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Biogenic Amines Modulate Olfactory Receptor Neurons Firing Activity in *Mamestra brassicae*

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Abstract

The modulatory effects of the biogenic amines octopamine and serotonin on pheromonal receptor neurons of *Mamestra brassicae* were investigated. The responses to sex pheromone components of two cells types (A and B) in single male long sensilla trichodea were monitored. Cell types A and B do not respond to the same compound. The response of type A to a pulse of the major sex pheromone component increased 5 min after octopamine injection. Responses of type B to other odorants increased after 30 min. In the absence of any pheromone stimulation the background firing activity of type A increased following octopamine injection. This background activity was used to evaluate the kinetics of octopamine and other biogenic amine effects on olfactory receptor neurons. Octopamine increased this background activity in a concentration- and time-dependent manner. Clonidine, an octopamine agonist, was shown to be more powerful in increasing the background activity of olfactory receptor neurons. The effects of octopamine and clonidine were hypothesized to arise from specific receptor activation as chlorpromazine (an octopamine antagonist) was shown to block the effect of octopamine. Serotonin, a known neuromodulator in most animal species, induced a reversible inhibition of spike firing. Altogether, these results indicate that biogenic amines can modulate the sensitivity of olfactory receptor neurons of moths either directly or by an action on adaptation.

Introduction

Octopamine (OA) is a major biogenic amine in insects, acting as a neuromodulator, neurohormone and neurotransmitter, modulating many physiological processes. OA modulates central as well as peripheral organs [for a review see Roeder (Roeder, 1999)]. OA is mainly released from neurosecretory endings and taken up by the haemolymph circulation. Elevated levels of OA have been described in relation to photoperiod, behaviour and especially stress (David *et al.*, 1985; Evans, 1985). Injection of OA into the haemolymph can modulate behaviour, such as flight activity in the locust (Stevenson and Kutsch, 1987).

OA improves the detection of and reactivity to pheromone blends in the male Oriental fruit moth and in the cabbage looper (Linn and Roelofs, 1984, 1986). Both species exhibit strong circadian variations in their sexual behaviour, including pheromone emission in females and pheromone orientation in males. These variations were correlated with OA levels in the haemolymph and in the central nervous system and were mimicked by OA injection (Linn *et al.*, 1992, 1994a,b,c). These authors suggested that the major role of OA is to modulate the activity of neurons located in the central nervous system. A direct effect of octopamine on the sensory system was not considered.

Previous studies indicated that OA, as well as other biogenic amines, acts directly on olfactory receptor neurons (ORNs). Küppers and Thurm found that serotonin raises the transepithelial potential in the whole antenna of the cockroach *Blabtica dubia* (Küppers and Thurm, 1975), suggesting a modulation of ORN sensitivity by this hormone. One support for this hypothesis, although indirect, arises from the observation that most insect species are equipped with an antennal heart containing neurosecretory endings that were shown to secrete high amounts of OA, for example in *Periplaneta* (Pass *et al.*, 1988). *In situ* hybridization experiments showed that a putative octopamine receptor is expressed in olfactory sensilla in *Bombyx mori* and *Heliothis virescens* (Nickisch-Rosenegk *et al.*, 1996) and receptors for tyramine, another related biogenic amine, have also been shown in the antenna of the *hono* mutant of *Drosophila* (Kutsukake *et al.*, 2000). Finally, a recent study (Pophof, 2000) showed that application of OA enhances the response of *Antherea polyphemus* pheromone receptor neurons. These authors concluded that OA acts directly on ORNs to increase their sensitivity to odour. In this later study ORN activity was only assessed in isolated antennal branches at least 30 min after OA injection into the insects.

In order to investigate the behaviour of neurons much earlier after OA injection we decided to monitor the firing activity of ORNs in the same sensillum of a living moth (*Mamestra brassicae*) before, during and shortly after OA injection. The firing activity of ORNs in response to olfactory stimulation was recorded 5 and 30 min after OA injection. We then evaluated the time course of the effect of OA injection by sampling the ORN activity every 5 min over a 60 min period post-injection. In addition to octopamine, we studied the effect of clonidine, an OA receptor agonist, of chlorpromazine (CP), an OA receptor antagonist, and serotonin (5HT). The data obtained in the present study show that the activity of ORNs is modulated by both OA, clonidine and 5HT within seconds after application of the compound. These results support the hypothesis that olfactory sensilla in *M. brassicae* bear OA as well as 5HT receptors.

Materials and methods

Insects

Mamestra brassicae (L.) moths were reared on a semi-artificial medium in the laboratory (Poitout and Bues, 1974). From the third to last instar larvae were reared individually. Male and female pupae were kept separately in groups of 20–30 at 20–24°C, 40–50% relative humidity, 16:8 h light:dark cycle. Adult moths were provided with a 10% sucrose solution and kept in plastic containers. Electrophysiological recordings were performed on adult males, 12–72 h after emergence, at room temperature.

Chemicals

All compounds injected were dissolved in haemolymph Ringer (Kaissling and Thorsson, 1980). DL-Octopamine hydrochloride was diluted in tenth steps to give 0.1–100 μg/μl (0.527–527 mM). The pH of these solutions was adjusted with NaOH to the same value as that of the haemolymph Ringer. Clonidine hydrochloride (clonidine) was used in tenth steps in the range 0.053–53 mM. 5HT was used in tenth steps in the range 0.414–414 mM. CP was used as a mixture of OA + CP (0.1 + 0.1 M). All compounds were purchased from Sigma. Control injections were done with haemolymph Ringer (Kaissling and Thorsson, 1980).

