The identification and distribution of major biogenic amines in the central nervous system of Aedes triseriatus (Say) (Diptera: Culicidae) and studies on their regulation of adult mosquito behavior

Mark G. Novak  
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Entomology Commons, Neuroscience and Neurobiology Commons, and the Neurosciences Commons

Recommended Citation

Novak, Mark G., "The identification and distribution of major biogenic amines in the central nervous system of Aedes triseriatus (Say) (Diptera: Culicidae) and studies on their regulation of adult mosquito behavior" (1992). Retrospective Theses and Dissertations. 10337. https://lib.dr.iastate.edu/rtd/10337

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
The identification and distribution of major biogenic amines in the central nervous system of *Aedes triseriatus* (Say) (Diptera: Culicidae) and studies on their regulation of adult mosquito behavior

Novak, Mark G., Ph.D.

Iowa State University, 1992
The identification and distribution of major biogenic amines in the central nervous system of *Aedes triseriatus* (Say) (Diptera: Culicidae) and studies on their regulation of adult mosquito behavior

by

Mark G. Novak

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1992
TABLE OF CONTENTS

ACKNOWLEDGMENTS iv
GENERAL INTRODUCTION AND LITERATURE REVIEW 1

PAPER I. IDENTIFICATION AND QUANTIFICATION OF MAJOR BIOGENIC AMINES IN THE CENTRAL NERVOUS SYSTEM OF THE MOSQUITO AEDES TRISERIATUS (SAY) (DIPTERA: CULICIDAE) 17

ABSTRACT 19
INTRODUCTION 20
MATERIALS AND METHODS 22
RESULTS 25
DISCUSSION 33
ACKNOWLEDGMENTS 38
LITERATURE CITED 39

PAPER II. EFFECTS OF α-METHYL-TYROSINE AND α-METHYL-TRYPTOPHAN ON BIOGENIC AMINE CONCENTRATIONS AND CIRCADIAN FLIGHT ACTIVITY OF AEDES TRISERIATUS (SAY) (DIPTERA: CULICIDAE) 42

ABSTRACT 44
INTRODUCTION 45
MATERIALS AND METHODS 47
RESULTS 50
DISCUSSION 62
LITERATURE CITED 65

PAPER III. SEROTONIN DEPLETION AFFECTS BLOODFEEDING BUT NOT HOST-SEEKING ABILITY IN AEDES TRISERIATUS (SAY) (DIPTERA: CULICIDAE) 67

ABSTRACT 69
INTRODUCTION 70
MATERIALS AND METHODS 73
RESULTS 76
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISCUSSION</td>
<td>81</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>85</td>
</tr>
<tr>
<td>GENERAL SUMMARY</td>
<td>88</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>91</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

I would like to thank the members of my committee, Drs. Wayne A. Rowley, Joel R. Coats, Philip G. Haydon, John A. Mutchmor, and Harold J. Stockdale for their guidance and assistance with this research project. Thanks to Dr. Donald Dyer for his generosity and willingness to share his HPLC and knowledge. I owe a particular debt of gratitude to my advisor Dr. Rowley, who provided a great deal of support and patience with this work and always allowed me the time and opportunity to pursue a variety of other research interests.

I am very grateful for the assistance of numerous student employees, most notably Lynn Goecke, Mary Minachi, Andrea and J. B. Silvers, and Jeff Smith. Thanks also to fellow graduate students Dave Bartholomew, Bill Berry, Beth Collins, and Michelle Sharon for their assistance, support, and friendship.

Finally, very special thanks to my entire family, particularly my parents and most of all to Stacey, my wife, for their extraordinary support, encouragement, and patience over many years.
GENERAL INTRODUCTION AND LITERATURE REVIEW

Classical transmitters such as acetylcholine, GABA (γ-aminobutyric acid), and glutamate have long been known to be important chemicals for relaying information in insect nervous systems. More recently, other compounds such as neuropeptides and biogenic amines have also been recognized as neuroactive. Biogenic amines are monoamine compounds that are important neurochemicals found in all major groups of invertebrates and vertebrates. In both invertebrate and vertebrate phyla, biogenic amines function as neurotransmitters, neuromodulators, and neurohormones. Many recent studies have focused on the identification and function of these compounds in insects. Two primary reasons for this intense research are 1) to more fully understand the underlying control mechanisms of insect physiological processes and behaviors, and 2) to provide alternative strategies for controlling insect populations; although biogenic amines are taxonomically ubiquitous, selective control methods may be possible by taking advantage of taxonomic differences in amine content and/or metabolic pathways.

Molecurally, biogenic amines are based on the phenylethylamine and indolamine structures (Fig. 1). The catecholamines, dopamine and noradrenaline (norepinephrine); the phenolamine, octopamine; and the indolalkylamine, serotonin (5-hydroxytryptamine) are the most readily identified amines in insects and the most intensively studied. Of these compounds, noradrenaline is the most enigmatic. It is either not found or, if present, found in much lower concentrations than the other amines. No positive evidence of specific noradrenergic neurons exists in insect tissues (Evans 1980). This compound may be present in a small number of neurons or it may be a metabolic mistake (Evans 1986).

Octopamine was originally isolated from octopus salivary glands by Erpsamer and Boretti in 1951 and has since been found in nervous tissues of all invertebrates
examined (Orchard 1982). Octopamine is present at much higher levels in invertebrates than vertebrates. While serotonin appears to be the quantitatively dominant amine in the central nervous systems of flatworms, annelids, and mollusks, octopamine occurs at higher concentrations than other monoamines in arthropods (Klemm 1985).

**Phenylethylamines**

![Phenylethylamines](image)

- **Compound** | **Substituent**<br>1. Tyramine | R<sub>1</sub> H H H<br>2. Octopamine | R<sub>2</sub> OH H<br>3. Dopamine | R<sub>3</sub> OH H<br>4. Noradrenaline | OH OH H<br>5. Adrenaline | OH OH OH

**Indolamines**

![Indolamines](image)

- **Compound** | **Substituent**<br>1. Tryptamine | R H<br>2. Serotonin | OH

Figure 1. Structures of the major biogenic amines (from Vaughan 1988)

Adrenaline, although important in vertebrate nervous systems, has not been found in any arthropod examined (Klemm 1985). Another biogenic amine, histamine, was recently identified in insects and may function as a neurotransmitter in the insect visual system (Elias and Evans 1983).

Our knowledge of the biosynthetic pathways of monoamines is incomplete in insects. The generally accepted pathways for the synthesis of catecholamines and octopamine begin with the amino acid tyrosine, while tryptophan is the amino acid precursor of serotonin (Fig. 2). Syntheses of dopamine and serotonin involve an initial hydroxylation of their amino acid precursors followed by a decarboxylation step. Noradrenaline is synthesized from dopamine by a second hydroxylation step (via
Figure 2. Steps in the biosynthesis of monoamines (modified from Vaughan 1988)
dopamine beta-hydroxylase). Octopamine pathways most likely involve an initial decarboxylation of tyrosine followed by a hydroxylation of the resultant tyramine compound.

The major inactivation strategy of biogenic amines in vertebrates is reuptake into the presynaptic nerve terminals via a high-affinity, sodium dependent mechanism. Similarly, high-affinity uptake systems have been described in a number of invertebrates (see Vaughan 1988) including a concentrative, sodium-dependent uptake mechanism for octopamine in the cockroach, *Periplaneta americana* (Evans 1978). A second strategy for amine inactivation in both vertebrates and invertebrates is by low-affinity uptake mechanisms which take up amines in non-neuronal tissue followed by enzymatic inactivation. In vertebrates, biogenic monoamines are enzymatically inactivated by monoamine oxidase and catechol-o-methyl transferase. Insect nervous tissue, in contrast, contains little, if any, of these enzymes. Alternatively, insects inactivate monoamines via N-acetylation (transferring acetyl groups from acetyl coenzyme A to the amino group on the monoamines) (Vaughan 1988).

**Localization of Biogenic Amines**

Histochemical and quantitative biochemical studies have been performed on several taxonomically varied insect species (see reviews by Klemm 1976, 1985, Evans 1980, Orchard 1982, David and Coulon 1985, Næssel 1988). The distribution of biogenic amine-containing cells and their release sites is widespread throughout the central and peripheral nervous systems of insects. Considerable interspecific variation exists with regard to the distribution and relative concentrations of monoamines. Specifically, these studies indicate the presence of one or more biogenic amines in various cell bodies throughout the brain as well as in nerve fibers in most neuropile areas (Fig. 3). Cell bodies containing the same biogenic amine are often aggregated in small clusters. A small number of amine-containing cells are regularly found in all ventral nerve cord
ganglia and also in the stomatogastric nervous system. Amine-containing fibers extend outside of the central nervous system to the corpora cardiaca, peripheral organs, and hypocerebral sympathetic ganglia (Raabe 1988). A plexus of serotonergic fibers from the sympathetic nervous system extend along the digestive tract of several insect species and have also been found in the periphery of mouthpart nerves (Nässel and Elekes 1985, Davis 1987, Orchard et al. 1988). Serotonergic fibers from the frontal ganglia innervate the Malpighian tubules, salivary glands, alimentary canal, genital organs, and somatic muscles (see review by Nässel 1988). In dipterans, direct
Innervation of these structures apparently does not occur. Instead, aminergic fibers terminate in a nerve sheath near the effector site; released amines function as local neurohormones (Nässel 1988).

Klemm (1976) reviewed studies on the distribution of biogenic monoamines in insects and concluded that amine distribution in the brain could be grouped into three categories: 1) structures in which no biogenic amines occur (e.g., primary photoreceptors, afferent and efferent fibers of the antennal nerve, the central fiber bundle in the antennal lobes, and the fiber bundles of the tractus olfactorio-globularis), 2) areas which contain biogenic monoamines in every insect studied to date (e.g., cell bodies in posterior region of the pars intercerebralis, α and β lobes of the mushroom bodies, the central body complex, and the tritocerebrum), and 3) structures in which the presence of monoamines varies interspecifically (e.g., cell bodies of the anterior pars intercerebralis, optic ganglia, calyx and peduncle of the mushroom bodies, pons cerebralis and antennal lobe). Based on comparative studies of monoamines in a variety of insects, Klemm (1976) concluded that there are no established relationships between 1) the occurrence or distribution of biogenic monoamines in visual centers of the insect brain and the visual abilities of insects 2) the occurrence of monoamines in the mushroom body and the "intelligence" of the insect 3) the occurrence of biogenic amines in the protocerebral ridge of the brain and either the visual abilities or the occurrence of ocelli, and 4) the occurrence of biogenic amines in the antennal lobes and the functional development of antennae. Although there are regularities in the occurrence and distribution of monoamines in some insect species, taxonomic relationships are not clear.

At a subcellular level, vertebrate studies have indicated that biogenic amines are typically stored in vesicles located in nerve endings (Vaughan 1988). Mobilization and release of catecholamines are stimulated by an influx of Ca$^{2+}$. Similarly, putative amine-containing vesicles and storage granules have been identified at intraneuronal
terminalizations in insect tissues (see Evans 1980). Evidence also exists for similar release mechanisms in insects (Lange et al. 1988).

The functional description of biogenic amines is dependent upon the effector site(s). If the receptor sites of a released amine are postsynaptic, the compound functions as a neurotransmitter. If, however, the compound affects receptors in a localized region (i.e. not limited to the post synaptic site), it is described as a neuromodulator. An amine functions as a neurohormone when it acts primarily on distant receptors, being transported by the hemolymph. Neuromodulator and neurohormone actions are relatively slow and long lasting in comparison to the action of neurotransmitters.

The mode of action of insect amine receptors has been principally studied at peripheral sites because of their accessibility (Evans 1986). Pharmacological studies of these sites has indicated that, as in the vertebrate nervous system, multiple receptor types with different modes of action exist for each transmitter (Evans 1986). Typically, amine receptors are linked to second messenger systems. Much of the knowledge of amine receptor sites is based on the octopaminergic dorsal-unpaired median neuron (DUMET1) and extensor tibiae muscle preparation of the locust hind leg. This model system has indicated the presence of two receptor types, one which mediates via increases in cyclic AMP levels and one which mediates actions via a release of calcium from intracellular stores (Evans 1986). Octopamine receptors have also been identified in the glandular lobe of the corpora cardiaca in Locusta (Pannabecker and Orchard 1986).

