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Electrophysiological responses of gustatory sensilla of *Mamestra brassicae* (Lepidoptera, Noctuidae) larvae to three ecdysteroids: ecdysone, 20-hydroxyecdysone and ponasterone A

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Abstract

Specialised phytophagous Lepidoptera such as *Bombyx mori* and *Pieris brassicae* have contact chemoreceptors that perceive ecdysteroids at very low concentrations. This sensory perception allows them to feed on substrates with a high content of phytoecdysteroids. We have evaluated if a polyphagous insect like *Mamestra brassicae* does possess contact chemoreceptor cells that are sensitive to these molecules. Electrophysiological recordings were performed from contact chemoreceptors located on the maxilla. These receptors were stimulated with some sugars, amino acids and salts and with three ecdysteroids. Our results demonstrate that a specific cell within the lateral sensilla responds to 20-hydroxyecdysone and ponasterone A but not to ecdysone.

Keywords: Ecdysteroids; Gustation; *Mamestra brassicae*; Electrophysiology

1. Introduction

Phytoecdysteroids are secondary metabolites widely distributed in the plant kingdom. Their structures are closely related to those found in ecdysteroids involved in the regulation of the moulting process in arthropods (Lafont, 1997). Over 200 ecdysteroids occurring in plants are yet described; the most common are 20-hydroxyecdysone and polypodine B. Their concentrations vary over a wide range (up to 10^{-3} M) and about 6% of the plants contain high amounts of ecdysteroids (Imai et al., 1969; Dinan, 1995). In plants, the role of these metabolites is not clear (Jones and Firn, 1978; Sláma, 1993). The most generally accepted hypothesis is that they protect against attacks by phytophagous insects, either by disturbing development or reducing food intake (Bergamasco and Horn, 1985; Camps, 1991; Lafont, 1997).

Reduction of food intake in the presence of ecdysteroids has been reported only in *Pieris brassicae* (Ma, 1969) and *Bombyx mori* (Tanaka et al., 1994). These authors demonstrated that feeding inhibition was due to a specialised sensory perception rather than to a poisoning effect. Both species have specialised diets, and their host plants contain only low levels of ecdysteroids (*Morus alba*: Takemoto et al., 1967; Blackford and Dinan, 1997) while plants containing high level of ecdysteroids are numerous (Lafont, 1997). What happens in species with a less specialised diet and thus a greater chance of exposure to plants containing high amounts of ecdysteroids?

We have tried to answer this question by investigating the sensory perception to three phytoecdysteroids in the cabbage moth *Mamestra brassicae* L. This species is relatively tolerant of ecdysteroids in the diet (Tanaka and Naya, 1995). Food choice tests conducted with different dosages of 20E (Ma, 1972), have shown that *P. brassicae* larvae were avoiding food treated with 20E 2×10^{-4} M (feeding ratio of treated substrate vs control: 4%). At the same dosage, feeding was much less affected in *M. brassicae* (feeding ratio: 50%), while higher doses of 20E yielded a maximal feeding ratio of 40%.

These observations suggest that *M. brassicae* larvae

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have a specific perception of 20E. In order to confirm this hypothesis, we have studied taste perception by *M. brassicae* larvae using electrophysiological recordings. Taste receptors were stimulated with compounds found in the diet, like fructose, leucine and proline and with the following ecdysteroids: ecdysone (E), 20-hydroxyecdysone (20E) and ponasterone A (ponA). These compounds have the same structural framework and differ only in the number and position of the hydroxyl groups located on the sterol side chain. Our findings suggest that ecdysteroids perception is mediated by a gustatory neurone (deterrent cell) located in the lateral sensilla, and with a lower sensitivity than those of *B. mori* and *P. brassicae*.

2. Material and methods

2.1. Biological material

Insects originated from a laboratory supply, and were reared on artificial diet (Poitout and Bues, 1970). They were maintained in transparent plastic boxes at 20°C under 16 h, 8 h light/dark photoperiod and 70% relative humidity. Recordings were performed on starved larvae, which had recently moulted to the third larval instar.

2.2. Chemicals

20E, potassium chloride, fructose, leucine and proline were purchased from SIGMA-ALDRICH company. Ecdysone and ponasterone A were gifts from R. Lafont (Ecole Normale Supérieure). Compounds were diluted in 10^{-2} M potassium chloride. Ecdysteroids were used at concentrations ranging from 10^{-8} M to 10^{-3} M. All solutions were kept at 4°C for less than one month. Ponasterone A was diluted within a solution of 5% ethanol.