The main component of the sex pheromone of *M.brassicae*, (*Z*)-11-hexadecenyl acetate (Z11–16:Ac), and the two other pheromone-related compounds, (*Z*)-9-tetradecenyl acetate (Z9–14:Ac) and (*Z*)-11-hexadecenol (Z11–16:OH), were synthesized in the laboratory by M. Lettere.

Injections

OA, clonidine, CP + OA and saline solutions were injected into the head of the animal, near the base of the antenna, using a fine tipped glass capillary connected to a Pressure Micro Injector (Cornerstone PMI-200; Dagan Corp.). The tip diameter of the injection pipette (~10 μm), the injection pressure (~20 p.s.i.) and the duration of injection (~200 ms) were adjusted so that the ejected volume approximated 1 μl. All injections are expressed in nanomoles injected.

Olfactory stimulation

The antenna was continuously flushed with a charcoal-filtered and humidified air stream (1.5 l/min) that was delivered through a glass tube (inner diameter 8 mm) ending 15 mm in front of the preparation. For stimulation, air (flowing at 0.5 l/min) was flushed for 0.5 s into the main stream through a Pasteur pipette containing a filter paper. This filter paper was loaded with 1 μl of a solution of either Z11–16:Ac, Z11–16:OH or Z9–14:Ac, diluted in hexane in tenth steps ranging from 1 to 1000 ng/μl. Odour cartridges were prepared just before the experiment. Consecutive stimulus presentations were separated by at least 3 min.

Single sensillum recordings

Insects were constrained in a Styrofoam block, leaving the head and the antennae free. A reference electrode filled with haemolymph saline was inserted into the eye. Just before recording several olfactory hairs were cut using sharpened forceps. Single sensillum recordings (SSR) were performed by covering one of these hairs with the recording electrode, filled with sensillum saline (Kaissling and Thorsson, 1980; Renou and Lucas, 1994).

The signal was amplified and filtered (Neurolog 102 D.C.) via two channels, the first to count spikes (×1000, 0.1–3 kHz filter) and the second to record sensillar potential (×10, d.c.–3 kHz filter). A third channel recorded the stimulus time course, using an anemometer inserted into the stimulus flow path (5 cm upstream of the odour cartridge). Data were sampled for 20 s (at 12 bits, 10 kHz per channel, with a DT3001; Data Translation) and stored in computer files.

Analysis

Spikes were detected and sorted by amplitude using AWAVE (Marion-Poll, 1995). Spikes originating from neuron A were usually larger than spikes originating from neuron B (Renou and Lucas, 1994). The intensity of the ORN responses was evaluated by counting the number of spikes elicited during presentation of the stimulus (0.5 s). Changes in 'background' firing activity were measured by counting the number of spikes detected over periods of 20 s, sampled at regular intervals. Unless otherwise noted, these data were evaluated by ANOVA followed by Bonferoni's *t*-tests on the means.

In addition to the spiking activity, we monitored the transepithelial potential (TEP) as the electrical potential between the recording and reference electrodes. We measured the TEP response (TEPR) as the difference between TEP before stimulation and the maximum amplitude of TEP reached during olfactory stimulation.

Experiments

Effect of OA on the responses to the pheromone components

Changes in the ORN sensitivity were evaluated by measuring the responses of a single sensillum to an olfactory stimulus at different concentrations before and 5 and 30 min after injection of 530 nmol OA (or a control solution). One group of insects was stimulated with Z11–16:Ac (OA injected, 12 insects; control, 10; 402 recordings analysed). A second group of insects was stimulated with Z11–16:OH, followed by Z9–14:Ac (OA injected, 6; control, 6; 231 recordings analysed). Each compound was delivered at 0, 1, 10, 100 and 1000 ng. On these recordings we measured both TEPR and firing activity.

Effect of OA on the background activity of ORNs

The background activity of ORNs was recorded under two conditions. In a first experiment one sensillum was sampled per insect and recorded while covering the hair down to the surrounding cuticle, for 20 s periods 5–10 min before injection and then every 5 min for 60 min after a 530 nmol OA (n = 7 insects) or control (n = 3) injection. This yielded 322 recordings. In a second experiment the same protocol was followed, except that the recording electrode left most of the hair shaft in free air. These observations were performed on five OA injected insects and three control injected insects. This yielded a total of 240 recordings.

Dose-dependent effect of OA and different pharmacological agents

We evaluated the effects of OA, an agonist (clonidine) and an antagonist (CP) on background firing activity of ORNs. This was performed using the same protocol, i.e. recording the spiking activity over 20 s periods from the same sensillum before injection and then every 5 min for 60 min. We used OA (0.53, 5.3, 53 and 530 nmol; n = 3, 5, 6 and 5 insects, respectively; 342 recordings), control saline (n = 6), clonidine (0.053, 0.53, 5.3, 53 nmol; n = 4, 5, 6 and 5, respectively; 422 recordings), a mixture of OA + CP (100 + 100 nmol; n = 5; 282 recordings) and 5-HT (0, 0.41, 4.1, 41 and 410 nmol; n = 6, 4, 6, 6 and 3, respectively; 631 recordings).

