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EFFECTS OF OCTOPAMINE ON CALCIUM ACTION POTENTIALS IN INSECT OOCYTES

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Summary

Our experiments show that octopamine receptors are present on the developing follicles of an insect, *Rhodnius prolixus*. Application of D,L-octopamine decreased the duration and overshoot of calcium-dependent action potentials (APs), and increased the intrafollicular concentration of cyclic AMP. The threshold concentration of D,L-octopamine for the reduction in electrical excitability was between 1 and 5×10^{-7} mol l^{-1}, and maximal effects of a 40–50 % reduction in AP overshoot and duration were apparent at 10^{-4} mol l^{-1}. At concentrations above 10^{-5} mol l^{-1}, a small (<10 %) hyperpolarization of the resting potential was also apparent. Effects of D,L-octopamine on oocyte excitability were independent of these small shifts in resting potential.

Current injection experiments, in which calcium entry was blocked by cobalt, demonstrated that D,L-octopamine reduced membrane resistance at both hyperpolarizing and depolarizing potentials. Octopamine did not affect the maximum rate of rise of the AP, dV/dt_{max}, which is an indicator of inward calcium current. It is suggested that octopamine may mediate its effects on excitability through an increase in a voltage-dependent potassium conductance.

Application of other phenolamines indicated a rank order of potency of D,L-octopamine > D,L-synephrine > tyramine. The α-adrenergic agonists clonidine, naphazoline and tolazoline were without significant effect at 10^{-5}–10^{-3} mol l^{-1}. Reduction of excitability by D,L-octopamine was effectively blocked by phentolamine and metoclopramide. Yohimbine and gramine were less effective as antagonists. Possible functions of octopamine receptors in insect follicles are discussed.

Introduction

Electrophysiological studies have demonstrated the presence of neurotransmitter receptors in the membranes of oocytes from several phyla. Membrane current and potential of *Xenopus* oocytes, for example, respond to cholinergic and catecholaminergic agents, but not to octopamine, histamine, or the amino acids aspartate, glutamate, γ-amino-*N*-butyric acid (GABA) and glycine (Kusano *et al.* 1982). Threshold concentrations for acetylcholine (ACh) effects in different

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batches of oocytes vary from 10^{-9} mol l^{-1} to more than 10^{-3} mol l^{-1}. Mouse and human oocytes are sensitive to GABA as well as to ACh, but not to catecholamines or amino acids (Eusebi *et al.* 1984; Dolci *et al.* 1985). In invertebrates, oocyte membranes of echinoderms and squid are sensitive to dopamine, serotonin and epinephrine, but not to ACh (Kusano, 1978).

Several possible functions for oocyte neurotransmitter receptors have been suggested. GABA released by the ovary and fallopian tubes may induce an increase in intracellular Ca^{2+} activity of mammalian oocytes, and may thereby alter Ca^{2+}-dependent cellular processes in eggs or early embryos (Dolci *et al.* 1985). Sperm-carried ACh may be involved in activation processes initiated by sperm–egg interaction (Eusebi *et al.* 1984). However, it has also been suggested that the presence of neurotransmitter receptors in the oocyte membrane is a consequence of a largely derepressed genome (Kusano *et al.* 1982). Nonetheless, oocytes, particularly those of *Xenopus*, have proved to be a useful model system for studies of second-messenger modulation of membrane responses (see Dascal *et al.* 1985).

We present here the first evidence for octopamine receptors in developing ovarian follicles. This study is the first to report modulation of excitable properties of an egg cell by a neurotransmitter, and is also the first report of a receptor for any neurotransmitter in an arthropod follicle. Ovarian follicles of the haematophagous insect *Rhodnius prolixus* produce action potentials (APs) of 2–3 s duration when depolarized. Excitability is a property of the oocyte, and not the surrounding follicle cells (O'Donnell, 1985). The rising phase of the AP is calcium-dependent (O'Donnell, 1985) and the repolarizing phase involves a voltage-dependent potassium conductance (O'Donnell, 1986). Application of potassium channel blockers increases the overshoot and duration of APs in *Rhodnius* oocytes (O'Donnell, 1986) and unmasks latent excitability in follicles of five other species from three other orders (O'Donnell, 1988). In contrast, excitability of *Rhodnius* oocytes is reduced by increasing the intracellular concentration of cyclic adenosine monophosphate (cyclic AMP) (O'Donnell & Singh, 1988). In this paper we report that D,L-octopamine also decreases electrical excitability and that this effect may be mediated through an accompanying increase in the intrafollicular concentration of cyclic AMP.

