STUDIES ON THE THORACIC STRETCH RECEPTOR ORGAN OF *MANDUCA SEXTA* AND EFFECTS OF OCTOPAMINE AND DEMETHYLCHLORDIMEFORM ON THE ACTIVITY OF THE STRETCH RECEPTOR

by

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INTRODUCTION

Stretch receptors are found in nearly all orders of the Insecta. Their discovery followed descriptions of the better known sensory end organs in the musculature of vertebrates, namely, the muscle spindles. Not until Alexandrowicz (1951) discovered the dorsal stretch receptor organs of Crustacea was it known that similar proprioceptive organs exist in the Anthropoda. Since then, stretch receptor organs have been found in many orders of insects, including locusts and grasshoppers (Slifer and Finlayson, 1956), saturniid moths (Finlayson and Lowenstein, 1955, 1956) and cockroach, dragonfly, honeybee and several species of giant silkmoths (Finlayson and Lowenstein, 1958), and seven other orders (Osborne and Finlayson, 1962).

Although dorsal stretch receptor organs have been identified in many orders of arthropods, those written about were primarily abdominal stretch receptor organs. Less is known about thoracic stretch receptor organs, although they have been found in the stick insect Carausius morusus (Slifer and Finlayson, 1956), the larva of the silkmoth Antheraea pernyi (Finlayson and Lowenstein, 1956, 1958) and the larva of Dictyopterygella recta (Osborne and Finlayson, 1962). Finlayson and Lowenstein were unable to find the receptor organ in thoracic segments of the pupal and adult stage of the silkmoth. In Acrididae (grasshoppers and locusts), Slifer and Finlayson did not find the intersegmental thoracic receptor organs either.

The purpose of this thesis is to determine if the stretch receptor organ is present in thoracic segments of the lepidopteran Manduca sexta

and if the stretch receptor organ remains functional through metamorphosis. The effects of the biogenic amine, octopamine (OA) and the formamidine insecticide, demethylchlordimeform (DCDM) on the sensory output of the stretch receptor was also determined.

The thoracic stretch receptor provides a convenient preparation for assessing the actions of biogenic amines and insecticides that affect behavior. Octopamine is found widely in the nervous system of invertebrates (Orchard, 1982) and is thought to be as important to the nervous system of insects as norepinephrine and epinephrine are to that of vertebrates (Evans, 1981). It is thought to be responsible for the general arousal of the insects by increasing the metabolic rate of the cells (Hollingworth and Lund, 1982).

Octopamine has been implicated as a neurotransmitter in the elicitation of the firefly lantern flashing (Nathanson, 1979) and possibly in the regulation of the release of hyperlipaemic hormone in the glandular lobe of the corpora cardiaca of the locust (Orchard and Loughton, 1981b). In the cockroach and locust, it functions as a neurohormone, stimulating lipid release from fat body (Gole and Downer, 1979; Orchard et al., 1982), stimulating glycogenolysis (Robertson and Steele, 1972, 1973) and increasing the rate of glucose oxidation (Candy, 1978). Octopamine also functions as a neuromodulator, modulating activity in the extensor-tibiae muscle of locust (Evans and O'Shea, 1977, 1978; O'Shea and Evans, 1979), the dorsal longitudinal muscle of Manduca sexta (Klaassen and Kammer, 1985; Fitch and Kammer, 1986; Klaassen et al., 1986), the stretcher and opener muscle of the crayfish (Jacobs and Atwood, 1981; Fischer and

Florey, 1983) and the crustacean heart (Florey and Rathmayer, 1978).

The formamidine insecticide, chlordimeform (CDM) has novel pesticidal activity primarily on Lepidoptera and Hemiptera. The behavioral effects are sublethal and in general increase the excitability and hyperactivity of the insects. In vivo, CDM is activated by N-demethylation and is converted readily to demethylchlordimeform (DCDM) (Atkinson and Knowles, 1974). In lepidopteran larvae, the increased excitability and locomotor activity will cause the larvae to fall from the treated plant, and death results through starvation and/or dehydration (Lund et al., 1979b; Streibert and Dittrich, 1977). In adult Manduca sexta, CDM induces wing beating and subsequently incoordinated attempts at flight (Kinnamon et al., 1984). This can go on for hours until the insect dies from exhaustion and stress (Lund et al., 1979b).

The action of CDM on behavior closely resembles that of OA. In crayfish, CDM induces a postural response like that activated by OA (Hollingworth and Lund, 1984). In the peripheral nervous system of insects, CDM or DCDM mimics the effect of OA in locust extensor-tibiae muscle (Evans and Gee, 1980), firefly light organ (Lund et al., 1979a; Hollingworth and Murdock, 1980), locust corpora cardiaca (Singh et al., 1981) and locust fat body (Orchard et al., 1982). Both CDM and DCDM elevate adenylate cyclase activity in the nerve cord of the American cockroach, an action also attributed to OA (Gole et al., 1983). Although the evidence seems to suggest that the formamidine CDM and its analog DCDM act on OA receptors, they have been known to mimic the effects of other biogenic amines (Osborne, 1985; Schmidt et al., 1981; Sauer and

Essenberg, 1984).

In the present study, the effects of OA and DCDM on the sensory output of the thoracic stretch receptor was investigated by superfusing the receptor organ with saline containing various doses of OA and DCDM. The results show that DCDM mimics the action of OA on the stretch receptor organ.

MATERIALS AND METHODS

Larvae of Manduca sexta were fed the diet of Baumhover et al. (1977). After pupation, the animals were maintained on a 16 hr light/8 hr dark cycle simultaneously with a 26°C day/23°C night temperature regime. The pupal stage lasted 18 to 20 days. The age of pharate moths was estimated on the basis of external morphological characteristics (Kammer and Kinnamon, 1979).

Larvae of the 4th and 5th instar, pupae of Day 0 through Day 10 and adults were used for this study.

Dissection

The thoracic stretch receptor organ of the larva of Manduca sexta was exposed by making a traverse cut through the body wall a few segments posterior to the last thoracic segment, pinning the animal right side down and cutting along the spiracles on the left side. After the gut has been removed, the IIN1b nerve from the mesothoracic ganglion was followed until the stretch receptor organ was detected. The lateral body wall of the thoracic region of the pupa was cut and the gut removed. The same procedure was used to look for the stretch receptor organ. In the adult, the thorax, after removing the abdomen, head, wings and legs, was cut slighty off the midline to produce two preparations. The IIN1b nerve was followed to see if the stretch receptor organ was present.

Methylene Blue Staining

Leuco-methylene blue solution was injected into live animals until the abdomen was slightly distended (Stark et al., 1969). The leuco-methylene blue solution, on being oxidised, will selectively stain the

nervous tissue a dark blue color. After 1 to 2 hours at room temperature or 12 to 24 hours in the refrigerator, the injected animals were dissected as described above. Tissues were fixed in cold, saturated ammonium molybdate solution for photography or for observations under the compound microscope. The tissues were mounted using Gurr's Hydromount.

