Octopamine receptors on spider peripheral mechanosensilla

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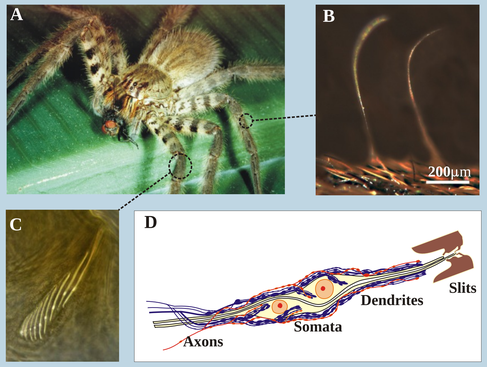
Introduction

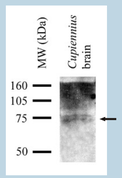
Spider mechanosensory neurons receive extensive efferent innervation. In the tropical wandering spider, *Cupiennius salei*, most of the efferent neurons are GABAergic and the mechanosensory neurons respond to agonists of ionotropic and metabotropic GABA receptors. Other possible neurotransmitters at these peripheral synapses include glutamate and acetylcholine. Octopamine, an invertebrate analog of noradrenaline, is a neuromodulator that acts as a neurotransmitter or a neurohormone. It is known to affect several peripheral sensory organs, including mechanoreceptors in insects and crustaceans. Moreover, octopaminergic neurons have been found in the central nervous system of *Cupiennius salei*.

Here we characterized the distribution of octopamine receptors (OctopR) in the mechanosensory VS-3 slit-sense organ and tactile hairs, and performed extracellular recordings from tactile trichobothria sensilla in the spider tibia to assess the physiological role of OctopR.

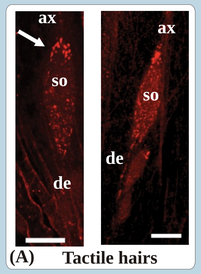


A: A female tropical wandering spider (*Cupiennius salei*). The electrophysiological recordings were performed from trichobothria hairs (B) on the tibia. The mechanosensory lyriform VS-3 slit organ (C) was also used for immunocytochemistry.
D: Efferent fibers make extensive contacts with the dendrites, somata and axons of mechanosensory neurons in the VS-3 organ (modified from Fabian-Fine et al. (2000) J Comp Neurol 420:195-210).

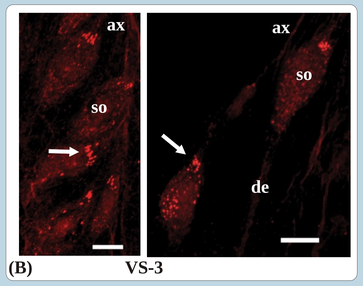
Western Blot analysis and Immunocytochemistry

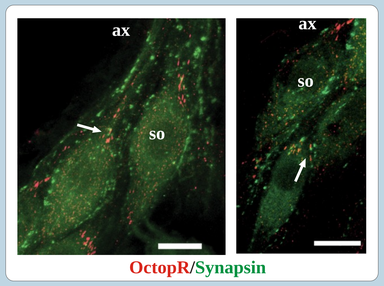


For Western blot analysis we used a polyclonal unpurified antibody (OAMB, 1:2,000) that was developed against a cloned *Drosophila* OctopR. In spider brain tissue extract a band was observed at approximately 72 kDa, which is the expected molecular weight of OctopR calculated from the *Drosophila* amino acid sequence.



Immunocytochemistry using OAMB antibody (1:1,000) and CY-3 as the secondary antibody. Immunoreactivity was strongest in the proximal parts of the somata (arrows) in neurons innervating the tactile hairs (A) and the VS-3 organ (B), but it was also present in other parts of the cell bodies and the axons (Scale bars 20 µm).

