

OCTOPAMINE ACTS CENTRALLY TO MODULATE THE
VENTILATORY PATTERN OF CORYDALUS CORNUTUS

by

KARIL LYNNE BELLAH

B.A., Bethany College, 1980

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

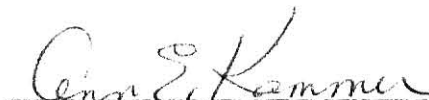
MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1982

Approved by:


Major Professor

Spec
Coll
ID
2668
.T4
1982
B44
c.2

A11202 247366

TABLE OF CONTENTS

List of Figures and Tables	ii
Acknowledgments	iii
Introduction	1
Materials and Methods	3
Results	6
Discussion	23
References	27
Abstract	31

LIST OF FIGURES AND TABLES

Figure 1.	Diagrammatic representation of aminergic neurons in abdominal ganglia 1 - 4	8
Figure 2.	Transient effect of octopamine on ventilatory frequency	10
Figure 3.	Graphic representation of dose-dependent frequency increase	13
Figure 4.	Recording from animal with abdominal ganglia isolated from sensory inputs	16
Table 1.	Octopamine actions on specific ganglia containing different oscillators	17
Figure 5.	Effect of cyproheptadine on frequency	19
Figure 6.	Effect of dopamine on frequency	21

ACKNOWLEDGMENTS

I am sincerely grateful to Dr. Ann Kammer for her ideas and encouragement throughout the course of this project. Her suggestions and support were invaluable.

I also thank Dr. Fred Wilson and Dr. Roger Fedde for their excellent advice and assistance. I appreciate the exchange of ideas from Greg Fitch and Dr. Sue Kinnamon and the technical assistance of Dale Claassen. Special thanks to Dr. Richard Beeman and Lee Klaassen for their time and materials for the biochemical assay.

Financial support for much of the work reported was provided by a grant (BNS-79-23096) from the National Science Foundation to Dr. Kammer.

INTRODUCTION

Biogenic amines are known to modulate the functions of insect nervous systems (for review see Evans, 1980). Most studies of biogenic amines have examined effects on peripheral effectors. Much less is known about the actions of biogenic amines on the insect central nervous system, although some information is available on the central actions of biogenic amines in other invertebrate nervous systems. The latter studies include the effects of serotonin and octopamine on posture in lobsters and crayfish (Livingstone et al., 1980), of octopamine on cricket auditory interneurons (O'Shea and Murphey, 1978), of serotonin on the leech swimming motor pattern (Willard, 1980), and of dopamine on the crustacean cardiac ganglion (Miller et al., 1981) and on the feeding motor pattern of Helisoma (Barker and Trimble, 1981). There is also considerable evidence for the presence of biogenic amines in various regions of the nervous system in several insects, suggesting a physiological role of biogenic amines in these systems (Evans, 1980).

We chose to investigate the central actions of biogenic amines by examining the effects of octopamine on the ventilatory motor pattern of an aquatic insect, Corydalis cornutus. This rhythmic behavior is exhibited by seven pairs of tracheal gills, which beat in a metachronal wave beginning with abdominal segment 3 followed in order by segments 4,5,6,7,2, and 1 (Kinnamon et al., 1980). Frequency of the rhythm varies from 0 - 120 gill beats/minute. Each gill retraction is caused by contraction of a gill retractor muscle, which is excited during ventilation by a motor neuron in the corresponding segmental ganglion. The ventilatory motor pattern can be generated by abdominal ganglion 2 or 3 after complete

isolation from other ganglia and peripheral inputs. Because this system has a simple motor pattern generated centrally without sensory inputs in dissected animals, it provides an excellent opportunity to study the effects of octopamine on rhythmic behavior.

In this paper we report that 1) monoaminergic neurons and octopamine are present in abdominal ganglia, 2) octopamine acts centrally to initiate ventilatory activity or increase its frequency, 3) octopamine exerts its effect only on chains of ganglia that contain certain oscillators, and 4) dopamine opposes the action of octopamine and decreases ventilatory frequency.

MATERIALS AND METHODS

Corydalis cornutus larvae (hellgrammites) were collected locally from streams and rivers and stored in tap water at room temperature without food. Large animals (head width of 8 - 12 mm) were acclimated to those laboratory conditions for two to four weeks before use.

Aminergic neurons in ganglia were stained with neutral red dye (Stuart et al., 1974). A portion of the nerve cord containing the last thoracic ganglion and all of the abdominal ganglia was removed from five animals. Tissues were stained in .02 mg/ml neutral red for 12 - 18 hours at 6° C and then examined under a dissecting microscope. Drawings of the stained somata in selected ganglia were made with the aid of a camera lucida.

Octopamine in abdominal ganglia 1-4 was detected by radioenzymatic assay. The method used to assay octopamine was a modification of the procedure developed by Molinoff et al. (1966) and modified by Evans (1978). This method entailed the enzymatic methylation of octopamine with a labeled methyl group from S-adenosyl methionine. Radioactive synephrine, the product of this reaction, was measured by counting the radioactivity of the samples. Abdominal ganglia 1-4 from five animals (20 ganglia) were pooled in 100 μ l 0.01 N formic acid to provide enough tissue for the sensitivity of the assay (1 ng octopamine). Each reaction tube contained the following: 100 μ l 0.1 M Tris buffer, pH 8.6, 10 μ l .1 nCi/ μ l [methyl- H^3] S-adenosyl-methionine, 10 μ l internal standard (1 ng DL-octopamine, Sigma) or 10 μ l Tris buffer, 10 μ l tissue sample, and 20 μ l Phenylethanolamine-N-methyl transferase (0.5 units, Sigma). After a 30 minute incubation at 37° C, reactions were ended by addition of 0.5 ml of 0.1 mg synephrine/ml borate buffer, pH 10. Two ml

toluene-isoamyl alcohol (3:2, v/v) were added, the tubes were vortexed, and the borate solution removed. The organic phase was then washed with 0.8 ml borate buffer lacking synephrine. One ml of sample in organic phase was extracted with 0.7 ml 0.1 N HCl and vortexed. A 0.5 ml aliquot of HCl solvent was placed in a scintillation vial and heated to dryness at 80-100° C. Dried extracts were redissolved in 0.5 ml 95% ethanol, and 10 ml scintillation fluid added. Radioactivity was counted for 10 minutes with a Searle Isocap/300 Liquid Scintillation Counter. Each sample was compared to simultaneous controls, and octopamine content determined from a standard curve.