Electrophysiological Recording

To determine the electrical activity of the stretch receptor in response to stretch, a loop of the exposed IIN1b nerve near the ganglion was sucked up a glass electrode. Peripheral branches of the nerve, except the one to the stretch receptor, were cut. The connection to the mesothoracic ganglion was also cut to eliminate action potentials coming from the central nervous system.

The stretch receptor organ was stretched by pulling the body wall in a direction parallel to the muscle fibers of the stretch receptor organ by using hooks attached to a glass rod mounted on a Narishige manipulator. The amount of stretch was increased or kept constant by adjusting the manipulator. All the experiments were performed at room temperature (23-25°C). The dissected preparations were kept moist with a saline solution (pH 7.0) composed of 25 mM NaCl, 25 mM potassium methanesulphonate, 4 mM $CaCl_2$, 33 mM $MgCl_2$, and 150 mM Tris methanesulphonate (modified after Rheuben, 1972).

Effects of Octopamine

The effect of OA on the stretch receptor was examined by recording the frequency changes after application of 10^{-5} M OA dissolved in saline. The recording procedure described above was employed. After the

frequencies at different amounts of stretch were determined, the relaxed preparation was superfused with 10^{-5} M OA for 10 minutes at a flowrate of 1.5 ml/min. Then the frequencies were again determined at the previous amounts of stretch. For control, the same procedure was done on other preparations, only this time larval saline was superfused for 10 minutes instead of the 10^{-5} M OA.

To examine the effects of different concentrations of OA, the same procedure as above was employed, with the preparation being superfused at three concentrations: 10^{-8} M, 10^{-7} M, and 10^{-5} M. The same preparation was exposed to all three concentrations, always in this order.

Effects of DCDM

The effects of DCDM on the stretch receptor output was studied using the same procedure as that used in the octopamine study. The preparation was superfused with 10^{-8} M and 10^{-5} M DCDM. For control, larval saline was used.

RESULTS

Disappearance of Thoracic Stretch Receptor Organ During Metamorphosis

Anatomical studies using a dissecting microscope and methylene blue staining showed that the stretch receptor organ was present in the larval stage and up to Day 4 of the pupal stage of Manduca sexta. In Day 5 pupae, the stretch receptor organ seemed to be in the process of disintegrating. Examination of the thorax of Day 6 to Day 10 pupae revealed no stretch receptor organs present. Adults gave the same negative results as the Day 6 to Day 10 pupae. The number of animals dissected for this study were over 20 larvae, 29 pupae, and 12 adults. Details of these observations are summarized in the following paragraphs.

Nomenclature for nerves of larvae is an adaptation of that for the adult (Nüesch, 1957), and the thoracic muscles were identified according to the scheme of Tsujimura (1983). The description of the thoracic larval muscles of Manduca sexta by Eaton (1982) did not correspond to the muscles I observed.

In the larva, there is a pair of stretch receptor organs in the meso- and metathoracic segments. Each one is found against the lateral body wall and there is always a lobe of fat-body connected to the central region of the stretch receptor organ. This connection to a fat-body lobe has also been observed in Antheraea pernyi (Finlayson and Lowenstein, 1958).

Each stretch receptor organ consists of a modified muscle fiber with sensory and motor innervation. The muscle fiber is anchored to the body wall of the larva by connective tissue on either end and is parallel to

the longitudinal muscles (Figs. 1 and 2). Under the compound microscope, the muscle fiber showed cross striations although the striations are less conspicuous than those in ordinary muscle fibers.

The sensory and motor innervation of the muscle fiber can be seen using the dissecting microscope on the methylene blue stained preparations. The IIN1b nerve from the ventral nerve cord can be seen to branch a short distance after traversing over a large trachea (Figs. 1, 2, and 3). One branch, designated the superficial branch in relation to a medial view of the body wall, provides the motor innervation of the modified muscle fiber; it joins the muscle fiber near its posterior end. The nerve branch that goes deep under the dorsal internal oblique muscle provides the sensory innervation near the anterior end of the muscle fiber. The cell body of the sensory neuron is enlarged and lies in a body of connective tissue which is silvery white and easily seen in the stained preparation (Fig. 3).

In Day 0-4 pupae the meso- and metathoracic stretch receptor organs are similar to those found in the larvae, although the nearby muscles are disintegrating. The disintegration can be inferred by the increased presence of fat and viscous fluid in the animal, and the decrease in the number of intact muscles. In Day 5 pupae, a muscle fiber was seen in the exact position of the muscle fiber of the stretch receptor organ in younger pupae, but no nervous innervation was observed. The two branches of the IIN1b nerve can still be seen but they are not connected to the muscle fiber. The muscle fiber is attached to the body wall by connective tissue and can be seen clearly amid the disintegrating larval

Fig. 1. Portion of the right side of the mesothoracic segment of a larva of Manduca sexta.

