Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 1333-1335

Received: 06-10-2020
Accepted: 04-12-2020

Mehere PV
Department of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Maharashtra, India

Patodkar VR
Department of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Maharashtra, India

Pangaokar LA
Department of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Maharashtra, India

Corresponding Author:
Mehere PV
Department of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Maharashtra, India

Examination of catecholamines in body extracts by positive-ion electrospray tandem mass spectrometry in mosquito

Mehere PV, Patodkar VR and Pangaokar LA

Abstract
Neurotransmission is a very important physiological process, which is characterized by three distinct stages: synthesis of the neurotransmitters; storage of the neurotransmitters, and release of these neurotransmitters (dopamine, serotonin, and octopamine). Once they are released, neurotransmitters bind to activate receptors in postsynaptic membranes. Ultimately insects must deactivate of these neurotransmitters—either by the reuptake mechanism, or via enzymatic processing. In insects the monoamine oxidase which deactivates neurotransmitter has not been found and thus the role of arylalkylamine N-acetyltransferase has been studied by many scientists. The study was designed to understand whether N-acetylation is the main pathway for deactivating the neurotransmitters. In the present study under our conditions melatonin and N-acetylserotonin were not detected from the mosquito body extracts, but in the serotonin-fed samples, an increase in the level of dopamine was observed under qualitative conditions.

Keywords: neurotransmitter, catecholamine, mosquitoes, mass spectrophotometry

Introduction
Neurotransmission is a very important physiological process, which is characterized by three distinct stages: synthesis of the neurotransmitters; storage of the neurotransmitters, and release of these neurotransmitters (dopamine, serotonin, and octopamine). Once they are released, neurotransmitters bind to activate receptors in postsynaptic membranes. Ultimately insects must deactivate of these neurotransmitters—either by the reuptake mechanism, or via enzymatic processing. In mammals, enzyme-catabolizing biogenic amines, i.e., monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) play a major role in their inactivation. MAO and COMT are not present in the insect nervous system (1). Inactivation of biogenic amines may be achieved by alternative metabolic pathways, such as *N*-acetylation and *O*-sulfation [2, 3]. It has been hypothesized that N-acetylation is the pathway in the insect brain, but there have been no studies to date that corroborate that conjecture. This study attempted to demonstrate whether N-acetylation is the main pathway in the brain and body of *Ae. Aegypti.*

Materials and Methods
Dopamine, serotonin, and melatonin were purchased from Sigma Aldrich. *Ae. aegypti* mosquitoes were reared as per paper published Mehere *et al*., 2011 [5], after which the females of this species were separated from the males. The female mosquitoes were divided into three groups one group was either fed on dopamine (50 mM) or one group on serotonin (25 mM) for 5 hours and other group was fed on 10% sugar solution as a control. Standards for dopamine and N-acetyldopamine were prepared in water on the same day of the experiments.

HPLC analysis of dopamine from tissue extracts
The brains of the *Ae. aegypti* females were separated from the bodies and placed in 0.4 M formic acid and 0.05 M Na2-EDTA. All the tissues were transferred into vial containing 200 µl of 0.1 mM formic acid with antioxidants, and placed on ice. Tissues were sonicated and centrifuged at 14,000 g for 20 min at 4 ºC, and then the supernatant was collected and stored at −20 ºC prior to analysis. Supernatants were chromatographed by HPLC with a reverse-phase column (C18 of 5 µm particles, 4.6 X 1100 mm), and resolved substrate and product were detected by HPLC with electrochemical detection n (HPLC-ED).

The biogenic amines were chromatographed in the presence of 6% acetonitrile and 50 mM of a NaH2PO4 monobasic buffer.

Agilent LC pump with reverse phase column (C18, 2.1x50mm) was used to separate the *N-acetyldopamine* and dopamine. LC separation was done using organic solvent comprised of 0.1% formic acid, 60% methanol, and 40% water as a mobile phase. The system was equilibrated for 2 min. 100 µL of prepared samples were injected for HPLC-MS analysis. *N-acetyldopamine* and dopamine were introduced into the 3200 Q-TRAP (Applied Biosystems / MDS SCIEX).

