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Inhibitory and Excitatory Effects of Iodobenzene on the Antennal Benzoic Acid Receptor Cells of the Female Silk Moth *Bombyx mori* L.

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Abstract

As shown in single-sensillum recordings, iodobenzene has a bimodal effect on the receptor cell tuned to benzoic acid (BA) of the female silk moth *Bombyx mori*. Exposure to iodobenzene causes an inhibition of the response to BA. With stimulation by iodobenzene alone, a reduction of basic nerve impulse firing during exposure is followed by a transient post-stimulus excitation (rebound). We suggest that inhibition suppresses excitation during exposure but fades afterwards more rapidly than excitation. Due to the spatial equivalence of the iodine and the acid residue, these effects might indicate opposing interactions of iodobenzene with the specific site for the key compound BA. This is supported by the fact that substitutions by smaller halogens are less effective in both inhibition and rebound. The inhibitory effect but not the rebound with iodobenzene alone was also observed in receptor cells tuned to key compounds other than benzoic acid, e.g. in the cell most sensitive to 2,6-dimethyl-5-heptene-2-ol (DMH-cell) occurring in the same sensillum as the BA-cell, or in the bombykol- and bombykal-cells of the male. At least in these cells the inhibitory effect might reflect the action of iodobenzene on a general site, e.g. the lipid matrix of the plasma membrane of the receptor cells.

Key words: aniline, benzoic acid receptor cell, *Bombyx mori*, inhibition, iodobenzene, olfactory receptor

Introduction

Insect olfactory receptors do not always respond in an excitatory manner to olfactory stimuli. Even the earliest recordings from single olfactory receptor cells in insects showed inhibitory as well as excitatory responses (Boeckh, 1962, 1967). In extracellular recordings from single sensilla, excitation, i.e. increased rate of nerve impulse firing, is usually accompanied by a decrease in the transepithelial potential (TEP; its change is called the receptor potential) and the transepithelial resistance, indicating depolarization of the receptor cell (Kaissling and Thorson, 1980). In contrast, inhibition of nerve impulse firing is accompanied by a positive receptor potential, detected as an increase of the TEP, indicating hyperpolarization of the cell. The presence of excitatory and inhibitory responses in the same receptor cell was regarded as a way of olfactory coding, especially when found in the so-called generalist cells.

Odor generalists are cells that may be found in sensilla basiconica and coeloconica and that respond to different but overlapping spectra of stimulus compounds. The same odorant can produce excitatory responses in one cell and inhibitory responses in another cell within the same sensillum (Schneider *et al.*, 1964; Schneider, 1969, 1984, 1992; Mustaparta, 1975; Selzer, 1984; Pophof, 1997; Dobritsa *et al.*, 2003; Hallem *et al.*, 2004). Inhibitory effects of general odorants were observed also in odor specialists, i.e. in cells specialized to a key compound and less sensitively responding to identical spectra of 'key-related' compounds. In moths, these cells are found in long-hair sensilla called sensilla trichodea. Examples of inhibitors for odor specialists are geraniol for pheromone receptors in the moth *Antheraea polyphemus* (Schneider *et al.*, 1964), (±)-linalool for the bombykol receptor cell of the male of the silk moth *Bombyx mori* (Kaissling *et al.*, 1989) or aniline for the receptor cell for

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benzoic acid (BA) of the female of *B. mori* (Priesner, 1979; Kaissling, 1987; Heinbockel and Kaissling, 1990; Ziesmann *et al.*, 2000; de Brito Sanchez, 1996). Interestingly, (±)-linalool, the above-mentioned inhibitor of a specialist pheromone receptor cell in the male of *B. mori*, strongly excites a cell occurring together with the BA receptor cell in the same sensillum of the female (Heinbockel and Kaissling 1996).

The cell sensitive to (±)-linalool [(±)-3,7-dimethyl-1,6-octadiene-3-ol] is somewhat more sensitive to the 'shortened' derivative of (±)-linalool, 2,6-dimethyl-5-heptene-2-ol (henceforth, DMH), and is therefore called DMH-cell. Thus the same compound [(±)-linalool] is a powerful excitatory stimulant of one cell type (DMH-cell) and an inhibitor of another cell type (bombykol-cell).

