EFFECTS OF BIOGENIC AMINES AND FORMAMIDINE INSECTICIDES ON THE CENTRAL PRODUCTION OF FLIGHT BY *MANDUCA SEXTA*

by

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GENERAL INTRODUCTION

Biogenic amines are abundant throughout the animal kingdom including the arthropods in which serotonin, dopamine and octopamine are thought to act as transmitters or as modulators of transmitter action. Although a number of studies have confirmed the actions of biogenic amines at neuromuscular junctions and other peripheral sites, few investigations in invertebrates have demonstrated specific central effects of biogenic amines. The studies that are available indicate that monoamines affect behaviors--but the mechanisms involved are not always clear.

Several problems exist in determining the central actions of biogenic amines. In insects the CNS is surrounded by a barrier that is impermeable to charged compounds. Very high concentrations must be injected into the hemolymph or superfused over the nerve cord in order to see any behavioral response. Crustaceans have less developed blood-brain barriers and in lobster and crayfish an arterial system infiltrates the CNS. Although this system has been used to demonstrate effects of biogenic amines in the CNS, the general application makes actions difficult to localize and mechanisms hard to discern.

The purpose of this thesis is to describe a system in an insect Manduca sexta in which the central actions of biogenic amines and putative agonists and antagonists were investigated. This preparation allowed for the direct application of octopamine, dopamine, serotonin, and formamidine insecticides to specific locations of the CNS under various conditions. Effects of these compounds on a complex behavior was studied via a single nerve recording of flight motor neuron activity. Results from this study demonstrate specific effects of biogenic amines and formamidines on the production of flight, and provide evidence for their mechanisms of action.

CHAPTER I

EFFECTS OF OCTOPAMINE, DOPAMINE AND SEROTONIN ON PRODUCTION OF FLIGHT MOTOR OUTPUT BY THORACIC GANGLIA OF *MANDUCA SEXTA*

INTRODUCTION

Various biogenic amines influence the neural mechanisms that generate motor programs for behavior. Serotonin intensifies feeding motor output in Aplysia (Kupfermann and Weiss, 1981) and Limax (Gelperin, 1981) and initiates locomotion in Aplysia (Mackey and Carew, 1983) and leeches (Willard, 1981). Dopamine evokes the feeding motor pattern in Limax (Wieland and Gelperin, 1983) and enhances pyloric motor output in lobsters (Anderson and Barker, 1977, 1981). In locusts, octopamine elicits motor patterns associated with flight or walking, or it suppresses the oviposition digging pattern, depending on the site of iontophoresis in metathoracic or abdominal ganglia (Sombati and Hoyle, 1984b). In some cases, two biogenic amines have similar effects on a motor program. Both L-DOPA, a precursor of dopamine and norepinephrine, and 5-HTP, a precursor of serotonin, initiate walking motor patterns in spinal cats (Grillner, 1969, 1976; Ahlman et al., 1971) and spinal rabbits (Viala and Buser, 1969), and both dopamine and serotonin activate motor patterns associated with feeding in Helisoma (Trimble and Barker, 1984; Granzow and Kater, 1977). In other cases, two amines have opposite effects on a motor program. Octopamine evokes tonic extension of the extremities in lobsters and suppresses tonic flexion, an opposing postural movement enhanced by serotonin (Livingston et al., 1980). In crayfish, octopamine intensifies walking and optokinetic responses, whereas serotonin suppresses both activities (Arnesen and Olivo, 1983). Octopamine also intensifies phasic flexion of the abdomen, a movement that contributes to the crayfish escape response; flexion of the abdomen is inhibited by serotonin (Glanzman and Krasne, 1983).

In the present study, the effects of dopamine, octopamine and serotonin on flight motor output were investigated in the moth, Manduca sexta. These biogenic amines can be synthesized by prothoracic and abdominal ganglia of

Manduca (Maxwell et al., 1978). Octopamine content of Manduca thoracic ganglia is substantial (Klaassen, 1983); quantities of the two other compounds have not been determined. Two questions were addressed in this study: 1) What are the effects of the three amines on the centrally generated flight pattern? 2) Do the amines that have similar effects on flight activity have similar mechanisms of action, or do they differ in their site and mode of action? To answer these questions, it was necessary to develop a dissected preparation in which activation of the flight program could be identified and monitored easily. During the study, it became apparent that the number of intact sensory nerves altered the effect of octopamine on the production of flight. Additional experiments were then designed to analyze the importance of sensory input in the action of biogenic amines.

METHODS

Larvae of Manduca sexta were reared on a carrageen-based artificial diet and maintained on a long-day (16 h), short-night (8 h) cycle. Adult moths, 1-2 days following eclosion, were dissected ventrally to expose the thoracic ganglia. In experiments studying the role of sensory input, sensory nerves and connectives to the head and abdomen were severed, thus isolating thoracic ganglia from peripheral and central inputs. In some experiments, fine-wire recording electrodes were placed in two antagonistic flight muscles of the mesothorax, the dorsal longitudinal depressor (dl_1) and the dorsal oblique elevator (dl_2) (nomenclature according Nüesch, 1953). In all experiments, either a suction recording electrode was placed on IIN1b, a nerve that branches to dl_1 and dl_2 (Nüesch, 1957; Eaton, 1974), or a pair of electrodes was positioned on the IIN1b branches innervating dl_1 and dl_2, designated here as Ndl1 and Ndl2, respectively.

Dopamine, DL-octopamine or serotonin (Sigma Chemical Company) dissolved in saline ($1\text{-}10 \times 10^{-2}$ M) was pressure injected into medial or lateral regions of thoracic ganglia following treatment of the sheath with 3% pronase for about 1.5 min. Controls were injected with equimolar concentrations of glucose in saline or with saline alone. Saline composition was 53 mM NaCl, 9.3 mM KCl, 6.1 mM $CaCl_2$, 7.9 mM $MgCl_2$, 114 mM Na-methanesulfonate and 27.8 mM Tris-methanesulfonate, pH 7.0. The pressure pulse was supplied by a picospritzer (General Valve Corporation) to a 1.2 mm O.D. glass micropipet containing the injection solution. The pipet was calibrated using an ocular micrometer to measure the diameter of the droplet ejected during a pulse. The volume of injections ranged from 5-100 nl. In some experiments, larger volumes (0.1-8.0 µl) were delivered using a 12 ml syringe to supply the pressure pulse (Kinnamon et al., 1984). All injection experiments were at 20-23 C.

The influence of sensory input on the effect of octopamine was examined by stimulating electrically a wing sensory nerve (IIN1c) before and after injection. A 200 ms stimulus train consisting of 30 pulses, each of 2 ms duration, was given every 2.5 sec at a voltage (1-4 V) that elicited short bursts of motor activity in untreated preparations.

Motor neurons innervating flight muscles dl_1 or dl_2 were visualized by backfilling their respective branches, Ndl1 and Ndl2, with 0.25 M $CoCl_2$ followed by ammonium sulfide development (Pitman et al., 1973) and silver intensification (Bacon and Altman, 1977). Although sensory afferents and neurosecretory axons are present in the nerve (Wasserman, 1982), only the axons of flight motor neurons were large enough to be backfilled successfully.

