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Activation and Inhibition of the Transduction Process in Silkmoth Olfactory Receptor Neurons

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Abstract

Electrophysiological responses of olfactory receptor neurons in both male and female silkmoths (*Bombyx mori*) were investigated. In both sexes, the G-protein activator sodium fluoride and 1,2-dioctanoyl-sn-glycerol, a membrane-permeable analog of the protein kinase C activator diacylglycerol, elicited nerve impulse responses similar to those elicited by weak continuous stimulation with odorants. Therefore, G_q-proteins and diacylglycerol-activated ion channels seem to be involved in the transduction process in both pheromone-sensitive neurons in males and general odorant-sensitive neurons in females. Decyl-thio-trifluoro-propanone is known to inhibit electrophysiological responses of male moths to pheromones, but has no effect in females. Application of this inhibitor reduced the frequency, but not the amplitude of elementary receptor potentials. It had no inhibitory effect on nerve impulse responses elicited by sodium fluoride or 1,2-dioctanoyl-sn-glycerol. This supports the idea that decyl-thio-trifluoro-propanone acts on a prior step of the transduction cascade, e.g. on the pheromone receptor molecules. General odorants, such as (±)-linalool and 1-heptanol, excite olfactory receptor neurons in females, but inhibit the pheromone-sensitive neurons in males. Both (±)-linalool and 1-heptanol inhibited the responses of male neurons elicited by sodium fluoride or 1,2-dioctanoyl-sn-glycerol. (±)-Linalool reduced the amplitude of elementary receptor potentials. In contrast to decyl-thio-trifluoro-propanone, (±)-linalool and 1-heptanol seem to interfere with later processes of the transduction cascade, possibly the opening of ion channels.

Introduction

Olfactory reception in moths starts with the adsorption of odorant molecules on the olfactory hairs of the antenna (Kanaujia and Kaissling, 1985). The hydrophobic odorant molecules pass the hydrophilic sensillum lymph inside the olfactory sensilla bound to odorant-binding proteins (Van den Berg and Ziegelberger, 1991; Kaissling, 1996, 2001). Arriving at the dendritic membrane, they stimulate the odorant receptors localized in the dendritic membrane of the olfactory receptor neurons. These molecules are still unknown in moths, but in *Drosophila* it has been shown that insect olfactory receptor proteins are, as in vertebrates, 7-transmembrane G-protein-coupled receptors (Clyne *et al.*, 1999; Vosshall *et al.*, 1999). G-proteins of the class $G_{q/11}$ were localized in the membrane of moth olfactory receptor neurons (ORNs) and the G-protein activator sodium fluoride (NaF) elicited nerve impulses in pheromone-sensitive ORNs of male moths (Laue *et al.*, 1997). The G_q-proteins probably activate PLCβ (Maida *et al.*, 2000), which leads to equimolar production of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. Rapid increase of IP_3 after pheromone stimulation was observed in moth antennal homogenates (Breer *et al.*, 1990; Kaissling and Boekhoff, 1993). IP_3-receptors were localized in the dendritic membrane of moth ORNs (Laue and Steinbrecht, 1997) and IP_3-activated Ca-currents were found in cultured moth ORNs (Stengl, 1993, 1994). In excised inside-out patches of the dendritic membrane from silkmoth ORNs IP_3 was ineffective, whereas activators of protein kinase C (PKC), such as diacylglycerol and phorbolester, activated ion channels (Zufall and Hatt, 1991). The diacylglycerol analog 1,2-dioctanoyl-sn-glycerol (DOG) elicited nerve impulses in moth pheromone-sensitive ORNs (Maida *et al.*, 2000). It could activate ion channels via increase of the activity of PKC, which activated cation currents in cultured moth ORNs (Stengl, 1993). A 4-fold increase in PKC-activity after pheromone stimulation was measured in antennal homogenates of male moths (Maida *et al.*, 2000).

Most of the above experiments were performed solely on male pheromone-sensitive ORNs. In the male silkmoth *Bombyx mori*, the long sensilla trichodea contain two ORNs responding to the female pheromone components *E,Z*-10,12-hexadecadienol, bombykol (Kaissling and Priesner,

1970) and *E,Z*-10,12-hexadecadienal, bombykal (Kaissling *et al.*, 1978). The long sensilla trichodea of the female contain two ORNs responding to benzoic acid and to 2,6-dimethyl-5-heptene-2-ol as well as (±)-linalool (Priesner, 1979; Heinbockel and Kaissling, 1996, Van der Goes van Naters, 2001). In the present paper, the male and female ORNs were investigated to show whether there are differences in olfactory transduction between the reception of pheromones and of general odorants.

Some compounds, such as the general odorant (±)-linalool and the esterase inhibitor decyl-thio-trifluoropropanone (DTFP), are known to inhibit the reception of pheromones in *B. mori* males (Kaissling *et al.*, 1989; Pophof *et al.*, 2000). In this paper we have investigated the points at which these and other compounds disrupt the transduction cascade. In particular, we studied the influence of the inhibitors on the elementary receptor potentials (ERPs), which are expected to reflect ion channel openings in response to the excitation of the receptors by single odorant molecules (Kaissling, 1994; Redkozubov, 1999).

Materials and methods

Pupae of *B. mori* L. (Bombycidae) were obtained from the Istituto Sperimentale per la Zoologia Agraria (Padova, Italy), INRA–Unité nationale séricicole (LaMulatiére, France) and Worldwide Butterflies (Dorset, UK). The animals were sexed at the pupal stage and allowed to emerge at room temperature. The moths were then stored in a refrigerator at 12°C and used for experiments between the ages of 1 and 4 days.