Tandem mass spectrometry was used in the positive-ion interfaced with a Turbo Ion Spray ™ the parameters used during this experiment. TurboIonSpray was operated at 600 ºC to introduce the LC eluent into the mass spectrometer. An enhanced product ion (EPI) scan was used to trace the *N*-acetyldopamine at (m/z 196.20). The collision gas we utilized in this experiment was nitrogen with a cell pressure of 1.1 Pa. The mass transition-dependent collision energy was 20 V for transitions m/z 154-137, 196 137 for the semi quantitative analysis of N-acetylated compounds in the brain of *Ae. aegypti*. Data were processed using Analyst 1.4.2.

Results

Detection of *N*-acetyldopamine in the brain and body extracts of *Ae. aegypti* was not successful using the HPLC-electrochemical detection. Further, we used LC-MS/MS in positive mode to detect levels of dopamine and *N*-acetyldopamine. Based on the retention time, dopamine and *N*-acetyl-dopamine were detected in body extracts of *Ae. aegypti*. N-acetyl-dopamine in the brain body extracts (Figure 1 and Figure 2) of mosquitoes were detected five hours after dopamine feeding. The *N*-acetyldopamine was traced using enhanced product ion scan (linear ion trap scan mode) at m/z 196. An accumulation of dopamine and an increase in the level of the *N*-acetylated compound was also detected compared to the control (sugar) after 5 hours of feeding, thus indicating the presence of an *N*-acetylation pathway in the brain of *Ae. aegypti*. It is important to note that an attempt was made to detect serotonin, *N*-acetylserotonin, and melatonin from the body extracts of *Ae. aegypti* using similar methods, but we were unable to detect these compounds in the extracts under the conditions used in this study. It should also be noted that under our conditions melatonin and *N*-acetylserotonin were not detected from the mosquito body extracts, but in the serotonin-fed samples, an increase in the level of dopamine was observed under qualitative conditions.

