Aromatic Amino Acid and Amine Levels in the Hemolymph of Parasitized and Unparasitized Larvae of *Mythimna separata*

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The levels of aromatic amino acids and amines in the hemolymph of larvae of the common armyworm, *Mythimna separata,* were analyzed after parasitization by the braconid wasp, *Apanteles kariyai.* Methyldopa, homovanillic acid, tryptophan and tyrosine remained unchanged in unparasitized and parasitized larvae. Dopamine and DOPA showed characteristic changes at pupation that were associated with the hardening and darkening of the cuticle; Dopamine in the hemolymph of unparasitized last instar larvae increased before pupation. Large increases in kynurenine and octopamine were detected in parasitized larvae. The increased level of octopamine in the hemolymph may be stress related.

When *Periplaneta americana* is subjected to mechanical, thermal or chemical stress, the concentration of octopamine (OCT) in the blood increases (Davenport and Evans, 1984a). Aromatic amino acid levels in the hemolymph also change after various stressors (Woodring *et al.,* 1988; Shimizu and Takeda, 1991; Hirashima and Eto, 1993a, b). Nappi *et al.* (1991, 1992) have reported that tyrosine (TYR) levels and dopa (DOPA) decarboxylase activity in the hemolymph are changed after parasitization. However, a little are known about aromatic amino acid levels in the hemolymph during parasite infection.

In the present report, aromatic amino acid and amine levels in the hemolymph of parasitized and unparasitized larvae of *Mythimna separata* were analyzed with a three-dimensional HPLC system-coulometric electrochemical detectors (ECD).

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Materials and Methods

Insects

The name of genus, *Mythimna, Leucania* and *Pseudalatia,* of the common armyworm is the same species. Larvae of *M. separata* cited in this paper were reared on an artificial diet (Yakult Co., Japan) at 25 ± 1 °C under a 16:8 L:D photoperiod. Adult *A. kariyai,* a gregarious parasite, were maintained in glass tubes with a cotton pad soaked with 30% honey solution. Parasitization was carried out on day 3 of the 5th instar larvae.

Sampling of hemolymph

Hemolymph was rapidly collected at day 3 and 7 in the unparasitized last instar larvae and day 3, 7 and 9 in the parasitized last instar larvae from a pin hole made in the first pair of abdominal prolegs in 50 µl microcaps lined with a silicone film. The hemolymph (50 µl) was mixed with 150 µl 0.4 N perchloric acid (PCA) solution and then centrifuged at 10,000×*g* for 10 min to remove cell debris and precipitated protein. The supernatant was filtered and then 80 µl solution was injected onto the column (Shimizu and Takeda, 1991; Takeda *et al.,* 1991).

HPLC with electrochemical detector (ECD)

A three-dimensional HPLC system (Coulochem Electrode Array System, Neurochem ESA) was used (see Shimizu and Takeda, 1991; Shimizu *et al.,* 1991; Takeda *et al.,* 1991), and it consisted of a gradient HPLC system and 16 high-sensitivity coulometric electrochemical detectors coupled with a compatible computer. A ratio accuracy for peak purity and chemicals for analyses were identical to those described by Takeda *et al.* (1991).

Results and Discussion

Table I shows aromatic amino acid and amine levels in the hemolymph of parasitized and unparasitized *M. separata.* Methyldopa (3-OMD), dopamine (DA), homovanillic acid (HVA), L-dopa (DOPA), tryptophan (TRP), tyrosine (TYR) and kynurenine (KYN) were detected in the hemolymph of both the parasitized and unparasitized host. These aromatic amino acid levels are rela-

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Table I. Aromatic amino acid and amine levels in the hemolymph of parasitized and unparasitized last instar larvae of *Mythimna separata.*

	ng/ml hemolymph*				
	Unparasitized		Parasitized		
	Day 3 (n = 5)	Day 7 (n = 5)	Day 3 (n = 4)	Day 7 (n = 5)	Day 9 (n = 5)
3-OMD	7.639 ± 5.06	13.373 ± 1.64	6.511 ± 3.19	8.598 ± 4.69**	15.755 ± 2.53
DA	35.538 ± 3.28	160.775 ± 52.13	35.836 ± 3.48	54.962 ± 12.82	29.772 ± 11.41
HVA	0.625 ± 0.12	1.175 ± 0.77**	0.928 ± 0.70	0.756 ± 0.76	0.667 ± 0.35
DOPA	19.248 ± 19.16	not detected	38.603 ± 14.02	125.667 ± 118.70	54.431 ± 34.75
TRP	1.903 ± 0.47***	1.576 ± 0.33***	1.834 ± 0.41***	1.219 ± 0.70***	0.138 ± 0.03***
TYR	5.504 ± 1.64***	4.616 ± 0.02***	5.479 ± 1.71***	5.371 ± 1.64***	3.413 ± 0.93***
KYN	395.965 ± 57.99**	267.013 ± 29.32**	956.883 ± 658.72	2148.718 ± 704.50	3245.105 ± 882.76
OCT	6.387 ± 1.75	6.833 ± 0.00**	13.192 ± 6.42**	24.545 ± 7.47	82.385 ± 18.87

Parasitization was carried out on day 3 of the 5th instar larvae. The staging in "days" means days after last larval molt. 3-OMD, methyldopa; DA, dopamine; HVA, homovanillic acid; DOPA, L-dopa; TRP, tryptophan; TYR, tyrosine; KYN, kynurenine; OCT, octopamine.

* Accuracy (see Takeda *et al.,* 1991): over 0.60.

** Accuracy: under 0.59.

*** μg/ml hemolymph.

tively equal in the each developmental stage of parasitized and unparasitized host hemolymph except for DA (just before pupation), DOPA (day 7 of parasitized last instar larvae) and KYN.

Among the aromatic amines, only OCT, and KYN levels, which is an aromatic amino acid, gradually increased from day 3 to day 9 parasitized last instar larvae, but their levels did not change in the unparasitized host hemolymph.

Levels of DA in the hemolymph just before pupation (day 7) increased, a change undoubtedly associated with cuticular sclerotization (Brunet, 1980). In the parasitized larvae, levels of DA did not increase, because there is no surge of ecdysteroid hormones (Tanaka *et al.,* 1987). Levels of DOPA in the hemolymph day 7 of parasitized larvae increased. Nappi *et al.* (1992) reported that the melanotic encapsulation response was compromised in hosts with reduced levels of DOPA decarboxylase (DDC) using a temperature-sensitive DDC mutant of *Drosophila melanogaster* when infected by the wasp parasitoid *Leptopilina boulardi.* In the present experiments, DDC activity was not measured after parasitization. However, DOPA was maintained at high levels. Accordingly we speculate that DDC activity might be low through lack of ecdysteroids in the parasitized larvae. This would explain the increasing DOPA and low DA.

Only OCT and KYN levels gradually increased in each developmental stage after parasitization. The elevation of blood OCT in response to exercise stress has been reported in *Acheta domestica* (Woodring *et al.,* 1988), in *Periplaneta americana* (Davenport and Evans, 1984a; Hirashima and Eto, 1993a, b) and in *Schistocerca americana gregaria* (Davenport and Evans, 1984a, b). This is the first report that KYN increases after parasitization. Previously we reported that levels of TYR and DOPA in the hemolymph of *Mamestra brassicae* increase following injection of octopaminergic insecticides as stressors (Shimizu and Takeda, 1991). Therefore, aromatic amino acid levels, besides OCT and KYN, are changed by the type of stressors, *i.e.,* chemical stressor as found previously by Hirashima and Eto (1993a, b) or stress by parasitization.

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