

The Effects of Octopamine on the EPSPs of *Procambarus clarkii* are linked to IP₃ Receptors.

BEN SHIRAR, QUINN UNDERRINER, AND SAHAR NAHIB
Department of Biology, Grinnell College, Grinnell, Iowa

ABSTRACT

The neurotransmitter octopamine raises the amplitude of excitatory post-synaptic potentials (EPSP) in crayfish (*Procambarus clarkii*). Previous studies have ruled out a connection between octopamine's effects and cAMP or cGMP systems, suggesting another secondary messenger system is responsible for the increase in EPSP amplitude. The IP₃ system, which is responsible for the release of intracellular calcium, has been suggested as a possible mechanism by which octopamine's effects on EPSP are generated. Using intracellular recording, the EPSPs of crayfish in three different solutions were measured; a baseline ringer's solution, a solution containing octopamine, and a solution containing octopamine and 2APB, an IP₃ receptor blocker. We found that the solution with the IP₃ receptor blocker had a much lower EPSP, supporting our hypothesis that octopamine acts via the IP₃ system.

INTRODUCTION

The biogenic amine octopamine has been demonstrated to increase EPSP amplitude in crustaceans (Djokaj S et al, 2001). Later research on octopamine and serotonin established that serotonin's effects on crustacean EPSPs are directly linked to cAMP (Araki et al, 2005) while octopamine's effects were not regulated by cAMP. Octopamine's effects, therefore, are not linked with those of serotonin and so the response mechanism must be a different system. Prior research has indicated that in insects, octopamine's effects may be linked to the IP₃ system, which is responsible for releasing intracellular calcium and thus raising EPSP amplitude (Baines and Downer, 1994). Further research also indicated that similar biogenic amines exhibited similar effects on both insects and crustaceans (Jahagirdar et al, 1987). Based on this research, we intend to examine the effects of octopamine on crayfish EPSPs and isolate the system responsible for the increase in EPSP amplitude. We hypothesized that octopamine's effects will be linked to the IP₃ system and that, specifically, octopamine-induced changes will respond to the blocking of IP₃ receptors. Our experimental data supports this hypothesis.

MATERIALS AND METHODS

Crayfish and its preparation

For this experiment we used *Procambarus clarkii* which were supplied by Carolina Biological supply company (North Carolina, USA). They were cooled in ice as a form of anesthetic which serves the ethical purpose of negating the pain the animal would have felt during experimental preparation, as well as preventing the chemical changes associated with pain. In order to be able to measure the EPSP in the tail muscle fibers, we cut off the tail and then cut along the ridges that runs parallel to the length of the tail on both sides. After this we separated the two sides and pushed off the viscera which was not needed for the experiment. We then pinned the remaining portion of the tail containing the long extension muscles onto a silicon layer in a dissection dish.

Saline solutions

The solutions needed for this experiment are a standard crayfish saline, a saline solution with octopamine and a solution of saline with 2APB and octopamine. Standard saline solution is composed of 5.4mM KCl, 196mM NaCl, 2.6mM MgCl₂ 6H₂O, 10mM sodium hepes buffer and 13.5mM CaCl₂ 2H₂O. It has a pH of 7.4. The solution of 1mM octopamine in 100μl aliquots was added to 100 ml of standard solution to produce 1:1000 dilution of solution of saline with octopamine to achieve final concentration of 100μM. In order to produce the final concentration of 100μM of 2APB and octopamine with crayfish saline we needed 50 mM 2APB in 300μl aliquots, 1mM octopamine in 100 μl aliquots, and 100ml of crayfish saline solution. We had around 100 ml of saline in the silicon dissection dish. We first injected the

octopamine followed about five seconds later by the 2APB.

Microelectrode and set-up of apparatus

We made the microelectrode from 1.2 mm glass pipettes which were heated in the microelectrode puller for about 7 seconds producing two pipettes with very narrow tips. We filled the microelectrode and microelectrode holder with 3M KCl solution and then rinsed it in crayfish solution. The crayfish nerve was sucked up by a suction electrode which was connected to a Grass SD-9 Stimulator. The stimulator was used to stimulate the nerve to generate an action potential creating EPSPs detected by the microelectrode. We use a micromanipulator to move the microelectrode holder. To measure the EPSPs we used a computer program called Scope. We first measured the voltage of the saline and then made that our zero point on the scale so our next measurement is taken with that as the starting point. We then checked the resistance which should be over 5 M Ω if the tip has not broken or there are no bubbles in the microelectrode. If the resistance was above 5 M Ω we then recorded the EPSPs caused by the stimulator.

