Melatonin in the Testis of the Cabbage Armyworm, *Mamestra brassicae*

Toshiaki Shimizu* and Naokuni Takeda

Laboratory of Applied Entomology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan
Department of Biomedical Science, COSMO Research Institute, Gongendo, Satte, Saitama, 340-01, Japan

Z. Naturforsch. **53c,** 927–931 (1998);
received May 14/June 12, 1998

Melatonin, *Mamestra brassicae,* Testis, HPLC-ECD, Metabolic Pathways, Diapausing Pupae

N-Acetyl-5-methoxytryptamine (melatonin; MEL) was detected in the testis of non-diapausing pupae and in the testis of post-diapause pharate adults of the cabbage armyworm, *Mamestra brassicae,* by use of a three-dimensional HPLC system with multiple coulometric electrochemical detectors.

Intermediates providing evidence of various metabolic pathways were identified, as follows: tryptophan (TRP) → 5-hydroxytryptophan (5-HTP) → 5-hydroxytryptamine (5-HT) → *N*-methyl-5-hydroxytryptamine (*N*-MET)
└→ 5-hydroxyindoleacetic acid (5-HIAA)
└→ *N*-acetyl-5-hydroxytryptamine (*N*-ACET-5-HT)
└→ melatonin (MEL).

The possible physiological roles of melatonin in the testis of both diapausing and non-diapausing pupae are discussed.

Introduction

Melatonin, *N*-acetyl-5-methoxytryptamine, has been found in a number of insects, such as the locust, *Locusta migratoria* (Vivien-Roels *et al.,* 1984), the face fly, *Musca autumnalis* (Wetterberg *et al.,* 1987), the fruit fly, *Drosophila melanogaster* (Finocchiaro *et al.,* 1988), damselflies, *Ischnura verticalis* and *Enallagma civile* (Tilden *et al.,* 1994), the silkworm, *Bombyx mori* (Takeda *et al.,* 1991; Itoh *et al.,* 1995) and the pea aphid, *Acyrthosiphon pisum* (Gao and Hardie, 1997). The production of melatonin in insects shows a circadian rhythm (Wetterberg *et al.,* 1987; Tilden *et al.,* 1994; Itoh *et al.,* 1995) and it has been proposed that melatonin might be an evolutionarly conserved molecule that transduces photoperiodic information (Vivien-Roels and Pevet, 1993).

During our analysis of indolalkylamines, we detected melatonin in the testis of non-diapausing pupae and of diapausing pharate adults of the cabbage armyworm, *Mamestra brassicae.* We examined possible associated metabolic pathways and considered the possible physiological functions of melatonin in the testis of the armyworm.

Materials and Methods

Insects

Larvae of the cabbage armyworm, *Mamestra brassicae,* were reared on an artificial diet (Silkmate; Nihonnosan Kogyo Co., Tokyo) at 25 °C under a 16 h light and 8 h dark (16L:8D) photoperiod to obtain nondiapausing pupae and at 20 °C under a 12L:12D photoperiod to obtain diapausing pupae, respectively. We utilized the nondiapausing pupae on days 1 and 8, diapausing pupae on day 100, and pharate adults (ey-epigmented pupae), respectively.

Preparation of samples

Since production of melatonin is associated with a nocturnal peak in the level of melatonin (Wetterberg *et al.,* 1987), testes were dissected from animals during the scotophase. Isolated testes were transferred to a 0.9% solution of NaCl to eliminate contamination by biogenic amines in the haemolymph. Testes were gently homogenized in a cooled manual microhomogenizer in 300 µl of 0.4 N perchloracetic acid. The homogenate was centrifuged at 10,000×*g* for 10 min at 0 °C and the supernatant was filtered through a Millipore filter (UFC 3 OHV; Nihon Millipore Ltd., Tokyo). Aliquots of 80 µl of supernatant were then injected onto the column for HPLC.