To determine the effects of exogenous octopamine on the ventilatory pattern, the ventral nerve cord was exposed while the ventilatory frequency was measured. For ventral dissections, animals with legs removed were pinned ventral side up to the bottom of a small recording chamber containing 100 ml of physiological saline (Kinnamon, unpublished). A ventral incision was made through the cuticle; care was taken not to cut the major tracheae, especially those supplying the ganglia. Dorsal dissections were performed in the same manner except that a cut was made in the dorsal midline, and the gut was reflected to expose the ventral nerve cord. To prevent the animals from becoming hypoxic, air was bubbled through the saline during experiments.

Dissected animals were left undisturbed for at least one hour, or until the ventilatory rate decreased below 100 beats/minute, before data were collected. To achieve the desired concentration of octopamine in the saline bath (10^{-3} - 10^{-6} M), a measured amount of a concentrated solution of octopamine was introduced to the chamber and mixed throughout by the turbulence created by aeration.

Data were collected by counting gill beats or by recording with a saline-filled suction electrode from the ventral motor nerve (V1) supplying the gill retractor muscle of abdominal segment 3. Recordings were stored on magnetic tape and then transferred to a Brush 260 (Gould) chart recorder. Most animals tested were exposed to one to three applications of octopamine; at least one hour was allowed between treatments. Ventilatory frequency was monitored for 10 minutes before octopamine was applied, and data were collected each minute after application for 15 minutes or until ventilatory rate returned to the pretreatment frequency. Data were accepted as significantly different when $p < .05$ in a paired t-test. Experiments with dopamine and cyproheptadine were of similar design.

To demonstrate the central action of octopamine, abdominal ganglia 1 - 3 (A1 - A3) were isolated from the rest of the nerve cord by severing the connectives anterior to A1 and posterior to A3. Sensory inputs were eliminated by severing all peripheral nerve roots of these ganglia. To examine the effects of octopamine on oscillators in different ganglia, a ganglion or groups of ganglia were isolated by severing selected connectives of the nerve cord. Some of these animals were given more than one treatment of octopamine, and data were collected and analyzed as before.

RESULTS

Neurons with an affinity for neutral red dye (which stains monoaminergic cells) were observed in the last thoracic ganglion and in abdominal ganglia 1 - 7 in all five animals examined. In abdominal ganglion 1 (A1), somata of approximately 10 stained neurons were observed in a midline, posterior cluster (Fig. 1A1). Ten to 14 somata were distributed equally into anterior and posterior groups in both ganglion 2 (A2) and ganglion 3 (A3) (Fig. 1A2, 1A3). A cluster of approximately 10 neurons was found in the center of ganglion 4 (A4), and the lateral posterior regions of A4 also contained stained somata (Fig. 1A4).

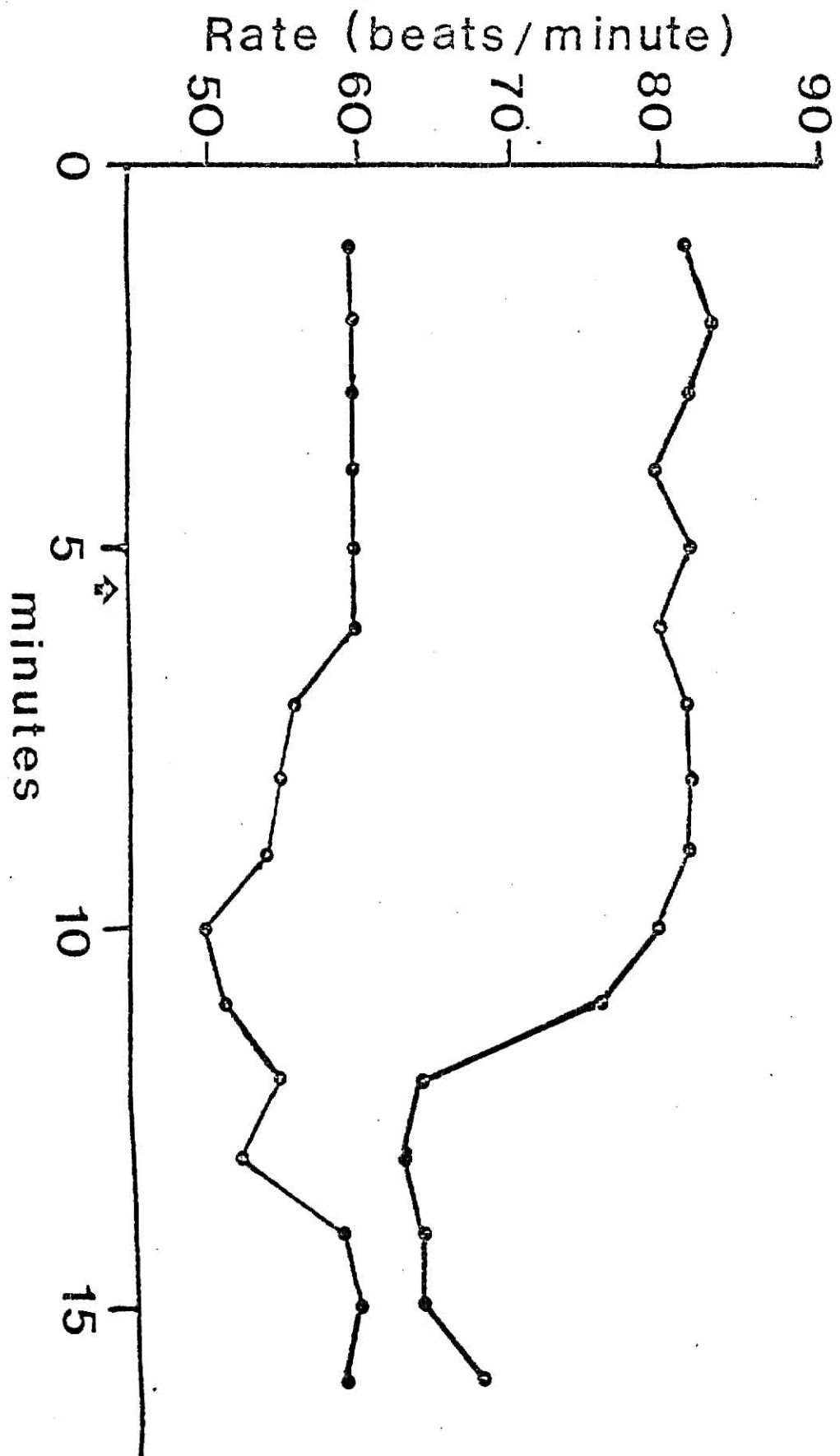
Octopamine was detected by radioenzymatic assay in the first four abdominal ganglia. Octopamine in other ganglia was not determined. The radioactive derivative of octopamine measured in sample reaction tubes was compared to simultaneous controls (reaction tubes without tissue sample or without enzyme) and to samples with an internal standard (1 ng octopamine). Radioactive counts in the samples were significantly above the background counts of both controls. Compared with the internal standard and a standard curve, approximately 1 ng of octopamine was detected in each of five samples from two tissue homogenates (10 samples total). The data indicated that each abdominal ganglion contained approximately 0.0125 pmole of octopamine.

