

Catecholamines and related *o*-diphenols in cockroach hemolymph and cuticle during sclerotization and melanization: comparative studies on the order Dictyoptera *

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Summary. Catecholamines and related *o*-diphenols extracted from the cuticle and hemolymph of adult cockroaches during sclerotization and pigmentation of the cuticle were analyzed by reverse phase HPLC with electrochemical detection. At ecdysis, dopamine (DA) *o*-conjugates predominated in the hemolymph of *Periplaneta americana*, *P. australasiae*, *P. fuliginosa*, *P. brunnea*, and *Blatta orientalis* (Blattidae); *Blattella germanica* (Blattellidae); and *Gromphadorhina portentosa* and *Blaberus craniifer* (Blaberidae). *N*-Acetyldopamine (NADA) conjugates were second in abundance in these species, but were major in the hemolymph of the other blaberoid species, *Leucophaea maderae* and *Nauphoeta cinerea*. After ecdysis NADA became the major hemolymph catecholamine in all species as DA decreased rapidly. *N*- β -Alanyldopamine (NBAD) concentrations in the hemolymph remained low in all species, although NBAD and its metabolite, *N*- β -alanyl norepinephrine (NBANE), were generally the major catecholamines in tanning cuticle. Catechol (1,2-dihydroxybenzene) occurred mainly as a conjugate(s) at high levels in the hemolymph of nymphs and adults of all blattid species. Only trace amounts were detected in *B. germanica* and *Cryptocercus punctulatus* (Cryptocercidae), and none was found in any of the blaberoid species. High concentrations of

NBANE and NBAD accumulated in tanning cuticle of *B. germanica*, *G. portentosa*, and all blattid species, whereas NADA and DA predominated in cuticle from the other blaberoid species, particularly *L. maderae* and *N. cinerea*. However, cockroaches as a group appear to utilize both the *N*-acetyl and *N*- β -alanyl catecholamines for stabilization of the exoskeleton. The Blattidae differed most from the other families in having considerably higher concentrations of catecholamines in hemolymph and cuticle, as well as the large amounts of catechol conjugates in the hemolymph.

Key words: Cockroaches – Catecholamines – Diphenols – Cuticle – Hemolymph – Sclerotization – Melanization – Pigmentation

Introduction

N-Acetyldopamine (NADA) and *N*- β -alanyldopamine (NBAD) are major precursors for sclerotizing agents in insect cuticle (Karlson and Sekeris 1962; Hopkins et al. 1982, 1984), whereas dopamine (DA) is an important precursor not only for synthesis of *N*-acylated catecholamines but also for insect melanins (Kramer et al. 1984; Hiruma et al. 1985; Roseland et al. 1987). The oxidation of the *N*-acyl catecholamines by phenoloxidases produces reactive quinonoid compounds that form adducts and crosslinks with the chitin-protein matrix of the cuticle (Andersen 1979, 1985; Brunet 1980; Lipke et al. 1983; Kramer and Hopkins 1987), whereas oxidized DA polymerizes to form melanin (Swan 1974; Hiruma et al. 1985).

The abundance of catecholamine derivatives in insect cuticle varies with species, developmental stage and the type or functional properties of the cuticle. NBAD and its β -hydroxylated derivative, *N*- β -alanyl norepinephrine (NBANE), were the major catecholamines extracted from dark brown cuticle, such as that of *Periplaneta americana*, as well as the pupal cuticle of *Manduca sexta*,

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Abbreviations: AMD α -methyldopa; DA dopamine; DOBA 3,4-dihydroxybenzoic acid; DOPA 3,4-dihydroxyphenylalanine; DOPAC 3,4-dihydroxyphenylacetic acid; DOPET 3,4-dihydroxyphenylethanol; DOPKET 3,4-dihydroxyphenylketoethanol; HPLC high performance liquid chromatography; NADA *N*-acetyldopamine; NANE *N*-acetyl norepinephrine; NBAD *N*- β -alanyldopamine; NBANE *N*- β -alanyl norepinephrine; NE norepinephrine

puparial cuticle of *Musca domestica* and elytra of *Tribolium castaneum* (Hopkins et al. 1984; Kramer et al. 1984; Roseland et al. 1985; Morgan et al. 1987; Czapla et al. 1988). NADA and its β -hydroxylated derivative *N*-acetylnorepinephrine (NANE) were the major catecholamines in the colorless head capsule cuticle of *M. sexta* (Hopkins et al. 1984; Morgan et al. unpublished) and the grayish-brown pronotal cuticle of *Leucophaea maderae* (Czapla et al. 1989).

These catecholamine profile differences between *P. americana* and *L. maderae* prompted us to determine if catecholamine and *o*-diphenols differ between families and species of cockroaches. In this study, we identified the major catecholamines and related *o*-diphenols that occur in the hemolymph and cuticle from ten species of cockroaches representing three families of the order Dictyoptera during sclerotization and melanization of cuticle. *o*-Diphenols in hemolymph and cuticle of adult *Cryptocercus punctulatus* of undetermined age also were determined.

Materials and methods

Insects. Cockroaches, *Periplaneta americana* (L.), *P. fuliginosa* (Serville), *P. australasiae* (F.), *P. brunnea* Burmeister, and *Blatta orientalis* L. (Blattidae); *Blattella germanica* (L.) (VPI strain, Blattellidae); and *Gromphadorhina portentosa* (Schaum), *Blaberus craniifer* Burmeister, *Nauphoeta cinerea* (Oliver) and *Leucophaea maderae* (F.) (Blaberidae) were reared in containers bedded with wood shavings at $28 \pm 2^\circ\text{C}$ with a photoperiod of 16L:8D. Water and Purina® "Lab Chow" were provided ad libitum. Last-stage nymphs were selected from the colonies, sexed and placed in square plastic petri dish rearing cages. Wood cockroaches, *Cryptocercus punctulatus* Scudder (Cryptocercidae), were collected by Christine Nalepa, North Carolina State University, Raleigh, NC, and were maintained in decaying wood. Family designations are according to McKittrick (1964) and Huber (1974).

