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# OCTOPAMINERGIC MODULATION OF THE MEMBRANE CURRENTS IN THE CENTRAL FEEDING SYSTEM OF THE POND SNAIL *LYMNAEA STAGNALIS**

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Octopamine is released by the intrinsic OC interneurons in the paired buccal ganglia and serves both as a neurotransmitter and a neuromodulator in the central feeding network of the pond snail *Lymnaea stagnalis* [19].

The identified B1 buccal motoneuron receives excitatory inputs from the OC interneurons and is more excitable in the presence of 10 μM octopamine in the bath. This modulatory effect of octopamine on the B1 motoneuron was studied using the two electrode voltage clamp method.

In normal physiological saline depolarising voltage steps from the holding potential of –80 mV evoke a transient inward current, presumably carried by $Na^+$ ions. The peak values of this inward current are increased in the presence of 10 μM octopamine in the bath. In contrast, both the transient ($I_A$) and delayed ($I_K$) outward currents are unaffected by octopamine application.

Replacing the normal saline with a $Na^+$-free bathing solution containing $K^+$ channel blockers (50 mM TEACl, 4 mM 4AP) revealed the presence of an additional inward current of the B1 neurons, carried by $Ca^{2+}$. Octopamine (10 μM) in the bath decreased the amplitudes of this current.

These results suggest that the membrane mechanisms which underlie the modulatory effect of octopamine on the B1 motoneuron include selective changes of the $Na^+$- and $Ca^{2+}$-channels.

*Keywords: Lymnaea* – octopamine – modulation – membrane current – voltage clamp

## INTRODUCTION

Octopamine is a feeding modulator in the central nervous system of *Lymnaea stagnalis* provided by the three octopamine-containing buccal interneurons of the feeding network [19]. OC neurons form direct synaptic connections with all the buccal feeding neurons studied. OC neurons are also considered to be intrinsic modulatory interneurons, as their firing activity leads to long-lasting (polycyclic) enhancement of the CPG activity, presumably by modulating the intrinsic membrane characteris-

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tics of both interneurons and motoneurons involved [8]. All the cellular responses – both synaptic and modulatory – are mimicked by extracellular application of octopamine into the bath suggesting that octopamine serves as both neurotransmitter and neuromodulator in the central feeding network of *Lymnaea stagnalis*.

Some of the direct synaptic effects – like the inhibitory inputs on B3 motoneurons are selectively blocked by "classical" octopaminergic antagonists, such as phentolamine or epinastine [20]. The longer-lasting modulatory effect like the excitatory inputs on B1 motoneurons, however, are not affected by these antagonists, suggesting the involvement of a different receptor mechanism. These effects on the B1 are modulated by the cyclic phosphodiesterase inhibitor IBMX, indicating a role for cAMP [12].

Our current studies concern the cellular mechanisms by which octopamine acts on the B1 protraction phase buccal motoneurons. B1 motoneurons are directly excited by the OC interneurons, and by octopamine application [19]. We have now used the effect of octopamine on B1 feeding motoneurons as a model for detailed studies of the background membrane mechanisms of the modulatory effect of octopamine.

To obtain more data on the membrane effects of octopamine we performed traditional current clamp 2-electrode intracellular recording and then two electrode voltage clamp experiments on the B1 motoneurons in the isolated CNS of *Lymnaea stagnalis*.

## MATERIALS AND METHODS

Experiments were performed on the isolated central nervous system (CNS) of *Lymnaea stagnalis* including the paired buccal ganglia. After dissection the isolated CNS was pinned onto a Sylgard-lined dish containing standard *Lymnaea* saline (Table 1) The outer layer of the connective tissue was removed mechanically from the dorsal surface of the buccal ganglia, then the inner layer was softened by protease (SIGMA type XIV) before electrode impalement. During the experiments the chamber was continuously perfused with saline (flow rate 1 ml/min), and octopamine application was performed either by the general perfusion system (100 μM octopamine) or by fast, local perfusion (10 μM) from a separate tube positioned close to the paired buccal ganglia [20].

