Preliminary Report on Olfactory Neurons Specific to the Sex Pheromone of the American Cockroach. Minoru Yamada* (Fisheries Laboratory, Faculty of Agriculture, Nagoya University, Nagoya) Shoziro Ishii, Yasunawa Kuwahara (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto) Received February 15, 1968, Boiyn-Kagaku, 33, 37, 1968.

Introduction

Although the chemical structure of the sex pheromone of the female American cockroach, Periplaneta americana (L.) still remains in question1,2,3, the male adult of this species can detect the sex pheromone with its olfactory sense organ in the antennae4. However, the recording of the summed receptor potentials (electroantennograms) from the American cockroach showed that the sex attractant elicited electrical responses not only in the antennae of the male but also in those of the female and nymph5. The present experiments have been undertaken in order to study preliminary whether there are neurons specific to the sex pheromone in the olfactory center of the cockroach.

Materials and Methods

The adults of the American cockroach bred in the laboratory were used. An unanaesthetized cockroach was fixed with adhesive tapes and wire hooks on a cork plate so that the head could not be moved. The brain was approached dorsally through the frons, and the minimum amount of the overlying tracheation was removed. Recording of the electrical responses was made extracellularly by means of a glass capillary electrode filled with 3M KCl solution. The electrical resistance of the electrode ranged between 30MΩ and 50MΩ.

* Present address: Medical Center Duke University, Durham, North Carolina, U.S.A.

The electrical activity of the neurons in the deutocerebrum was fed into a cathode follower and then into a conventional d-c and a-c amplifier. Under these conditions it was possible to record the nerve activity for many hours or even one day. Stimulation was accomplished by puffing odorous substances contained in polyethylene squeeze-bottles.

The prepurified sex pheromone used in the present experiment was extracted by the following procedure modified from Wharton et al6. Some one hundred virgin females were fed on dog biscuits and water in a glass pot in which filter papers were placed. After two weeks the filter papers were soaked into 500ml of water. The water extract was steam-distilled until the distillate reached to 1000ml. After salting out the sex pheromone was extracted with 500ml of ether. The ether layer separated was kept at -20°C until use. This ether solution of the prepurified sex pheromone showed strong activity to elicit sexual response of the male cockroach when a filter paper impregnated with this solution was brought close to the cockroach, whereas no response occurred to the female and nymph. The concentrated crude sex pheromone dissolved in ether was dropped onto a filter paper and used as a stimulant after evaporation of the solvent. Odor stimuli were applied at regular intervals during the course of electrode penetration into the brain in order to ensure that the electrode did not miss the units that were not spontaneously active.
Results and Discussions

Figure 1 shows the examples of the single unit activity of the male cockroach. Propionic acid induced a positive slow potential (upward deflection) which was sustained during the stimulation, while the discharge of impulses was depressed. After the cessation of stimulation the units exhibited an increased activity (Fig. 1 A). It is interesting to note, on the other hand, that the prepurified sex pheromone (Fig. 1 B) and methylethyl ketone (Fig. 1 C) produced a negative slow potential (downward deflection) accompanied by a burst of activity which was followed by a silent period and a gradual increase to the normal background activity. These changes in activity may be explained in terms of hyperpolarization or depolarization of the membrane. These units that are sensitive to both the sex pheromone and other odors were frequently found in both male and female of this insect.

Figure 2 shows the responses of the highly specific neurons to odor stimuli. The recordings were made from the same male cockroach as that used in Figure 1. The unit was not spontaneously active, but responded vigorously to the sex pheromone with a slow negativity accompanied by a train of impulses during the

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Fig. 1. Responses of a neuron in the deutocerebrum of the male cockroach to propionic acid (A), prepurified sex pheromone (B), and methylethyl ketone (C). Upper tracings are AC recordings (calibration 2mV), and lower tracings DC recordings. Time calibration 200msec. The downward deflection represents negative potential at the recording electrode. The black bars below each tracing indicate the duration of the stimulation.

Fig. 2. Responses of a neuron in the deutocerebrum of the male cockroach to the prepurified sex pheromone (A) and methylethyl ketone (B), and those of the female cockroach to the prepurified sex pheromone (C) and methylethyl ketone (D). Calibration: 1mV, 200msec. in A and B; 0.5mV, 200msec. in C and D.
stimulation (Fig. 2 A). However, it failed to respond to other types of odor stimuli tested, for examples, propionic acid, acetic acid, benzyl acetate, p-dichlorobenzene, 2-methyl-2-butanol, acotophenone, geraniol, 1,2-dichloroethane, trichloroethylene, cycloheptanone, diethylsulphate, cyclopentanone, ethyl ether, methyl alcohol, methylethyl ketone. A neuron of the female also showed such a high specificity to the prepurified sex pheromone as in the male (Fig. 2 C and D). However, this unit showed spontaneous discharges. Because of the stimulations with the prepurified sex pheromone and the small number of odorous chemicals tested, it is difficult to draw the conclusion about the "Sex pheromone neuron". Although the present experiments were failed to elucidate that the sex pheromone elicits sexual response only to the male and not to the female and nymph by electrophysiological method in the neuron of deutocerebrum of both sexes, it is of interest that the present finding together with the responses at the receptor level, suggests a possibility that these extremely specific units in both sexes may play an important role in response to the sex pheromone.

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References