When 10^{-5} M octopamine was applied to dissected animals with intact nervous systems, ventilatory rate increased (Fig. 2). A gradual increase in ventilatory frequency occurred within minutes after octopamine was added to the saline bath. The effect was transient. The mean percent increase in ventilatory rate [(final rate - initial rate)/initial rate $\pm$ SEM] for 11 animals tested was $17.1 \pm 2.00\%$. Nine animals

FIGURE 1. Camera lucida drawings of hellgrammite abdominal ganglia 1 through 4 (A1 - A4). Somata of aminergic neurons were stained with neutral red dye. In A1 the motor neuron (MN) innervating the gill retractor muscle and its axon in nerve V1 are shown. That neuron did not stain with neutral red, but was backfilled with cobalt chloride (Kinnamon, unpublished).



FIGURE 2. Transient effect of 10^{-4} M octopamine on ventilatory rates (gill beats per minute) of two animals. Time of addition of octopamine is indicated by arrow.

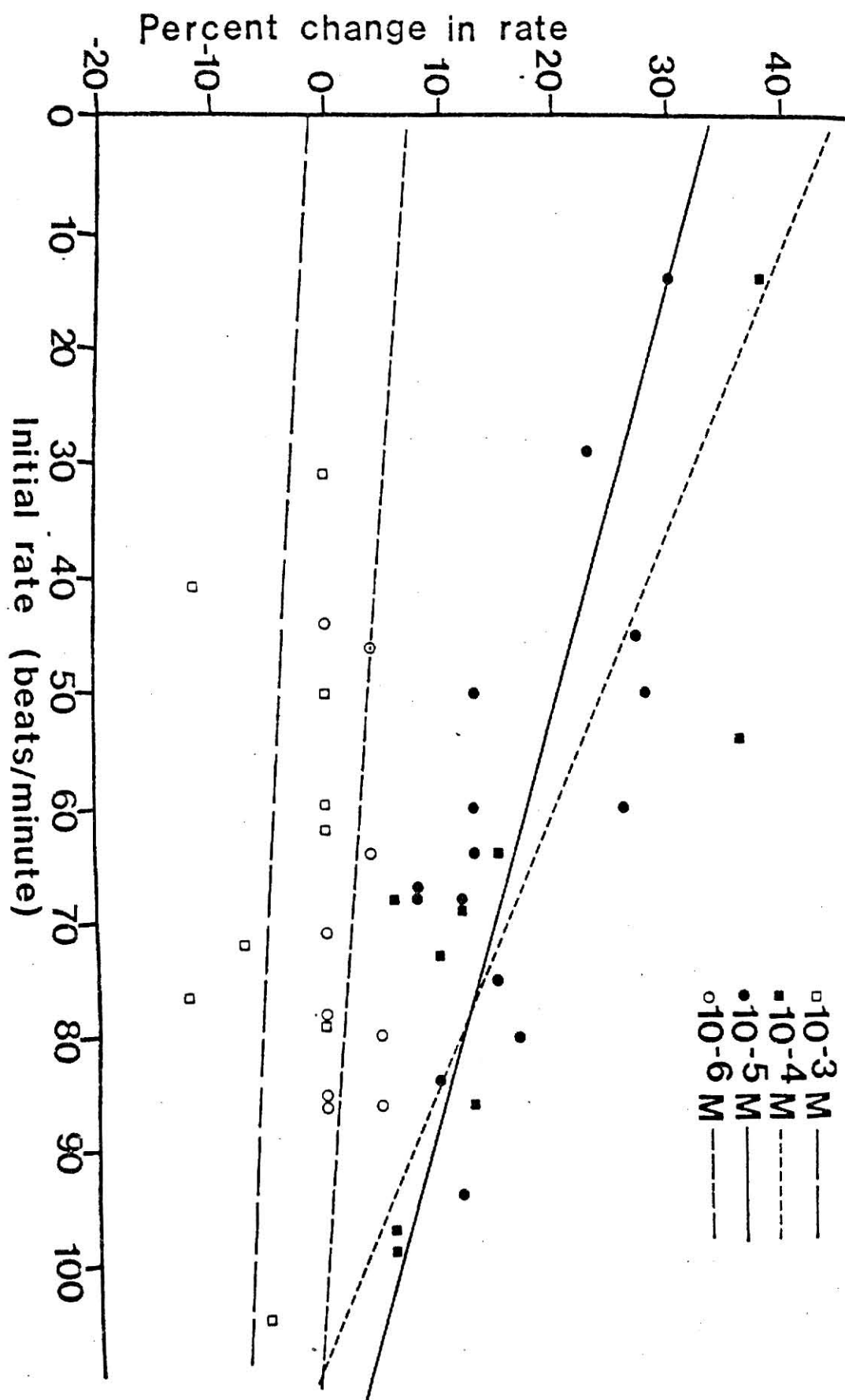


tested with 10^{-4} M octopamine responded with a mean percent increase of $15.8 \pm 4.20\%$. There was no significant difference between the percent changes in ventilatory rate with 10^{-5} and 10^{-4} M octopamine. With 10^{-6} M octopamine, however, only four of nine animals showed an increase in rate; the mean percent increase for those four animals was only $6.7 \pm 0.92\%$. Octopamine concentrations of 10^{-3} M had no effect on five of nine animals tested. In the remaining four animals, 10^{-3} M octopamine caused an $8.9 \pm 1.60\%$ decrease in the ventilatory frequency.

With both 10^{-4} and 10^{-5} M octopamine, there was a significant inverse correlation between the percent increase in ventilatory rate after octopamine and the ventilatory rate before octopamine (Fig. 3). A similar relationship did not exist with 10^{-3} or 10^{-6} M octopamine, and the mean responses at these concentrations were significantly smaller than those at 10^{-4} and 10^{-5} M octopamine. In subsequent analyses, data obtained with 10^{-5} and 10^{-4} M octopamine are combined as responses at those two concentrations are not significantly different.

Ventilatory behavior was initiated by octopamine. Six of seven dissected animals showing no ventilatory activity started to produce the ventilatory pattern within minutes after application of 10^{-4} or 10^{-5} M octopamine. The mean frequency of this activity was 20.2 ± 2.40 beats/minute. Ventilatory activity initiated by octopamine lasted longer than octopamine-induced increase in frequency of ongoing ventilatory rate. Of the six animals that responded in this experiment, three had intact CNSs, one had abdominal ganglia 1 - 3 isolated from the rest of the nerve cord, and two animals had A1 - A3 isolated from peripheral inputs also (all nerve roots of these ganglia were severed). No

FIGURE 3. Significant inverse correlation between percent change in ventilation rate after octopamine and ventilatory rate before octopamine was added to saline bath. Graph shows percent change $[(\text{final rate} - \text{initial rate})/\text{initial rate}]$ versus initial frequency for different doses of octopamine. An inverse relationship between initial rate and percent change exists with 10^{-5} M ($r = -.706$, $n = 15$) and 10^{-4} M ($r = -.831$, $n = 9$), but not with concentrations of 10^{-3} M ($r = -.152$, $n = 9$) or 10^{-6} M ($r = -.062$, $n = 9$).



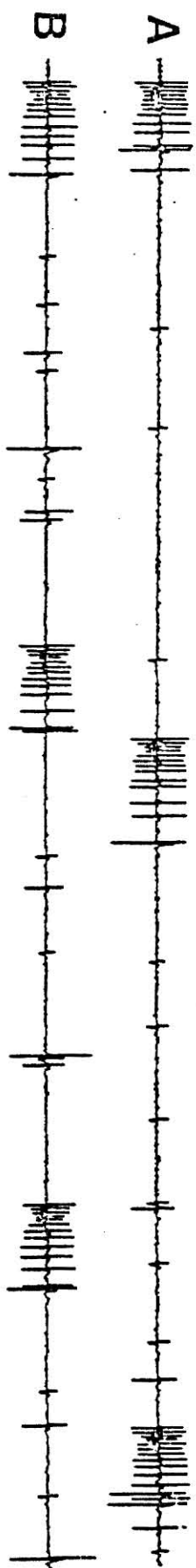
differences were noted among the responses of these different preparations.

Octopamine acted centrally on the nervous system. As mentioned above, octopamine turned on behavior when A1 - A3 were isolated from ascending and descending inputs and from all peripheral inputs. In all five animals with A1 - A3 isolated, octopamine increased ventilatory frequency $13.7 \pm 1.97\%$. There were no gill movements and no intact sensory inputs in these preparations. Activity was recorded from the motor root that innervates the gill retractor muscle of abdominal segment 3 (Fig. 4).

Octopamine affected specific ganglia that contain oscillators possessing different properties (Table 1). Octopamine affected only chains of two or more ganglia. Octopamine had no effect on any single ganglion except in two of 11 animals in which ventilatory frequency in segment 3 increased. Octopamine exerted the greatest effect on chains containing A3 and had less effect on chains containing A2 but not A3. The mean percent increase in ventilation for chains containing A3 was not significantly different from that for intact animals.