Hemolymph and cuticle extraction. Cockroaches were quick-frozen in dry ice powder to prevent clotting of the hemolymph and stored at -20°C until analyzed. After thawing in a desiccator (approx. 5 min), each insect was placed in a spring steel clip that applied light pressure to the abdomen. The insect was suspended head down for 1 min, the coxa of a front leg was severed and hemolymph was collected with microcapillary pipettes (5–10 μl) and diluted tenfold in 1.2 M HCl containing 5 mM ascorbic acid and 6 $\mu\text{g}\cdot\text{ml}^{-1}$ internal standard α -methyl dopa (AMD). The extracts were centrifuged at $6500 \times g$ for 10 min and the supernatants collected for HPLC analysis.

The pronotal cuticle of each species was analyzed, except in the smaller *B. germanica* in which whole body cuticle was examined. Integument was dissected and placed in distilled water with a few crystals of phenylthiourea. Cuticle was cleaned of adhering muscle and fat body, and the inner surface was scraped and rinsed with distilled water to remove epidermis. Pieces of cuticle were blotted dry on absorbent tissue, weighed (0.5–5.0 mg), and then homogenized in a ground glass tissue grinder in 0.3 ml 1.2 M HCl and 5 mM ascorbate containing 6 $\mu\text{g}\cdot\text{ml}^{-1}$ AMD. The homogenate then was centrifuged at $6500 \times g$ for 10 min and the supernatants collected for HPLC analysis.

Analysis of catecholamines by HPLC. Aliquots of the hemolymph supernatants (0.25 ml) were heated at 100°C under nitrogen for 10 min to release catecholamines from their acid labile conjugates. Samples of both heated and unheated extracts (0.05–0.1 ml) were first adsorbed on alumina at pH 8.6 and then recovered in 1 M

acetic acid after washing (Czapla et al. 1988). The primary mobile phase for hemolymph analysis consisted of methanol (17.5% v/v), sodium octyl sulfate (SOS) (0.34 mM), sodium EDTA (0.90 mM), and 0.1 M H_3PO_4 adjusted to pH 3.1 with NaOH. The primary mobile phase for cuticle samples consisted of methanol (15% v/v), SOS (0.16 mM), sodium EDTA (0.09 mM), and 0.1 M H_3PO_4 adjusted to pH 2.9 with NaOH. A third mobile phase consisted of acetonitrile (26% v/v), 1.1 mM sodium dodecyl sulfate, 0.05 mM sodium EDTA, and 0.1 M H_3PO_4 adjusted to pH 3.3 with NaOH. The retention times of *o*-diphenol standards were compared to unknown electroactive peaks in hemolymph and cuticle extracts using the different mobile phases. Quantities of individual catecholamines were calculated by comparing peak heights with that of the internal standard in each extract and then correcting for recoveries established using standard compounds. The percent conjugation of each compound was calculated from the difference in amounts present in the heated and unheated samples.

Chemicals. Tyrosine, DA, DOPA, NADA, NE, DOPAC, DOBA and AMD were obtained from Aldrich Chemical Company or Sigma Chemical Company. DOPET and DOPKET were gifts from Dr. Peter Sorter, Hoffmann-La Roche Inc. and Dr. S.O. Andersen, University of Copenhagen, Denmark, respectively. NBAD, NBANE and NANE were synthesized as previously described (Czapla et al. 1988).

Results

Catecholamines and other *o*-diphenols in hemolymph

DA was the major catecholamine present at ecdysis in the hemolymph of all species examined except *L. maderae* and *N. cinerea*, in which NADA was predominant (Figs. 1A, 2A). The blattid species (*Periplaneta* and *Blatta*) had the highest total DA concentrations (conjugated plus free) in the following order: *B. orientalis*, 0.6 mM; *P. americana*, *P. brunnea* and *P. fuliginosa*, ≥ 0.3 mM; and *P. australasiae*, 0.15 mM. The blattellid and the blaberoid cockroaches had lower levels of DA (< 0.15 mM), with *L. maderae* and *N. cinerea* having the lowest concentrations, < 0.04 mM (Fig. 2A).

NADA concentrations in the hemolymph of newly-ecdysed insects were highest in *N. cinerea* (0.19 mM), followed by *P. fuliginosa* (0.13 mM), *L. maderae* (0.08 mM), *B. orientalis* (0.07 mM), and *P. americana* (0.06 mM, Figs. 1A, 2A). NBAD levels in the hemolymph were always less than 0.05 mM (Figs. 1 and 2), whereas DOPA and NE levels were never greater than 0.01 mM (data not shown).

Other *o*-diphenols identified in hemolymph after acid hydrolysis were DOPET, DOPKET, DOPAC, and catechol (Table 1). Concentrations of these compounds were generally low except for catechol in the blattid species, which exceeded 0.2 mM. Most of the catechol in *P. americana* appeared to be in the form of a glucoside conjugate (Czapla et al. unpublished data). Catechol was also found in trace amounts in *B. germanica* and *C. punctulatus* hemolymph, but none was detected in the blaberoid cockroaches. DOPET also increased to relatively high levels by 24 h in *P. fuliginosa* and *B. orientalis*.

