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The Levels of Biogenic Amines in the Corpora Allata, Corpora Cardiaca and Frontal Ganglion in the Cricket, *Gryllus bimaculatus*

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ABSTRACT—Secretory activity of the corpora allata (CA) and corpora cardiaca (CC) in the pars-intercerebralis complex has been suggested to be controlled by biogenic amines, despite a lack of quantitative information. The situation is similar to the frontal ganglion (FG), a member of the same complex. Hence, the levels of biogenic amines and related substances in these organs were measured in cricket, *Gryllus bimaculatus*, by high performance liquid chromatography with electrochemical detection. All organs examined contained considerable amounts of major biogenic amines, such as octopamine (OA), dopamine, and 5-hydroxytryptamine (5-HT). They also included epinephrine. The amounts of OA and 5-HT were higher than those of other biogenic amines whose presence was detected in the examined organs. The distributional patterns of biogenic amines were different among the CA, CC and FG, *e.g.,* the highest content of OA in the CC. The presence of precursors and metabolites indicates that biogenic amines are synthesized and metabolized in neuronal elements in the examined organs. It is probable that they are functional and also involved in the neural control of hormone secretion in CA and CC, because their turnover rates estimated from the present analysis are comparable to those in the nervous system.

INTRODUCTION

The corpora allata, corpora cardiaca and frontal ganglion are important components of the pars-intercerebralis complex. Biogenic amines in this complex are considered to be involved in the control of various physiological phenomena in insects, either directly or possibly through controls of endocrine organs such as the corpora allata (CA) and the corpora cardiaca (CC). Fluorescence histochemistry demonstrated the presence of dopamine (DA) in the CA of the locust, *Schistocerca gregaria* (Klemm, 1971; Lafon-Cazal and Arluison, 1976), and 5-hydroxytryptamine (5-HT) in the CC of the cockroach *Periplaneta americana* (Colhoun, 1963; Migliori-Natalizi *et al.,* 1970).

The CA have been investigated as the organs which secrete juvenile hormone (JH) (Wigglesworth, 1964). Radioenzymatic assays demonstrated the presence of octopamine (OA) in the CA of the locusts *S. gregaria* (Evans, 1978) and *Locusta migratoria* (David and Lafon-Cazal, 1979), and the cockroach *P. americana* (Evans, 1978). These studies suggested that OA may be involved in the control of JH biosynthesis. This idea was supported by the study that OA enhanced the release of JH from the CA of *L. migratoria* (Lafon-Cazal and Baehr, 1988).

The CC secretes a neurosecretory factor, adipokinetic hormone (AKH), which stimulates a release of diglycerides from the fat body a few minutes after the onset of flight in *S. gregaria* (Goldsworthy *et al.*,1972). The level of OA in the locust hemolymph was elevated during the first minutes of flight in *S. gregaria* (Goosey and Candy, 1980). Based on the studies on secretion of AKH by OA, Orchard and Loughton (1981a, b) suggested that the neurotransmitter involved in the synapses between axons of the nervi corporis cardiaci (NCC) II and the cells releasing hyperlipemic hormone is aminergic, possibly octopaminergic. This possibility was supported by the presence of 5-HT, OA and DA in the glandular lobe, and 5-HT and OA in the NCC II of the locust *L. migratoria* (Orchard *et al.*, 1986).

The frontal ganglion (FG), which is situated in front of the brain, is innervated by three nerves arising from the brain. In the cockroach, frontal ganglionectomy abolishes

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circadian rhythms of the levels of 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA) in the CA and CC (Jagota and Habibulla, 1992). In the male cricket *Acheta domesticus,* the FG is reported to be concerned with the male sexual behavior (Beck, 1974). Klemm *et al.* (1986) found that the FG in the crickets *Gryllus bimaculatus* and *A. domesticus* contains many 5-HT immunoreactive neurons, although there is few quantitative information, which is also not sufficient in the CA and CC.

Our understanding of the function of the amines in endocrine organs would be enhanced by quantitative information, in particular by information about the occurrence of possible precursors and metabolites. It is similarly crucial to determine precursors and metabolites of biogenic amines, because biologically important amines are usually actively synthesized and metabolized where they are released. To clarify these problems, we applied the method of Nagao and Tanimura (1988) to simultaneously determine small amounts of biogenic amines, their precursors and metabolites in the cricket CA, CC and FG by use of high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The CA is an organ which is related to development and it is important to compare developmental stages, sex or physiological changes. As a first stage, we determined amounts of biogenic amines of the adult male cricket and will discuss whether biogenic amines are synthesized or metabolized in the CA, CC and FG.