Tip recordings were performed from single sensilla trichodea of isolated male and female antennae using glass capillary Ag–AgCl electrodes. The reference electrode was filled with hemolymph Ringer solution and inserted into the antennal base; the recording electrode was filled with sensillum-lymph Ringer solution and slipped over the cut sensillum tip (Kaissling, 1995). The preparation was held in a permanent airstream (1 m/s) filtered through charcoal and humidified by percolation through distilled water.

For the application of the G-protein-activating fluoride (Antonny *et al.*, 1993), the standard recording pipette was exchanged for a pipette filled with a modified sensillum-lymph Ringer solution, in which sodium chloride was equimolarly replaced by 20 mM NaF (Sigma) (Laue *et al.*, 1997). 1,2-Dioctanoyl-sn-glycerol (DOG, Sigma), a membrane-permeable PKC activator, was applied to the outer dendrite via the recording electrode, which contained 0.1 mM DOG in sensillum-lymph Ringer solution with 0.005% DMSO (Maida *et al.*, 2000). This concentration of DMSO had no effect in control experiments performed on 11 male and five female ORNs. A fresh DOG solution was prepared before each experiment from a stock solution of 20 mM DOG in sensillum-lymph Ringer with 1% DMSO, which was kept frozen.

Two methods were used to apply volatile odorants and inhibitors to the ORNs of sensilla trichodea:

1. Capillary stimulation: to elicit weak continuous pheromone stimulation, we used a capillary containing a cotton thread (2 cm long) loaded with 10^{-3} µg of either bombykol or bombykal (synthesized by H.-J. Bestmann, University Nürnberg-Erlangen). The tip of the capillary (diameter 40 µm) was positioned a few micrometers below the antennal preparation, within the horizontal permanent airstream, and the pheromones were allowed to evaporate. To apply high doses of the inhibitor decyl-thio-trifluoropropanone (DTFP, synthesized by A. Guerrero, Barcelona), 10 µg were loaded onto a cotton thread positioned in a capillary and a short (200 ms) strong air puff was blown through the capillary over the sensillum (Kaissling, 1995).
2. Cartridge stimulation: to apply excitatory or inhibitory stimuli of intermediate strength, the chemicals were loaded on pieces of filter paper (1 cm^2 area) placed in glass cartridges (inner diameter 7 mm). During stimulation the continuous airstream was passed through the chemical-containing cartridge. Stimulus load per filter paper was: 0.1 µg bombykol, 100 µg DTFP, 5 mg and 50 µl (±)-linalool (Sigma) and 1-heptanol (Sigma).

The signals were amplified using a custom-made amplifier with a band pass DC to 2 kHZ. The unfiltered data were sampled off-line using the data acquisition program SuperScope (GW Instruments). The parameters measured under the influence of the different chemicals were the transepithelial potential, the transepithelial resistance, the nerve impulse frequency of the single ORNs and the amplitude of the elementary receptor potentials (ERPs). To measure the resistance of the preparation, an alternating current (0.2 V, 1 Hz) was injected into the sensillum (Kodadová and Kaissling, 1996). The amplitude of an ERP was defined as the difference between the transepithelial potential before the onset of the ERP and the minimum potential before the nerve impulse occurred (Figure 3A). Amplitudes of the ERPs eliciting one or two nerve impulses and separated by at least 100 ms, were measured. Groups of more than two nerve impulses were probably elicited by several overlapping ERPs; the amplitudes of such events were not measured. For statistical analysis, StatView (SAS Institute Inc.), Excel (Microsoft) and Prism (GraphPad Software) were used. Because of the non-Gaussian distribution of the data and great variability between the individuals, the nonparametric Wilcoxon paired signed rank test was used to compare values measured in the same animals before and after treatment.

Results

Both the G-protein activator NaF (20 mM) and the diacylglycerol analog DOG (0.1 mM) elicited nerve impulse

responses in a considerable proportion of male pheromone-sensitive ORNs and female general odorant-sensitive ORNs of *B. mori* (Figures 1 and 2; Table 1). The nerve impulse activation started ~1–3 min after the capillary containing the agents contacted the sensilla. In males, neither compound had any effect on the resistance of the preparation; they caused a slight, but significant decrease of the transepithelial potential (Table 2). Both agents significantly increased the nerve impulse frequency of both male ORNs (Table 2). The effects elicited by NaF and DOG in females were similar to those in males and resembled responses to weak continuous odorant stimulation (Figures 1 and 2).

In males, but not in the females, the nerve impulses were accompanied by small deflections of the transepithelial potential, similar to ERPs but with larger amplitudes of 1 mV or more. Typically, this effect was more pronounced after application of DOG than after application of NaF (Figure 3). Frequency distributions of the amplitudes of spontaneous ERPs of the bombykol cell, compared with ERPs elicited by NaF and DOG, showed that under application of the activators, in addition to ERPs with normal amplitude larger depolarizations occurred (Figure 4A,C). The mean ERP amplitude of the bombykol cell increased significantly after application of DOG, but not after application of NaF (Table 3).

The number of ERPs in the bombykal cell—either spontaneous or elicited by NaF or DOG—was lower than in the bombykol cell, which corresponds to the lower nerve impulse frequency measured in this cell type (Table 2). The distribution of ERP amplitudes in the bombykal cell was broader during application of DOG in comparison to spontaneous ERPs (Figure 4D). The increase of the mean ERP amplitude after application of DOG was in about the same range as in the bombykol cell, but was not significant due to the lower number of measurements (Table 3). Application of NaF had no apparent effect on the ERP amplitudes of the bombykal cell (Figure 4B; Table 3).

