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Caffeine inhibits serotonin's enhancement of EJP amplitude in crayfish deep extensor muscle.

SARAH MIRK and BRIAN WERMCRANTZ
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

Caffeine is commonly considered a psychoactive drug due to its stimulant properties present both in the behavioral increase in attention and at synapses throughout the body. At the neuromuscular junction, caffeine likely achieves its psychomotor stimulation by increasing EJP amplitude. However, research in the past year found that caffeine has a paradoxical effect at the flexor muscle: it inhibits serotonin-induced synaptic enhancement. We tested whether this behavior also occurs at the superficial extensor muscle. As previously observed at the flexor muscle, recorded EJP amplitudes in the crayfish extensor muscle returned to control levels once caffeine was introduced to a serotonin-rich saline solution.

INTRODUCTION

The psychomotor stimulatory effects of caffeine are all too familiar to busy researchers. While caffeine has differing physiological effects at different concentrations, it typically enhances synaptic transmission, observable as an increase in amplitudes of excitatory junction potentials (EJPs) (Nehlig et al., 1992). Olson et al (1988) found that rats fed with caffeine-rich food exhibit higher levels of serotonin as well as cyclic AMP in the brain. Serotonin is an important neurotransmitter used to control mood and attention level, hallmark symptoms of caffeine consumption, and, therefore, may play role in caffeine's psychomotor stimulation (Beat et al. 2007). Caffeine's stimulatory effects, therefore, may be explained, at least in part, by modulating serotonin levels. For these reasons, in addition to its more obvious behavioral effects, caffeine is commonly considered a stimulant. Fisone et al (2004) found that caffeine psychoactive effects rely on the drug antagonizing adenosine receptors. However, more recent research demonstrates that caffeine depresses serotonin's excitatory effect on EJPs in the crayfish superficial flexor muscles (Celenza et al 2007).

Our research tested whether this recently discovered paradoxical effect of caffeine found in the flexor muscles occurs also in the crayfish deep extensor muscles. Specifically, our project addressed how the increased presence of extracellular caffeine and serotonin impacts synaptic transmission. We investigated whether Celenza et al's (2007) findings are unique to the crayfish's SFM group or whether the same unexpected depressive effect of caffeine occurs in the crayfish's deep extensor muscles as well. If the depressive effect of caffeine on serotonin is repeated in the deep extensors as it the SFM, it will indicate that the unexpected interaction is not due to unique physiological aspects of the crayfish SFM, but instead due to the interaction of the two substances in the prototypical synapse model. If this conclusion is made, further research can be done to determine exactly how caffeine inhibits serotonin EJP stimulation.

Because overwhelming research demonstrated that caffeine typically acts a stimulant, we hypothesized that caffeine would increase serotonin-induced enhancement of synaptic transmission at the crayfish neuromuscular junction. However, our research supported the findings of Celenza et al. (2007) and suggested that our hypothesis was incorrect. We recorded control EJPs with an average displacement amplitude of 16mV. After serotonin application to the extracellular fluid, EJPs increased to approximately 25mV and then dropped down back to 17mV once caffeine was introduced to the solution, contradicting our predictions.

MATERIALS AND METHODS

Dissection and Recordings

This experiment used crayfish species *Procambaris clarkii*, which were chilled on ice. We removed the upper part of the shell and muscle tissue from the crayfish's tail, exposing the deep extensor muscles (DEM) and connected nerves. We then placed the tail in a standard crayfish saline solution. Using a 1.2 mm glass electrode filled with 3M KCl and attached to a manipulator, change in voltage across the cell membrane was recorded in muscle cells while simulating the DEM nerves between 3Hz and 8Hz. The resulting 10ms-long fluctuations in amplitude were known as excitatory junction potentials (EJPs). We continually monitored each

electrode's resistance and discarded those with resistances outside the standard 5-20MΩ range. Resistances between these values insured that the recording electrodes were neither too small (creating high electrical noise) or too large (interfering with the measurement of accurate membrane potentials). In carrying out our experiment, the crayfish system was first exposed to standard crayfish saline solution to obtain a baseline EJP. The saline solution was then removed gradually and replaced with the experimental serotonin solution and then, in turn, the experimental caffeine solution was added without removing the serotonin solution. EJPs were monitored over time, with between ten and forty taken in the presence of each solution. The results for each solution were then averaged.

Experimental Solutions

The standard crayfish saline solution contains a mixture of 5.4 mM KCl, 196 mM NaCl, 2.6 mM MgCl2.6H2O, 10.0 mM Na Hepes Buffer, and 13.5 mM CaCl2.2H20, with an overall pH of 7.4. To the crayfish system was added 100 ml of 10 µM concentration of serotonin and an equal amount of 10 µM concentration caffeine, both obtained from Fisher Pharmaceutical.

