The Journal of Experimental Biology 206, 4487-4496

doi:10.1242/jeb.00705

Loss of gustatory responses to pyrrolizidine alkaloids after their extensive ingestion in the polyphagous caterpillar *Estigmene acrea*

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Accepted 4 September 2003

Summary

Electrophysiological recordings from taste sensilla of the caterpillar *Estigmene acrea* with the pyrrolizidine alkaloid (PA) seneciphylline *N*-oxide demonstrated that extensive feeding on plants rich in PAs caused a loss in response of the PA-sensitive cell in the lateral styloconic sensillum on the galea. The effect could be repeated using pure PAs fed to the insect in synthetic diets and by injection of PA into the hemolymph. The sensitivity loss lasted for approximately two hours and was less pronounced in individuals that had been reared on PA-containing food. Behavioral experiments and field observations demonstrate a parallel reduction in responsiveness to PAs and to PA-containing plants.

Key words: neural sensitivity, pyrrolizidine alkaloid, taste threshold, effect of experience, *Estigmene acrea*, caterpillar.

Introduction

Although there are examples of labile sensory responses to chemicals in insects (Blaney et al., 1986; Schoonhoven et al., 1998), responses falling to zero after experience are not known. For example, olfactory sensitivity of mosquitoes to lactic acid is reduced following a blood meal (Davis, 1984), and gustatory sensitivity to inositol and other plant chemicals was shown to be reduced by about 20% in caterpillars of the tobacco hornworm *Manduca sexta* if they were reared on diet containing those chemicals (Schoonhoven, 1969). Glendinning and co-workers demonstrated a more marked reduction in gustatory responses of *M. sexta* to salicin and caffeine that translated into reduced levels of deterrence (Glendinning et al., 1999, 2001). Reduced sensory input to sugars or amino acids, which occurs when the individuals are satiated with carbohydrates or proteins, respectively, is sometimes more extensive (Simpson et al., 1991).

Estigmene acrea (Lepidoptera; Arctiidae) has highly polyphagous larvae that sequester pyrrolizidine alkaloids (PAs) as precursors of pheromone (Rothschild et al., 1979; Krasnoff and Roelofs, 1989; Weller et al., 1999) and presumably for defense. The importance of PAs to *E. acrea* is suggested by the caterpillar's great gustatory sensitivity to them (Bernays et al., 2002a) and by the dedication of a high proportion of taste cells to their detection (Bernays et al., 2002b).

We have previously shown that *E. acrea* caterpillars are extremely sensitive to PAs, with gustatory receptors responding to concentrations as low as 10^{-12} mol l^{-1}. Here, we show that a dramatic reduction in sensitivity to PAs can occur following ingestion of large amounts as a result of (1) recent experience of feeding on plants rich in PAs, (2) diets containing large proportions of powdered plants containing PAs or (3) diets containing pure specific PAs. The biological significance of these findings is discussed.

Materials and methods

Insects

In southern Arizona, *Estigmene acrea* Drury is found at elevations of 1000–2000 m in grassland and savanna habitats associated with drainages where scattered trees occur. Caterpillars favor habitats close to tree canopies and forage on a variety of larger herbaceous plants and a few woody plants. They are typically found on foliage up to 2 m above the ground, where they move from plant to plant, sometimes encountering plants containing PAs, especially *Senecio longilobus* (Asteraceae) or *Crotalaria pumila* (Fabaceae).

E. acrea caterpillar cultures were obtained from caterpillars collected at Gardner Canyon and Box Canyon in southern Arizona. The cultures were reared in the laboratory on a wheat-germ-based artificial diet (Yamamoto, 1969). Insects were reared individually or in pairs in 200-ml plastic cups containing

a small cube of diet that was replaced daily. The cups were kept in an environment chamber with a 14 h:10 h light:dark cycle and temperature was kept constant at 25°C. Recordings were made on insects in day 2 of the final larval stadium, at which time they feed actively.

Chemicals

Pyrrolizidine alkaloids (PAs) were fed to insects at various concentrations prior to tests by adding them to synthetic diet. Weighed blocks of diet were warmed until the agar melted, and weighed amounts of PAs were added. We used the PAs monocrotaline (Sigma Chemicals, St Louis, MI, USA) and retrorsine (Carl Roth GmbH, Karlsruhe, Germany).

In all cases, the PA used to test electrophysiological responses was 10^{-7} mol l^{-1} seneciphylline *N*-oxide. The other chemicals used were 10^{-3} mol l^{-1} serine and 10^{-2} mol l^{-1} protocatechuic acid. All chemicals were dissolved in 0.05 mol l^{-1} KCl.

Electrophysiology

Electrophysiological recordings were made from the lateral styloconic sensilla on the galea of the caterpillar with the tip-recording method (Hodgson et al., 1955) using live insects immobilized by immersion in a vial of 0.1 mol l^{-1} KCl with a rubber gasket around the neck so that the head was exposed (Gothilf and Hanson, 1994). The indifferent electrode was sealed through the glass of the vial so that it made contact with the KCl in which the insect was immersed. Immediately prior to each stimulation, the stimulating solution was drawn from the tip of the recording electrode with absorbent paper to reduce concentration increases due to evaporation. After each stimulation, the insect's mouthparts were rinsed with distilled water and then wiped with absorbent tissue. A Johnson baseline-restoring preamplifier was used to provide high input resistance to reduce the stimulus artifact (Frazier and Hanson, 1986), and the signal was amplified and filtered with a band width set at 130–1200 Hz. Recordings of the first 1 s of the response were made directly onto a computer in the spike analysis program SAPID (Smith et al., 1990). Only records from one side of each insect and only a single record of the response by an insect to each chemical or combination of chemicals were used for analysis. At least 3 min were allowed to elapse between successive stimulations to ensure complete disadaptation of the receptor cells.

Subsequent analysis was made either in the VIEWDAT part of the SAPID program or in the spike train analysis STA program version 3.0 (courtesy of E. Städler, Eidgenössische Forschungsamstalt, Switzerland), which permitted examination of the records at different degrees of temporal resolution. We did not use those parts of the programs that automatically classify action potentials because this was clearly not appropriate with these data, where spike amplitude often changed with time or concentration. The cell responding to PAs in the lateral galeal sensilla produced very large action potentials that were distinct from those of any of the other cells (Bernays, 2002b). We used spike number in the first 500 ms in all analyses.

