Levels of Biogenic Amines in the Brain during Pupal and Adult Development of the Silkworm, *Bombyx mori*

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Levels of a wide range of biogenic amines and related metabolites were determined in the brain of the silkworm, *Bomby mori,* during pupal and adult development using a three-dimensional HPLC system with multiple coulometric electrochemical detection.

In the brain of the female adults, metabolic pathways such as tyrosine (TYR-4)->dihydroxyphenylalanine (*L*-DOPA)-dopamine (DA), TYR-4->tyramine (TYRA), and tryptophan (TRP)->5-hydroxytryptamine (5-HT) were identified. At this sstage, 3,4-dihydroxyphenylethylene (DOPAC) was also detected.
Metabolic pathways of biogenic amines in the brain from pupal to adult stages are discussed.

Introduction

In efforts to obtain quantitative information about biogenic amines in tissues, high-performance liquid chromatography (HPLC) has proved to be a useful technique and has been applied widely (cf. Klemm, 1985). HPLC, in particular, HPLC with electrochemical detection (ECD), has allowed accurate detection of various biogenic amines and has been used in analyses of some insects (cf. Hopkins *et. al.,* 1984; Sloley and Orikasa, 1988; Czapla *et al.,* 1990; Puiroux *et al.,* 1990; Krueger *et al.,* 1990). Further improvements in ECD led to development of a dual coulometric detection system consisting of 16 electrodes in series been developed (Matson *et al.,* 1984). Such a system has been used to analyze invertebrate nervous systems and haemolymph (Shimizu and Takeda, 1991; Shimizu *et al.,* 1991; Takeda, 1991; Takeda and Svendsen, 1991; Sparks and Geng, 1992; Geng *et al.,* 1993). For example, we previously quantified a wide range of biogenic amines and related metabolites in the brain and suboesophageal ganglion of *Bombyx mori* at the larval stage using such an HPLC system (Takeda *et al.,* 1991). In this study we analyzed the biogenic amines and related metabolites of the brain of the silkworm during its pupal and adult development.

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Materials and Methods

Insects

Larvae of *Bombyx mori,* C 146×J 137, were reared on artificial diet (Yakuruto Co., Ltd., Tokyo, Japan) at 20 °C with a photoperiod of 16L:8D. We utilized animals at three stages, namely female pupae (day 6), pharate male and female adults in cocoons and female adults (after oviposition) for our experiments.

Preparation of sample

The brains were dissected from animals, and the suboesophageal ganglion and optic lobes were removed from the brain. Each set of pooled brains (N=5) from three stages in a 0.4 N solution of perchloracetic acid (PCA) that contained 100 mg/100 ml EDTA and 50 mg/100 ml sodium metabisulphite was homogenized with a Physcotron (Nich-On, Tokyo, Japan). Homogenates of these samples were centrifuged at 10,000 × g for 10 min at 0 °C. Aliquots of 80 µl of supernatant, after filtration, were injected onto the column for analysis.

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 *et al.* (1991), Shimizu and Takeda (1991) and Shimizu *et al.* (1991). The analyzer with multiple electrochemical detector electrodes was capable of assessing the amounts of several compounds at once in a single sample. The 16 serial electrodes were

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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 found 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 measurement of "ratio accuracy" (Matson *et al.*, 1984).

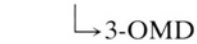
Chemicals

Chemicals for use as standards were all of analytical reagent grade. All compounds were purchased from Sigma (St Louis, MO, U.S.A.). The compounds were tyrosine-4 (TYR-4), *L*-dihydroxyphenylalanine (*L*-DOPA), dopamine (DA), 3-methoxy-tyramine (3-MT), norepinephrine (NE), epinephrine (E), metanephrine (MN), normetanephrine (NMN), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), 4-hydoxy-3-methoxy-mandelic acid (VMA), vanillic acid (VA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), tyramine (TYRA), methyldopa (3-OMD), octopamine (OCT) and epinine in the catecholamine system; and tryptophan (TRP), 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), 5-hydroxyindolacetic acid (5-HIAA), melatonin (MEL) and *N*-methyl-5-hydroxytryptophan (*N*-MET) in the indolalkylamine system. Standard chromatograms of these compounds have already been published (Takeda *et al.*, 1991).