HPLC with electrochemical detection (ECD)

A Neurochem HPLC neurochemical analyzer (ESA, Inc., Chelmsford, MA, U.S.A.) was used. Details of the operation of the analyzer and the mobile phase were reported previously by Takeda

* *Present address:* Dr. T. Shimizu, Zennba 380, Isehara, Kanagawa 259–11, JAPAN.
Reprint requests to Dr. T. Shimizu.
Fax: (81) 0463 94 0984.

0939–5075/98/0900–0927 $ 06.00

et al. (1991), Shimizu and Takeda (1991) and Shimizu *et al.* (1991). The analyzer with multiple electrochemical detector electrodes was capable of simultaneously sessing the amounts of several compounds in a single sample. The 16 serial electrodes were set in an incremental 60-mV array that ranged from 0 to 900 mV. Typically, each compound yielded an average ratio of peak heights between electrodes of 1:6:1. However, the exact ratio was specific for each compound and could be used to establish the purity of compounds in unidentified peaks that eluted from the column at the same time as known standards. Unidentified peaks in the chromatogram of the sample were matched with those of standards by reference to both retention times and the oxidation electrodes. Since nearly all compounds were spread over at least two electrodes, ratios could be calculated by reference to peaks of unknown compounds, giving a measurements of "ratio accuracy" (Matson *et al.*, 1984).

Chemicals

Chemicals used as standards were all of analytical reagent grade. All compounds were purchased from Sigma (St. Louis, MO, U.S.A.). The compounds were tryptophan (TRP), 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), 5-hydroxyindolacetic acid (5-HIAA), *N*-acetyl-5-hydroxytryptamine (*N*-ACET-5-HT), melatonin (MEL) and *N*-methyl-5-hydroxytryptophan (*N*-MET). Standard chromatograms of these compounds have been published elsewhere (Takeda *et al.*, 1991).

Results

Testis from non-diapausing pupae

Melatonin was detected in extracts of the testis from non-diapausing pupae together with the following precursors: TRP, 5-HTP, 5-HT, *N*-MET, 5-HIAA and *N*-ACET-5-HT (Table I). Levels of TRP per testis were very high and the accuracy for determination of peak purity was also high. Accuracies for peak purities of 5-HT and *N*-ACET-5-HT on day 1 and day 8 were low or not significant.

Testis from diapausing pupae

No MEL was detected in the testis of diapausing pupae on day 100, but it was detected in the testis of diapausing pharate adults (Table I and Fig. 1B). Levels of MEL in the testis of the pharate adult

Table I. Levels of indolalkylamines, melatonin, and its precursors in the testis of diapausing and non-diapausing pupae of the cabbage armyworm, *Mamestra brassicae*.

Compounds	pg/testis*		
	Non-diapausing pupae (day 1)	Non-diapausing pupae (day 8)	Diapausing pupae (day 100)
TRP	95,599 ± 37,711	250,982 ± 26,072	126,437 ± 2,920
5-HTP	2,330 ± 1,786	(36 ± 8)	not detected
5-HT	(3,457 ± 2,018)	(233 ± 272)	221 ± 17
N-MET	not detected	(121 ± 29)	(52 ± 0)
5-HIAA	56 ± 60	(80 ± 29)	(36 ± 0)
N-ACET-5-HT	(27 ± 9)	(50 ± 10)	(104 ± 4)
MEL	(1,190 ± 0)	677 ± 346	not detected

The data (means ± S.D.) correspond to more than 0.60 and the data in parentheses to below 0.59 in terms of ratio accuracies for peak purity (see Takeda *et al.*, 1991; Shimizu and Takeda, 1994). Analysis of samples was performed four times in each case.

* Presented in pg per a fused single testis (pair of testes).