Statistical analyses were carried out using the JMP 3.2.1 Software (SAS Institute, Cary, NC, USA) program.

Recent feeding on PA-containing plant material or diets

Insects were fed on *Senecio longilobus* collected from the field in southern Arizona. This plant population contains total PAs at concentrations of 0.3–0.9% dry mass. The major alkaloids are retrorsine and usaramine, followed by integerrimine and seneciphylline. In the plant, all PAs are stored as *N*-oxides. It is likely that concentrations and compositions varied between populations (Johnson et al., 1985; Witte et al., 1992), but in all experiments we used young foliage and inflorescences collected at a single site in the Santa Rita experimental ranges, Pima County, Arizona, and all experiments were in late summer and autumn of 2002.

In the first set of experiments, insects in the first day of the final larval stage had their rearing diet replaced with sprigs of *S. longilobus*. They fed avidly on this host plant. At 6 h, 24 h or 48 h, individuals were taken from their cups and the response of the PA-sensitive neuron in the lateral sensillum to 10^{-7} mol l^{-1} seneciphylline *N*-oxide was recorded. One experiment compared control and 6-h exposed insects; another experiment compared control, 24-h and 48-h exposed insects.

In the second set of experiments, the feeding regime was similar but the individual caterpillars were continuously observed for 6 h prior to recording to enable us to examine feeding parameters of individual caterpillars in relation to electrophysiological response. We monitored the duration of all feeding bouts and examined the relationship between actual time spent feeding in periods of time prior to the test, the duration of the final feeding bout prior to the test, and the time between the last feeding bout and the test.

To determine if changes monitored as a result of feeding on *S. longilobus* were due to the presence of PAs, insects were reared as usual and then fed on synthetic diet containing PAs for 24 h prior to testing. Chemicals added to the diet prior to testing were moncrotaline at 1% or 2% dry mass and retrorsine at 1% dry mass.

In the course of these experiments, we tested the lateral styloconic sensilla with KCl alone at intervals.

Rearing on synthetic diets with or without a source of PAs

If the loss of the PA response is due to toxic feedback, there is the possibility that it occurs because the insects had not experienced any PAs during their development, prior to the sudden ingestion of relatively large quantities. To examine the possible effects of experience, one family of insects was reared on plain diet with or without additional diet containing 0.1% monocrotaline. Twenty-four hours before testing, individuals received a new block of diet containing 0%, 0.001%, 0.05%, 0.1%, 1% or 2% monocrotaline.

Insects were reared in cups with 10 larvae per cup for 5 days, 5 larvae per cup for the succeeding 2 days and then individually until the time of testing on day 2 of the last larval stage. Those caterpillars receiving diet containing 0.1% monocrotaline also received a block of plain diet, to enable

them to self-select food and not be forced to eat only test diets. On day 1 of the final larval stage (i.e. just after the final larval ecdysis), individual insects from each rearing condition received new food (either plain diet or a diet containing monocrotaline at one of the test concentrations). All insects ate the diets in apparently normal amounts during the 24-h period, and a measure of this was obtained by counting fecal pellets produced by the time of testing.

In all cases, the insects were taken directly from the diet and tested without any period of food deprivation. The response of the PA-sensitive neuron in the lateral sensillum to 10^{-7} mol l^{-1} seneciphylline *N*-oxide was recorded.

Experiment on adaptation to S. longilobus *sap*

We examined the possibility that a loss of sensitivity to PA was due to peripheral adaptation of the PA cell in the sensilla. *S. longilobus* leaves were ground in distilled water (50 mg in 10 ml), and the resultant material filtered through tissue. The filtrate was placed over an individual lateral styloconic sensillum in a normal recording electrode for 2 min, 3 min, 10 min or 20 min. During the initial 1 s, the response of the cells was recorded. After both the 2-min and 3-min exposures, the electrode was removed and replaced immediately to get a measure of the extent of adaptation to the chemicals in the plant sap; the response was initially dominated by the response of the PA cell. After the 10-min exposure, the sensillum was tested with the usual 10^{-7} mol l^{-1} seneciphylline *N*-oxide at 3–4-min intervals to examine the recovery from adaptation.

A test of hemolymph feedback on sensory response

A number of species of arctiid caterpillars sequester PAs (Boppré, 1990; Hartmann and Witte, 1995; Weller et al., 1999) and store these chemicals exclusively in the form of their *N*-oxides in the hemolymph and integument. Ingested PA *N*-oxide is reduced in the gut and passively absorbed as free base into the hemolymph, where it is enzymatically converted into its *N*-oxide (Hartmann, 1991; Lindigkeit et al., 1997). It appeared possible that large quantities of such materials in the hemolymph of *E. acrea* could provide a basis for reducing the gustatory sensitivity to them. Such feedbacks are indicated in the reduced gustatory sensitivity of receptors to sugars and amino acids in various insects (Simpson et al., 1991).

To test the feedback hypothesis, we injected a PA into the hemolymph and monitored the response of the PA cell in the lateral styloconic sensilla with 10^{-7} mol l^{-1} seneciphylline *N*-oxide 1 h, 2 h and 3 h after injection. We injected each individual with 5 μl of a solution of 1% monocrotaline. To make up the solution, 30 mg was dissolved in 100 μl ethanol, and insect saline was added to make the volume up to 3 ml. Control insects were injected with 5 μl saline containing similar quantities of ethanol.

Each insect was tested first with 10^{-7} mol l^{-1} seneciphylline *N*-oxide in the usual way. Monocrotaline was then injected through the membrane between two segments of the first thoracic leg as it rested in its recording vial. Because the insect was anaesthetized and flaccid, there was rarely any leakage; in cases where leakage was noticed, the caterpillar was discarded. The success of the injection (i.e. into circulating hemolymph) was indicated by including 0.5% amaranth (w/v) in the injectate. In all cases, the red color could be seen spreading anteriorly and ventrally and was presumed to represent the movement of monocrotaline in the hemolymph. Each insect was retested approximately 1 h, 2 h and 3 h later. Nine control-injected and 10 test-injected insects were monitored.

