Guanosine 3',5'-Cyclic Monophosphate Reduces the Response of the Moth's Olfactory Receptor Neuron to Pheromone

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Abstract

The effects of the membrane-permeable dibutyryl guanosine 3',5'-cyclic monophosphate (db-cGMP) on the bombykol-elicited receptor current and nerve impulse activity were studied using the open sensillum recording technique. db-cGMP was applied to the outer dendritic membrane of the olfactory receptor neuron of the moth *Bombyx mori*. db-cGMP reduced the amplitude of the overall receptor current activated by a pulse of strong pheromone stimuli as well as diminished the nerve impulse frequency elicited by continuously applied weak pheromone stimuli. The observed inhibition of the response to pheromone was due to size reduction of an elementary receptor current that elicits the nerve impulses and underlies the overall receptor current. It is suggested that cGMP is a factor which may adjust cell sensitivity to odour and play a role in olfactory adaptation.

Introduction

Odour perception is a result of complex biochemical and electrophysiological reactions occurring in olfactory receptor neurons (ORNs) (Lancet, 1986; Shepherd, 1993; Kaissling, 1996) which involve as second messengers cyclic nucleotide monophosphates. Cyclic adenosine monophosphate (cAMP) contributes to an excitatory signal transduction pathway (Nakamura and Gold, 1987; Breer *et al.*, 1990) as well as to an inhibitory pathway (Michel and Ache, 1992). Cyclic guanosine monophosphate (cGMP) contributes to adaptation processes (Zufall *et al.*, 1991c; Boekhoff *et al.*, 1993; Shepherd, 1993; Leinders-Zufall *et al.*, 1996, 1998; Zufall and Leinders-Zufall, 1997) in the olfactory receptor cell. The role of cyclic nucleotides in signal transduction in insect ORNs still remains to be elucidated, although pheromone transduction has been studied intensively in insects (Villet, 1978; Breer *et al.*, 1990; Ziegelberger *et al.*, 1990; Zufall and Hatt, 1991; Zufall *et al.*, 1991b; Boekhoff *et al.*, 1993; Stengl, 1993, 1994; Redkozubov, 1996; Krieger *et al.*, 1997; Laue *et al.*, 1997; Wegener *et al.*, 1997). Although some data concerning the contribution of cyclic nucleotides to pheromone transduction have been collected (Villet, 1978; Ziegelberger *et al.*, 1990; Zufall and Hatt, 1991; Zufall *et al.*, 1991b), our understanding of these processes is still incomplete. A possible involvement of cGMP in pheromone transduction is suggested, because the cyclic nucleotide and its synthetic enzyme are both present in moth antennae and the amount of cGMP is increased there after pheromone stimulation (Ziegelberger *et al.*, 1990; Boekhoff *et al.*, 1993). The present work was undertaken to elucidate the role of cGMP in pheromone transduction using electrophysiological recording *in situ*. Here we report an attenuating effect of the membrane-permeable dibutyryl cGMP on the response of the olfactory receptor cell of the moth *Bombyx mori* to its sex pheromone bombykol. The observed attenuation of the response to pheromone is due to size reduction of an elementary receptor current which elicits the nerve impulses and underlies the overall receptor current. A contribution of cGMP to an adjustment of the receptor cell sensitivity to odour is suggested.

Materials and methods

The method of single sensillum recording (Kaissling, 1995) from the antennal sensilla trichodea of male *Bombyx mori* was employed. Receptor currents were recorded *in situ* from the open sensillum under transepithelial voltage-clamp conditions, where a recording capillary electrode filled with receptor-lymph solution (Kaissling and Thorson, 1980) was slipped over the sensillum tip and a reference electrode filled with hemolymph solution was inserted into the antennal stem. Dibutyryl guanosine 3',5'-cyclic monophosphate (db-cGMP; D3510, Sigma, St Louis, MO) was applied to the outer dendrite via the recording electrode containing the cyclic nucleotide. This electrode was exchanged for the recording electrode without the nucleotide. The pheromone stimuli were applied to the sensillum via an airstream blown

through a cartridge with filter paper, which comprised 100 or 0.001 µg of bombykol as an odour source. Strong pheromone stimuli were applied in a single pulse of 500 ms duration. Weak pheromone stimuli were applied continuously for 2–6 min.

Currents were recorded by a patch-clamp amplifier ROK-3 (VKNZ, Moscow, Russia). The data were filtered at 1 kHz (low-pass Bessel), digitally sampled at 0.25 ms per point and analysed using the Igor Pro software package (Wave Metrics, Lake Oswego, OR). Receptor current amplitude was measured as a difference between the baseline and the maximum current (around the most negative value) reached during the pheromone stimuli. The receptor currents are shown so that a downward deflection of the track represents a current flowing into the sensillum from the recording pipette; that is, from the sensillum lymph space to the hemolymph space. Kinetics of the rising phase of the receptor current was evaluated by fitting with a single exponential function over the rising phase (in the range between the baseline and the maximum current), resulting in a time constant τ. The fitting was performed with an iterative Levenberg–Marquard nonlinear least squares fitting algorithm of the Igor Pro software.