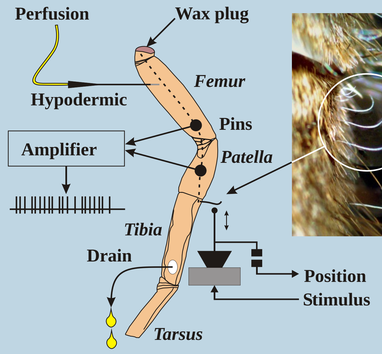




Double immunostaining using OAMB antibody (red) and an antibody against *Drosophila* synapsin (1:100) with Alexafluor 488 as the secondary antibody (green). Some of the synaptic vesicles on the efferent fibers were located close to OctopR (arrows). However, OAMB labeling was exclusively found on mechanosensory neurons and was not present on the efferent fibers (Scale bars 20 µm).

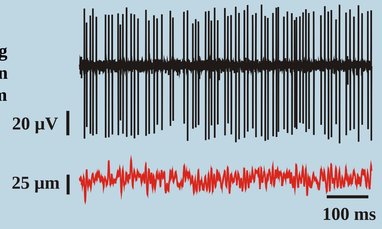
Electrophysiology

Experimental Set Up

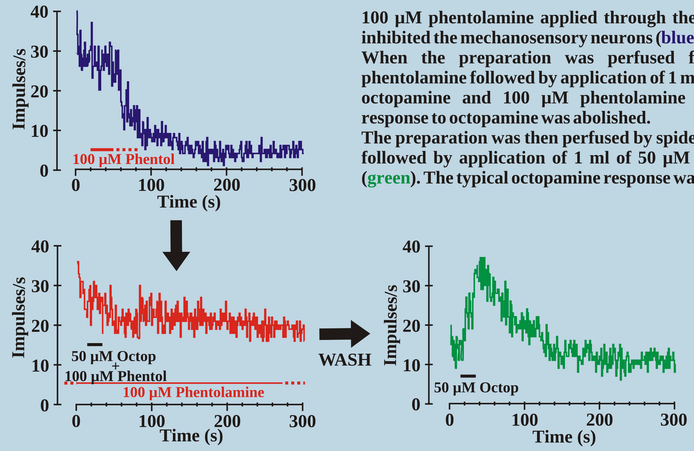


Action potentials were recorded extracellularly from autotomized legs perfused with spider saline. Drugs were applied directly into the saline flow just before it entered the leg. Two silver wire electrodes placed in the femur and patella allowed recordings from small trichobothria sensilla (~700 µm long) situated at the proximal end of the tibia. A stimulator consisting of a tungsten wire mounted on a loudspeaker was attached to one of the hairs with petroleum jelly. The hair was stimulated by pseudorandom noise displacement with a maximum amplitude of ~50 µm.

A typical recording showing the response of a neuron (black) to a pseudorandom noise stimulus (red).

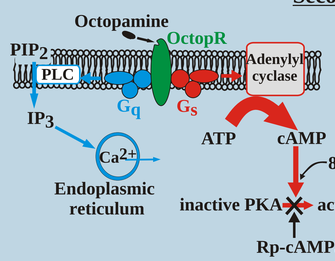


Phentolamine blocked the octopamine effect



100 µM phentolamine applied through the perfusion system inhibited the mechanosensory neurons (blue).
When the preparation was perfused for 12 min with phentolamine followed by application of 1 ml mixture of 50 µM octopamine and 100 µM phentolamine (red) the typical response to octopamine was abolished.
The preparation was then perfused by spider saline for 12 min followed by application of 1 ml of 50 µM octopamine alone (green). The typical octopamine response was observed.

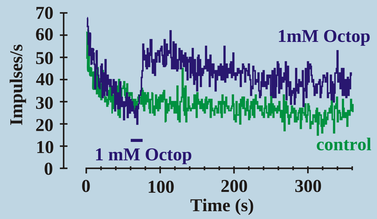
Second messenger system



At least two second messenger pathways are known to transduce octopamine responses. The most common is a Gs-Adenylyl cyclase pathway (red). Another one is a Gq-PLC pathway (blue).