2.3. Electrophysiological recordings

Recordings were performed in larvae on the lateral and medial sensilla, located on the galea of the maxillary palps. A grounded silver electrode (0.8-mm diameter) was introduced in the cephalic cavity after separation of the abdomen. An electrode containing the test compound covered the tip of the sensilla to stimulate it. Operations were performed using a dissecting microscope (Leica Wild M10, France). Borosilicate glass electrodes (o.d. 2 mm, tip diameter about 20 μm) were made with a P77 electrode puller (Sutter Instruments Co., USA) and filled with the stimulating solution, just before recording. Electrophysiological activities of the sensilla were recorded in D.C. mode using a TastePROBE amplifier (Syntech, Holland; Marion-Poll and Van der Pers, 1996).

These potentials were amplified and filtered (CyberAmp 320, Axon Instrument, USA: gain: 1000–2000; Bessel filter; DC/0.1–4000 Hz). Data were recorded and stored on a computer with a data acquisition card (DT 2821, Data Translation, 12 bits precision, sampling rate: 10 kHz) using a custom program, ATLSPK (Marion-Poll and Tobin, 1991). Each recording lasted 2.5 s and was triggered by a pulse delivered by TastePROBE on the initial contact of the electrode with the sensilla. Under these conditions, the background noise was 200 μV peak-to-peak.

The corresponding data files were analysed with custom software, Awave (Marion-Poll, 1995, 1996). Spikes were detected after filtering the original data with a low-pass filtered derivative (Marion-Poll and Tobin, 1991). They were sorted when possible, by measuring the amplitude of each spike or according to their shape. However, the signal to noise ratio of these recordings did not allow this sorting with a complete accuracy. The results were analysed with a spreadsheet (Excel, Microsoft™). Responses were quantified by the number of spikes elicited during the first second of each recording. The response shape was evaluated by post-stimulus histograms, computed by counting the number of spikes occurring in consecutive bins of 50 ms.

Each set of recordings was performed by stimulating the gustatory sensilla of the larvae first by standard stimuli, followed by increasing concentrations of the tested phytoecdysteroids. Each stimulus was applied twice, keeping a time interval of about one minute between successive stimulations. All the stimulations were done on ten larvae per phytoecdysteroid. A total of 835 recordings were made.

3. Results

Under our experimental conditions, the medial and lateral gustatory sensillae of *M. brassicae* responded to stimulatory substances, by a downwards D.C. shift reaching 100–300 mV, similar to a receptor potential. This parameter was not taken into account in our analysis. Responses were evaluated according to the total number of action potentials elicited during the stimulating period, and to the shape of the spikes or the distribution of their amplitudes.

The total number of detected action potentials is given in Fig. 1. Since all test substances were diluted in 10^{-2} M KCl, this solution served as a control. Two sets of stimulations stand out: responses to 20E from the lateral sensilla for two concentrations (10^{-4} M and 10^{-3} M) and the reaction to PonA for the 10^{-4} M (this compound is not entirely soluble in the 5% ethanolic solution at 10^{-3} M). In contrast, the medial sensilla did not respond to either ecdysteroids. None of the cells responded to E over the range of concentrations tested.

Such an analysis does not take into account the temporal parameters of the response. Responses to 20E and