Before testing the effects of inhibitors on ORNs excited by NaF and DOG (Table 1), the modulation of responses to bombykol by (±)-linalool and 1-heptanol were tested. Of 62 tested ORNs of *B. mori* males, 54 (87%) were inhibited (Figure 5B) and eight (13%) excited by (±)-linalool. In all ORNs, the responses to bombykol were inhibited by (±)-linalool (Figure 5B,C). In two ORNs, (±)-linalool first caused excitation, but then, 40 min later, inhibition of the nerve impulse response (Figure 5D). After the end of (±)-linalool application, often off-responses were observed; these were stronger after previous pheromone stimulation (Figure 5C,D). In nine ORNs inhibited by (±)-linalool, 1-heptanol was also tested and had a strong inhibitory effect on the response to bombykol (Figure 5B); three ORNs were excited by (±)-linalool and 1-heptanol.

(±)-Linalool reduced the frequency of ERPs elicited by pheromone stimulation slightly in the bombykol cell (Figure 6A) and strongly in the bombykal cell (Figure 6B).

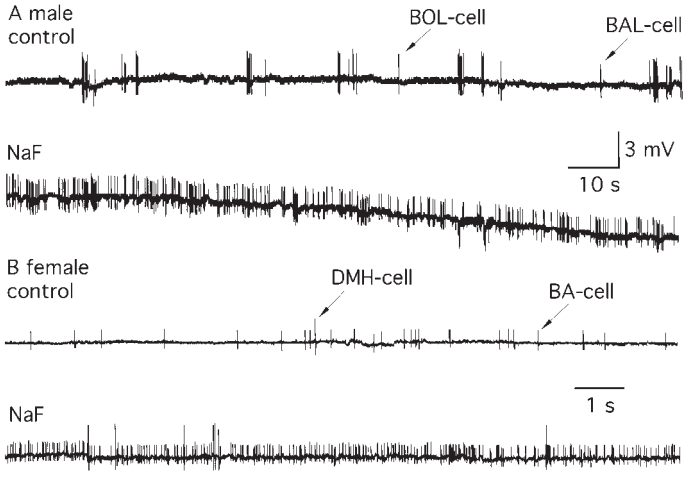

Figure 1 Excitation of ORNs of *B. mori* male **(A)** and female **(B)** by the G-protein activator sodium fluoride (NaF, 20 mM) applied for ~3 min via the recording electrode, in comparison to spontaneous activity recorded under sensillum-lymph Ringer solution (control). Arrows indicate the nerve impulses of the different types of ORNs responding best to bombykol (BOL-cell) and bombykal (BAL-cell) in males, and to 2,6-dimethyl-5-heptene-2-ol (DMH-cell) and benzoic acid (BA-cell) in females.

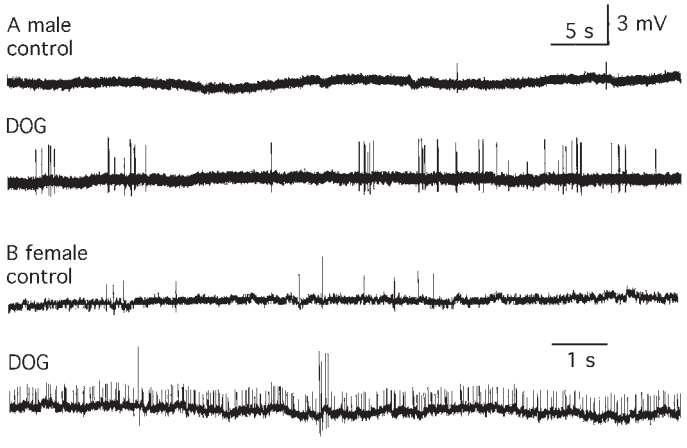

Figure 2 Excitation of ORNs of *B. mori* male **(A)** and female **(B)** by 1,2-dioctanoyl-sn-glycerol (DOG, 0.1 mM) applied for ~3 min via the recording electrode, in comparison to spontaneous activity recorded under sensillum-lymph Ringer solution (control).

During application of (±)-linalool, frequency distributions of the ERP amplitudes showed a shift towards lower values (Figure 6A,B), which was much more apparent in the bombykol cell. The average ERP amplitude decreased significantly in the bombykol cell, but not in the bombykal cell (Table 3). After the end of (±)-linalool application the amplitude and the frequency of ERPs returned to previous values (Figure 6A,B; Table 3). In the bombykol cell, the ERP frequency increased after the end of inhibition (Figure 6A).

The vapor of decyl-thio-trifluoro propanone (DTFP)

Table 1 Summary of the excitatory effects of sodium fluoride (NaF, 20 mM in sensillum-lymph Ringer solution) and 1,2-dioctanoyl-sn-glycerol (DOG, 0.1 mM in sensillum-lymph Ringer solution) and subsequent inhibitory effects of DTFP (100 μg, cartridge), (±)-linalool and 1-heptanol (both 50 μl, cartridge) on nerve impulse responses recorded from olfactory sensilla of *B. mori* males and females

Animals tested	DOG		NaF	
	15 males	1 female	15 males	4 females
Sensilla tested	29	8	33	4
Excited	23 (90%)	6 (75%)	21 (64%)	3 (75%)
Air (control)	13 tested: 13 no effect		8 tested: 8 no effect	
DTFP	5 tested: 5 no effect		8 tested: 8 no effect	
(±)-Linalool	10 tested: 10 inhibited		16 tested: 10 inhibited; 6 no effect	
1-Heptanol	5 tested: 5 inhibited		13 tested: 9 inhibited; 2 excited; 2 no effect	

Inhibition or excitation means a nerve impulse frequency decrease or increase, respectively, by at least a factor of two.

