an inhibitory response suggests that dopamine became inhibitory upon the introduction of methylphenidate, supporting our hypothesis.

Methylphenidate, in vertebrates, is a dopamine reuptake inhibitor. More generally, methylphenidate is a catecholamine reuptake inhibitor, which includes norepinephrine and dopamine. Methylphenidate, via the inhibition of dopamine reuptake, could effectively cancel out a chain of chemical reactions involving a protein kinase that modulates dopamine receptors to be excitatory in nature. For instance, by introducing methylphenidate, a catecholamine other than dopamine could be binding to the postsynaptic cell and use a g-protein complex to activate a protein kinase which makes dopamine receptors inhibitory. Another plausible explanation is that methylphenidate is a sigma-1 receptor agonist, which binds to the signal receptor and modulates calcium signaling in the presynaptic cell through the IP3 receptor. If the modulation causes a decrease in either calcium concentration or calcium's ability to activate SNARE proteins responsible for exocytosis in the presynaptic cell, the rate of neurotransmitter release is decreased and the postsynaptic response would be inhibited.

As shown in Dougan et. al (1987), after dextroamphetamine (an analog to methylphenidate) is applied, the effect of dopamine is switched, which supports our hypothesis of the dopamine receptors on the postsynaptic cell being modulated. The implications this has is that where there is an excess of dopamine in a person, a localized treatment using methylphenidate may be plausible to help lower the effects of the condition. Further research may lead to more answers as to how the receptors are modified, or even at what point methylphenidate begins to significantly affect dopamine receptors.

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