Cyproheptadine, at a concentration of 10^{-4} M, drastically reduced ventilatory frequency (Fig. 5). Ventilatory frequency did not return to the pretreatment frequency until cyproheptadine was removed and the preparation flushed with saline. Seven of eight animals showed a mean decrease of $38.0 \pm 7.05\%$ a few minutes after cyproheptadine was added. Application of equimolar octopamine did not eliminate the action of cyproheptadine. Three of the seven animals were also tested with 10^{-5} M cyproheptadine, and none responded.

FIGURE 4. Octopamine increased the ventilatory rate in animals with isolated ganglia. Recording from nerve VI of abdominal ganglion 3 in an animal in which abdominal ganglia 1 - 3 were isolated from the rest of the nerve cord and all peripheral nerve roots were severed. Each burst of spikes represents activity in the gill retractor motor neuron and would result in gill retraction in animals with intact nerve roots. Trace A is a recording before octopamine. Trace B, recorded after 10^{-5} M octopamine, shows shorter interburst intervals and, therefore, an increased ventilatory frequency.



250 ms

TABLE 1. Effects of octopamine on dissected animals with isolated abdominal ganglia. Responses to 10^{-4} and 10^{-5} M octopamine have been combined. The percent increase in ventilatory frequency for each preparation is reported as the mean of those animals responding $\pm$ SEM. R/N is the ratio of animals responding per number of animals tested.

<u>Abdominal Ganglia</u>	<u>Percent Change</u>	
	<u>in Frequency</u>	<u>(R/N)</u>
ant. - 7	16.6 $\pm$ 1.95	(16/16)
1-3	15.4 $\pm$ 2.51	(7/9)
ant. - 2	6.6 $\pm$ 1.16	(4/8)
3-7	18.8 $\pm$ 2.56	(5/6)
2-3	15.3 $\pm$ 1.31	(5/5)
4-7	0	(16/16)
1	0	(16/16)
2	0	(10/10)
3	17.9, 5.5	(2/11)

FIGURE 5. Effect of 10^{-4} M cyproheptadine on ventilatory rate.
Application of cyproheptadine is indicated by arrow.

