Octopamine-induced increase in sensitivity in spider VS-3 mechanosensory neurons is mediated by Ca^{2+}/calmodulin dependent protein kinase II

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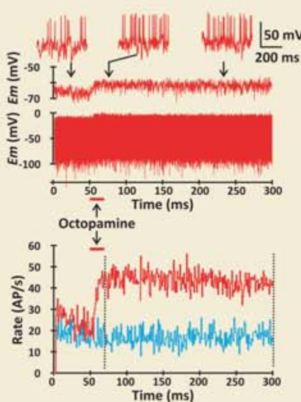
INTRODUCTION

Octopamine is a biogenic amine that modulates numerous physiological functions in invertebrates, similar to those associated with adrenaline or noradrenaline in vertebrates. Several octopamine receptors have been cloned and tested in expression systems. They are G-protein coupled and most can be classified as α- or β-adrenergic-like octopamine receptors (OctαR and OctβR, respectively), or octopamine/tyramine receptors. OctαRs generate intracellular Ca-signals and, to a lesser extent, raise cAMP levels, while OctβRs increase cAMP with no effect on calcium. Octopamine regulates cAMP levels also in many intact invertebrate cells, but less is known about its effects on calcium and very little is known about the signaling pathways that are activated or inhibited by calcium or cAMP. Octopamine also modulates various ionic currents in neurons, but it is not obvious how this modulation leads to changes in excitability. These changes may be excitatory or inhibitory even in the same neurons, suggesting that several receptor types may be present, or diverse signaling mechanisms are activated by the same receptors.

Mechanosensory neurons in the legs of the spider *Cupiennius salei* were immunoreactive to an antibody against *Drosophila* OAMB receptor. Some efferent neurons that synapse with mechanosensory afferents were labeled with an antibody against octopamine, and octopamine application enhanced the sensitivity of mechanosensory neurons (Widmer et al. 2005). The present study investigates how octopamine modifies the sensitivity of neurons innervating the VS-3 slit sensilla on the patella of *C. salei*. Major goals of this study were to identify the second messenger molecules that are regulated by octopamine, to learn what types of signaling mechanisms are triggered by these molecules and how they lead to the physiological effects.

OCTOPAMINE EFFECTS ON SPIKE RATE



VS-3 neuron response to electrical pseudorandom noise stimulation is shown with and without action potentials (AP). Upper traces show the spikes before and after octopamine application on a different scale. Octopamine was ejected iontophoretically at the time indicated. The lower traces show the AP firing rate from the recording above (red) and the AP rate of a control recording from the same neuron without octopamine application (blue).

OCTOPAMINE EFFECT ON FREQUENCY RESPONSE

Frequency response analysis was performed from the excitatory period following octopamine application and during the corresponding period in control recording. Frequency response functions were fitted by the power-law relationship

$$G = Af^{k}$$

where f is frequency; A is a constant describing overall sensitivity (gain at 1 Hz); k is the fractional exponent that describes the rate of adaptation. The phase relationship was fitted by:

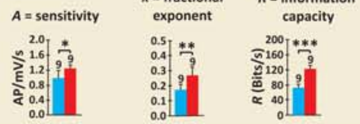
$$P(f) = k\,90° - \Delta t(f)\,360°$$

where $P(f)$ is the phase lag as a function of frequency; and Δt is a time delay.

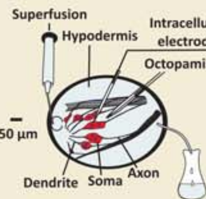
The coherence functions, $\gamma^2(f)$ between the input and output were calculated and plotted against log frequency. Linear information capacity, R, was calculated from the coherence function using the Shannon formula (Shannon & Weaver, 1949).

$$R = \int \log_2\{1/(1-\gamma^2(f))\}df$$

Gain (AP/s/mV) · Phase · Coherence · Frequency (Hz) · Control · Octopamine



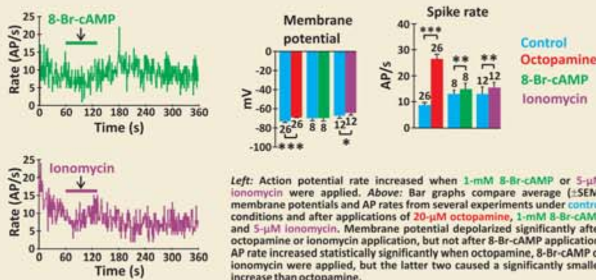
METHODS



20 mV / 500 ms · 2 nA / 500 ms

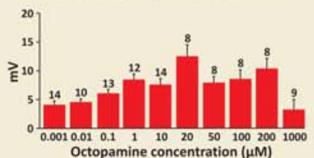
Legs from adult tropical wandering spiders (*Cupiennius salei*) were autotomized. A small piece of the patellar cuticle containing VS-3 slit sense organ was dissected and the organ was removed from cuticle, mounted on a coverslip and placed in an experimental chamber as a hypodermis preparation. Preparations were continuously superfused with spider saline (in mM: 223 NaCl, 6.8 KCl, 8 $CaCl_2$, 5.1 $MgCl_2$, and 10 HEPES, pH 7.8). Drugs were ejected close to the neurons either using an iontophoretic drug ejection system or manually with a syringe via tubing. Sharp borosilicate glass microelectrodes were filled with 3 M KCl. Recordings were made in discontinuous current- or voltage-clamp mode using a SEC-10L amplifier (npi electronic, Tamm, Germany). For frequency response recordings pseudorandom white noise stimulation was applied as current injection through the recording electrode (green trace) and resulting action potentials (blue trace) were recorded.