**Roles of Biogenic Amines in Insects**

Numerous investigations have examined the effects of biogenic amines at the organismal level. Several studies have correlated biogenic amine levels to changes in insect activity, circadian rhythms, diapause and other behaviors. Some specific examples include the following:
**Locomotor activity/behavior**

Tunnicliff et al. (1969) compared two populations of *Drosophila melanogaster*, selected for differences in spontaneous motor activity, and reported higher dopamine concentrations in the inactive line and lower concentrations in the active line. Conversely, the noradrenaline concentration was higher in the active line and lower in the inactive line. Decreased spontaneous activity in *D. melanogaster* was observed when gamma hydroxybutyric acid (a compound that increases dopamine levels) was included as a food additive (Connolly et al. 1971). Hemolymph serotonin levels were related to intensity of locomotor activity in *Acheta domesticus*, but no correlation was noted between serotonin levels in the brain and locomotor activity (Muszynska-Pytel and Cymborowski 1978). The heads and bodies of hyperactive ants (*Lasius niger*) contained significantly higher octopamine levels than hypoactive individuals (David and Verron 1982). Increases in hemolymph octopamine levels have been observed in response to flight or stress in several insects including the locust, *Schistocerca americana* (Goosey and Candy 1980), *Periplaneta americana* (Bailey et al. 1984), and *A. domesticus* (Woodring et al. 1988). Harris and Woodring (1992) demonstrated differences in amine concentrations of *Apis mellifera* with respect to season (highest amine levels corresponded to period of greatest foraging activity), adult age, and colony of origin.

Amine concentration changes have also been associated with behavioral modification. Increased serotonin levels and low noradrenaline levels were observed in ants (*Formica rufa*) displaying aggressive behavior (Kostowski et al. 1975). Direct injection of amines into *A. mellifera* brains affected unconditioned and conditioned responses to olfactory stimuli (Mercer and Menzel 1982). Octopamine, but not dopamine or serotonin, enhanced responsiveness to unconditioned olfactory stimuli in *A. mellifera*. Dopamine and serotonin reduced the response (by blocking information retrieval) to a conditioned stimulus. Fuzeau-Braesch and David (1979) reported
differences in octopamine content of solitary and migratory *Locusta migratoria*, but no
differences were noted in similar phases of the locust *Schistocerca americana gregaria*
(Morton and Evans 1983).

**Circadian changes**

Evidence of circadian changes in amine levels is ambiguous. Hinks (1967) suggested
a relationship between the release of serotonin from brain neurosecretory cells of
noctuid moths and the onset of circadian nocturnal flight. However, Owen et al. (1987)
maintained that changes in tryptophan (a serotonin precursor), not serotonin, were
actually observed. Fowler et al. (1972) found that serotonin levels varied over a 24-hour
period in all stages of *Drosophila melanogaster* and serotonin concentration was
elevated in the hemolymph and cerebral ganglia of *A. domesticus* at transitions of a
12:12 hr light-dark cycle (Muszynska-Pytel and Cymborowski 1978). Circadian
serotonin concentration changes were observed in the frontal ganglia of *P. americana*
(Pandey and Habibulla 1982). However, Owen et al. (1987) also measured serotonin and
dopamine levels in the cerebral ganglia of *P. americana* and found no correlation
between circadian activity and amine levels, amine precursors, or metabolic products.
A circadian pattern of octopamine concentration changes was observed in
*A. domesticus* brains and continued when the insects were held in constant light
(Woodring et al. 1988). This is the first report of a free-running rhythm of a biogenic
amine in an insect.

**Feeding behavior**

Octopamine and octopamine agonists induced hyperphagia of sucrose in *Phormia
regina* (Long and Murdock 1983), while pharmacologically induced amine depletion
increased the sucrose concentration acceptance threshold in this species (Brookhart et
al. 1987). Similarly, serotonin depletion in *Rhodnius prolîcus* led to reduced size of
blood meals and prevented cuticle plasticization (Cook and Orchard 1990). An increase
In hemolymph serotonin was associated with feeding activity in *R. prolixus* (Lange et al. 1989).

**Diapause**

Large increases of octopamine were observed in the brain of *Mamestra configurata* following termination of diapause (Bodnaryk 1979) and diapause in *Gryllus campestris* was eliminated by daily administrations of octopamine (Ismail and Fuzeau-Braesch 1982). Dopamine, or a dopamine metabolite, was suggested as an important compound in the induction or termination of diapause in *Ostrinia nubilalis* (Houk and Beck 1977). Purroux et al. (1990) demonstrated differences in dopamine, serotonin, and their respective metabolites in the brains of diapausing and nondiapausing *Pieris brassicae* pupae; they suggested that dopamine and serotonin are involved in the maintenance and termination of the diapausing state in this insect.

**Physiological effects**

Numerous examples of monoamine involvement in physiological processes have been described. Probably the example cited most frequently is the role of octopamine in modulating the lighting of the firefly lantern (Robertson and Carlson 1976). Biogenic amines are also recognized modulators at neuromuscular junctions. Serotonin increased the frequency of oviduct contractions in *Tabanus* (Cook 1981) and *Gryllus*, but the strongest contractions in *Gryllus* were obtained with octopamine (Seflan 1987). Serotonin stimulated uterine contraction in *Blabera* but not in *Glossina fuscipes* (Raabe et al. 1985). Norepinephrine and serotonin increased the frequency of myogenic contractions and enhanced the amplitude of neurally-evoked contractions in *Locusta* oviduct (Lange and Orchard 1984), while octopamine reduced the amplitude of neurally-evoked contractions. Octopamine increased contraction strength and stimulated the oxidation of substrates in locust thoracic muscle (Candy 1978). Biogenic amines affected heart and digestive tract contractions in several insect species (see Raabe 1989).
As mentioned above, the modulation of the myogenic rhythm in locust extensor tibiae muscle has been extensively studied. Octopamine works as a modulatory local neurohormone at this site, facilitating the amount of neurotransmitter released by the DUMETI neurons (Evans and O'Shea 1977, 1978). Release of octopamine from the dorsal unpaired median cell (DUMDL) in the metathoracic ganglia is an important modulatory factor influencing the kinetics of contraction and may also be an energy saving adaptation (Whim and Evans 1988).

Serotonin has been shown to stimulate Malpighian tubule activity in many species (see Raabe 1989), including the mosquito *Aedes taeniorhynchus* (Madrell and Phillips 1978). Only serotonin was observed to modify both fluid secretion and transepithelial voltage in *Aedes aegypti* Malpighian tubules (Veenstra 1988). Fluid absorption by the midgut was enhanced by serotonin in *Rhodnius* (Farmer et al. 1981) while dopamine, but not octopamine, acted as a stimulant in *Locusta* (Rafaeli et al. 1984).

Examples of octopamine functioning as a neurohormone have been well documented. In the locust *Schistocerca americana*, hemolymph octopamine levels increased after 2 minutes of flight and reached a 5-fold peak after 10 minutes (Goosey and Candy 1980). Subsequently, the octopamine concentration declined to near normal (non-flown) levels after 60 minutes of flight. It was determined that the octopamine concentration in the hemolymph was sufficient to stimulate flight muscle metabolism (Goosey and Candy 1982, Whim and Evans 1988) and had hyperlipemic effects on the fat body *in vivo* (Orchard et al. 1981). No concurrent decrease in octopamine concentration in the brain or thoracic ganglia occurred during flight of *S. americana*; however, octopamine levels in the dorsal longitudinal nerves and dorsal-ventral nerve/muscle tissues decreased significantly in flown locusts (Goosey and Candy 1982). The increase of hemolymph octopamine shortly after flight begins could be accounted for by release from thoracic nerves. It is believed that increased octopamine concentration is responsible for stimulation of glucose oxidation by the
flight muscles during the initial period of flight. After ten minutes of flight, adipokinetin hormone (responsible for lipid oxidation) is released from the corpora cardiaca and takes over as the flight mediating neurohormone. Interestingly, release of adipokinetin hormone from the glandular lobe of the corpora cardiaca of *S. americana* is regulated by octopamine (Pannabecker and Orchard 1986).

Octopamine concentration also increased in cockroach (*P. americana*) hemolymph following commencement of flight or in response to handling (Bailey et al. 1984). After 20 min. of flight, the octopamine concentration was about 40% above normal. The concentration rapidly declined to the resting level when flight ceased.

Hypertrehalosemia, in response to stress, was induced by increased octopamine levels (Downer 1979). Octopamine increased the glycogen phosphorylase content of *Periplaneta* fat body, although the effect was not observed in *Locusta* (Van Marrewijk et al. 1983).

**Biogenic Amines in Diptera**

Although biogenic amines have been widely studied in insects, relatively few taxa have been examined. Most of the primary work has concentrated on large orthopteran and lepidopteran species, owing to their large size and hemolymph volume. Information on biogenic amines in Diptera remains sparse.

Several early studies investigated amines in *Drosophila* but most of these assays used whole body or head homogenates and techniques which have been criticized for lack of specificity. Evidence presented by Trimmer (1985) indicates that, in *Calliphora*, serotonin may function neurohormonally to control secretory activity of salivary glands. Pimley (1984) reported that dopamine, adrenaline, noradrenaline and octopamine inhibited *in vitro* lipid synthesis by *Glossina morsitans* fat cells, while octopamine stimulated synthesis of proline. It was not determined, however, which amines are present in *G. morsitans*. Biogenic amine levels have been reported for *Sarcophaga* and *Musca* (Clarke and Donellan 1982) and *Calliphora* (Nässel and Laxmyr
Noradrenaline was not found in measurable amounts in *Calliphora* (Nässel and Laxmyr 1983), but Clarke and Donellan (1982) reported noradrenaline in *Musca*. Octopamine, dopamine and serotonin are present in the central nervous system of *Calliphora* but in different proportions than in other insects (Nässel and Laxmyr 1983).

As yet, virtually no information exists on biogenic amines in mosquitoes or any other nematoceran Diptera. Since there is considerable evidence linking biogenic amines and behavior, determining the presence and functions of these compounds in mosquitoes would prove interesting. Mosquitoes provide numerous, unexplored opportunities for this type of research. Despite the wealth of knowledge about certain facets of mosquito physiology and behavior, significantly less is known about underlying control mechanisms, particularly with respect to sensory physiology and the interplay of external and internal stimuli that regulate behavior. For example, preliminary experiments with the mosquito *Aedes triseriatus* indicated decreased spontaneous flight activity following injection of reserpine, a general amine-depleting compound (Novak, unpublished). It would be desirable to identify which amine(s) are most important in modulating flight activities in this mosquito. While numerous behaviors of mosquitoes have been extensively described, their underlying controls are not well understood. Mosquitoes possess distinct, circadian activity rhythms (Jones et al. 1974) which may include host-seeking, mating, oviposition, and other behaviors. Although expression of these behaviors involves flight activity, they are not necessarily related. As pointed out by Bowen (1990), spontaneous flight activity and host-seeking behavior may both be expressed in a circadian pattern in many species, but they are not tightly coupled temporally; flight activity peaks may precede and/or lag behind host-seeking. Different mechanisms must exist to regulate each of these activities.

Each of these behavioral patterns have themselves been described as a composite of behavioral steps, thus adding to the complexity of each process. Edman (1989)
diagramed the host-seeking and feeding processes in mosquitoes (Figs. 4 and 5),

describing host-seeking as a "sustained response to a continuum of reinforcing
stimuli." These complex behaviors may be separated experimentally, providing
numerous opportunities to explore the underlying control mechanisms at each step.

Given the relative importance of biogenic amines in controlling processes in other
insects, it is likely that they exert modulatory regulation at one or more points.

However, as Edman (1989) summarized the state of mosquito research, "Unfortunately,
the task of experimentally dissecting away and understanding this neuro-behavioral
system has been painfully slow with little substantial progress."

The goals of this research project were to 1) identify and quantify the major
biogenic amines in the central nervous system of the mosquito *Aedes triseriatus* (Say);
2) determine whether the amine concentrations changed circadianly or with respect to
age, and 3) to determine if changes in specific amine concentrations could be related to
changes in spontaneous flight activity, host-seeking, or bloodfeeding behaviors of this
mosquito.