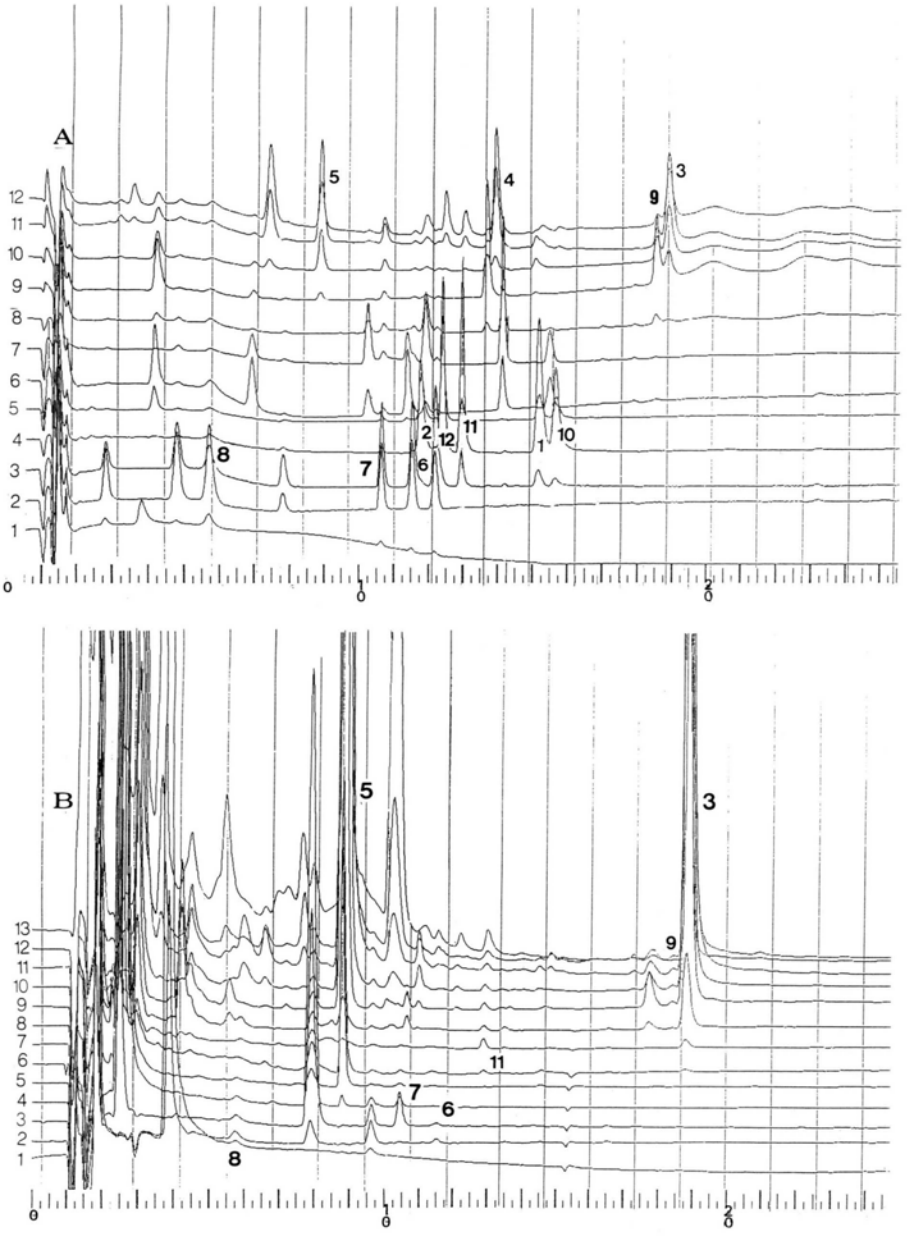

Fig. 1. HPLC-ECD chromatograms. A, peaks generated by standards; B, sample from the testis of post-diapause pharate adults (post-diapausing pupae). Abscissa, retention time (min), Ordinate, no. of channels.
Full-scale current, 2 μA (A), 5 μA (B).
1. 5-HT, 2. 5-HTP, 3. TRP, 4. TYRA (tyramine), 5. TYR-4 (4-tyrosine), 6. DA (dopamin), 7. DOPAC (3,4-dihydroxyphenylacetic acid), 8. DOPA (L-dopa), 9. MEL, 10. *N*-MET, 11. *N*-ACET-5-HT, 12. 5-HIAA.
See text for abbreviations.

were lower (ca. 100 pg/testis) than those in the testis of non-diapausing pupae.