A. Gut removed with no muscles cut. Nerve IIN1b can be readily identified as it passes medial to a large tracheal trunk.

B. Dil and dio muscles cut to reveal stretch receptor organ, which lie lateral to dil and dio.

T - Trachea

IIN1b - IIN1b nerve

MF - Muscle fiber of stretch receptor organ

SR - Stretch receptor

D - Deep

S - Superficial

dil - Dorsal internal longitudinal muscle

dio - Dorsal internal oblique muscle

lip_1 - Lateral internal posterior muscle

lia_1, lia_2 - Lateral internal anterior muscle

Fig. 1A

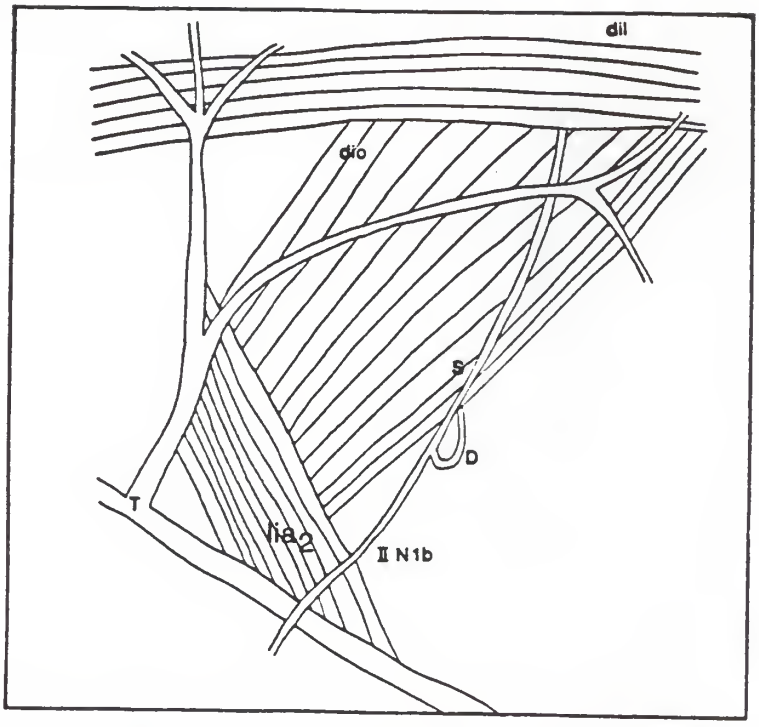


Fig. 1B

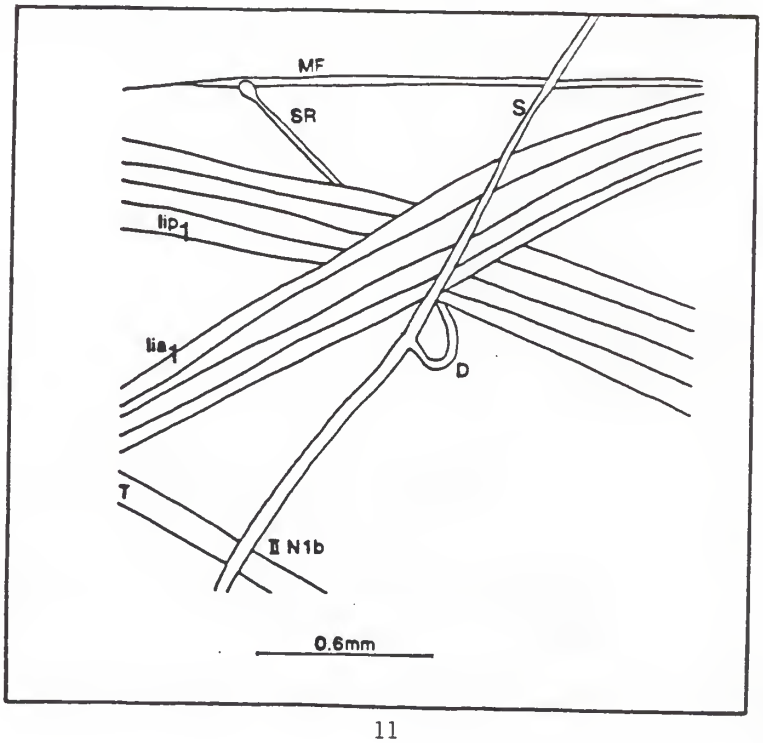


Fig. 2. Portion of the right side of the mesothoracic segment with all the muscles omitted to reveal the stretch receptor organ and its sensory and motor innervations.

T - Trachea

MG - Mesothoracic ganglion

MF - Muscle fiber

CB - Cell body

MN - Motor neuron

SN - Sensory neuron

D - Deep

S - Superficial

Fig. 2

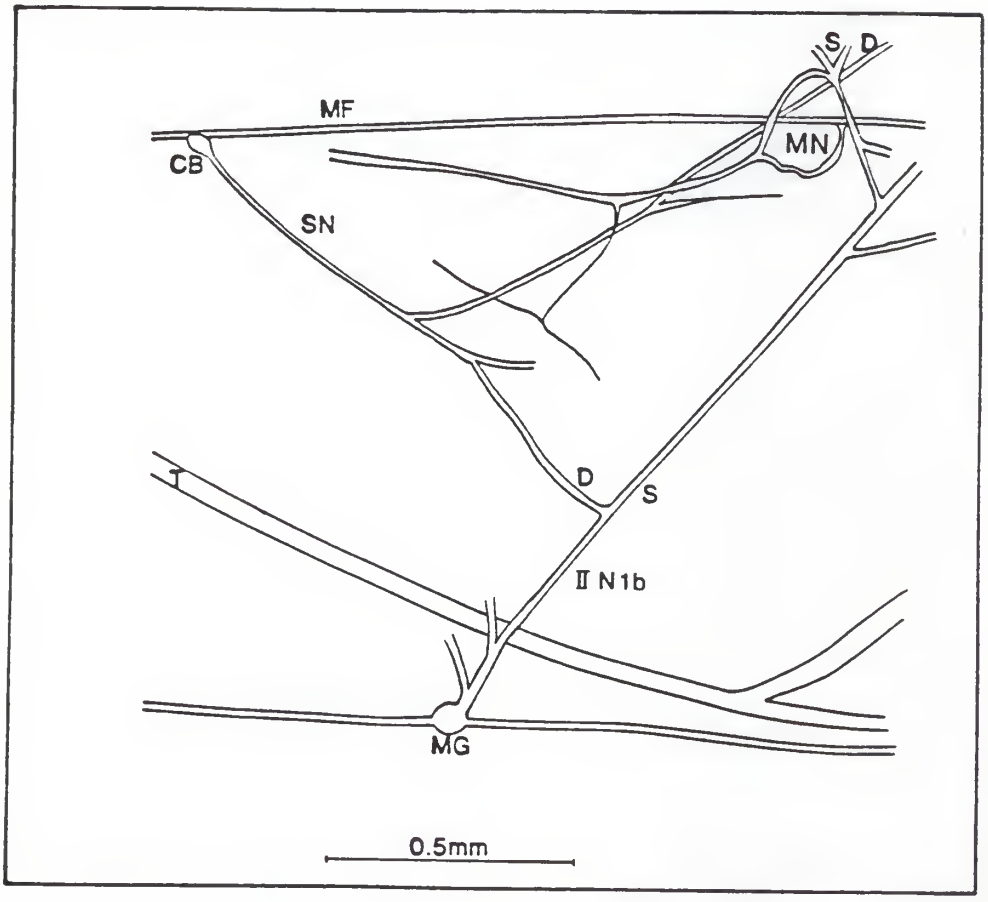


Fig. 3. A stretch receptor organ of a 5th instar Manduca sexta.

