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Cocaine increases synaptic transmission at the crayfish neuromuscular junction

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ABSTRACT

Cocaine is an alkaloid that stimulates the nervous system. It has high affinity for the 5-HT transporter, blocking the re-uptake of serotonin. Therefore, manipulation of the serotonergic system may reveal the mechanisms of action of cocaine. We hypothesized that cocaine would increase EJPs amplitude in the neuromuscular junction of a crayfish. Moreover, we predicted that cyproheptadine hydrochloride, a 5-HT receptor antagonist, would decrease EJPs amplitude in presence of cocaine. To test our hypothesis, we exposed a crayfish tail to different solutions of 5-HT, cocaine and cyproheptadine hydrochloride and through intracellular recording we measured EJPs amplitudes. As we expected, cocaine enhanced EJPs in the neuromuscular junction of a crayfish. Moreover, we observed that when the 5-HT2C blocker was added to the solution, the EJP amplitude decreased.

INTRODUCTION

Cocaine is a powerful alkaloid that stimulates the central nervous system (Filip, M., Frankowska, M., Zaniewska, M., Golda, A., Przegalinski, E; 2005). It has a high addictive potential given the way it affects the mesolimbic reward pathway (Mateo, Yolanda; Budygin, Evgeny A; John, Carrie E; Jones, Sarah R; 2004). For these reasons, cocaine addiction has become a major public health problem worldwide; unfortunately, completely effective pharmacological treatments have not yet been created (Filip, M., Frankowska, M., Zaniewska, M., Golda, A., Przegalinski, E; 2005). Laboratories worldwide are currently investigating the mechanisms of action of cocaine to determine which pathways it follows in 1) creating the reward sensation in the user and 2) in making the user addicted. Recent research has shown that cocaine has a correlation with the serotonergic system in the brain (Rocha, Beatriz A; Goulding, Evan H; O'dell, Laura E; Mead, Andy N; Coufal, Nicole G; et al; 2002). More specifically, it has been suggested that cocaine binds with high affinity to the dopamine (DA) and serotonin (5-HT) transporters, blocking dopamine and serotonin reuptake and therefore increasing the extracellular concentrations of the mentioned monoamines in the brain (Filip, M., Frankowska, M., Zaniewska, M., Golda, A., Przegalinski, E; 2005). High concentrations of DA and 5-HT are responsible for the medical effects of cocaine in the user, which include euphoria, hyperactivity, and a feeling of well-being, amongst other "positive" effects. These feelings are what induce the user to want to take cocaine again, which may explain its addictive properties (Filip, M., Frankowska, M., Zaniewska, M., Golda, A., Przegalinski, E; 2005).

Existing knowledge on the mechanisms of action of cocaine demonstrates that in general, the activation of the serotonergic system has an inhibitory effect on the reinforcing effects of cocaine (Filip, M., Frankowska, M., Zaniewska, M., Golda, A., Przegalinski, E; 2005). Also, it is known that different subtypes of 5-HT receptors, when activated, can have different effects on the reinforcing effects of cocaine (Rocha, Beatriz A; Goulding, Evan H; O'dell, Laura E; Mead, Andy N; Coufal, Nicole G; et al; 2002). In general, current research still focuses heavily on understanding all of the mechanisms of action of cocaine because the contribution of different pathways is unclear. Since research on cocaine is fairly recent, there is much to be studied. For instance, some subtypes of 5-HT receptors have no effect on regulation of cocaine whereas other subtypes (most notably 5-HT2C) do have an important effect (Filip, M., Frankowska, M., Zaniewska, M., Golda, A., Przegalinski, E; 2005).

Our study attempts to test the current understanding of the involvement of the serotonergic system in cocaine addiction by using the neuromuscular junction of a crayfish. We chose to work with crayfish because they have a simple nervous system that is easy to manipulate. Specifically, our study looked at the 5-HT2C subtype receptor and its role in the effect of cocaine in synaptic transmission. As we expected, cocaine enhanced EJPs in the neuromuscular junction of a crayfish. Moreover, we observed that when the 5-HT2C blocker was added to the solution, the EJP amplitude decreased, thereby supporting our proposed model as to the mechanism of cocaine action and the potential that pharmacological agents have in becoming effective treatments for cocaine addiction.

MATERIALS AND METHODS

Dissection:

The crayfish were placed in ice baths prior to the experiment for sedation. Once sedated, the tail of the crayfish was separated from the torso, and lateral incisions were made along the tail to facilitate the removal of excess muscle allowing exposure of the superficial extensor muscles. The tail fin was removed as well. The dissection was then pinned to the sylgard bottom of a small dish lined with wax that required less solution. This was necessary because of to the small amount of cocaine that was available.

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Solutions:

We prepared four different preparations with three different solutions in which the crayfish tail was immersed. The first, the control solution, was 100mL of Ringer's solution, a replica of natural crayfish saline, with 20μL of 10mM 5-HT. The Ringer's solution contained:

NaCl	205.0 mM
KCl	5.4 mM
CaCl	13.5 mM
MgCl	2.6 mM
Tris Buffer pH 7.4	10.0 mM

The second solution was the control solution with 0.01 mg/ml cocaine added. For the third solution, 100 μL of A 10 μM 5-HT_2 antagonist, cyproheptadine hydrochloride, was added to the control solution with cocaine. In between the third and fourth solution, the dissection was flushed with saline to wash the antagonist from the extensor muscles. The fourth preparation was composed of the second solution: Ringer's solution, 5-HT, and cocaine.