Here we study the effects of iodobenzene (I-benzene) on the response of the BA receptor cell of the female *B. mori*. To this end we extracellularly recorded receptor potentials and spike responses of the BA receptor cell under different regimes of chemical stimulation. I-benzene was chosen as it produces both excitation and inhibition in the BA-cell (de Brito Sanchez, 1997, 2000). We also study its effects on other odor specialists, the DMH-cell in the same sensillum, and the bombykol- and bombykal receptors of the male.

Studying the bimodal effects of iodobenzene, we hope to contribute to a better understanding of olfactory inhibition, especially of the cellular sites of inhibitory actions for inhibitory chemicals. We discuss our observations in the light of recent findings in *Drosophila* showing that the mode of response to various odorants (excitation or inhibition) depends on the receptor molecules (Hallem *et al.*, 2004).

Materials and methods

Adult females and males of *B. mori* were obtained from pupae from the Instituto Sperimentale per la Zoologia Agraria (Padova, Italy), the INRA Unité Nationale Séricole (La Mulatière, France) and Worldwide Butterflies (Sherbourne, UK). The pupae were segregated by sex and maintained at room temperature. Adult moths were kept in refrigerators at 12°C until they were used for experiments.

Stimuli

Isolated antennae of *B. mori* moths were stimulated with the following chemical substances (Figure 1):

1. Benzoic acid (BA), at stimulus loads of 1, 3.3, 10 and 100 μg per filter paper (f.p.). Acetone was used as a solvent for carrying out dilutions. It was evaporated before stimulation.
2. Linalool (3,7-dimethyl-1,6-octadiene-3-ol), at a stimulus load of 500 μg/f.p., diluted in paraffin oil.
3. Bombykol [(*E*,*Z*)-10,12-hexadecadiene-1-ol], at a stimulus load of 0.1 μg/f.p., with the solvent (hexane) evaporated.
4. Bombykal [(*E*,*Z*)-10,12-hexadecadienal], at a stimulus load of 0.1 μg/f.p., with the solvent (hexane) evaporated.

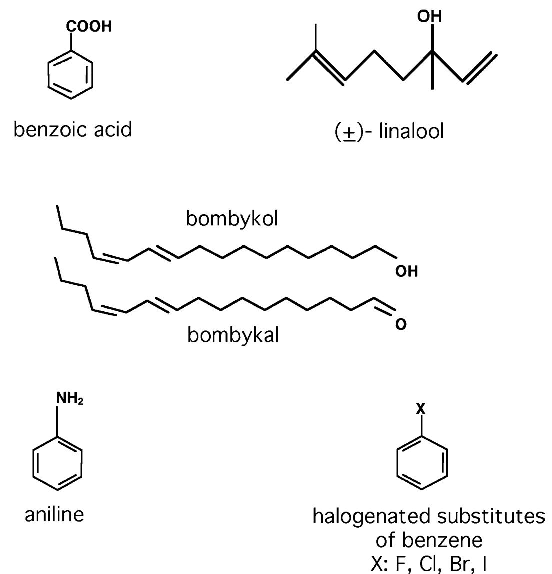

Figure 1 Chemical substances tested.

5. Aniline, at a stimulus load of 50 μl/f.p.
6. Undiluted halogen-substituted benzene (F-, Cl-, Br- and I-benzene), at a stimulus load of 50 μl/f.p.

Females of *B. mori* were stimulated either with BA + I-benzene, with (±)-linalool + I-benzene or with aniline + I-benzene, at the respective stimulus loads mentioned above. Males were stimulated with bombykol + I-benzene or bombykal + I-benzene.

Chemical substances were added to pieces of filter paper (7.2 × 14 mm), placed in small glass tubes (inner diameter 7 mm; length 5 cm) and mounted at a distance of 5 cm from the antenna. For simultaneous stimulations, two pieces of filter paper containing the key compound and the undiluted I-benzene, respectively, were placed one after the other in the glass tube.

Procedure

Briefly, transepithelial recordings were made from the cut tips of single sensilla trichodea, using glass capillaries with Ag–AgCl electrodes. Recording electrodes had a diameter at the tip of 10–15 μm (DMZ-Universal Microelectrode Puller, Zeitz Instruments, Augsburg, Germany). The reference electrode was filled with hemolymph ringer solution and the recording electrode with sensillum-lymph ringer solution [technique and solutions described by Kaissling (1995)].

