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, Bombyx 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 stage, 3,4-dihydroxyphenylethylamine (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; Crapla 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 (Maison 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.

Materials and Methods

Insects

Larvae of Bombyx mori, C 146 x 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 x 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 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-hydroxy-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>
<thead>
<tr>
<th>Compound</th>
<th>Pupa female (pg/brain)</th>
<th>(Ratio accuracy)</th>
<th>Pherase adult male + female (pg/brain)</th>
<th>(Ratio accuracy)</th>
<th>Adult female (pg/brain)</th>
<th>(Ratio accuracy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-OMD</td>
<td>699.6±5.8*</td>
<td>(0.67, ERR)</td>
<td>109.1±8.2*</td>
<td>(0.83, ERR)</td>
<td>312.2±2.8*</td>
<td>(0.63, 0.96)</td>
</tr>
<tr>
<td>5-HT</td>
<td>_**</td>
<td>_**</td>
<td>337.5±4.4*</td>
<td>(0.00, 0.99)</td>
<td>500.2±11.0*</td>
<td>(0.00, 0.88)</td>
</tr>
<tr>
<td>5-HTP</td>
<td>_**</td>
<td>_**</td>
<td>290.0±0.6*</td>
<td>(0.88, 0.87)</td>
<td>78.3±0.9*</td>
<td>(0.65, 0.36)</td>
</tr>
<tr>
<td>DA</td>
<td>1.2±0.0*</td>
<td>(0.00, 0.71)</td>
<td>14.0±1.6*</td>
<td>(0.96, 0.96)</td>
<td>8.0±0.0*</td>
<td>(0.96, 0.96)</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3.6±0.7*</td>
<td>(0.61, 0.62)</td>
<td>14.0±1.6*</td>
<td>(0.66, 0.00)</td>
<td>78.3±0.9*</td>
<td>(0.65, 0.36)</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>18.9±1.1*</td>
<td>(0.72, 0.93)</td>
<td>14.0±1.6*</td>
<td>(0.96, 0.96)</td>
<td>61.5±0.2*</td>
<td>(0.95, 0.98)</td>
</tr>
<tr>
<td>MN</td>
<td>_**</td>
<td>_**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCT</td>
<td>_**</td>
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<tr>
<td>TRP</td>
<td>120.1±3.0*</td>
<td>(0.88, 0.60)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TYRA</td>
<td>_**</td>
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<td></td>
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<tr>
<td>TYR-4</td>
<td>_**</td>
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</tbody>
</table>

* 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 \(L-DOPA \rightarrow DA \rightarrow DOPAC\) was present.

**Adult stage**

We detected TYRA and OCT in the brains of adult females after oviposition. These metabolic pathways appeared to be present: \(TYR-4 \rightarrow L-DOPA \rightarrow DA \rightarrow DOPAC\); \(TRP \rightarrow 5-HTP \rightarrow 5-HT\); and \(TYR-4 \rightarrow TYRA \rightarrow OCT\). 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:

\[
TYR-4 \rightarrow L-DOPA \rightarrow DA \rightarrow DOPAC \quad \text{\textasciitilde}{3-OMD} \quad TYRA \rightarrow OCT
\]

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 *Bombyx 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.

**Acknowledgement**

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