The results for peak purities for the samples at the various stages indicated that the following metabolic compounds were present: TRP, 5-HTP, 5-HT, *N*-MET, 5-HIAA, *N*-ACET-5-HT and MEL.

Discussion

Metabolic pathways

The main metabolic pathways identified in the present study were as follows:

TRP → 5-HTP → 5-HT → *N*-MET
↳ 5-HIAA
↳ *N*-ACET-5-HT → MEL.

We previously obtained evidence for these metabolic pathways in the central nervous system and hemolymph of the silkworm, *Bombyx mori* using the same analytical system (Takeda *et al.,* 1991). This is the first report, to our knowledge, of the metabolic pathway to melatonin in the testis of an insect.

At all stages examined, levels of the amino acids TYR-4 (with the exception of the pupal stage) and TRP were high, and these amino acids are precursors to catecholamines and indolalkylamines, respectively (Table I). In the corpus cardiacum of the American cockroach, *Periplaneta americana,* levels of both amino acids are also high (Shimizu *et al.,* 1991).

Detected indolalkylamines

There are many reports of detection of 5-HT, but only one report, to our knowledge, of an analysis of 5-HT in the testis of an insect, namely the cockroach, *Periplaneta americana* (Sloley *et al.,* 1986). The limit for detection of 5-HT was less than 25 pg/μg protein, and no 5-HT was detected in the testis of this cockroach (Sloley *et al.,* 1986). *N*-MET was not detected in the brain of *Manduca sexta* (Geng *et al.,* 1993) when the same 16-sensor electrochemical detection HPLC system was used as the one that we used in the present study.

5-HIAA was also not detected in the brain of *Manduca sexta* (Geng *et al.,* 1993), but was detected in the terminal ganglion of the same insect (Sparks and Geng, 1992). In the testis of the armyworm, the amount of 5-HIAA was fairly low (Table I).

N-ACET-5-HT was detected in the brain of *Manduca sexta* (Sparks and Geng, 1992; Geng *et al.,* 1993), in the central nervous system of the male cockroach, *Blaberus craniifer* (Barreteau *et al.,* 1993) and in the subesophageal ganglion of the silkworm, *Bombyx mori* (Takeda *et al.,* 1991).

MEL has been found in a number of insects, as described previously, but has not previously been found in the reproductive system.

Possible functions of MEL

Melatonin was not detected in the testis of diapausing pupae (day 100), but it was detected in the testis of post-diapausing pharate adults and of non-diapausing pupae. Peristaltic movements of the upper vas deferens in *Mamestra brassicae* are initiated at the eye-pigmented pupal stage in both diapausing and non-diapausing pupae (Shimizu *et al.,* 1986) and the diameter of the upper vas deferens remains about 350 μm (Shimizu, 1989). Accordingly, if MEL is involved in peristaltic movements of the upper vas deferens, production of MEL might provide a physiological signal for initiation of such movement before the adult stage. There may be other reasons also for activation of the production of MEL after diapause.

Movements of sperm ducts of the gypsy moth, *Lymantria dispar* (Giebultowicz *et al.,* 1988) show a circadian rhythm and are evident during the scotophase, while production of MEL tends to be higher during the scotophase than during the photophase in some insects (Wetterberg *et al.,* 1987; Tilden *et al.,* 1994; Itoh *et al.,* 1995). It may be of interest to investigate whether MEL is directly involved in the peristaltic movements of the sperm duct.

Barreteau H., Goudey-Perriere F., Perriere C., Jacquot C., Gayral P., Grosclaude J. M. and Brousse-Gaury P. (1993), Influence of fasting and isolation on biogenic amine levels in the nervous system of the male cockroach, *Blaberus craniifer* Burm (Dictyoptera: Blaberidae). Comp. Biochem. Physiol. **105C**, 11–16.