Single elementary receptor currents (ERCs) eliciting one action potential (insets in Figure 2) were taken for analysis. Pieces of 0.2 s duration (0.1 s before and 0.1 s after the nerve impulse) were sampled from recordings for the following ERC averaging. An averaged ERC was obtained by using the tip of nerve impulses to align the average (Kaissling and Thorson, 1980). The averaged ERCs were used for the following analysis because the original ERCs vary greatly in their amplitude and duration (see ERCs in inset in Figure 2). The following parameters of the current were measured on the averaged ERC: amplitude and duration. An ERC amplitude was measured as the difference between the baseline and the maximum current, which was actually counted as the mean value within a 3 ms range around the most negative merit, reached during the prepulse portion of ERC. ERC duration was measured as the time between onset (downward track deflection) of the ERC before the spike and its offset (upward track deflection) after the spike at the level of the half amplitude of the ERC.

Results

db-cGMP inhibits the response to strong pheromone stimuli. Bombykol (100 µg at odour source) elicited the receptor current generated by a bombykol-sensitive receptor neuron. Mean amplitude of the current was 54.4 ± 14.1 pA (n = 4). db-cGMP (0.1 mM) reduced the response to bombykol up to 13.4 ± 2.3 pA (n = 4), i.e. the receptor current decreased by ~75% of the control value. The observed decrease in the receptor current amplitude was significant according to the paired t-test (P = 0.044). db-cGMP also prolonged the rising phase of the receptor
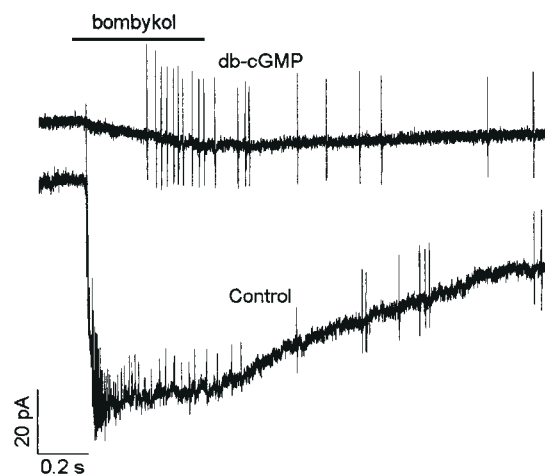

Figure 1 Effect of db-cGMP (0.1 mM) on the overall receptor current elicited by strong bombykol (100 µg at odour source) stimuli. The amplitude of the receptor currents was estimated as 71 pA (control) and as 8 pA (db-cGMP). The time constants of the rising phase of the receptor current are 14 ms (control) and 453 ms (db-cGMP). The horizontal bar above indicates the duration of the pheromone stimuli. db-cGMP was applied for 3 min before testing the pheromone response.

current from a time constant of τ = 46.7 ± 36.6 ms (n = 4) in the control to τ = 442.7 ± 162.7 ms (n = 4) with db-cGMP (P = 0.043). Figure 1 shows the effects of db-cGMP on the receptor cell response (the overall receptor current) to strong pheromone stimuli. Note that an increasing cGMP level in the outer dendrite of the ORN reduces the amplitude and slows down the rising kinetics of the response of the receptor cell to pheromone, so that the overall receptor current elicited by strong bombykol stimuli becomes as small and as slow as the response to an odour concentration which is several orders of amount smaller.

Weak pheromone stimuli applied continuously elicit usually a tonic increase in the action potential activity. Figure 2 and Table 1 illustrate the action potential activity elicited in the bombykol-sensitive ORN by weak bombykol stimuli (0.001 µg). db-cGMP (0.1 mM) reduced the bombykol-activated nerve impulse frequency by ~87% of the control value. This implies that the action potential activity elicited by weak bombykol stimuli decreases after db-cGMP application to such low values and reaches the spontaneous frequency observed without pheromone stimuli.