Materials and methods

Methods for intracellular stimulation and recording have been described previously (O'Donnell, 1985). A follicle was placed in the bottom of a small chamber which contained about 2 ml of saline, continuously perfused at a rate of 4–8 ml min^{-1}. Two microelectrodes were passed through the follicular epithelium and into the oocyte. Current was injected through one microelectrode while membrane potential was measured with the other. D,L-Octopamine and all other drugs were obtained from Sigma Chemical Company and were dissolved in control

saline (CS) which consisted of (in mmol l^{-1}): NaCl, 129; KCl, 8·6; $MgCl_2$, 8·5; $CaCl_2$, 2; glucose, 34; and Bistris, 15. Saline was adjusted to pH 6·8 by addition of 5 mmol l^{-1} HCl.

Action potential duration was measured from the point at which membrane potential was rising maximally until the potential had returned to within 5 mV of the resting value. Overshoot was measured as the difference between the peak of the AP and the zero potential. Afterpotentials were measured as the difference between the resting potential prior to the action potential, and the potential 0·4 s after repolarization. The latter time corresponds to the point of maximal undershoot of hyperpolarizing afterpotentials in oocytes exposed to forskolin or cyclic AMP (O'Donnell & Singh, 1988).

Cyclic AMP was assayed using a commercial kit (Diagnostic Products Corporation, Los Angeles, California) which measured competitive binding of nucleotide to cyclic-AMP-dependent protein kinase (Tovey *et al.* 1974). Pooled samples of three follicles were frozen and homogenized in 1 ml of ice-cold 5 % trichloroacetic acid. Samples of the homogenized follicles were then assayed for protein (Bramhall *et al.* 1969) and cyclic AMP.

Results

Recordings of APs evoked by depolarizing current injection indicated a decline in oocyte excitability in response to D,L-octopamine (Fig. 1). Both duration and overshoot of the APs decreased by as much as 40–50 %. The threshold concentration for reducing excitability was between 1 and 5×10^{-7} mol l^{-1}, and maximal effects were reached in 7–10 min. At higher concentrations (10^{-3} mol l^{-1}), the maximal response was reached within 5 min and the decline in excitability was partially reversed by a further 5 min of exposure to D,L-octopamine (Fig. 1A,B). The basis for this apparent desensitization is unknown.

Mean values of the afterpotential ranged from 1 to 4 mV in control saline for the groups of follicles used in the experiments of Fig. 1, and the mean value for all follicles was $+3·0 \pm 0·5$ mV. The effects of octopamine on afterpotential were also variable, although paired *t*-tests indicated a small but significant reduction at two of the concentrations tested (Fig. 1C). Only in 2 of 30 experiments did octopamine produce undershooting (i.e. hyperpolarizing) afterpotentials.

At D,L-octopamine concentrations of 10^{-5} mol l^{-1} or higher, the resting potential hyperpolarized 5–10 % relative to the control value of $-52·4 \pm 1·0$ mV ($N = 30$) (Fig. 1D). A number of experiments suggested that the effects of D,L-octopamine on excitable properties were independent of these small shifts in resting potential. First, addition of 5×10^{-7}–10^{-6} mol l^{-1} octopamine reduced AP overshoot and duration (Fig. 1A,B) but did not significantly alter the resting potential. Second, there were dramatic differences in the time course of reversal of octopamine effects on resting properties. AP overshoot and duration returned to initial values within 10 min after removal of 10^{-3} mol l^{-1} D,L-octopamine, but the resting potential remained hyperpolarized by 10 ± 4 % ($N = 5$). Third, the