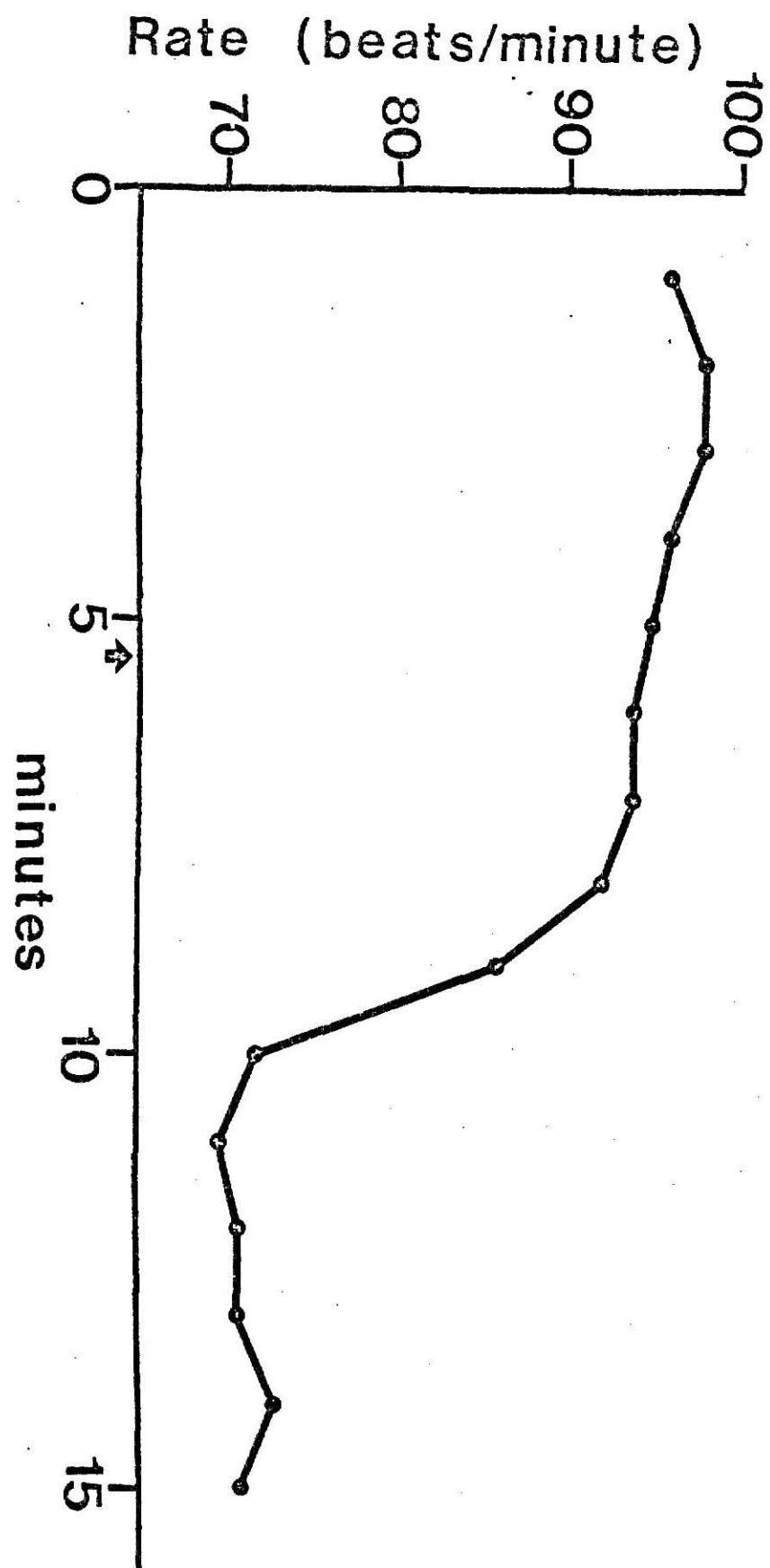
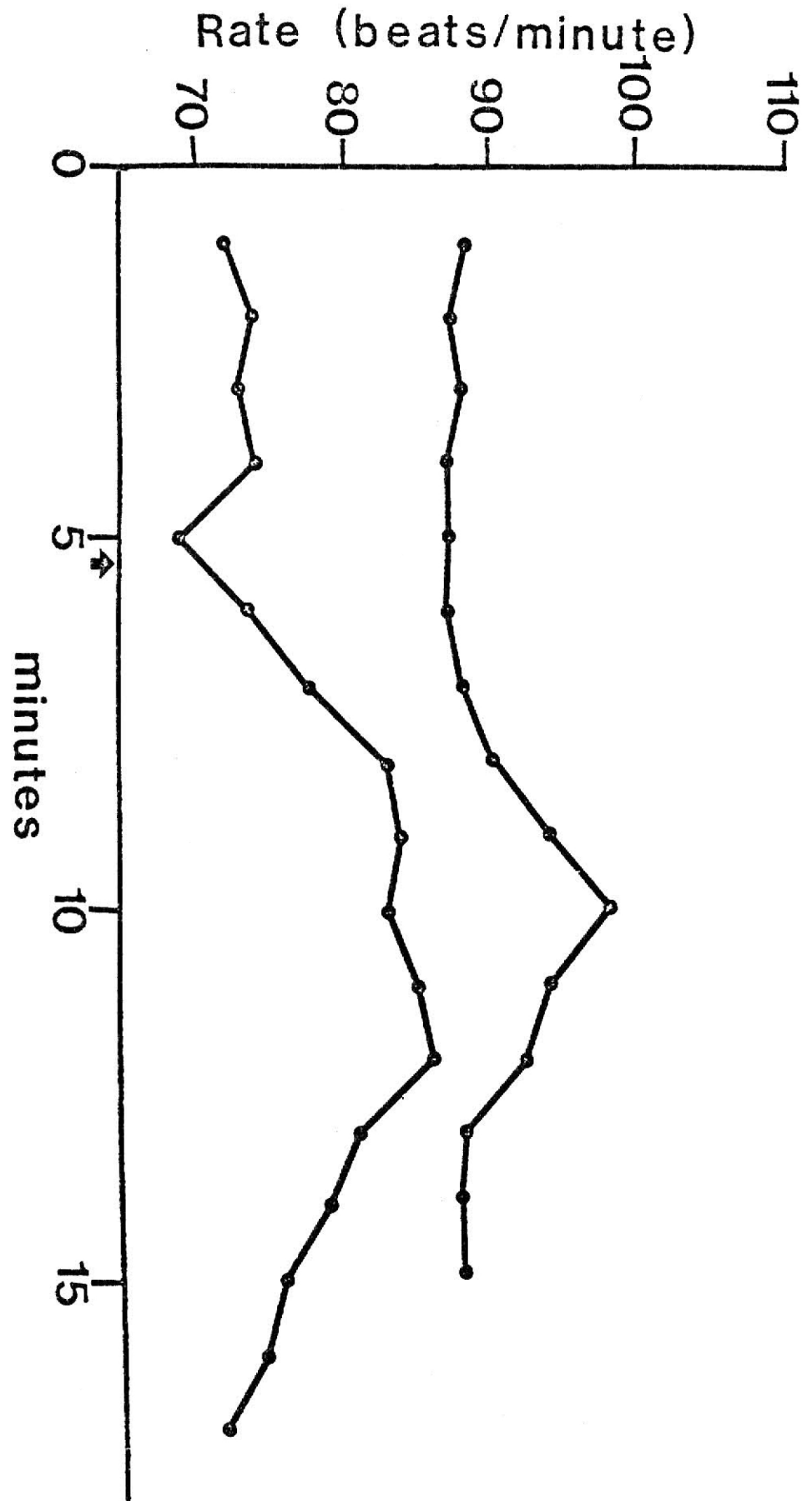


FIGURE 6. Effect of 10^{-4} M dopamine on ventilatory frequencies of two animals. Application of dopamine is indicated by arrow.



Exogenous dopamine (10^{-4} or 10^{-5} M) decreased ventilatory frequency $16.7 \pm 4.04\%$ in seven of nine animals (Fig. 6). Decrease in ventilatory rate was gradual and transient; ventilatory rate returned to the pre-treatment frequency within minutes after the decrease in rate.

DISCUSSION

That octopamine occurs in the abdominal ganglia of Corydalis cornutus is consistent with the finding that octopamine and other biogenic amines occur in many portions of insect nervous systems (Evans, 1980). Evidence for monoaminergic neurons was provided by the observation of neutral red-stained somata in the last thoracic ganglion and in all of the abdominal ganglia. Not all of those cells necessarily contain octopamine as neutral red stains monoamines nonspecifically (Stuart et al., 1974). The amount of octopamine in the first four abdominal ganglia was approximately 0.0125 pmole/ganglion. Evans (1978) reported 1.04 ± 0.11 pmole/abdominal ganglion in locust and 1.56 ± 0.52 pmole/abdominal ganglion in cockroach. The amount of octopamine in hellgrammite ganglia was approximately 100 times less than amounts in other insect ganglia. A difference in ganglia sizes may account for the difference in octopamine content as the approximate volume of a Corydalis abdominal ganglion ($4.2 \times 10^6 \mu\text{m}^3$) was about 100 fold less than that of a cockroach abdominal ganglion ($2.7 \times 10^8 \mu\text{m}^3$, calculated from a linear dimension (Evans, 1980) assuming the ganglion is a sphere). O'Shea et al. (1979) reported 0.14 ± 0.02 pmole octopamine per soma from locust metathoracic ganglia, an amount greater than in the entire hellgrammite ganglion. The available data did not allow an estimate of the number of octopaminergic neurons in Corydalis ganglia.