Table 2 Effect of sodium fluoride (NaF, 20 mM) and 1,2-dioctanoyl-sn-glycerol (DOG, 0.1 mM) applied via the recording electrode on the transepithelial resistance (*R*), transepithelial potential (TEP) and the nerve impulse frequency (SF) of the pheromone-sensitive ORNs of *B. mori* males

	Ringer	NaF	n	Sig.	Ringer	DOG	n	Sig.
R (MΩ)	266 ± 57	269 ± 82	9	n.s.	271 ± 61	258 ± 45	13	n.s.
TEP (mV)	35.97 ± 7.59	32.35 ± 8.17	18	**	31.78 ± 9.01	27.68 ± 9.44	22	**
SF BOL (imp./s)	0.19 ± 0.29	2.6 ± 2.11	9	**	0.14 ± 0.26	1.34 ± 1.45	18	**
SF BAL (imp./s)	0.04 ± 0.06	0.98 ± 1.13	5	*	0.02 ± 0.03	0.97 ± 1.36	15	**

BOL, bombykol cell; BAL, bombykal cell; *n*, number of sensilla tested. The parameters were measured after application of the chemicals for 3–5 min; nerve impulse frequency was monitored for 1 min. Means ± SD are given. Sig., significance: $*P < 0.05$; $**P < 0.01$; n.s., not significant (Wilcoxon nonparametric paired signed rank test).

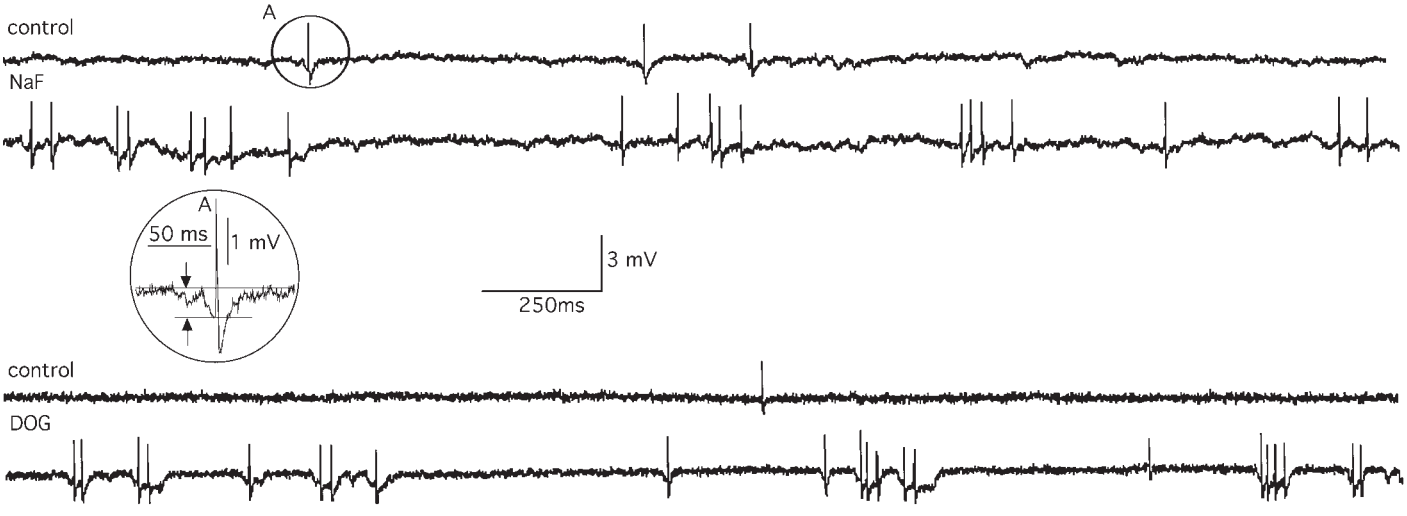

Figure 3 Spontaneous ERPs recorded under sensillum-lymph Ringer solution (control) and ERPs elicited by NaF (20 mM) and DOG (0.1 mM) in *B. mori* males. As an inset **(A)**, an enlarged ERP with nerve impulse is shown. Arrows indicate the amplitude of the ERP.

inhibited nerve impulse responses elicited in ORNs of *B. mori* males by pheromone stimulation (Figure 7); the frequency of the pheromone-elicited ERPs was strongly reduced in both male ORNs during application of DTFP (Figure 6C,D). Neither the distribution of ERP amplitudes (Figure 6C,D) nor their average amplitude (Table 3) were modulated by application of DTFP.