Stimulation was carried out by means of an air current (100 ml/s) that passed through the glass tube (inner diameter 7 mm) with the stimulus and lasted 1 s if not stated otherwise. The duration of the stimulus was regulated by means of an electric valve. Between stimulations, the antennae were exposed to a current of clean air at the same speed but coming from a direction at 90° angle.

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For some substances, we established the dose–response curve by recording the response of the cell under study to increasing stimulus concentrations. The cell was first stimulated with all substances in their weakest concentration. Thereafter, a control stimulation with air alone was performed. Then, the cell was stimulated with all substances in the next concentration step. In this way, the concentration of the test substances was progressively increased with control stimulations interspersed between concentration steps. For each concentration step, stimuli were presented in the same sequence. Between stimulus presentations, filtered air was blown for 30 s towards the antennal preparation.

Electrophysiological responses were recorded with the help of a preamplifier (input resistance 10^{12} Ω), an oscilloscope (Tektronix), a paper recorder (Schwarzer) and a tape recorder (Racal). During recordings, responses were observed on an oscilloscope screen, while the occurrence of nerve impulses was monitored through speakers. Responses were evaluated using commercially available software (Superscope, GW Instruments).

We evaluated the cell response in terms of the frequency of action potentials (i.e. the number of nerve impulses during one-second stimulation). We used analysis of variance (ANOVA) for repeated measurements (Winer, 1971; Zar, 1984).

Results

The effect of halogenated substitutes of benzene on the response of the BA-cell

During stimulation with the larger halogen substitutes of benzene, the BA receptor cell showed a hyperpolarization with an absence of nervous impulses. This response was followed by a post-stimulus depolarization (rebound) and a transient increase in the rate of nerve impulses (Figure 2). Iodobenzene produced the strongest effects and was therefore used in further experiments. Figure 2a shows an example of the bimodal responses of a single BA-cell stimulated with the halogen-substitutes of benzene. Figure 2b shows the average impulse rates of BA-cells before, during and after stimulation. The control treatment and the stimulation with substitutes of smaller size (F-benzene and Cl-benzene) did not produce significant responses of the BA-cell, as no significant differences were found between the activity before, during and after stimulation [see Figure 2b; control: $F(2,4) = 0.23$, NS; F-benzene: $F(2,6) = 0.56$, NS; Cl-benzene: $F(2,10) = 2.60$, NS]. Stimulation with substitutes of larger size (Br-benzene and I-benzene) changed significantly the spike frequency of the BA-cell during and after stimulation (see Figure 2b; Br-benzene: $F(2,12) = 14.17$, $P < 0.001$; I-benzene: $F(2,14) = 88.92$, $P < 0.0001$]. For both Br- and I-benzene, inhibition during stimulus exposure and subsequent activation of the BA receptor cell could be observed.

Comparison of the responses of the BA-cell to I-benzene + BA and to BA alone

The BA receptor cell of the female of *B. mori* was stimulated simultaneously with I-benzene (50 μl/f.p.) and BA (1, 3.3, 10, 100 μg/f.p.), or with BA alone. Dose–response curves were obtained both for the stimulation period (Figure 3a) and for the post-stimulation period (rebound activity) (Figure 3b). The measurements are from two sensilla of two animals which gave exceptionally large responses.

During stimulation with 1–10 μg/f.p. of BA alone, the BA-cell showed excitatory responses (Figure 3a, black circles). In comparison, the responses to BA + I-benzene (white circles)