RESULTS

Flight motor pattern (FMP)

In Manduca sexta, rhythmic contractions of flight muscles are observed in both whole animals and dissected preparations. Simultaneous recordings from wing depressor and wing elevator flight muscles during flight-like, large-amplitude wing movements of a tethered moth show a high frequency (20 Hz), alternating pattern of muscle potentials (Fig. 1A). The same pattern of activity can be recorded from flight muscles in the dissected preparation (Fig. 1B). Although lower in frequency (10 Hz), the flight pattern recorded from exposed flight muscles remains appropriately phase-locked with wing movements.

The output of motor neurons innervating two mesothoracic muscles that generate wing movements was correlated with flight activity. These two flight muscles, the depressor dl_1 and the elevator dl_2, are active in antiphase during flight (Kammer, 1971) and are innervated by branches (Nd11 and Nd12) of a mesothoracic motor nerve, IIN1b (Nüesch, 1957; Eaton, 1974). Extracellular nerve recordings from IIN1b reveal a motor pattern that is in phase with the alternating rhythmic excitation of dl_1 and dl_2 during flight (Fig. 2A). Simultaneous recordings of activity in the nerve branches Nd11 and Nd12 demonstrate that the motor pattern has two anti-phasic components, one in each branch of IIN1b (Fig. 2B). Combined, the activity from both branches forms a flight motor pattern (FMP) that alternately excites the dl_1 and dl_2 antagonistic flight muscles to contract in antiphase during flight.

To determine the number and distribution of motor neurons that produce the FMP, Nd11 and Nd12 were backfilled with cobalt chloride. Seven cell bodies in the thoracic ganglia were stained consitently (Fig. 3). Five of the seven were stained by backfilling Nd11, the IIN1b branch that innervates the depressor muscle dl_1. Four of these neurons have cell bodies in the

Fig. 1. Extracellular recordings from antagonistic flight muscles (dl_1 and dl_2) during flight activity. (A) Flight pattern from a tethered moth. Muscle potentials were recorded during fixed flight by inserting fine copper wires through the cuticle into depressor (d) and elevator (e) muscles. Flight frequency: 20 Hz. (B) Flight pattern from a dissected preparation. Muscle potentials were recorded as in A with wire electrodes placed into exposed depressor (d) and elevator (e) muscles. Flight frequency: 10 Hz.

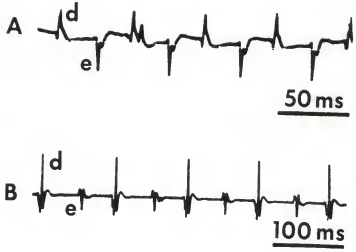

A
d
e
50 ms
B
d
e
100 ms

Fig. 2. The flight motor pattern (FMP) recorded from nerve IIN1b. (A) Top trace, muscle potentials recorded from a wing depressor muscle, dl_1, during flight activity. Bottom trace, simultaneous recording from nerve IIN1b showing the activity of motor neurons that innervate dl_1 and a wing elevator muscle, dl_2. (B) Top trace, dl_1 muscle potentials as in A. Middle trace, a simultaneous recording of depressor motor neuron activity from Ndl1, the branch of IIN1b that innervates dl_1. Bottom trace, a simultaneous recording of elevator motor neuron activity from Ndl2, the branch innervating dl_2. The combined output of both branches forms the FMP. A and B are different preparations that were treated with 5×10^{-9} mol dopamine.

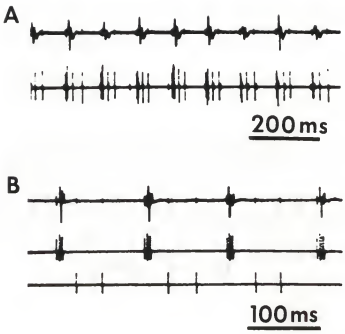
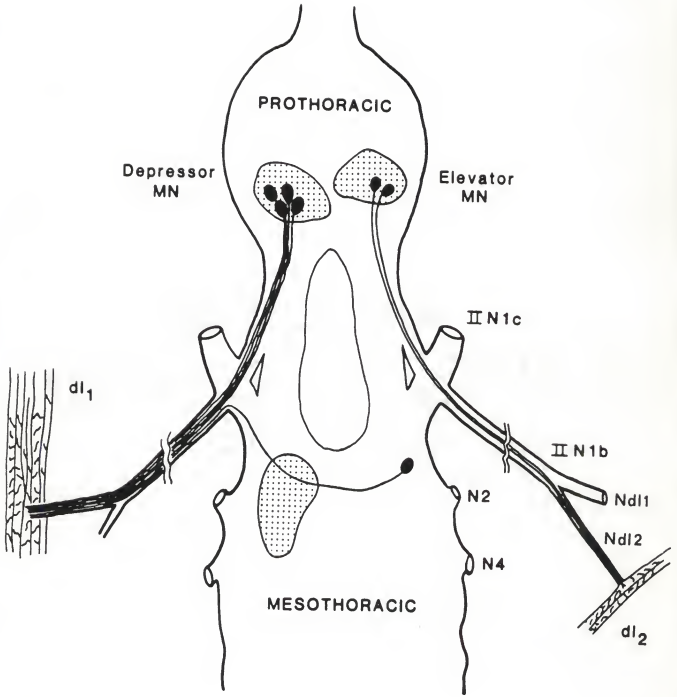

A
200ms
B
100ms

Fig. 3. A diagrammatic representation of the prothoracic and mesothoracic ganglia and IIN1b flight motor neurons. The flight motor nerve IIN1b innervates both dl_1 and dl_2. Cobalt backfills of the dl_1 branch (Ndl1) stained five motor neurons (left). Backfills of the dl_2 branch (Ndl2) stained two motor neurons (right). Shaded areas represent dendritic arborizations of the filled neurons. All motor neurons are located bilaterally in the thoracic ganglia. Nerve IIN1c is a sensory nerve from the wing.


PROTHORACIC
Depressor MN
Elevator MN
II N1c
dl1
II N1b
N2
Ndl1
Ndl2
N4
MESOTHORACIC
dl2

prothoracic ganglion ipsilateral to the filled nerve with the fifth having a contralateral soma in the mesothoracic ganglion. Cobalt backfills of Nd12, the branch that innervates the elevator muscle dl_2, stained two cells in the prothoracic ganglion. These neurons were ipsilateral to the filled nerve and adjacent to the dl_1 prothoracic motor neurons.

The motor neurons of nerve IIN1b produce a pattern of rhythmic bursting that alternately excites antagonistic flight muscles. Thus, monitoring IIN1b for the flight motor pattern is a convenient method for detecting flight activity and for studying the effects of biogenic amines on the flight program of Manduca sexta.

