Electroantennogram Responses of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) Male Moth to Two Female Sex Pheromone Components

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(Received September 14, 1984)

Electroantennogram (EAG) responses of the *Spodoptera litura* male to female sex pheromone components, (Z, E)-9, 11-tetradecadienyl acetate (compound A) and (Z, E)-9, 12-tetradecadienyl acetate (compound B) were recorded. The order of amplitude of the EAG responses were compounds A+B (9:1) $\geq$ compound A > compound B. No increase in response was observed when the mixture ratio of the compounds was changed. In adaptation experiments, continuous exposure of an antenna to air containing compound A strongly suppressed the subsequent EAG response to both compounds A and B. Air containing compound B similarly suppressed the EAG response to compound B, though the responsiveness to compound A was only partially suppressed.

INTRODUCTION

Sex pheromone components of the common cutworm moth, *Spodoptera litura*, were identified as (Z, E)-9, 11-tetradecadienyl acetate (compound A) and (Z, E)-9, 12-tetradecadienyl acetate (compound B) (TAMAKI et al., 1973). Biological activity of these two compounds was tested under both laboratory and field conditions and their optimum mixture ratio was confirmed as 9:1 (TAMAKI and YUSHIMA, 1974; YUSHIMA et al., 1974). TAMAKI et al. (1976) analyzed the chemicals released from virgin female moths and listed those relating to the sex pheromone components such as (Z)-9-tetradecenyl acetate (compound C), (E)-11 tetradecenyl acetate, (compound D) and other chemicals. The behavioral roles of these two sex pheromone components were analyzed and compound A was found to be the principal attractant to males (NAKAMURA and KAWASAKI, 1977; KAWASAKI, 1981). Responses of receptor cells to these two sex pheromone compounds were analyzed and mixing of the two compounds increased the frequency of impulses as compared with that of compound A alone (AIHARA and SHIBUYA, 1977).

In this experiment, the effect of dose of pheromonal chemicals, mixture ratio, and receptor saturation on electroantennogram (EAG) responses were analyzed.

MATERIALS AND METHODS

S. litura larvae were reared on an artificial diet (FUJIE and MIYASHITA, 1973) at 25°C under a 16 light–8 dark condition. Pupae and male moths which emerged were

kept at 20°C under continuous light and provided with water. Experiments were made 2 to 5 days after emergence.

Two sex pheromone compounds, A and B, and two related chemicals, (*Z*)-9-tetradecenyl acetate (compound C) and (*E*)-11-tetradecenyl acetate (compound D), were purified by TLC (thin layer chromatography) or preparative GLC (gas liquid chromatography). Gas chromatographic analysis of test chemicals showed that purities of compounds A, B, C, and D were 93.6, 96.7, 97.1, and 97.9%, respectively. Known amounts of these chemicals were serially diluted with *n*-hexane for experiments.

The EAG responses were recorded from the antenna of an intact living male moth placed in a glass tube. Its head was fixed to the edge of the tube by bees wax. One antenna extruding from the tube was fixed on a copper wire with sticky paste (Fig. 1-B); the other antenna was cut off. The insect was then, put in an air flow system (Fig. 1-A). The air was purified with silica gel and activated charcoal and was humidified by passing through distilled water. Except in the adaptation experiment, 3 l/min of the purified air was passed over the antenna through flow 1 in Fig. 1-A. In the adaptation experiment, 0.5 l/min of air with pheromone was added through flow 2 to 2.5 l/min of the purified air through flow 1. Thus, 3 l/min of the air was passed over the antenna. The outlet for the purified air was 8 mm in i.d. and the antenna was 6 cm from the outlet. The air containing the chemicals was exhausted from the system (Fig. 1-A).

A glass microelectrode filled with insect RINGER's solution was inserted into the base of the antenna through an intersegmental membrane as an indifferent electrode. The tip of the antenna was put in contact with the solution in the glass capillary for recording (Fig. 1-B). Ten microliters of each serially diluted chemical was coated on the inside of a glass tube (9.5 mm i.d. × 20 mm). After the solvent had evaporated, the tube was

Fig. 1. Diagram of the air flow system and the way of fixation of a moth and antenna. A: Airflow system; P: pump, Si: silica gel, C: activated charcoal, W: water, F: flow meter, S2: sample holder, V: three-way valve, Sy: syringe, S1: sample holder, Ex: exhaust. B: fixation of a moth and antenna; A: antenna, cw: copper wire, I: indifferent electrode, R: recording electrode.