Figure 4. Example of a mosquito host-seeking process from initial flight to arrival at
host (modified from Edman 1989)
Explanation of dissertation format

This dissertation consists of three papers that will be submitted for publication. These papers are preceded by a general introduction and literature review and are followed by a general summary. References that are cited in the general introduction, literature review, and general summary follow the general summary. Paper I identifies the major biogenic amines in *Aedes triseriatus* and evaluates changes in brain amine concentrations with respect to age and circadian periodicity. Paper II examines the effects of two pharmacological agents, α-methyl tyrosine (AMT) and α-methyl tryptophan (AMTP) on amine concentrations. Changes in the spontaneous flight activity of *A. triseriatus* were also monitored in association with drug treatment. Paper III evaluates the effect of selective amine depletions on the host-seeking and bloodfeeding behaviors of *A. triseriatus*. Papers I and II will be submitted to *Physiological Entomology* for publication. Paper III will be submitted to the *Journal of Insect Behavior*. The primary author is responsible for the design and performance of all experimental work included.
In this dissertation. All results and conclusions from these experiments were interpreted and written into manuscript form by the primary author.
PAPER I. IDENTIFICATION AND QUANTIFICATION OF MAJOR BIOGENIC AMINES IN THE CENTRAL NERVOUS SYSTEM OF THE MOSQUITO AEDES TRISERIATUS (SAY) (DIPTERA: CULICIDAE)
Identification and quantification of major biogenic amines in the central nervous system of the mosquito *Aedes triseriatus* (Say) (Diptera: Culicidae)

Mark G. Novak and Wayne A. Rowley
Dept. Entomology, Iowa State University, Ames, IA 50011
The major biogenic amines of the insect nervous system (dopamine, octopamine, and serotonin) were identified and quantified by high performance liquid chromatography in the brain and thoracic ganglia of female *Aedes triseriatus* mosquitoes. Octopamine was quantitatively dominant in both tissues while dopamine and serotonin were found in smaller but generally similar amounts. Noradrenaline was not found in measurable quantities. Amine levels in the brain and thoracic ganglia increased significantly between days 1-14 of adult life while total protein content remained unchanged. Statistically significant circadian changes in brain amine levels were not observed. When brains were divided by region (midbrain, optic lobes, subesophageal ganglion), octopamine was more abundant in the optic lobes, relative to the distribution of dopamine and serotonin.
INTRODUCTION

Biogenic amines are neuroactive compounds that have been widely studied in both vertebrates and invertebrates. These monoamine compounds have been identified in both the central and peripheral nervous systems of insects and have been demonstrated to function as neurotransmitters, neuromodulators, and neurohormones.

Biogenic amines are involved in a wide variety of behavioral and physiological processes including modulation of the firefly light organ (Robertson and Carlson 1976), flight metabolism in Locusta (Whim and Evans 1988), and feeding behavior in both the blow fly Phormia regina (Long and Murdock 1983, Brookhart et al. 1987) and the blood-feeding bug Rhodnius prolixus (Lange et al. 1989, Cook and Orchard 1990). Recently, environmental and genetic factors were shown to affect amine concentrations in Apis mellifera (Harris and Woodring 1992).

Studies of biogenic amines in Diptera have generally been limited to cyclorrhaphan species. In addition to experiments on blow fly feeding behavior (cited above), identification and quantification of amines have been reported for Calliphora erythrocephala (Nässel and Laxmyr 1983), Musca domestica and Sarcophaga barbata (Clarke and Donnellan 1982). Other studies have identified and mapped serotonin-immunoreactive neurons in Calliphora (Nässel and Elekes 1985, Nässel 1988), Sarcophaga bullata (Nässel and Cantera 1985), and Drosophila (Nässel 1988).

In contrast, few studies have examined the presence or roles of monoamines in nematocerous Diptera. Nijhout (1977) suggested that a catecholamine or phenolic amine is involved in antennal hair erection in male mosquitoes. Serotonin, but not other amines, modified fluid secretion and transepithelial voltage in the Malpighian tubules of Aedes aegypti (Veenstra 1988). Johansson et al. (1986) reported on serotonin distribution in the larval nervous system of the midge Chironomus tentans. The paucity of information on biogenic amines in mosquitoes is especially surprising.
given the influence of these insects on human and animal health, and the extensive research on mosquito behavior and physiology.

The present study was undertaken to determine the presence and concentration of major biogenic amines (dopamine, noradrenaline, octopamine and serotonin) in portions of the central nervous system (brain and fused thoracic ganglia) of female *Aedes triseriatus* (Say) and to determine the change in concentrations of these amines with respect to age and circadian periodicity. High performance liquid chromatography with electrochemical detection (HPLCED) was used for biogenic amine quantification. The information gained from this study will serve as a basis for further research on the role of monoamines in modifying the physiology and behavior of this mosquito.
MATERIALS AND METHODS

All *Aedes triseriatus* used in this study were reared by conventional laboratory methods. Approximately 250 first instar larvae were placed in distilled water in 40 x 24 cm enamel pans and fed Tetramin® ad lib. Pupae were separated by sex and size using a mechanical separator. Newly emerged females, of similar size, were held in groups of 20 in 0.5 l cartons with constant access to .3 M sucrose. All stages were kept at 27°C, 80% relative humidity, with a 16:8 hr light/dark regimen.

To determine whether amine concentrations change with age, brain samples (includes optic lobes, midbrain, and subesophageal ganglia) were dissected from pupae and various aged adults (days 1, 3, 7, 10, 14, and 28 post-emergence) of the same cohort. Thoracic ganglia (includes fused pro-, meso-, and metathoracic ganglia) were dissected from adults on days 1, 10, and 21 post-emergence.

Whole brains were removed from 14-day-old females and divided into optic lobe, midbrain, and subesophageal samples to compare the relative abundance of monoamines in these brain regions. Midbrains were pooled in groups of four while subesophageal ganglia and optic lobe pairs were pooled in groups of four to eight.

Whole brains from 14-day-old females were also analyzed for circadian changes in amine levels. These tissues were dissected at three intervals corresponding to periods of maximum and minimum mosquito flight activity. Dissections were made during mid-photophase (6-8 hr after lights on), 1 hr before lights off to lights off, and mid-scotophase (4-5 hr after lights off). Mosquitoes used in the night sampling period were cold-inactivated while still in darkness (handling unanesthetized mosquitoes was done under a red light to minimize flight activity) and held in the dark until immediately before their dissection.

Brain and thoracic ganglia samples were prepared for HPLCED analysis as follows. Adult female *A. triseriatus* were cold-inactivated and mounted in modeling clay under a dissecting microscope. Heads were immersed in ice-cold *Aedes* saline (Hayes 1953) for
brain dissections. The dorsal portion of the head capsule was cut away and the cerebral ganglia were quickly removed. Brains were dissected from pupae that were cold inactivated when immersed in ice-cold Aedes saline on a glass depression slide. The fused thoracic ganglia of adult mosquitoes were dissected by mounting the cold-inactivated mosquito in clay, removing the legs at the coxae and pulling apart the ventral sclerites until the fused ganglia were exposed. The ventral nerve cord, peripheral nerves, and trachea were dissected away and the ganglia were removed.

Neural tissue samples were homogenized in 25 µl of 0.15M perchloric acid with 5.0 mM sodium metabisulfite and 0.1mM EDTA. The dissecting and homogenization procedure for each brain or thoracic ganglia was routinely completed in less than 2 minutes. Dopamine (DA), noradrenaline (NA), and serotonin (5HT) quantifications could be determined from the same sample. The samples for whole brain determinations consisted of 2 cerebral ganglia or 3-4 thoracic ganglia per 25 µl perchloric acid solution. Octopamine (OA) quantifications were made from pooled samples of 3 cerebral ganglia or 3-4 thoracic ganglia per 25 µl perchloric acid solution. The homogenated samples were centrifuged at 10,000 g for 5 min. The supernatant was removed and stored at -70° C prior to HPLC analysis. Samples were thawed and centrifuged again immediately before injection. Injection sample size was 12.5 or 15 µl.

The HPLC unit consisted of a Waters 6000A solvent delivery system with a single channel electrochemical detector (BAS model LC4B) and a Rheodyne (model 7125) sample injector with a 200 µl sample loop. Chromatographic separations were performed by a 250 x 4.6 mm C18 column (Bioanalytical Systems), 5 µm particle size. The mobile phase consisted of 75 mM NaH2PO4, 1 mM octyl sodium sulfate, 0.05 mM EDTA and 13% acetonitrile for dopamine and serotonin samples; acetonitrile concentration was reduced to 8.5% for octopamine determinations. Isoproterenol was included in some samples as an internal standard to serve as an indicator of volume consistency. The electrochemical detector was operated at .65 V for dopamine,
noradrenaline, and serotonin or .95 V for octopamine samples. Pump flow rate was maintained at 1ml/min. for all runs.

Identification and quantification of amines was by comparison with known standards (obtained from Sigma Chemical Co.). Peak identity was initially confirmed by spiking samples with standards and observing a single, symmetrical peak, and altering buffer conditions (pH and % acetonitrile) to confirm peak identities with standards. Amines were quantified by peak height analysis. Sample order was randomly assigned with respect to sample days within age study, time of day within circadian study, and brain region within brain partition experiment.

Total protein content of brains and thoracic ganglia was estimated (micro-Bradford assay, Sigma Chemical Co.) to determine if A. triseriatus neural tissue changed in size as a function of age. Brain samples were taken from pupae (day 2 post-pupation) and on days 1, 7, and 14, post-emergence. Thoracic ganglia protein content was determined on days 1, 7, 10, and 14, post-emergence. Protein content was expressed relative to bovine serum albumin (BSA) standards.

Analysis of variance was used to evaluate amine and protein content of samples with respect to age and circadian periodicity. Linear regression with a quadratic model was used to describe differences in amine content as a function of age.
RESULTS

Analysis of cerebral and thoracic ganglia samples of *A. trisertatus* indicated that dopamine, octopamine and serotonin were present in readily measurable quantities in both of these tissues. Minimal sensitivity to compound standards were approximately 20 pg of dopamine and serotonin and 50 pg of octopamine. Measurable quantities of noradrenaline were not detected in this study (Fig. 1). Dopamine, octopamine, and serotonin levels were considerably higher in cerebral ganglia as compared to thoracic ganglia; however, these differences are reduced when amine content is expressed on a pmol per mg protein basis (Table 1). The relative abundance of the three monoamines was similar in both the cerebral and thoracic ganglia. Octopamine was present in the highest concentration in both tissues. Dopamine and serotonin were found at levels that were approximately 3 to 5-fold lower than octopamine levels.

The brain levels of all three monoamines were lowest in the pupal and young adult samples but increased significantly between 1-14 days post-emergence (Fig. 2) (p<0.01 for DA, OA and 5HT; based on a pairwise contrast for means of pupae and 14-day-old adults). Biogenic amine concentrations plateaued after the first two weeks of adult life; 4-week-old females had similar amine levels to 14-day-old mosquitoes. Similarly, significant increases in all amine levels were observed in thoracic ganglia during the first three weeks of the adult stage (p<0.01 for DA, OA and 5HT; based on a pairwise contrast for means of pupae and 14-day-old adults). Although monoamine levels increased during the experimental period in both cerebral and thoracic ganglia, a significant change in total protein content of these tissues was not observed (Fig. 3) (brain p = .85, thoracic ganglia p = .31).
Figure 1. Chromatograms of biogenic amines. (A) Standard solution containing 75 pg of dopamine (DA), noradrenaline (NA), and serotonin (5HT) with detector electrode set at .65 V. (B) Cerebral ganglia sample from 10-day-old *Aedes triseriatus* females. (C) Standard solution containing 100 pg of octopamine (OA) with detector electrode set at .95V. (D) Cerebral ganglia sample from 10-day-old *Aedes triseriatus* females.
Table 1. Comparison of biogenic amine levels in whole brains and thoracic ganglia of 10-day-old *Aedes triseriatus*

<table>
<thead>
<tr>
<th>Structure</th>
<th>DA</th>
<th>OA</th>
<th>5HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pg/tissue (SE)</td>
<td>42.8 (2.4)</td>
<td>135.2 (9.5)</td>
<td>53.5 (2.2)</td>
</tr>
<tr>
<td>pmol/mg protein</td>
<td>84.5</td>
<td>267.0</td>
<td>91.8</td>
</tr>
<tr>
<td>Thoracic ganglia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pg/tissue (SE)</td>
<td>14.9 (.36)</td>
<td>51.7 (3.4)</td>
<td>9.8 (.53)</td>
</tr>
<tr>
<td>pmol/mg protein</td>
<td>63.7</td>
<td>220.9</td>
<td>36.4</td>
</tr>
</tbody>
</table>

Whole brain means based on 5 pooled samples for DA and 5HT and 4 pooled samples for OA. Thoracic ganglia means based on 4 pooled samples for each amine.
Figure 2. Biogenic amine concentrations in the cerebral and thoracic ganglia of female pupae and adult A. triseriatus (Vertical bars = SEM, brain means based on 5 pooled samples per age group, thoracic ganglia means based on 4 pooled samples per age group)
The relative proportions of dopamine, octopamine and serotonin in partitioned brains of 14-day-old *Aedes triseriatus* females are presented in Table 2. Highest levels of dopamine and serotonin were present in the midbrain with approximately 2-3 times lower amounts in the optic lobes and subesophageal ganglia. In comparison, octopamine levels in the optic lobe and midbrain regions were similar.