A. Photograph of the stretch receptor organ.

B. Diagramatic representation of the stretch receptor organ in the above photograph.

MF - Muscle fiber

CB - Cell body

SN - Sensory neuron

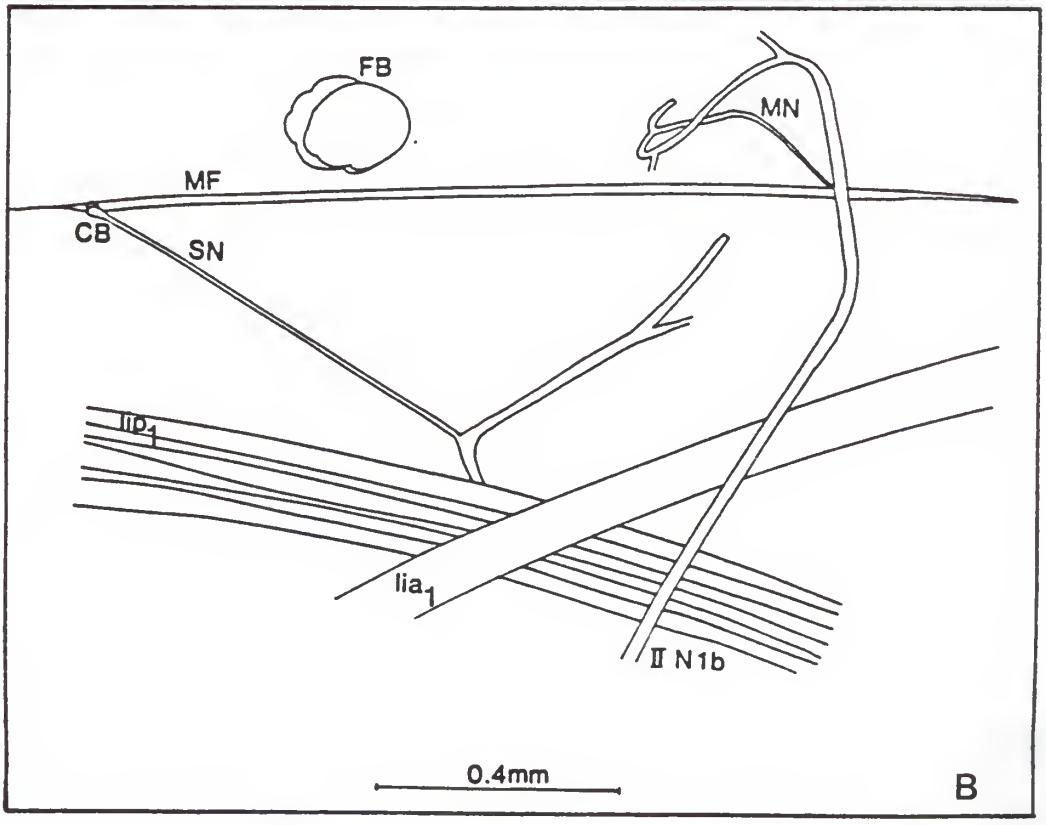
MN - Motor neuron

FB - Fat body

lia_1 - Lateral internal anterior muscle

lip_1 - Lateral internal posterior muscle


A


FB
MN
MF
CB
SN
IIp1
IIa1
II N1b
0.4mm
B

muscles. These observations suggest that the innervation of the stretch receptor organ disintegrates first, leaving the muscle fiber, which then disintegrates by Day 6.

It was found that the amount of time between injecting the animal and dissecting it to give the optimal amount of staining is related to the condition of the body muscles. In early pupae, in which the larval muscles are still intact, and in late pupae, in which the adult muscles are formed, treatment with methylene blue for about 20-24 hours in the refrigerator is required to get a good stain. In pupae in which the muscles are disintegrating, treatment for 24-28 hours is required to get the optimum staining.

During the early pupal stage, the thoracic ventral nerve cord shortens. The distances between the three thoracic ganglia decrease at different rates. There does seem to be a relationship between the time of disappearance of the thoracic stretch receptor organs and the decrease in distances between the ganglia. The thoracic stretch receptor organ is not found in Day 6 pupae while the mesothoracic and metathoracic ganglia appear to fuse on Day 6 also. There is no measurable separation between the mesothoracic and metathoracic ganglia in the adult.

Thoracic Stretch Receptor Output

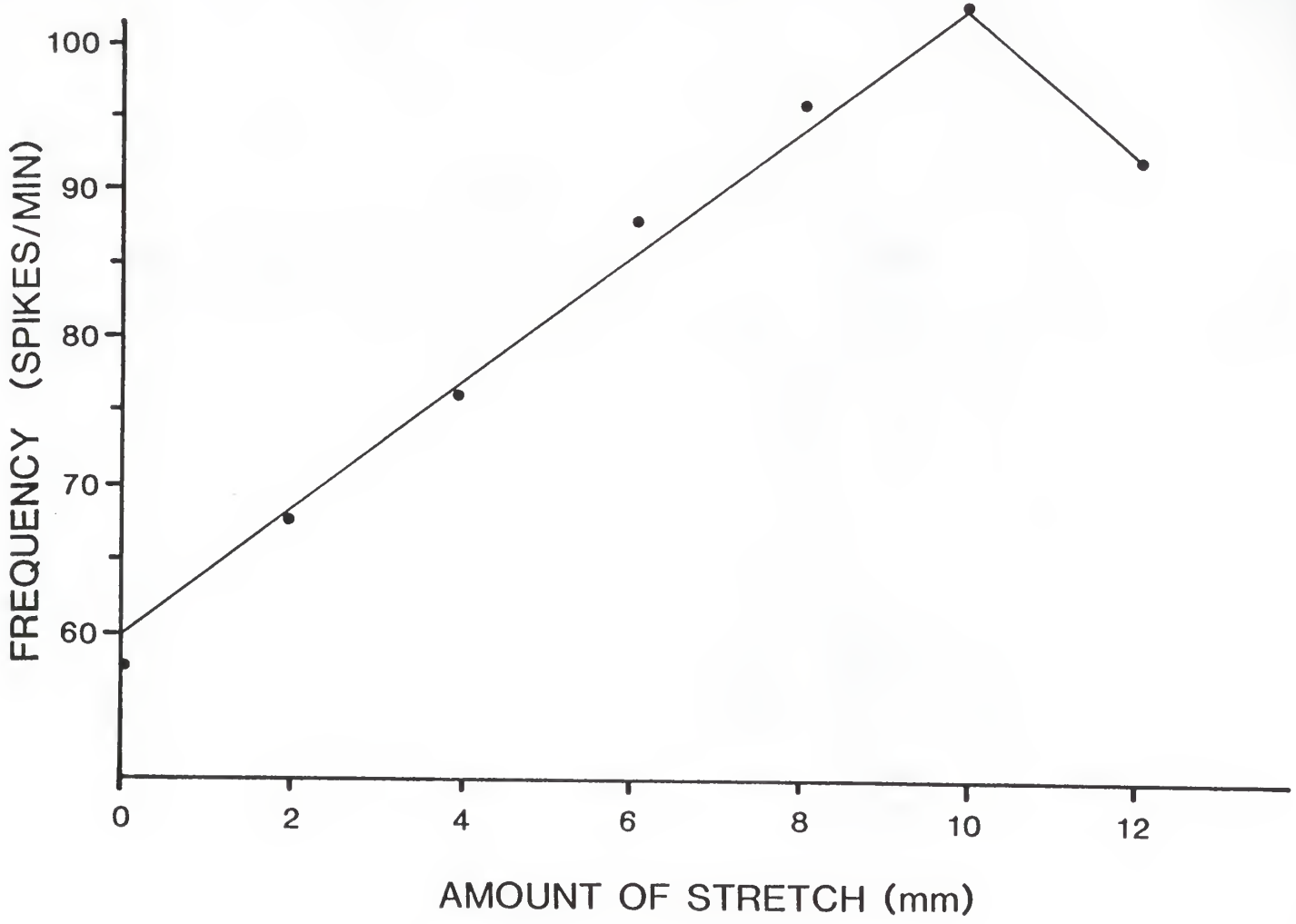
In Manduca sexta larvae, the body wall had to be stretched to a minimum tension before there was a regular discharge frequency. Since this stretch appeared to be different in the individual larvae, the initial stretch, 0 stretch, was defined as the minimum stretch that gave a reasonably regular discharge frequency. Subsequent frequency readings

were then taken at different lengths relative to the initial position.