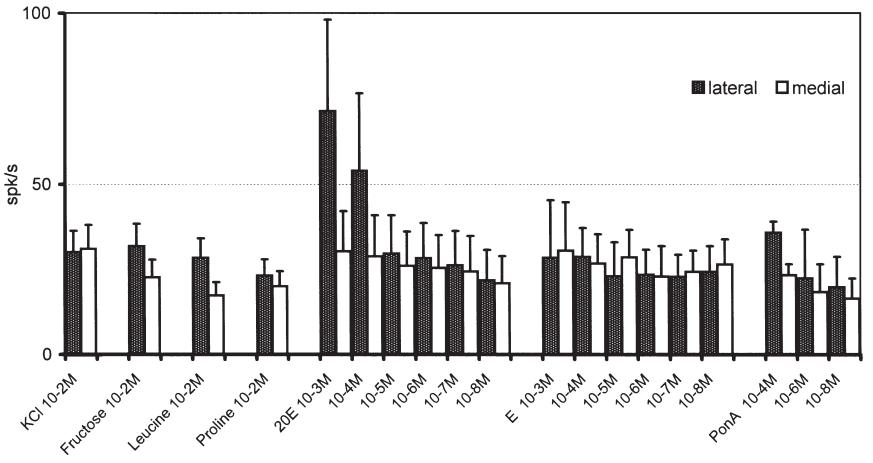

Fig. 1. Responses of gustatory sensilla stimulated by different chemicals. Abscissa: chemicals and respective concentrations. Ordinates: response amplitudes. Dark bars: lateral sensilla, open bars: medial sensilla. Each bar represents the average number of action potentials detected during 2.6 s (expressed in spikes/s; *n*=10–20 recordings). Vertical lines show the standard error to the mean. Abbreviations: 20E, 20-hydroxyecdysone; ponA, Ponasterone A; E, ecdysone.

PonA started after a concentration-dependent delay, and slowly decayed over the stimulation period. Responses to the other compounds, KCl, fructose and the two amino acids, were distinctly more phasic, with a burst of spikes during the first 200 ms followed by a slow decay (Fig. 2).

A more accurate analysis of the data indicates that the extracellular action potentials recorded originate from different cells. Three criteria support this hypothesis: the distribution of the action potential amplitudes, the time course of the responses and the presence of overlapped action potentials such as depicted in Fig. 3. In this study, we measured the amplitude of each spike and computed their frequency distribution. If the nervous activity is due to a single cell category, these values are distributed around a single mode. If the activity is due to different cells with different amplitudes, the frequency distribution shows two modes.

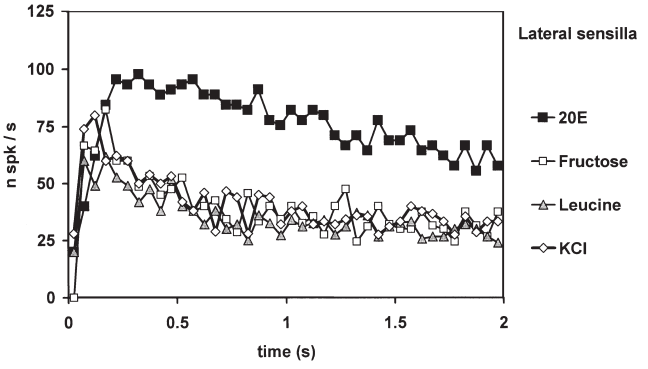

Fig. 2. Post-stimulus histograms (PSTH) obtained in response to a series of compounds on the lateral sensilla. Abscissa: time (s). Ordinates: average number of action potentials recorded during consecutive 50 ms bins (*n*=10–20 recordings). Fructose, KCl and leucine 10^{-2} M elicit a phasi-tonic response. 20E responses are tonic and start after a 50–100 ms delay at 10^{-3} M.

In the medial sensilla, spike amplitudes were distributed along two overlapping classes of amplitudes, which means that two different cells were generally active during the stimulations. In the lateral sensilla, the responses to KCl, proline, leucine and fructose were bimodal, with a major mode at 0.5 mV and a second at 0.75 mV. When stimulated with increasing concentrations of 20E, spikes with higher amplitude were elicited. Their contribution increased with the concentration, with a mode of 0.86 mV at 10^{-3} M (Fig. 4). Similar results were obtained with PonA. It is not clear if the large amplitude cell firing in presence of KCl (or at low concentrations of 20E) is the same that responds to 20E at 10^{-4} and 10^{-3} M. At the same time that the contribution of this cell increased, the activity of the small spike cell apparently decreased (Fig. 4). Given the high firing rate of the large cell at 10^{-3} M, this observation might represent a bias in the detection method.

These observations indicate that large action potentials originate from one cell, specifically stimulated by 20E or PonA, but not by E. In the recordings where the action potentials originating from this large cell could be separated by amplitude criteria, we analysed their responses separately [Fig. 5(a)]. The activity of this cell increased with the concentration of 20E, and was maintained during the 2.5 s of the recordings [tonic response, Fig. 5(a)]. The activity of the other cells was high in the absence of 20E (50–25 spikes/s), with an initial burst of activity followed by a rapid decay (phasic response). The firing activity of these cells was depressed in the presence of 20E (20–5 spikes/s). In contrast, the nerve cells housed within the medial sensilla responded phasically to the presentation of the stimuli and the firing level was not affected by the presence of 20E [Fig. 5(b)].