The B1 buccal motoneuron can be easily identified visually on the dorsal surface of the buccal ganglia by its size and position [13]. This neuron was impaled by 12–15 Mohm resistance borosilicate glass microelectrodes filled with a mixture of 4M K-acetate and 0.3 M KCl. Its intracellular activity and the voltage responses after octopamine application was monitored in bridge mode using an Axoclamp 2B amplifier. For membrane resistance tests the same neuron was impaled with a second microelectrode, and (–3–5 nA) intracellular current pulses were injected into the cell body. Digitized voltage data with a sampling rate of 3 KHz were visualized using the Dasylab (Dasytech GmbH, Full Version 5.6) software. Data were stored on a PC connected with a National Instruments (PC-6035E) AD-DA converter. For recording

voltage-evoked membrane currents the B1 neurons were impaled with a second microelectrode filled with the same solution but pulled to a resistance of 3–5 Mohms before switching the amplifier to TVC (two electrode voltage clamp) mode. Strathclyde Electrophysiological Software, Version 3.26 was used to generate voltage clamp protocols and to record and analyse the currents. A standard P/N leak subtraction protocol was used during data acquisition, while further data analysis was performed after leakage subtraction of 3000 Hz filtered signals. Isolation of individual membrane currents was achieved by either using different test protocols or by perfusing the recording chamber with modified salines (Table 1).

*Table 1*
Composition of the *Lymnaea* salines (pH = 7.9)

| | Normal saline [mM] | $Na^+$-free 1/2 $Ca^{2+}$ saline [mM] | $Na^+$-free– K blocker saline [mM] |
|---|---|---|---|
| NaCl | 24 | – | – |
| KCl | 2 | 2 | 2 |
| $CaCl_2 \cdot 2H_2O$ | 4 | 2 | 4 |
| $MgCl_2 \cdot 6H2O$ | 2 | 2 | 2 |
| $NaH2PO_4 \cdot 2H_2O$ | 0.1 | 0.1 | 0.1 |
| NaOH | 35 | –* | –* |
| HEPES | 50 | 50 | 50 |
| Tetraethyl-ammonium cloride | | | 50 |
| 4-aminopyridine | | | 4 |
| N-Methyl-D-glucamine | | 50 | – |

* pH set by adding saturated KOH solution.

## RESULTS

### *Octopamine responses of the buccal feeding neurons*

Application of 100 µM octopamine (in normal saline) locally over the buccal ganglia depolarizes the B1 motoneuron (Fig. 1A), and a similar response is seen when octopamine is perfused through the whole bath [12]. Application of octopamine in the bath at a lower concentration (10 µM) has a similar depolarizing effect. Injecting current pulses through a second electrode shows that a small reduction in membrane resistance occurs. However, the traces show that the B1 motoneuron has become much more excitable, firing many more action potentials, and this increase in excitability lasts long after the octopamine has been washed out and the membrane potential has returned to normal (Fig. 1B). In further tests, a fixed depolarizing current was injected into a B1 motoneuron through one electrode and the action poten-