Responses elicited by NaF or DOG were clearly not

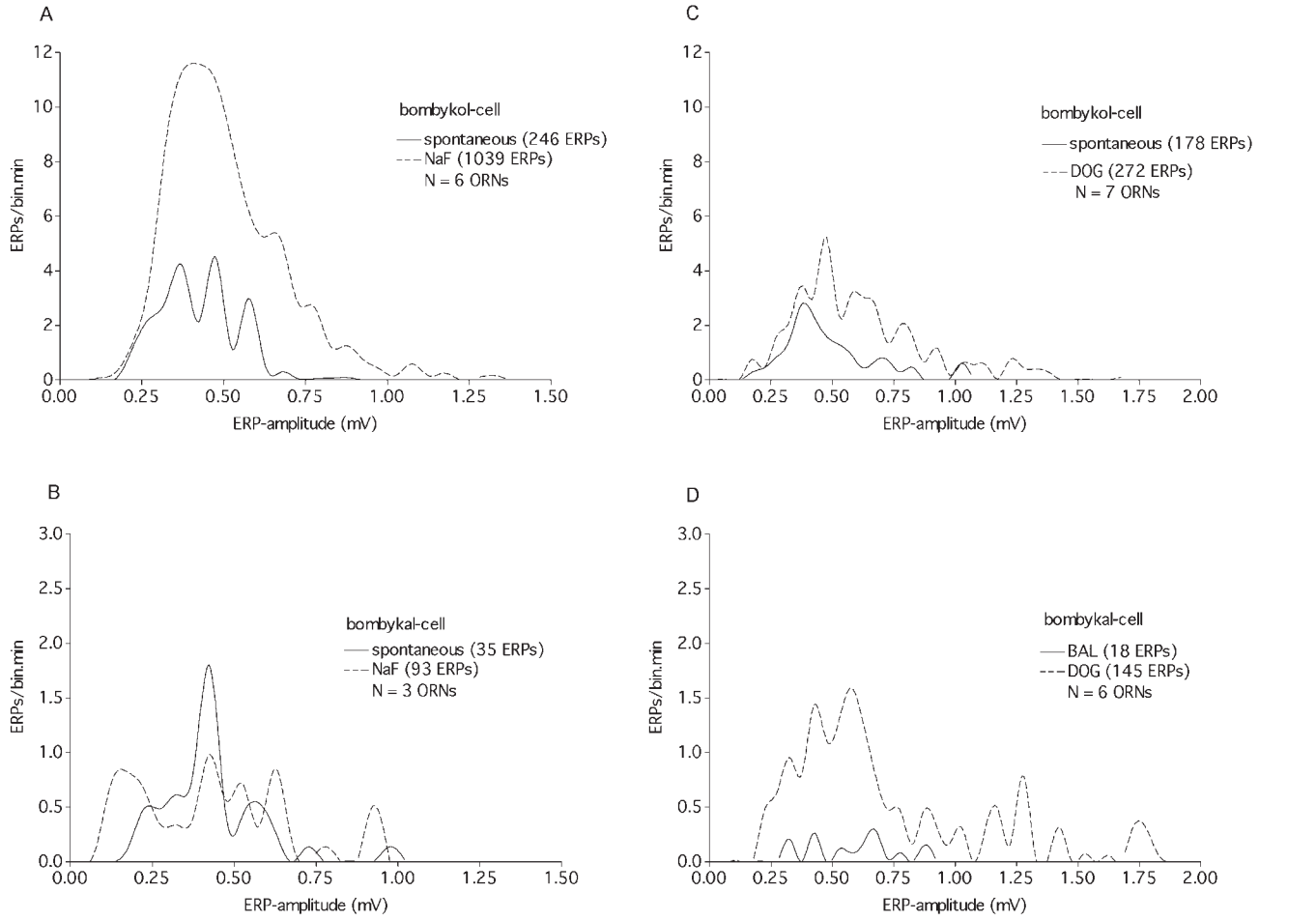

Figure 4 Distribution of the amplitudes of ERPs in the bombykol cell **(A, C)** and bombykal cell **(B, D)** of *B. mori* males occurring spontaneously under sensillum-lymph Ringer solution and elicited by sodium fluoride (NaF, 20 mM, A, B) or 1,2-dioctanoyl-sn-glycerol (DOG, 0.1 mM, C, D). Relative frequencies of ERPs per bin (0.05 mV) were determined for each ORN, averaged and then scaled according to the average number of ERPs per minute measured in the same set of ORNs; n = number of ORNs tested. For different ORNs, the recording time varied from 1 to 9 min and the frequency of ERPs from 1 to 30 per min. Total number of ERPs evaluated (not corrected for cell activity and recording duration) is given in brackets.

Table 3 Amplitudes of the ERPs (mean ± SD) occurring spontaneously (under sensillum-lymph Ringer) and under the influence of the various chemicals

	Bombykol cell (mV)	n	Figure	Bombykal cell (mV)	n	Figure
Spontaneous	0.42 ± 0.07	6	4A	0.36 ± 0.22	3	4B
NaF	0.49 ± 0.12			0.41 ± 0.19		
Spontaneous	0.48 ± 0.15	7	4C	0.58 ± 0.17	6	4D
DOG	0.64 ± 0.14*			0.73 ± 0.23		
Pheromone	0.63 ± 0.14			0.43 ± 0.06		
Pheromone + (±)-linalool	0.53 ± 0.09*	7	6A	0.42 ± 0.05	2	6B
Pheromone after (±)-linalool	0.58 ± 0.07			0.48 ± 0.07		
Pheromone	0.56 ± 0.12	6	6C	0.59 ± 0.18	6	6D
Pheromone + DTFP	0.57 ± 0.15			0.57 ± 0.11		

NaF, sodium fluoride (20 mM); DOG, 1,2-dioctanoyl-sn-glycerol (0.1 mM); pheromone, bombykol or bombykal (10^{-3} μg); DTFP, decyl-thio-trifluoro propanone (100 μg); (±)-linalool (50 μl). Calculated from same data as the frequency distributions shown in Figures 4 and 6. Average ERP amplitudes were first calculated for each ORN and then averaged between the ORNs; n, number of ORNs. *Significant effect of a chemical ($P < 0.05$, Wilcoxon paired signed rank test). Note the variability between the experiments (different sets of animals).