A detailed analysis of the shape of the bombykol-elicited nerve impulses showed that the impulses are preceded by a small prepulse receptor current (see inset in Figure 2). It is known that with weak pheromone stimuli the nerve impulses are accompanied by preceding elementary receptor potentials which are the putative responses to single pheromone molecules (Kaissling, 1994). Summation of such responses in space and time makes up the overall receptor potential which is evoked by a single strong stimulus (Kaissling, 1987). The effect of db-cGMP on the parameters of the prepulse elementary receptor current, underlying
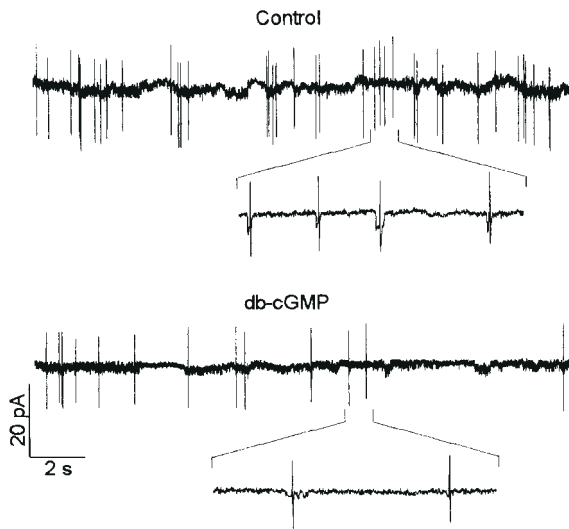
Control

db-cGMP

Figure 2 Effect of db-cGMP (0.1 mM) on nerve impulse activity elicited by weak bombykol (0.001 μg at odour source) stimuli. Upper (control) and lower (db-cGMP) traces were recorded just before and after start of db-cGMP application, respectively. In insets parts of the recording are shown at an expanded (tenfold) time scale in order to illustrate the presence of the ERCs. The data were digitally filtered at 300 Hz for illustrating purposes only.

Table 1 Effect of db-cGMP (0.1 mM) on the bombykol-elicited nerve impulse frequency and the ERC parameters

	Frequency (imp/s)	ERC amplitude (pA)	ERC duration (ms)
Control	2.4 ± 1.03	1.46 ± 0.21	29.2 ± 5.5
db-cGMP	0.4 ± 0.3	0.82 ± 0.09	33.1 ± 2.5
Paired *t*-test	$P = 0.097$	$P = 0.036$	$P = 0.24$

The data are shown as mean ± SD for four cells. Statistical significance of the db-cGMP effect was estimated by paired *t*-test for $\alpha = 0.05$.

the above-mentioned elementary receptor potential, was examined to elucidate why the overall receptor current was reduced by the nucleotide. It is shown that db-cGMP reduces the size of the ERC. Figure 3 illustrates the effect of db-cGMP on the averaged ERC elicited by weak pheromone stimuli. Table 1 summarizes the parameters of the averaged bombykol-elicited ERC before and after db-cGMP (0.1 mM) application. From the data presented in Table 1 it follows that db-cGMP reduces the amplitude of the averaged ERC by 44%. db-cGMP does not appear to influence the duration of the averaged ERC because it remained almost unaltered (a non-significant increase). Thus, db-cGMP in the outer dendrite reduces the responsiveness of the moth ORN to odour by decreasing the size of the averaged ERC.

Discussion

It was demonstrated here for the first time that db-cGMP attenuates the ORN response in *Bombyx mori* to phero-
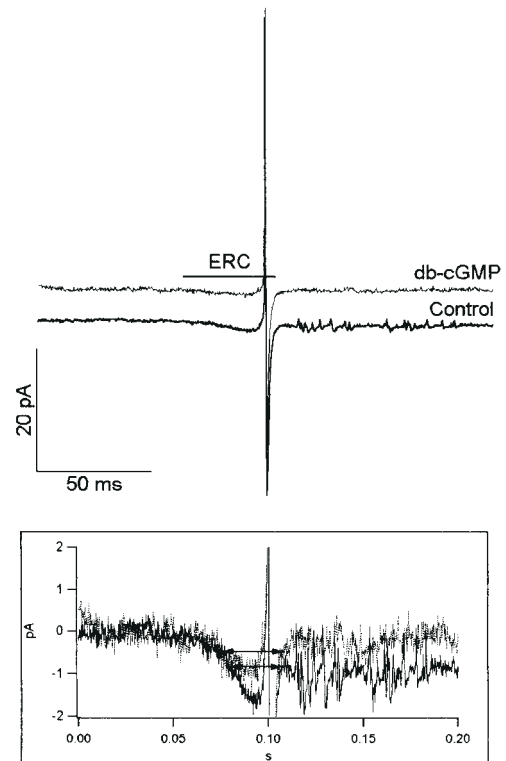