Immediately after an increase in length was applied to the stretch receptor organ, there was an increase in discharge frequency. The discharge frequency quickly dropped to a lower frequency during maintained tension, i.e. the receptor organ adapted. On return to the 0 stretch position, the discharge frequency was lower than that originally obtained at 0 stretch. These responses are typical of the abdominal stretch receptors in the Lepidoptera, Odonata, Orthoptera and Hymenoptera studied by Finlayson and Lowenstein (1958).

In the Manduca thoracic stretch receptor organ, the adapted stimulus-response relationship was linear up to a certain amount of stretch (Fig. 4). Beyond this position, the discharge frequency began to fall. A similar response was observed by Finlayson and Lowenstein (1958), and they attributed it to 'over-stretch', i.e., the receptor had become extended beyond its physiological range. The linear relationship was observed only in preparations in which the CNS connections were cut. This behavior has been observed previously in many studies of the physiology of the stretch receptor organ in insects where the stretch receptor organs were isolated from the insect (Finlayson and Lowenstein, 1958) or isolated from the CNS (Weevers, 1966; Pearson et al., 1983). In preparations where the CNS was intact, input from the ganglia alters the tension in the muscle fiber of the stretch receptor organ, which therefore produces impulses at a frequency that is not linearly related to the stimulus.

Fig. 4. Frequency of spiking activity in IIN1b in response to stretch of the stretch receptor organ. Note linearity up to a maximum amount of stretch and subsequent fall in discharge activity due to 'over-stretch'. Data, which are from one experiment, are representative of results from five similar experiments. Each point is a mean of five readings, with a minute range of variation.


100
90
80
70
60
FREQUENCY (SPIKES/MIN)
0
2
4
6
8
10
12
AMOUNT OF STRETCH (mm)

Recordings from the sensory nerve of the thoracic stretch receptor organ in the Day 1-4 pupae were similar to those from larvae in which no stretch was applied. The effect of stretch on the stretch receptor organ in the pupa was not studied because of the difficulty of stretching the thoracic body wall, which is now covered with inextensible cuticle.

Effects of Octopamine (OA) on the Thoracic Stretch Receptor Output

To see if OA has any effect on the output frequency of the stretch receptor in response to stretch, 10^{-5} M OA was applied to the relaxed preparation. Before the application of OA, the output frequency was determined at different stretches after superfusion with larval saline. Subsequent changes in stretch included going back to the relaxed position between readings. Finlayson and Lowenstein (1958) found that this method is complementary to the method in which the stimulus was applied in a step-by-step increase in stretch, giving results which are comparable.

OA at a concentration of 10^{-5} M decreased the discharge frequency of the Manduca thoracic stretch receptor in response to stretch with occasional bursting activity (Fig. 5) in five preparations. At 0 stretch, OA resulted in a decrease of between 46 percent to 89 percent in the discharge frequency. A t-test comparing the responses before and after the application of OA at 0 stretch indicates that the difference in discharge frequency is significant at the $p < 0.001$ level. At a stretch of 2 mm, the decrease in discharge frequency is significant at the $p < 0.025$ level, whereas at a 4 mm stretch the decrease is significant at $p < 0.05$ level. Again, it should be pointed out that the 0 stretch is arbitrary and comparisons between preparations have to be made with the

caution that the 0 stretch might be at any point on the physiological range.

Figure 6 shows the response to OA at three different molarities. As can be clearly seen, increasing the molarity of OA further decreases the response of the stretch receptor to a given stretch. The decrease in output frequency was consistent at different amounts of stretch; this effect was seen in seven preparations. Since the same preparation was superfused with the three molarities of OA, it is possible that there could be a cumulative effect at each increment of molarity. No attempt was made to wash the preparation with saline after superfusion with each molarity because the process would have taken too long and the preparation would have deteriorated. As it was, many preparations were discarded because of irregular or no responses after some time.

An analysis of variance test done on the results of the experiments at 0 stretch indicated that the decreases in response are significant at the $p < 0.0006$ level. T-tests (Least Significant Difference) done on the results at 0 stretch indicate that the OA treatment was significantly different from the saline treatment at all three molarities of OA. The responses to the treatments with 10^{-8} M OA and 10^{-5} M OA were significantly different. There was no significant difference between the responses to treatment with 10^{-8} M OA and 10^{-7} M OA. Neither was there a significant difference between the responses to treatment with 10^{-7} M OA and 10^{-5} M OA.

Fig. 5. Spiking activity in IIN1b in response to stretch of the stretch receptor organ before and after superfusing with 10^{-5} M OA. OA decreased the response to stretch. Data are representative of seven similar experiments. Each point is a mean of five readings, with a minute range of variation.