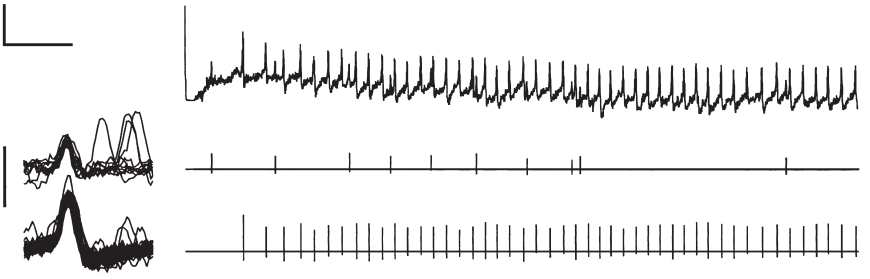

Fig. 3. Sample recording from a lateral sensilla stimulated by 20E at 10^{-3} M. Action potentials detected from the raw data (top row) could be separated in two classes, which have a consistent amplitude and shape. Middle and lower rows show respectively the shape of the spikes (superimposed on the left side) and the activity of each cell. Sometimes, the two cells fire almost simultaneously. Top row scale: vertical bar=2 mV, horizontal bar=50 ms, duration shown=500 ms; middle and lower row scale: vertical bar=1 mV; spike window=6 ms.

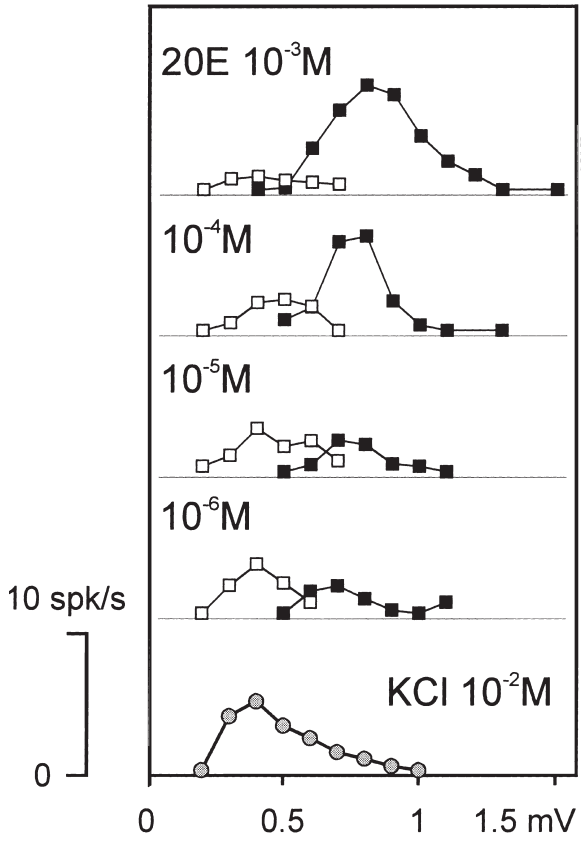

Fig. 4. Distribution of the spike amplitudes according to stimulus and spike class in the lateral sensilla. Abscissa: spike amplitude (100 μV bins ranging from 0 to 1.5 mV). Ordinates: number of spikes/s. Each dot represents the average number of spikes/s within the corresponding amplitude bin (*n* records=10). Solid squares=large spikes; open squares=small spikes; grey circles=KCl spikes. Large and small spikes were separated by means of amplitude threshold (see text). No separation was attempted for spikes elicited with KCl.

4. Discussion

In this study, we have investigated the response of medial and lateral gustatory sensilla to molecules similar to the moulting hormones of arthropods. These responses were compared with those obtained for compounds found in the insect diet.

Medial and lateral sensilla have different sensitivity profiles and kinetic responses. The main difference concerns the phasic character of the response recorded on the medial sensilla. Our results clearly demonstrate that the lateral sensilla specifically respond to 20E and PonA. None of the sensilla responded to E over the whole range of concentrations tested. This result is surprising if we consider the structural similarities between these compounds. The presence of a hydroxyl group in C-20 (present in 20E and PonA but absent in E) is necessary to stimulate the gustatory cells. The hydroxyl in C-25 (present in 20E but not in PonA) seems to play a role, if we consider the increase of effectiveness observed with 20E over PonA.

