Short Communication

Biogenic Amines in Periplaneta americana L.: Accumulation of Octopamine, Sympathrine, and Tyramine by Stress

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The nervous system of the American cockroach contains a high level of OA, which plays an important role in regulating the nervous system, and acting as a neurotransmitter, neurohormone, and neuromodulator. When Periplaneta was subjected to mechanical, thermal or chemical (insecticidal) stress, the concentration of OA in the blood increased. However, the effects of stressors on the levels of OA precursors and metabolites in Periplaneta have not been described. Hence, this present study represents a preliminary evaluation of the effect of various stressors, including vibration, optical, and thermal stress, on the levels of OA and related substances in the American cockroach, using a convenient procedure for simultaneous analysis of these substances. This procedure involves direct injection of the sample into a C_18 reversed-phase HPLC column, the eluted fractions being monitored electrochemically by an amperometric detector.

OA hydrochloride, SOS, PCA, Tyr, TA, methanol for HPLC, and EDTA sodium salt dihydrate were obtained from Nacalai Tesque; DHBA hydrobromide was from Sigma Chemical Co., and SN from Tokyo Chem. Ind. Co. All water was distilled and deionized.

Investigations were done on the American cockroach (P. americana L.) of both sexes. The insects were reared under crowded conditions in this laboratory at 28°C with a photoperiod of 12 hr light and 12 hr dark at a relative humidity of 65–70%; they were provided with an artificial mouse diet (Oriental Yeast Co.) and water ad libitum. Adult cockroaches weighing approx. 1 g aged at least four weeks post ecysis were removed from the colony, and nervous tissues were dissected during the second quarter of the light cycle.

The insects were mechanically stressed in a 50 ml tube (3.5 cm in diameter) by vibrating at 100 Hz and 10 W for 15 min, using an SC-8302 transducer (Body Sonic Co.) attached to an FG-272 function generator (Kenwood Co.) and an A-535 stereo amplifier (Pioneer Electronic Co.) at 28°C maintained by a CU-255 chamber (Tomy Seiko Co.). Optical stress was applied by an MS-60D flash light (Shimadzu Co.) at 40 Hz and 10 W for 15 min, and thermal stress, by immersing the insect in water at 60°C for 30 s before sampling to heat-fix the blood.

Supraesophageal ganglion (brain) and whole-thoracic nerve cord including subesophageal, mesothoracic, and metasthathoracic ganglia were dissected out of a single insect anaesthetised by cold exposure without saline. Haemolymph samples (20 μl) were rapidly collected by using a microcapillary tube through an incision made to sever the metathoracic legs of an animal. The tissues thus obtained were immediately transferred to 200 μl of ice-cold 0.2 m PCA containing 200 μM EDTA and DHBA (100 ng/ml) as an internal standard in an Eppendorf polypropylene microtube (1.5 ml), and homogenized with a UCD-200TM Bioruptor (Cosmo Bio.) ultrasonic cell disrupter at 20 kHz and 200 W for 30 s. The homogenate was centrifuged at 15,000 × g and 4°C for 30 min by a KM-15200 micro-centrifuge (Kubota Co.). The supernatant (20 μl) filtered through a 0.2 μm filter (HLC-DISK 13, Kanto Chem. Co.) was directly injected into an HPLC column.

Chromatography was carried out by using a CCPD solvent-delivery pump (Tosoh Co.), an EICOM DG-100 degasser, and a PL-100 damper (Eicom Co.). Samples were injected through a valve (Rheodyne 7125) fitted with a 20-μl sample loop. OA and related substances were separated through a C_18 reverse-phase column (4.6 × 250 mm) that was protected by a pre-column (4.6 × 10 mm) packed with Cosmosil 5 C_18 AR of 5 μm average particle size (Nacalai Tesque). An EYELA CTP-100 thermo-controlled cooling pump (Tokyo Sci. Chem. Co.) was used to maintain the column oven at 25.0±1°C. An EICOM ECD-100 amperometric detector with a graphite electrode was set at an oxidizing potential of 870 mV (relative to an Ag/AgCl reference electrode). Signals from the electrochemical detector were recorded and integrated by a Chromatopac C-R1A data processor (Shimadzu Co.). A running buffer consisting of 0.1 M citric acid–0.1 M sodium acetate (pH 3.5), 15% of methanol, and SOS (270 mg/liter) as an ion-pair reagent was run isocratically (0.8 ml/min). The mobile phase was filtered under vacuum through a 0.45 μm-pore filter (Millipore SJLHM4710) to remove the particulate matter.