Total catecholamine concentrations generally decreased in hemolymph of all species as the new cuticle

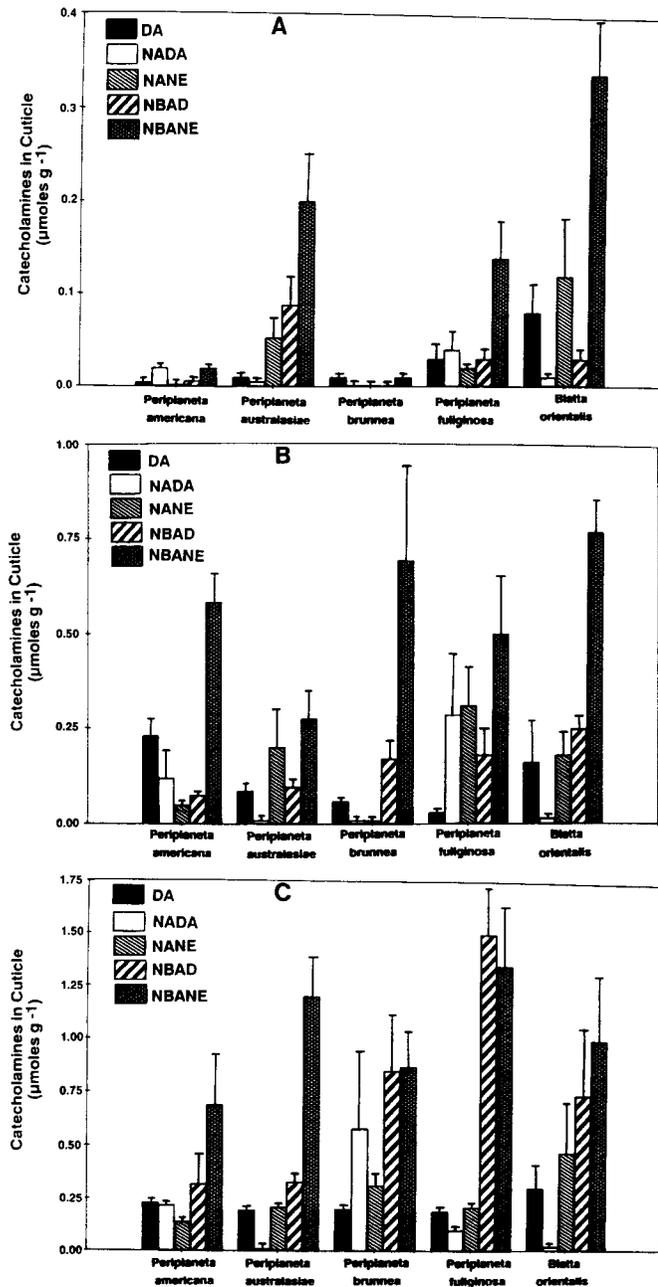


Fig. 3A–C. Catecholamine concentrations ($\mu\text{moles} \cdot \text{g}^{-1}$) extracted by cold acid from the cuticle of Blattellidae. A, at ecdysis; B, 6 h after ecdysis; C, 24 h after ecdysis. Data are the means of 3–5 insects \pm SE

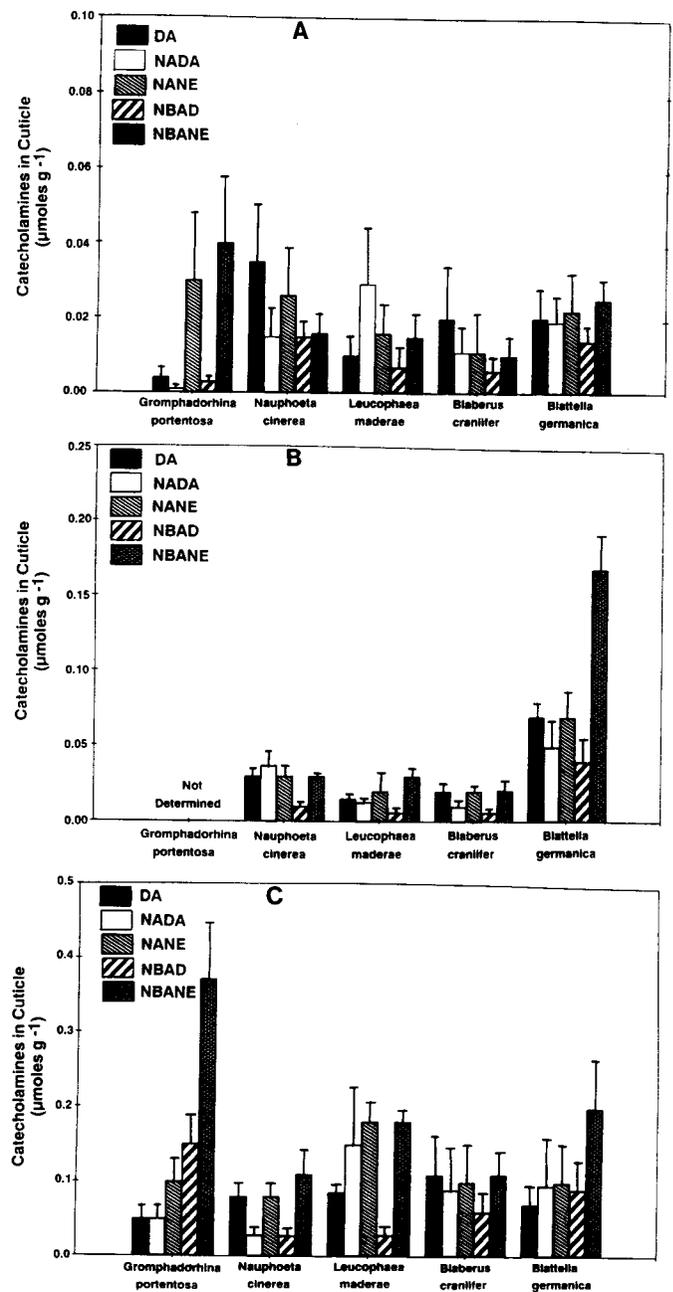


Fig. 4A–C. Catecholamine concentrations ($\mu\text{moles} \cdot \text{g}^{-1}$) extracted by cold acid from the cuticle of Blaberidae and Blattellidae. A, at ecdysis; B, 6 h after ecdysis; C, 24 h after ecdysis. Data are the means of 3–5 insects \pm SE