MATERIALS AND METHODS

Animals

Crickets, *Gryllus bimaculatus* DeGeer, were reared at 27–28°C with relative humidity of 45–50% under a 14:10 light-dark cycle. They were fed on artificial insect diet (Oriental Yeast) and carrot slices. Adult male crickets were taken from the colony after the imaginal moult and maintained in another container for ten days. They were anesthetized with ice and dissected in a cold saline solution consisting of 140 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$ and 44 mM glucose, buffered with 2 mM N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid (Dotite) and NaOH to pH 7.3.

Since the CA, CC and FG are tiny organs, five glands were pooled in a microglass homogenizer cooled with ice, and were stored at –70°C before use. They were homogenized in 40 μl of 0.1 M perchloric acid containing 50 ng/ml 3,4-dihydroxybenzylamine (DHBA) as the internal standard. The homogenate was transferred to a 1.5 ml polypropylene tube (Eppendorf), and was shaken for 30 min at 4°C. Following centrifugation at 15,000 *g* for 30 min at 0°C, 5 μl of the supernatant was injected onto the HPLC column.

The HPLC systems

The HPLC system was composed of a pump, an injection valve, and C_{18} reversed-phase column (Shiseido type 250 mm × 4.6 mm i.d.) heated at 30°C in a column oven. A glassy carbon electrode (EICOM, WE-GC) was used for an electrochemical detection. The detector potential was set at 0.88 V versus an Ag/AgCl reference electrode. They were also maintained at 30°C in a column oven. The mobile phase contained 0.18 M monochloroacetic acid, 40 μM ethylenediamine tetra-acetic acid disodium salt, 1.66 mM sodium-1-octanesulfonic acid as the ionpair reagent, and 8.0 % (v/v) CH_3CN as the organic modifier, and was adjusted to pH 3.6 with NaOH. The mobile phase was filtered with a 0.22 μm filter and degassed. The flow rate was kept at 0.7 ml/min.

Chromatograms were analyzed by a Maxima 820 (Waters) chromatography workstation and quantified by comparing the peak height in the sample with the peak height in the standard solution of known content.

RESULTS

Using the HPLC system, we detected the presence of three major biogenic amines of insects, OA, DA and 5-HT, and epinephrine (E) in CA, CC and FG. Their precursors and metabolites were also detected in the same run of HPLC. Particular peaks were identified by comparing both the retention times and hydrodynamic voltammograms with those of the standards. Figure 1A shows a typical chromatogram of a standard mixture of 2 ng each of biogenic amine and related substance. Typical chromatograms of the examined organs are shown in Fig. 1: the corpora allata (Fig. 1B), corpora cardiaca (Fig. 1C) and frontal ganglion (Fig. 1D).

The contents of biogenic amines and related substances in the examined organs are shown in Table 1. All organs included considerable amounts of OA, DA, 5-HT and E; their precursors, tyramine (TA), tryptophan and 5-hydroxytryptophan (5-HTP) ; and metabolites, N-acetyltyramine (Nac-TA), 3,4-dihydroxyphenylacetic acid (DOPAC) and N-acetyldopamine (Nac-DA). N-acetyl-5-hydroxytryptamine (Nac-5-HT) was detected in the CC and FG, but not in the CA. Synephrine (OA metabolite), 5-HIAA (5-HT metabolite), and metanephrine (E metabolite) were not detected in any organs.

Among the precursors found in the examined organs, the level of tryptophan, 5-HT precursor, was highest. In the CA, the amount of 5-HT was largest followed by OA, while OA in the CC was most abundant compared to other biogenic amines detected in the pars-intercerebralis complex. The levels of DA in the FG was higher than those in the other organs. In all organs, N-acetylated metabolites, Nac-TA and Nac-DA, were detected in large quantities. N-acetyl-octopamine were not detected by the present procedure. The level of Nac-DA was higher than that of DA in all organs, the ratio of Nac-DA/DA was 6 : 1 in the CA, 5 : 1 in the CC, and 3 : 1 in the FG.