Monoamine concentrations of cerebral ganglia samples, taken at three time intervals over a 24 hr period, are presented in Fig. 4. No statistically significant changes in brain amine content were observed (p = 0.92, 0.93, and 0.53 for DA, 5HT, and OA).
Table 2. Comparison of biogenic amine levels in three brain regions of *Aedes triseriatus*

<table>
<thead>
<tr>
<th>Amine</th>
<th>Midbrain</th>
<th>Optic lobes</th>
<th>Subesophageal</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA pg/tissue ± SE (n)</td>
<td>35.3 ± 3.3 (6)</td>
<td>12.6 ± 2.0 (4)</td>
<td>10.4 ± 3.1 (6)</td>
</tr>
<tr>
<td>relative proportion</td>
<td>.60</td>
<td>.22</td>
<td>.18</td>
</tr>
<tr>
<td>OA pg/tissue ± SE (n)</td>
<td>47.4 ± 3.0 (3)</td>
<td>50.0 ± 3.6 (3)</td>
<td>19.1 ± 2.2 (3)</td>
</tr>
<tr>
<td>relative proportion</td>
<td>.41</td>
<td>.43</td>
<td>.16</td>
</tr>
<tr>
<td>5HT pg/tissue ± SE (n)</td>
<td>22.8 ± 3.2 (3)</td>
<td>10.0 ± .18 (3)</td>
<td>8.5 ± .22 (3)</td>
</tr>
<tr>
<td>relative proportion</td>
<td>.55</td>
<td>.24</td>
<td>.21</td>
</tr>
</tbody>
</table>

All three amines were determined by separate runs of pooled samples (4 midbrains per sample, 4-8 optic lobe pairs or subesophageal ganglia per sample) from 14-day-old adult *Aedes triseriatus*.

Figure 4. Biogenic amine levels in the brain of *Aedes triseriatus* sampled at 3 periods over 24 hr. (mean values based on 5 pooled samples for each amine, error bars = SEM)
DISCUSSION

Dopamine, octopamine and serotonin have been the three most often identified and studied monoamines in insects as well as other invertebrates. Octopamine is more abundant in arthropods relative to vertebrates or other invertebrate phyla (Klemm 1985) and it is often the quantitatively dominant amine in insect nervous systems. However, the ratio of these three compounds varies considerably from species to species without distinct phylogenetic trends. Noradrenaline is typically present in smaller quantities or not found in insect tissues. Adrenaline has not been conclusively demonstrated in insects or other invertebrates (Klemm 1985).

Most previous studies that identified and quantified biogenic monoamines in insect nervous systems have focused on large orthopteroid and lepidopteran species. The advent of more sensitive analytical techniques such as HPLC-ED and radioenzymatic assays has allowed greater sensitivity and the ability to analyze smaller amounts of tissue. Still, few reports exist for species in other insect orders including Diptera. Identification and quantification of biogenic amines have been reported for Calliphora erythrocephala (Nässel and Laxmyr 1983), Musca domestica and Sarcophaga barbata (Clarke and Donnellan 1982).

To our knowledge this study is the first to report on the identification and quantification of major monoamines in the central nervous system of a mosquito species. Dopamine, octopamine and serotonin were found in readily measured quantities with minimal pooling of tissue samples. Noradrenaline was not found under these conditions. Noradrenaline is either not present in the central nervous system of A. triseriatus or found in much smaller quantities than the other major amines. Octopamine was present in greatest concentrations in both cerebral and thoracic ganglia. Serotonin and dopamine were found in lower and generally similar concentrations. The relative proportions of these amines in the brains of 10-day-old A. triseriatus (.19 DA: .58 OA: .23 5HT, pg/tissue) is
similar to those reported for whole brains of 4 to 14-day-old *Calliphora erythrocephala* (.29 DA: .49 OA: .22 5HT, pmol/tissue) by Nässel and Laxmyr (1983).

Direct comparisons to amine concentrations reported in other studies are often difficult due to the variety of methods used to quantify amine levels (e.g. by unit weight, by tissue, by unit protein, etc.) as well as size and age differences of the insects. Evans (1978) suggested expressing amine content as picomoles per piece of tissue analyzed rather than on a unit wet weight basis since it was found that weight of some adult insect nervous tissues increases with time from the final moult. Different ganglia of the locust were shown to have different rates and degrees of growth through adult life (Sbrenna 1971). The amine concentrations in this study were expressed per tissue as well as per unit protein (relative to BSA standards) due to the great difficulty in accurately weighing or estimating volume of mosquito neural tissue. The brain concentrations of dopamine and serotonin (expressed per unit protein) are of the same order of magnitude as reported by Clarke and Donnellan (1982) for *Musca domestica*. However, their octopamine concentration in *M. domestica* brain was an order of magnitude lower than that of *A. triseriatus* (this study) as well as their measurements in *Locusta*. Similarly, the serotonin and octopamine concentrations of *A. triseriatus* in this study were approximately equal to those concentrations in *Apis mellifera* (Harris and Woodring 1992) when expressed per unit protein. Serotonin and dopamine concentrations, expressed per unit protein, in adult *A. mellifera* (Fuchs et al. 1989) were approx. 3 and 7.5 fold higher than respective amounts in *A. triseriatus* reported here.

The octopamine content of the optic lobes of *A. triseriatus* appeared higher, relative to dopamine and serotonin levels. High relative concentrations of octopamine have been reported in the optic lobes of various insect species such as *Mamestra configurata* (Bodnaryk 1980), *Periplaneta americana*, and *Schistocerca americana* (Evans 1978), and *Manduca sexta* (Davenport and Wright 1986), but not in *Calliphora* (Nässel and Laxmyr 1983). It was suggested that octopamine may be an important modulator of sensory
information in *M. configurata*, particularly for input from the compound eyes (Bodnaryk 1980).

The levels of all 3 monoamines were lowest in pupal and day 1 adult samples and then increased substantially during the first two weeks of adult life. This is assumed to be an actual increase in amine concentrations, rather than corresponding to an increasing mass of nervous tissue, since protein content did not increase during the first two weeks of adult life. Changes in neuroactive substances have been reported for numerous insect nervous tissues yet the patterns of change are not consistent. Octopamine was found to peak during the middle of larval life and then increase again throughout early adult life of *Locusta migratoria* (first 15 days monitored) (Fuzeau-Braesch et al. 1979). No appreciable increase in brain octopamine levels occurred during the first several days of adult life in the moth *Mamestra configurata* (Bodnaryk 1980). However a 30-fold increase was observed during the last ten days of metamorphosis. When changes in brain weight were taken into account, the rise in octopamine was 10-fold. The octopamine content of brains in *Acheta domesticus* did not increase throughout larval or adult life (Woodring et al. 1988). Since the brain volume of *A. domesticus* increased by about 30% during the first twelve days of adult life, the brain concentration of octopamine actually decreased. (A significant rise in octopamine levels in hemolymph was noted during the last days of the last larval instar of *A. domesticus* (Woodring et al. 1988). Similarly, Evans (1978) reported growth in ganglia of *Schistocerca americana gregaria* without an increase in octopamine levels. Dopamine, octopamine, and serotonin were all found in higher concentrations in adult *Calliphora erythrocephala* when compared to larvae, while DOPA was found in much higher concentrations in larvae (Nässel and Laxmyr 1983). Dopamine, octopamine and serotonin concentrations were significantly higher in adult *Apis mellifera* (of mixed ages) in comparison to newly emerged (< 24 hr old) bees (Harris and Woodring 1992). Protein content was not significantly different in the two groups, indicating no changes in brain size. Seasonal differences in amine levels were also noted in this study. Fuchs et al. (1989)
also examined neuroactive compounds during adult life of A. mellifera but did not find changes in dopamine and serotonin levels in brain tissue. However, norepinephrine concentrations decreased during the first week of adult life and remained at lower levels through day 40 (norepinephrine was not reported by Harris and Woodring), while glutamate and GABA showed obvious peaks around day 10. No change in brain protein content was observed during adult life.

It is not understood whether changes in amine concentrations (when they occur) are part of the normal development/maturation process of the neural tissue or a reflection of changes in behavioral or physiological patterns. Bodnaryk (1980) suggested that the enhanced octopaminergic system in adult M. configurata may be required to process and/or modulate the increased sensory input, particularly from the compound eyes. It is interesting to note that flight ability of A. triseriatus peaks during the second and third weeks of adult life (Clarke 1988) corresponding to the period of highest amine levels measured in this study.

Because mosquitoes have well defined peaks of circadian flight activity it was desirable to determine if daily fluctuations in amine levels occurred in the brain of A. triseriatus. Changes in amine levels have been correlated with activity of various insects, including flight activity of A. triseriatus (Novak in prep). Aedes triseriatus exhibits distinct peaks of flight activity under laboratory conditions (Clarke 1988) preceding lights off and just after lights on. However, no changes in monoamine levels were evident in the cerebral or thoracic ganglia of A. triseriatus in samples taken just prior to lights off (during the period of peak flight activity), approximately three hours into the scotophase or during mid-day. Several previous studies have investigated circadian changes in insect tissues with various results. A broad peak in octopamine levels in both the brain and blood of Acheta domesticus was evident during the first four hours of lights off (Woodring et al. 1988). This rhythm was demonstrated to be free running, as evidenced by 2 day entrainment to a light dark cycle followed by constant darkness. A broad octopamine peak
was present during the day in the brain of *P. americana* and a peak in hemolymph octopamine occurred about 3hr after lights off (Davenport and Evans 1984). Circadian changes in serotonin content have been reported in *Drosophila* (Fowler et al. 1972), hemolymph of *A. domesticus* (Muzynska-Pytel and Cymborowski 1978), and frontal ganglion of *P. americana* (Pandey and Habibulla 1982). However, Owen et al. (1987) found no circadian changes in levels of dopamine and serotonin in *P. americana*. Although changes in brain *A. triseriatus* amine levels were not evident in the present study, amine changes in other tissues or hemolymph of mosquitoes may be found to be related to circadian patterns.

The demonstration of dopamine, octopamine, and serotonin in the central nervous system of *A. triseriatus* is not, in itself, surprising. However, the information provided by this study will be useful in further investigation into the roles that biogenic amines play in the regulation of behavioral and physiological processes of this mosquito.
ACKNOWLEDGMENTS

The authors would like to thank Dr. Donald C. Dyer for his technical assistance and use of the HPLC system. We also thank Mary Minachi for her help with mosquito dissections and sample preparations.
LITERATURE CITED


PAPER II. EFFECTS OF α-METHYL TYROSINE AND α-METHYL TRYPTOPHAN ON BIOGENIC AMINE CONCENTRATIONS AND CIRCADIAN FLIGHT ACTIVITY OF Aedes Triseriatus (SAY) (DIPTERA: CULICIDAE)
Effects of α-methyl tyrosine and α-methyl tryptophan on biogenic amine concentrations and circadian flight activity of Aedes triseriatus (Say) (Diptera: Culicidae)

Mark G. Novak and Wayne A. Rowley
Dept. Entomology, Iowa State University, Ames, IA. 50011
ABSTRACT

A single, oral dose (approximately 4 μg) of α-methyl tyrosine (AMT) or α-methyl tryptophan (AMTP) reduced biogenic amine concentrations in the central nervous system of the mosquito *Aedes triseriatus*. AMTP treatment selectively lowered the brain concentration of serotonin through the 14 days monitored following treatment. During the first week, serotonin levels in AMTP-treated mosquitoes were approximately 10% of control levels. Dopamine and octopamine concentrations were not significantly altered by AMTP. Administration of AMT significantly depleted dopamine through 14 days post-treatment; dopamine was reduced by 50% in AMT-treated mosquitoes during the first week. Octopamine concentration was also lowered by AMT (determined on Day 3 post-treatment), but not to the degree of dopamine depression. A statistically significant decrease in the spontaneous, circadian flight-activity of *A. triseriatus* was observed for several days following treatment with AMT or AMTP. However, flight activity returned to control levels prior to evidence of amine pool repletion. Amine reductions did not alter the circadian pattern of flight activity.
INTRODUCTION

Biogenic amines such as dopamine, serotonin, and octopamine have been widely documented in the nervous systems of invertebrates (see review by Klemm 1985). These compounds play proven and putative roles as neurotransmitters, neuromodulators and neurohormones (reviewed by Evans 1980). Their activities have been linked to numerous physiological processes and behaviors in insects such as altering *Phormia regina* responsiveness to food stimuli (Long et al. 1986, Brookhart et al. 1987), regulating bloodfeeding in *Rhodnius prolixus* (Cook and Orchard 1990), and modulating hyperlipaemic response to stress in *Locusta migratoria* (Orchard et al. 1981).

Determining the roles that biogenic amines play in behavioral modification has proven challenging. Several approaches have been used including injection of amines in vivo (Mercer and Menzel 1982, Kamyshev et al. 1983), use of agonists or antagonists of amine receptors (Hollingworth and Murdock 1980, Long and Murdock 1983) or through the use of amine-altering compounds. Compounds such as reserpine and amphetamine have been used in insect behavioral studies (Brookhart et al. 1987), but these compounds are general depleters of amine concentrations. Perhaps more useful are those chemicals that can selectively deplete a specific biogenic amine, rather than the entire pool of amines. Compounds with such selective action have received considerable attention in vertebrate behavioral studies, but few have been used in invertebrate research or have demonstrated the same selectivity (Sloley 1989).

