Effects of Venom from the Joro Spider on Insects

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It is said that a web spinning spider such as the joro spider (Nepila clavata) injects venom into a prey insect to paralyze it before predation. An active ingredient called Japanese spider toxin (JSTX) has been isolated from the venom glands of the joro spider and shown to be an irreversible suppressor of glutamate excitation in the neuro-muscular junction of lobster (Pilinurus japonicus) (Kawai et al., 1984). The structural study of JSTX so far has shown that it is a compound with a molecular weight of less than 1,000 (Yoshoka et al., 1984).

We were interested in the physiological activity of JSTX in real insects, mosquito larvae, German cockroach, tobacco cutworm and green rice leafhopper, which are kept in our laboratories.

MATERIALS AND METHODS

Venom glands collected from joro spiders were homogenized in equivalent volumes of water (1 gland/1 ml) by ultrasonification and the supernatant was lyophilized to give a JSTX sample.

The quantity of JSTX sample is expressed as the gland equivalent (GE) in numbers of glands.

From the stock cultures of 4 insect species maintained in our laboratory, insects at the following stages were selected and used for the experiments: first instar larvae of the mosquito, Culex pipiens molestus, male adults of German cockroaches, Blattella germanica, fifth instar larvae of the tobacco cutworm, Spodoptera litura, and adults of the green rice leafhopper, Nephotettix cincticeps.

Fig. 1. Method of electronic measurement of sucking motions of the green rice leafhopper. CW: copper wire, GR: glass ring, GW: fine gold wire, LH: green rice leafhopper, PF: stretched parafilm, RS: recording system, S: sucrose solution containing venom, SP: silver paste.

Five mosquito larvae were released into a glass tube (7 mm in diameter, 60 mm in height) containing 0.2 ml of aqueous solution of appropriate GE of JSTX. Dead and heavily intoxicated individuals were counted 3 hr after the release. Each test was repeated 3 times.

Cockroaches were administered with JSTX through three routes, i.e., injection, topical and oral applications. For injection, a solution of JSTX in 1 μl of water was injected into the thoracic body cavity of the cockroach under CO2 anesthesia using a microsyringe. For topical application, a solution of JSTX in 1 μl of 50% aqueous acetone was topically applied on the tergum of the thorax of the insects. For oral application, 1 μl of aqueous solution of JSTX was dropped on the mouthpart of the unanesthetized adult cockroach. The solution was soon swallowed. After the application, the cockroaches were confined individually in a polyethylene cup (6 cm in diameter, 4.5 cm in height), so that poisoning symptoms were easily observed.

A solution of JSTX in 1 μl of water was injected into the thoracic body cavity of an anesthetized tobacco cutworm larva. It was placed in the cup and its poisoning symptoms were observed.

A green rice leafhopper was placed on the para-
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Fig. 2. Effect of venom on the sucking motions of the green rice leafhopper. a: control, b1 and b2: 10 GE/ml solution, c: 50 GE/ml solution. S: salivation, I: ingestion of solution, R: resting, R’: abnormally prolonged rest.

film membrane covering a 5% sucrose solution containing JSTX and an electric charge of 1 V, 500 Hz was loaded between the insect and the solution using the apparatus as depicted in Fig. 1. As the insect inserted its stylet into the solution for sucking, electric current flowed through the circuit of the apparatus and was recorded on the charts shown in Fig. 2. The effects of JSTX on the insect’s sucking motion were analyzed by comparing the waveforms of the charts.

All experiments were performed at 23–25°C.

RESULTS AND DISCUSSION

In Calex mosquitoo, movement of larvae was depressed soon after the release into the venom solution. Five and 25 GE/ml of JSTX gave 100% mortality but 1 GE/ml, 0% mortality.

When 1 GE of JSTX was injected into the adult German cockroach under CO₂ anesthesia, apparent symptoms of intoxication appeared in all the individuals (ten adults in every dosage) as follows. As soon as the cockroaches recovered from CO₂ anesthesia (10 min following the injection), paralysis appeared in all the hind legs and the animals stayed motionless with legs folded for 30–60 min. This quiescence was a characteristic of the symptoms. They resumed normal posture 90 min after the injection and their normal movements by 120 min. On injection of 0.1 GE of JSTX, the same symptoms appeared but disappeared in a short time compared to the injection of 1 GE of JSTX. All insects resumed normal movement 90 min after the injection. No distinctive symptom was seen with injection of 0.01 GE of JSTX.