Table 2. Percent conjugation of catecholamines in hemolymph of cockroaches during adult cuticle tanning^a

Species	DA			NADA			NBAD		
	0 h	6 h	24 h	0 h	6 h	24 h	0 h	6 h	24 h
<i>B. craniifer</i>	78 \pm 12	94 \pm 3	93 \pm 3	99 \pm 1	92 \pm 4	85 \pm 5	75 \pm 15	92 \pm 4	80 \pm 10
<i>B. orientalis</i>	99 \pm 1	ND	ND	76 \pm 5	ND	ND	69 \pm 5	ND	ND
<i>G. portentosa</i>	67 \pm 11	ND	88 \pm 8	69 \pm 15	ND	90 \pm 5	44 \pm 6	ND	86 \pm 8
<i>L. maderae</i>	ND	100	100	35 \pm 20	75 \pm 11	88 \pm 11	79 \pm 10	73 \pm 2	50 \pm 15
<i>N. cinerea</i>	52 \pm 8	80 \pm 10	74 \pm 2	90 \pm 10	88 \pm 7	72 \pm 5	90 \pm 2	76 \pm 5	75 \pm 10
<i>P. americana</i>	85 \pm 10	80 \pm 10	75 \pm 10	70 \pm 10	60 \pm 20	85 \pm 20	67 \pm 20	80 \pm 15	50 \pm 20
<i>P. brunnea</i>	90 \pm 7	95 \pm 5	ND	ND	90 \pm 10	85 \pm 10	ND	ND	ND