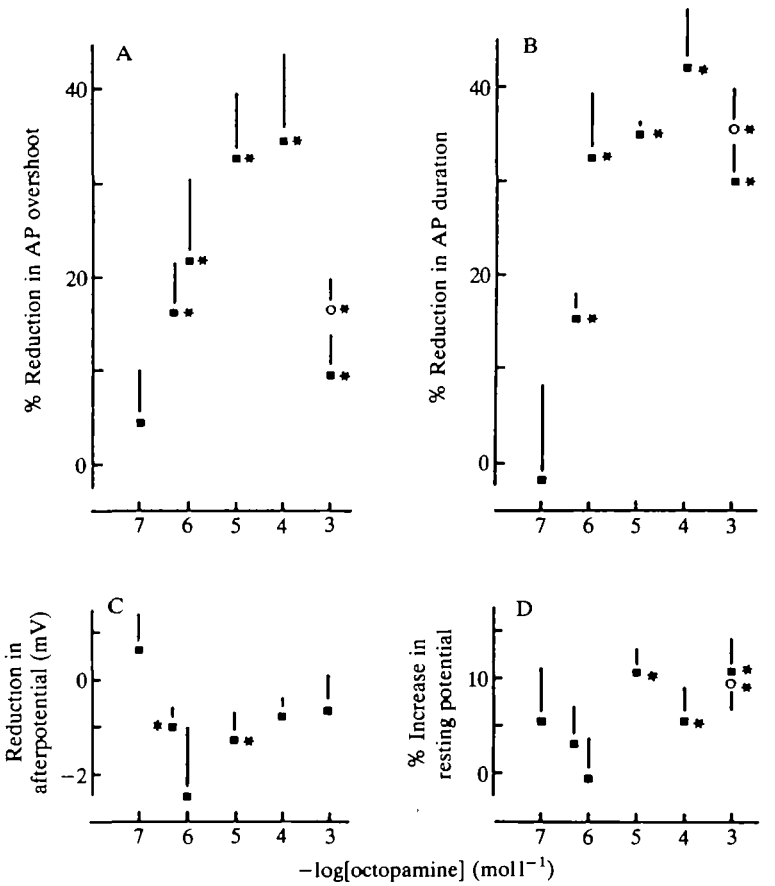

Fig. 1. Dose–response graphs for the effects of D,L-octopamine on action potential characteristics and resting potentials in *Rhodnius* follicles. Each point indicates the mean + 1 S.E.M. for five follicles. The asterisks indicate points which differ significantly ($P < 0{\cdot}05$) in a paired *t*-test from the corresponding values in control saline (CS). Action potentials were evoked 1, 3, 5, 7 and 10 min after application of octopamine. The values presented are those at 10 min (filled squares). For 10^{-3} mol l^{-1} D,L-octopamine, the values after 5 min (open circles) are also shown (see text).

dose–response curves of Fig. 1 show that significant hyperpolarization of the resting potential after a 10-min exposure to 10^{-3} mol l^{-1} D,L-octopamine was associated with significantly smaller reductions in AP overshoot and duration compared to the effect of 10^{-6} mol l^{-1} D,L-octopamine. Lastly, if the current-injection electrode was used manually to voltage-clamp the oocyte resting potential to the value measured in control saline, application of 10^{-4} mol l^{-1} D,L-octopamine nonetheless produced a 33 ± 5 % reduction in AP duration ($N = 4$).

Although we have not examined in detail the basis for the octopamine-induced changes in resting potential, preliminary experiments suggest that the hyperpolarization is not dependent upon an increase in electrogenic sodium pumping, an

increase in resting potassium permeability, or a decrease in sodium permeability. Addition of ouabain (10^{-4} mol l^{-1}) 10 min before and during application of 10^{-4} mol l^{-1} D,L-octopamine did not reduce the hyperpolarization (9 ± 2 mV; $N = 4$) relative to the values in the absence of ouabain, suggesting that the hyperpolarization is not due to an increase in electrogenic Na^+/K^+-ATPase activity. In *Periplaneta*, octopamine mediates a reduction in potassium sensitivity of the glial cells which form the blood–brain barrier (Schofield & Treherne, 1986). However, a 10-fold elevation of the bathing saline potassium concentration depolarized the follicle membrane potential by $32{\cdot}5 \pm 2{\cdot}1$ mV ($N = 4$) before application of 10^{-4} mol l^{-1} octopamine and by $34{\cdot}2 \pm 2{\cdot}2$ mV 10 min after addition of octopamine. Similar results were obtained with 10^{-5} mol l^{-1} D,L-octopamine. Depolarizations with and without D,L-octopamine did not differ significantly, suggesting that the potassium permeability at the resting potential is not increased by octopamine. All salines contained 10 mmol l^{-1} cobalt, a calcium channel blocker, to prevent action potentials during depolarization by potassium-enriched saline. Reduction of saline sodium concentration from 129 to 29 mmol l^{-1} by replacement with choline produced a change in resting potential of less than 2 mV before or 10 min after addition of 10^{-4} mol l^{-1} octopamine ($N = 3$), suggesting that the hyperpolarization produced by these high concentrations of octopamine is not due to a change in sodium permeability.