Recently, Sloley and Orikasa (1988) and Sloley (1989) reported on the long-term, selective depletion of dopamine and serotonin in *Periplaneta americana* by α-methyl tyrosine (AMT) and α-methyl tryptophan (AMTP) respectively. The mode of action of these compounds is generally believed to be by inhibition of tyrosine hydroxylase by AMT and tryptophan hydroxylase by AMTP, enzymes responsible for the synthesis of the respective amines from their amino acid precursors (Sloley 1989). Amine depletions were similar in cockroaches that were injected with AMT and AMTP or
when these compounds were mixed in sucrose and administered per os. These findings led to the suggestion that AMT and AMTP may be successful tools for elucidating the physiological roles of amines in insects or possibly aid pesticide development (Sloley 1989).

The objectives of the present study were to determine the effects of a single oral dose of α-methyl tryptophan or α-methyl tryrosine on amine concentrations (dopamine, octopamine, serotonin) in the cerebral ganglia of the mosquito *Aedes triseriatus* (Say) and to evaluate the consequences of AMT and AMTP administration on the mosquito's spontaneous, circadian flight-activity.
MATERIALS AND METHODS

All *Aedes triseriatus* used in this study were reared by conventional laboratory methods. Approximately 250 first instar larvae were placed in distilled water in 40 x 24 cm enamel pans and fed Tetramin® ad lib. Pupae were separated by sex and size using a mechanical separator. Newly emerged females of similar size were held in 0.5 l cartons without access to sucrose. On day 4 or 5 post-emergence, starved, virgin female *A. triseriatus* were provided access to either 10% sucrose (controls), or 10% sucrose containing AMT (0.15%), or AMTP (0.15 and 0.75%). The mean dose ingested per mosquito was estimated by weighing 10 mosquitoes in each treatment group before and after feeding.

Following this initial meal, all mosquitoes were held in groups of 25-30 in cartons with constant access to sucrose (without further exposure to AMT or AMTP). Cerebral ganglia from treated (0.15% AMT or 0.15% AMTP) and control mosquitoes were analyzed by high performance liquid chromatography with electrochemical detection (HPLCED) for amine concentration changes. The spontaneous, circadian flight-activity of treated and control mosquitoes was evaluated with an acoustic activity chamber system for 13 days following drug administration.

**HPLC Analysis**

Cerebral ganglia samples (includes midbrain, optic lobes, and subesophageal ganglia) were prepared for HPLCED analysis as follows: Mosquitoes were cold-inactivated, mounted in modeling clay under a dissecting microscope and their heads immersed in ice cold *Aedes* saline (Hayes 1953). The dorsal portion of the head capsule was then cut away and the cerebral ganglia were quickly removed. This neural tissue was homogenized in 25 μl of 0.15M perchloric acid with 5.0 mM sodium metabisulfite and 0.1mM EDTA. The dissecting and homogenization procedure for each brain was routinely completed in less than 2 minutes. Dopamine (DA) and serotonin (5HT) quantifications were determined from the same sample. These
samples consisted of 2 cerebral ganglia per 25 µl perchloric acid solution. Octopamine (OA) quantifications were made from separate samples consisting of 3 cerebral ganglia per 25 µl perchloric acid solution. Homogenated samples were centrifuged at 10,000 g for 5 min, and the supernatant was removed and stored at -70°C prior to HPLC analysis. Samples were thawed and centrifuged again immediately before injection. Injection sample size was 12.5 or 15 µl. Tissue samples were taken on days 1, 7, and 14 post-treatment for dopamine/serotonin measurements and on day 3 post-treatment for octopamine measurements.

The HPLC unit consisted of a Waters 6000A solvent delivery system with a single channel electrochemical detector (BAS model LC4B) and a Rheodyne (model 7125) sample injector with a 200 µl sample loop. Chromatographic separations were performed by a 250 x 4.6 mm C18 column (Bioanalytical Systems), 5 µm particle size. The mobile phase consisted of 75 mM NaH₂PO₄, 1 mM octyl sodium sulfate, 0.05 mM EDTA and 13% acetonitrile for dopamine and serotonin samples; acetonitrile concentration was reduced to 8.5% for octopamine determinations. Isoproterenol was included in some samples as an internal standard to serve as an indicator of volume consistency. The electrochemical detector was operated at .65 V for dopamine and serotonin and .95 V for octopamine samples. Pump flow rate was maintained at 1 ml/min. for all runs.

Identification of amines was by cochromatography with known standards (obtained from Sigma Chemical Co.). Peak identity was initially confirmed by spiking samples with standards and observing a single, symmetrical peak and altering buffer conditions (pH and % acetonitrile) to confirm peak identities with standards. Amines were quantified by peak height analysis in comparison to standard curves. Samples of the three treatments (AMT, AMTP, control) were run in succession. Sample order was randomly assigned with respect to sample days and treatment within days. Six to eight
samples were run per treatment per day. A randomized block design with analysis of variance was used to compare treatment means by day.

**Flight Activity**

The spontaneous flight activity of treated and control mosquitoes was evaluated by a microcomputer-monitored flight activity system (Rowley et al. 1987). Immediately after feeding on AMT or AMTP solutions, 32 mosquitoes (treated and controls) were individually placed into acoustic activity chambers made from modified reagent bottles (Jones et al. 1967). The individual bottles were mounted over microphones (sensitive to wing beat frequency) interfaced with a microcomputer. The number of flights, flying time and an activity score were recorded for each chamber by 30 min. periods. Mosquitoes had constant access to sucrose and their flight activity was monitored for 13 days following drug administration. The experiment was repeated twice for the AMT treatment and three times for AMTP treatments. Analysis of variance was used to evaluate the total daily flight data (activity scores, number of flights, and flight duration) by group for each day post-treatment. The activity score represented the number of minutes within each 30-min period in which a mosquito made at least 1 flight of any duration (Jones et al. 1967). Any mosquitoes that died during the run were eliminated from the entire data analysis. Preliminary analysis of flight data indicated that a square root transformation was required for "number of flights" data.
RESULTS

AMTP solutions were readily accepted by feeding mosquitoes. There were no differences in the average meal size between either AMTP concentration treatment and the control group. The mean quantity ingested (2.71 ±0.33 μl SD, n=30) resulted in approximated doses of 4.06 and 20.3 μg of AMTP per mosquito fed on the 0.15% and 0.75% AMTP solutions, respectively. AMT solutions were less readily imbibed. Approximately 50% of starved A. triseriatus fed noticeably on the 0.15% AMT solution. Of those mosquitoes that fed, the quantity of AMT solution ingested was often visibly less than that of the AMTP or control group. Only mosquitoes that fed to at least 50% repletion were used in experimental treatments. This resulted in a range of dose between approximately 2-4 μg AMT per mosquito. There was no overt behavioral modification or statistically significant mortality associated with either drug treatment.

Concentrations of dopamine and serotonin were determined simultaneously by HPLC/ESD. Minimal detectable limits (twice background noise) were approximately 20 pg for the dopamine and serotonin standards and approximately 50 pg for the octopamine standard. Sample chromatograms of standards and A. triseriatus cerebral ganglia are presented in Fig. 1. Noradrenaline was initially screened for but, under the described operating conditions, measurable quantities were not found in the cerebral ganglia samples.

Orally administered doses of AMT and AMTP significantly reduced cerebral ganglia amine concentrations in A. triseriatus; some selectivity of amine depletion was evident (Fig. 2). The dopamine concentrations of AMT-treated mosquitoes were significantly lower than the control group on all 3 days sampled (p values = 0.0003, 0.0005, and 0.0295 for days 1, 7, and 14, respectively). Dopamine levels were reduced by about 50% on days 1 and 7, but a partial recovery was evident by day 14 post-treatment.
Figure 1. Chromatograms of biogenic amines. (A) Standard solution containing 75 pg of dopamine (DA), serotonin (5HT), and internal standard isoproterenol (ISP) with detector electrode set at .65 V. (B) Cerebral ganglia sample from 10-day-old *Aedes triseriatus* females. (C) Standard solution containing 100 pg of octopamine (OA) with detector electrode set at .95 V. (D) Cerebral ganglia sample from 10-day-old *Aedes triseriatus* females.
(Fig. 2 top). No significant alterations in dopamine levels were evident in AMTP-treated mosquitoes (p values = 0.23, 0.30, 0.22).

The cerebral ganglia serotonin-content of AMTP-treated mosquitoes was drastically depleted within 24 hr of drug ingestion (Fig. 2, middle). Average serotonin concentration was reduced to less than one-tenth of control levels on days 1 and 7 post-treatment; serotonin was undetectable in several samples taken on these days. Some replenishment of serotonin content was evident by day 14 post-treatment, but levels were still significantly reduced (p = 0.0003). The serotonin content of cerebral ganglia in AMT-treated *A. trisertatus* was slightly reduced on all three days sampled, but no statistically significant differences were detected (p = 0.20, 0.13, and 0.20 for days 1, 7, and 14 respectively).

Octopamine content of cerebral ganglia in treated and control mosquitoes was compared on day 3 post-administration (Fig. 2, bottom). Octopamine content was significantly reduced in the AMT-treated mosquitoes (p = 0.0403) but not in the AMTP-treated mosquitoes (p = 0.5210).

Spontaneous circadian flight-activity parameters were reduced in conjunction with the depression of amine concentrations. Activity chamber data comparing flight parameters of AMTP-dosed mosquitoes (0.15 and 0.75% treatments) vs controls is presented in Fig. 3 and Table 1. While it is clearly evident that all 3 flight variables (activity score, flights, duration) increased during the first 10-11 days of monitoring, activity parameters of both AMTP-treated groups were substantially reduced during the first week in comparison to the control group. A decrease in the spontaneous flight activity occurred within 24 hr of drug ingestion. Statistically significant differences in the mean flight duration/day occurred on days 1-8 and on days 1-5 for the number of flights and activity score means. Depression of spontaneous flight activity was reversible. By the end of the experimental period, flight activity was generally similar for treated and control groups. Surprisingly, flight activity parameters were inversely
Figure 2. Comparison of biogenic amine concentrations in cerebral ganglia of *Aedes triseriatus* orally dosed with .15% AMT or .15% AMTP (n= 6-8 pooled samples per treatment, vertical bars = SEM)
Figure 3. Flight activity of *Aedes triseriatus* dosed with .15% and .75% AMTP (administered per os) on days 1-13 post-treatment: a) Activity scores; b) Number of flights; c) Total flying time; Vertical lines = SEM; * denotes p<0.05
Mean total activity score, total flights, and mean total duration of flights across different treatments and days post-treatment.
Table 1. F values and p > 0.05 from univariate analysis results of AMTP-treated Aedes triseriatus flight activity (See Fig. 3)

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity score</th>
<th>Flights</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.80, .0002</td>
<td>5.94, .0039</td>
<td>10.04, .0001</td>
</tr>
<tr>
<td>2</td>
<td>12.26, .0001</td>
<td>6.12, .0034</td>
<td>12.89, .0001</td>
</tr>
<tr>
<td>3</td>
<td>8.94, .0003</td>
<td>5.18, .0077</td>
<td>9.15, .0003</td>
</tr>
<tr>
<td>4</td>
<td>5.78, .0045</td>
<td>3.34, .0387</td>
<td>7.32, .0012</td>
</tr>
<tr>
<td>5</td>
<td>5.12, .0081</td>
<td>3.52, .0344</td>
<td>9.17, .0003</td>
</tr>
<tr>
<td>6</td>
<td>2.86, .0634</td>
<td>2.90, .0608</td>
<td>5.99, .0038</td>
</tr>
<tr>
<td>7</td>
<td>2.22, .1149</td>
<td>1.71, .1874</td>
<td>5.17, .0078</td>
</tr>
<tr>
<td>8</td>
<td>1.64, .2013</td>
<td>1.27, .2861</td>
<td>3.89, .0246</td>
</tr>
<tr>
<td>9</td>
<td>1.85, .1638</td>
<td>1.30, .2788</td>
<td>2.81, .0661</td>
</tr>
<tr>
<td>10</td>
<td>1.44, .2430</td>
<td>1.11, .3349</td>
<td>1.75, .1800</td>
</tr>
<tr>
<td>11</td>
<td>3.39, .0387</td>
<td>2.32, .1050</td>
<td>2.54, .0850</td>
</tr>
<tr>
<td>12</td>
<td>2.81, .0664</td>
<td>1.55, .2187</td>
<td>1.21, .3039</td>
</tr>
<tr>
<td>13</td>
<td>2.20, .1172</td>
<td>1.46, .2392</td>
<td>1.82, .1692</td>
</tr>
</tbody>
</table>

^ Analysis based on VV transformed values

related to AMTP dose during the second week. Aedes triseriatus dosed with 0.75% AMTP had the lowest activity levels during the first 4 days following treatment.