Effects Of Biogenic Amines On The FMP

In the first set of experiments, dopamine, octopamine or serotonin were injected into prothoracic and mesothoracic ganglia of preparations with intact sensory nerves. The output of flight motor neurons was monitored in nerve IIN1b. In all preparations, no activity was present in IIN1b for at least 10 min before an injection.

Effects of dopamine

Injection of dopamine (3×10^{-8} mol) into the prothoracic ganglion evoked single, large-amplitude spikes within 2 min in 4 of 5 trials (Fig. 4A). These action potentials in IIN1b were constant in amplitude and were produced at a cycle time of 20-35 msec. The single spikes were the only activity elicited by prothoracic injection; at no time was bursting activity or the FMP observed.

Injection of dopamine into the mesothoracic ganglion had different effects on production of the FMP depending on the region injected. Dopamine ($2\text{-}4 \times 10^{-8}$ mol) injected into lateral regions elicited no activity in IIN1b in 5 of 6 trials (Fig. 4B), although leg and abdomen movements were detected.

However, smaller doses (1-5 x 10^{-9} mol), when injected into the medial region of the mesothoracic ganglion, triggered the FMP (N = 5; Fig. 4C). Flight motor output occurred within seconds after injection with little or no non-flight activity preceding or following the FMP. Duration of the dopamine-induced FMP was dose-dependent for injections greater than 5 x 10^{-11} mol (Fig. 5). At the highest dose tested (10^{-8} mol), dopamine maintained the FMP 20 min, at which time the experiment was terminated.

Effects of octopamine

Injection of octopamine (2 x 10^{-8} mol) into the prothoracic ganglion of preparations with intact sensory nerves elicited multiple large-amplitude action potentials in IIN1b within 2 min of injection in 4 of 5 trials (Fig. 6A). The unpatterned activity appeared to involve only prothoracic motor neurons as demonstrated by severing the axon of the mesothoracic contralateral motor neuron and observing no change in IIN1b activity. Unpatterned motor output was the only response elicited by prothoracic injection of octopamine.

The effect of octopamine in the mesothoracic ganglion varied with the region of injection. Octopamine (4-6 x 10^{-8} mol), when injected into the medial region, elicited no activity in IIN1b within 2 min in 7 of 9 preparations (Fig. 6C); large-amplitude, single spikes were produced in the other two cases. Injections of a lower dose (4 x 10^{-9} mol) into lateral regions of the mesothoracic ganglia elicited single, large-amplitude action potentials within 2 min in 6 of 8 trials (Fig. $6B_1$). When the axon from the mesothoracic motor neuron was severed in 4 animals producing large amplitude spikes, activity continued unabated indicating that the lateral mesothoracic injection of octopamine activates a depressor motor neuron in the prothoracic ganglion.

Fig. 4. Effects of dopamine in the lateral and medial regions of thoracic ganglia. Shaded areas outline regions of injections. Traces are extracellular records of activity in nerve IIN1b. (A) Prothoracic (PRO) injection of dopamine (3×10^{-8} mol) elicited single, large amplitude spikes; the FMP was not produced. (B) Mesothoracic (MESO) injections ($2\text{-}4 \times 10^{-8}$ mol) into lateral regions had no effect. (C) Mesothoracic injection ($1\text{-}5 \times 10^{-9}$ mol) into the medial region triggered the FMP.

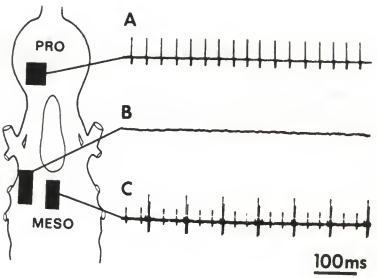

A
PRO
B
C
MESO
100ms

Fig. 5. Duration of the FMP induced by injecting dopamine into the medial region of the mesothoracic ganglion. Maximum observation time was 20 min. Controls were injected with saline or glucose dissolved in saline. N for each dose is shown above bar. Error bar indicates one standard deviation.

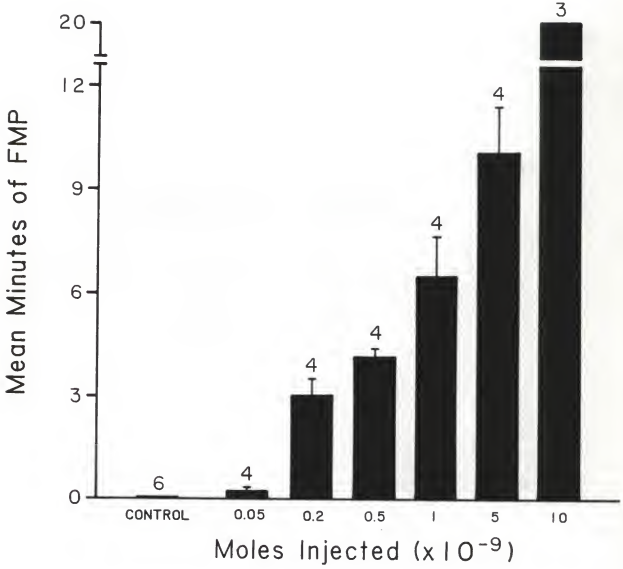
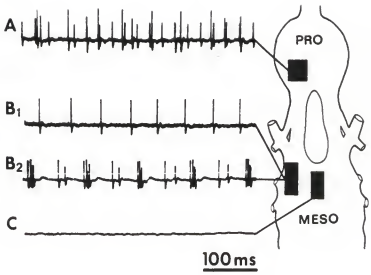

Mean Minutes of FMP
20
12
9
6
3
0
6
4
4
4
4
4
3
CONTROL
0.05
0.2
0.5
1
5
10
Moles Injected (x 10⁻⁹)

Fig. 6. Effects of octopamine in thoracic ganglia. Shaded areas outline regions of injections. Traces are recordings from nerve IIN1b. (A) Prothoracic (PRO) injection of octopamine (2×10^{-8} mol) activated multiple spikes from prothoracic motor neurons; the FMP was not produced. (B_1) Mesothoracic (MESO) injections (4×10^{-9} mol) at lateral regions elicited single, large-amplitude spikes. (B_2) At doses exceeding 10^{-8} mol, the spiking activity in B_1 was often followed by the FMP. (C) Mesothoracic injection ($4\text{-}6 \times 10^{-8}$ mol) at the medial region had no effect.