Results

Effect of OA on the responses to the pheromone components

Stimulation with increasing loads of Z11–16:Ac elicited dose-dependent changes in TEPR, which reached –4 mV at the highest dosage. Following the injection of 530 nmol OA or control saline no changes in the TEPR dose–response curves could be detected (Figure 1). We also checked whether TEP remained stable following injection. This was possible, however, in only those few cases where the electrical contact was not interrupted by mechanical effects following injection. After the control saline injection there were appreciable shifts in TEP in six of nine insects, with variations of amplitude of 30–50 mV (in absolute value) observed over the 10 s period following injection. Following injection of 530 nmol OA only three of nine insects exhibited such large shifts.

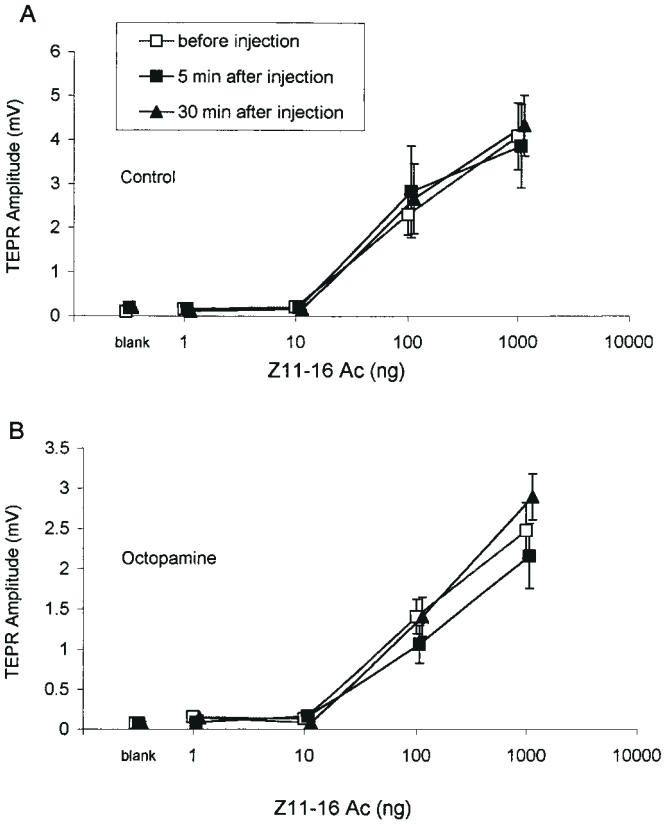

Figure 1 Transepithelial potential response (TEPR) evoked in trichoid sensilla by 0.5 s puffs of Z11–16:Ac. **(A)** Following control injection (n = 10 insects). **(B)** Following injection of 530 nmol OA (n = 12). Error bars, SEM.

Each long sensillum trichodeum of male *M. brassicae* hosts two olfactory receptor neurons (Renou and Lucas, 1994). The first cell (cell A) responds to Z11–16:Ac, the second (cell B) to Z11–16:OH and Z9–14:Ac. Under our recording conditions the amplitude of extracellularly recorded spikes from cell A are about twice that of spikes from cell B.

In response to presentation of Z11–16:Ac cell A increased its spiking activity (Figure 2A), reaching 55 spikes/s at the highest load. Injection of saline before odorant stimulation does not change the dose–response curve (Figure 2A, insert). Injection of 530 nmol OA enhanced the responses to Z11–16:Ac measured 5 min after injection. This effect was still visible 30 min after OA injection. The main observed effect was a significant increase (P = 0.05) in the responses to the different loads of Z11–16:Ac by 10–15 spikes/s. The same increase was observed with a blank stimulus. The slopes of the dose–response curves remained unchanged.