Finocchiaro L., Callebert J., Launay J. M. and Jallon J. M. (1988), Melatonin biosynthesis in *Drosophila*: its nature and its effects. J. Neurochem. **50**, 382–387.

Gao N. and Hardie J. (1997), Melatonin and the pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol. **43**, 615–620.

Geng C., Sparks T. C., Skomp J. R. and Gajewski R. P. (1993), Biogenic amines in the brain of *Manduca sexta* during larval-pupal metamorphosis. Comp. Biochem. Physiol. **106C**, 275–284.

Giebultowicz J. M., Bell R. A. and Imberski R. B. (1988), Circadian rhythm of sperm movement in the male reproductive tract of the gypsy moth, *Lymantria dispar*. J. Insect Physiol. **34**, 527–532.

Itoh M. T., Hattori A., Nomura T., Sumi Y. and Suzuki T. (1995), Melatonin and arylalkylamine *N*-acetyltransferase activity in the silkworm, *Bombyx mori*. Mol. Cell. Endocrinol. **115**, 59–64.

Matson W. R., Langlais P., Volicer L., Gamache P. H., Bird E. D. and Mark K. A. (1984), *n*-Electrode three-dimensional liquid chromatography with electrochemical detection for determination of neurotransmitters. Clin. Chem. **30**, 1477–1488.

Shimizu T. (1989), Development of spermiduct and seminal vesicle during pharate adult of the cabbage armyworm, *Mamestra brassicae*, (Lepidoptera; Noctuidae). Invert. Reprod. Dev. **15**, 221–224.

Shimizu T. and Takeda N. (1991), Biogenic amine levels in the hemolymph of the cabbage armyworm larvae (*Mamestra brassicae*) following injection of octopaminergic insecticides. Z. Naturforsch. **46C**, 127–132.

Shimizu T. and Takeda N. (1994), Aromatic amino acid and amine levels in the hemolymph of parasitized larvae of *Mythimna separata*. Z. Naturforsch. **49C**, 693–695.

Shimizu I., Shimizu T. and Fukami J. (1986), Peristaltic movement and its suppression by chlordimeform, iproniazid and 2-amino-4-phosphonobutyric acid of the spermiduct in the cabbage armyworm, *Mamestra brassicae*. App. Ent. Zool. **21**, 186–189.

Shimizu T., Mihara M. and Takeda N. (1991), HPLC analysis of biogenic amines in the corpus cardiacum of the American cockroach, *Periplaneta americana*. J. Chromat. **539**, 193–197.

Sloley B. D., Downer R. H. G. and Gillott C. (1986), Levels of tryptophan, 5-hydroxytryptophan, and dopamine in some tissues of the cockroach, *Periplaneta americana*. Can. J. Zool. **64**, 2669–2673.

Sparks T. C. and Geng C. (1992), Analysis of the biogenic amines in the central nervous system of the tobacco hornworm by high-performance liquid chromatography with 16-sensor electrochemical detection. Anal. Biochem. **205**, 319–325.

Takeda N., Takaoka H., Shimizu T., Yazawa M. and Yagi S. (1991), Biogenic amine levels in the central nervous system and haemolymph of the silkworm, *Bombyx mori*. Comp. Biochem. Physiol. **100C**, 677–682.

Tilden A. R., Anderson W. J. and Hutchison V. H. (1994), Melatonin in two species of damselfly *Ischnura verticalis* and *Enallagma civile*. J. Insect Physiol. **40**, 775–780.

Vivien-Roels B. and Pevet P. (1993), Melatonin: presence and formation in invertebrates. Experientia **49**, 642–647.

Vivien-Roels B., Pevet P., Beck O. and Fevre-Montange M. (1984), Identification of melatonin in the compound eyes of an insect, the locust (*Locusta migratoria*), by radioimmunoassay and gas chromatography-mass spectrometry. Neurosci. Let. **49**, 153–158.

Wetterberg L., Hayes D. K. and Halberg F. (1987), Circadian rhythms of melatonin in the brain of the face fly, *Musca autumnalis*. De Geer. Chronobiologia **14**, 377–381.