Testing other chemicals/cells

No planned experiments were carried out with different chemicals to examine whether the reduction in response to PA was accompanied by a reduction in responsiveness of other cells to other compounds. However, in many instances we performed control tests with KCl alone. We tested several control and PA-fed treated insects for response in the medial galeal PA cell in individuals in the experiment in which 2% monocrotaline was fed to insects before tests. At intervals, we also tested 10^{-2} mol l^{-1} protocatechuic acid, which stimulates a cell in the lateral sensillum, and 10^{-3} mol l^{-1} serine, which stimulates a sucrose/amino acid cell in the medial sensillum (Bernays et al., 2002b). These tests were carried out especially on individual insects that lost PA sensitivity, and results are presented as combined data from these miscellaneous trials.

Behavioral correlates of sensory change

In the laboratory, one-day-old final-stage larvae were placed into fresh cups with either plain synthetic (control) diet or with control diet together with sprigs of *S. longilobus*. They were maintained at 25°C (14 h:10 h L:D) for 14–18 h. Observations were made on individuals from each pretreatment presented with synthetic diet containing 0.01% monocrotaline (Table 1, test food 1). We recorded the behavioral response to first contact with this test food in each case. In most cases, this response was the duration of the first feeding bout. A few caterpillars, however, contacted the food repeatedly with their mouthparts and walked away. These rejections were scored as bout durations of '0' in the analysis. After contact or feeding on the PA test food, this food was replaced with a similar synthetic diet in which PAs and cellulose were replaced with sucrose and casein (Table 1, test food 2) to determine if any differences between treatments were responses specific to PAs or to food generally. The durations of feeding bouts on test foods 1 and 2 were each compared with Kruskal–Wallis tests. Because we explicitly predicted reduced feeding responses to the PA-containing food by PA-experienced caterpillars, we used a one-tailed Kruskal–Wallis test to analyze the responses to test food 1.

In the field, we observed individual caterpillars foraging. Last-stage caterpillars were observed in their natural habitats in east Gardner Canyon, Santa Rita Mountains, Pima County in late August 2001 and in Box Canyon, Santa Rita Mountains, Pima County in late August and early September 2002. We recorded by hand, with the aid of digital watches, all walking, feeding and resting bouts and the substrates upon which they occurred. We also recorded all plants on which the head was

Table 1. *Composition of synthetic diets used in the laboratory behavioral experiment*

Ingredients	Amounts	
	Test food 1	Test food 2
Casein	**0 g**	**10.0 g**
Sucrose	**0 g**	**6.8 g**
Cellulose	**38.44 g**	**21.64 g**
Salt mix	0.96 g	0.96 g
Linoleic acid	0.2 ml	0.2 ml
Cholesterol	0.2 g	0.2 g
Ascorbic acid	0.12 g	0.12 g
B vitamin mix	0.21 ml	0.21 ml
Choline chloride	0.3 ml	0.3 ml
Agar	5.12 g	5.12 g
Water	160 ml	160 ml
Monocrotaline	**0.0052 g**	**0 g**

Rows in bold indicate diet components different in the two test foods.

lowered to the plant surface with or without an apparent bite. These events were called tastes. Rejections are defined as tastes followed by walking or resting instead of feeding.

Observations lasted from 10 min to 6 h on a total of 50 insects. On no occasion were individuals found on *Crotalaria* initially. In 2001, individuals had to be transferred to the region where *Crotalaria* occurred. In 2002, we initiated observations early in the day when feeding appeared to be just beginning and insects were roosting on other, taller, plant species. On six host-plant species, there were multiple contacts and one or more series of feeding bouts by at least 10 individuals. Rejections also occurred on all of them but appeared to increase on *Crotalaria* relative to the other species. We compared rejection rates on first encounter with each plant species and rejection rates on the following four encounters in a sequence of contacts with the same species. The data include more than one sequence on a plant by a single individual if the sequences were separated by at least one hour and multiple feeding bouts on different species.

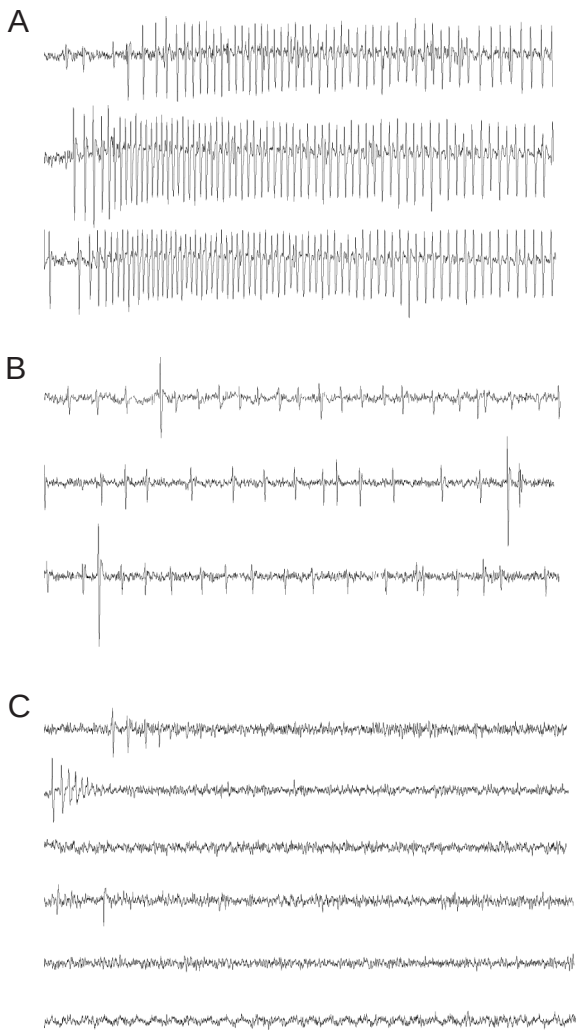

Fig. 1. Examples of recordings from the lateral galeal sensillum. (A) Three recordings illustrate the normal response of the pyrrolizidine alkaloid (PA) cell to 10^{-7} mol l^{-1} seneciphylline *N*-oxide. (B) Three recordings of normal responses to the electrolyte alone, showing principally the response of a salt cell. (C) Six recordings to show the lack of response to 10^{-7} mol l^{-1} seneciphylline *N*-oxide after recent feeding on *Senecio longilobus*. First 500 ms shown.