In response to Z9–14:Ac and Z11–16:OH cell B increased

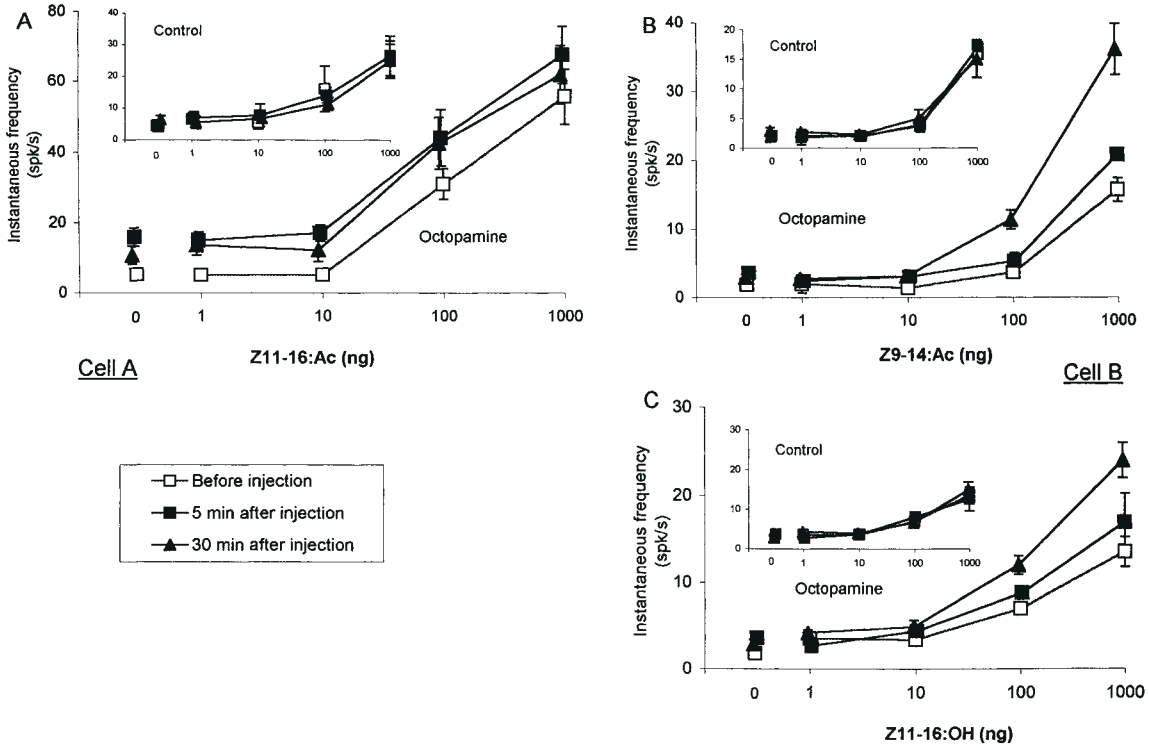

Figure 2 Firing activity evoked in trichoid sensilla by 0.5 s puffs of odours. **(A)** Responses evoked by Z11–16:Ac in the large spike cell ($n = 10$). **(B)** Small spikes cell responses to Z9–14:Ac ($n = 6$). **(C)** Small spike cell responses to Z11–16:OH ($n = 6$). Each point represents the mean number of spikes observed during the first 0.5 s of the response, expressed as spikes/s. Error bars, SEM. (Insets) Responses before and after control injection. (Main graphs) Reponses before and after 530 nmol (100 μg) octopamine injection.

its activity (Figure 2B and C). When a saline solution was injected the dose–response curve remained unchanged (Figure 2B and C, insert). Five minutes following 530 nmol OA injection the responses to 1000 ng Z9–14:Ac and Z11–16:OH were increased by 4–5 spikes/s ($P = 0.01$). Thirty minutes following injection the responses to 100–1000 ng Z9–14:Ac and Z11–16:OH were significantly higher ($P < 0.01$). No increase in responses to the blank stimulus could be detected in these recordings. The main effect observed was thus a change in the slopes of the dose–response curves.

Responses of cell A to Z11–16:Ac were analysed using a peristimulus histogram (PSTH) with 100 ms bins. This histogram was computed over a period of 6 s, including 2 s before stimulation (Figure 3). It showed a burst of spikes during stimulation, followed by a progressive relaxation of the excitation. Following 530 nmol OA injection the firing activity before stimulation increased by 5 spikes/s. The mean PSTH of responses obtained before and following OA injection were subtracted (Figure 3, insert). The resulting curve is almost linear, with no marked difference during or after the stimulus presentation.

Effect of OA on the background activity of ORNs

Following 530 nmol OA injection the background firing activity of cell A significantly increased after 10 min, reached a maximum of 12 spikes/s at 25–30 min and returned to 3 spikes/s after 60 min (Figure 4C). When the recording electrode entirely covered the sensillum under investigation cell A increased its firing activity up to 7 spikes/s and this firing enhancement followed a similar time course (Figure 4A) to that of uncovered sensilla.

In turn, the activity of cell B remained unaffected under free sensillum conditions (Figure 4D), as well as in covered sensilla (Figure 4B). In contrast to OA injections, background firing of both cells A and B remained unaffected after saline injection (Figure 4A–D). Samples of recordings are presented in Figure 5.

Dose-dependent effect of OA and different pharmacological agents

The intensity of cell A background firing and the time course of its variation depended on the quantity of OA injected at the base of the antenna (Figure 6). Following 5.3 nmol firing of cell A did not increase significantly. Following 53 nmol OA cell A background activity reached its maximum (7 spikes/s) 15 min after injection and returned to the level before injection after 30 min. Following 530 nmol OA cell A background activity reached its maximum (12 spikes/s) 25 min after injection and returned to the level before injection after 60 min.

Following agonist (clonidine) injection the background activity of cell A increased, depending upon the concen-