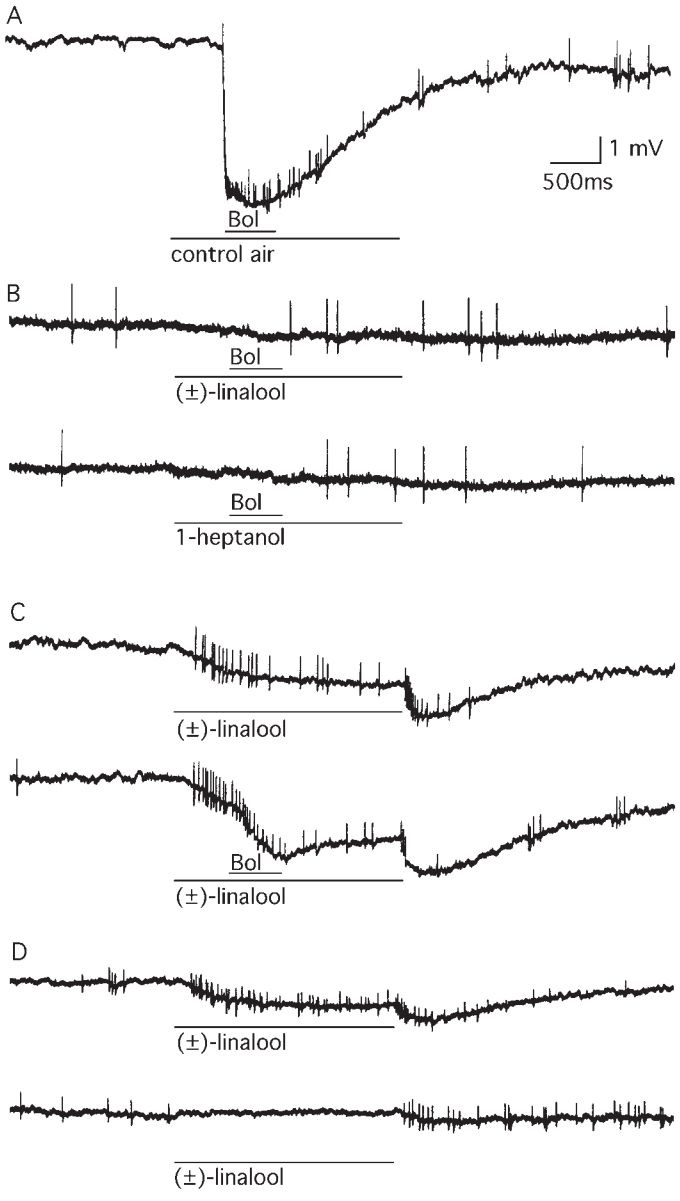

Figure 5 Effect of (±)-linalool on ORNs of *B. mori* males. **(A)** Response to bombykol presented over a background air current (control). **(B)** Inhibition of the response to bombykol by (±)-linalool and 1-heptanol. **(C)** (±)-Linalool excited this ORN, but inhibited the response to bombykol. **(D)** Excitation and inhibition by (±)-linalool recorded in one individual ORN within 40 min. Traces under (A) and (C) are from the same sensillum. Stimulus load (cartridge) 0.1 μg bombykol, 5 mg (±)-linalool or 1-heptanol.

inhibited by DTFP (Table 1; Figure 7); however, they were inhibited by the application of (±)-linalool or 1-heptanol (Table 1; Figure 8b–e). In a few cases of NaF-elicited nerve impulses, these compounds had no effect; in two sensilla treated with NaF, 1-heptanol even activated nerve impulse responses (Table 1; Figure 8f). DOG-elicited nerve impulses were inhibited, in all tested cases, by both (±)-linalool and 1-heptanol (Table 1).

Discussion

The olfactory transduction cascade in moths starts with the activation of receptors by odorants. The resulting activation of G-proteins leads to the activation of PLCβ, followed by equimolar production of IP_3 and diacylglycerol. There is experimental evidence that both of them might open ion channels (Stengl *et al.*, 1999)—see Figure 9. Here, the G-protein activator NaF and the diacylglycerol analog DOG were used to activate the transduction cascade in both male and female silkmoth ORNs. Their effects were already known for the male pheromone-sensitive ORNs (Laue *et al.*, 1997; Maida *et al.*, 2000) and here they have been demonstrated for the first time in the female general odorant-sensitive ORNs (Figures 1 and 2). Furthermore, the class of G_q-proteins was found in both male and female antennal homogenates (Laue *et al.*, 1997). We conclude that in moths the same transduction cascade is involved in pheromone reception and in general odorant reception. This contrasts with the situation in mammals, where the IP_3/diacylglycerol-dependent transduction cascade is involved in pheromone reception in the vomeronasal organ, but cyclic nucleotides transduce the reception of general odorants in the main olfactory epithelium (Zufall and Munger, 2001).

DTFP, which inhibited responses to pheromone, did not inhibit nerve impulse responses elicited by DOG or NaF. This supports previous evidence (Pophof *et al.*, 2000) that DTFP inhibits an earlier step of the transduction cascade, the activation of the pheromone receptor molecules (Figure 9). DTFP did not modulate the amplitude of pheromone-induced ERPs, but strongly reduced their frequency. ERPs are thought to represent a small number of ion channel openings driven by the activation of single odorant receptor molecules (A.V. Minor and K.-E. Kaissling, personal communication). DTFP may merely reduce the number of activated receptor molecules.

In contrast to DTFP, (±)-linalool reduced not only the number but also the amplitude of ERPs in the bombykol cell. Furthermore, the nerve impulse discharges elicited by DOG and NaF were inhibited by both (±)-linalool and 1-heptanol. This suggests that the mechanism of action of these odorants differs from that of DTFP. They might interfere with the lipids of the plasma membrane and reduce the number of ion-channel openings per ERP, and they could affect ion channels directly (Figure 9), as is known for ion channels involved in olfactory and visual transduction of vertebrates (Kawai and Miyachi, 2000).