Although only the day 11 activity score was statistically significant, their activity scores and numbers of flights were greater than the 0.15% AMTP-treated group and generally greater than or equal to control group activity during the last 4-5 days of the experimental period.

While overall activity was depressed in AMTP-treated mosquitoes, it was determined that drug treatment did not affect the 24-hr flight activity pattern of A. triseriatus females. The mean number of flights per hour of AMTP-treated and control group mosquitoes are depicted in Fig. 4 for a selected day during which the AMTP group activity was still significantly depressed. There was no indication of a change in the activity pattern of AMTP-treated A. triseriatus, only an obvious dampening of flight...
activity. This activity pattern was representative of treated and control mosquitoes throughout the experimental period.

Similar to the AMTP trials, flight activity was significantly depressed following AMT administration. All flight parameters of treated A. *triseriatus* rapidly declined (beginning on Day 1) to activity levels significantly below those of the control group (Fig. 5). Although all flight parameters of AMT-treated mosquitoes were lower than those of the control group through day 8 post-treatment, statistically significant differences were observed through day 5 for mean flight duration and through day 4 for flight and activity score measurements (Table 2). The slightly shorter duration of depressed activity (in comparison with AMTP groups) may have been due to the lower average dose of AMT initially ingested by the mosquitoes. Flight activity of treated mosquitoes returned to control levels by day 9, and similar to the 0.75% AMTP-treated mosquitoes, surpassed control activity levels for the remaining days of the experimental period. As with AMTP-treated mosquitoes, there was no indication that AMT affected the circadian pattern of spontaneous flight activity.
Figure 5. Flight activity of *Aedes triseriatus* dosed with .15% AMT (administered per os) on days 1-13 post-treatment: a) Activity scores; b) Number of flights; c) Total flying time; Vertical lines = SEM; * denotes p< 0.05
Table 2. F values and p > 0.05 from univariate analysis results of AMT-treated *Aedes triseriatus* flight activity (See Fig. 5)

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity score</th>
<th>Flights&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.40, .0091</td>
<td>3.87, .0549</td>
<td>8.37, .0057</td>
</tr>
<tr>
<td>2</td>
<td>11.98, .0012</td>
<td>3.90, .0541</td>
<td>8.44, .0055</td>
</tr>
<tr>
<td>3</td>
<td>9.47, .0034</td>
<td>5.23, .0267</td>
<td>9.44, .0035</td>
</tr>
<tr>
<td>4</td>
<td>7.97, .0069</td>
<td>5.62, .0218</td>
<td>9.79, .0030</td>
</tr>
<tr>
<td>5</td>
<td>1.82, .1841</td>
<td>2.72, .1058</td>
<td>6.42, .0146</td>
</tr>
<tr>
<td>6</td>
<td>1.26, .2664</td>
<td>0.77, .3837</td>
<td>3.66, .0618</td>
</tr>
<tr>
<td>7</td>
<td>0.24, .6286</td>
<td>0.08, .7788</td>
<td>1.35, .2518</td>
</tr>
<tr>
<td>8</td>
<td>0.34, .5617</td>
<td>0.08, .7825</td>
<td>1.36, .2500</td>
</tr>
<tr>
<td>9</td>
<td>0.14, .7073</td>
<td>0.39, .5353</td>
<td>0.12, .7332</td>
</tr>
<tr>
<td>10</td>
<td>1.97, .1664</td>
<td>2.46, .1235</td>
<td>0.16, .6918</td>
</tr>
<tr>
<td>11</td>
<td>3.46, .0689</td>
<td>3.02, .0887</td>
<td>0.74, .3944</td>
</tr>
<tr>
<td>12</td>
<td>2.30, .1362</td>
<td>3.29, .0760</td>
<td>0.79, .3772</td>
</tr>
<tr>
<td>13</td>
<td>3.74, .0590</td>
<td>5.27, .0262</td>
<td>2.66, .1131</td>
</tr>
</tbody>
</table>

<sup>a</sup> Analysis based on √y transformed values
DISCUSSION

As documented in *P. americana* (Sloley 1989), oral administration of either AMT or AMTP reduced biogenic amine concentrations in the central nervous system of *A. triseriatus*. These observations are in contrast to those of Omar et al. (1982) who determined that AMT (at 500mg/kg) failed to significantly affect dopamine or serotonin levels in the cerebral ganglia of *P. americana*: they observed no overt abnormal behavior or changes in excitability following treatment.

The selectivity of AMT did not appear to be as great as that of AMTP. AMT administration depleted dopamine most significantly but octopamine concentrations were also significantly reduced on day 3 post-administration, and serotonin levels were slightly lower on all 3 days sampled. In comparison, AMTP appeared more selective and quite effective in its depletion of serotonin. Serotonin concentrations were reduced to about one tenth of control levels on days 1 through 7 post-treatment. AMT and AMTP supposedly inhibit the enzymatic hydroxylation of the amine precursors, tyrosine and tryptophan (Sloley 1989). Perhaps enzymatic selectivity of AMT is not as great as AMTP, leading to partial inhibition of serotonin and octopamine synthesis.

Both drugs significantly depressed amine concentrations throughout the 14 day experimental period. Although AMT and AMTP persistence was not determined in the present study, Sloley (1989) reported that both AMT and AMTP were poorly metabolized by *P. americana*: the estimated half-lives were 12 and 21 days respectively. Similarly, Lancaster and Sourkes (1969) found that *M. domestica* did not catabolize injected AMTP. Eventual disappearance of these drugs may be due to auto- or photoxidation (Sloley 1989).

Depression of spontaneous flight activity of *A. triseriatus* was similar with both drugs was and was correlated with decreased amine concentrations. The depressive effects observed with AMT and AMTP administration were similar to effects reported by use of general amine inhibitors. Preliminary experiments with *A. triseriatus* also
indicated decreased spontaneous flight activity following injection of reserpine, a
general amine-depleting compound (Novak, unpublished). Amphetamine and reserpine
were used by Brookhart et al. (1987) to study feeding behavior of the blow fly Phormia
regina. Both compounds depleted amine concentrations generally (onset and duration
were greater for reserpine) and lowered responsiveness to sucrose solutions. However,
when concentrated sucrose solutions were offered, the amine-depleted flies took larger
than normal meals. While amphetamine-treated flies exhibited near normal behavior,
reserpine-treated flies were lethargic and relatively unresponsive to external stimuli.
The return of sucrose responsiveness in amphetamine-treated flies mirrored the return
of amine concentrations to control levels.

In this study, the depression of spontaneous flight activity in AMT- and
AMTP-treated A. triseriatus was reversible but the subsequent return of normal flight
levels was apparently not correlated with complete replenishment of amine
concentrations. The evaluated flight parameters remained depressed for up to a week
following drug administration but returned to, and even exceeded, control levels prior
to a complete replenishment of amine pools. Restoration of normal flight activity after
several days may be due to development of receptor supersensitivity to depleted amine
concentrations, analogous to the well documented observations of neurotransmitter
supersensitivities developing in denervated muscles. Alternatively, there may be a
critical amine pool concentration required for "normal" flight activity, but amines are
typically present in excess of this level. A third possibility is that the depression and
return of flight activity are associated with secondary effects of AMT and AMTP and
not related to amine depletion. However, the observed depressive effects of general
amine depleters suggests that this is not the case.

While the surpassing of control flight levels was not statistically significant with
all parameters, it was evident in both 0.15% AMT and 0.75% AMTP groups. This higher
level of activity may represent an excess in nutrient stores (i.e. unexpended energy)
compared to the control group. In neither case was the 24-hr flight activity pattern modified by drug administration.

Spontaneous flight activity of mosquitoes may be initiated by a host of environmental factors (light/dark regimen, temperature, humidity, response to other external stimuli) or physiological cues (appetential, reproductive, etc.). The flight activity system used in these experiments isolates individual mosquitoes in containers with little external stimuli other than the light regimen. Therefore, it was not possible to ascertain the effects of differential amine depletion on specific behavioral patterns. Additional experiments which can isolate behaviors may provide clues to the specific roles of serotonin or dopamine.

The demonstration of the ability of AMT and AMTP to selectively reduce amine concentrations in the central nervous system of *P. americana* has led to the suggestion that these compounds may be useful tools for evaluating neural controls of various insect behaviors (Sloley 1989). The persistence of these compounds for several weeks in insect tissue indicates that these compounds are poorly metabolized or excreted; thus, behavioral modification or sublethal effects may also persist for long periods and be more easily studied. Persistence of these compounds also suggests that they may provide information on development of novel insecticides. Indeed, long-lasting behavioral modification without necessarily producing mortality may contribute insect population control (see review by Haynes 1988). It is not clear what degree of depressed behavior would significantly affect survival. However, reversal of effects is a key disadvantage of most sublethal insecticides studied thus far, and it appears that the modifications of flight activity elicited by AMT and AMTP in *A. triseriatus* are negated prior to amine repletion.
LITERATURE CITED


PAPER III. SEROTONIN DEPLETION AFFECTS BLOODFEEDING BUT NOT HOST-SEEKING ABILITY IN AEDES TRISERIATUS (SAY) (DIPTERA: CULICIDAE)
Serotonin depletion affects bloodfeeding but not host-seeking ability in
*Aedes triseriatus* (Say) (Diptera: Culicidae)

Mark G. Novak and Wayne A. Rowley

Dept. Entomology, Iowa State University, Ames, IA 50011
ABSTRACT

The host-seeking and bloodfeeding behaviors of *A. triseriatus* were evaluated following treatment with the amine depleting compounds α-methyl tyrosine (AMT) and α-methyl tryptophan (AMTP). AMTP was previously shown to selectively deplete serotonin levels in *A. triseriatus* cerebral ganglia while AMT reduced dopamine levels most significantly. Host-seeking ability (evaluated with an olfactometer) was not altered by treatment with either AMT or AMTP. Bloodfeeding success was significantly reduced by either oral administration or injection of AMTP. AMTP-treated mosquitoes responded positively when placed in close proximity to a host (rabbit) but fewer mosquitoes fed to repletion or fed at all. Those that fed to repletion took longer to do so. No differences were observed between control and AMT-treated mosquitoes. These observations suggest that serotonin plays a role in modulating the bloodfeeding ability of *A. triseriatus*. 
INTRODUCTION

Serotonin, an indolalkylamine, is an important neuroactive compound found in both vertebrates and invertebrates. Serotonin, along with octopamine and dopamine, is one of the major biogenic amines in insects. Relatively small numbers of serotonergic neurons occur in the insect nervous system, but large areas of the central and peripheral nervous systems are innervated by these fibers (Nässel 1988). Serotonergic cell bodies are typically found in small aggregations in different regions of the brain or segmental ganglia of insects (Nässel 1988). While most serotonergic neurons are interneurons, serotonergic networks of fibers have been observed on the surface of peripheral nerves and organs in Calliphora (Nässel and Elekes 1985), Periplaneta (Davis 1987) and Rhodnius (Orchard et al. 1988, Lange et al. 1988). These networks of varicose fibers suggest a neurohormonal function for serotonin (Nässel and Elekes 1985), and those associated with mouthpart nerves and salivary glands indicate possible involvement with feeding activity (Davis 1987).

Serotonin, as well as dopamine and octopamine, has been reported to modulate a variety of physiological and behavioral functions in insects. One or more of these biogenic amines have been implicated in the control of locomotory behavior, circadian activity, diapause and feeding activity (see reviews by Evans 1980, Klemm 1985, and Nässel 1988). Specifically, serotonin concentration was reported to vary circadianly in the cerebral ganglia and hemolymph of Acheta domesticus (Muzyńska-Pytel and Cymborowski 1978) and the frontal ganglia of Periplaneta americana (Pandey and Habibulla 1982). However, Owen et al. (1987) found no correlation between circadian activity and serotonin content (or dopamine content) of cerebral ganglia in P. americana. No statistically significant circadian changes in serotonin, dopamine, or octopamine concentrations were observed in the cerebral ganglia of Aedes triseriatus (Novak, in prep). Increased locomotor activity was induced by serotonin injection.
(Kaminshev et al. 1983) and serotonin, along with dopamine, has been suggested to be involved in regulating diapause in *Pteris brassicae* pupae (Puiroux et al. 1990). Serotonin modulated oviduct muscular contractions of *Tabanus* (Cook 1981) and *Locusta* (Lange and Orchard 1984), uterine contractions in *Blaberia* (Raabe et al. 1985), and foregut and hindgut contractions in several insect species (Raabe 1989). Increased fluid secretion and altered transepithelial voltage of Malpighian tubules were induced by serotonin in the mosquito *Aedes aegypti* (Veenstra 1988).

In addition to these functions, biogenic amines have been implicated in the control of feeding behaviors in insects. General amine depletion, induced by reserpine or D-amphetamine, increased the response threshold to sucrose in the blow fly *Phormia regina* (Brookhart et al. 1987). Serotonin induced salivation in the blowfly *Calliphora vicina* (formerly *C. erythrocephala*) (Trimmer 1985), and feeding is the natural stimulus for release of serotonin into the hemolymph of the blood-sucking bug, *Rhodnius prolixus* (Lange et al. 1989). Serotonin depletion led to reduced blood meal size in *R. prolixus* (Cook and Orchard 1990).