Results

Recent feeding on PA-rich plant material or diets containing PAs

Many of the insects that had fed on *S. longilobus* for the previous 6 h, 24 h or 48 h had no response to the seneciphylline *N*-oxide stimulus. Fig. 1A illustrates three normal responses to the 10^{-7} mol l^{-1} seneciphylline *N*-oxide stimulus. The dominant cell responds strongly and other cell activity is largely hidden. Fig. 1B illustrates the response of cells in the lateral sensillum to KCl alone. An occasional firing of the PA cell occurs, but the principal cell responding to the salt is much smaller. From the pattern of firing, one other cell appears to be firing occasionally. Fig. 1C illustrates six examples of the loss of PA sensitivity. In most cases, no spikes could be determined but occasionally there was a single spike or a very short series of spikes that faded quickly. The data are distinctly bimodal: insects had no or very few spikes or the frequency was more or less within the normal range.

After having the *S. longilobus* food available for 6 h, almost half the insects showed the loss of sensitivity (Fig. 2A). By 24 h, only three out of 13 insects showed any response. At 48 h the result was similar: four out of 12 insects had a response and these responders had relatively low spike frequencies (Fig. 2B).

In experiments where we obtained precise feeding times prior to sensillum testing we found that the sum of durations of feeding bouts during the 2 h prior to testing showed the strongest correlation with numbers of insects showing loss of sensitivity (Fig. 3A). The insects needed to have been feeding for approximately 30 min or more during the previous 2 h for

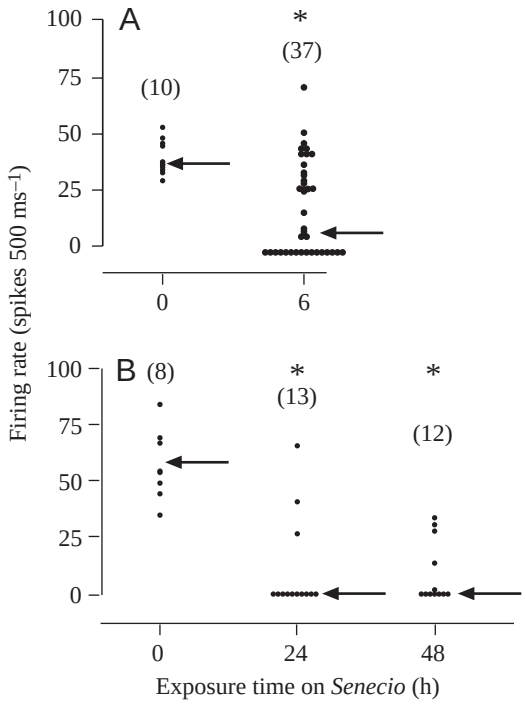

Fig. 2. The response of the pyrrolizidine alkaloid (PA) cell to 10^{-7} mol l^{-1} seneciphylline *N*-oxide after recent feeding on *Senecio longilobus*: (A) control *vs* 6-h exposure, (B) control *vs* 24- or 48-h exposure. Numbers in parentheses indicate number of replicates in each treatment. Arrows indicate median values. Asterisks indicate significant difference from controls using Dunnett's test at $P<0.05$.

there to be a marked effect (Fig. 3B). There was no relationship with duration of the last feeding bout prior to testing (Fig. 3C) or time between the last bout and the time of the test (Spearman's rank correlation, $P>0.1$).

Experiments in which the PAs monocrotaline or retrorsine were added to diet and the insects given only that diet for 24 h before testing showed that the loss of sensitivity could be caused by these compounds alone at 1% or 2% dry mass (Fig. 4). Retrorsine appeared to be less effective but, because amounts of the chemical were limited, diet blocks were small and in some cases there was almost nothing left at the time of testing, so that insects may not have been fully fed.

Rearing on synthetic diets with or without a source of PA

Insects reared with both control diet and 0.1% dry mass monocrotaline diet available had higher responses to 10^{-7} mol l^{-1} seneciphylline *N*-oxide than those reared on control diet alone (Fig. 5A). There was a trend, however, in both rearing treatments for responding insects to have a somewhat reduced spike frequency after 24 h feeding on the diets with highest concentration of monocrotaline. After feeding on the three highest concentrations of monocrotaline (0.1–2%), the sensory loss was observed. The percentage of individuals showing this sensory loss increased with concentration of monocrotaline fed upon and was markedly higher in individuals that had not previously experienced monocrotaline in the rearing food