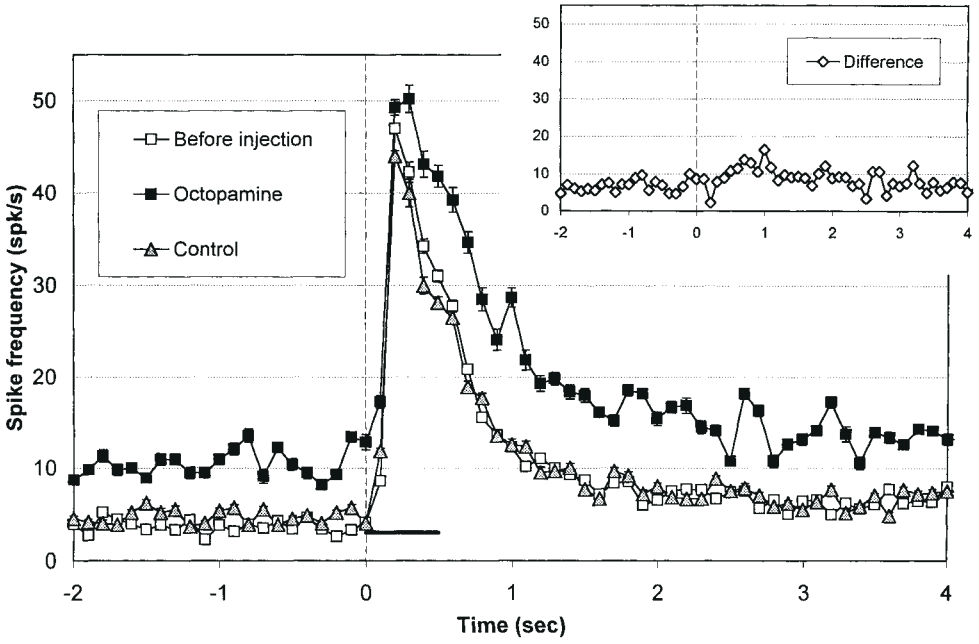

Figure 3 Peristimulus time histogram of the response of the large spike ORNs to 1 μg Z11–16:Ac before treatment and 20 min after injection of control or 530 nmol OA. Each curve results from the mean of 13 (OA) or 5 (control) replicates. (Inset) To allow comparison between pre- and post-injection PSTH the differences in values before injection from values after octopamine injection are presented. The stimulus was applied at time $t = 0$ and lasted 0.5 s.

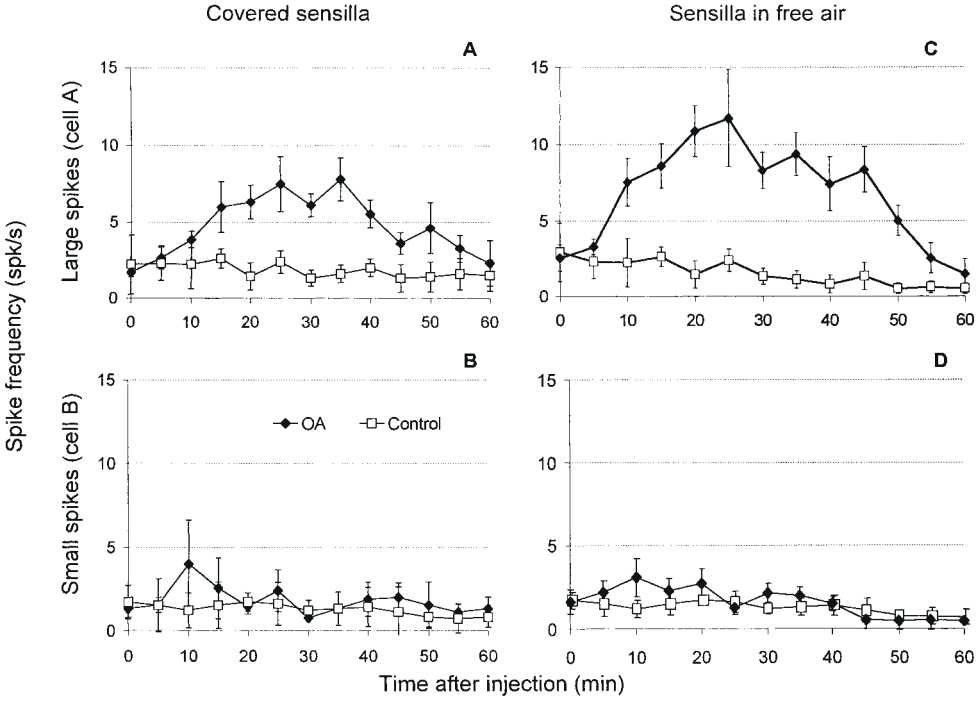

Figure 4 Changes in firing activity of ORNs following 530 nmol OA or control injections. **(A, B)** Sensilla completely covered by the recording electrode (OA, $n = 7$; control, $n = 3$). **(A)** Large spike cells; **(B)** small spike cells. **(C, D)** Sensilla not covered (OA, $n = 5$; control, $n = 3$). **(C)** Large spike cells; **(D)** small spike cells. Spikes were counted over 20 s periods, sampled every 5 min over 60 min. Each square represents the mean of several individuals. Error bars correspond to the SEM.

tration injected (Figure 7A). At 0.53 nmol clonidine background firing increased significantly after 5 min, reached a maximum at 10 min and then decreased to its initial level after 25 min. After injection of 5.3 nmol clonidine background activity increased after 5 min, reached a maximum after 10 min and then decreased to a plateau value