A
B1
B2
C
PRO
MESO
100 ms

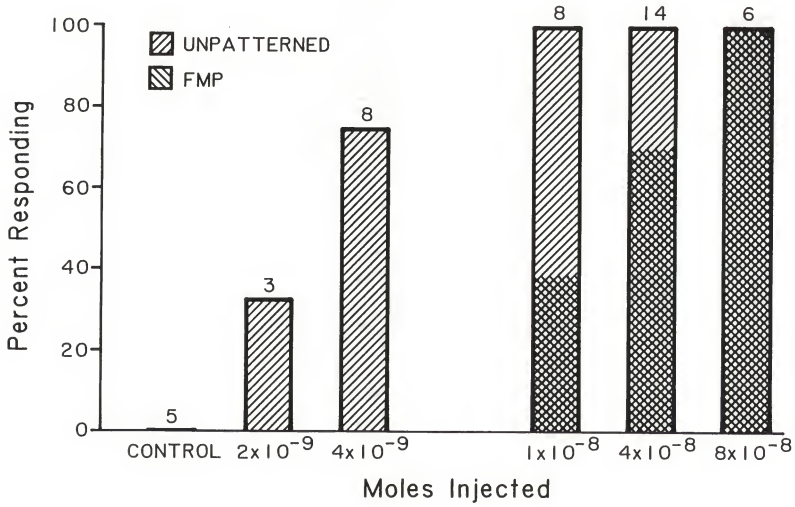
At doses exceeding 10^{-8} mol, injections of octopamine into lateral regions of the mesothoracic ganglion elicited one, then several large-amplitude spikes that often became organized as the FMP (Fig. $6B_2$). The effect of octopamine on the production of non-flight and flight motor output was dose-dependent (Fig. 7). At 8×10^{-8} mol, the FMP was elicited in 6 of 6 preparations. Duration of the octopamine-induced FMP was variable (2-20 min) and appeared related to the number of intact peripheral nerves. Upon termination of the FMP, preparations remained active and produced unpatterned activity. Tactile stimulation of the wing or head increased motor output and often reinitiated the FMP.

Effects of serotonin

Injecting serotonin into thoracic ganglia did not elicit motor activity in nerve IIN1b. To determine whether serotonin inhibited flight output, serotonin was injected after the FMP had been initiated by dopamine or octopamine. Medial injection of serotonin (5×10^{-10} mol) into the mesothoracic ganglion terminated the dopamine-induced (5×10^{-10} mol) FMP within 60 sec in 8 of 8 trials. In contrast, lateral mesothoracic injection of serotonin did not inhibit the FMP. In control experiments, medial injection of 0.1 µl saline had no effect on the dopamine-induced FMP in 5 of 6 trials.

Serotonin had little effect on motor activity elicited by 10^{-8} mol octopamine. Although serotonin (10^{-8} mol) decreased the number of spikes per burst or stopped activity in 8 of 13 trials, the effect was only temporary with non-flight motor output returning within 4-6 min in 50% of those cases. Injection of an equal volume (1 µl) of saline also temporarily suppressed activity in 3 of 3 trials.

Fig. 7. Effect of injected octopamine on the production of non-flight and flight motor activity in IIN1b. Increasing doses of octopamine injected into lateral regions of the mesothoracic ganglion increased the occurrence of unpatterned, large-amplitude spiking (right diagonal lines). At higher doses, octopamine increased the number of preparations that also produced the FMP (left diagonal lines). Controls were injected with saline or glucose dissolved in saline. N for each dose is shown above bar.


100
80
60
40
20
0
Percent Responding
UNPATTERNED
FMP
5
3
8
8
14
6
CONTROL
2x10⁻⁹
4x10⁻⁹
1x10⁻⁸
4x10⁻⁸
8x10⁻⁸
Moles Injected

Influence of sensory input on the amine-induced FMP

To determine whether the effect of octopamine or dopamine on the FMP required sensory input, all sensory nerves and connectives to the head and abdomen were cut, thereby isolating thoracic ganglia from peripheral and central inputs. In these preparations, octopamine ($2\text{-}4 \times 10^{-8}$ mol) injected into lateral mesothoracic regions elicited no IIN1b activity within 10 min ($N = 5$; Fig. 8). This is in contrast to the response of sensory-intact thoracic ganglia, where octopamine elicited motor output within 2 min following lateral mesothoracic injection.

Low doses of dopamine ($2\text{-}10 \times 10^{-10}$ mol) injected into ganglia isolated from sensory input elicited activity similar to that induced in intact ganglia; injection at the medial mesothoracic region triggered the FMP ($N = 6$; Fig. 8). Thus, unlike octopamine, the effect of dopamine on the FMP appears to be independent of sensory input.

Effect of octopamine on the response to electrical stimulation

To determine whether octopamine increases the response of thoracic ganglia to sensory input, the wing sensory nerve IIN1c was stimulated electrically before and after injecting low doses of octopamine (4×10^{-10} mol) into lateral regions of thoracic ganglia. Such doses are below the threshold for eliciting spontaneous activity.

In the untreated preparation, a high frequency stimulus train delivered every 2.5 sec elicited low frequency bursting in nerve IIN1b (Figs. 9,10). After injecting octopamine, stimulation of the wing sensory nerve increased bursting activity within 2 min in all preparations and evoked low-frequency FMP within 10 min in 5 of 6 preparations (Figs. 9,10).

Fig. 8. Effects of octopamine (OCT) and dopamine (DA) injections in lateral (L) and medial (M) regions of mesothoracic ganglia in the presence and absence of sensory input. In intact thoracic ganglia, octopamine injected into lateral regions (4×10^{-9} mol) elicited a response more often than did injections into the medial region ($4\text{-}6 \times 10^{-8}$ mol). In contrast, injections of dopamine evoked more responses in the medial region ($1\text{-}5 \times 10^{-9}$ mol) than in lateral regions ($2\text{-}4 \times 10^{-8}$ mol). In isolated thoracic ganglia, lateral injections of octopamine elicited no response ($2\text{-}4 \times 10^{-8}$ mol), whereas dopamine remained effective in the medial region ($2\text{-}10 \times 10^{-10}$ mol). Response to injection was defined as appearance of motor output in nerve IIN1b within 2 min following injection. N for each set of experiments is shown above bar.

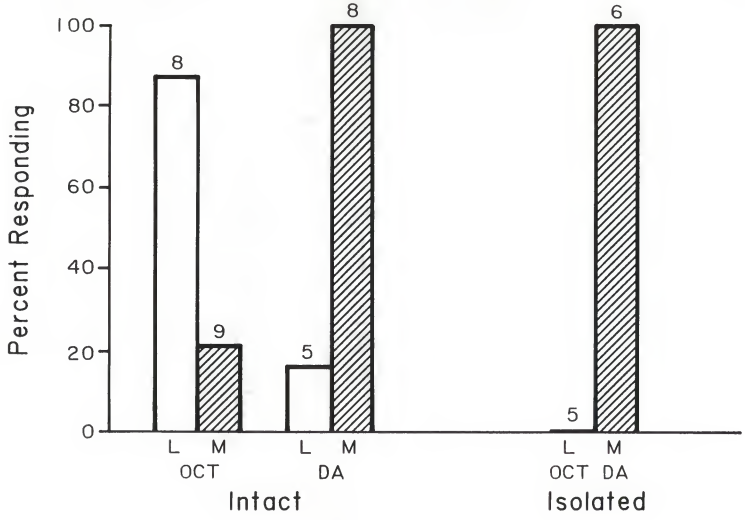

Percent Responding
100
80
60
40
20
0
8
9
5
8
5
6
L M
OCT
L M
DA
Intact
L M
OCT DA
Isolated

Fig. 9. Recordings of IIN1b activity generated by electrical stimulation of a wing sensory nerve before and after injecting octopamine. Each trace begins immediately following stimulation. Top trace, before octopamine injection; few spikes were produced in response to stimulation. Middle trace, 2 min after injection; irregular spiking activity followed electrical stimulation. Bottom trace, 5 min after injection; the FMP was produced following stimulation.