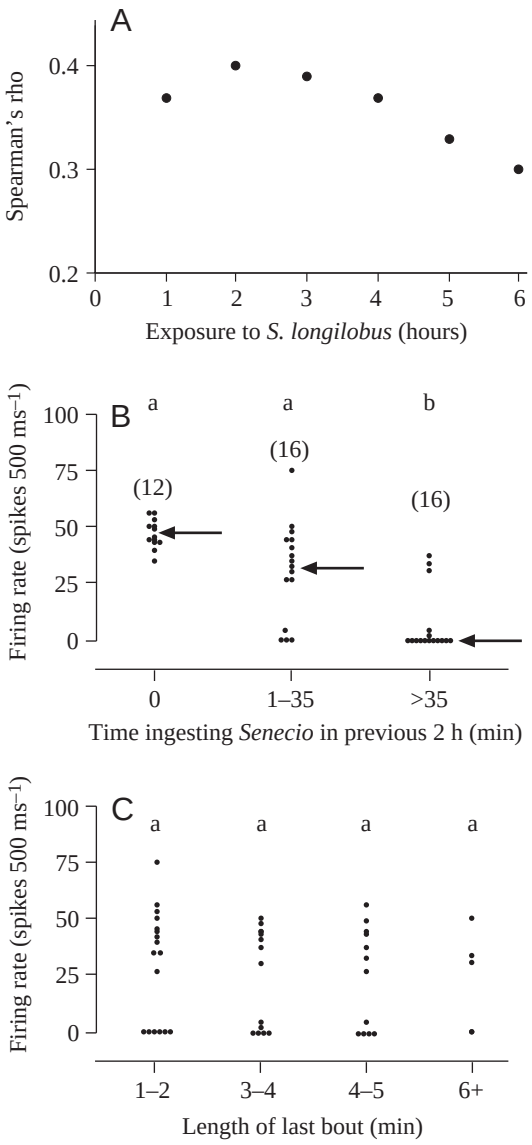

Fig. 3. The response of the pyrrolizidine alkaloid (PA) cell to 10^{-7} mol l^{-1} seneciphylline *N*-oxide in relation to feeding parameters on *Senecio longilobus*. (A) Spearman's rank correlation (relating duration of actual ingestion and resultant sensory response) shown for different durations of exposure to *S. longilobus*. (B) Response of the PA cell to 10^{-7} mol l^{-1} seneciphylline *N*-oxide for control insects and individuals that had fed for shorter times (1–35 min) or longer times (>35 min) during the previous 2 h. (C) Response of the PA cell to 10^{-7} mol l^{-1} seneciphylline *N*-oxide in insects that had different final bout durations on *S. longilobus*. Numbers in parentheses indicate number of replicates in each treatment. Arrows indicate median values. Different letters indicate significant differences among groups using Tukey tests at $P<0.05$.

(Fig. 5B). Amounts of feeding on the different foods were not different, as measured by fecal pellet production (Fig. 5C).

Experiment on adaptation to S. longilobus sap

Exposure of the lateral sensillum to the sap of *S. longilobus* resulted in sensory adaptation of the response. An example of

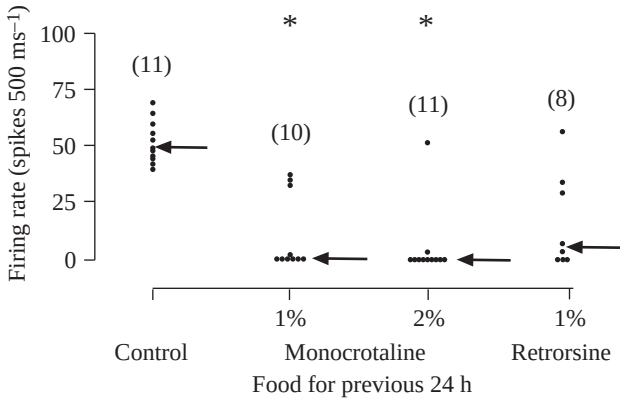

Fig. 4. Response of pyrrolizidine alkaloid (PA) cells in the lateral galeal sensillum to 10^{-7} mol l^{-1} seneciphylline *N*-oxide after 24 h of exposure to synthetic diets in the laboratory. The foods were control diet, diet containing 1% or 2% dry mass monocrotaline or diet containing 1% dry mass retrorsine. Numbers in parentheses indicate number of replicates in each treatment. Arrows indicate median values. Asterisks indicate significant difference from controls using Dunnett's test at $P<0.05$.

the initial response to the sap and declining input at 2-min and 3-min exposure is shown in Fig. 6A. The presumed PA cell (largest spikes) no longer fired after exposure of 1 min, and a second cell was almost silent after 2 min. The third cell firing did not appear to change. A similar pattern of change was found for six insects (Fig. 6B).

Insects tested with 10^{-7} mol l^{-1} seneciphylline *N*-oxide after 10 min exposure to the sap of *S. longilobus* showed a gradual recovery to the original sensitivity to this PA by 20 min following removal of the plant sap (Fig. 7).

Hemolymph feedback on sensory response

Over a period of 3 h, insects injected with saline showed no change in response to 10^{-7} mol l^{-1} seneciphylline *N*-oxide (Fig. 8, broken line). However, those injected with monocrotaline all showed some decline. Seven of the 10 insects still had no sensitivity after 1 h. Two more insects showed a major decline after 2 h, while the remaining insect showed a minor reduction after 2 h. Recovery was close to complete in nine of the 10 insects 3 h after injection (Fig. 8).

Effects on other cells

Fig. 1 shows that the loss of sensitivity also involves a loss of sensitivity of the response of a salt cell in the lateral sensillum to KCl alone. The sporadic tests of the lateral sensillum to protocatechuic acid also showed a similar loss of activity in a deterrent cell. Tests on the medial sensillum with seneciphylline showed that response in the PA-specific cell was greatly reduced by recent experience of PA, and response to serine showed a significant but much less marked effect on the sucrose/amino acid cell (Fig. 9).