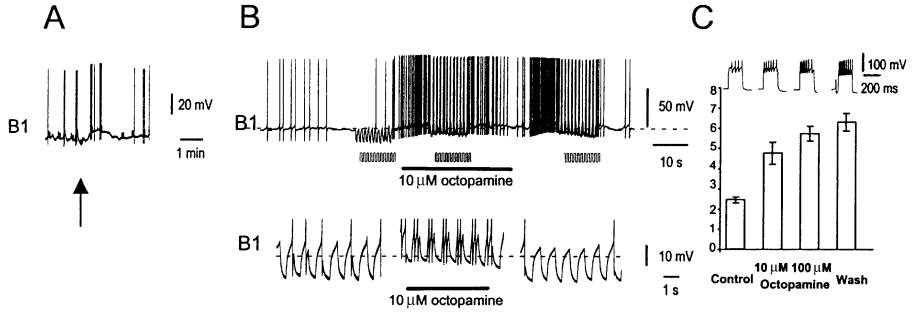


*Fig. 1.* Intracellular responses of the B1 buccal feeding motoneuron after application of octopamine into the bath. A. Transient microperfusion of 100 μM octopamine adjacent to the buccal ganglia (marked by arrow) evokes a small depolarization on the B1 motoneuron. B. Octopamine (10 μM) in the bath applied by general perfusion increases the firing activity of the B1 motoneuron. The lower trace displays the membrane voltage changes during hyperpolarizing test pulses injected by a second microelectrode inserted into the same cell. Octopamine depolarizes the B1 neuron and slightly decreases the amplitude of the voltage steps suggesting a decreased conductance during octopamine application. C. The number of action potentials in the B1 motoneuron evoked by constant depolarizing current injection pulses are significantly higher in 10–100 μM octopamine applied to the bath. The effect is long-lasting, cannot be washed out in ten minutes by normal saline

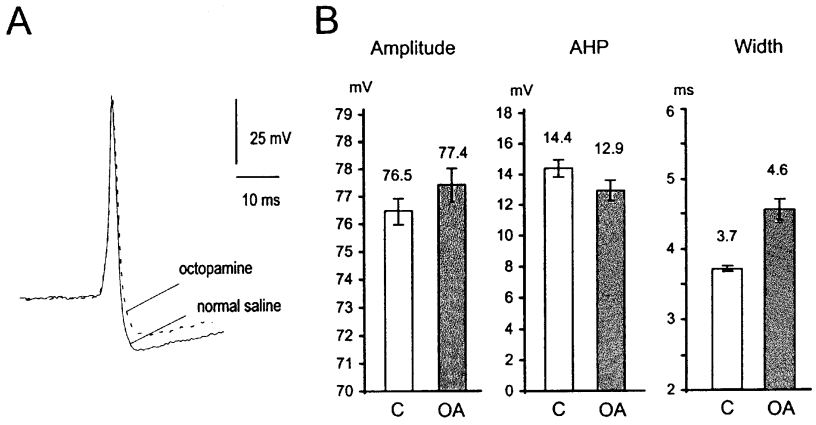


*Fig. 2.* Octopamine (10 μM) in the bath changes the shape of the B1 neuron action potential. A. Individual action potentials recorded in control saline (solid trace) and after octopamine microperfusion (dotted line). B. Action parameter measurements show an increase of action potential width and decrease of the after hyperpolarization (AHP) component of the action potentials, while the amplitudes measured from the resting membrane potential up to the peak does not significantly change in octopamine. C: action potential parameter values measured in normal saline (empty bars), OA: values measured in octopamine (filled bars)

tials recorded with a second electrode. This showed that the number of action potentials elicited increased significantly with octopamine concentration (Fig. 1C).

The B1 action potential shape is also changed, with the afterhyperpolarization reduced by octopamine (Fig. 2A). In a representative sample the height of the individual action potentials are the same in control and in 10 μM octopamine (76.5 ± 0.44 mV and 77.4 ± 0.58 mV; n = 14, 17). In these cells, the afterhyperpolarization is smaller (12.9 ± 0.67 mV n = 13) and the width of the action potential (measured at half amplitude) is increased (4.6 ± 0.15 ms n = 13) in octopamine compared to the normal saline controls (14.4 ± 0.56 mV, n = 17; and 3.7 ± 0.04 ms, n = 13) (Fig. 2B).

## *Membrane currents of B1 motoneuron in normal saline*

To investigate the effect of octopamine in more detail, we applied a 2-electrode voltage clamp to the B1 motoneuron in normal saline. From a –80 mV holding potential, depolarizing voltage steps to –60 mV to +45 mV revealed at least three components of membrane currents (Figs 3, 4). Above about –45 mV, a fast inward current is activated reaching its peak between –25 and –15 mV (Figs 3A, B, 4Bi).