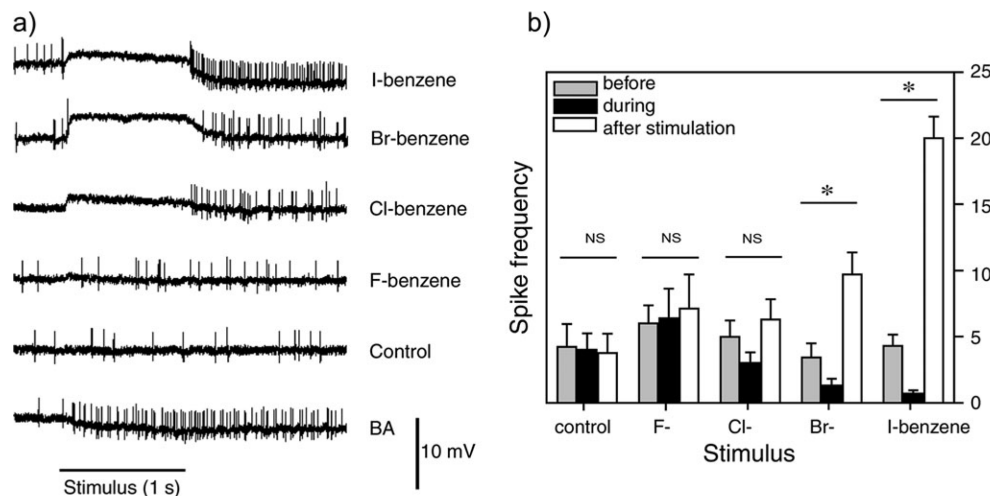

Figure 2 **(a)** Recordings from an individual sensillum trichodeum of the female *B. mori* antenna. The BA receptor cell responds to stimulation with halogenated substitutes of benzene (50 μl/f.p.) and to 10 μg/f.p. of benzoic acid (BA, bottom trace). Control stimulus with clean air. Substitutes of larger atomic size (Br and I) generated inhibition during stimulation followed by post-stimulus excitation (rebound). **(b)** Average spike frequency (number of nerve impulses per s ± SEM) for control (n = 3 cells; N = 2 individuals), F-benzene (n = 4; N = 2), Cl-benzene (n = 6; N = 3), Br-benzene (n = 7; N = 3) and iodobenzene (n = 8; N = 4), before (grey bars), during (black bars) and after stimulation (white bars). NS: non-significant effects; *$P < 0.05$.

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were significantly reduced, obviously due to inhibition by I-benzene. At a concentration of 100 µg of BA, the two response curves coincided, indicating a lack of inhibition at this BA concentration. During the post-stimulus phase (Figure 3b), the response to the combined stimulation was higher than the response to the stimulation with BA alone. Obviously, the rebound effect of I-benzene was added to the response to BA. When the two curves coincided at the load of 100 µg/f.p. of BA, the rebound was no longer visible.

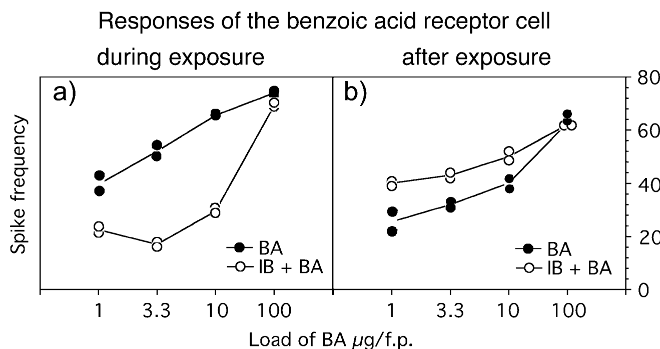

Figure 3 Dose–response curves of the BA receptor cell stimulated with BA alone (black circles) or with BA + iodobenzene (50 µl/f.p.) (white circles). Spike frequencies (number of nerve impulses per s) measured from two sensilla of two different animals. The load of BA per filter paper was 1, 3.3, 10 and 100 µg/f.p. **(a)** Responses during ones stimulation. **(b)** Responses during 1 s post-stimulation.

The effect of I-benzene on the response of other olfactory receptor cells

To determine whether the bimodal effect (inhibition followed by excitation) of I-benzene is a general phenomenon also observable in other types of olfactory receptor cells of male and female *B. mori,* or is specific to the BA receptor cell of the *B. mori* female, the DMH-cell of females (also known as the linalool cell) and the bombykol and bombykal cells of males were studied under similar conditions.

Figure 4a shows an example of the responses of the DMH receptor cell of *B. mori* females, which occurs together with the BA-cell in the same sensillum. Control (first trace, top) shows a basal activity of the BA-cell (small spikes) and a single spike of the mostly silent DMH-cell. The second trace shows a response of the BA-cell to I-benzene with a reduction of the basal spiking activity during exposure followed by the typical rebound. The DMH-cell, however, did not respond to I-benzene, either during or after stimulation. Note that the response pattern exhibited by the BA-cell to I-benzene was not so clear as that shown in Figure 2a (first trace) but was within the range of variability of the responses.