Fig. 2. EAG responses to two pheromone components, 9: 1 mixture of two components, and related chemicals. Squares, hollow triangles, solid triangles, hollow circles, and solid circles indicate 9: 1 mixture of compounds A and B, compound A, compound B, compound C, and compound D, respectively. Vertical bars show 95% confidence intervals.

put in a 5 ml syringe. Stimulation was caused by the introduction of a 1 ml air puff laden with the chemical from a syringe through a needle into the air flow system (Fig. 1-A). The responses were monitored on a cathode-ray oscilloscope via high impedance pre-amplifier and photographed or pen-recorded for analysis.

The amount of a chemical applied to the glass tube ranged from 1×10^{-5} to 100 μg for the dose-response experiment with compounds A, B, C, D, or a mixture of compounds A+B. In the mixture ratio experiment, the total amount on the glass tube was either 0.1 μg or 0.001 μg . The response to each mixture ratio was recorded sequentially and the order of stimulations was systematically changed. In the receptor adaptation experiment, the amount of chemical on the tube was varied from 1×10^{-4} to 1 μg , and 0.1 or 1 mg of a chemical absorbed on a 6×4 cm filter paper (Toyo No. 2) as hexane solution was set in the sample holder in flow 2 as shown in Fig. 1. In the adaptation experiment, before recording EAG response to each chemical, the antenna was continuously exposed to the air containing the chemical for 3 min and the exposure continued during the recording of EAG response. Relative concentration of the chemicals for adaptation was roughly estimated by a comparison with the result of the dose-response relationships and the EAG responses recorded at the initial exposure to the pheromone containing air. One milligram and 0.1 mg of chemicals adsorbed on a filter paper in flow 2 were roughly respectively equivalent to 4×10^{-2} – 1×10^{-3} μg and 2×10^{-2} – 1×10^{-3} μg of chemicals coated inside the glass tube.

In each experiment, the interval of each stimulation was over 10 sec and replication was done at least 15 times. EAG amplitudes were calculated after the responses to air were subtracted.

RESULTS AND DISCUSSION

1. Dose-response curve

Figure 2 shows the dose-response curves using the various components or mixture. Among the individual pheromone components and the mixture of the two components, the amplitude of EAG response in decreasing order was compounds A+B, A, and B, even though differences in the responses were not notable in higher doses, especially between compounds A+B and A. The responses to compound C were intermediate between those of compounds A and B but the responses to compound D were lower than the other compounds. Biological activities of these two related chemicals were analyzed in the field and neither had any synergistic effect (YUSHIMA et al., 1974; TAMAKI et al., 1976), but rather, an inhibitory effect on trap capture. The inhibitory effect was stronger in compound C than compound D (TAMAKI et al., 1976). This result coincides with the amplitude of EAG response obtained in this experiment.

AIHARA and SHIBUYA (1977) reported that compound B alone did not induce the single olfactory receptor cell response. But in this experiment, EAG responses were recorded with compound B and this result suggested the existence of receptor cells responding to that compound. It is possible that the reason they did not detect the receptors is that there is another type of sensory hair responding to compound B that is different from the trichodeum type sensory hair they examined.

2. Effect of mixture ratio

MINKS et al. (1974) reported that the amplitude of EAG response changed with the

Fig. 3. Effect of mixture ratio of compounds A and B on EAG response. Solid circles and hollow circles indicate total fixed amounts of 0.1 μg and 0.001 μg , respectively. Vertical bars show 95% confidence intervals.

Fig. 4. Effect of receptor adaptation by compound A or B on the EAG responses to the various amounts of each compound. A: response to compound A, B: response to compound B. Hollow circles, solid circles, hollow triangles, solid triangles, and squares indicate adaptation with 0.1 mg of compound B, with 1 mg of compound B, with 0.1 mg of compound A, with 1 mg of compound A, and without chemicals, respectively.

mixture ratio of two sex pheromone components in *Adoxophyes orana* and *Clepsia spectrana*. The highest responses were obtained at the mixture ratio corresponding to optimum attractancy ratio in the field in both species.