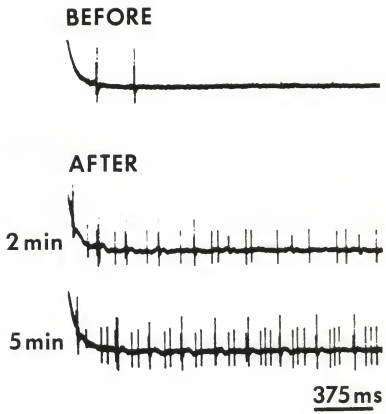
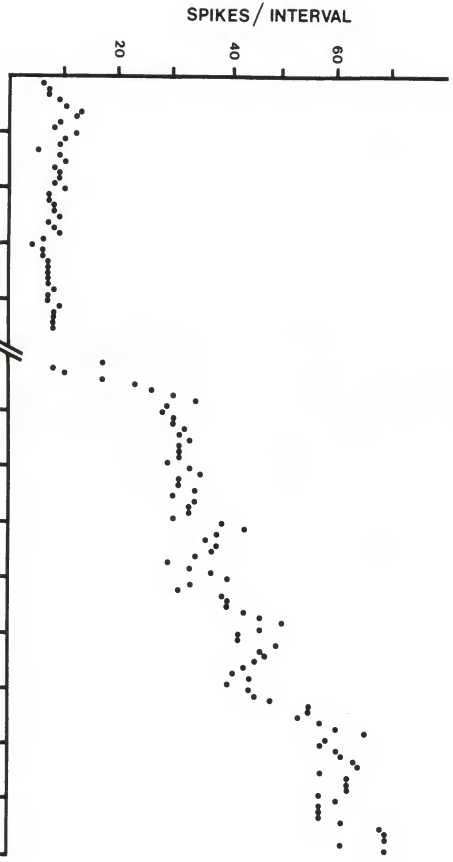

BEFORE
AFTER
2 min
5 min
375 ms

Fig. 10. Spiking activity in IIN1b in response to electrical stimulation of a wing sensory nerve before and after injecting octopamine. The number of spikes produced during the 2.5 sec interval following each stimulus train was counted. Octopamine injected at arrow increased response to stimulation. Data, which are from one experiment, are representative of results from four of five similar experiments.


SPIKES / INTERVAL
20
40
60
20
40
STIMULUS NO.
20
40
60
80
25 sec

DISCUSSION

Results from this study provide evidence that dopamine, octopamine and serotonin act on the central nervous system to initiate, modulate, or terminate patterned motor output associated with flight in Manduca sexta. Serotonin inhibits the FMP, an effect opposite to that of dopamine or octopamine. The inhibitory action of serotonin is most effective on the FMP induced by dopamine. Serotonin acts within the medial region of the mesothoracic ganglion, where dopamine exerts an excitatory action.

Although both dopamine and octopamine elicit the FMP in sensory-intact preparations, results from isolated thoracic ganglia show that the two amines differ in their mechanisms of action. When sensory nerves are cut, dopamine can activate the FMP whereas octopamine cannot. The action of octopamine requires sensory input.

Dopamine and octopamine also differ in that their actions on the FMP are exerted at different locations in intact mesothoracic ganglia. Dopamine triggers the FMP when injected into the medial region, whereas octopamine elicits the flight pattern when injected into lateral regions (Fig. 8). Localization of action to either lateral or medial regions applies to motor activity in nerve IIN1b and does not exclude actions of these amines in other regions of the CNS.

Dopamine

Flight motor output in Manduca is activated more effectively by dopamine than by octopamine. Dopamine can elicit the FMP at lower concentrations than can octopamine, and in a shorter response time. The pattern induced by dopamine is produced abruptly following injection, is maintained at a relatively constant burst frequency for the duration, and is terminated abruptly. Little or no unpatterned motor activity precedes or follows the dopamine-induced flight pattern. These characteristic effects of dopamine on

the generation of the FMP in either the presence or absence of sensory input suggest that dopamine acts on the flight generator, possibly as the transmitter for neurons that normally command the behavior.

Dopamine initiates motor patterns in other systems isolated from sensory input. In Limax (Wieland and Gelperin, 1983) and Helisoma (Trimble and Barker, 1984), isolated buccal ganglia are triggered by dopamine to produce output similar to the centrally generated feeding motor pattern. In lobsters, dopamine induces a pattern, similar to the pyloric motor pattern, from isolated stomatogastric ganglia in which the spontaneous pyloric rhythm has been inactivated by TTX (Raper, 1979; Anderson and Barker, 1981).

Octopamine

In Manduca, octopamine increases the probability that the FMP will be produced, but only when injected into mesothoracic ganglia with intact sensory nerves. The effect of octopamine on these preparations is unlike that of dopamine because non-flight motor output often precedes and follows production of the FMP. A similar non-flight spiking pattern is observed when subthreshold doses of octopamine are applied to the mesothoracic ganglion. In addition, octopamine injected into the prothoracic ganglion elicits unpatterned activity from prothoracic motor neurons. It appears that octopamine can activate motor output via multiple pathways. Suprathreshold doses of octopamine injected into the mesothoracic ganglion may activate the flight pattern generator since the FMP is elicited. Moreover, since non-flight motor output is also observed, octopamine can activate IIN1b motor neurons by other pathways in both prothoracic and mesothoracic ganglia.

Sensory input

That octopamine increases the efficacy of sensory input has recently been shown in locusts (Sombati and Hoyle, 1984a). In the present study, motor activity generated in response to a fixed electrical stimulus was

increased up to 600% after injecting octopamine. Enhancement of the response to sensory input by octopamine could result from either increased release of transmitter from sensory afferents or increased responsiveness of postsynaptic pre-motor interneurons or motor neurons. In crayfish, octopamine increases the response of the lateral giant escape reaction to electrical stimulation of a sensory nerve by acting presynaptically to the lateral giant command neurons (Glanzman and Krasne, 1983). In lobsters, a presynaptic action of octopamine increases the excitability of abdominal extensor motor neurons by enhancing input to those neurons (Harris-Warrick and Kravitz, 1984).

The present study demonstrates the importance of considering sensory input in the action of neuroactive compounds. Dopamine and octopamine appear to have similar effects on flight production until the role of sensory input is examined. The results suggest that octopamine elicits flight activity by modulating sensory transmission, whereas dopamine acts directly on the central pattern generator.

Although the centrally generated motor pattern for flight can be produced without sensory input, it is likely that the central program is influenced by peripheral and central inputs, and that neuroactive compounds such as biogenic amines modulate that influence, as well as directly regulate production of the motor program.