The relative abundances of E, OA, DA and 5-HT were calculated to examine tissue specific distribution of biogenic amines (Fig. 2A). The three organs showed different distributional patterns of biogenic amines. In the FG, four biogenic amines were contained in rather equal amounts. To determine the relationships of the biogenic amines with their precursors and metabolites, the relative abundances of OA, TA and Nac-TA, DA, DOPAC and Nac-DA, and 5-HT, 5-HTP and Nac-5-HT in all organs were also calculated (Fig. 2B, C and D). The amount of OA was higher than that of TA and Nac-TA in the CA and CC, but in the FG, the level of Nac-TA exceeded that of OA. The ratio of TA was apparently low compared to OA and Nac-TA in all organs examined. The

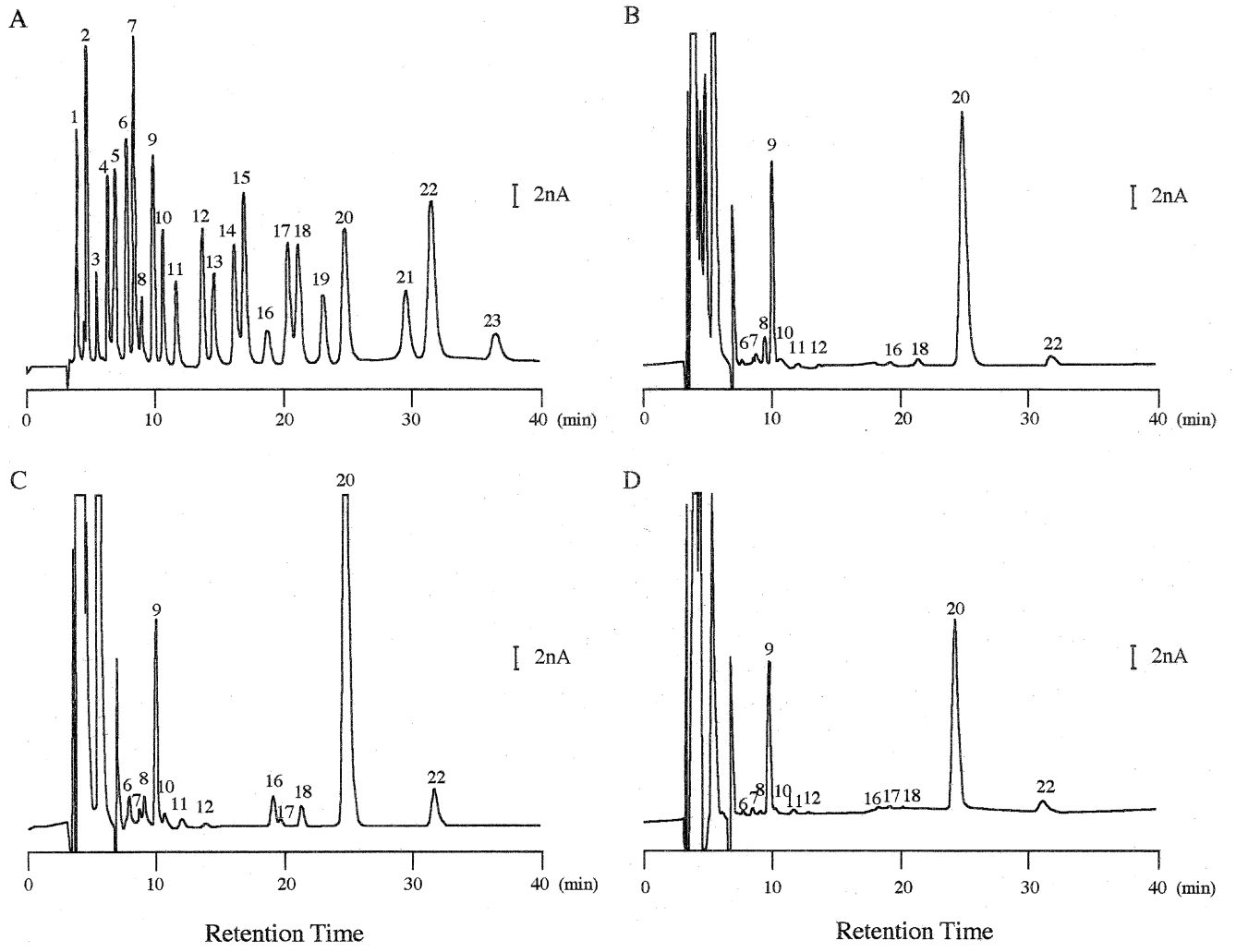

Fig. 1. A. Typical chromatogram of a standard mixture of 2 ng of each biogenic amine and related substance. Peak identification: 1, 3,4-dihydroxymandelic acid; 2, 3-methoxy-4-hydroxymandelic acid, 3,4-dihydroxyphenylalanine; 3, tyrosine; 4, 3-methoxy-4-hydroxyphenylglycol; 5, norepinephrine; 6, 5-hydroxytryptophan; 7, E ; 8, OA ; 9, DHBA; 10, DOPAC; 11, Nac-DA; 12, DA; 13, metanephrine; 14, epinine; 15, 5-HIAA; 16, Nac-TA; 17, Nac-5-HT; 18, TA; 19, 3-methoxy-4-hydroxyphenylacetic acid; 20, tryptophan; 21, 3-methoxytyramine; 22, 5-HT; 23, 6-hydroxymelatonin. B-D. Chromatograms of biogenic amines and related substances of the organs. B, corpora allata; C, corpora cardiaca; D, frontal ganglion. Peak identification is as A.