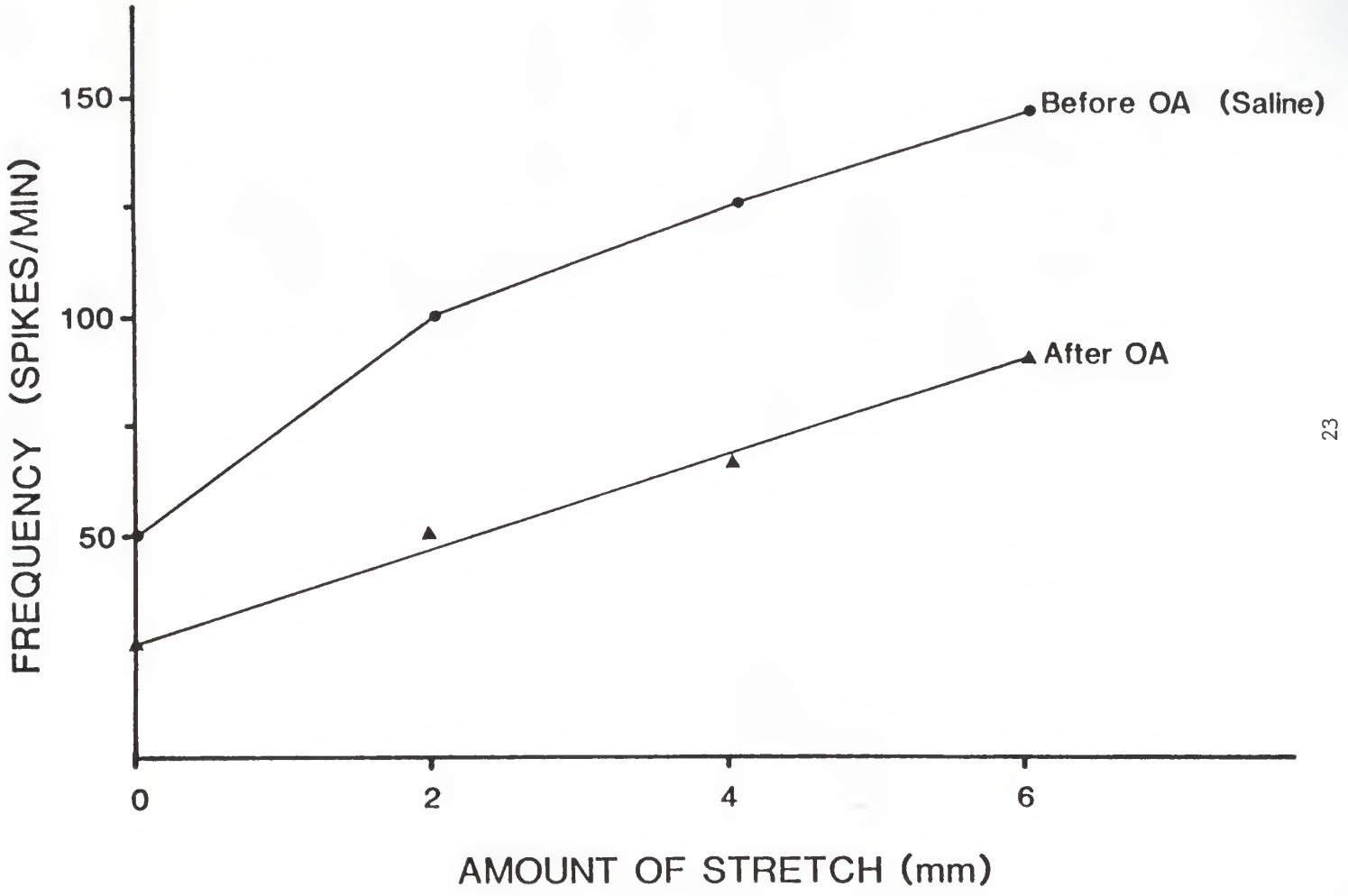

150
100
50
FREQUENCY (SPIKES/MIN)
Before OA (Saline)
After OA
0
2
4
6
AMOUNT OF STRETCH (mm)

Fig. 6. Effect of OA on the response of the stretch receptor organ to stretch. Increasing doses of OA decreased the response. Increased stretch resulted in increased response, which is consistent at the different molarities of OA. For control, the preparations were superfused with saline. Numbers above the bars indicate the number of preparations used to calculate the mean frequency.

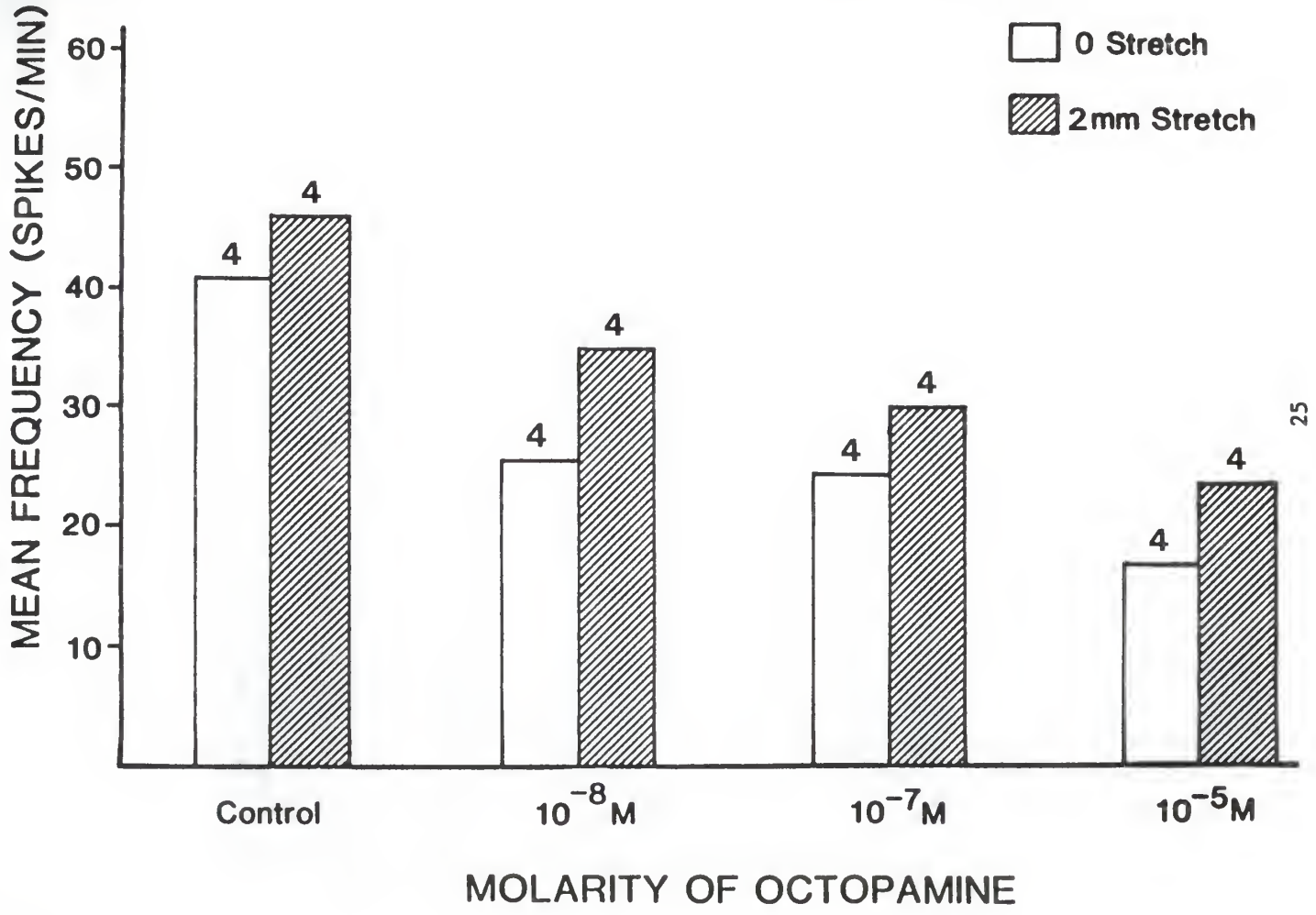
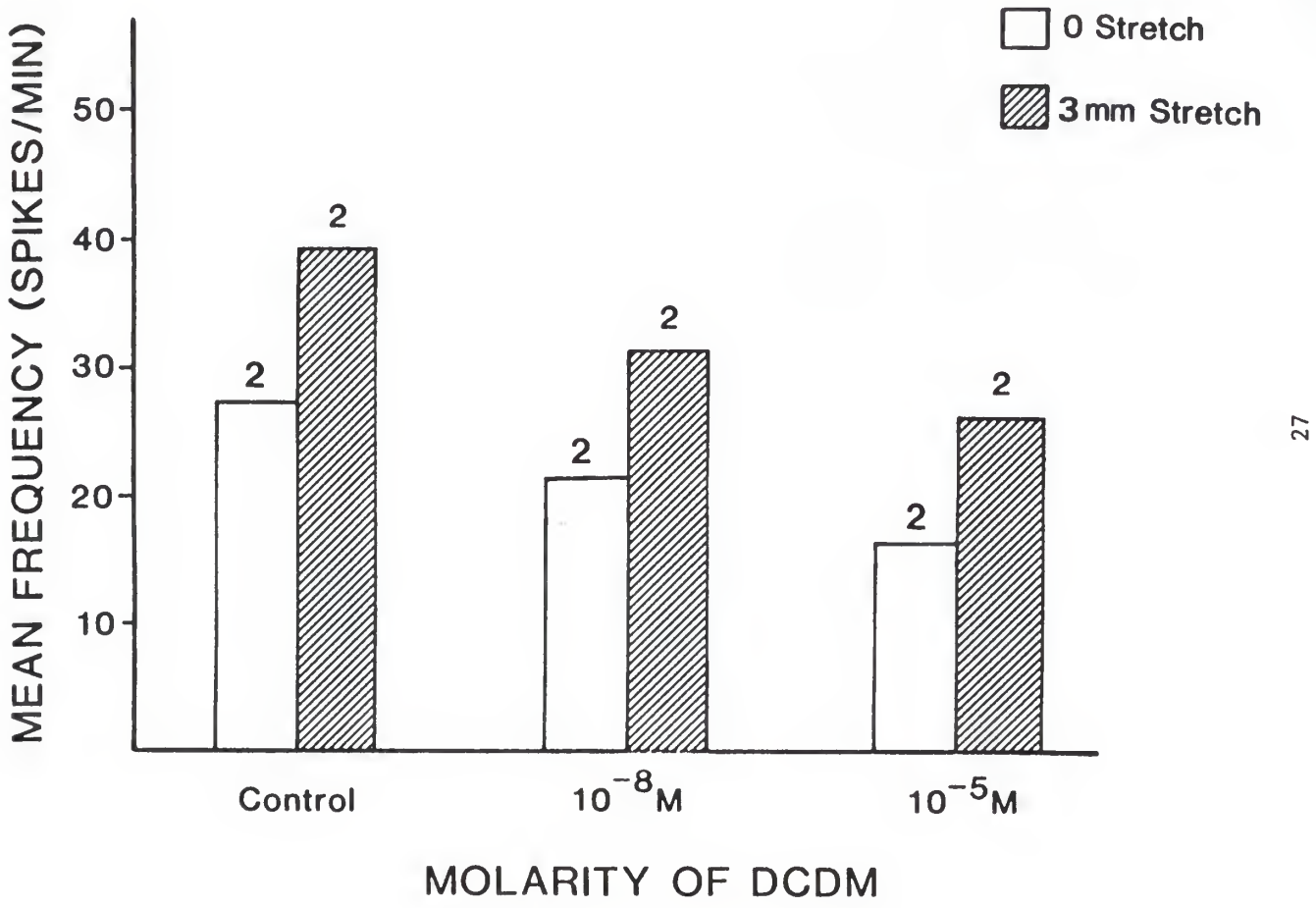