^a Data are means of 3 samples \pm SE; ND, not determined

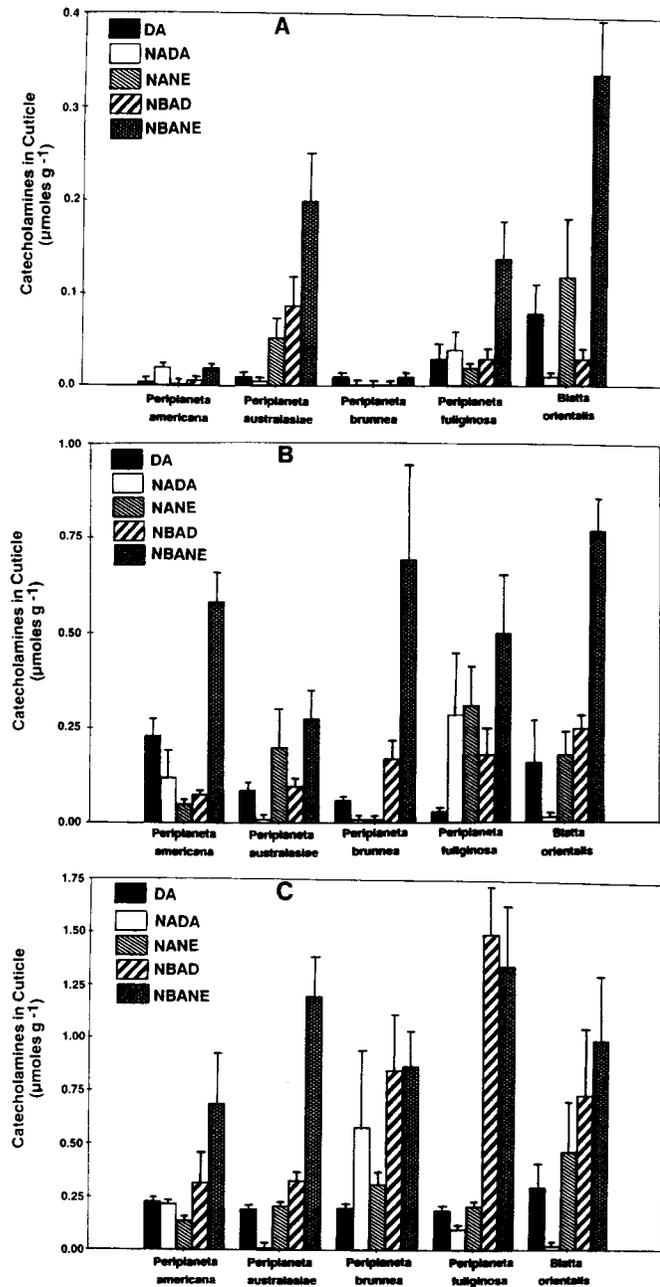


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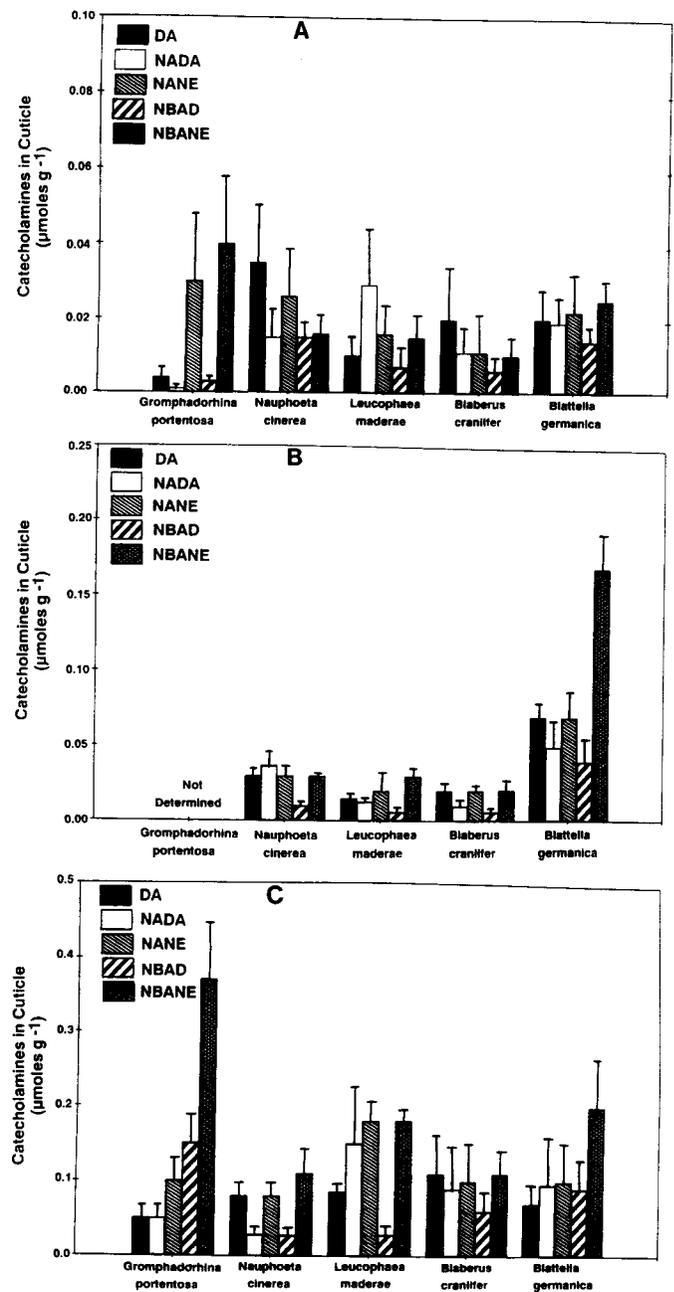


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	0 h	6 h	24 h	0 h	6 h	24 h	0 h	6 h	24 h
<i>B. craniifer</i>	78 \pm 12	94 \pm 3	93 \pm 3	99 \pm 1	92 \pm 4	85 \pm 5	75 \pm 15	92 \pm 4	80 \pm 10
<i>B. orientalis</i>	99 \pm 1	ND	ND	76 \pm 5	ND	ND	69 \pm 5	ND	ND
<i>G. portentosa</i>	67 \pm 11	ND	88 \pm 8	69 \pm 15	ND	90 \pm 5	44 \pm 6	ND	86 \pm 8
<i>L. maderae</i>	ND	100	100	35 \pm 20	75 \pm 11	88 \pm 11	79 \pm 10	73 \pm 2	50 \pm 15
<i>N. cinerea</i>	52 \pm 8	80 \pm 10	74 \pm 2	90 \pm 10	88 \pm 7	72 \pm 5	90 \pm 2	76 \pm 5	75 \pm 10
<i>P. americana</i>	85 \pm 10	80 \pm 10	75 \pm 10	70 \pm 10	60 \pm 20	85 \pm 20	67 \pm 20	80 \pm 15	50 \pm 20
<i>P. brunnea</i>	90 \pm 7	95 \pm 5	ND	ND	90 \pm 10	85 \pm 10	ND	ND	ND

^a Data are means of 3 samples \pm SE; ND, not determined

Table 3. Concentrations of *o*-diphenols ($\mu\text{moles}\cdot\text{g}^{-1}$) other than catecholamines in cockroach cuticle during adult cuticle tanning^a

Species	DOPKET		DOPET		DOPAC	
	0 h	24 h	0 h	24 h	0 h	24 h
<i>B. craniifer</i>	tr	0.02 ± 0.01	tr	tr	tr	tr
<i>B. germanica</i>	tr	0.02 ± 0.01	tr	0.03 ± 0.01	tr	tr
<i>B. orientalis</i>	0.02 ± 0.02	0.18 ± 0.7	0.19 ± 0.1	0.24 ± 0.06	tr	0.07 ± 0.2
<i>L. maderae</i>	0.03 ± 0.02	0.19 ± 0.06	0.04 ± 0.01	tr	0.03 ± 0.02	tr
<i>N. cinerea</i>	0.01 ± 0.01	0.05 ± 0.02	tr	tr	tr	tr
<i>P. americana</i>	tr	0.06 ± 0.02	tr	0.30 ± 0.05	tr	0.15 ± 0.1
<i>P. australasiae</i>	tr	0.24 ± 0.04	tr	0.11 ± 0.02	tr	tr
<i>P. brunnea</i>	tr	0.20 ± 0.05	tr	0.26 ± 0.08	tr	tr
<i>P. fuliginosa</i>	0.03 ± 0.01	0.13 ± 0.04	0.02 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	tr

^a Data are means of 2–3 samples ± SE; tr = trace (<0.01 $\mu\text{moles}\cdot\text{g}^{-1}$)

tanned (Figs. 1 and 2). NADA increased to peak levels in *P. americana* by 6 h (0.25 mM) and had declined by 24 h (0.05 mM) (Figs. 1B, 1C). It became the most abundant catecholamine in the hemolymph of all species, except for *P. fuliginosa*, by 24 h because of the decrease of DA (Figs. 1C, 2C). DA levels fell by a factor of 60 in *B. orientalis* by 24 h, whereas in *P. americana*, *P. brunnea*, and *P. fuliginosa*, they decreased almost 30-fold (Fig. 1C). The conjugates of DOPET and DOPKET generally increased in hemolymph during the first 24 h (Table 1).