In *B. mori* females, at much lower doses as used here for inhibition, (±)-linalool excited strongly and 1-heptanol weakly the ORN specialized to 2,6-dimethyl-5-heptene-2-ol; the responses to (±)-linalool seem to consist of an excitatory and an inhibitory component (Van der Goes van Naters, 2001). This could indicate simultaneous receptor activation and ion channel inhibition by (±)-linalool in female ORNs. The off-effects observed after the end of (±)-linalool

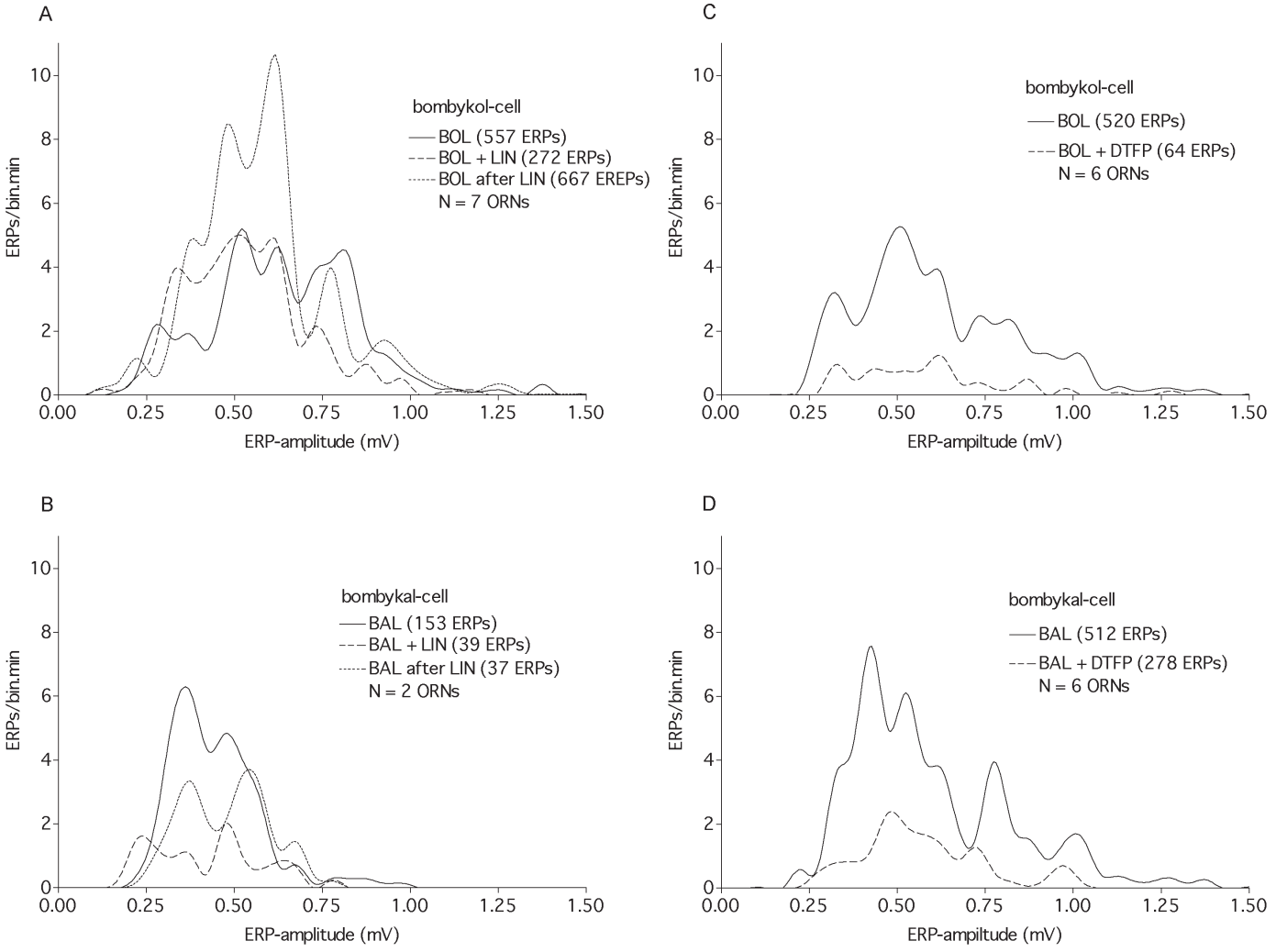

Figure 6 Distribution of the amplitudes of ERPs in the bombykol cell **(A, C)** and bombykal cell **(B, D)** of *B. mori* males elicited by weak continuous pheromone stimulation (bombykol or bombykal, 10^{-3} µg, evaporation from capillary) before, during and after the end of inhibition by (±)-linalool (50 µl, cartridge; A, B) and before and during inhibition by DTFP (10 µg, capillary; C, D); *n* = number of ORNs tested. For different ORNs, the recording time varied from 1 to 7 min and the frequency of ERPs from 6 to 216 per minute. For explanation of the evaluation see Figure 4.

application in male ORNs could be explained by a combined excitatory and inhibitory effect, where the inhibition might have a shorter time-course (Stange and Kaissling, 1995).

In the silkmoth *Antheraea pernyi*, inhibitory effects of geraniol on pheromone-sensitive ORNs were described (Schneider *et al.*, 1964). Other workers (Kaissling *et al.*, 1989) found in *B. mori* that ~70% of the bombykol-sensitive ORNs were inhibited, 10% excited and 20% unaffected by (±)-linalool. This corresponds well with our experiments, where 87% of the ORNs responding to bombykol were inhibited and 13% excited by (±)-linalool. These observations show an unexpected inhomogeneity of pheromone receptors. There are even time-dependent variations, since some ORNs were first excited and later inhibited by (±)-linalool (Figure 5D). Furthermore, after excitation of nerve impulse responses by the G-protein activator NaF, male ORNs were usually inhibited but in a few cases excited