RESULTS

Our experiment was designed to investigate the interaction between caffeine and serotonin in the neuromuscular junction of a crayfish. We hypothesized that caffeine would increase the enhancement effect of serotonin, since caffeine is also a stimulant. However, recent research by Celenza, et al (2007), found that, paradoxically, caffeine depressed the enhancement effect of serotonin in the crayfish SFM. Our average of 22 recordings per organism revealed a 21% decrease in EJP amplitude obtained from the deep extensor muscles of two different crayfish in the presence of serotonin and caffeine versus those obtained in the presence of the serotonin solution alone (Fig. 1 $p<0.05$ and Fig. 2). Our study shows that, initially, the serotonin increased the amplitude of the EJPs by an average of 62 percent above the control level. Upon the addition of the caffeine solution to the system, the amplitude of the EJPs returned to approximately the control level with EJPs averaging 17mV in amplitude.

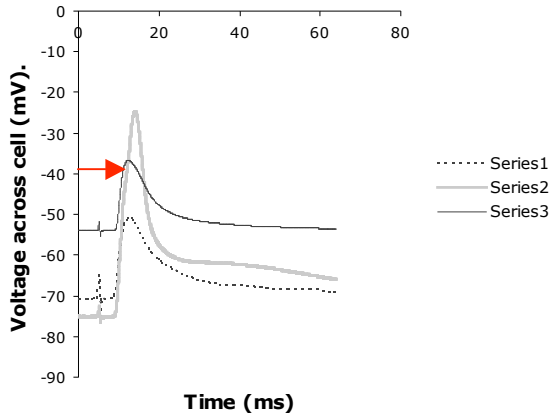

Fig. 1 Comparison of the change over time in the voltage across the cell membrane (EJPs, measured in mV) of the deep extensor muscles in the presence of three solution: standard saline (series 1), serotonin (series 2) and serotonin and caffeine (series 3). Two sets of data from two crayfish was used and averaged. Initially, the average control EJP had an amplitude of 16 mV. With the addition of serotonin, the average amplitude increased to 25.5 mV, with an arrow denoting the point where the EJP became an action potential. When the caffeine solution was added, the average amplitude decreased to an average of 16.5mv.

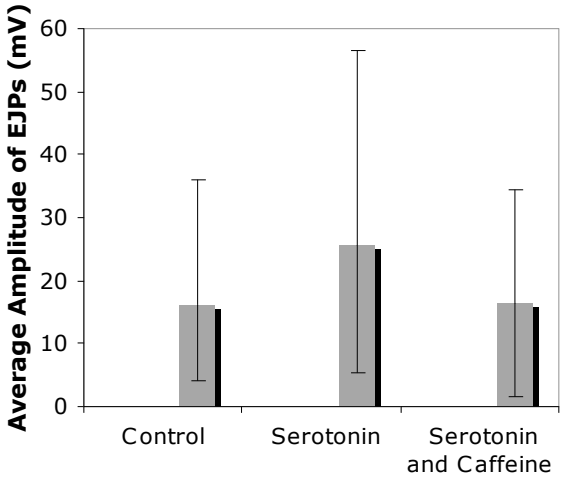

Fig. 2 Comparison of average amplitude of EJPs (in mV) measured from two crayfish in the presence of a control saline solution, serotonin and serotonin and caffeine. The average control amplitude was 16 mV, rising to 25.5 mV in the presence of serotonin and decreasing to 16.5 mV with the addition of caffeine (n=2). The error bars represent the range of the data between two crayfish, while from each crayfish an average of 22 recordings per solution were made.

DISCUSSION

Our data disagrees with our hypothesis that caffeine would further increase EJP amplitude once applied to the extracellular solution with the presence of serotonin. A significant decrease in EJP amplitude was recorded after the addition of caffeine with EJP amplitude returning almost completely to control levels. EJP amplitude differed between control and serotonin and caffeine levels by one percent, while serotonin application increased EJP amplitude by 62%. The rapidity and magnitude of the EJP amplitude decrease suggests that caffeine not only depresses serotonin-induced enhancement of EJP amplitude, but almost completely inhibits this enhancement in the crayfish deep extensor muscles.

While the explanation for this paradoxical event is not clear, caffeine probably depresses the enhancement effect of serotonin via interaction with one of the mechanisms through which either caffeine or serotonin typically act. Given that at these concentrations caffeine antagonizes adenosine receptors, (Fisone et al. 2004) and serotonin acts on sub-receptors that increase cyclic AMP (Bear et al. 2007), it is probable that the explanation for the results seen here can be found through further research on the effects of caffeine on serotonin receptors or the effect of serotonin on adenosine receptors. Furthermore, serotonin typically modulates synaptic transmission by working on a number of sub-receptors that modulate cyclic AMP levels (Bear et al. 2007). The effect of increased cyclic AMP levels on caffeine may also account for the paradoxical effect observed. Whether this interaction occurs throughout the crayfish or within other organisms as well definitely warrants further study as it contradicts the typically understood nature of caffeine acting as a stimulant.

Nevertheless, the fact that findings of Celenza et al. (2007) were confirmed in another muscle group of the crayfish, indicates that this paradoxical interaction is not due to physiologically unique to one part of the crayfish, but instead due to the interaction of the caffeine and serotonin in the synapse. It is likely that this paradoxical effect also occurs in humans, yet more study in humans and other animals may challenge the paradoxical effect of caffeine in crayfish. Further research may reveal a more complicated understanding of how caffeine we consume daily achieves its psychomotor stimulation.

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