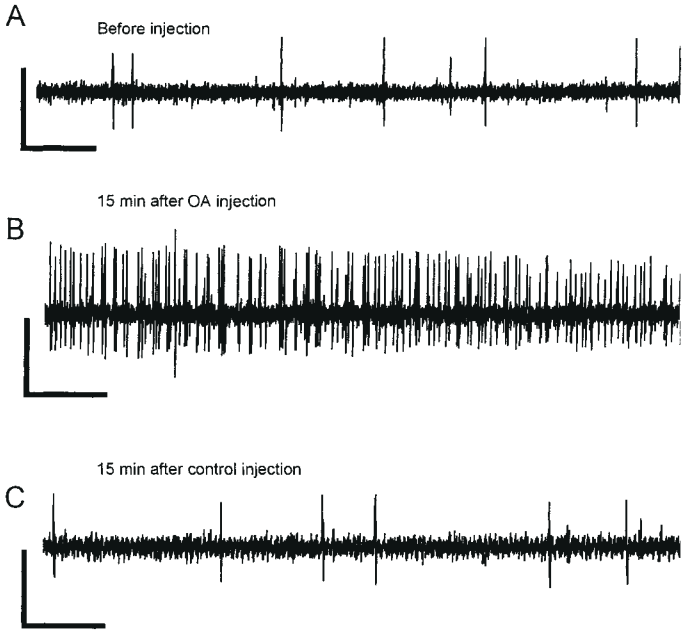

Figure 5 Examples of changes in firing activity following OA or control injection. **(A, B)** 530 nmol OA injection, same sensillum, before and 15 min following, respectively, OA injection. **(C)** Typical activity in a different sensillum, 15 min after a control injection. Scale: vertical bar, 1 mV; horizontal bar, 1 s.

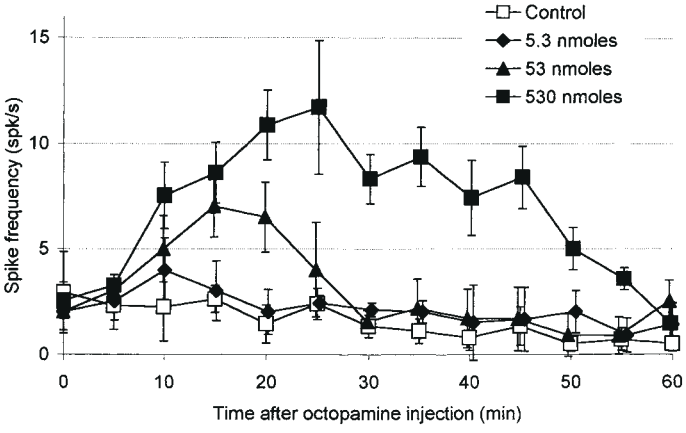

Figure 6 Changes in firing activity of ORNs following injection of different concentrations of OA: 0 (n = 6), 0.53 (n = 3), 5.3 (n = 5), 53 (n = 6) and 530 nmol (n = 5) (0.1, 1, 10 and 100 μg). Spikes from the large spike cell were counted over 20 s periods, sampled every 5 min over 60 min. Each point represents the mean of several individuals. Error bars represent the SEM.

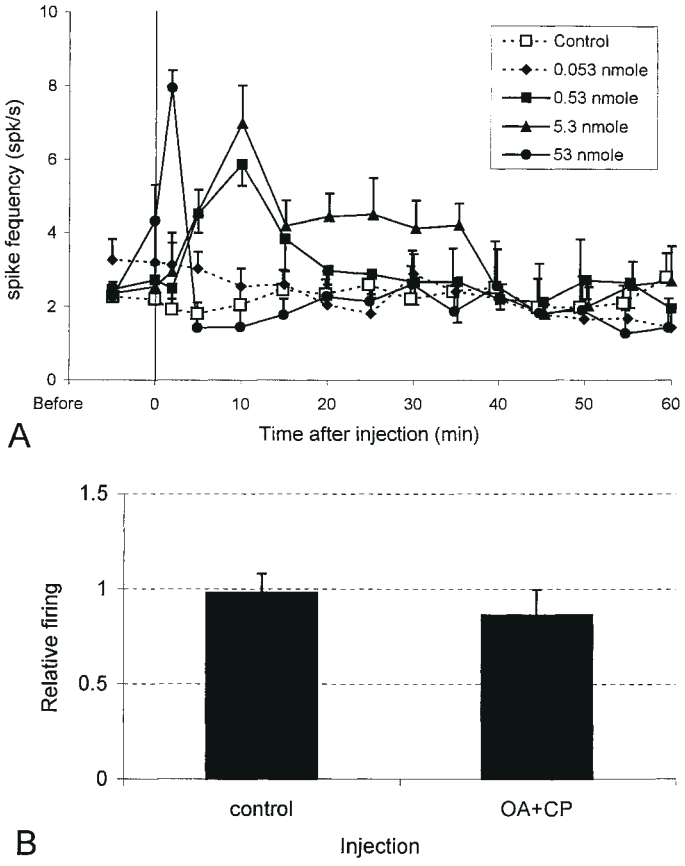

Figure 7 **(A)** Changes in firing activity of ORNs following injection of different concentrations of clonidine: 0 (n = 6), 0.053 (n = 4), 0.53 (n = 5), 5.3 (n = 6) and 53 nmol (n = 5). Spikes from the large spike cell were counted over 20 s periods, sampled every 5 min over 60 min. Each point represents the mean of several individuals. Error bars represent the SEM. **(B)** Changes in firing activity of ORNs following injection of 100 nmol OA + 100 nmol CP (OA + antagonist, n = 5) (control, n = 6). Each bar represents the ratio of the firing activity computed over 20 s periods before and after injection. Error bars correspond to the SEM. Control and OA + CP are not significantly different (t-test).

after 15 min. The background activity decreased to its initial level after 40 min. The highest concentration of clonidine (53 nmol) elicited a 3.5-fold increase in cell A background activity, a value similar to that measured after 530 nmol OA. At this dose background firing was enhanced immediately after injection, i.e. within 20 s, reached its maximum after 5 min and then decreased rapidly to reach its initial level after 10 min.