Two other phenolamines also reduced oocyte excitability. 10^{-4} and 10^{-5} mol l^{-1} D,L-synephrine reduced AP duration by 54 ± 7% ($N = 4$) and 23 ± 3% ($N = 4$), respectively, after 10 min. Tyramine reduced AP duration by 15 ± 7% ($N = 4$) after 10 min at 10^{-4} mol l^{-1}, but was ineffective at 10^{-5} mol l^{-1}. These results suggest a rank order of potency of D,L-octopamine > D,L-synephrine > tyramine.

With three compounds related to octopamine – phenylethylamine, methyl phenyl ethanolamine and metanephrine – there was no significant effect on excitability at 10^{-5} mol l^{-1}. An unexpected result was that the α-adrenergic agonists clonidine, naphazoline and tolazoline, which are effective agonists of octopamine 1, octopamine 2A and octopamine 2B receptors, respectively, in locust skeletal muscle (Evans, 1981), were without significant effect on oocyte excitability at 10^{-5}–10^{-3} mol l^{-1}. Epinephrine, norepinephrine, dopamine, serotonin, ACh or GABA had no significant effect after 5–15 min at 5×10^{-5}–10^{-3} mol l^{-1}.

The effects of a number of aminergic antagonists are summarized in Table 1. At 10^{-5} mol l^{-1} or higher, phentolamine and metoclopramide (Fig. 2) completely inhibited the effects of 10^{-5} mol l^{-1} D,L-octopamine on AP duration. However, yohimbine was also 100% effective at 10^{-4} mol l^{-1} and 39% effective at 10^{-5} mol l^{-1}. In contrast, the sensitivity of octopamine 1 and 2 receptors in insect skeletal muscle to metoclopramide and yohimbine differs by a factor of 50 or more (Evans, 1981). Gramine, a highly effective antagonist of octopamine 2 receptors in locust oviduct (Orchard & Lange, 1985) and corpora cardiaca (Pannabecker & Orchard, 1986), was only 38% effective at 10^{-4} mol l^{-1}. Although these experiments do not provide a detailed pharmacological profile of the oocyte octopamine

Table 1. *Action of antagonists on octopamine-induced reduction in action potential duration*

Drug	Blocking effect (%) at 10^{-4} mol l^{-1}	10^{-5} mol l^{-1}	10^{-6} mol l^{-1}
Metoclopramide	100	100	0
Yohimbine	100	39 ± 19	0
Phentolamine		100	
Gramine	38 ± 18		

Results are expressed as a mean percentage reduction in response ± S.E.M. at the end of a 10 min exposure to 10^{-5} mol l^{-1} D,L-octopamine.

Action potentials were evoked by depolarizing current injection in control saline and 1, 3, 5, 7 and 10 min after the start of octopamine application. Each antagonist was applied for a 5-min prewash period as well as during the octopamine pulse. The follicle was then washed for 10 min in control saline, followed by a 10–15 min reapplication of D,L-octopamine in the absence of antagonist.

A value of 100 % indicates that a paired *t*-test showed no significant difference ($P < 0{\cdot}05$) in AP duration in the presence and absence of octopamine. A value of 0 % indicates that there was no significant difference in AP duration in the presence or absence of the antagonist.

$N = 3$–5 for each drug at each concentration.

