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SECOND MESSENGERS OF OCTOPAMINE RECEPTORS IN THE SNAIL *LYMNAEA**

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We describe octopamine responses of 3 large buccal neurons of *Lymnaea* and test the hypothesis that these are cAMP-dependent.

The B1 neuron is excited by octopamine and the depolarisation is significantly enlarged ($P < 0.05$) by application of the blocker of cAMP breakdown, 3-isobutyl-1-methylxanthine (IBMX). The B1 neuron is also depolarised by forskolin, an activator of adenylyl cyclase.

The B2 and B3 neurons are inhibited by octopamine, and the response is not affected by IBMX. Both cells are excited by forskolin.

We conclude that the B1 neuron response to octopamine is likely to be mediated by cAMP, while the B2 and B3 responses are cAMP-independent.

Keywords: Lymnaea stagnalis – feeding – octopamine – receptor – second messenger

INTRODUCTION

Although octopamine has been widely described as a transmitter and modulator in arthropods, especially insects and crustacea (see review by [11]), it has not been well characterised in other phyla. However, in the last five years, octopamine has been found to have a major role in the feeding system of a model gastropod *Lymnaea stagnalis*.

Antagonists that block octopamine actions in insects impede *Lymnaea* feeding [17]. Feeding is controlled and modulated by the buccal ganglia, which have 3 OC (octopamine-containing) interneurons. These make synaptic and modulatory outputs onto all the known feeding neurons in the buccal ganglia [16]. These connections can be mimicked by octopamine action [3] and include chemically mediated actions on three of the largest neurons: excitation of the B1 and inhibition of the B2 and B3 neurons.

Most octopamine responses in insects (e.g. neuronal [10]; muscular [6] [19]) are mediated by adenylyl cyclase-dependent mechanisms (see [11] for review). In a few

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cases (e.g. the leg myogenic rhythm, [7] or haemocytes, [2]) inositol-phosphate pathways have been implicated, suggesting at least two types of G-protein linked responses are present in arthropods. However, no direct ion-channel receptors have been reported. The aim of these experiments has been to test the hypothesis that the responses seen in the buccal neurons of *Lymnaea* are mediated by production of cAMP.

MATERIAL AND METHODS

The CNS was isolated as described previously. It was then placed in a saline bath [5], and the B1, B2 and B3 neurons identified visually [14]. Impalement with K^+ acetate-filled glass micro-electrodes was facilitated by digestion of the connective tissue sheath with protease (Sigma XIV). The bath was perfused at 1 ml/min with *Lymnaea* High Mg^{++}/High Ca^{++} (Hi-Di) saline, or with *Lymnaea* High Mg^{++}/low Ca^{++} saline (solutions described in [5]). Octopamine (Sigma) was dissolved in saline to make a 0.1 M solution and frozen until the day of use. It was diluted immediately after thawing and then perfused into the bath. 3-isobutyl-1-methylxanthine (IBMX, Sigma) and forskolin (Sigma) were dissolved in saline and DMSO (respectively) and stock solutions frozen. Freshly thawed solutions were diluted with saline, and delivered to the bath via the perfusion system. Upon arrival, the pump was stopped and only restarted when the wash was required.

RESULTS

Octopamine has direct effects on buccal neurons

Bath perfusion of octopamine in normal saline causes activation of fictive feeding [17], so we dissolved octopamine in High Mg^{++}/low Ca^{++} saline which eliminates synaptic connections. In this saline, direct responses to octopamine are still seen. These include a depolarisation of the B1 motoneuron and hyperpolarisation of the B2

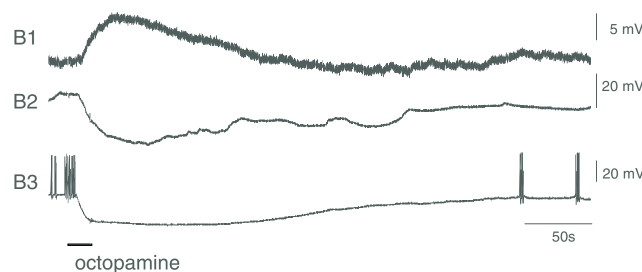

Fig. 1. Effect of 500 µM octopamine dissolved in High Mg^{++}/low Ca^{++} saline on buccal neurons. Simultaneous recordings from a B1 motoneuron, excited by octopamine and a B2 and a B3 neuron, both inhibited by octopamine