The fast inactivating inward currents are followed by an outward current, which appears to have two components. The initial outward component is more pronounced after the membrane is prehyperpolarized for 300 ms to –120 mV before the voltage steps (Fig. 3B). Subtraction of the current traces taken at –80 mV holding potential shows the transient outward current (Fig. 3C). This outward current inactivates rela-

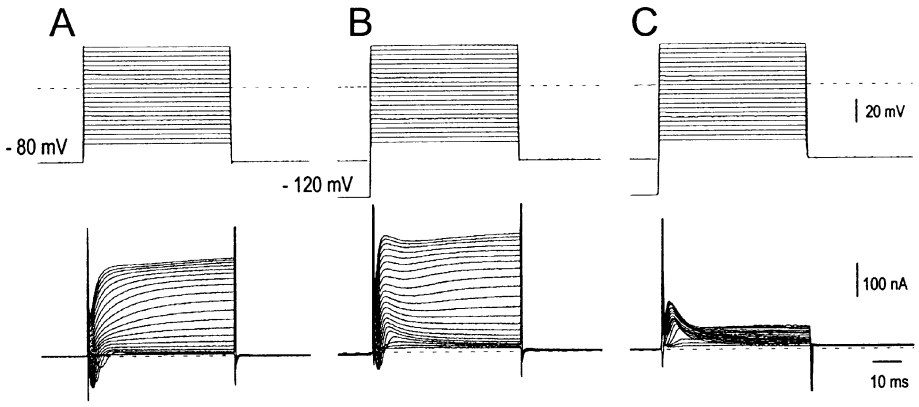


*Fig. 3.* Characterization of the voltage-evoked membrane currents of the B1 motoneuron in normal saline. A. Voltage steps from –60 mV up to +45 mV (upper trace) evokes a series of early inward currents followed by a series of non-inactivating outward currents (lower trace). B. After the prehyperpolarization of the B1 membrane to –120 mV, the same test voltages were applied, making the early transient component of the outward currents more visible. C. Subtracting the two sets of currents evoked from different holding potentials (A and B) reveals the fast inactivating transient outward component

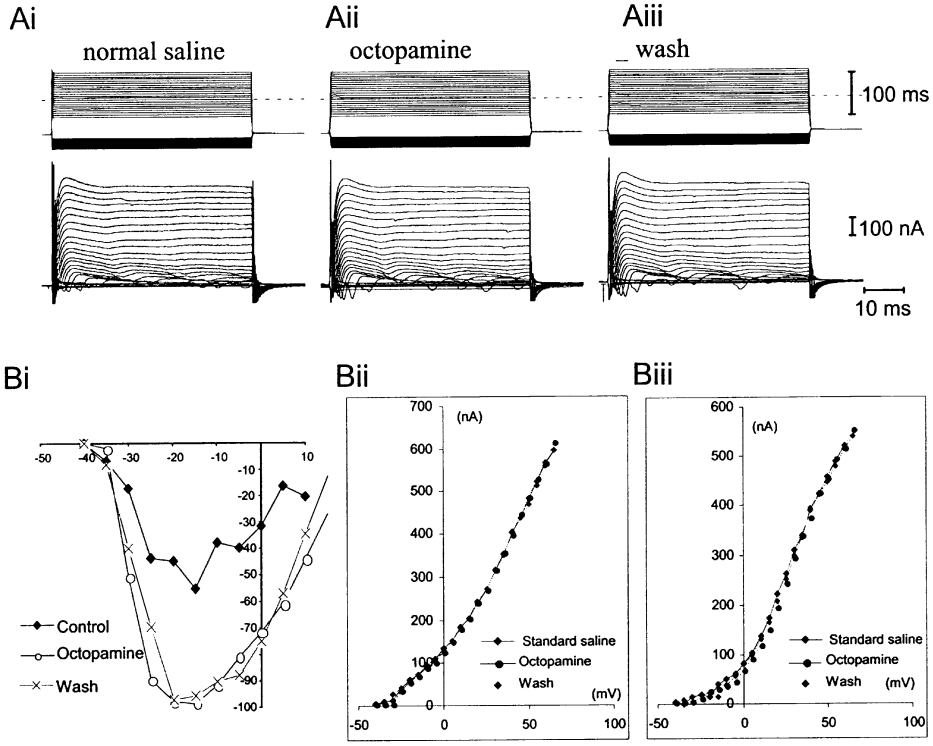


*Fig. 4.* Current evoked voltage responses of the B1 motoneuron are modulated by octopamine. A. Series of voltage-activated membrane currents using the same voltage protocol in normal saline (Ai) in the presence of 10 µm octopamine in the bath (Aii) and after wash (Aiii). B. IV-curves recorded on B1 neuron reveal a long-lasting enhancement of the fast inward currents (Bi) without substantial changes of either the transient outward (Bii) or the delayed outward currents (Biii)