Table 1. Amounts of biogenic amines and related substances in the cricket corpora allata, corpora cardiaca and frontal ganglion estimated by HPLC-ECD. The amounts are expressed in pmol/organ.

	Corpora allata	Corpora cardiaca	Frontal ganglion
Epinephrine	0.11 ± 0.01	0.10 ± 0.02	0.27 ± 0.05
Octopamine	0.29 ± 0.06	2.61 ± 0.31	0.25 ± 0.07
Dopamine	0.02 ± 0.01	0.05 ± 0.03	0.15 ± 0.02
5-HT	0.77 ± 0.06	0.99 ± 0.13	0.41 ± 0.07
Tyramine	0.02 ± 0.02	0.12 ± 0.07	0.06 ± 0.01
5-HTP	0.13 ± 0.07	0.31 ± 0.15	0.09 ± 0.02
Tryptophan	5.93 ± 1.81	10.30 ± 0.44	4.76 ± 0.60
DOPAC	0.15 ± 0.03	0.12 ± 0.01	0.07 ± 0.01
N-acetyldopamine	0.12 ± 0.01	0.26 ± 0.03	0.49 ± 0.08
N-acetyltyramine	0.26 ± 0.11	2.13 ± 0.36	0.46 ± 0.07
N-acetyl-5-HT	nd	0.23 ± 0.11	0.44 ± 0.01

Mean ± S. E. (n=5). nd, not detectable.

amount of DA was much less than that of metabolites in all organs. The level of 5-HT was eight times higher than that of 5-HTP in the CA. The CC and FG contained Nac-5-HT in a small amount, so that the relative abundance of 5-HT, as the ratio of a biogenic amine to its metabolite, was much greater than that of other biogenic amines.

DISCUSSION

Fulfillment of several criteria is required to conclude that biogenic amines are physiologically active chemicals in certain tissues. Evidences of synthesis and degradation, in addition to their presence, are considered to be such requisites. The present study showed that the CA, CC, and FG of the cricket *G. bimaculatus* contained major insect biogenic amines: OA, 5-HT and DA; their precursors TA, tryptophan

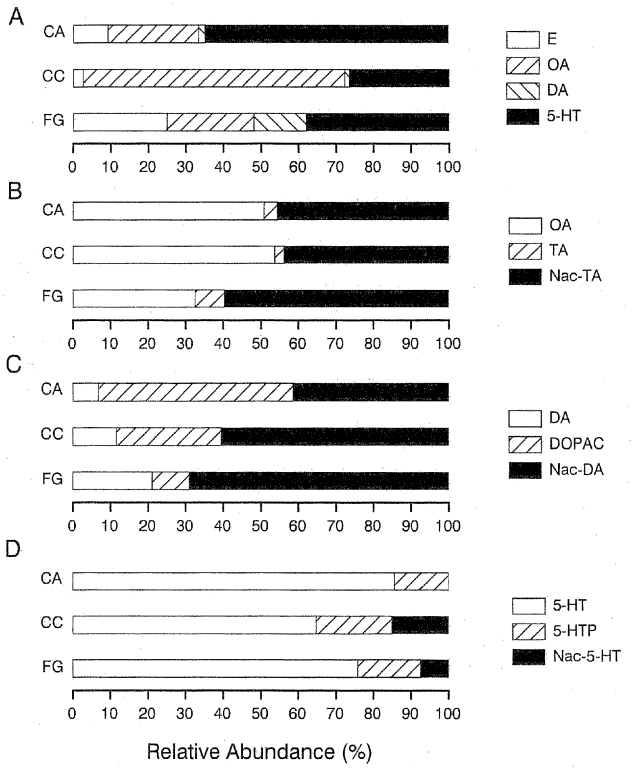

Fig. 2. Relative abundances of biogenic amines and related substances in the CA, CC and FG of the cricket. A, Relative abundances of E, OA, DA and 5-HT; B, Relative abundances of OA, TA and Nac-TA; C, Relative abundances of DA, DOPAC and Nac-DA; D, Relative abundances of 5-HT, 5-HTP and Nac-5-HT.

and 5-HTP; and metabolites Nac-TA, Nac-DA and DOPAC. In all organs, considerable amounts of OA and 5-HT were detected. The levels of OA and Nac-TA in the CC were one order higher than those in the other organs. All the organs also contained E. The presence of E in the insect tissues has been controversial, since most of the previous studies could not detect E in the insect nervous tissues. Nonetheless, a small amount of E was detected in the subesophageal, mesothoracic and terminal ganglia of the same cricket species in this study (Nagao and Tanimura, 1988) and also in the ant *Formica rufa* (Kostowski *et al.*, 1975).