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CHAPTER II

EFFECTS OF FORMAMIDINES ON FLIGHT MOTOR OUTPUT PRODUCED BY THORACIC GANGLIA OF *MANDUCA SEXTA*: COMPARISONS WITH EFFECTS OF OCTOPAMINE

INTRODUCTION

Chlordimeform (CDM) and related formamidines induce marked behavioral changes in some arthropods. For example, hyperactivity and increased locomotion have been observed in a number of insects and crustaceans following CDM treatment (Lund et al., 1979a,b; Hollingworth and Lund, 1982). One suggested mode of formamidine action on invertebrate behavior is the activation of aminergic receptors in the central nervous system (CNS). However, few effects of formamidines on the CNS are known to mimic the effects of biogenic amines. One exception is in crayfish, in which CDM induces a postural response that is activated by octopamine and to a lesser degree by dopamine (Hollingworth et al., 1984).

Most of the evidence that formamidines are aminergic agonists comes from *in vitro* studies on amine-sensitive adenylate cyclases and from studies of formamidine effects on peripheral systems. In insect CNS, demethylchlordimeform (DCDM), the N-demethylated analog of CDM, stimulates octopamine-sensitive adenylate cyclase activity in *Periplaneta americana* nerve cord (Gole et al., 1983) and *Manduca sexta* thoracic ganglia (Hollingworth and Lund, 1982). In the periphery, CDM or DCDM mimic the effect of octopamine on locust extensor tibiae muscle (Evans and Gee, 1980), firefly light organ (Lund et al., 1979a), locust corpora cardiaca (Singh et al., 1981) and locust fat body (Orchard et al., 1982). Although the accumulated evidence suggests that CDM and its analog DCDM act on octopamine receptors, formamidines also duplicate the effects of other biogenic amines. For example, in the ixodid tick, DCDM mimics the effect of dopamine by inducing salivary gland secretion (Schmidt et al., 1981; Sauer and Essenberg, 1984).

In *Manduca*, CDM elicits nearly continuous flight when applied topically to whole moths, and it induces a motor pattern associated with flight when superfused over the CNS of dissected preparations (Kinnamon et

al., 1984). Recent work has shown that the biogenic amines octopamine and dopamine elicit flight motor output upon injection into Manduca thoracic ganglia; however, the effects of octopamine were localized to lateral regions and required the presence of sensory input, whereas dopamine was effective at the medial region of the mesothoracic ganglion either with or without sensory input (Chapter 1).

In the present study, the central actions of CDM and DCDM on flight motor output in Manduca sexta were investigated by micro-injecting the formamidines into various regions of intact and isolated thoracic ganglia. Effects of CDM and DCDM on motor activity are compared with the effects of octopamine and dopamine. Results show that formamidines mimic the central effects of octopamine rather than dopamine. The findings provide evidence of CDM and DCDM specificity and suggest a mechanism by which formamidines induce flight.

METHODS

Larvae of Manduca sexta were reared on a carrageen-based cornmeal diet (Bell and Joachim, 1976) and maintained on a long-day, short-night (16:8) cycle. Within 2 days following eclosion, the moth thorax was detached from the head and abdomen, and dissected ventrally to expose thoracic ganglia. In some experiments, ganglia were isolated from sensory input by severing peripheral nerves. In all experiments, a suction recording electrode was placed on IIN1b, the motor nerve that innervates two antagonistic mesothoracic flight muscles, the dorsal longitudinal depressor (dl_1) and the dorsal oblique elevator (dl_2) (nomenclature according to Nüesch, 1953). Recordings of IIN1b output during flight-like activity of the moth show a distinctive bursting motor pattern (Fig. 1); this flight motor pattern (FMP) has been associated with the central production of flight in Manduca sexta.

Chlordimeform [N-(2-methyl-4-chlorophenyl)-N′,N′-dimethylformamidine] or the N-demethylated analog, demethylchlordimeform, was dissolved in saline (10^{-2} M) and pressure injected into medial or lateral regions of thoracic ganglia. Controls were injected with equimolar concentrations of glucose in saline or with saline alone. Saline composition was 53 mM NaCl, 9.3 mM KCl, 6.1 mM $CaCl_2$, 7.9 mM $MgCl_2$, 114 mM Na-methanesulfonate and 27.8 mM Tris-methanesulfonate, pH 7.0. The pressure pulse was supplied by a picospritzer (General Valve Corporation) to a 1.2 mm O.D. glass micropipet as previously described (Chapter 1). Volume of injections ranged from 10-400 nl. All experiments were at 20-23 C.

The influence of sensory input on the effect of chlordimeform was examined by stimulating electrically a wing sensory nerve (IIN1c) before and after injection. A 200 ms stimulus train consisting of 30 pulses, each of 2 ms duration, was given every 2.5 sec at a voltage (0.4-1.0 V) that elicited several IIN1b action potentials from the untreated preparation.

RESULTS

Effects of Formamidines on Flight Motor Output

CDM and DCDM were injected into Manduca sexta at three locations: a lateral region in the prothoracic ganglion, and a lateral and a medial region in the mesothoracic ganglion (Fig. 1). Effects on thoracic motor activity were tested in preparations with intact sensory nerves and were monitored via nerve IIN1b.

Effects of CDM

Injection of chlordimeform (10^{-9} mol) into the prothoracic ganglion, elicited unpatterned, large-amplitude action potentials in IIN1b within 6 min in 3 of 4 preparations (Fig. 2A). The multiple spiking activity was from prothoracic motor neurons as demonstrated by cutting the prothoracic-mesothoracic connective and observing no activity from the mesothoracic ganglion (N = 3). Prothoracic injection of chlordimeform did not elicit patterned activity in any preparations.

Responses to injection of CDM into the mesothoracic ganglion varied with location of injection. At the medial region, CDM (10^{-9} mol) elicited no IIN1b activity within 12 min in 3 of 3 preparations (Fig. 2C). Injections of an equal dose into the mesothoracic lateral region elicited single action potentials within 6 min (Fig. $2B_1$) followed by several large-amplitude spikes that organized into the FMP within 12 min (N = 6; Fig. $2B_2$). In 3 of 3 animals, the single spiking activity preceding the flight pattern was associated with dl_1 activity and was not affected by severing the connective from the mesothoracic ganglion, thereby indicating that lateral mesothoracic injection of CDM initially activates a prothoracic depressor motor neuron.

Fig. 1. A diagrammatic representation of prothoracic (PRO) and mesothoracic (MESO) ganglia and motor nerve IIN1b that innervates the antagonistic flight muscles dl_1 and dl_2. Suction electrode recordings from IIN1b proximal to branching show a bursting motor pattern during flight-like activity of the moth (trace). IIN1c is a large wing sensory nerve and was stimulated electrically in some experiments. Darkened areas outline regions of injections.