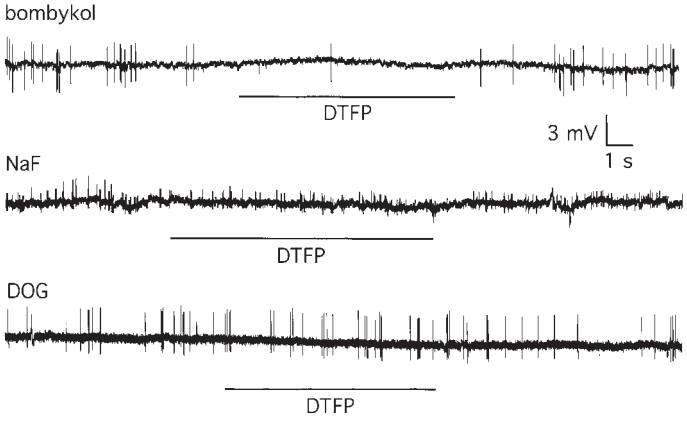

Figure 7 Decyl-thio-trifluoro-propanone (DTFP, 100 µg, cartridge) inhibited the nerve impulse response of a male ORN to weak continuous bombykol stimulation. DTFP did not inhibit nerve impulses elicited by sodium fluoride (NaF, 20 mM) or 1,2-dioctanoyl-sn-glycerol (DOG, 0.1 mM) applied via the recording pipette.

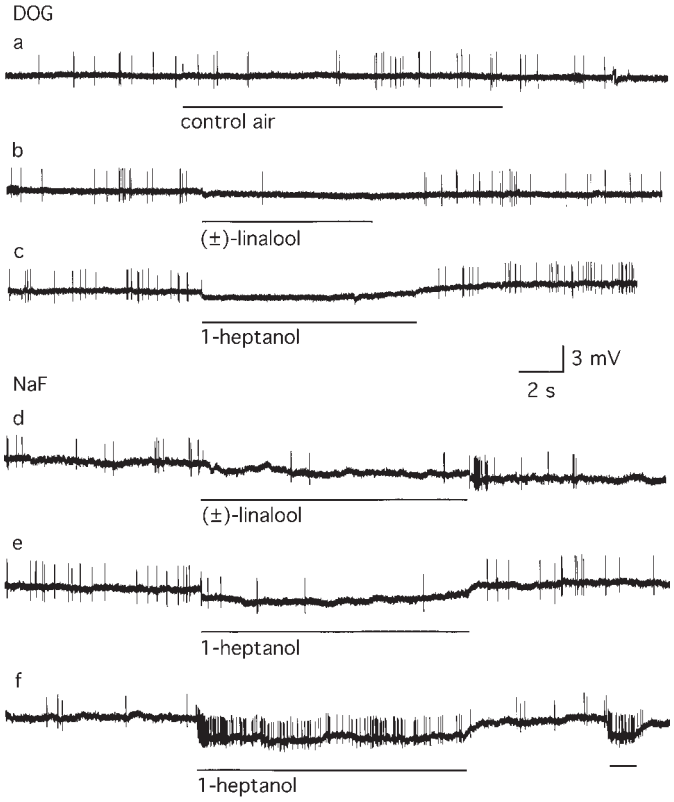

Figure 8 (±)-Linalool **(b)** and 1-heptanol **(c)** (50 µl, cartridge) blocked the nerve impulse responses elicited in the ORNs of *B. mori* males by application of 1,2-dioctanoyl-sn-glycerol (DOG, 0.1 mM, **a–c**) via the recording electrode; a charcoal-filtered airstream **(a)** had no effect. If nerve impulses were elicited by sodium fluoride (NaF, 20 mM, **d–f**), (±)-linalool **(d)** and 1-heptanol **(e)** had inhibitory effects in most cases (see also Table 1); in some ORNs, however, excitation by 1-heptanol **(f)** was observed after NaF application. Recordings originate from three sensilla: a–c, d–e, f.

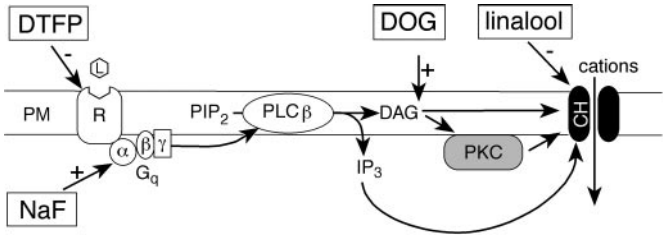

Figure 9 Schematic drawing of the olfactory transduction cascade, as it is expected to operate in insects, modified from earlier work (Steinbrecht, 1999). The ligand (L) activates the receptor (R) coupled to a G-protein (G_q) and the activated G-protein regulates phospholipase Cβ (PLCβ). PLCβ metabolizes phosphatidylinositol-4,5-bisphosphate (PIP_2) to inositol-1,4,5-trisphosphate (IP_3) and diacyglycerol (DAG). DAG and IP_3 activate ion channels (CH) via different pathways. The expected mode of action of the agents investigated in the present paper is indicated by arrows (+ excitation; – inhibition). Sodium fluoride (NaF) was used to activate the G-protein and 1,2-dioctanoyl-sn-glycerol (DOG) mimicked the activation by DAG. Decyl-thio-trifluoro-propanone (DTFP) inhibited responses to pheromones, but did not affect activation by NaF or DOG. Therefore, DTFP acts at an early step of the transduction cascade and probably blocks the pheromone receptors. (±)-Linalool and 1-heptanol inhibited the responses to pheromones as well as the activation of nerve impulses by NaF or DOG; therefore, they could inhibit ion channels.

by 1-heptanol (Table 1; Figure 8f). Such variable effects of (±)-linalool and 1-heptanol were observed only after excitation by pheromone or the G-protein activator NaF. If the transduction process was activated at a later step by DOG (Figure 9), both (±)-linalool and 1-heptanol always had an inhibitory effect (Table 2). It might be that (±)-linalool and 1-heptanol inhibit the diacylglycerol-dependent part of the transduction process, but do not affect the IP_3-dependent part, or even activate it in some cases.

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