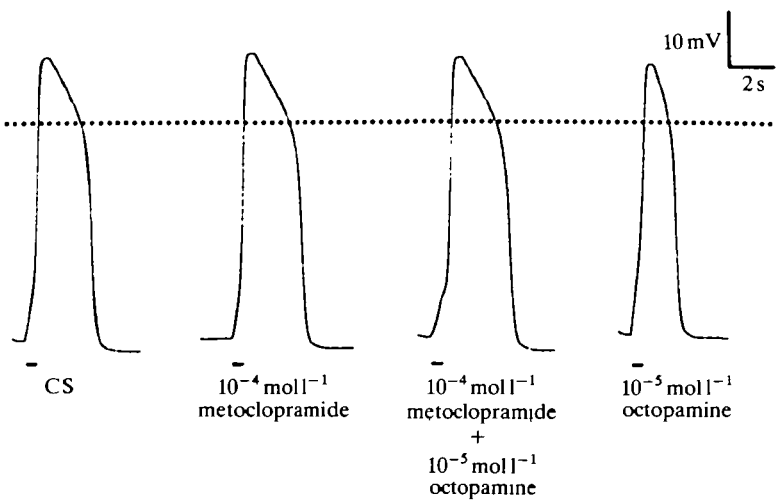

Fig. 2. Blockage of octopamine effects by metoclopramide. The dotted line indicates the zero potential, and the bar below each recording indicates the duration of the current pulses ($1–2 \times 10^{-7}$ A) used to evoke the AP. Recordings were made in control saline (CS) (left), after 5 min in 10^{-4} mol l^{-1} metoclopramide (second from left), and after 10 min in 10^{-4} mol l^{-1} metoclopramide and 10^{-5} mol l^{-1} octopamine (third from left). The follicle was then washed for 10 min in CS, and the recording on the right was made after 15 min in 10^{-5} mol l^{-1} octopamine.

receptors, they do suggest differences in agonist and antagonist sensitivity relative to the multiple types of octopamine receptors in locust tissues.

Effects of D,L-octopamine are generally mediated through an increase in the intracellular concentration of cyclic AMP (Evans, 1985). Application of exogenous cyclic AMP, the adenylate cyclase activator forskolin or phosphodiesterase inhibitors produces effects on oocyte AP overshoot and duration comparable to those produced by octopamine (O'Donnell & Singh, 1988). Concentrations of cyclic AMP in *Rhodnius* follicles increased threefold after 10 min in saline containing 10^{-5} mol l^{-1} D,L-octopamine, from a basal level of $0{\cdot}04 \pm 0{\cdot}02$ pmol mg protein^{-1} ($N = 9$) in control saline to $0{\cdot}13 \pm 0{\cdot}04$ pmol mg protein^{-1} ($N = 7$). Intrafollicular concentrations of cyclic AMP after 10 min in 10^{-4} mol l^{-1} D,L-octopamine ($0{\cdot}12 \pm 0{\cdot}01$ pmol mg protein^{-1}, $N = 8$) were not significantly higher than the latter values. Concentrations of cyclic AMP are also increased by application of forskolin, an activator of adenylate cyclase, or 3-isobutyl-1-methyl xanthine, a phosphodiesterase inhibitor (O'Donnell & Singh, 1988). We did not examine whether octopamine elevates intracellular concentrations of cyclic GMP, primarily because application of a phosphodiesterase-resistant derivative, 8-bromocyclic GMP, does not alter oocyte excitability (O'Donnell & Singh, 1988).

These results suggest that octopamine may exert its effects on oocyte excitability through an increase in the intracellular concentration of cyclic AMP. However, the ionic basis for the reduction in excitability is unknown. Indeed, cyclic AMP may modulate more than one type of ionic conductance, even in a single cell (Lotshaw *et al.* 1986). Although the effect of changing bathing saline potassium concentration indicated that D,L-octopamine did not alter the potassium permeability which contributes to the resting potential; nonetheless, an octopamine-mediated increase in a voltage-dependent potassium conductance seems a likely explanation for the changes observed in the oocyte AP. A reduction in AP overshoot and duration could result from a decrease in inward calcium current during the rising phase of the AP (O'Donnell, 1985), or an increase in outward potassium current during repolarization (O'Donnell, 1986). The latter change would be detectable as a decrease in membrane resistance. Indeed, D,L-octopamine reduced membrane resistance at both hyperpolarizing and depolarizing potentials (Fig. 3). Cyclic AMP produces similar changes in oocyte current/voltage relationships (O'Donnell & Singh, 1988), whereas potassium channel blockers such as tetraethylammonium produce the opposite effect, i.e. slope resistance increases (O'Donnell, 1986). Calcium entry was blocked with 10 mmol l^{-1} cobalt in these experiments, so it appears unlikely that a decrease in calcium conductance is responsible for the decline in excitability in response to octopamine. In support of the latter view is the observation that D,L-octopamine did not decrease the maximum rate of rise of the AP, dV/dt_{max}, which is an indicator of calcium current. The magnitude of dV/dt_{max} in the presence of 10^{-5} mol l^{-1} D,L-octopamine, $1{\cdot}4 \pm 0{\cdot}1$ V s^{-1} ($N = 5$), did not differ significantly from the value in control saline, $1{\cdot}5 + 0{\cdot}1$ V s^{-1}.