The use of pharmacological agents for modifying amine concentrations have become useful tools in many of these behavioral studies. Recent demonstrations of selective depletion of amines by α-methyl-tyrosine (AMT) and α-methyl-tryptophan (AMTP) have led to the suggestion that these compounds may be useful in determining the roles that biogenic amines play in regulating insect behaviors (Sloley 1989). In *Periplaneta americana*, AMTP selectively depleted serotonin and AMT reduced dopamine concentrations most significantly (Sloley and Orikasa 1988, Sloley 1989). Previous research with *A. triseriatus* (Novak in prep) has demonstrated similar, significant, and selective amine depletions with both drugs in the cerebral ganglia of this mosquito. Additionally, it was demonstrated that both drugs induced a decrease in the spontaneous circadian flight-activity of *A. triseriatus*. Because of their ability to affect amine concentration and spontaneous flight activity in *A. triseriatus*,
It was desirable to investigate the effects of amine depletion on behaviors such as host-seeking and bloodfeeding.
MATERIALS AND METHODS

All *A. triseriatus* used in this study were reared by conventional laboratory methods. Approximately 250 first instar larvae were placed in distilled water in 40 x 24 cm enamel pans and fed Tetramin® ad lib. Pupae were separated by sex and size using a mechanical separator. Mechanical separation of pupae also provided mosquitoes of uniform size. Newly emerged females were held in groups of 20-25 in 0.5 l cartons. Female mosquitoes that were to be orally dosed with AMT or AMTP were held without access to sucrose until treatment on day 3 or 4 post-emergence. Mosquitoes that were treated with AMT or AMTP by injection were held with constant access to sucrose. AMT and AMTP were obtained from Sigma Chemical Co. All stages were kept at 27 C, 80% relative humidity, with a 16:8 hr light/dark regimen.

On days 3 or 4 post-emergence, female *A. triseriatus* were offered a 10% sucrose solution containing AMT (0.15%), or AMTP (0.15, or 0.75%). Mosquitoes in the control group received 10% sucrose. Virtually 100% of the mosquitoes in the control and AMTP groups fed successfully. The estimated mean volume of 0.75% AMTP solution ingested by the mosquitoes was 2.7 μl (approx. 20 μg of AMTP ingested). Slightly less than half of the mosquitoes fed on the AMT-treated sucrose. Mosquitoes that did not feed to, or near repletion (> 50%), were removed from the cups. All *A. triseriatus* were then held with access to 10% sucrose until 24 hr prior to bloodfeeding or hostseeking trials.

Since *A. triseriatus* fed less readily on the AMT-treated sucrose solutions, it was desirable to be certain that the mosquitoes were receiving equal doses of AMT and AMTP. In this experimental group, *A. triseriatus* were held, upon emergence, with constant access to sucrose. On days 3 or 4 post-emergence, females were injected with 4 μg AMT or AMTP, dissolved in 1 μl of *Aedes* saline (Hayes 1953). Control group mosquitoes received a 1 μl injection of saline. Injections were made with a micro-syringe inserted through the cervical membrane and under the dorsal surface of
the prothorax. These mosquitoes were offered a host on day 3 post-injection. Sucrose was removed 24 hr prior to bloodfeeding trials.

Host-seeking ability of orally dosed AMT- and AMTP-treated *A. triseriatus* was evaluated with an olfactometer (Fig. 1) on days 3 or 4 post-treatment. Humidified air was pulled through the olfactometer by a 4 in electrical exhaust fan mounted at the downwind end of the apparatus. Treated and control groups (16-18 mosquitoes per group) were run alternately. Mosquitoes were initially acclimated in the downwind entrance chamber for 10 min. A screen separator was then removed, allowing the mosquitoes to fly upwind toward host stimuli (human arm and breath). Response to host stimuli was monitored for 10 min. Mosquitoes that flew upwind and entered an end-tube adjacent to the source of host odors were identified as host-seeking. Host-seeking behavior was confirmed by observing the random dispersal of mosquitoes in the absence of host stimuli and comparing the response of bloodfed and non-bloodfed mosquitoes.

![Figure 1. Schematic diagram of mosquito olfactometer: A] entrance port and acclimation chamber; B) end tube for host-seeking mosquitoes and port for arm and breathing tube (host stimuli), C) electric fan and air flow exhaust. D) entrance tube for humidified air flow (olfactometer based on designed of R. Hancock and W. Foster, Ohio State University, unpublished)
On day 3 post-treatment, bloodfeeding ability was assessed by placing cups of mosquitoes (15-18 mosquitoes/cup) in contact with the ears of restrained rabbits for 10 min periods. After host exposure, mosquitoes were grouped as replete, partially fed, or unfed. **Bloodfeeding success was evaluated by determining the percentage of replete females in each cup.** Additionally, bloodfeeding success in some trials was evaluated by weighing all bloodfed mosquitoes. Non-bloodfed mosquitoes were also routinely weighed to confirm consistency of size between treatments. Host-seeking and bloodfeeding data were analyzed with contingency tables. Observational comparisons were also made between the groups while in contact with the host. These observations included the time to approach the host, amount of probing, and time to acquire blood.
RESULTS

It was previously demonstrated that oral administration of AMT and AMTP elicited significant, long-term reductions in biogenic amine concentrations in *A. triseriatus* nervous tissue (Novak, in prep). The selectivity and degree of reduction was greater for AMTP. Serotonin levels in *A. triseriatus* cerebral ganglia were reduced by AMTP treatment to approximately 10% of control levels by day 1 post-administration and remained significantly reduced at day 14 post-treatment. Dopamine and octopamine levels were not significantly altered. Administration of AMT led to a significant decrease (approximately 50% depletion) in dopamine concentration. Additionally, octopamine levels were significantly reduced on day 3 post-treatment but to a lesser extent (approximately 20%) than dopamine depletion.

Depression of amine levels did not affect the host-seeking ability of *A. triseriatus*. Eighty-three percent of AMT-treated *A. triseriatus* responded positively to host stimuli compared to 84% of the control group mosquitoes ($X^2 = 0.07$, $p > 0.75$) (Table 1). Similarly, little difference was observed between host-seeking of 0.15% AMTP-treated compared to controls (73% vs 71% respectively, $X^2 = 0.14$, $p > 0.50$). *Aedes triseriatus* treated with a higher dose of AMTP also showed no significant impairment of host-seeking ability compared to their respective controls ($X^2 = 1.64$, $p > 0.25$).

Evidence that *A. triseriatus* were indeed host-seeking is provided by the observation of a significant difference in the response of bloodfed (fed 24 hrs prior to testing) and non-bloodfed mosquitoes ($X^2 = 48.9$, $p << 0.001$) as well as preliminary observations that less than 10% of the mosquitoes entered the end-tube when host stimuli were not present. Additionally, it was observed that both control and treated mosquitoes that entered the end tube (host-seekers) would consistently land on the screen adjacent to the source of host-stimuli and vigorously probe through the mesh.
Table 1. Results of olfactometer trials comparing the host-seeking activity of AMT- or AMTP-treated Aedes triseriatus with controls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Host-seeking</th>
<th>Total</th>
<th>% host-seeking</th>
<th>$X^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15% AMT</td>
<td>205</td>
<td>247</td>
<td>83</td>
<td>0.07 NS a</td>
</tr>
<tr>
<td>Control</td>
<td>225</td>
<td>267</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>0.15% AMTP</td>
<td>135</td>
<td>185</td>
<td>73</td>
<td>0.14 NS</td>
</tr>
<tr>
<td>Control</td>
<td>135</td>
<td>191</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>0.75% AMTP</td>
<td>110</td>
<td>136</td>
<td>82</td>
<td>1.64 NS</td>
</tr>
<tr>
<td>Control</td>
<td>123</td>
<td>141</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Non bloodfed *</td>
<td>46</td>
<td>53</td>
<td>87</td>
<td>43.9 **</td>
</tr>
<tr>
<td>Bloodfed</td>
<td>11</td>
<td>53</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

a $X^2_{0.05,1} = 3.84$

* comparison between starved (24 hr) and bloodfed (24 hr prior) mosquitoes

** $p < 0.001$

Bloodfeeding success was not significantly affected by AMT treatment in A. triseriatus (Table 2). A slightly greater proportion of AMT-treated mosquitoes bloodfed to repletion in comparison to control mosquitoes (70% vs 64%) but a comparison of non-bloodfed, partial bloodfed, and replete females between the AMT-treated and control groups was not statistically significant ($X^2 = 1.93, p > 0.25$). In contrast, similar comparisons between controls and AMTP-treated A. triseriatus indicated that feeding success was significantly lowered by AMTP ($X^2 = 34.1$ and 36.3 for .15% and .75% AMTP treatment, respectively, $p << 0.001$). Forty-nine percent of 0.15% AMTP-treated mosquitoes fed to repletion in comparison to 70% of the control group. Bloodfeeding success was reduced even further in 0.75% AMTP treated mosquitoes. The percent feeding to repletion (33%) was less than half of the control group (74%).
Table 2. Bloodfeeding success comparisons of control and AMT- or AMTP-treated *Aedes triseriatus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replete</th>
<th>Partial Blooded</th>
<th>Non-Blooded</th>
<th>Total Exposed</th>
<th>% replete</th>
<th>$X^2$</th>
<th>$X$ weight (mg) of blooded</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15% AMT</td>
<td>159</td>
<td>23</td>
<td>46</td>
<td>228</td>
<td>70</td>
<td>6.79 ± 0.22 (21)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>129</td>
<td>23</td>
<td>51</td>
<td>203</td>
<td>64</td>
<td>1.93 NS a</td>
<td></td>
</tr>
<tr>
<td>0.15% AMTP</td>
<td>181</td>
<td>56</td>
<td>134</td>
<td>371</td>
<td>49</td>
<td>6.39 ± 0.15 (86) b</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>240</td>
<td>39</td>
<td>65</td>
<td>344</td>
<td>70</td>
<td>34.1 **</td>
<td></td>
</tr>
<tr>
<td>0.75% AMTP</td>
<td>35</td>
<td>26</td>
<td>44</td>
<td>105</td>
<td>33</td>
<td>5.18 ± 0.14 (47) b</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80</td>
<td>12</td>
<td>16</td>
<td>108</td>
<td>74</td>
<td>36.3 **</td>
<td></td>
</tr>
</tbody>
</table>

*a $X^2 0.05,2 = 5.99$  
** $p < 0.001$  
$b F < 0.05$, ANOV

In addition to evaluating bloodfeeding success by determining the number of mosquitoes feeding to repletion, subsamples of mosquitoes that fed to any degree were weighed. AMTP-treated mosquitoes took, on average, significantly less blood than control mosquitoes. There was no significant difference in the weights of AMT-treated mosquitoes and their respective controls.