Microelectrodes:

Using a micropipette puller, we made a microelectrode with a thin tube of glass by heating up the tube in the middle and then pulling each side in opposite directions. This leaves two micropipettes with very fine tips. Then, we filled the micropipettes with 3MKCl to make the microelectrodes.

Data Collection:

The amplitudes of the EJPs at the neuromuscular junction of the crayfish were measured in each solution. The voltages around the membrane of the muscle cells were measured by comparing the voltage between a reference electrode placed in the bath and the tip of the microelectrode after inserting it into the muscle cell; the resistance ranged from 5MΩ-20 MΩ. Electric pulses were delivered to the nerve through a suction electrode attached to a micromanipulator. Any resulting EJPs were measured via the microelectrode and displayed through the Scope program. The amplitudes of the EJPs in each solution were recorded, and the average amplitudes for each solution were then calculated.

RESULTS

The purpose of the experiment was to determine whether cocaine has an effect on synaptic transmission at the neuromuscular junctions of crayfish. To determine this, the amplitudes of the EJPs in the control solution were measured as a "baseline" from which to compare the resulting EJPs in the other three preparations. These results were obtained by measuring the change in the muscle's cell membrane voltage as an electrical pulse was delivered to the nerve.

We found that the average of the amplitudes of the resulting EJPs in the 5-HT and cocaine solution was 19 mV, as opposed to an amplitude average of 14 mV in the control solution. With the addition of the antagonist to the cocaine and 5-HT solution, the average amplitude was 8 mV. The average amplitude of the EJPs increased to 11 mV once the preparation was washed with saline and the cocaine and 5-HT solution were added. This average, however, was not as high as the first cocaine and 5-HT preparation.

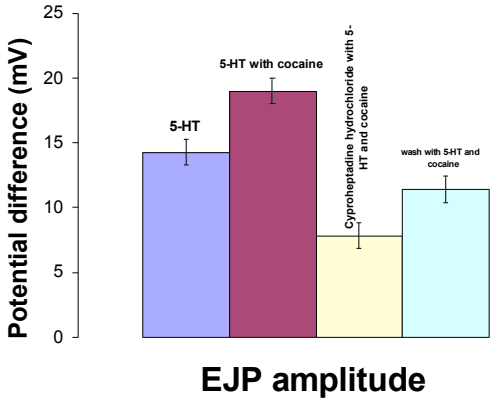

Figure 1. Effect of cocaine on synaptic transmission of muscles in a crayfish tail. Error bars represent ±1 S.E. The bars represent the mean EJP amplitude under different preparation. With 5-HT the mean is 14.3 mV. For 5-HT with cocaine the mean is 19 mV (P= 0.050, comparing 5-HT with 5-HT and cocaine). For 5-HT with cocaine and cyproheptadine hydrochloride the mean is 7.9 mV (P= 0.025, comparing 5-HT with cocaine and cyproheptadine hydrochloride) and with the wash that has cocaine and 5-HT the mean is 11. mV (P= 0.036, comparing the wash that has cocaine and 5-HT).

DISCUSSION

The data indicate that EJP amplitude increases with the addition of cocaine to the control solution. In the first cocaine/5-HT solution, we saw an average increase in the EJP amplitude. When the antagonist was added, the overall amplitude decreased dramatically. Once the preparation was washed and the cocaine was added, the EJP amplitude increased, but not to the levels of the first cocaine preparation. This may suggest that the wash was not completely effective and that some residual antagonist may have remained, decreasing the overall amplitude. These findings support our hypothesis because the solutions with cocaine registered a higher EJP, and the wash solution increased once the antagonist was removed.

We believe that the cocaine specifically affected the amount of serotonin transmitted. Our data shows that the presence of a 5-HT antagonist is correlated with a decrease in EJP amplitude. This may indicate that cocaine affects the serotonin levels causing the increase in synaptic transmission. These results are consistent with the studies of Filip, et al. (2005) that claim that cocaine has "a high affinity for the 5-HT transporter," and therefore, blocks the reuptake of the serotonin, resulting in increased levels in the synaptic gap. Researchers believe that the manipulation of the serotonin levels in the brain may have beneficial effects on those who engage in cocaine regularly. This would decrease levels of serotonin transmitted to post-synaptic cells and would thus, decrease the rewarding sensation that some cocaine users experience. Future research could study different antagonists for multiple 5-HT subtype receptors to determine how the levels of synaptic transmission vary and if there is any relationship within the different receptor subtypes.

The manipulation of serotonin levels may have serious side effects considering its importance as a major neurotransmitter. These possible effects should be further examined to ensure safety and effectiveness. This necessitates future work that would identify specific 5-HT subtype receptors so that the side-effects are not as great.

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