Results

Brains during pupal and adult stages

The biogenic amines and their precursors detected in extracts of the brain from pupal to adult stages were as follows: 3-OMD, 5-HT, 5-HTP, DA, DOPAC, *L*-DOPA, MN,OCT, TRP, TYRA and TYR-4 (Table I). Three metabolic pathways were deduced to be present: 1) TYR-4 ->*L*-DOPA ->DA->DOPAC;



2) TRP->5-HTP->5-HT; and 3) TYR-4->TYRA->OCT. Through the three developmental stages, levels of TRP and *L*-DOPA per brain decreased gradually.

Pupal stage

We detected 3-OMD, DA, DOPAC, *L*-DOPA and TRP in extracts from the brains of female pupae. Among these compounds, levels of *L*-DOPA were particularly high. It appeared that

Table I. Levels of biogenic amines and their precursors in the brain of *Bombyx mori* at pupal and adult stages.

Compound	Pupa female (pg/brain)	(Ratio accuracy)	Pharate adult male+female (pg/brain)	(Ratio accuracy)	Adult female (pg/brain)	(Ratio accuracy)
3-OMD	699.6±5.8	(0.67, ERR)	–**		109.1±8.2	(0.83, ERR)
5-HT	–**		–**		312.2±2.8	(0.63,0.96)
5-HTP	–**		–**		–**	
DA	1.2±0.0*	(0.00,0.71)	–**		440.8±3.5	(0.00,0.78)
DOPAC	3.6±0.7*	(0.61,0.62)	53.3±27.9	(0.64,0.00)	337.5±4.4	(0.00,0.99)
L-DOPA	18.9±1.1*	(0.72,0.93)	2.3±1.1*	(0.82,0.99)	500.2±11.0	(0.00,0.88)
MN	–**		11.5±0.0	(0.00,0.73)	–**	
OCT	–**		not detected		290.0±6.6	(0.88,0.87)
TRP	120.1±3.0*	(0.88,0.60)	14.0±1.6*	(0.83,0.93)	8.0±0.0*	(0.96,0.96)
TYRA	–**		33.9±0.0*	(0.66,0.00)	78.3±0.9	(0.65,0.36)
TYR-4	–**		141.3±17.6*	(0.83,0.75)	61.5±0.2*	(0.95,0.98)
	(*N*=4)		(*N*=8)		(*N*=2)	

* ng/brain, ** both ratio accuracies for peak purity were less than 0.60, nonsignificant, see text. Mean ± SD. Analysis of the sample is replicated and shown in parentheses. ERR: Error.

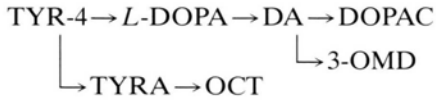
the metabolic pathway of *L*-DOPA–>DA–>DOPAC was present.

Adult stage

We detected TYRA and OCT in the brains of adult females after oviposition. These metabolic pathways appeared to be present: TYR-4–> *L*-DOPA–>DA–>DOPAC; TRP–>5-HTP–>5-HT; and TYR-4–>TYRA–>OCT. There were no clear differences in levels between male and female in pharate adults in cocoons.

Discussion

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



We previously reported evidence for these metabolic pathways in the central nervous system and haemolymph of the silkworm, *Bombyx mori* (Takeda *et al.*, 1991). The presence of 3-OMD in the insect brain had not been reported until we detected it in many insects, including the silkworm *Bombys mori* using our analytical system.

At all three stages examined, levels of the amino acids TYR-4 (without the pupal stage) and TRP remained high, and these amino acids are precursors to catecholamine and indolalkylamine, respectively. In the corpus cardiacum of the American cockroach, *Periplaneta americana*, levels of both amino acids are also high (Shimizu *et al.*, 1991). Levels of TYR-4, a precursor for catecholamines such as a OCT, might be changed by chemical (Shimizu and Takeda, 1991), physical and biological stresses (Shimizu and Takeda, 1994). By changing levels of catecholamines or their precursors, insect might be physiologically able to resist some stresses.

OCT, detected in extracts of the female adult brain, has been called the "fight or flight" hormone, and the concentration of OCT is known to respond to stress in the field cricket (Adamo *et al.*, 1995). OCT seems to be an important neurohormone not only in mating behavior (Linn and Roelofs, 1986), with wing vibration associated with the release of pheromones, but also in responses to pheromone (Linn and Roelofs, 1986) and the production of pheromones (Christensen *et al.*, 1991) and the juvenile hormone biosynthesis in the corpora allata (Kaatz *et al.*, 1994). In male moths, *Trichoplusia ni*, OCT also has modulatory effects on the sensitivity and periodicity of responses to the sex pheromone (Linn and Roelofs, 1986). OCT detected in the brains of female adults in the present study gave a high ratio accuracy (about 0.87), which is a measure of peak purity, and the amount of OCT was 290 pg per brain. This high level of OCT content might modulate behavior including mating behavior at the adult stage.