There was a dose-dependent increase in ventilatory frequency over a narrow range of octopamine concentrations (10^{-4} - 10^{-5} M). Increases in frequency were gradual and transient; ventilatory rates returned to pretreatment values within minutes after increase in frequency occurred.

The central action of octopamine on the nervous system of the hellgrammite is one of the few examples of a biogenic amine acting centrally in insect preparations. Octopamine modulates the sensitivity of interneurons involved with auditory responses in the cricket Acheta domesticus (O'Shea and Murphey, 1978). In the moth Manduca sexta, chlordimeform, an octopamine agonist (Evans and Gee, 1980; Hollingworth and Murdock, 1978), acts centrally to modulate motor output (Kinnamon et al., 1980). In the hellgrammite, octopamine increased the ventilatory activity of a preparation consisting of an isolated chain of ganglia with all peripheral nerve roots cut. Hence the octopamine is acting centrally.

Octopamine exerted its greatest effect when chains of ganglia containing abdominal ganglion 3 were isolated from other ganglia. There was no significant difference in the increases in frequency between octopamine-treated animals with isolated chains of two or more ganglia containing A3 and octopamine-treated animals with intact nervous systems. Abdominal ganglion 3 is the locus of the dominant oscillator for the ventilatory rhythm because it initiates the metachronal beating of the gills, produces the rhythm in isolation from other oscillators at a frequency nearest that of intact preparations (Kinnamon et al., 1981), and is driven and reset by phasic stimulation (Fitch and Kammer, 1981). Chains of ganglia containing a secondary oscillator in A2 but not connected to A3 showed only a slight increase in ventilatory frequency in response to exogenous octopamine. No difference in increase in frequency was observed between octopamine-treated animals with chains containing abdominal ganglion 3 and those containing both A3 and A2. That octopamine increased the frequency in animals with chains of two or more ganglia may be due to the low level of

general excitation, decreased concentration of octopamine receptors, or lack of timing cues between oscillators; however, there is no evidence supporting any one of those ideas.

Cyproheptadine, an aminergic antagonist, drastically decreased ventilatory frequency. Most aminergic antagonists are not specific for a single biogenic amine; such is the case with cyproheptadine, which blocked octopamine-sensitive and dopamine-sensitive adenylate cyclase activity in the cockroach brain (Harmer and Horn, 1977). Roberts and Walker (1981) reported that cyproheptadine was a potent octopamine antagonist in cockroach abdominal ganglia. Because cyproheptadine produced the opposite effect of octopamine in Corydalis, we propose that cyproheptadine inhibited the action of endogenous octopamine in modulating ventilatory frequency.

Octopamine and dopamine had opposing actions on ventilatory frequency. With the same concentrations of octopamine and dopamine, octopamine increased, whereas dopamine decreased ventilatory rate; the magnitude of change was similar in both cases. The opposing action of two other biogenic amines has previously been demonstrated in lobster and crayfish (Livingstone et al., 1980). Octopamine and serotonin produce opposite postural behaviors in those invertebrates by either increasing or decreasing firing of specific neuronal units innervating the flexor and extensor muscles. As more invertebrate systems are examined, there may emerge a widespread dependence on the opposing action of biogenic amines similar to the opposing effects of norepinephrine and acetylcholine in vertebrate nervous systems.

That the central action of octopamine in Corydalis has physiological significance is supported by several observations. First, octopamine exerted its greatest effect on the dominant oscillator, which determines

the frequency of the other oscillators. This is the expected site of action for physiological control. Second, octopamine was found in the ganglia generating the ventilatory pattern. It is possible that octopamine is released from interneurons as a neurotransmitter or neuromodulator that affects components of the central pattern generator. Third, cyproheptadine, an octopamine blocker, reduced ventilatory frequency. This compound may exert its effect by blocking the action of endogenous octopamine. These results suggest that, in the central nervous system, there is an ongoing release of octopamine that continuously modulates ventilatory frequency. Finally, the antagonistic actions of octopamine and dopamine offer a possible mechanism for the fine control of ventilatory frequency by two biogenic amines.

These findings are consistent with evidence in other insect nervous systems that octopamine may affect a response preparing the animal for increased activity. Octopamine inhibits the intrinsic rhythm of myogenic leg muscles in locust (Evans and O'Shea, 1978), stimulates heart rate in cockroach (Collins and Miller, 1977), activates glycogenolysis in cockroach fat body (Gole and Downer, 1979), mediates hormone release in locust (Orchard and Loughton, 1981), stimulates oxidation of glucose and other fuels in locust thoracic flight muscles (Candy, 1978), causes a cAMP-mediated activation of phosphorylase activity in cockroach nerve cord (Robertson and Steele, 1972), and increase ventilatory frequency in Corydalis. Octopamine may mediate a generalized stress-response in insects analogous to the action of norepinephrine and epinephrine in mammals.