Figure 3 shows the effect of mixture ratio of compounds A and B on the EAG response. Compound B (0:10) resulted in lower response than the other mixture ratio. But the effect of mixture ratio on the EAG response was not notable and there was no difference between the most effective mixture ratio in the field and the others. In this experiment, no difference was observed between response to compound A and a mixture of compounds A and B in any ratio. This is inconsistent with the result in Fig. 1. The reason is not clear but it is possible that some effect was caused by sequential stimulations with equal doses of chemicals. AIHARA and SHIBUYA (1977) analyzed the effect of mixture ratio of compounds A and B on the single olfactory receptor response and reported that a 9:1 mixture elicited the highest impulse frequency. But at the EAG level, such a phenomenon was not observed in the present experiment.

DEN OTTER (1977) analyzed the single cell responses to the two sex pheromone components in *Adoxophyes orana* and concluded that there were two types of cells mainly

Fig. 5. EAG responses to compound A or B after continuous exposure to compound A or B air at two concentrations.

responding to each component. At the receptor cell level, there was no increase in the spike frequency at a given mixture ratio when the ratio was changed, and it was concluded that the mechanism distinguishing the mixture ratio depended on the central nervous system (DEN OTTER, 1977). In *Adoxophyes orana*, an increase in response was observed at the optimum attractancy ratio at the EAG level (MINKS et al., 1974) but not at the single cell level (DEN OTTER, 1977). On the other hand, it was observed at the single cell level (AIHARA and SHIBUYA, 1977) but not in the EAG level in *S. litura*.

3. Receptor adaptation experiment

Figure 4 shows the effect of continuous exposure of the antenna to the compound A or B air on the subsequent EAG response to the compounds themselves; in both cases, exposure resulted in a decreased response. The effect of a respective compound was stronger with 1 mg than with 0.1 mg. Compound A air suppressed the EAG response to compound B as well as that of compound A, but compound B air suppressed only the response to compound B. The effect of the compound B air on the EAG response to compound A was not clearly observed. The EAG response to compound A was increased with the amount of the compound when the antenna was exposed to the compound B air, but the responses were less than with no treatment at all (Figs. 4-A, 5).

The results showing that compound A or B in the air suppressed the EAG responses suggested that the receptor had been saturated by the compounds. The difference in effect of the two compounds was observed in their cross application. BAKER and ROELOFS (1976) analyzed the cross effects of each of the three components of the sex pheromone on EAG response in *Argyrotaenia velutinana* and suggested the existence of at least two different cells responding to two pheromonal components. Their conclusion coincided with the results of single cell recording (O'CONNELL, 1975).

In *Spodoptera litura*, the results indicated that there are two types of receptor cells which individually respond to compounds A and B. Both receptors were saturated by the respective compounds and were saturated by compound A; however, the receptor for compound A was not completely saturated by compound B. This indicated that a considerable number of receptors responding to compound A remained available when they were exposed to compound B, but that compound B receptors were saturated by both compound B and by compound A. AIHARA and SHIBUYA (1977) concluded that compound B alone hardly has the intrinsic activity to generate impulses

in the olfactory cells, but that it does have a synergistic effect on the perception of compound A at a single cell level. However, EAG responses were recorded by compound B in the present experiment, showing that there are receptor cells which respond to it; this therefore contradicts their conclusion. But the result of the cross application experiment showing that the compound B receptor was completely saturated by compound A indicates the possibility that the B receptor does not exist completely independent of the A receptor. It is possible that these results were caused by the chemical structures of the two compounds, but there is also the possibility of a cross effect between compounds A and B in the olfactory perception.

ACKNOWLEDGEMENTS

I am grateful to Drs. T. SHIBUYA and Y. AIHARA for their suggestions in the conduct of this experiment. Thanks are also due to Drs. Y. TAMAKI and J. INOUCHI for criticizing the manuscript.

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