As expected, the DMH-cell was strongly activated by a stimulus load of 500 µg/f.p. of (±) linalool (Figure 4, third trace). During stimulation with (±)-linalool + I-benzene, the response of the DMH-cell was strongly but not fully inhibited (fourth trace). A rebound was visible for both cells: the typical rebound after I-benzene of the BA-cell (cf. second trace), and a brief rebound of the DMH-cell (five of the large spikes). The slow receptor potential during this double

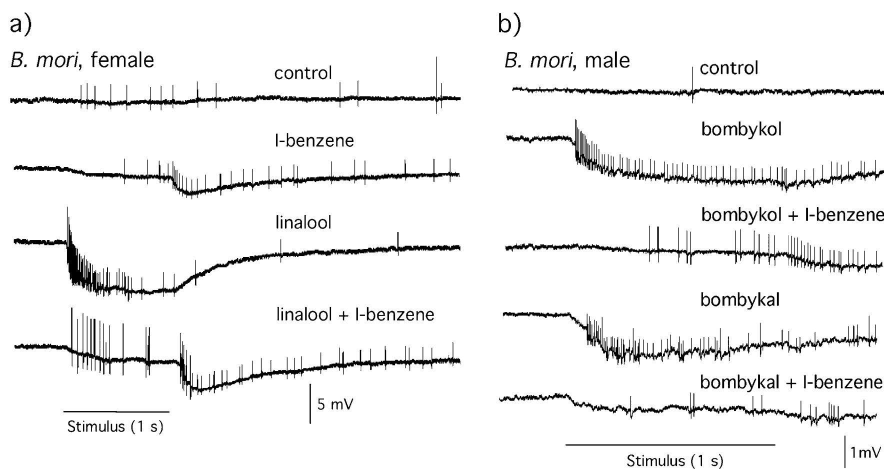

Figure 4 **(a)** Recordings from one individual sensillum trichodeum of the female *B. mori* antenna. Trace 1: basic activity of the BA receptor cell (small spikes). At the end a single large spike from the DMH receptor cell was fired. Trace 2: iodobenzene elicits a rebound activity in the BA receptor cell only. Trace 3: response of the DMH receptor cell upon stimulation by (±)-linalool (0.5 µl/f.p.). Note the progressive reduction of spike amplitude during the response, typical for strong responses. Trace 4: admixture of iodobenzene reduced the response to (±)-linalool. The rebound of the impulse response of the BA-cell was similar to that observed for iodobenzene alone (cf. trace 2); the added rebound of the DMH-cell (five large spikes) is also reflected in the amplitude of the receptor potential enlarged in comparison with I-benzene alone (trace 2). **(b)** Recordings from one individual sensillum trichodeum of the male *B. mori* antenna. The bombykol-cell produces larger spikes, the bombykal-cell smaller ones. The spike amplitude progressively reduces during the response of the bombykol-cell. Trace 1: control with clean air. The response to bombykol (trace 2) is largely inhibited by adding iodobenzene (trace 3), with a rebound of the bombykol-cell. Similarly the response to bombykal (trace 4) is inhibited by iodobenzene (trace 5), with a very weak rebound of the bombykal-cell. Note a few large spikes of the bombykol-cell (five in trace 4, one in trace 5). Iodobenzene alone does not produce a rebound in any of these cells (not shown).

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rebound was larger than the one of the BA-cell after I-benzene alone, indicating the additional depolarization of the DMH-cell. In summary, I-benzene also inhibits the DMH receptor cell whereas I-benzene given alone does not produce a rebound in this cell type.

Figure 4b shows examples of the responses of the bombykol and the bombykal receptor cells of *B. mori* males. As expected, both the bombykol and the bombykal cells were activated by bombykol and bombykal, respectively (stimulus loads: 0.1 μg/f.p.; second and fourth trace). Combined stimulation with I-benzene resulted in clear inhibition in both cases (third and fifth traces) with rebound, the latter being very weak in the depicted bombykal cell. As in the DMH-cell, I-benzene alone did not produce a rebound in these cells (not shown).