Since it was evident that *A. triseriatus* did not feed as readily on AMT as AMTP solutions, it was desirable to ensure that differences in bloodfeeding ability were not a result of unequal dosage. Therefore, a sample of mosquitoes was injected with 4 µg doses of AMT or AMTP and their bloodfeeding success compared to saline-injected controls. Significantly fewer of the AMTP-injected *A. triseriatus* fed to repletion than did the control mosquitoes ($X^2 = 17.02, p < 0.001$) (Table 3). Bloodfeeding success of AMT-injected mosquitoes was slightly lower than that of the control group but the difference was not statistically significant ($X^2 = 2.98, p > 0.05$).
Table 3. Bloodfeeding success comparisons between AMT- or AMTP-injected *Aedes triseriatus* and controls (saline-injected)

<table>
<thead>
<tr>
<th>Injection</th>
<th>Replete Bloodfed</th>
<th>Partial Bloodfed</th>
<th>Non-Bloodfed</th>
<th>Total Exposed</th>
<th>% replete</th>
<th>(X^2) (vs control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMT (4 ng)</td>
<td>20</td>
<td>1</td>
<td>10</td>
<td>31</td>
<td>67</td>
<td>2.98 NS (a)</td>
</tr>
<tr>
<td>AMTP (4 µg)</td>
<td>10</td>
<td>4</td>
<td>17</td>
<td>31</td>
<td>32</td>
<td>17.02 *</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>26</td>
<td>1</td>
<td>5</td>
<td>32</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) \(X^2_{0.05,1} = 3.84\)
\(*\) \(p < 0.001\)

Observations of bloodfeeding behavior revealed notable differences between AMTP-treated mosquitoes and AMT-treated or control groups of *A. triseriatus*. By the end of the first minute of a trial, most mosquitoes (> 80%) in all treatment groups were in contact with the host (rabbit ear). Typically, more than half of the control group and AMT-treated mosquitoes already had imbibed a visible amount of blood. Others were either stationary with their proboscis embedded or were walking around on the screen in contact with the host and intermittently probing. Virtually all control and AMT-treated mosquitoes that exhibited interest in the host were replete with blood within 5-6 min. In contrast, although most AMTP-treated mosquitoes also responded positively to the host within the first minute of exposure, few had imbibed a visible amount of blood. Several similar behaviors of AMTP-treated mosquitoes were commonly observed. AMTP-treated *A. triseriatus* would often probe repeatedly without apparent success. Others would remain stationary with their proboscis embedded for 1-2 minutes but without visibly acquiring blood. These unsuccessful mosquitoes would either eventually lose interest in feeding and fly from the host or would continue probing and eventually acquire blood. However, most of the successful mosquitoes would terminate feeding prior to repletion and leave the host with only a partial...
bloodmeal. As a result, a higher proportion of AMTP-treated mosquitoes took partial bloodmeals in comparison to control or AMT-treated mosquitoes, as evident from the comparison of bloodfed mosquito weights. AMTP-treated mosquitoes that did feed to repletion took longer than control mosquitoes. Generally, treated mosquitoes would feed throughout most of the 10 min. exposure period.
DISCUSSION

The choice of AMT and AMTP as tools for modifying amine concentrations of *A. triseriatus* in this study was based on their ability to selectively deplete amine levels. Serotonin concentrations in the central nervous system of *A. triseriatus* were substantially and selectively reduced by AMTP treatment, while neither dopamine nor octopamine levels were significantly altered (Novak, in prep). In contrast, treatment with AMT significantly reduced the dopamine concentrations but not to the extent that serotonin levels were reduced by AMTP administration. Octopamine content of AMT-treated mosquitoes was also reduced but less than dopamine depletion. Sloley (1988) and Sloley and Orikasa (1989) demonstrated similar selective amine depletions using these drugs in *Periplaneta americana* and suggested that their selectivity may make them useful tools to elucidate the roles of biogenic amines in insects.

Most of the previous studies relating biogenic amines to insect behavior have relied on the direct injection of biogenic amines, their agonists, or general amine depleters. With these methods, biogenic amines have been variously implicated in modulating feeding activities of several insects. Enhanced proboscis extension in response to water and sucrose was exhibited by adult blow flies (*Phormia regina*) following injection of octopamine or octopaminergic drugs (Long and Murdock 1983). Injected flies became hyperphagic when imbibing sucrose solutions. Similarly, injection of octopamine into cerebral ganglia of *Apis mellifera* increased proboscis extension responses to non-conditioned olfactory stimuli (Mercer and Menzel 1982). In contrast, dopamine and serotonin reduced the percentage of bees responding to a conditioned response. Depleting amine levels by injection of reserpine or d-amphetamine increased the mean acceptance threshold to sucrose solutions in *P. regina* (Brookhart et al. 1987). However, the functional significance of any one amine is unclear since these drugs are general amine depleters; dopamine, octopamine and serotonin levels were all significantly
reduced. Likewise, a significant disadvantage to the injection of biogenic amines themselves is their rapid metabolism *in vivo* and presumably ephemeral effects.

In spite of the significant, selective reductions in amine levels by AMT and AMTP neither drug affected the host-seeking activity *A. triseriatus*. In contrast, previous behavioral studies using AMT and AMTP found that both drugs led to decreased spontaneous flight activity of *A. triseriatus* (Novak, in prep). These observations are not necessarily contradictory. Spontaneous flight activity was monitored in the absence of external stimuli, except for the light/dark regimen and constant access to sucrose. Even though *A. triseriatus* is considered to be a diurnally active mosquito, most spontaneous flight activity of this species occurs during the early morning and late evening and is circadianly controlled (Clarke 1988). In the absence of external stimuli, little flight activity occurs during the mid-day period. Therefore, while overall spontaneous flight activity may be depressed, it is not necessarily an accurate predictor of activity in response to external stimuli. When presented with host stimuli, most *A. triseriatus* (both amine-depleted and control mosquitoes) responded positively and without significant differences.

*Aedes triseriatus* that had reduced concentrations of serotonin (AMTP-treated) were less successful at obtaining a bloodmeal than were control group mosquitoes. This suggests that serotonin is integral in modulating some aspect(s) of bloodfeeding activity in *A. triseriatus*. Depletion of dopamine, and to a lesser extent octopamine, did not affect the ability of the mosquitoes to acquire a bloodmeal. It is difficult to make a direct comparison between behavior of AMT and AMTP-treated mosquitoes since the degree of amine reduction appears less for AMT. However, the same dosage of AMT used in this study, without effect on host-seeking or bloodfeeding behavior of *A. triseriatus*, was enough to significantly reduce the spontaneous flight activity of this mosquito for several days following drug administration. Additionally, the flight activity levels of AMT-treated mosquitoes were similar to those treated with AMTP (Novak, in prep).
Studies with other insects have specifically implicated serotonin as an important modulator of feeding activities. Trimner (1985) provided evidence that in the blow fly Calliphora vicina, serotonin is released into the hemolymph, functioning as a neurohormone to stimulate salivation. Similarly, serotonin appears to be integrally involved in feeding activities of the blood-sucking bug Rhodnius prolixus. Serotonin increased rapidly in the hemolymph of feeding R. prolixus (Lange et al. 1989) and has also been identified as the cuticle plasticizing factor in this bug (Orchard et al. 1988). Injection of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into R. prolixus led to depletion of peripheral serotonergic stores of serotonin (Cook and Orchard 1990). Insects treated with 5,7-DHT consumed significantly smaller blood meals than controls, and did not undergo cuticle plasticization. These observations, together with ultrastructural studies indicating serotonergic processes innervating salivary glands, digestive tract, and epidermis in R. prolixus (Lange et al. 1988, Orchard et al. 1988), provide evidence of the importance of serotonin in regulating bloodfeeding in this insect.

The association of serotonin with feeding activities has also been recognized in other arthropods, as well as in, the medicinal leech, Hirudo medicinalis (Lent et al. 1989) and the mollusk Lymnaea (Kemenes et al. 1990). In Lymnaea, 5,6-DHT and 6-OH-DA were used to deplete serotonin and dopamine stores respectively. Both serotonin and dopamine depletion led to reduced biting movements in response to sucrose, suggesting that both serotonin and dopamine are involved in this feeding activity. An observation made by Kemenes et al. (1990) on the feeding behavior of amine depleted snails was strikingly similar to the observation of the behavioral response of amine-depleted A. triseriatus. These authors noted that “at time periods when biting responses were inhibited or reduced by drug injection, the increased general exploratory activity associated with the introduction of sucrose into the experimental dish still persisted.” Similarly, amine-depleted mosquitoes displayed
increased exploratory activity when offered a host and, as noted previously, when arriving in proximity to the host stimuli in olfactometer trials, all mosquitoes displayed a general excitedness and vigorous probing activity through the mesh screen separating them from the prospective host.

While it was evident that serotonin-depleted mosquitoes could effectively find a host but were less successful at obtaining a complete bloodmeal, the exact reason(s) for this inhibition are unclear. Although hemolymph serotonin levels are known to rise at the onset of feeding in *Rhodnius* and peripheral depletion of serotonin reduces bloodfeeding success, the mechanisms controlled by serotonin are not known (Cook and Orchard 1990). Perhaps as demonstrated in *Calliphora* (Trimmer 1985), serotonin controls salivation in *A. triseriatus*. Thus, inhibition of salivation would make it difficult for the mosquito to probe effectively and ingest a bloodmeal. Casual observations of AMTP- and AMT-treated *Aedes triseriatus* indicated little trouble in feeding on sucrose.

The identification of more drugs that selectively alter amine concentrations would be increasingly helpful in identifying roles that biogenic amines play in regulating insect behavior. AMT and AMTP are particularly easy to work with because they can be administered orally, facilitating the simultaneous treatment of large numbers of individuals. One disadvantage to their use is that the reduction of amine concentrations occurs in both the central and peripheral nervous systems (Sloley 1989). The use of these drugs in combination with other selective effectors such as 5,7-DHT, should be even more effective in identifying the level(s) of biogenic amine control of insect behavior.
LITERATURE CITED


GENERAL SUMMARY

The primary objectives of this research project were to determine the presence and abundance of the major biogenic amines in the mosquito *Aedes triseriatus* and to identify mosquito behaviors that may be modulated by these amines. Knowledge gained from this research may be viewed as significant for two reasons: First, basic information on the abundance and distribution of biogenic amines in the CNS of a very significant family of insects, Culicidae, has been previously lacking. Second, identifying amine-modulated behaviors and observing behavioral changes resulting from amine alteration could provide insight into alternative insect-control methods.

The first phase of this study identified dopamine, serotonin, and octopamine (but not noradrenaline) as abundant in the cerebral and thoracic ganglia of *A. triseriatus*. No changes in brain amine-levels were evident when samples were taken at periods corresponding to maximum and minimum circadian flight activity. However, all 3 major amines increased during the first two weeks of adult life. It is not known whether this increase represents only a physical maturation process or whether it may also correspond to a change or increase in the behavioral repertoire. Reports of similar circadian and age-related amine changes in other insect species have been inconsistent.

The second phase of this study employed two pharmacological agents, AMT and AMTP, that had been previously shown to induce selective depletions in the cockroach *Periplaneta americana*. Administration of these compounds to *A. triseriatus* resulted in similar amine reductions confirming their depressive selectivity in this mosquito. Spontaneous circadian flight-activity was investigated to determine whether these compounds would elicit differential effects. A decrease in the spontaneous flight activity but not in the circadian activity pattern of *A. triseriatus* was observed following amine depletions induced by both AMT and AMTP. However, spontaneous flight activity returned to normal levels prior to repletion of amine pools. If the depression of flight activity is indeed a manifestation of amine reduction, a
compensating mechanism for amine loss must occur. This observation presents a possible disadvantage to the efficacy of insect control strategies which rely on behavioral modification rather than lethality, as postulated by Haynes (1988). Still, the manifestation of flight-activity modification suggests that the use of pharmacological agents such as AMT and AMTP can be useful tools for elucidating roles of biogenic amines in behavioral and physiological processes.

The final phase of this research project used the same strategy of amine-alteration to examine the effects of selective amine depression on the host-seeking and bloodfeeding abilities of *A. triseriatus*. While neither AMT or AMTP treatment impaired host-seeking ability, AMTP-treated mosquitoes were less likely to bloodfeed and, if they did feed, they were less likely to bloodfeed to repletion. This finding suggests that serotonin is integral to the bloodfeeding ability of *A. triseriatus* and complements the observations of serotonin’s importance to blood and sucrose feeding in other insect species such as *Rhodnius prolixus* and *Calliphora erythrocephala*, and in other invertebrate species such as mollusks and leeches.

Pharmacological agents used to manipulate amine concentrations have proven to be important tools in investigating putative roles of biogenic amines in other insect species: both AMT and AMTP were effective in *A. triseriatus*. Particular advantages of AMT and AMTP are their long-term selective effects. Their prolonged action is particularly appreciated when working with compounds, such as biogenic amines, that are rapidly metabolized *in vivo*. Although some previous studies have provided evidence of behavioral modification elicited by direct amine injection, it is generally recognized that the ephemeral presence of amines *in vivo* presents significant obstacles to their use or to interpretation of their effects in behavioral assays. Preliminary experiments with the direct amine-injection in *A. triseriatus* were generally unsuccessful.
Future studies of biogenic amines in mosquitoes should continue to integrate the use of quantitative techniques and behavioral assays to determine other roles of biogenic amines. Incorporating additional pharmacological agents, particularly those with selective actions, would enhance these investigations. More specifically, the importance of serotonin in bloodfeeding activities must be further defined. Additionally, the distribution of dopamine, serotonin, and octopamine should be determined throughout the entire central and peripheral nervous systems. Serotonin antibodies are commercially available and have been successfully used to identify serotonergic neurons, but effective dopamine and octopamine antibodies are not widely available yet. Immunohistochemical techniques for visualizing aminergic cells and fibers would be quite useful; of particular interest would be determining the presence or extent of serotonergic innervation of mouthparts, salivary glands, or digestive tract, as has been observed in other insects. In addition to simply mapping the distribution of aminergic neurons, it would be more desirable to determine changes in aminergic cells and fibers in relation to the developmental stage or age of the mosquito, physical stress such as exhaustive flight, or behavioral state such as adult diapause in Culex mosquitoes. Although tedious, it should be possible to identify biogenic amines in mosquito hemolymph by pooling samples of sufficient volume to analyze by HPLC or radioimmunoassay techniques. As demonstrated in other insect species, biogenic amines could also function as neurohormones modulating flight, feeding, or circadian activity of mosquitoes. And identifying these roles would enhance our understanding of mosquito behavior and physiology.
LITERATURE CITED