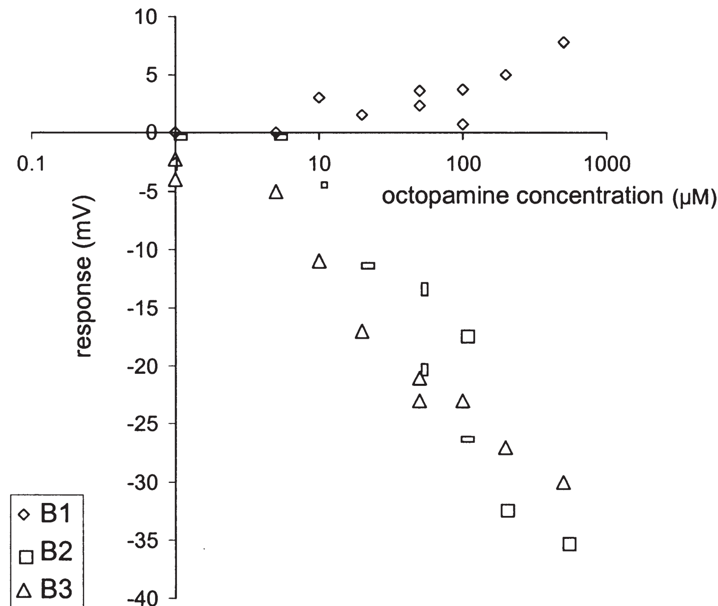

Fig. 2. Dose response curve for octopamine application to buccal neurons. Ten applications of different concentrations of octopamine dissolved in High Mg^{++}/low Ca^{++} saline were made to one preparation, with simultaneous recordinds from B1, B2, B3 neurons

and B3 motoneurons (Fig. 1). Analysis of repeated applications of octopamine showed that these three buccal neurons did not share the same dose-response curve (Fig. 2). The B3 hyperpolarising response is noticeable at 1 μM, an order of magnitude below the threshold for B1 and B3 responses (10 μM). As the concentration of octopamine is increased, the size of response increases, at least until 5 mM. At the higher concentrations, the B2 hyperpolarisation increases more quickly than the B3, with a crossover at about 100 μM. These differences in threshold and pattern of dose-response curves were seen consistently in the High Mg^{++}/low Ca^{++} and also in the Hi-Di saline (which reduces fictive feeding by raising the action potential threshold by about 10–15 mV [4]).

Effect of forskolin

The diterpenoid, forskolin, has been used in many systems to activate adenylyl cyclase. Application of forskolin in Hi-Di saline excites each of the B1, B2 and B3 neurons. It produces a long lasting (10 minute) depolarisation of the B1 neuron, on which occasional rapid EPSPs are seen (Fig. 3). The B2 neuron, which (as usual) is already firing steadily in the Hi-Di saline, fires faster with forskolin (Fig. 3). [We did check that this B2 had been hyperpolarised by octopamine.] Similar results were seen

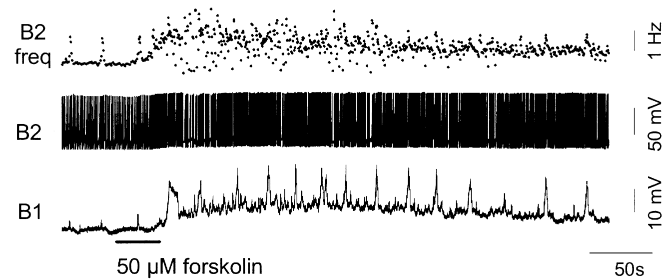

Fig. 3. Effect of forskolin on buccal neurons – simultaneus recordings from B1 and B2 in Hi-Di saline, with a plot of the firing rate of B2 added

in both Hi-Di saline and High Mg^{++}/low Ca^{++} saline, in 11 preparations. In control experiments with 1,9-dideoxyforskolin, which does not activate adenylyl cyclase, not effect on membrane potential or firing rate is seen.