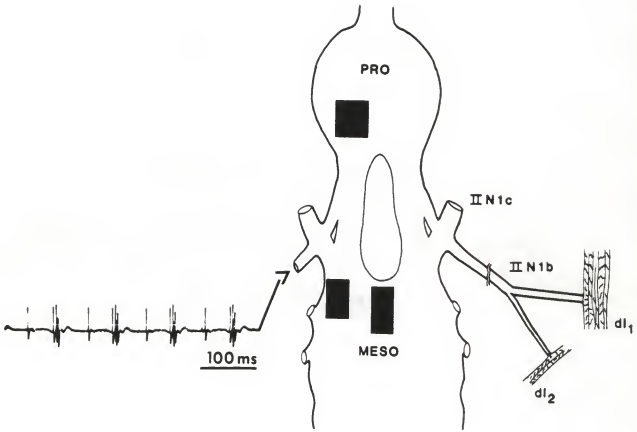
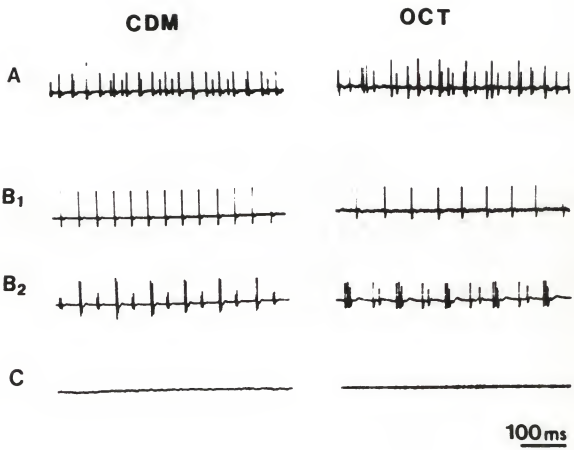

PRO
MESO
IIN1c
IIN1b
dl1
dl2
100 ms

Fig. 2. Effects of chlordimeform (CDM) and octopamine (OCT) injections into thoracic ganglia. Traces are recordings from nerve IIN1b. (A) Prothoracic injection of CDM (10^{-9} mol) activated multiple spikes from prothoracic motor neurons, but not the flight pattern. Prothoracic injection of OCT (2×10^{-8} mol) evoked similar activity. (B_1) Mesothoracic injection of CDM (10^{-9}) or OCT (10^{-8} mol) at the lateral region elicited single, large-amplitude spikes. (B_2) The spiking activity in B_1 was often followed by flight motor output. (C) Mesothoracic injection of CDM (10^{-9} mol) or OCT (4×10^{-8} mol) at the medial region had no effect.


CDM
OCT
A
B1
B2
C
100ms

Larger doses of CDM (4×10^{-9} mol) at the mesothoracic lateral region elicited single spiking activity in IIN1b within 2 min and the FMP within 6 min of injection (N = 3). Response to CDM injection was continuous for long periods; in 2 extended experiments, flight output continued for 30 min at which time the experiment was terminated.

CDM and octopamine (10^{-8} mol) were effective at the same regions of thoracic ganglia and elicited similar activity in nerve IIN1b (Fig. 2). Both compounds evoked mixed spiking activity from prothoracic motor neurons after prothoracic injection and activated a prothoracic depressor motor neuron followed by the FMP when injected into the mesothoracic lateral region. However, comparison with the octopamine data obtained previously (Chapter 1) showed that CDM was effective at lower doses and for longer periods than octopamine.

Effects of DCDM

Injection of DCDM, the N-demethylated analog of CDM, was effective at the same regions as CDM but at lower doses and with shorter response times. In the prothoracic ganglion, DCDM (10^{-10} mol) elicited mixed, unpatterned output within 5 min (N = 3) similar to that induced by CDM (Fig. 2A). DCDM (4×10^{-10} mol) had no effect within 12 min at the medial region of the mesothoracic ganglion (N = 3), but injection of 10^{-10} mol at the lateral region elicited large-amplitude single depressor spikes from the prothoracic ganglion within 5 min (N = 4). In 3 of the 4 preparations, the FMP was observed within 10 min of injection.

Influence of Sensory Input on Formamidine-Induced Activity

To determine whether the effects of CDM or DCDM required sensory input, all sensory nerves were cut, thereby isolating thoracic ganglia. In these preparations, CDM (2×10^{-9} mol) or DCDM (2×10^{-10} mol) injected into the

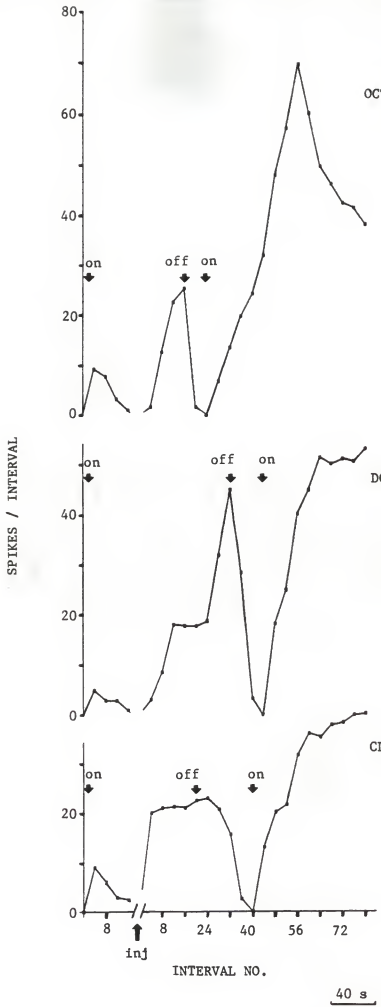
lateral region of the mesothoracic ganglion elicited no IIN1b activity within 12 min (N = 3 for each compound). Effects of octopamine on motor activity also require sensory input, whereas dopamine is effective in both intact and isolated ganglia (Chapter 1).

To determine whether CDM or DCDM increased the response of thoracic ganglia to sensory input, the wing sensory nerve IIN1c was stimulated electrically before and after injecting CDM or DCDM into thoracic lateral regions. Sensory nerves to the mesothoracic ganglion were severed to decrease the level of response in the untreated preparation. Relatively large doses of CDM (7×10^{-10} mol) were required to obtain within 4 min a gradual increase in the number of spikes responding to stimulation (Fig. 3). When electrical stimulation was terminated 4.3 min post-injection, activity in IIN1b decreased. The cessation of motor output suggests that the effect of CDM was dependent on stimulation. After 10 min, however, an electrical stimulus was not required to maintain increasing motor activity.

A greater rate of increase in the response to stimulation was induced by DCDM (Fig. 3). Effect of 10^{-10} mol DCDM 1-2 min after injection was similar to that of 10^{-7} mol CDM 6 min following injection. Absence of an electrical stimulus resulted in decreased motor output from DCDM-treated prepartions (Fig. 3).

Octopamine (10^{-10} mol) elicited a large and rapid response to stimulation (70 spikes/interval within 4 min), but the duration of the effect was much shorter than that of the formamidines. A decline in motor output in response to stimulation was observed 4 min after octopamine injection (Fig. 3).