Analysis of the recordings indicates that responses were due to the activity of several nervous cells with action potentials of different amplitudes. The small ones were associated with cells responding to amino-acids, fructose and KCl in both lateral and medial sensilla. The larger action potentials originated from an additional cell, located in the lateral sensilla, responding to 20E and PonA. This cell has also distinctive properties like the latency of the responses at the onset of the stimulation (Fig. 3) and its tonic character [Fig. 5(a)]. These criteria are not found in the cells responding to amino acids and fructose. The functional characteristics of this cell meet the criteria of the deterrent cell, as proposed by Peterson et al. (1993) in *Manduca sexta*. This hypothesis is compatible with the behavioural observations of Ma (1972), who found that diets containing 20E at 2×10^{-4} M inhibit the feeding behaviour of *M. brassicae*.

Up to now, perception of 20E by a deterrent cell has been found in two oligophagous or monophagous Lepidoptera species: *P. brassicae* (Ma, 1972), and *B. mori* (Tanaka et al., 1994). In these two species, the perception threshold of 20E by a deterrent cell was lower (10^{-5} M for *P. brassicae*, 10^{-6} M for *B. mori*), and associated with an inhibition of the food intake. Mulberry leaves, *Morus alba*, apparently contain 20E at concentrations too low (0.25 mg/kg fresh weight) to inhibit feeding behaviour or to intoxicate *B. mori* (Takemoto et al., 1967).

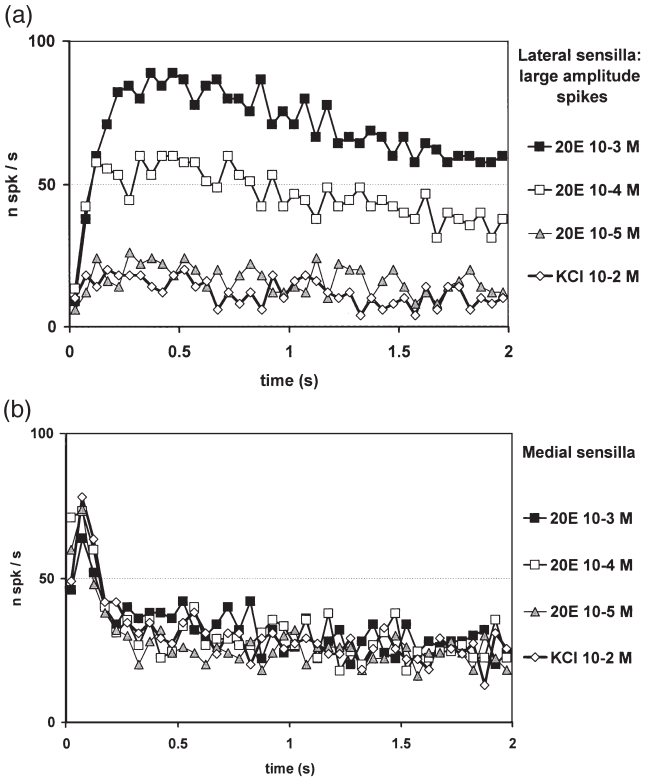

Fig. 5. PSTH in response to 20E and KCl. Abscissa: time (s) after the initial contact (bins=50 ms). Ordinates: average number of spikes detected. (a) Lateral sensilla (large amplitude spikes only). Action potentials were separated on the basis of their amplitude and only those of large amplitude were kept. (b) Medial sensilla. Responses exhibit a marked burst of activity during the first 100–200 ms followed by a tonic activity.

Although such a perception could allow Lepidoptera larvae to avoid plants containing high phytoecdysteroids levels, the picture remains unclear in the case of *M. brassicae*. In laboratory conditions (Tanaka and Naya, 1995), this species has been shown as tolerant to diet-containing ecdysteroids probably because it detoxifies these molecules into apolar metabolites, like many other Noctuidae (Robinson et al., 1987; Zhang and Kubo, 1993). In the wild, occurrence of phytoecdysteroids in host plants might act as an avoidance signal but not as efficiently as for more specialised species like *P. brassicae* and *B. mori*, i.e. only when very large concentrations in ecdysteroids are present (Jones and Firn, 1978; Blackford and Dinan, 1997).

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