Figure 3 Averaged ERC before (thick line) and after db-cGMP (0.1 mM) (thin line) application. Horizontal bar indicates ERC. Eighty-eight single ERCs contributed to the averaged ERC in control and 104 single ERCs under db-cGMP. An inset in the box below shows the averaged ERCs in a range of ±2 pA around their baseline. The size of the averaged ERC in control (solid line) is 1.6 pA, that under db-cGMP (dotted line) is 0.9 pA. A bottom horizontal line (double arrowheads) illustrates the duration (27 ms) of the averaged ERC in control measured at its half amplitude level. The upper horizontal line (double arrowheads) illustrates the duration (34 ms) of the averaged ERC under cGMP measured at its half amplitude level. The large amplitude irregular noise right of the nerve impulse (in control) is due to the following single ERCs which were activated close to the ERC taken for averaging. A weak bombykol stimulus (0.001 μg at odour source) was blown over sensillum continuously for 2 min in control and for 6 min under db-cGMP.

mone. The amplitude of the receptor current as well as the nerve impulse frequency in response to strong and weak pheromone stimuli were reduced by increasing the cGMP concentration in the outer dendrite of the ORN. The reduction of the pheromone response was due to a decrease in the ERC size. On the other hand, some inconsistency in the proportion of the response reduction indicates that a diminished activation probability of the ERCs may also contribute to the observed decrease in the pheromone response. The overall receptor current was reduced by db-cGMP to a larger extent (by 75%) than the size of the averaged ERC (by 44%). In case all ERCs were reduced in size by 44%, one would expect a similar decrease in the overall receptor current elicited by strong pheromone stimuli (saturating pheromone concentration like in present work). This should result in a proportional reduction of the

overall receptor current following the ERC size abatement in the presence of db-cGMP. However, the reduction of the overall receptor current did not follow exactly the decrease in the averaged ERC size. This is possibly due to a repressing effect of db-cGMP on ERC activation in addition to the ERC size abatement. The reduced probability of ERC activation might further decrease the impulse frequency.

It was reported previously that guanylate cyclase is present in moth antennae and that an increase in cGMP levels in the cell soma takes place upon pheromone stimulation (Ziegelberger *et al.*, 1990; Boekhoff *et al.*, 1993). db-cGMP decreases the frequency of ERC occurrence in ORNs of the moth, while activation of the components of the excitatory transduction pathway leads to an increase in the frequency and amplitude of ERCs (Redkozubov, 1995, 1996; Laue *et al.*, 1997). The down-regulating effect of cGMP on the pheromone response of the ORNs described here implies that a regulatory cascade, involving guanylate cyclase and cGMP, is probably present in the ORNs of the moth. Such a regulatory cascade is perhaps involved in sensitivity regulation in these cells. It appears that the cascade converges onto the same target, namely, the pheromone-activated ion channel, as does the excitatory transduction pathway, involving Gq protein, phospholipase C, diacylglycerol and protein kinase C (Breer *et al.*, 1990; Zufall and Hatt, 1991; Stengl, 1993, 1994; Redkozubov, 1995, 1996; Laue *et al.*, 1997). Such a regulating mechanism differs principally from the mechanism discovered recently in some animal species that use dual transduction pathways, consisting of independent excitatory and inhibitory paths, in ORNs (Breer *et al.*, 1990; Fadool and Ache, 1992; Michel and Ache, 1992). Two second messengers, cAMP and inositol 1,4,5-trisphosphate, regulate separately opposing ionic conductances.

The fact that under db-cGMP the response to strong pheromone stimuli becomes as small and as slow as a response to much weaker pheromone concentrations suggests that cGMP may adjust cell sensitivity to odour. An advantage of such a mechanism could be its contribution to adaptation of the olfactory receptor cell to strong stimuli, i.e. it reversibly reduces their sensitivity to odour (Zufall *et al.*, 1991c; Borisy *et al.*, 1992; Kramer and Siegelbaum, 1992; Kleene, 1993; Leinders-Zufall *et al.*, 1996, 1998; Zufall and Leinders-Zufall, 1997). Recently a role of cGMP in adaptation was clarified in salamander ORNs, where the processes leading to excitation and long-lasting adaptation are attributable to the activation of distinct, parallel second-messenger pathways, i.e. the cAMP cascade leading to transient activation of ion channels and excitation, and the cGMP cascade resulting in persistent ion channel activity and long-lasting adaptation (Zufall *et al.*, 1991a,c; Leinders-Zufall *et al.*, 1996, 1998; Zufall and Leinders-Zufall, 1997). Differently in the moth, cGMP attenuates the ORN excitation mediated by the protein kinase C cascade (Zufall and Hatt, 1991; Redkozubov, 1995, 1996; Laue *et al.*, 1997),

presumably affecting the ion conductance activated by PKC. Thus, the investigation of the regulatory cascade, that involves cGMP and controls the sensitivity of the receptor cells in moths, could help to gain insight into the transduction machinery and adaptation mechanisms of ORNs.

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