DA was previously detected in the larval brain (Geng *et al.*, 1993) and corpora allata (Granger *et al.*, 1996) of *Manduca sexta* with the same 16-sensor electrochemical detection HPLC system as the one that we used, and the amount in the brain is fairly low on day 0 but rises to a maximum on day 6 of the last instar. Similar increasing levels were noted in *Bombyx mori* in the previous report (Takeda *et al.*, 1991). In the brain of *Bombyx mori* from larval to adult stages, the maximum level was found at the post-spinning stage (27.6 ng/brain) (Takeda *et al.*, 1991). This peak in levels of DA might be involved in spinning behavior at the pharate pupal stage.

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Adamo S. A., Linn C. E. and Hoy R. R. (1995), The role of neurohormonal octopamine during "fight or flight" behavior in the field cricket *Gryllus bimaculatus*. J. Exp. Biol. **198,** 1691–1700.

Christensen T. A., Itagaki H., Teal P. E. A., Jasensky R. D., Tumlinson J. H. and Hildebrand J. E. (1991), Innervation and neural regulation of the sex pheromone gland in female *Heliothis* moths. Proc. Nat. Acad. Sci. USA **88,** 4971–4975.

Czapla T. H., Hopkins T. L. and Kramer K. J. (1990), Catecholamine and related *o*-diphenols in cockroach hemolymph and cuticle during sclerotization and melanization: comparative studies on the order Dictyoptera. J. Comp. Physiol. **160B,** 175–181.

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

Granger N. A., Sturgis S. L., Ebersohl R., Geng C. and Sparks T. C. (1986), Dopaminergic control of corpora allata activity in the larval tobacco hornworm, *Manduca sexta*. Arch. Insect Biochem. Physiol. **32,** 449–466.

Hopkins T. L., Morgan T. D. and Kramer K. J. (1984), Catecholamines in hemolymph and cuticle during larval, pupal and adult development of *Manduca sexta* (L.). Insect Biochem. **14,** 533–540.

Kaatz H., Eichmuller S. and Kreissle S. (1994), Stimulatory effect of dopamine on juvenile hormone biosynthesis in honey bees (*Apis mellifera*): Physiological and immunocytochemical evidence. J. Insect Physiol. **40,** 865–872.

Klemm N. (1985), The distribution of biogenic monoamines in vertebrates. In: Neurobiology (Giles R. and Balthazart J. ed.) Springer, Berlin, pp. 280–296.

Krueger R. R., Kramer K. J., Hopkins T. L. and Speirs R. D. (1990), *N*-alanyldopamine and *N*-acetyldopamine occurrence and synthesis in the central nervous system of *Manduca sexta* (L.). Insect Biochem. **20,** 605–610.

Linn C. and Roelofs W. L. (1986), Modulatory effects of octopamine and serotonin on male sensitivity and periodicity of response to the sex pheromone in the cabbage looper moth, *Trichoplusia ni*. Arch. Insect Biochem. Physiol. **3,** 161–171.

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.

Puiroux J., Moreau R. and Gourdoux L. (1990), Variations of biogenic amine levels in the brain of *Pieris brassicae* pupae during non-diapausing and diapausing develoopment. Arch. Insect Biochem. Physiol. **14,** 57–69.

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 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. and Orikasa S. (1988), Selective depletion of dopamine, octopamine, and 5-hydroxytryptamine in the nervous tissue of the cockroach (*Periplaneta americana*). J. Neurochem. **51,** 535–541.

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. (1991), Dynamics of monoamine metabolism in the central nervous system of some terrestrial isopods. In: Biology of Terrestrial Isopods (Chaigneau J., Mocquard J. P. and Juchault P. ed.) University of Poitiers Press, Poitiers, France, pp. 195–200.

Takeda N. and Svendsen C. N. (1991), Monoamine concentrations in *Hydra magnipapillata*. In: Coolenterate Biology: Recent Research on Cnidaria and Ctenophora (Williams R. B., Cornelius P. F. S., Hughes R. G. ad Robson E. A. ed. Kluwer Academic Press, Dordrecht, Boston, London, pp. 549–554.

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.