Catecholamine conjugates

DA, NADA, and NBAD were sequestered as acid-labile conjugates in cockroach hemolymph, with the extent of DA conjugation ranging from 50% in *N. cinerea* to almost 100% in *B. orientalis* at ecdysis (Table 2). NADA conjugation ranged from 35% in *L. maderae* to 99% in *B. craniifer*, whereas NBAD conjugation was 44% in *G. portentosa* to 90% in *N. cinerea*. No general trends in relative percent conjugation of the diphenolic compounds in the hemolymph of various species during sclerotization were observed.

Catecholamines and other *o*-diphenols in cuticle

NBANE was the major extractable catecholamine in the cuticle of Blattidae, Blattellidae, and *G. portentosa* (Blaberidae) 24 h after ecdysis (Figs. 3 and 4). NBANE concentrations were particularly high at ecdysis in extracts of unsclerotized cuticle of *B. orientalis*, *P. australasiae* and *P. fuliginosa* (0.30, 0.20, and 0.14 $\mu\text{mole}\cdot\text{g}^{-1}$, respectively) (Fig. 3A). A general increase also occurred in extractable NBAD, NANE, NADA, and DA in all species through the same period, with NBAD equaling NBANE concentrations in *P. brunnea*, *P. fuliginosa*, and *B. orientalis* by 24 h (Fig. 3C). *P. fuliginosa* had the highest concentrations of both NBAD and NBANE, 1.45 and 1.3 $\mu\text{mole}\cdot\text{g}^{-1}$, respectively, at that time. The extractable catecholamines tended to be lower in concentration in the Blaberoid species during cuticle sclerotization than in the Blattidae species (Fig. 4B).

The *N*-acetyl catecholamines in cockroach cuticle were usually less than 0.25 $\mu\text{mole}\cdot\text{g}^{-1}$ in all species, except when NADA and NANE accumulated to about 0.5 $\mu\text{mole}\cdot\text{g}^{-1}$ in *P. brunnea* and *B. orientalis*. In the blaberoid species, the *N*- β -alanyl catecholamines, NBAD and NBANE, were highest in *G. portentosa* (0.15 and 0.37 $\mu\text{mole}\cdot\text{g}^{-1}$, respectively, by 24 h, Fig. 4C). In the other blaberoid species and *B. germanica*, concentrations of both the *N*-acetyl and *N*- β -alanyl catecholamines were similar, ranging from 0.03 to 0.2 $\mu\text{mole}\cdot\text{g}^{-1}$ (Fig. 4C). DA generally increased in the cuticle of all species during the 24 h after ecdysis, with the blattids having more than two-fold higher levels than the other species (Figs. 4B, 4C). DOPET and DOPKET levels generally increased in sclerotized cuticle, particularly in the blattid species (Table 3). Only trace levels of DOPAC were extracted from the cuticle of most cockroaches.

Catecholamines in hemolymph and cuticle of *C. punctulatus*

NADA was the only catecholamine detected in hydrolyzed hemolymph extracts (0.01 mM), whereas DOPET and DOPKET levels were much higher (0.09 mM and 0.05 mM, respectively, Table 4). NBANE and NANE were the major catecholamines extracted from cuticle (0.24 and 0.28 $\mu\text{mole}\cdot\text{g}^{-1}$, respectively, Table 4), whereas DOPKET occurred at higher levels (0.40 $\mu\text{mole}\cdot\text{g}^{-1}$).

Table 4. Catecholamine and related *o*-diphenol concentrations in hemolymph and cuticle extracts from adult *Cryptocercus punctulatus*^a

Compounds	Hemolymph (mM)	Cuticle ($\mu\text{moles}\cdot\text{g}^{-1}$)
DA	tr	0.06 ± 0.01
NADA	0.01	0.07 ± 0.01
NBAD	tr	0.10 ± 0.02
NANE	0	0.28 ± 0.02
NBANE	0	0.24 ± 0.01
DOPKET	0.05	0.40 ± 0.02
DOPET	0.09	0.11 ± 0.01
CATECHOL	tr	0

^a Data are the means of 4 adults of unknown age ± SE; tr = trace (<0.01 mM)

Discussion

Cockroaches appear to sequester DA conjugates in the hemolymph during the nymphal feeding period for later use as precursors for sclerotization and melanization. However, two blaberoid species, *L. maderae* and *N. cinerea*, primarily stored a NADA conjugate(s), whereas *B. germanica* had nearly equal concentrations of DA and NADA. DA levels were about two-fold higher in the blattid species than in blattellid and blaberoid species. Extractable catecholamine concentrations in blattid cuticle were also higher than those in blaberoid and blattellid cuticle. DA and NADA were conjugated primarily as the 3-*o*-sulfate in *P. americana* hemolymph (Bodnaryk and Brunet 1974; Bodnaryk et al. 1974; Sloley et al. 1987; Czapla et al. 1988). DA was also found mainly as the 3-*o*-sulfate conjugate in *L. maderae* (Czapla et al. 1989). Conjugation has been suggested as a mechanism to protect catecholamines, especially DA, against premature oxidation to quinones (Bodnaryk and Brunet 1974).