Behavior

In the laboratory studies of behavior, insects that were given

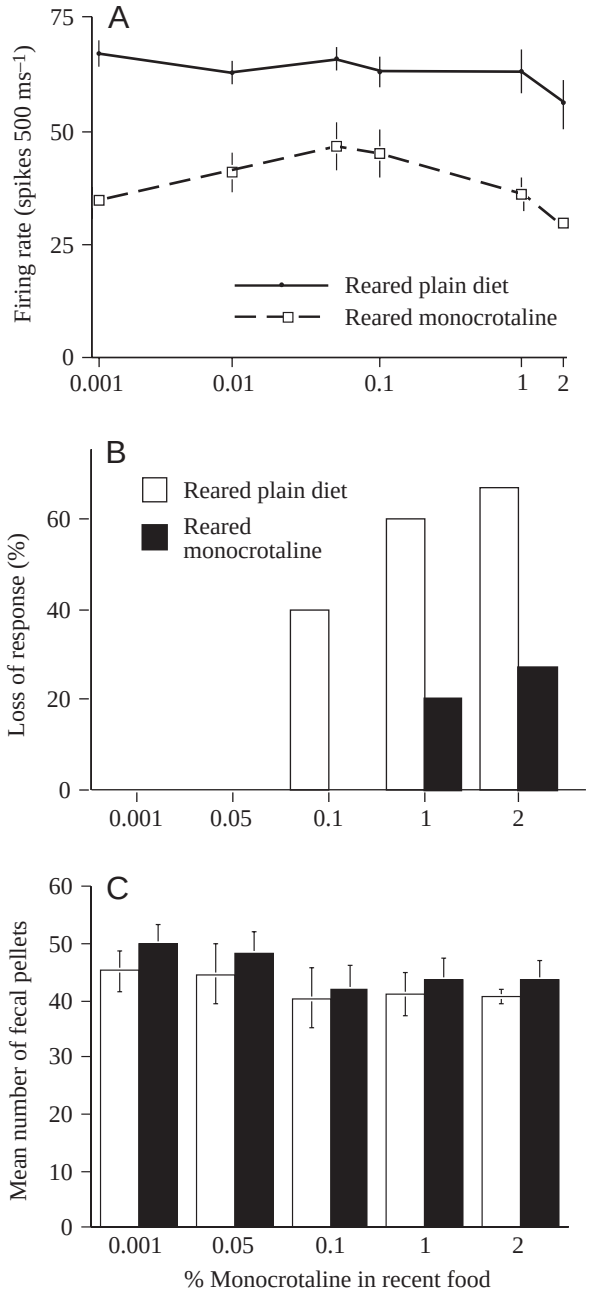

Fig. 5. Effects of rearing on diet containing low concentration of monocrotaline. (A) Response of pyrrolizidine alkaloid (PA) cells in the lateral galeal sensillum to 10^{-7} mol l^{-1} seneciphylline *N*-oxide after 24 h of exposure to synthetic diets containing different concentrations of monocrotaline. Only those individuals that responded in a typical manner are shown. (B) Percentage of insects in each category in which sensory shutoff of the PA cell was observed (% less than 10 spikes). (C) Numbers of fecal pellets produced in the 24-h test feeding period. Bars represent means, and vertical lines represent S.E.M.

control diet for 24 h had longer first feeding bouts on test food 1 (containing monocrotaline; median=98.6 s) than insects offered both control diet and *S. longilobus* (median=58.5 s) for the previous 14–18 h ($\chi^2=3.56$, d.f.=1, $P=0.03$). By contrast,

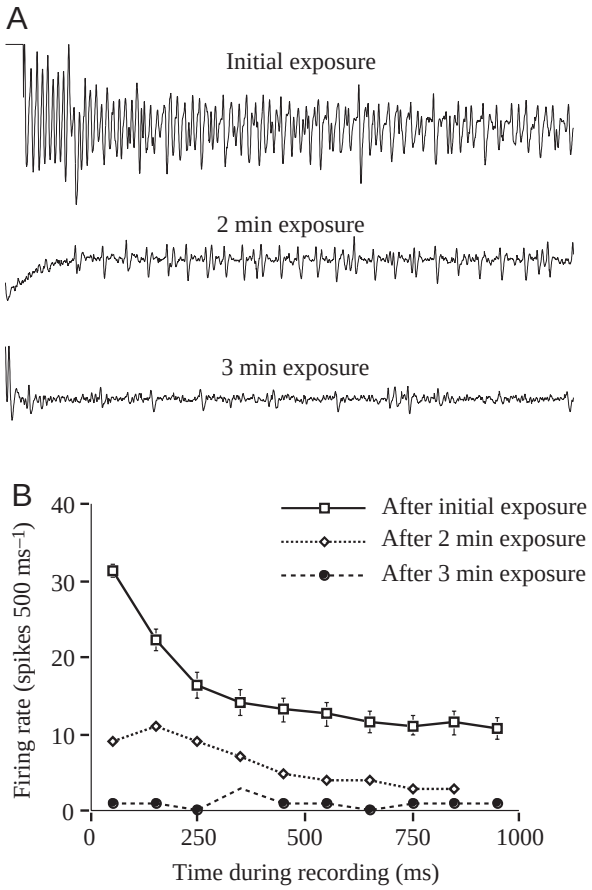

Fig. 6. Adaptation to sap from *Senecio longilobus* plants. (A) Examples of traces to show the initial response to the sap and the loss of response after two and three minutes of exposure to the sap. (B) Firing rate of the lateral galeal sensillum to 10^{-7} mol l^{-1} seneciphylline *N*-oxide throughout the second of exposure at the three different times. Values are means ± S.E.M. (*N*=6).

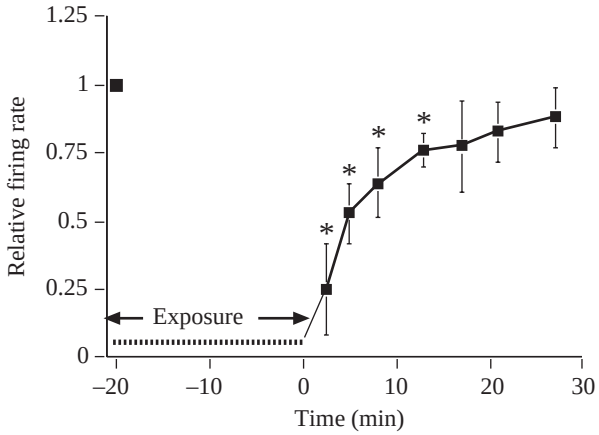

Fig. 7. Disadaptation of the pyrrolizidine alkaloid (PA) cells in the lateral galeal sensillum to 10^{-7} mol l^{-1} seneciphylline *N*-oxide after adaptation using exposure of the sensillum for 10 min to sap of *Senecio longilobus*. Asterisks indicate a significant decrease from initial level of response (P<0.05). The black square at time zero is the initial firing rate normalized to one.

subsequent feeding bouts of test food 2 did not differ between these treatments (χ^2=1.54, d.f.=1, P=0.21).

In field observations, all host plants were rejected occasionally. However, the likelihood of rejecting most of them either decreased or did not change with successive encounters. In the case of the PA host plant *Crotalaria pumila*, however, rejection rates increased during successive encounters (Table 2).