The combined effect of aniline and I-benzene on the response of the BA-cell

Aniline is an inhibitor of the BA receptor cell as it blocks the response to BA while aniline alone causes a small receptor potential sometimes eliciting a few nerve impulses of the DMH-cell (Kaissling, 1987). Simultaneous stimulation with I-benzene and aniline was performed in order to determine whether or not aniline also suppresses the rebound activity of the BA receptor cell elicited by I-benzene. The first trace (Figure 5) shows a nerve impulse response to a weak BA stimulus (equivalent to 0.1 μg/f.p.; see Heinbockel and Kaissling, 1996, their figure 8). This response was blocked by aniline, while aniline did not produce a rebound effect (second trace). The third and fourth traces show responses to I-benzene with clear rebound (cf. Figure 4a, trace two). The combined stimulation of the BA-cell with aniline and I-benzene (fifth and sixth traces) resulted in complete suppression of the weak BA-response during exposure and a clear reduction of the I-benzene rebound, both indicating the inhibitory effect of aniline (cf. traces three and four, without aniline).

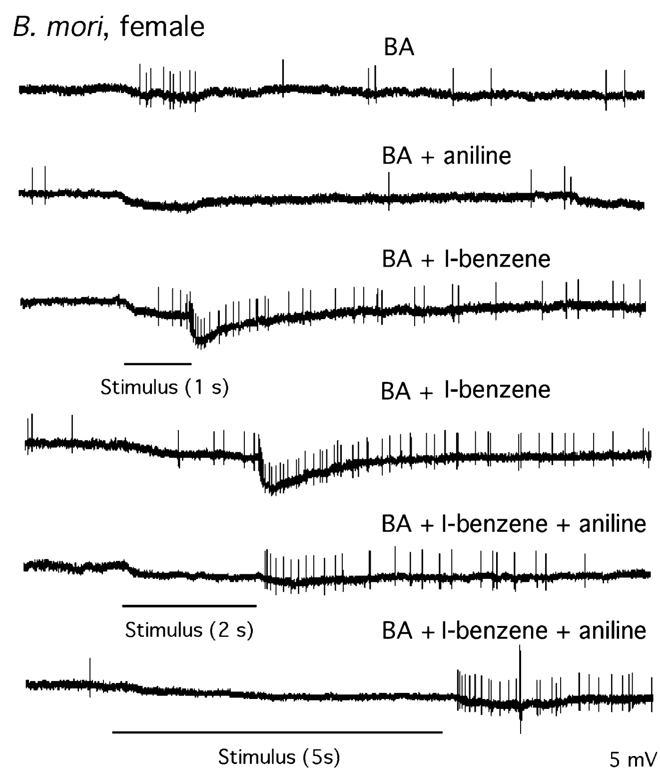

Figure 5 Recordings from one individual sensillum trichodeum of the female *B. mori* antenna. Trace 1: control stimulation with air containing a small concentration (equivalent to 0.1 μg/f.p.) of BA, producing a weak spiking response of the BA receptor cell. The same weak BA stimulation was used in traces 2–6. Trace 2: aniline inhibits the benzoic acid response, but without a rebound. Traces 3, 4: iodobenzene partially inhibits the benzoic acid response and produces a marked rebound. Traces 5, 6: aniline added to iodobenzene fully blocks the BA-response and reduces the rebound elicited by I-benzene. The DMH receptor cell is present, as indicated by one large spike in trace 6.

Discussion

The bimodal effect of iodobenzene on the BA receptor cell

We showed that olfactory stimulation of the BA receptor cell with halogenated substitutes of benzene generates a bimodal effect: inhibition during stimulation and post-stimulus excitation (rebound). These effects were not observed with F-benzene, hardly visible with Cl-benzene, significant with Br-benzene and maximal with iodobenzene. We interpret the bimodal effect as caused by different kinetics of excitation and inhibition. During exposure to these halogenated benzenes, inhibitory effects suppress excitatory ones. After the end of exposure the inhibitory effects fade more rapidly than the excitatory ones, so that the latter produce cell responses, receptor potential and nerve impulses.

Inhibition with poststimulus excitation was observed when general anesthetics and the key compound of an olfactory receptor cell were applied simultaneously (Stange and Kaissling, 1995). Here it was assumed that the inhibitory anesthetic (e.g. butane) evaporates rapidly, while the excitatory key compound (e.g. a pheromone) remains present in the sensillar lymph and is thus capable of stimulating the receptor cell for some time once the anesthetic had gone. These authors found that anesthetic and stimulant had to be applied at the same locus of the receptor cell dendrite in order to get inhibition. This shows that the sites for excitation and inhibition must be in close proximity, although they are probably not identical. Given their different chemical nature, it is unlikely that the anesthetic acts on the same site as the key compound.