To evaluate the effects of an OA antagonist, CP was mixed with OA to determine whether it antagonized the effects of OA on cell A background firing. The ratio of the maximum firing rate of cell A reached following injection to that before injection was computed. Following injection of an equimolar (100:100 mM) mixture of OA + CP background firing did not increase compared with its pre-injection level (Figure 7B).

Following 5HT injection background firing was reduced or even suppressed (Figure 8). The intensity and time course of the reduction depend on the 5HT concentration injected at the base of the antenna. Following 0.41 nmol 5HT injection firing of cell A did not significantly decrease. Following 4.1 nmol 5HT injection cell A background activity reached a minimum (1 spike/s) 10 min after injection and returned to

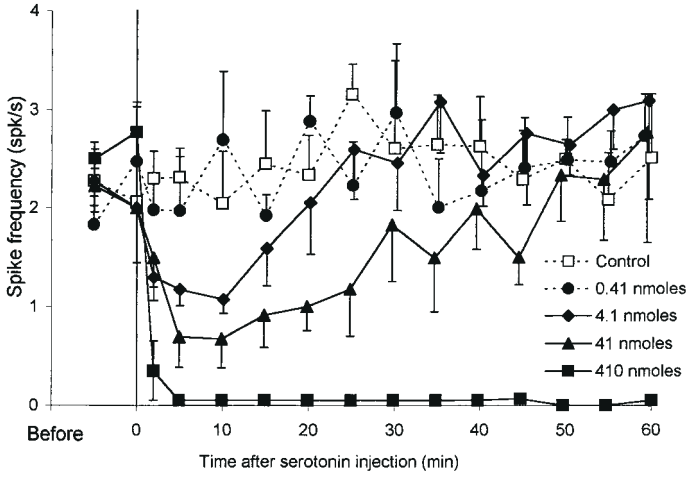

Figure 8 Changes in firing activity of type A ORNs following injection of different concentrations of serotonin: 0 ($n = 6$), 0.41 ($n = 4$), 4.1 ($n = 6$), 41 ($n = 6$) and 410 nmol ($n = 3$) (0.1, 1, 10 and 100 μg). Spikes from the large spike cell were counted over 20 s periods, sampled every 5 min over 60 min. Each point represents the mean of several individuals. Error bars represent the SEM.

its before injection level after 20 min. Following 41 nmol 5HT injection the minimum firing activity was lower (0.7 spikes/s) and was reached 10 min after injection. Return to the before injection level was reached later (40 min) compared with the pattern obtained for 4.1 nmol 5HT. Following 410 nmol 5HT injection cell A background activity was reduced and almost suppressed after 5 min. This effect lasted more than 60 min. For all the doses tested significant decreases in background firing were observed 2 min after injection.

Discussion

The effects of OA on the firing activity of two types of male *M. brassicae* ORNs were evaluated. Both olfactory receptor neurons are housed in the same sensillum and are involved in sex pheromone communication. Both cell types showed increased responses to olfactory stimulation after OA injection. In turn, no changes in amplitude of TEPR were detected. These results are similar to those found in another moth species, *Antheraea polyphemus*, by Pophof, who concluded that OA increases the sensitivity of pheromone receptor neurons (Pophof, 2000). A known OA agonist, clonidine, mimicked OA-induced activation of background firing in the same concentration range as OA (this work). With respect to OA antagonists, Pophof observed that epinastine reduces spike firing by moth ORNs (Pophof, 2000). We show herein that another antagonist, CP, also suppressed the effect of exogenous OA.

These pharmacological data are consistent with the involvement of an OA receptor. Nickisch-Rosenegk *et al.* cloned an OA receptor expressed in the antennal sensilla of two lepidoptera species, *B. mori* and *H. virescens* (Nickisch-Rosenegk *et al.*, 1996). We confirmed by RT–PCR that antennal tissues of *M. brassicae* express an OA receptor protein which has 98% homology to the OA receptor of *H. virescens* (Grosmaitre *et al.*, 2000). Thus it is likely, as suggested by Pophof, that the observed effects of OA on the activity of ORNs are mediated by receptors in the antenna rather than by indirect effects on the metabolism of the insects (Pophof, 2000). There are two possible mechanisms for the cellular effects of OA. First, OA might increase the expression of olfactory proteins through the activation of a transcriptional cascade. Second, OA might affect a more direct transduction pathway involved in spike generation. The second hypothesis is better supported by the short delay (<5 min for OA, 2 min for clonidine) before appearance of the effects, which includes the time of diffusion from the antennal base to the sensilla.