Discussion

The data presented here clearly show that recent feeding on pyrrolizidine alkaloids at relatively high concentration commonly causes a complete loss in response of the PA cell in the lateral styloconic sensillum of *Estigmene acrea* (Fig. 4) to a PA that normally causes very high firing rates and is a strong phagostimulant (Bernays et al., 2002a). The loss was also marked after feeding on the PA-rich *Senecio longilobus*, and in this case is presumed to be due to the ingestion of PAs in the host plant (Fig. 2). Thirty minutes of feeding on *S. longilobus* in the 2 h prior to testing was enough to eliminate the response in the majority of insects (Fig. 3). Monocrotaline (occurring in a number of *Crotalaria* spp. and several other PA-containing plant species) and retrorsine (occurring in relatively high concentrations in *S. longilobus*) were both effective in causing the sensory loss.

Table 2. *Rejection levels of common host plants by* E. acrea *caterpillars in the field*

	Encounter 1		Encounters 2–5		Change	
	N	% Rejection	*N*	% Rejection	Direction	χ^2(*P*)
Crotalaria pumila	**50**	**0**	**75**	**32**	**+**	**<0.001**
Viguiera dentata	46	46	61	20	–	<0.001
Bidens leptocephala	25	12	52	6	–	<0.001
Macheranthera tanacetifolius	10	30	27	15	–	<0.005
Dalea candida	18	28	29	28	0	–
Desmodium procumbens	19	68	13	46	–	<0.01

Encounter 1 is compared with successive encounters 2–5 on the same plant species.

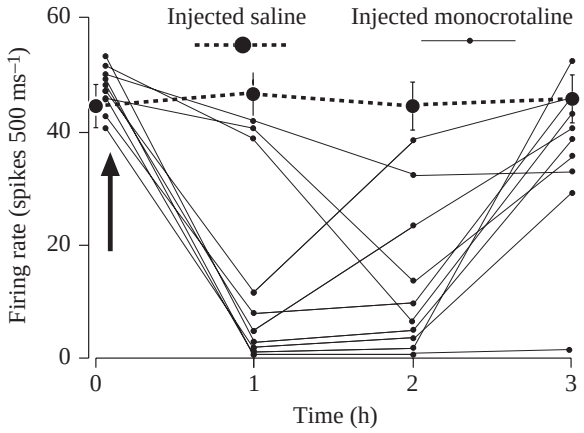

Fig. 8. Effects of injection. Response of pyrrolizidine alkaloid (PA) cells in the lateral galeal sensillum to 10^{-7} mol l^{-1} seneciphylline *N*-oxide after injection of saline or 5 µl of 1% monocrotaline. Data for the saline-injected individuals (*N*=9) are shown as means ± S.E.M. (broken line). Data for injected insects (*N*=10) are shown as individual trajectories. The arrow indicates injection time (within 10 min of the initial recording).

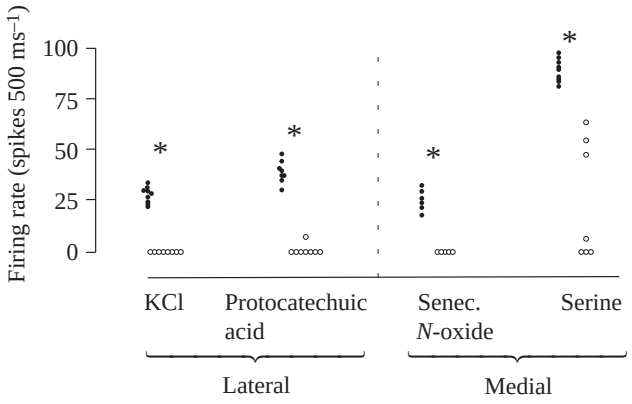

Fig. 9. Responses of cells other than the pyrrolizidine alkaloid (PA) cell in the lateral galeal sensillum in various insects with or without a sensory shutoff in the PA cell in the lateral galeal sensillum. Open symbols represent individuals with no PA response in the lateral sensillum; filled symbols represent individuals with a normal PA response in the lateral sensillum. Asterisks indicate a significant decrease in response by insects whose PA cell in the lateral galeal sensillum is non-responsive.

Much of the data presented here concerns insects that had been reared on synthetic diet without any experience of PA compounds until the last day before testing. Such deprivation of these chemicals, important in reproduction and presumably defense (Rothschild et al., 1979; Krasnoff and Roelofs, 1989; Weller et al., 1999), may be quite common in nature, as analyses of field-collected insects indicate that some individuals in the final larval stage are PA-free (T. Hartmann, unpublished data). Furthermore, field observations indicate that in some locations PA-containing plants can be relatively rare depending on seasonal conditions or other factors (M. S. Singer, unpublished observations).

Experiments on insects reared with PA-containing food available demonstrated that this experience reduced the number of insects showing a loss of the sensory response when they were subsequently fed high concentrations of PA-containing diet, although it was not eliminated (Fig. 5). This demonstrates that experience does moderate the effect. The reduced effect was not due to differential feeding on the high PA-containing diets provided in the 24 h before tests, as demonstrated by the similarity in fecal pellet production across all treatments. It may be in some way related to the generally elevated responses of insects with long-term experience of PA, a phenomenon fully described elsewhere (Chapman et al., in press). It may also be related to an enhanced ability to handle high levels of PA that are ingested and are potentially toxic. In PA-adapted arctiids, the alkaloid is absorbed as pro-toxic PA free base into the hemolymph, where it is efficiently *N*-oxidized and thus detoxified by senecionine *N*-oxygenase (Lindigkeit et al., 1997). In the host-specific PA-sequestering arctiid *Tyria jacobaeae* (cinnabar moth), this enzyme is expressed in the fat body and released as soluble enzyme into the hemolymph (Naumann et al., 2002; T. Hartmann, unpublished results). It needs to be verified whether the enzyme is constitutively expressed or whether its expression is induced or its release from the fat body enhanced in the presence of pro-toxic PA free base. Increased ability to detoxify (Glendinning and Slansky, 1995; Snyder and Glendinning, 1996) or excrete (Self et al., 1964) with experience has been documented in other insect species.