The sequestration of catecholamine conjugates in cockroach hemolymph during the nymphal feeding period is quite different from the storage of these compounds in the tobacco hornworm, *M. sexta*. In *M. sexta*, catecholamine conjugates accumulated in hemolymph only after apolysis to pharate stages of larvae, pupae and adult (Hopkins et al. 1982, 1984). However, β -D-glucopyranosyl-*o*-L-tyrosine (tyrosine glucoside) was sequestered in hemolymph during the final larval feeding period of *M. sexta* for subsequent metabolism to catecholamines during pupal cuticle tanning (Kramer et al. 1980; Ahmed et al. 1983). Tyrosine glucoside has been identified in several lepidopterous and dipterous species (Kramer and Hopkins 1987), whereas tyrosine glucoside or sulfate has not been found in *P. americana* (Kramer et al. 1980; Czapla et al. 1988).

Catechol (1,2-dihydroxybenzene) was present at high levels in the hemolymph of blattid species as an acid-labile conjugate, but it was absent in the hemolymph of blaberoid cockroaches. Only trace levels were detected in *B. germanica* and *C. punctulatus*. Catechol became the major *o*-diphenol in *P. americana* hemolymph shortly after ecdysis and remained so during larval and adult life (Czapla et al. 1988). The function of catechol in the hemolymph of the blattid cockroaches is unknown, but it may serve as an antimicrobial agent or as a substrate for melanization during wound healing or encapsulation of pathogens and parasites. Catechol appears to be conjugated primarily as a β -glucoside, but a sulfate conjugate also may be present (Czapla et al. unpublished data).

The *N*- β -alanyl catecholamines, NBAD and NBANE, were the major catecholamines extracted by cold acid from cuticle of all blattid species during sclerotization, although DA and the *N*-acetyl catecholamines also accumulated, but at a slower rate. The blaberoid and blattellid species had more equal concentrations of both the *N*-acetyl catecholamines and *N*- β -alanyl catecholamines than the other species, with the exception of *G. portentosa*.

The pronotal cuticular coloration of the blattid spe-

cies was red-brown to almost black. The high percentage of the *N*- β -alanyl catecholamines in these cuticles correlated with their general occurrence in stiff brown or dark cuticle (Hopkins et al. 1984). The pronotal cuticle of *L. maderae* and *N. cinerea* was a mottled gray over a light brown background and had a higher relative percentage of DA and the *N*-acetyl catecholamines than the *N*- β -alanyl catecholamines. In general, cockroaches appear to rely on both *N*-acetyl and *N*- β -alanyl catecholamines for sclerotization, because large quantities of each typically occur in tanning cuticle.

The black pigmentation of some blattid and blaberoid species and *B. germanica*, which is presumably due to melanin deposition, may correlate with the relatively high DA concentrations in these cuticles during pigmentation. *B. orientalis* and *P. fuliginosa* in particular have very dark pigmentation and high levels of DA in the cuticle at ecdysis. The detection of high levels of DA in the cuticle of black phenotypes of *Tribolium castaneum* (Kramer et al. 1984; Roseland et al. 1987), *M. sexta* (Hopkins et al. 1984; Hiruma et al. 1985) and *B. germanica* (Czapla et al. 1990) suggested that DA is an important precursor of insect melanins.

Unlike cockroach cuticle, the stiff brown or colorless cuticles of some holometabolous insects contained predominantly one type of tanning precursor (Hopkins and Kramer 1990). *Manduca sexta* pupal cuticle was sclerotized primarily by quinonoids derived from NBAD. The *N*-acetyl catecholamines were nearly undetectable in pupal cuticle at 6 and 24 h after ecdysis (Hopkins et al. 1984; Morgan et al. 1987). However, the major *o*-diphenol present in the stiff colorless larval head capsule of *M. sexta* was NADA, with only minor amounts of DA and NBAD detectable (Hopkins et al. 1984). Similar results were seen in *T. castaneum*, whose red-brown elytral cuticle contained mainly *N*- β -alanyl catecholamines during tanning (Kramer et al. 1984; Roseland et al. 1987; Morgan et al. 1987). The *N*- β -alanyl catecholamines were also predominant in puparial exuviae of the flies, *Stomoxys calcitrans* and *Musca domestica*, with only very small amounts of the *N*-acetyl catecholamines (Roseland et al. 1985). Therefore, the predominance of the *N*- β -alanyl catecholamines in the stiff brown cuticles of holometabolous species is quite different than the condition observed in the brown or dark cuticles of cockroaches, for which both types of tanning precursors appear to be important. The *N*- β -alanyl catecholamines in cockroach cuticle accounted for about 80% of total catecholamines in 24 h extracts, whereas these compounds accounted for approximately 95% of the total in holometabolous species.

The relatively low concentrations of NBAD (free and conjugated) in cockroach hemolymph contrasted to the high levels of the *N*- β -alanyl catecholamines (NBAD and NBANE) in tanning cuticle. *N*- β -Alanyl conjugation of DA, therefore, probably occurred during transport to the cuticle via the epidermis. Krueger et al. (1989) have demonstrated high rates of NBAD synthesis *in vitro* by *M. sexta* larval integument, when cultures were supplemented by DA. Cockroach epidermis is also a likely tissue for *N*- β -alanylation of dopamine. NBANE was

a cuticular oxidative metabolite of NBAD in *M. sexta* pupal cuticle (Morgan et al. 1987) and probably was produced by a similar mechanism in cockroach cuticle. The N-acetyl catecholamines also appear to be important substrates in the sclerotization of cockroach cuticle and, unlike NBAD, relatively large amounts of NADA conjugates are found in hemolymph during sclerotization. N-Acetylation of dopamine, therefore, may occur in fat body or other tissues, followed by conjugation and release back into the hemolymph.