Effect of IBMX

IBMX (3-isobutyl-1-methylxanthine) is a membrane permeable blocker of cyclic nucleotide phosphodiesterase(s). The response of the B1 neuron to octopamine is doubled by application of 10 µM IBMX for 20 minutes, compared to controls made

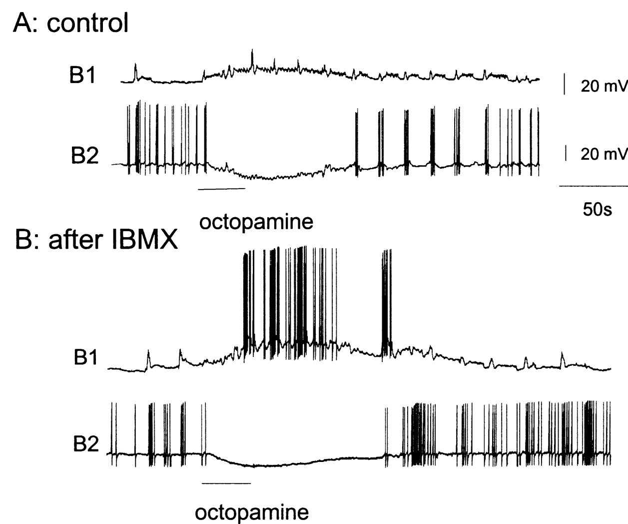

Fig. 4. Effect of IBMX on response of the buccal neurons B1 and B2 to 50 µM octopamine in Hi-Di saline. A: control application; B: after 20 minutes perfusion with 10 µM IBMX. The B1 depolarising response is stronger than in A and elicits action potentials, while the B2 is of similar size

on the same preparation before IBMX. In Hi-Di saline, 50 μM octopamine normally produces a small depolarisation in B1, but after IBMX treatment the response is much bigger and leads to B1 action potentials (Fig. 4). The enhanced response can be divided into two parts: a smooth depolarisation which is larger, and short-lasting discrete EPSPs which appear more frequently. The shape of these EPSPs is reminiscent of those produced by stimulating the N1 or SO interneurons [13–15]. Measurement of the smooth depolarising component produced by 10 mM octopamine shows it increased from 8.8 ± 1.6 mV to 19 ± 4 mV ($P < 0.05$). However, the B2, hyperpolarisation was not increased, and showed no significant change in size ($P > 0.05$), though more N1-like synaptic inputs were seen in some preparations.

DISCUSSION

Our work shows that the buccal neurons respond directly to octopamine but also indicates differences in the ways that the cells react. This indicates differences in the octopamine receptors.

B1

The B1 motoneuron is excited by octopamine. This corresponds with the excitatory input received following OC stimulation [16]. The size of the response is significantly increased by the cyclic phosphodiesterase blocker IBMX, suggesting that the cAMP pathway may be stimulated by octopamine. This is supported by the excitatory effect of the adenylyl cyclase activator, forskolin. A cAMP dependent octopamine receptor was described in *Aplysia* [3], but it is not known whether it is expressed in buccal neurons. From *Lymnaea,* Gerhardt et al. [8] extracted an octopamine receptor (Lym oa1) which increased both cAMP and inositol phosphates when expressed in HEK 293 cells.

B2 and B3

The B2 and B3 motoneurons are both inhibited by octopamine, as they are by OC stimulation [16]. The simplest explanation of the lower threshold in B3 than B2 is that the receptors may be nearer the surface of the buccal ganglia, probably on the cell soma. This would fit with iontophoretic experiments in which it is much easier to eject enough octopamine onto the B3 than B2 to obtain a response. The crossover in dose-response curves may occur because the B2 has endogenous membrane rhythmicity, which may be affected by octopamine action.

In the B2 and B3 neurons, if octopamine activated adenylyl cyclase, we would expect forskolin to inhibit them. However forskolin actually excites them. Application of IBMX to either B2 or B3 produces no increase in the response to

octopamine, which would be expected if octopamine activates adenylyl cyclase. We conclude that the B2 and B3 octopamine receptors are not likely to activate adenylyl cyclase and are therefore different to those in the B1 neuron. Gerhardt et al. [9] expressed a second octopamine receptor from *Lymnaea* which was independent of both cAMP and inositol phosphates in HEK 293 cells. The Lym oa2 receptor's second messenger was not determined, but their transfection experiments caused an increase in a Cl^- conductance. In the B2 and B3 neurons the very large hyperpolarisations suggest that K^+ conductance is increased, which would accord with Bahls observation [1] of K^+ dependent octopamine hyperpolarisations in buccal neurons of *Helisoma*.