0 Stretch
2mm Stretch
MEAN FREQUENCY (SPIKES/MIN)
60
50
40
30
20
10
4
4
4
4
4
4
4
4
Control
10^{-8}M
10^{-7}M
10^{-5}M
MOLARITY OF OCTOPAMINE
25

Fig. 7. Effect of DCDM on the response of the stretch receptor organ to stretch. Increasing doses of DCDM decreased the response. The preparations responded to increased stretch by increasing the output frequency. For control, the preparations were superfused with saline. Numbers above the bars indicate the number of preparations used to calculate the mean frequency.


0 Stretch
3 mm Stretch
MEAN FREQUENCY (SPIKES/MIN)
50
40
30
20
10
2
2
2
2
2
2
Control
10^{-8}M
10^{-5}M
MOLARITY OF DCDM

Effects of Demethylchlordimeform (DCDM) on the Thoracic Stretch Receptor Output

In several instances, the effects of DCDM on insects parallel that of OA. Therefore the application of DCDM to the thoracic stretch receptor organ would be expected to give similar results to those obtained by applying OA.

Figure 7 shows the response to DCDM at two different molarities. The control was again the application of larval saline for the same duration of time. 10^{-8} M DCDM decreases the output frequency of the stretch receptor organ in response to stretch. Increasing the molarity to 10^{-5} M further decreased the output frequency. The results of these experiments are similar to those in which OA was applied. Figure 7 also shows the results at two different amounts of stretch. Increasing the stretch increased the output frequency of the stretch receptor organ. This effect of DCDM was observed on five preparations.

Analysis of variance on the results at 0 stretch indicate that the differences were significant at the $p < 0.0002$ level. T-tests (Least Significant Difference) for the results at 0 stretch indicate that the treatment with 10^{-8} M DCDM and 10^{-5} M DCDM were both significantly different from the saline treatment but between the responses to the two molarities of DCDM, there were no significant differences.

DISCUSSION

Disappearance of the Thoracic Stretch Receptor Organ

The results from this study show that the thoracic stretch receptor organ of larval Manduca sexta does not persist through metamorphosis. The stretch receptor organ disappears by Day 6 of the pupal stage with the innervation gone before the muscle fiber disintegrates. The present observations do not support the suggestion by Rheuben (personal communication) that a thoracic stretch receptor is present in adult moths.

Casaday and Camhi (1976), although unable to follow the nerve branches of the larval IIN1b nerve through metamorphosis, showed that the sensory branch of this nerve to the stretch receptor organ disappears at around Day 4 of the pupal stage. The branch of IIN1b that innervates the muscle fiber of the stretch receptor organ and the dorsal longitudinal muscle in the larvae divides into three twigs in the adult to innervate the adult dorsal longitudinal muscle. This observation is in agreement with the one obtained in the present study which shows that the innervation is gone by Day 5.

A possible explanation for the disappearance of the thoracic stretch receptor organ involves the drastic change in the thoracic morphology during metamorphosis. In contrast to the soft, pliable cuticle of the larvae, the exoskeleton of the adult thorax is largely inextensible. Paralleling this change in cuticle is a change in the amount of longitudinal movement possible in the thorax itself, from a lot to none at all. Since the stretch receptor organ detects stretch during longitudinal movement of the thorax, there is no function for it in the

adult. Change in the cuticle correlates with change in the mode of locomotion of the animal from peristaltic crawling to flying. In flying, there are no massive distortions of the thoracic body wall; larval locomotion, however, does involve massive distortions. Thus, there is no effective stimulus in the adult for a receptor organ that detects stretch due to contraction of body wall muscles.