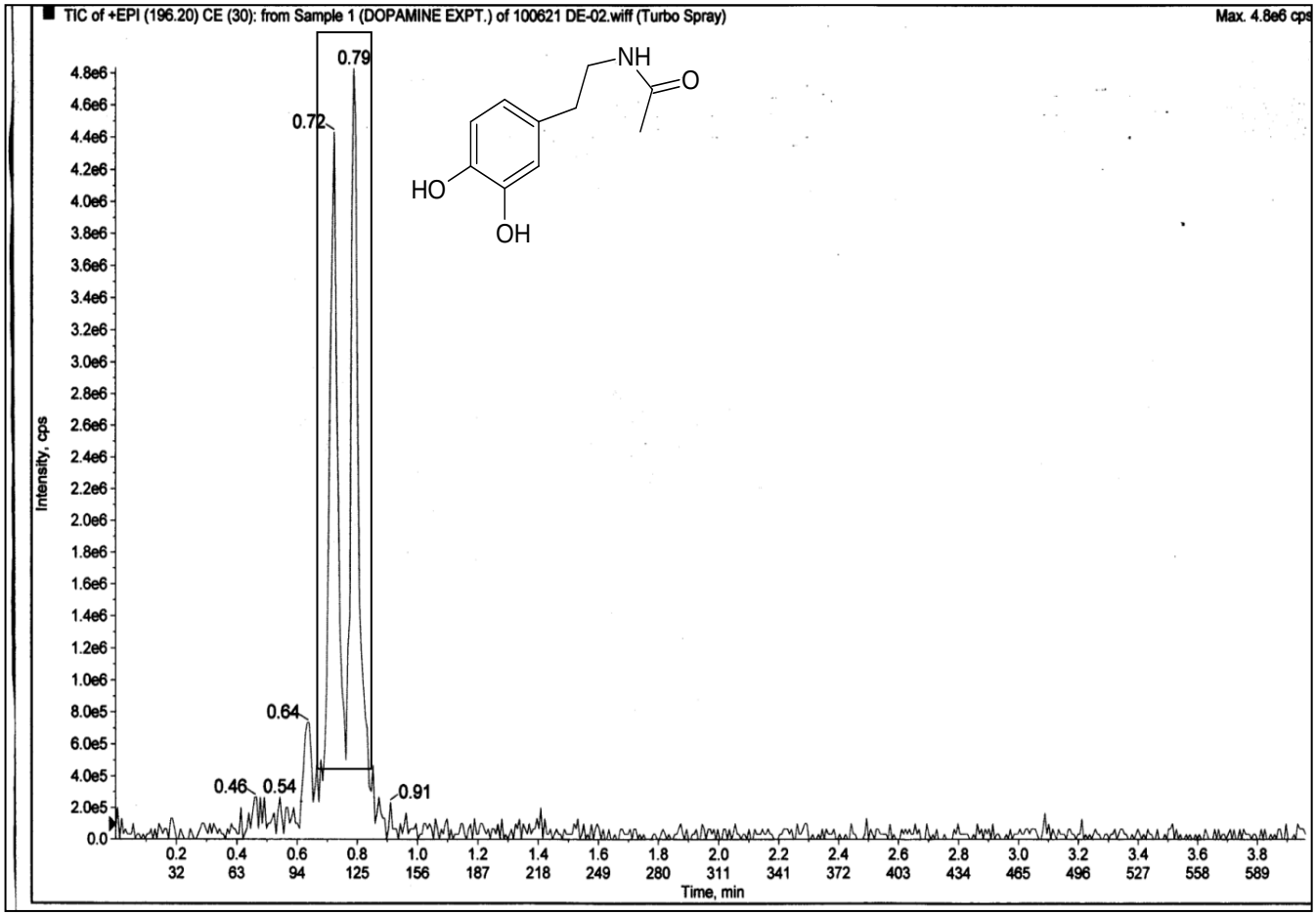

Fig 1: LC-MS-TIC chromatogram of *N*-acetyldopamine from body sample. In this figure X axis represents the time (min) and Y-axis represents the intensity in counts per seconds (CPS).