As is briefly described in the "Introduction", the previous studies on distribution of biogenic amines in the CA and CC showed that they are localized in innervating axons of the nervi corporis allati (NCA) and the NCC, which directly connect the CC to the brain. Recent immunohistochemical data further demonstrated that the NCA and the NCC include 5-HT-immunoreactive (ir) neurons, and 5-HT-ir axons innervate the surface area of the CC in the cricket *Teleogryllus commodus* and the cockroach *P. americana* (Pipa and Moore, 1988). In the CC of *L. migratoria,* 5-HT-containing axons associated with NCC II are confined to the anterior region of the glandular lobe (Orchard *et al.*, 1986). The OA content in the CA of *G. bimaculatus* measured in the present study by HPLC-ECD is very close to the values reported for the locusts *S. gregaria* (Evans, 1978) and *L. migratoria* (David and Lafon-Cazal, 1979) determined by radioenzymatic techniques, and the amount in the cockroach *Diploptera punctata* (Thompson *et al.*, 1990) detected by HPLC-ECD, although the OA content in the cricket CC is somewhat higher than those in locust and cockroach (Evans, 1978). The CC of cricket is structurally separated to two lobes: glandular (intrinsic) lobe and storage lobe, however, because of technical difficulty, we collected the CC without separation.

The levels of amines and their metabolites in the FG coincide with those reported in the central nervous system of the same cricket (Nagao and Tanimura, 1988), supporting that the present determination yielded reasonable values, and further indicating that the amines in the FG may have neuronal function. Although the amount of 5-HT were highest among four biogenic amines determined in the FG, and the FG of *G. bimaculatus* include a large number of 5-HT-ir neurons (Klemm *et al.*, 1986), we consider by the reason mentioned above that all of the four amines may have physiological roles, *e.g.*, in circadian rhythms (Jagota and Habibulla, 1992) and sexual behavior (Beck, 1974).

Physiological roles of biogenic amines in the CA and CC may include regulation of hormone secretion from these organs. Actually, OA enhanced the release of JH from the CA of *L. migratoria* (Lafon-Cazal and Baehr, 1988). In the cockroach, OA may have a direct effect on the intrinsic cells of the CA, or modulates release of neurotransmitters or neurohormones from many nerve terminals in the CA (Thompson *et al.*, 1990). Further, OA was identified as the most probable effector of AKH release from locust CC (Orchard and Lange, 1983). DA and 5-HT may modulate release of some other neurohormones or release of AKH (Orchard *et al.*, 1986). In the CC of the cockroach, it has been suggested that DA and 5-HT may influence the release of neurohormones from the CC (Downer *et al.*, 1984).

Our present study revealed that the ratio of the amount of OA to that of its metabolite was nearly the same in both the CA and the CC (Fig. 2B), indicating that OA is released and metabolized from nerve terminals in these organs at a reasonable rate. Compared to OA and its metabolite, the ratios of the amounts of DA metabolites to that of DA in the CA and CC (Fig. 2C) was very high. This fact suggests that dopaminergic nerve terminals very actively release, reuptake and metabolize DA. These results strongly support the idea that octopaminergic and dopaminergic nerves are involved in the physiological regulation of hormone secretion in both the CA and the CC. In this context, the physiological meaning of 5-HT is ambiguous particularly in the CA, since its metabolites were not detected despite the high ratio of 5-HT amount (Fig. 2A).

The presence of precursors, TA and 5-HTP, indicate that OA and 5-HT are synthesized in the all organs examined, although the metabolic rate of 5-HT seems to be very low. The lack of DA precursors may be due to high turnover

of DA which is mentioned above. N-acetylated metabolites, Nac-DA, Nac-TA and Nac-5-HT were detected in all the organs except for Nac-5-HT in the CA, however, synephrine and 5-HIAA were not detected. Although N-acetyloctopamine was not detected in all organs, N-acetyltransferase is probably included in the metabolic pathway of biogenic amines in the CA and the CC.

The present study provided information on the distribution of biogenic amines and related substances in the cricket pars-intercerebralis complex. Further studies on the distributional pattern of these substances in different developmental stages and physiological states will give us more information on the functional roles of biogenic amines in the control of hormone secretion from the CA and the CC.

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