No symptoms of the poisoning appeared when 0.1 GE was topically applied. With the oral application of 0.1 GE of JSTX, the same symptoms appeared as shown with the injection, although the severity of paralysis was lighter. Several minutes after the application, walking activity was hindered and the paralysis remained in the hind legs of all individuals. Two to 3 cockroaches out of 10 showed serious paralysis in all legs, while the others recovered in 30–50 min.

On injection of 1 GE/μl of an aqueous solution of JSTX into tobacco cutworm larvae, light poisoning symptoms appeared in all the larvae treated. The jointed legs of the larvae were paralyzed such an extent that the larvae could not crawl normally. They recovered from the intoxication in 120 min. On injection of 0.1 GE, paralysis in the joint legs also appeared in 3 larvae out of 10 treated.

Figure 2 shows the time course of electric current changes made by the green rice leafhopper, each of which was fixed in an apparatus as depicted in Fig. 1 and fed with 5% sucrose solution without or with JSTX as a co-solute. The relationships of the motions of leafhoppers with specific forms of electric current waves have been well analyzed (Kawabe and McLean, 1980): S form wave refers to the salivation, i.e., the insertion of the stylet through the paraffilm to the solution; I form wave refers to the sucking of the solution, and R form wave to an interruption in sucking when the stylet is inserted into the paraffilm membrane.

On feeding with 2 GE/ml of JSTX no change was induced in the sucking behavior of the leafhopper. On feeding with 10 GE/ml of JSTX, elongation in R form waves (R’ stands for an abnormally long one) frequently appeared and finally the sucking was interrupted intermittently for several minutes at a time as shown in Fig. 2b. Other behaviors such as walking, however, were not affected. On feeding with 50 GE/ml of JSTX, R’ form wave appeared sooner than on feeding with 10 GE/ml (Fig. 2c).

JSTX has been shown to be a potent suppressor of the excitation by glutamate in the neuromuscular junction of the lobster’s leg (Kawai et al.,
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Since it is well established that neuromuscular junctions in insects are also mediated by glutamate, it is reasonable to propose that the paralytic symptoms of insects with JSTX administrations as described above are ascribable to inhibition of the neuromuscular junction to the motor muscles (Usherwood and Duce, 1985).

Very recently various glutamate receptor antagonists were successively discovered from venom of spiders, Argojine from Argiope lobata (Grishin et al., 1986), Argotoxin 622 and 636 from Argiope trifasciata and Araneus gemma (Usherwood et al., 1987), JSTX-3, -4 and -5 from Nophila clasata (Aramaki et al., 1986), and NSTX (New Guinean spider toxin) from Nophila maculata (Aramaki et al., 1986). Although available information so far is limited, it is interesting to note that there are common features among these substances such as high solubility in water, fairly low molecular weight near 600 and, most of all, antagonistic activities against glutamate receptor. Since no activity on real insects has been reported for them, we are interested in the application of our assay on these toxins.

To summarize the present results, the Culex mosquito, the German cockroach and the green rice leafhopper are concluded to be feasible and handy tools for assaying spider venom, because of the distinctiveness of poisoning symptoms detectable on respective administrations of at least 1 GE/0.2 ml, 0.1 GE, 0.5 GE/50 ml of the venom to the insect.

REFERENCES


Effect of Rearing Humidity of Host Insects on the Spore Types of Entomophaga mainaiga Humber, Shimazu et Soper (Entomophthorales: Entomophthoraceae)

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In studies on entomophthoraceous fungi, it is essential to know their life cycles in order to elucidate the mechanism of their prevalences. To clarify their life cycles, an understanding of conditions influencing formation and germination of resting spores is necessary. I experienced that the spore types of Entomophaga mainaiga on the gypsy moth, Lymantria dispar, vary from one insect rearing container to another, and this led to the hypothesis that differences in humidity of the rearing containers may affect spore types. To confirm this hypothesis, the effects of humidity on spore types in infected host insects were investigated using dry and humid rearing chambers.

Gypsy moth, Lymantria dispar and three-spotted pluza, Acantholupia agnata were used in this study as the experimental insects. The larvae of L. dispers used for the experiment were a laboratory reared strain derived from Ibaraki Prefecture. Those of A. agnata were kindly supplied by Dr. Kenjiro Kawasaki of the National Institute of Agro-Environmental Sciences, and reared continuously in my laboratory. Two isolates of E. mainaiga, F-495 from Ishikawa Prefecture and F-541-2 from Ibaraki Prefecture were used for the