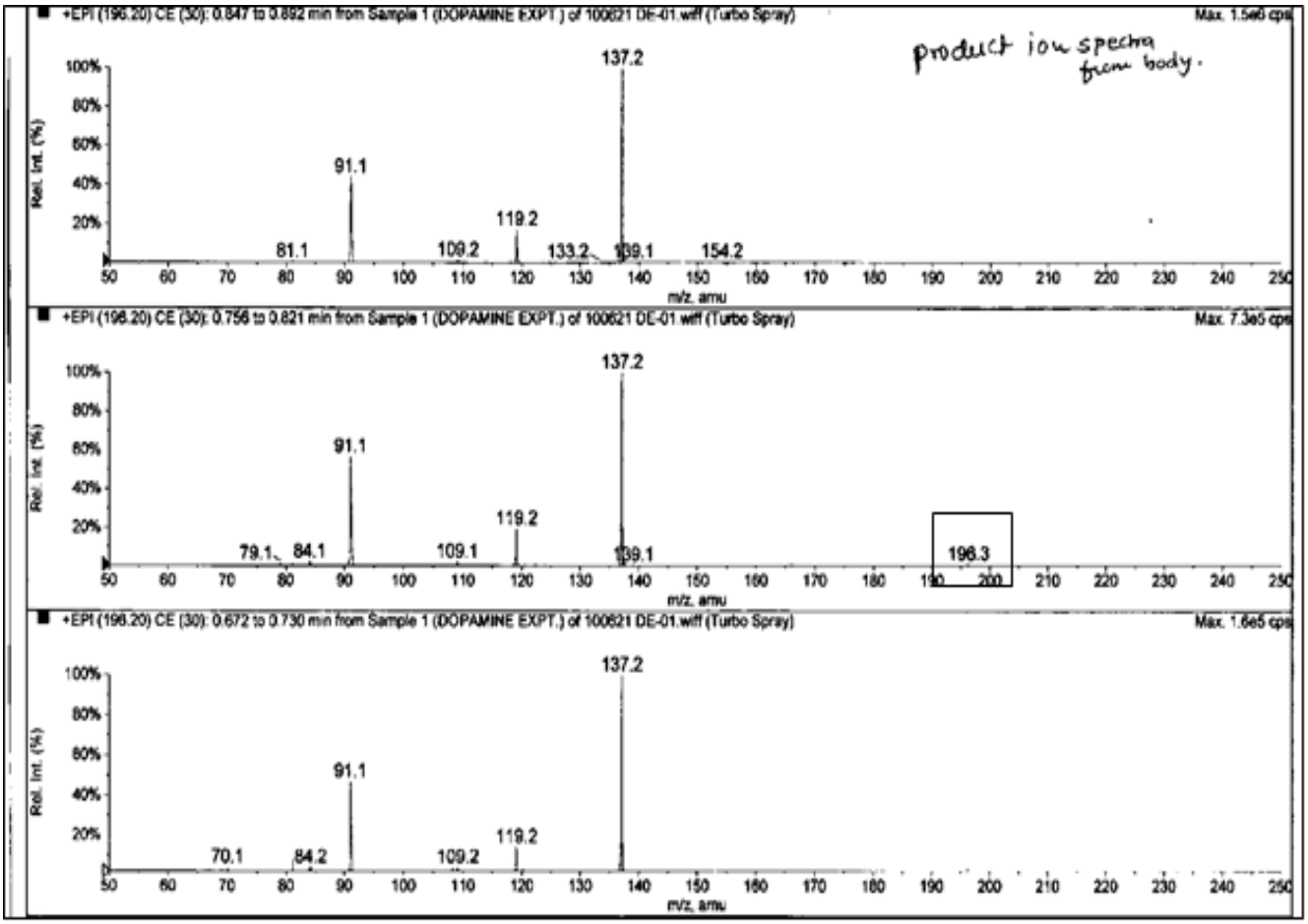

Fig 2: Detection of *N*-acetyldopamine from the body of the mosquitoes using the product ion spectra of protonated molecule. In Figure X-axis represents the time and Y-axis represents relative intensity in (%)

Discussion

This is the first study in where this kind of demonstration regarding *N*-acetyldopamine has been carried out. Trace amounts of *N*-acetyldopamine were detected using the EPI method from body extracts, suggesting the presence of *N*-acetylated pathways in the body of mosquitoes (Figure 1 and [5]. These results are in comparison with Mehere *et al*., 2011 where *N-acetyldopamine* was detected in the brain. Based on bioinformatics sequence analysis, thirteen hypothetical *N-acetyltransferases* have been found in *Ae. aegypti* (Mehere *et al*., 2011) [5]. Mehere *et al.*, 2011 [5] suggest that six AeAANATs were expressed in the head, and five of them showed activity toward arylalkylamines and some of them expressed in the mosquito body. These results provide persuasive evidence to suggest that AANATs may be the primary pathway for neurotransmitter inactivation in *Ae. aegypti*. This study was not able to detect *N*-acetylserotonin or melatonin in the extracts raised a question whether these compounds used in downstream pathways. This hypothesis further needs to be investigated. Similarly, melatonin was not detected in either brain (Unpublished data by Mehere *et al*) or body extracts from the *Ae. aegypti* species. This absence suggested to us that [1] AeAANATs in the brain may not play a role in circadian rhythm regulation, or [2] melatonin was further metabolized. This study was able to detect dopamine in the serotonin-fed sample using the MRM method (data not shown), which suggests that serotonin may regulate the release of dopamine in the brain and the body of *Ae. aegypti*. A similar phenomenon has been associated with human regulatory functions, where it has been suggested that serotonin does play an important role in controlling dopamine levels in the brain [4]. It is still not clear whether or not and how quick if melatonin or serotonin get metabolized in brain and body of the mosquitoes.

Acknowledgment

I am very much thankful to Dr. Jianyong Li, Professor Biochemistry, Virginia Polytechnic Institute and State University, USA for his guidance and letting me work in his laboratory.

References

1. Sloley BD. Metabolism of monoamines in invertebrates: the relative importance of monoamine oxidase in different phyla. Neurotoxicology 2004;25:175-183.
2. Wright TR. The genetics of biogenic amine metabolism, sclerotization, and melanization in *Drosophila melanogaster*. Advance Genetics 1987;24:127-222.
3. Roeder T, Seifert M, Kahler C, Gewecke M. Tyramine and Octopamine: antagonistic modulators of behavior and metabolism, Archives of Insect Biochemistry Physiology 2003;54:1-13.
4. Di Matteo V, Di Giovanni G, Pierucci M, Esposito E. Serotonin control of central dopaminergic function: focus on *in vivo* microdialysis studies. Progress in Brain Research 2008;172(7-44):137.
5. Mehere PV, Han Q, Christensen BM, Li J. Identification and characterization of two arylalkylamine *N*-acetyltransferases in the yellow fever mosquito, *Aedes aegypti*. Insect Biochemistry and Molecular Biology 2011;41(9):707-714.