REFERENCES

1. Bahls, F. H. (1990) Analysis of a long-duration hyperpolarization produced by octopamine in an identified effector neuron of *Helisoma. Neurosci. Lett. 120,* 131–133.
2. Baines, D., Downer, R. G. H. (1994) Octopamine enhances phagocytosis in cockroach hemocytes – involvement of inositol trisphosphate. *Arch. Insect Biochem. Physiol. 26,* 249–261.
3. Chang, D. J., Li, X. C., Lee, Y. S., Kim, H. K., Kim, U. S., Cho, N. J., Lo, X., Weiss, K. R., Kandel, E. R., Kaang, B. K. (2000) Activation of a heterologously expressed octopamine receptor coupled only to adenylyl cyclase produces all the features of presynaptic facilitation in *Aplysia* sensory neurons. *Proc. Natl. Acad. Sci. U. S. A, 97,* 1829–1834.
4. Elliott, C. J. H., Kemenes, G. (1992) Cholinergic interneurons in the feeding system of the pond snail *Lymnaea stagnalis.* II. N1 interneurons make cholinergic synapses with feeding motoneurons. *Philos. Trans. R. Soc. Lond B 336,* 167–180.
5. Elliott, C. J. H., Stow, R. A., Hastwell, C. (1992) Cholinergic interneurons in the feeding system of the pond snail *Lymnaea stagnalis.* I. Cholinergic receptors on feeding neurons. *Philos. Trans. R. Soc. Lond B. 336,* 157–166.
6. Evans, P. D. (1984) A modulatory octopaminergic neurone increases cyclic nucleotide levels in locust skeletal muscle. *J. Physiol. 348,* 307–324.
7. Evans, P. D. (1984) Studies on the mode of action of octopamine, 5-hydroxytryptamine and proctolin on a myogenic rhythm in the locust. *J. Exp. Biol. 110,* 231–251.
8. Gerhardt, C. C., Bakker, R. A., Piek, G. J., Planta, R. J., Vreugdenhil, E., Leysen, J. E., van Heerikhuizen, H. (1997) Molecular cloning and pharmacological characterization of a molluscan octopamine receptor. *Mol. Pharmacol. 51,* 293–300.
9. Gerhardt, C. C., Lodder, H. C., Vincent, M., Bakker, R. A., Planta, R. J., Vreugdenhil, E., Kits, K. S., van Heerikhuizen, H. (1997) Cloning and expression of a complementary DNA encoding a molluscan octopamine receptor that couples to chloride channels in HEK293 cells. *J. Biol. Chem. 272,* 6201–6207.
10. Nathanson, J. A., Greengard, P. (1973) Octopamine-sensitive adenylate cyclse: evidence for a biological role of octopamine in nervous tissue. *Science 180,* 308–310.
11. Roeder, T. (1999) Octopamine in invertebrates. *Prog. Neurobiol. 59,* 533–561.
12. Rose, R. M., Benjamin, P. R. (1981) Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. 1. Initiation of feeding cycles by a single buccal interneurone. *J. Exp. Biol. 92,* 187–201.
13. Rose, R. M., Benjamin, P. R. (1981) Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. 2. The interneuronal mechanism generating feeding cycles. *J. Exp. Biol. 92,* 203–228.
14. Rose, R. M., Benjamin, P. R. (1979) The relationship of the central motor pattern to the feeding cycle of *Lymnaea stagnalis. J. Exp. Biol. 80,* 137–163.
15. Vehovszky, A., Elliott, C. J. H. (1995) The hybrid modulatory/pattern generating N1L interneuron in the buccal feeding system of *Lymnaea* is cholinergic. *Invert. Neurosci. 1,* 67–74.

16. Vehovszky, A., Elliott, C. J. H. (2001) Activation and reconfiguration of fictive feeding by the octopamine-containing modulatory OC interneurons in the snail *Lymnaea. J. Neurophysiol. 86,* 792–808.
17. Vehovszky, A., Elliott, C. J. H., Voronezhskaya, E. E., Hiripi, L., Elekes, K. (1998) Octopamine: A new feeding modulator in *Lymnaea. Phil. Trans. Roy. Soc. Lond. B 353,* 1631–1643.
18. Vehovszky, A., Hiripi, L., Elliott, C. J. H. (2000) Octopaminergic synaptic connections between the identified buccal OC neurons and members of the buccal feeding network or the pond snail *Lymnaea stagnalis. Eur. J. Neurosci. 12,* 92.
19. Whim, M. D., Evans, P. D. (1991) The role of cyclic-amp in the octopaminergic modulation of flight-muscle in the locust. *J. Exp. Biol. 161,* 423–438.