RESULTS

Our experimental data displayed statistically significant differences in EPSP amplitude augmentation between each treatment group; statistical P-values for a 2-sample T-test of any combination of treatment groups (i.e. Control with Octopamine, Octopamine with Control, Octopamine with 2-APB+Octopamine) yielded P-values below .05, within our threshold for rejecting the null hypothesis that Octopamine would produce the same results with and without 2 APB. The exception was 2-APB and control, between which no statistical difference was found, indicating that these two findings are for all intents and purposes identical. The alternative hypothesis, that octopamine's effects on EPSP amplitude would be dampened by the addition of 2-APB, an IP3 receptor blocker, was supported, suggesting a link between octopamine's augmentation of EPSP amplitude and the IP3 system. Figure 1 shows the mean amplitude of each treatment, which allows us to see that the octopamine + 2 APB treatment averaged a similar value to the baseline treatment, suggesting that the IP3 blockers canceled out octopamine's effects.

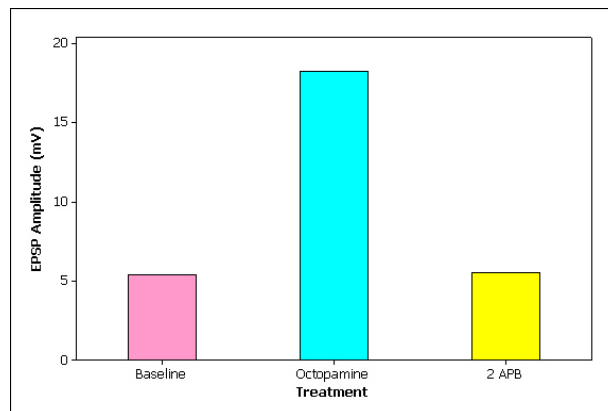


Figure 1. Mean EPSP amplitudes for each treatment. Baseline Mean= 4.5, Octopamine Mean= 18.2, and Octopamine + 2 APB Mean= 4.6.

DISCUSSION

One factor that slightly hampered our experimental data was the fact that our data came from multiple preparations, and different crayfish, depending on any number of factors, could be more or less responsive—this concern is muted some by the fact that all of our data came from multiple preparations, meaning that the net difference should. Additionally, the validity of the data for the octopamine + 2 APB sample is slightly confused by the fact that octopamine and 2 APB were introduced immediately following one another, without allowing the crayfish to soak in 2 APB for a significant period of time. Our sample size was rather small and, as with any experiment, additional repetitions of the experiment would better solidify the validity of our results.

Another possible oversight was that we never tested the effects of 2 APB by itself on the EPSP amplitude, therefore we can not make definitive claims about whether the data gathered from the 2-APB+Octopamine treatment was demonstrative of 2-APB's effects in relation to octopamine, or whether 2-APB elicited the same reaction in the absence of octopamine. Further research could focus on the long-term effects of octopamine and 2 APB in regards to synaptic plasticity. An additional branch for further research could be whether other secondary messenger blockers or antagonists could diminish octopamine's effectiveness in a similar way. This would help further determine the degree of specificity that octopamine possesses, beyond that suggested by prior research (Araki et al, 2005)

ACKNOWLEDGEMENTS

We thank Clark Lindgren, our professor, for his immeasurable patience and his ability to inspire us to continue to try and collect data long after we wanted to give up and go get lunch. From the bottom our hearts we thank Sue Kolbe, our lab assistant, for always making sure that we had all the instruments and chemicals that we needed exactly when they were necessary. We would also like to thank Grace Hazeltine and Molly Wingfield for assuring us that lab work is a painstaking process for everyone and helping us find nerves when there were seemingly none to be found.

REFERENCES

Djokaj S, Cooper RL, and Rathmayer W. 2001. Presynaptic effects of octopamine, serotonin, and cocktails of the two modulators on neuromuscular transmission in crustaceans. *J Comp Physiol [A]* 187: 145–154.

Jahagirdar AP, Milton G, Viswanatha T, and Downer RGH. 1987. Calcium involvement in mediating the action of octopamine and hypertrehalosemic peptides on insect haemocytes. *FEBS Lett* 219: 83–87.

Baines D and Downer RGH. 1994. Octopamine enhances phagocytosis in cockroach hemocytes: involvement of inositol trisphosphate. *Arch Insect Biochem Physiol* 26: 249–261.

Araki M and Nagayama T. 2003. Direct chemically mediated synaptic transmission from mechanosensory afferents contributes to habituation of crayfish lateral giant escape reaction. *J Comp Physiol [A]* 189: 731–739.

Makoto Araki, Toshiki Nagayama, and Jordanna Sprayberry. 2005. Cyclic AMP Mediates Serotonin-Induced Synaptic Enhancement of Lateral Giant Interneuron of the Crayfish. *J Neurophysiol*, 94: 2644 - 2652.

Christian Walther and Klaus E. Zittlau. 1998. Resting Membrane Properties of Locust Muscle and Their Modulation II. Actions of the Biogenic Amine Octopamine. *J Neurophysiol*,; 80: 785 - 797.