Fig. 3. Spiking activity in IIN1b during (on) and between (off) periods of electrical stimulation of IIN1c, and before and after injecting (inj) chlordimeform (CDM), demethylchlordimeform (DCDM), or octopamine (OCT). A 200ms stimulus train was delivered every 2.5 sec during the "on" periods. The number of spikes produced over 4 consecutive intervals of 2.5 sec was averaged and plotted. After injecting the compounds, spikes per interval were not recorded until an increase in the average number was observed (break in curve). For CDM, the first point plotted is 3.5 min post-injection; for DCDM, 1.5 min; for OCT, 1 min. Intervals thereafter were successive. Data, which are from one experiment for each compound, are representative of results of similar experiments (N = 3 for CDM, DCDM; N = 5 for OCT).


OCT
DCDM
CDM
on
off
on
SPIKES / INTERVAL
0
20
40
60
80
8
8
24
40
56
72
inj
INTERVAL NO.
40 s

DISCUSSION

In Lepidoptera, the insecticide CDM induces hyperactivity that results in behavioral changes such as increased locomotion (Lund et al., 1979a,b; Hollingworth and Lund, 1982). Kinnamon et al. (1984) showed that CDM-induced hyperactivity in Manduca sexta was at least partially due to an increase in responsiveness of the CNS to sensory input. The present study provides additional evidence that CDM and a related formamidine, DCDM, modulate excitation by enhancing the efficacy of sensory input, and that these compounds exert their effects by binding octopaminergic receptors.

Effects of CDM and DCDM on flight behavior in Manduca were investigated by observing motor output from thoracic ganglia. Earlier work with this preparation showed that two biogenic amines, octopamine and dopamine, elicit the flight pattern by different mechanisms and at different locations (Chapter 1). Results presented here demonstrate that CDM and DCDM mimic the effects of octopamine rather than dopamine. The formamidines and octopamine evoke similar motor output when injected into lateral regions of thoracic ganglia but are inactive at the mesothoracic medial region or in ganglia isolated from sensory input. In contrast, dopamine is effective at the mesothoracic medial region in both intact and deafferented thoracic ganglia.

Effects of CDM and DCDM on responsiveness of the CNS to sensory input also are similar to effects of octopamine. Motor activity generated in response to a fixed electrical stimulus is increased after injecting formamidines or octopamine. However, the compounds differ in the dynamics of their effects. Octopamine exhibits a rapid activation and deactivation; CDM is slower to act, but its effect is longer lasting; DCDM shows rapid activation and has an extended effect. Delay in the initial effects of CDM has been observed in studies on the firefly light organ (Hollingworth and Murdock, 1980) and is thought to involve metabolism of CDM to the more active

demethylated form, DCDM (Knowles, 1984). The long-lasting effects of formamidines are probably due to an inability of the insect to deactivate the synthetic compounds (Salvisberg, 1980).

Results from the present investigation support existing evidence that CDM and DCDM are octopamine agonists. However, unlike in previous studies, the octopamine-like actions of formamidines were demonstrated on insect behavior rather than in biochemical assays. The similar effects of formamidines and octopamine on Manduca flight behavior show more directly that the hyperactivity in insects treated with formamidine insecticides results from activation of octopaminergic mechanisms.

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GENERAL CONCLUSION

Effects of octopamine, dopamine and serotonin on the central nervous system of Manduca sexta provide additional evidence that biogenic amines are a part of the central mechanisms involved in behavior. Octopamine is ubiqituous in invertebrates but relatively rare in vertebrates, and it may be similar functionally to vertebrate norepinephrine and epinephrine. Dopamine and serotonin are present throughout the animal kingdom and are involved in numerous neural functions including the generation and control of complex movements.

Understanding the central nervous system is a formidable task even in the "simpler" invertebrates. The ultimate result of brain functioning is behavior, and some elements involved in producing behavior are now being identified. Biogenic amines appear to play an important role in behaviors--in the initation, intensity and termination. An investigation of these roles would seemingly require a system in which neurons, transmitters and neural connections are understood; however, mapping of behavioral neural circuitry is extremely difficult at present. The Manduca flight system described in this thesis demonstrates that with relatively little understanding of circuitry, effects of biogenic amines can be characterized and their mechanisms of action hypothesized.

The flight system was also proved useful for determining the mode of action of other neuroactive chemicals. Chlordimeform was shown to mimic the effects of octopamine--possibly, the mechanism by which the insecticide induces flight and disrupts normal behavior in whole insects. The fact that chlordimeform acts like octopamine and not the other monoamines suggests that formamidines may be potent octopamine agonists. The system described in this study may be beneficial as an assay to identify other putative agonist and antagonists of octopamine and dopamine.

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EFFECTS OF BIOGENIC AMINES AND FORMAMIDINE INSECTICIDES ON THE CENTRAL PRODUCTION OF FLIGHT BY *MANDUCA SEXTA*

by

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B. A., Kansas State University, 1982

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1985

The effects of biogenic amines on production of the flight motor pattern in the moth Manduca sexta were examined by pressure injecting nanomolar to micromolar amounts of octopamine, dopamine or serotonin into thoracic ganglia. Flight motor output was monitored by extracellular recordings from a pair of antagonistic flight muscles of the mesothorax and from their motor nerve.

In mesothoracic ganglia with sensory nerves intact, octopamine (4×10^{-9} mol) injected into lateral regions evoked regular firing of a single motor neuron, whereas a higher dose (4×10^{-8} mol) often elicited the flight motor pattern. In the absence of sensory input, these doses of octopamine had little effect. Low doses (4×10^{-10} mol) greatly enhanced the response to electrical stimulation of a wing sensory nerve.

Dopamine injected into the medial region of the mesothoracic ganglion elicited the flight motor pattern in the presence or absence of sensory input. The dopamine-induced flight pattern was suppressed by injecting serotonin into the same region.

The effects of two formamidine insecticides, chlordimeform (CDM) and demethylchlordimeform (DCDM), were compared with the effects of octopamine and dopamine on flight motor activity. Nanomole amounts of each compound were dissolved in saline and injected into various regions of the CNS in the presence or absence of sensory input. The effects of CDM (10^{-9} mol) and DCDM (10^{-10} mol) mimicked the effects of octopamine. The formamidines elicited unpatterned motor activity when injected into the prothoracic ganglion and activated large-amplitude single spikes followed by the flight motor pattern when applied to lateral regions of the mesothoracic ganglion, but were inactive at the medial region of the mesothoracic ganglion. As with octopamine, the effects of CDM and DCDM required sensory input. In addition,

the action of formamidines on the efficacy of sensory input was similar to that of octopamine. Both CDM (7×10^{-10} mol) and DCDM (10^{-10} mol) enhanced the response of flight motor neurons to electrical stimulation of a wing sensory nerve.

These findings demonstrated that dopamine, octopamine and serotonin have different effects on flight motor output in Manduca and suggest that biogenic amines are involved in initiating, maintaining and terminating flight behavior. Octopamine may modulate sensory input to the flight pattern generator by enhancing the efficacy of synaptic transmission.

Similarites between the actions of chlordimeform and those of octopamine suggest that the insecticide acts on octopamine receptors in the CNS of Manduca.