tively rapidly, similar to a relatively fast activating $K^+$ outward current, named transient A currents already described in snail neurons [6] as well as in other neural and non-neural tissues [14]. The second outward component does not seem to inactivate during the course of the 50 ms voltage steps, and very likely corresponds to the delayed outward potassium currents described first in squid giant axon [10] then found in the somata of different snails (*Anisodoris:* 5, *Tritonia*: 17, Aplysia: *2*).

When the standard *Lymnaea* saline is replaced by a medium made without $Na^+$ and containing half normal $Ca^{2+}$ (Table 1), only the outward current components can be evoked by standard voltage protocol steps. This suggests that the dominant current is carried by sodium ions. In a saline made without $Na^+$ and containing 50 mM TEACl and 4 mM 4AP (Table 1) most of the fast inward and outward currents are greatly reduced but a residual inward current is seen (Fig. 5A). This has slower kinetics than the $Na^+$ current, and is probably a $Ca^{2+}$ current. It activates at –25 mV and has a peak about 0 mV, resembling the HVA currents reported in molluscan neurons [7, 11].

## *Membrane currents of B1 motoneurons are modulated by octopamine*

Holding at –80 mV, and applying depolarizing command steps between –60 and +45 mV showed that octopamine increased the initial fast inward current while the outward currents – both the transient and delayed components are unaffected (Fig. 4A). This is best shown on the current/voltage (I–V) curves of the individual currents, plotting the peak current against the command step potentials (Fig. 4B). Octopamine (10 μM) application in the bath is followed by a long-lasting increase of the amplitude of the inward currents all along their activation range without a shift in peak value (Fig. 4Bi). This increase of the inward current often persisted even after 10 minutes washing with normal saline. However, the outward currents evoked by the same step pulses did not change in octopamine, and no shift of the I–V curves were visible either for the outward A currents (Fig. 4Bii) or delayed potassium currents (Fig. 4Biii).

In the saline made without $Na^+$ and containing the outward current blockers TEACl and 4-AP (Table 1), the rapid $Na^+$ current and both outward currents disappear, making more visible a slowly activating and slightly inactivating inward current, presumably carried by $Ca^{2+}$ (Fig. 5A). Octopamine (10 μM) application in the bath greatly reduced the amplitude of this remaining $Ca^{2+}$ current (Fig. 5B), which were slower to activate and took longer to reach their peak. Furthermore, I–V plots revealed a shift of the peak values of the inward current along the x-axis to more positive values (Fig. 5C).

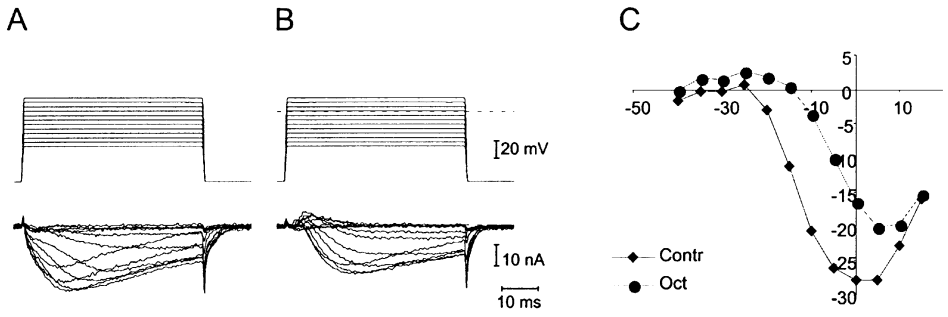


*Fig. 5.* Octopamine decreases $Ca^{2+}$ currents on B1 motoneuron. In a saline free of $Na^+$ but containing 50 mM TEACl and 4 mM 4-AP, the inward ($Ca^{2+}$) currents (A) are decreased in the presence of 10 μm octopamine in the bath (B). The amplitude of the slowly inactivating inward current evoked by a single voltage step from –80 mV holding potential to +15 mV is decreased in octopamine (C)