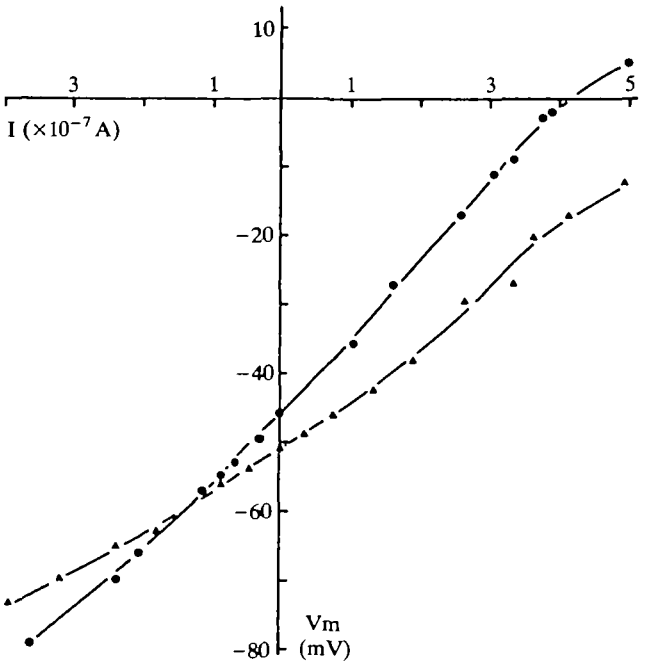

Fig. 3. Steady-state current–voltage curves for a *Rhodnius* follicle perfused with control saline (●) and 10 min after application of 10^{-4} mol l^{-1} D,L-octopamine (▲). Both salines contained 10 mmol l^{-1} Co^{2+}. Each point indicates the membrane potential at the end of a 2–3 s current pulse. Three other follicles gave similar results.

Discussion

The results suggest that octopamine receptors are present on the ovarian follicles of *Rhodnius*. Although excitability is a property of the oocyte and not the follicle cells (O'Donnell, 1985), octopamine receptors might be present on the follicle cells, the oocyte, or both. Because *Rhodnius* oocytes are well coupled to the follicle cells, cyclic AMP produced in the follicle cells may diffuse into the oocyte through connecting gap junctions (Huebner, 1981). It is notable that responses of *Xenopus* oocytes to catecholamines but not ACh disappear or are greatly reduced after removal of the inner ovarian epithelium and follicular cell layer (Kusano *et al.* 1982). We could not determine the effects of octopamine on denuded *Rhodnius* oocytes because attempts to remove the follicular epithelium with fine forceps ruptured the oocyte.

Although a function of octopamine receptors on *Rhodnius* follicles is not readily apparent, octopamine is known to modulate the contraction of locust oviducal muscles, and its presence has been demonstrated both in the muscles and in nerves which project to them (Orchard & Lange, 1985). Octopamine is also present in significant quantities in the oviducts of the moths *Manduca* and *Spodoptera* (Davenport & Wright, 1986). The results of the present study raise the possibility that octopamine might exert effects not only on the muscles of the reproductive

tract, but on the egg cells within it. If so, the electrical effects of octopamine described here may be incidental to other consequences of changes in follicular cyclic AMP content. Effects of cyclic AMP on development of amphibian oocytes have been well documented (reviewed by Maller, 1983), although the possible roles of cyclic AMP in the development of insect oocytes and eggs have received much less attention. Irrespective of the possible developmental functions of octopamine receptors in *Rhodnius* follicles, the large size of these cells, and the ease with which they can be isolated in large numbers, suggests that they will provide a useful model system for further biochemical and electrophysiological studies of octopamine actions.

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