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References

- Ahmed RF, Hopkins TL, Kramer KJ (1983) Tyrosine and tyrosine glucoside titres in whole animals and tissues during development of the tobacco hornworm *Manduca sexta* (L.). *Insect Biochem* 13:369–374
- Andersen SO (1979) Biochemistry of insect cuticle. *Ann Rev Entomol* 24:29–61
- Andersen SO (1985) Sclerotization and tanning in cuticle. In: Kerut GA, Gilbert LI (eds) *Comparative insect physiology, biochemistry, and pharmacology*. Pergamon Oxford, vol 3, pp 59–74
- Bodnaryk RP, Brunet PCJ (1974) 3-O-Hydrosulphato-4-hydroxyphenethylamine (dopamine 3-O-sulphate), a metabolite involved in the sclerotization of insect cuticle. *Biochem J* 138:463–469
- Bodnaryk RP, Brunet PCJ, Koeppe JK (1974) On the metabolism of N-acetyldopamine in *Periplaneta americana*. *J Insect Physiol* 20:911–923
- Brunet PCJ (1980) The metabolism of the aromatic amino acids concerned in the cross-linking of insect cuticle. *Insect Biochem* 10:467–500
- Czapla TH, Hopkins TL, Morgan TD, Kramer KJ (1988) Diphenols in hemolymph and cuticle during development and cuticle tanning of *Periplaneta americana* (L.) and other cockroach species. *Arch Insect Biochem Physiol* 7:13–26
- Czapla TH, Hopkins TL, Kramer KJ (1989) Catecholamines and related o-diphenols in the hemolymph and cuticle of the cockroach *Leucophaea maderae* (F.) during sclerotization and pigmentation. *Insect Biochem* 19:509–515
- Czapla TH, Hopkins TL, Kramer KJ (1990) Catecholamines in the cuticles of four strains of the German cockroach *Blattella germanica* (L.) during sclerotization and melanization. *Arch Insect Biochem Physiol* 12:145–156
- Hiruma K, Riddiford LM, Hopkins TL, Morgan TD (1985) Roles of dopa decarboxylase and phenoloxidase in the melanization of the tobacco hornworm and their control for 20-hydroxyecdysone. *J Comp Physiol B* 155:659–669
- Hopkins TL, Kramer KJ (1990) Catecholamine metabolism and the integument. In: Retnakaran R, Binnington K (eds) *The physiology of the insect epidermis*. Inkata Press, Victoria, Australia. In Press
- Hopkins TL, Morgan TD, Aso Y, Kramer KJ (1982) N- β -Alanyldopamine: major role in insect cuticle tanning. *Science* 217:354–366
- Hopkins TL, Morgan TD, Kramer KJ (1984) Catecholamines in haemolymph and cuticle during larval, pupal, and adult development of *Manduca sexta*. *Insect Biochem* 14:553–540
- Huber I (1974) Taxonomic and ontogenetic studies of cockroaches (Blattaria). *Univ Kans Sci Bull* 50:233–332
- Karlson P, Sekeris CE (1962) N-Acetyldopamine as sclerotizing agent of the insect cuticle. *Nature (Lond)* 195:183–184
- Kramer KJ, Hopkins TL (1987) Tyrosine metabolism for insect cuticle tanning. *Arch Insect Biochem Physiol* 6:279–301
- Kramer KJ, Hopkins TL, Ahmed RF, Mueller D, Lookhart G (1980) Tyrosine metabolism for cuticle tanning in the tobacco hornworm, *Manduca sexta* (L.) and other Lepidoptera: identification of β -D-glucopyranosyl-O-L-tyrosine and other metabolites. *Arch Biochem Biophys* 205:146–155
- Kramer KJ, Morgan TD, Hopkins TL, Roseland CR, Aso Y, Beeman RW, Lookhart GL (1984) Catecholamines and β -alanine in the red flour beetle *Tribolium castaneum*. *Insect Biochem* 14:293–298
- Krueger RA, Kramer KJ, Hopkins TL, Speirs RD (1989) N- β -Alanyldopamine levels and synthesis in integument and other tissues of *Manduca sexta* (L.) during larval-pupal transformation. *Insect Biochem* 19:69–175
- Lipke H, Sugumaran M, Henzel W (1983) Mechanisms of sclerotization in dipterans. *Adv Insect Physiol* 17:1–85
- McKittrick FA (1964) Evolutionary studies of cockroaches. *Cornell Univ Agric Expt Sta Mem* 389:1–197
- Morgan TD, Hopkins TL, Kramer KJ, Roseland CR, Czapla TH, Tomer KB, Crow FW (1987) N- β -Alanyl norepinephrine: biosynthesis in cuticle and possible role in sclerotization. *Insect Biochem* 17:255–263
- Roseland CR, Grodowitz MJ, Kramer KJ, Hopkins TL, Broce AB (1985) Stabilization of mineralized and sclerotized puparial cuticle of muscid flies. *Insect Biochem* 15:521–528
- Roseland CR, Kramer KJ, Hopkins TL (1987) Cuticular strength and pigmentation of rust-red and black strains of *Tribolium castaneum*. *Insect Biochem* 17:591–596
- Sloley BD, Downer RG (1987) Dopamine, N-acetyldopamine, and dopamine-3-O-sulphate in tissues of newly ecdysed and fully tanned adult cockroaches (*Periplaneta americana*). *Insect Biochem* 17:591–596
- Swan GA (1974) Structure, chemistry and biosynthesis of the melanins. *Fortschr Chem Org Naturst* 31:521–582