In the abdomen, the stretch receptor organ persists in the adult giant silkmoths (Finlayson and Lowenstein, 1958). The muscle of the stretch receptor organ does not degenerate even when the motor innervation is severed experimentally (Finlayson, 1956). The stretch receptor muscle does undergo changes in the number and sizes of nuclei during metamorphosis (Finlayson and Mowat, 1963). The functional significance of this persistance can easily be seen in that the movements of the adult abdomen are similar to those of the larvae, primarily involving contractions and stretches of the abdominal intersegmental folds. Therefore a stretch receptor organ may persist when metamorphosis does not alter body movements and thus the function of a stretch receptor organ, but the stretch receptor may disappear when metamorphosis alters body shape and movements.

In the present study, sensory output from the periphery was recorded from nerve IIN1b of adult moths when the wing was moved. This observation indicates that mechanoreceptors such as wing stretch receptors may be present in the adult. This possibility was not pursued. The wing stretch receptor would be expected to resemble that of the locust. The locust wing stretch receptor is found at the wing hinge and consists of a

single neuron associated with connective tissue (Gettrup, 1962). In contrast, the stretch receptor organ found in the thorax of Manduca larvae and pupae in this study includes a muscle fiber, the stretching of which stimulates the sensory neuron. The locust receptor responds to the positional angle of the wing and the rate of wing movement (Gettrup, 1962, 1963; Wilson, 1961). These responses contribute to the determination of wingbeat frequency (Möhl, 1979; Wilson and Gettrup, 1963).

Another difference between the thoracic stretch receptor organ examined in this study and the wing hinge stretch receptor of the locust is that since muscle cells are absent, the locust is unable to adjust the length of the sense organ (Gettrup, 1963), while inputs from the CNS in moths alter the length of the muscle fiber such that the response is not linearly related to the stimulus (Weevers, 1966).

Effects of OA and DCDM on the Stretch Receptor Output

Results from this study provide evidence that OA and DCDM act peripherally, to modulate the output of the stretch receptor organ of Manduca sexta, both at the arbitrary 0 position and upon stimulation by stretch. OA and DCDM significantly decrease the output frequency of the stretch receptor organ at the concentrations studied.

The hemolymph levels of OA in Manduca are 10^{-7} M in adults, 10^{-8} M in pupae, and unknown in larvae (Klaassen, 1983). The concentrations used in this study are therefore probably within the physiological range.

Although OA is believed to be responsible for the "general arousal" of invertebrates by increasing metabolic rates of cells (Hollingworth and Lund, 1982), specific functions may be decreased by OA. In the neurohemal

organ of the stick insect, applications of OA at doses of 10^{-4} M or higher result in the suppression of nervous activity (Osborne, 1985). Application of 10^{-5} M OA to preparations of sense organs of the blowfly larvae is followed by a decline in the firing rate of the sense organ accompanied by occasional bursting activity (Osborne, 1985). This result is similar to the one obtained in this study in which 10^{-5} M OA caused a decline in the output frequency with occasional bursting activity.

Other examples of decrease in functions by OA are a decline of maintained or basal tension in the extensor-tibiae muscle (O'Shea and Evans, 1979; Hoyle, 1978a; Evans and Siegler, 1982) and a reduction of the frequency and amplitude of the myogenic contractions in the locust (Evans and O'Shea, 1978). This latter effect is dose-dependent with a total inhibition of the rhythm at 10^{-7} M. In the present study, total inhibition was not observed at the concentrations studied.

Although in Manduca OA may have a "general arousal" effect on the whole animal, its inhibitory effect on the output of the stretch receptor is not unexpected in light of the inhibitory effects of OA discussed above. As in the vertebrate autonomic nervous system where one can not predict the effect of epinephrine and acetylcholine on a particular system, it is possible that the effect of OA on a specific invertebrate function may be difficult to predict.

The fact that actions of CDM mimic those of OA in many instances in both the CNS and PNS of insects suggests that CDM acts on OA receptors (Claassen, 1985; Gole et al., 1983; Hollingworth and Lund, 1982; Evans and Gee, 1980; Lund et al., 1979a; Singh et al., 1981; Orchard et al.,

1982). A comparison of the effects of OA and DCDM on the stretch receptor output further supports this hypothesis. In this study, DCDM, the N-demethylated analog of CDM, mimicked OA in decreasing the output of the stretch receptor organ. Surprisingly DCDM was less potent than OA, in that the same molarity of DCDM caused a smaller percentage decrease than OA. The finding that DCDM mimics OA in decreasing the output frequency adds to the evidence that DCDM acivates OA receptors.

SUMMARY

1. The thoracic stretch receptor organ of Manduca sexta does not persist through metamorphosis. It is present in the larvae and up to Day 4 of the pupal stage and completely gone by Day 6, at which time the meso- and metathoracic ganglia are fused.
2. The thoracic stretch receptor of larvae responds to stretch in a manner typical for a slowly adapting mechanoreceptor of the static type.
3. Application of the biogenic amine, OA, resulted in a decrease in the output frequency of the stretch receptor organ at a given stretch.
4. The formamidine insecticide, DCDM, mimicked OA in lowering the output frequency, although the effect is less than that of equal concentrations of OA.

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STUDIES ON THE THORACIC STRETCH RECEPTOR ORGAN OF *MANDUCA SEXTA* AND EFFECTS OF OCTOPAMINE AND DEMETHYLCHLORDIMEFORM ON THE ACTIVITY OF THE STRETCH RECEPTOR

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ABSTRACT

The morphology of the larval and early pupal thoracic muscle stretch receptor organ of the lepidopteran, Manduca sexta, was described on the basis of methylene blue stainings and dissection. In the late pupal and adult stage, the thoracic stretch receptor organ could not be found. In response to stretch, the larval receptor produced impulses in a pattern that is typical for a slowly adapting mechanoreceptor of the static type. In particular there was a large initial drop in output frequency. The stimulus-response relationship was linear up to a certain maximum of stretch, beyond which the output frequencies fell. The effects of the biogenic amine DL-Octopamine and the formamidine insecticide Demethylchlordimeform on the stretch receptor output were studied. Octopamine is important to arthropods where it fulfills many of the functions of adrenalin and noradrenalin in vertebrates, whereas the behavioral disruptions caused by DCDM include increased excitability and locomotor activity. Contrary to expectations, octopamine lowered the output frequency of the stretch receptor in response to stretch. DCDM, which normally parallels octopamine in its effects, also decreased the output frequency of the stretch receptor in response to stretch although the effect is less than that of equimolar concentrations of octopamine.