REFERENCES

- Candy, D.J. (1978). The regulation of locust flight muscle metabolism by octopamine and other compounds. Insect Biochem. 8, 177-181.
- Collins, C., and Miller, T. (1977). Studies on the action of biogenic amines on cockroach heart. J. exp. Biol. 67, 1-15.
- Evans, P.D. (1978). Octopamine distribution in the insect nervous system. J. Neurochem. 30, 1009-1013.
- Evans, P.D. (1980). Biogenic amines in the insect nervous system. Adv. Insect Physiol. 15, 317-473.
- Evans, P.D. and Gee, J.D. (1980). Action of formamidine pesticides on octopamine receptors. Nature 287, 60-62.
- Evans, P.D. and O'Shea, M. (1978). The identification of an octopaminergic neurone and the modulation of a myogenic rhythm in the locust. J. exp. Biol. 73, 235-260.
- Fitch, G.K. and Kammer, A.E. (1981). Interactions of ventilatory rhythm generators and sensory modulation of their outputs. Soc. Neurosci Abst. 7, 411.
- Gole, J.W.D. and Downer, R.G.H. (1979). Elevation of adenosine 3',5'-monophosphate by octopamine in fat body of the American cockroach, Periplaneta americana L. Comp. Biochem. Physiol. 64C, 223-226.
- Harmer, A.J. and Horn, A.S. (1977). Octopamine-sensitive adenylate cyclase in cockroach brain: effects of agonists, antagonists, and guanylyl nucleotides. Mol. Pharmacol. 13, 512-520.
- Hollingworth, R.M. and Murdock, L.L. (1980). Formamidine pesticides: octopamine-like actions in a firefly. Science 208, 7476.

- Kinnamon, S.C., Klaassen, L.W., and Kammer, A.E. (1980). Habituation and effects of an octopamine agonist in the developing moth flight control system. Soc. Neurosci. Abst. 6, 627.
- Kinnamon, S.C., Fitch, G.K., Bellah, K.L., and Kammer, A.E. (1981). Segmental distribution of ventilatory control mechanisms in an aquatic insect. Soc. Neurosci. Abst. 7, 411.
- Livingstone, M.S., Harris-Warrick, R.M., and Kravitz, E.A. (1980). Serotonin and octopamine produce opposite postures in lobsters. Science 208, 76-79.
- Miller, M.W., Sullivan, R.E., Benson, J.A., and Berlind, A. (1981). Dopamine action on the crustacean cardiac ganglion. Soc. Neurosci. Abst. 7, 317.
- Molinoff, P.B., Landeberg, L., and Axelrod, J. (1969). An enzymatic assay for octopamine and other p-hydroxyphenylethylamines. J. Pharmac. exp. Ther. 170, 253-261.
- Orchard, I. and Loughton, B.G. (1981). Is octopamine a transmitter mediating hormone release in insects? J. Neurobiol. 12, 143-153.
- O'Shea, M. and Murphey, R.K. (1978). Octopamine modulates sensitivity of identified insect interneurons. Soc. Neurosci. Abst. 4, 203.
- O'Shea, M., Goodman, C.S., McCaman, R., and Spitzer, N.C. (1979). Embryonic development of identified neurons: temporal pattern of morphological and biochemical differentiation. Science 204, 1219-1222.
- Roberts, C.J. and Walker, R.J. (1981). Octopamine receptors on Limulus and Periplaneta central neurons. Comp. Biochem. Physiol. 69C, 301-306.
- Robertson, H.A. and Juorio, A.V. (1976). Octopamine and some related noncatecholic amines in invertebrate nervous system. Int. Rev. Neurobiol. 19, 173-224.

- Robertson, H.A. and Steele, J.E. (1972). Activation of insect nerve cord phosphorylase by octopamine and adenosine 3',5'-monophosphate. J. Neurochem. 19, 1603-1606.
- Stuart, A.E., Hudspeth, A.J., and Hall, Z.W. (1974). Vital staining of specific monoamine-containing cells in the leech nervous system. Cell Tiss. Res. 153, 55-61.
- Trimble, D.L. and Barker, D.L. (1981). Activation of the feeding motor pattern of Helisoma by dopamine. Midwest Neurobiol. Abst. 4, 7.
- Willard, A.L. (1980). Serotonin increases the probability that the leech nervous system will produce the swim motor pattern. Soc. Neurosci. Abst. 6, 27.

OCTOPAMINE ACTS CENTRALLY TO MODULATE THE
VENTILATORY PATTERN OF CORYDALUS CORNUTUS

by

KARIL LYNNE BELLAH

B.A., Bethany College, 1980

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1982

ABSTRACT

The central effects of octopamine and its physiological role in the modulation of the ventilatory pattern were examined in an aquatic insect, Corydalis cornutus. Monoaminergic neurons in the abdominal ganglia were revealed by staining the ventral nerve cord with neutral red dye. Ten to 14 somata were stained in each of the first four abdominal ganglia. Octopamine was detected by radioenzymatic assay in the first four abdominal ganglia; approximately 0.0125 pmole octopamine per ganglion was found.

Octopamine was applied to dissected animals in saline baths, and gill beats were counted or activity recorded from the motor root innervating the gill retractor muscle. With 10^{-4} and 10^{-5} M octopamine, ventilatory rate increased $16.6 \pm 1.95\%$ (mean $\pm$ SEM) in all 16 animals tested, but with concentrations of 10^{-3} and 10^{-6} M, little or no response was observed. The effect of octopamine lasted only a few minutes, after which ventilatory rate usually returned to the pretreatment frequency. The magnitude of the increase in frequency was inversely related to the pretreatment frequency.

Octopamine initiated ventilation or increased the ventilatory frequency in both intact preparations and dissected preparations with abdominal ganglia 1 - 3 isolated from other ganglia and sensory inputs. Octopamine had no effect when any single ganglion was isolated from the others. It exerted the greatest effect on chains of ganglia containing abdominal ganglion 3 and a lesser effect on chains containing ganglion 2 but not ganglion 3.

Cyproheptadine, an aminergic antagonist, drastically decreased ventilatory frequency $38.0 \pm 7.05\%$ in seven of eight dissected animals

with intact nervous systems. Dopamine produced an effect opposite to that of octopamine and decreased the ventilatory frequency in 10 of 12 animals tested. The average decrease in ventilatory rate was $16.7 \pm 4.04\%$.

The results show that octopamine acts centrally to modulate ventilatory frequency and that dopamine antagonizes the action of octopamine.