The firing activity of one cell type in *M. brassicae* showed OA-dependent activation in the absence of any direct odorant stimulation. This activation was clearly OA dose dependent: at higher doses of OA we observed a decrease in latency, an increase in peak firing activity and an increase in duration of the effect. These results seem to indicate that OA increases spontaneous activity of ORNs in the absence of stimulation. Several arguments suggest that OA enhances the sensitivity of ORNs towards traces level of pheromone. We propose that ORNs are exposed to a continuous low level stimulation, as suggested recently by Ziesman *et al.* (Ziesman *et al.*, 2000). Firstly, the OA-induced enhancement of background firing was decreased by more than 50% when the sensillum was completely covered by the recording electrode. Covering the sensillum should prevent external odorant molecules gaining access to the ORNs. However, the reduction was not complete. It is possible that molecules were still present within the sensillum lymph or adsorbed to the cuticle before the sensillum was covered. Secondly, this excitation was only observed in cell A, which responds to Z11–16:Ac. This molecule is routinely used in our laboratory for pheromone physiology studies. Therefore, traces of Z11–16:Ac might remain on the set-up. The background firing activity of cell B remained unaffected. This cell is tuned to Z9–14:Ac and Z11–16:OH, which are less frequently used in our laboratory. Thirdly, control recordings performed in a different laboratory, where Z11–16:Ac had never been used before, showed that OA did not increase firing activity in *M. brassicae* cell type A (J. Dolzer, personal communication).

It is not clear, however, by which means OA enhances the sensitivity of ORNs. As found by Pophof, OA increases spike firing of both ORNs in response to pheromone stimulation (Pophof, 2000). This effect was interpreted as a modulation of receptor neuron sensitivity. Since the receptor potential was not modified, this author suggested that OA directly modulates spike generation by ORNs. According to this conclusion we would expect both olfactory cell types to change their gain, i.e. change the slope of the dose–response curve, rather than shifting the curve along the horizontal

axis. This is consistent with our observations on cell B responding to Z9–14:Ac and Z11–16:OH. However, this is not consistent with the increase (vertical shift) observed in the dose–response curve of cell A responding to Z11–16:Ac.

In turn we suggest that the main effect of OA is to change the adaptation state of ORNs. When neurons are adapted they fire a reduced number of spikes in response to the same stimulus strength, as shown by Whun-Yu Mok Zack (Whun-Yu Mok Zack, 1979). If ORNs are exposed to a continuous low level of Z11–16:Ac then cell A would be in a 'desensitization' or a 'long-lasting adaptation' state, according to the terminology proposed by Zufall and Leinders-Zufall (Zufall and Leinders-Zufall, 2000). Dis-adapting the ORNs with OA would reveal this low level stimulation and provoke a vertical shift in the dose–response curve. In cell B, which, due to lower exposure to its key compounds is not desensitized, we would not expect OA to increase background firing. However, adaptation of cell B could have occurred later during the experiment. In our stimulation protocol we apply the odorant in increasing order of concentration. Although the stimuli were presented a few minutes apart, it is possible that adaptation could occur after a number of stimulations, especially when presenting the highest doses of odour at the end of a series of stimulations. Accordingly, OA should mostly enhance responses to high loads of odour: these loads are expected to be more affected by adaptation. This would explain the changes in shape of the dose–response curve of cell B.

Alternatively, it is possible that both cells do not adapt in the same way. Kodadova has showed that the ORNs of *A. polyphemus* differ in their dynamic properties, the small spike cell recovering faster from adaptation than the large spike cell (Kodadova, 1996). It is striking to observe that the dose–response curve of the large cell is shifted vertically after OA injection, whereas the slope of the dose–response curve of the small spike cell becomes steeper after injection (Pophof, 2000). These different effects of OA might be correlated with the different adaptation properties of the cells.

It is unlikely, however, that OA changes short-term adaptation (Zufall and Leinders-Zufall, 2000) since a careful examination of the time course of the responses did not reveal any modification in their shape. Further experiments are certainly needed to elucidate the mechanisms by which OA might interfere with adaptation processes.

The effect of 5HT on background spike firing was opposite to that of OA, as 5HT induced a dose-dependent decrease in background firing of *M. brassicae* ORNs. It remains to test the effect of 5HT on the sensitivity of cells towards high loads of odour and if adaptation mechanisms could be suggested, as for OA. Opposite effects of 5HT and OA have been described in a number of different systems, for example in neurons of the optic lobe of the honeybee (Erber *et al.*, 1993) and in other invertebrates neurons. In lobsters injection of the monoamines OA and 5HT into the haemolymph causes the animals to adopt opposing static postures: OA causes tonic extension of all extremities, while 5HT induces a tonic flexion (Kravitz, 1988). Concerning sensory systems, whereas 5HT was shown to modulate central olfactory responses to odorants (Kloppenburg *et al.*, 1999) as well as the firing properties of central olfactory neurons (Mercer *et al.*, 1996), the effect of OA in central neurons remains unknown.

Until recently the responses of moth pheromone receptor neurons were considered stable and independent of insect physiology. The described effects of OA and 5HT on spike firing of ORNs of *M. brassicae* show that neurohormones with opposite effects might have modulatory functions and ensure precise regulation of the olfactory sensilla. Since the sensillum is a complex system, comprising neurons and accessory cells, biogenic amines might affect different cell types bearing different receptor subtypes. Thus an important issue in understanding the neuromodulatory role of these biogenic amines in insect pheromonal responses will be the investigation of the location and subtypes of receptors as well as the components in the signalling pathways implicated in this action.

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