## DISCUSSION

### *Octopamine modulates B1 excitability as well as the action potential shape*

Voltage recording experiments revealed that the depolarizing effect of octopamine applied to the bath evokes a small depolarization and a long-lasting increase of the excitability on the B1 motoneurons. Additionally, the action potential parameters (its width and after hyperpolarization amplitude) are changed, suggesting that octopamine modulates voltage-gated membrane channels which are responsible for action potential generation.

Neuromodulators may control excitability by changing action potential shape. One of the most studied example is the *Aplysia* sensory-motor synapse where serotonin broadens the presynaptic action potentials through modifying voltage-activated $K^+$ conductances [4]. However, even this serotonergic modulation is multi-component, with separate mechanisms providing an increase in excitability and in spike broadening [16].

### *Octopamine modulates voltage-activated membrane currents*

Our main conclusion is that on B1 motoneurons the fast sodium currents are increased (nearly doubled) by octopamine while the (HVA) type Ca-currents are slowed and decreased by octopamine.

The increased sodium conductance at least partly explains the increased excitability of the B1 neuron during the octopamine effect. Current-voltage analysis reveals an increase in the amplitude of the fast sodium-dependent inward current without any significant shift of the I–V curves. The increase of this current in octopamine may mean a lowered threshold for action potential generation.

The $Ca^{2+}$ current which is modified in the presence of octopamine is the high voltage activated type, activating at –25–15 mV and reaching its peak value around 0–5 mV. This potential range is reached by the membrane only during the action potentials on B1 neuron which have an amplitude of about 80 mV with a 20 mV overshoot.

If part of the outward $K^+$ current is $Ca^{2+}$-dependent, then a reduction in the HVA $Ca^{2+}$ inward current will account for the smaller afterhyperpolarization (AHP) seen in octopamine. The smaller AHP means that the cell can fire again more quickly. Therefore both the decrease in $Ca^{2+}$ current and increase in $Na^+$ current in octopamine contribute to the increased firing frequency of the B1 neurons.

Enhanced excitability is a well-known mechanism for neuromodulators which provide a facilitatory influence in a pattern-generating system. The membrane mechanisms (i.e. the voltage-activated membrane currents which are modulated) underlying the facilitation are, however, rather diverse in different snails and identified neurons. Excitatory modulation by conopressin involves enhanced $Na^+$ and $Ca^{2+}$ inward currents, in *Lymnaea* [15] but only increased $Ca^{2+}$ current in *Helisoma* buccal neu-

rons [21]. In *Helix* buccal ganglia M neurons, which are probably homologous with the *Lymnaea* B1 motoneurons, the excitatory modulator serotonin affects both $Na^+$ and $Ca^{2+}$ currents [9]. In many examples, however, modulation involves changes of both outward ($K^+$) and inward $Ca^{2+}$ currents, e.g. SCPb modulation in *Hermissenda* [1], or dopaminergic modulation in the respiratory system in *Lymnaea* [3].

## *Functional consequence of the current modulation on B1 motoneurons*

Our work on the large motoneuron B1 may also serve as a model for the effects of octopamine on other protraction phase neurons. Octopamine in the bath also enhances monosynaptic connections between the protraction phase interneurons (SO, N1L) and their monosynaptic followers, including the B1 motoneurons [18]. At least part of this facilitation of synaptic transmission results from an increase in excitability of the presynaptic neurons (SO and N1L). Our current results on the octopamine effect on the protraction phase B1 motoneurons suggest a novel cellular mechanism, increased $Na^+$ influx for the facilitatory modulation by octopamine in the central feeding network of *Lymnaea stagnalis*.

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