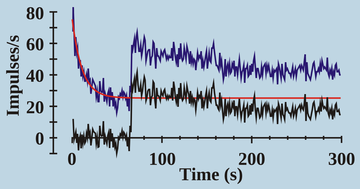
We used a cAMP analog (8-Br-cAMP) and a competitive inhibitor of PKA type I and II (Rp-cAMPS) to test whether the octopamine response was mediated via the Gs-Adenylyl cyclase pathway.

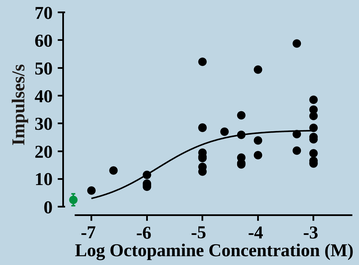
Octopamine increased the firing frequency of the mechanosensory neurons



60 seconds after the start of the recording 0.5 ml of 1 mM octopamine was applied to the perfusion solution (blue). The firing frequency increased rapidly. At this concentration the octopamine effect was still seen more than 5 min after application. The green trace shows a control recording where octopamine was not applied.
Tyramine, the precursor of octopamine, had a similar effect (not shown).

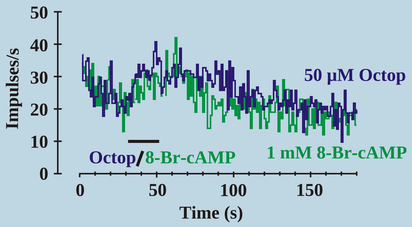
A dose-response function was calculated from 36 responses to different octopamine concentrations. An exponential decay (red) was fitted to the first 30 seconds of each recording before drug application. This function was then subtracted from the original recording (blue). The peak response was calculated by averaging the 20 highest points of the resulting trace (black). The peak values were used to plot the dose-response curve.

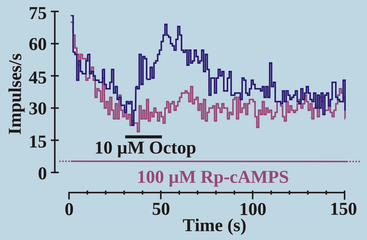




The dose-response curve was fitted by a Hill function that indicated EC_{50} = 1.5 µM. The green point represents the peak value (mean ± s.d.) of five control recordings where no drug was applied.

8-Br-cAMp mimicked the effect of octopamine.
1 ml of either 1 mM 8-Br-cAMP (green) or 50 µM octopamine (blue) was applied to the preparation. The amplitude and time course of the responses were similar.





Rp-cAMPS blocked the octopamine effect.
A cell responding normally to 10 µM octopamine (blue) was perfused with 100 µM Rp-cAMPS. After 15 min, a new application of 10 µM octopamine had no effect on the firing frequency of the mechanosensory neuron (purple).

Summary and Conclusions

- Western blot analysis and immunocytochemistry using an antibody against *Drosophila* OctopR (OAMB) suggested that octopamine receptors are present in spider peripheral mechanosensory neurons and especially strongly concentrated on the proximal parts of the cell bodies.
- Double staining using OAMB and an anti-synapsin antibody indicated that synaptic contacts could occur between efferent fibers and OctopR on the mechanosensory neurons.
- Octopamine application significantly increased the firing frequency of mechanosensory neurons in response to a mechanical stimulus.
- The effect of octopamine was reversibly blocked by the octopamine receptor antagonist phentolamine. However, high phenotlamine concentration alone inhibited the firing frequency, suggesting an additional mechanism of action.
- 8-Br-cAMP mimicked the effect of octopamine and Rp-cAMPS blocked the effect, suggesting that OctopR are positively coupled to adenylyl cyclase and act via a PKA mediated pathway.

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