8-Br-cAMP AND IONOMYCIN EFFECTS ON VS-3 NEURONS



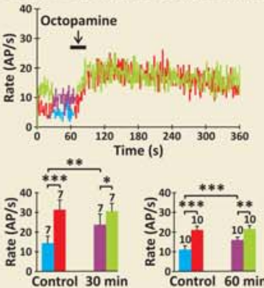
Left: Action potential rate increased when 1-mM 8-Br-cAMP or 5-µM ionomycin were applied. *Above:* Bar graphs compare average (±SEM) membrane potentials and AP rates from several experiments under control conditions and after applications of 20-µM octopamine, 1-mM 8-Br-cAMP and 5-µM ionomycin. Membrane potential depolarized significantly after octopamine or ionomycin application, but not after 8-Br-cAMP application. AP rate increased statistically significantly when octopamine, 8-Br-cAMP or ionomycin were applied, but the latter two caused a significantly smaller increase than octopamine.

DOSE RESPONSE RELATIONSHIP



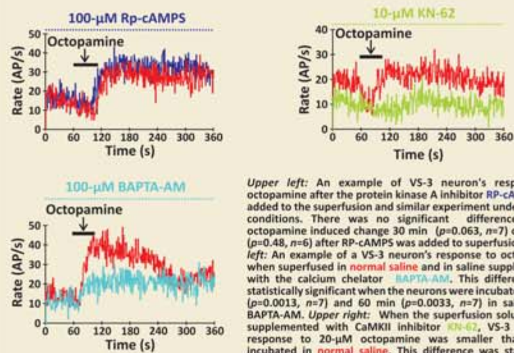
Bar graphs show the synaptic potentials elicited when octopamine was applied at various concentrations while the neurons were held at -90 mV holding potential. Maximum synaptic potential was recorded when 20 µM octopamine was applied. Numbers of experiments at each concentration are indicated above each bar.

OCTOPAMINE INDUCED A LONG LASTING INCREASE IN FIRING RATE



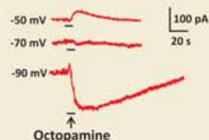
Above: An example showing AP rates from two recordings from the same VS-3 neuron where 20 µM octopamine was applied first time or 60 minutes after the first experiment. *Below:* Bar graphs show the AP rates (mean ± SEM) before octopamine was applied (blue bars) and during the maximum rate after octopamine application (red bars) when there was no previous exposure to octopamine (*control*). The purple bars are spike rates 30 and 60 min after first octopamine application and the green bars are maximum spike rates during the second octopamine application.

SIGNAL TRANSDUCTION PATHWAY INHIBITORS



Upper left: An example of VS-3 neuron's response to octopamine after the protein kinase A inhibitor RP-cAMPS was added to the superfusion and similar experiment under control conditions. There was no significant difference in the octopamine induced change 30 min (p=0.063, n=7) or 60 min (p=0.48, n=6) after RP-cAMPS was added to superfusion. *Lower left:* An example of a VS-3 neuron's response to octopamine when superfused in normal saline and in saline supplemented with the calcium chelator BAPTA-AM. This difference was statistically significant when the neurons were incubated 30 min (p=0.0013, n=7) and 60 min (p=0.0033, n=7) in saline with BAPTA-AM. *Upper right:* When the superfusion solution was supplemented with CaMKII inhibitor KN-62, VS-3 neuron's response to 20-µM octopamine was smaller than when incubated in normal saline. This difference was statistically significant 20 min after KN-62 was added to the superfusion (p=0.0001, n=12).

CURRENT-VOLTAGE RELATIONSHIP



I (pA) · E_m (mV)

Top: When a VS-3 neuron was held under voltage-clamp at -70 mV, application of 10-µM octopamine produced a small inward current. Larger inward current was recorded at -90 mV and an outward current at -50 mV holding potential. *Bottom:* Peak currents from several experiments plotted against voltage indicate that the reversal potential for octopamine induced current was -63 mV.

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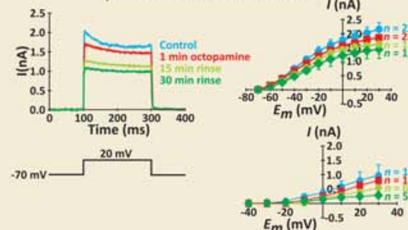
Octopamine effects on voltage-activated potassium outward currents



20 mV · -70 mV

Left: When inward currents were blocked with 1-µM TTX and 100-µM Ni^{2+}, voltage steps to 20 mV produced a slowly deactivating outward K-current. 1 min after octopamine application, amplitude of this current was reduced and it continued to diminish after octopamine was removed from the bath solution. *Right:* Amplitudes of the steady state (*above*) and transient (*below*) K-current components plotted against test potentials under control conditions and after octopamine application.

SUMMARY AND CONCLUSIONS

- Octopamine enhanced the sensitivity of VS-3 neurons especially at high stimulation frequencies.
- This enhancement lasted at least for one hour after octopamine application.
- Changes in sensitivity were also detected when Ca-ionophore ionomycin or cAMP analog 8-Br-cAMP were applied.
- The cAMP pathway was unlikely to mediate the octopamine effect, since the protein kinase A inhibitor, RP-cAMPs, did not diminish this effect.
- In contrast, the octopamine induced sensitivity enhancement was significantly reduced by KN-62, an inhibitor of CaMKII and with the Ca^{2+} chelator BAPTA-AM.
- Octopamine also depolarized the neurons by 3.8 mV and reduced the amplitudes of voltage-activated K-currents.
- We propose that octopamine receptors in VS-3 neurons activate IP_3 leading to Ca^{2+} release from intracellular stores. The Ca-surge turns on CaMKII, which modulates voltage-activated K-channels, resulting in persistent enhancement in excitability.