In the case of halogenated benzene (iodobenzene discussed here), the same compound produces both excitation and inhibition, obviously with different kinetics. After stimulation, inhibition disappears at a faster rate than excitation, here not to explain by different evaporation rates. It seems inevitable

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to postulate opposing effects of iodobenzene with different kinetics of the termination, but not necessarily mediated by different sites of action.

Sites of action of iodobenzene

Inhibition and rebound caused by the iodobenzene stimulus of 50 μl/f.p. occurs over a wide range of concentrations of BA. Combined with the most intense BA-stimulus used (100 μg/f.p.; see Figure 3), iodobenzene neither inhibits nor produces a rebound. The lack of a rebound might be due to the fact that the excitation by BA reached saturation and no further increase of the response was possible. The lack of inhibition, however, might indicate that the key compound successfully displaced the halogenated benzene. This suggests competition of iodobenzene and BA at least in the site of inhibition.

Since iodobenzene presented alone did not produce (post-stimulus) excitation in any of the tested cell types (specialized to bombykol, bombykal or DMH) except the BA receptor cell, and since substitution with smaller halogens produced a weaker or no rebound, we suggest that the excitatory effect of iodobenzene on the BA-cell occurs via an agonistic interaction with the specific receptor site for benzoic acid. The iodine atom may occupy the recognition site of the BA carboxyl group.

It should be noted that iodobenzoic acid with iodine in any ring position was ineffective when applied to the female sensilla trichodea. It did not induce excitation, inhibition or a rebound effect, whereas substitutions with small halogens strongly excited the BA-cell. 3-Fluorobenzoic acid was even more effective than BA (de Brito Sanchez and Kaissling, 2005). This supports the notion of a precise spatial interaction at the BA receptor site for excitation of the BA-cell, as well as for its inhibition.

Interestingly, aniline does not produce a rebound if presented alone. It inhibits the excitation of the BA-cell whether the latter was elicited by BA (Kaissling, 1987; Heinbockel and Kaissling, 1990) or by iodobenzene (Figure 5). Thus aniline, too, could also act on the BA receptor site, but as an antagonist.

It seems tempting to interpret our results using the model proposed by Hallem *et al.* (2004) for excitation and inhibition of *Drosophila* olfactory receptor cells on the basis of extensive experiments with ectopic expression of receptor molecules in mutant receptor cells (Dobritsa *et al.*, 2003). Hallem *et al.* conclude that the receptor molecule exists in an active and an inactive conformation, the active one triggering the spontaneous nerve impulse firing via G-protein coupling (Figure 6). Some odorants bind to the active form and cause excitation by shifting the equilibrium between the two forms towards the active one. Other odorants bind to the inactive form, shifting the equilibrium in the other direction and thus inhibiting firing. In this model BA would bind to the active receptor, aniline would bind to the inactive form and

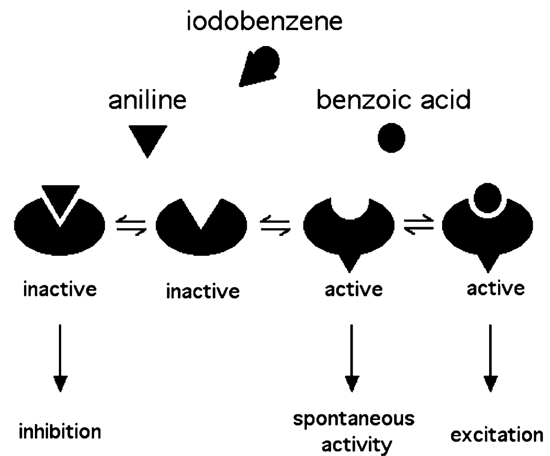

Figure 6 Model of excitation and inhibition of olfactory receptor cells, modified after Hallem *et al.* (2004). The key stimulus, BA, excites the cell, aniline inhibits it and iodobenzene acts bimodally. All three compounds might compete with each other at the same receptor site. The receptor molecule occurs in two conformations accessed differently or influenced by the various agents.

iodobenzene would bind to both forms. This would require differential interactions of the various agents with the two conformers or conformational differences in their binding sites. For the model of Hallem *et al.* (2004), one second-messenger pathway would suffice. This is different from a mechanism of excitation and inhibition found in crustacean olfactory cells by Hatt and Ache (1994), where two second messenger types (cAMP and IP_3) mediate opposite response modes within the same receptor cell. It is still unknown whether the two pathways are triggered by the same receptor molecule or separate ones.