The loss of sensory response appears to be something additional to normal sensory adaptation. Limited adaptation to PA compounds does occur over a period of 1 s (Bernays et al., 2002a), and in the present study we have evidence that exposure to PA-containing plant sap continuously for one minute reduces the sensory response of the PA cell to zero (Fig. 6). Thus, 10 min of exposure would certainly have resulted in zero input from the PA cell. Tests of insects with seneciphylline *N*-oxide immediately after such a 10-min exposure was terminated indicated that sensory recovery, or disadaptation, was quite rapid and was complete after just 20 min (Fig. 7). Such a time frame is not unusual in studies of adaptation (Torre et al., 1995).

That the sensory loss involves more than peripheral sensory adaptation is indicated by the fact that preparation of insects for recording after feeding on the high PA-containing diets involved times of up to 40 min and typically averaged 15 min, and yet at these times large numbers of individuals still had no response to the test PA. In addition, in experiments combining feeding observations and electrophysiology there was no evidence that the period between the last feeding bout and the time of testing was correlated with the presence of the sensory loss.

It appears that the loss of sensory response to PAs by the PA cell is largely generated by postingestive feedback. It was mimicked by injection of 50 µg monocrotaline into the hemolymph to give quantities that are biologically realistic (Fig. 8). In fact, in caterpillars of *E. acrea* actively feeding

on *S. longilobus*, hemolymph PA levels corresponding to 30–250 μg were determined (T. Hartmann et al., unpublished). Unlike peripheral sensory adaptation and disadaptation, the effect was relatively long-lived, generally lasting a couple of hours. The injection was a single event and, presumably within a very short time, the injected pro-toxic monocrotaline was detoxified to its *N*-oxide and distributed within the body or deposited in the integument (Nickisch-Rosenegk and Wink, 1993). The precise duration of detoxification upon injection needs to be determined. In an insect feeding for a period of hours on a PA-containing food, there is likely to be more or less continuous uptake of the compounds and, if the concentrations are high, hemolymph concentrations of the more toxic free base may continue to rise.

We know that the two common PA-containing plants used by *E. acrea* in southern Arizona can be noxious for this polyphagous caterpillar. Rearing on *C. pumila* alone caused all individuals to die (E. A. Bernays, unpublished results) and rearing on *S. longilobus*, even in a mixture, severely reduced survival (M. S. Singer and D. Rodrigues, unpublished results). We suggest that *E. acrea*, while requiring PAs in the food, can also suffer from ingesting too high a level. The loss of the sensory response to PAs may thus be adaptive – hosts containing these chemicals should become less phagostimulatory and therefore less likely to be eaten. Further studies are needed to confirm this suggestion. Presently, no other example is known where a PA-sequestering insect species has been shown to suffer from levels of PAs that are noxious, but most studies so far have concentrated on species that have a closer affiliation with their PA-containing host plants.

In laboratory behavioral tests, we found that insects offered *S. longilobus* and control diet or control diet alone for 14–18 h showed differences in feeding bout length when offered synthetic diet containing monocrotaline – caterpillars previously given control diet alone had longer bouts than those that had been offered this food and *S. longilobus*. This result, coupled with the lack of a difference in feeding bouts on test food 2, demonstrates a change in feeding response to PAs rather than to food generally. It is important to note that caterpillars in this experiment could freely regulate their intake of PAs (by ingesting *Senecio* or control diet) prior to the behavior test and were therefore less likely to experience a complete loss of sensory activity. In nature, we have found that *E. acrea* caterpillars foraging in a habitat with *C. pumila* did show an increased likelihood of rejecting it after a succession of feeding bouts on it, while this did not occur with a series of host plants that do not contain PAs (Table 2). Results from both the laboratory experiment and field observations support the idea that sensory changes following ingestion of large amounts of PAs serve to temporarily curtail feeding on foods with PAs.

The mechanism of sensory loss is not known. Fig. 1 demonstrates that it involves more than just the PA cell. That is, the effect is not specific to the PA cell. One cell in the lateral sensillum regularly responds to the electrolyte (KCl) alone at 20–30 spikes s^{-1} but it is also obliterated. Furthermore, we found that responses to protocatechuic acid, a third cell in the lateral sensillum, were not measurable in insects that did not respond to the PA stimulus. From preliminary microscopy we know that there are the usual four dendrites at the tip of the sensillum but have not yet discovered what stimulates the fourth cell. However, that three of the cells all become unresponsive suggests a whole-sensillum response. Work is needed to determine if this is a pore-closing process (Bernays et al., 1972) or a change in the structure of the fibrous material just within the pore (Shields, 1996), but the fact that the noise level doesn't change suggests that overall resistance due to pore closure is not a major factor. There may be some change in the sensillum liquor (Pietra et al., 1979) or a change in the neurons themselves. The diminishing spike amplitude in some traces may indicate that current-carrying channels in the neuronal membrane of the taste cells are closing down, so the current is eventually below the threshold for initiation or propagation. Such changes could arise in diverse ways (e.g. Wolbarsht and Hanson, 1965; Kijima et al., 1995).

Effects in the medial sensillum were not the focus of this study. There is a specific PA cell present (Bernays, 2002b) and we found that, in some individuals, its response to PAs was reduced also. However, in most of those tested with serine, we found that the sugar/amino acid cell responded in a normal way, though with more than usual amounts of noise. This effect could suggest that there is some alteration in pore properties such that only some types of stimulant are able to enter. Possible differences include solubility, molecular size/charge and affinity for proteins present in the medium. In any case, it would appear that the medial sensillum is less affected and may continue to respond to certain stimulating nutrients at least.

Conclusions

Loss of sensitivity by a cell or whole gustatory sensillum occurs when presumed overdoses of a needed plant secondary metabolite, pyrrolizidine alkaloid, are ingested. It is most pronounced in insects naïve to PAs until a day or two before testing and it can be mimicked by injection of PA. It has a behavioral role in reducing further feeding on PA-containing foods for a few hours, perhaps allowing physiological handling mechanisms time to deal with the materials.

M.S.S. was funded by the Center for Insect Science through NIH Training Grant 1K12 Gm 00708. D.R. was supported by a grant from CAPES Foundation/Brazil. Dr Frank Hanson provided insights on receptor physiology. Dr Erich Städler made the STA program available.

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