REFERENCES

Peristaltic Movement and Its Suppression by Chloridimeform, Ipronia-i'd and 2-Amino-4-Phosphonobutyric Acid of the Spermiduct in the Cabbage Armyworm, Mamestra brassicae (Lepidoptera: Noctuidae)\(^1\)

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Peristalsis of the spermiduct (vasa deferentia) is an important function, as it is required to propel the mature, elongated cysts of spermatozoa into storage organs (SZÖLLÖSI and LANDUREAU, 1977). This peristaltic movement was observed in vivo at the end of the pupal stage in Samia cynthia (SZÖLLÖSI and LANDUREAU, 1977) and in Ephesia kühniella (NOWOCK, 1973). In the previous report, SHIMIZU (1986) observed that the occurrence of myogenesis and muscle differentiation by cultured tissue derived from the cultivated spermiduct of diapausing Mamestra brassicae pupae, and suggested that peristalsis of the spermiduct seems to be a myogenicity. However, it is not clear whether peristaltic movements of the spermiduct are controlled by the nervous system in vivo (neurogenicity).

In the present in vivo study, we observed peristaltic movements of the spermiduct in the adult cabbage armyworm, Mamestra brassicae, and found that two monoamine oxidase inhibitors suppressed its peristalsis.

MATERIALS AND METHODS

Insect. Larvae of the cabbage armyworm, Mamestra brassicae, were reared on an artificial diet (AGUI et al., 1975; SHIMIZU and FUKAMI, 1983) at 25°C under a long day photoperiod to obtain non-diapausing male pupae. Day 0 pupae to adults at day 4 after eclosion were used in the present experiments.

Chemicals. L-Glutamic acid and two monoamine oxidase inhibitors were used; chloridimeform (KUWAHARA, 1978; BEEMAN and MATSUMURA, 1973; AZIZ and KNOWLES, 1973; BENEZET and KNOWLES, 1980; GRIFONI et al., 1977), iproniazid (KUWAHARA, 1978; HOLDEN and HADFIELD, 1975; BEEMAN and MATSUMURA, 1973). L-Glutamic acid monosodium salt, chloridimeform hydrochloride, iproniazid phosphate and 2-amino-4-phosphonobutyric acid were prepared at 1,000, 500, 100, 1 and 0.1 ppm with RINGER solution (pH 6.5). These prepared solutions were introduced into a small assay vessel. Chloridimeform hydrochloride was obtained from Nihon-Nohyaku Co., Ltd., Tokyo, Japan. L-Glutamic acid monosodium salt and iproniazid phosphate were purchased from Wako Pure Chemical Industry Co., Ltd., Japan. Two-amino-4-phosphonobutyric acid which acts as a glutamate antagonist (CULL-CANDY et al., 1976) was purchased from Sigma Chemical Co.

Measurement of spermiduct movements. The experimental animals were dissected from dorsal side to the terminal abdominal segments under the RINGER solution, where the testis and its spermiduct were removed and transferred into a small assay vessel. The testis was inserted and fixed with an insect pin. The local movements of a port of entrance of spermiduct (proximal part of the vasa deferens; indicated by an asterisk in Fig. 1) were counted.

Fig. 1. Schematic representation of the adult spermiduct of Mamestra brassicae. T = testis, S.P = spermiduct, L = part of ligation.

Fig. 2. Peristaltic movements of the spermiduct in Mamestra brassicae at different developmental stages. P₀ = just pupation, EP = eye pigmentation, A₀ = just after eclosion (adult). Each value is mean of 10 animals.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Non treatment</th>
<th>Concentrations (ppm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Chlordimeform</td>
<td>7.5±0.5</td>
<td>8.0±1.4</td>
</tr>
<tr>
<td>Iproniazid</td>
<td>8.3±1.1</td>
<td>7.0±1.0</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>13.5±0.7</td>
<td>17.0±0.0</td>
</tr>
<tr>
<td>2-Amino-4-phosphonobutyric acid</td>
<td>7.5±0.7</td>
<td>9.0±0.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> The spent time per 10 peristaltic counts. Mean±S.D. was represented by three separate determinations.

<sup>b</sup> N.M.(R): non movement, but recovered by washing with drug-free RINGER solution. Very slow movements were indicated as N.M.

under a microscope and the total time per 10 peristaltic counts was measured. Measurements were carried out three times on the same sample and repeated on three or four separate preparations. The movements of the whole spermiduct (enlarged spermiduct in Fig. 1) were recorded by a transducer by tying the spermiduct with a thread to an arm of a transducer.

RESULTS AND DISCUSSION

Figure 2 shows the relationship between developmental stage and the peristaltic movements of the spermiduct in pupae and adults. Just after pupation to before eye pigmentation, the movements and enlargement of the spermiduct were not observed. Later, active peristaltic movements of the well developed spermiduct were observed in all individuals during eye pigmented pupal stage. The imaginal differentiation of the vasa deferentia depends on endogenous ecdysone (Nowcock, 1973). This differentiation in the presence of ecdysone has been demonstrated in vitro (Szöllös and Landureau, 1977; Shimizu, 1986).

Local peristaltic movements of the spermiduct in the adult (indicated as an asterisk in Fig. 1) were inhibited by chlordimeform and iproniazid. Chlordimeform and iproniazid showed inhibitory movements of the spermiduct at concentrations
of 500 and 1,000 ppm, respectively (Table 1). L-Glutamic acid and its antagonist, 2-amino-4-phosphonobutyric acid solution also inhibited the local peristaltic movements.

The movement of complete spermiduct of the adult was about 7–8 times/min (Fig. 3), while the mean number of peristaltic movements per minute of the newly formed muscles on the glass surface of the culture vessel was 10.5 ± 6.1 (SHIMIZU, 1986). This number in vitro corresponded with that in Fig. 3, in vivo. As indicated in Table 1, the local movement of the spermiduct was 9.8 ± 0.8 sec/10 times. This number may induce a peristaltic movement of the entire spermiduct.

Next, suppression effects of monoamine oxidase inhibitors on peristaltic movements of the intact spermiduct in the adult were investigated. As shown in Fig. 4, spontaneous peristaltic movements of the spermiduct were suppressed by 1,000 ppm chlordimeform or 1,000 ppm iproniazid solution, but they recovered after being washed with drug-free Ringer solution. Similar results were observed in suppressive peristaltic movements of the isolated gut of the American cockroach, Periplaneta americana, after treatment with chlordimeform (1 × 10−3 M) (SHIMIZU et al., 1985). Chlordimeform and iproniazid suppressed spermiduct movement and are well known inhibitors of monoamine oxidase. However, the inhibition of monoamine oxidase is not considered to be the primary action (target) of chlordimeform in producing toxicity in insects (BEEMAN, 1982). In addition, since 500 ppm of chlordimeform is a high concentration, the inhibition of gut (SHIMIZU et al., 1985) and spermiduct movements are probably not important toxicity-action.

Although L-glutamic acid has been proposed as a candidate for excitatory transmitter at the neuromuscular junctions of insects (BRERANEK and MILLER, 1968; Usherwood and Machili, 1968), it is not clear whether autonomy of peristaltic movements depends on a neurogenicity or myogenicity and both in the present experiment. Nevertheless, chlordimeform, iproniazid and 2-amino-4-phosphonobutyric acid could inhibit both local movements in the proximal part of the vas deferens and total peristaltic movements of the spermiduct of Mamestra brassicae. If iproniazid is injected into the male adult’s abdomen, they might be failure to transport the sperm to the female.

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