It remains open as to which processes underlie the different kinetics of excitation and inhibition in the BA-cell, and other recptor cells. According to a model of Kaissling (2001), the kinetics of receptor cell responses are determined by the hypothetical deactivation of the odorant in the sensillum lymph rather than its binding to the receptor molecules. Odorant deactivation is necessary in flux detectors (Kaissling, 1998), where the stimulant accumulates in the perireceptor compartment during exposure. This could apply to inhibitors as well, except in cases where they are volatile and evaporate readily. Thus, for inhibitors that do not evaporate quickly, a deactivation process which could determine the kinetics of the inhibition seems necessary. It shoud be noted that benzoic acid adsorbed on the female antenna is rapidly metabolized to N-benzoylserine (Oldenburg *et al.*, 2001). Whether this process is fast enough to account for the termination of the response of the BA-cell remains to be shown.

Inhibition of olfactory receptor cells other than the BA-cell

The fact that, besides the BA-cell, other types of olfactory receptor cells—the DMH-cell (occurring in the same

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sensillum as the BA-cell), and the bombykol- and bombykal-cells—of the male were inhibited by iodobenzene indicates that the inhibitory site in these cells is relatively unspecific. In view of the structure of iodobenzene, it seems unlikely that it acts on the site of the key compounds other than BA. Conceivably iodobenzene could interfere with the lipid matrix of the cell membrane, as has been suggested for the action of general anesthetics (Stange and Kaissling, 1995). From our experiments it is not excluded that in the BA-cell iodobenzene or aniline act also on the lipid matrix, or on both targets.

Except in the BA-cell, iodobenzene alone did not produce a rebound in the various cell types. However, in all cases of inhibition by simultaneous exposure to iodobenzene and a key compound, there was a rebound of the cell due to excitation by the respective key compound (Figure 4). This means that also in the other three cell types the inhibition by iodobenzene disappeared faster than the excitation of the respective cell by its key stimulus. Different kinetics of excitation and inhibition of olfactory receptor cells elicited by various odorants was already described by Boeckh (1962). It remains to be shown which mechanisms are responsible for deactivation or removal of odorants as well as inhibitors.

Possible mechanisms of inhibition

In general, more than one site for the action of inhibitors in olfactory receptor molecules can be distinguished: the receptor molecule, the lipid matrix and the ion channels of the plasma membrane. A typical inhibitor acting on the site of the key compound is decyl-thio-trifluoro-propanone (DTFP), inhibiting various receptor cells tuned to straight-chain pheromones (Pophof *et al.*, 2000). Its peripheral action is supported by the finding that it does not inhibit nerve impulse firing elicited by administering NaF or 1,2-dioctanoyl-sn-glycerol (DOG), agents which act in the transduction cascade downstream of the receptor molecules (Maida *et al.*, 2000; Pophof and Van der Goes van Naters, 2002).

General odorants, such as geraniol, (+/−)-linalool and 1-heptanol, might excite or inhibit by acting on receptor molecules [e.g. (±)-linalool exciting the DMH-cell] but might in addition also interfere with other targets. For instance, in contrast to DTFP, (±)-linalool inhibits responses to NaF and DOG, suggesting direct effects on ion channels (Pophof and Van der Goes van Naters, 2002). Interestingly, (±)-linalool does not always have the same effect on the bombykol-cells in the male of *B. mori* (Kaissling *et al.*, 1989). Some of these cells did not respond to (±)-linalool, and some even responded with spiking or were excited first and inhibited later (Pophof and Van der Goes van Naters, 2002). This shows an unexpected inhomogeneity of the bombykol receptor cells.

Certain compounds inhibit but also damage the cell when applied at high concentrations. This is the case for many amines that inhibit pheromone receptor cells as well as other receptor cell types. Some compounds inhibit cells at low concentrations but excite them at higher doses. These compounds could reduce membrane conductance at low doses possibly by interference with the lipid matrix but could increase conductance at higher doses, thereby destabilizing the membrane. Stimulus recovery can be incomplete, indicating an irreversible damage to cellular function (Kaissling, 1977).

Finally, mutual inhibition of receptor cells innervating the same sensillum has been found to be important for coding pheromone blends (Nikonov and Leal, 2002). The mechanism of this inhibition, most likely an interaction between adjacent cells, still awaits clarification.

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