Standard solutions were prepared at a concentration of 1 mg/ml in the mobile phase, and the working concentration was achieved by serial dilution in the same solution. Samples and standards were injected alternately for identification and quantitative analysis. Each peak was identified by comparing both the retention times and hydrodynamic voltammograms with those of the standards. Concentrations were obtained by a comparison of ratios, based on the peak area of the chromatograms, between the sample and standard DHBA.

The amounts of OA (13 nmol/ml in the haemolymph and 254 pmol/tissue for the thoracic nerve cord) were higher than those of TA (a precursor in the biosynthetic pathway of OA, Fig. 1, 9 nmol/ml and 11 pmol/tissue, respectively) and SN (N-methyl OA, 6 nmol/ml and 93 pmol/tissue, respectively), whereas TA (692 pmol/tissue) was at the

Abbreviations: OA, octopamine; HPLC, high-performance liquid chromatography; SOS, sodium 1-octanesulfate; PCA, perchloric acid; Tyr, L-tyrosine; TA, tyramine; EDTA, ethylenediaminetetraacetic acid; DHBA, 3,4-dihydroxybenzylamine; SN, DL-synephrine.
Biogenic Amines in Stressed Cockroach

Fig. 1. Pathway for Phenolamine Production from Tyrosine.
The reactions are catalyzed by the following enzymes: DD, DOPA decarboxylase; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine-N-methyltransferase.

Table I. Concentrations of OA, TA, SN, and Tyr in Stressed Thoracic Nerve Cords of Periplaneta americana L.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vibration (100 Hz)</th>
<th>Heat (60°C)</th>
<th>Flash light (4 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr</td>
<td>113.9 ± 12.0</td>
<td>154.0 ± 8.1*</td>
<td>128.9 ± 11.3</td>
</tr>
<tr>
<td>TA</td>
<td>957.4 ± 174.0*</td>
<td>821.7 ± 173.1*</td>
<td>521.4 ± 117.8*</td>
</tr>
<tr>
<td>OA</td>
<td>145.0 ± 18.0</td>
<td>165.1 ± 16.7*</td>
<td>351.8 ± 28.9*</td>
</tr>
<tr>
<td>SN</td>
<td>351.4 ± 34.5*</td>
<td>433.6 ± 75.5*</td>
<td>731.6 ± 45.4*</td>
</tr>
</tbody>
</table>

See the text for details of the experimental procedure; data are the mean ± SE of 5 experiments with two replicates. In the control, the concentrations of Tyr, TA, OA, and SN were 139.3 ± 131.5, 11.0 ± 2.6, 253.7 ± 24.3, and 93.0 ± 22.1 pmol/tissue, respectively.

* Significantly different from the control at p=0.05 according to Duncan’s multiple-range test.10

The highest level and followed by OA (354 pmol/tissue) and SN (99 pmol/tissue) in the brain. The distribution of TA and SN in different tissues was not in parallel with that of OA. An increase of Tyr, TA, and SN, in addition to that of OA, was observed in the whole-thoracic nerve cord of stressed P. americana (Table I), whereas the concentrations of almost all of the other 18 biogenic amines and related substances decreased, including dopamine, serotonin, epinephrine, and norepinephrine (data not shown). A similar tendency was observed for the levels of biogenic amines and related substances in the haemolymph and brain of stressed Periplaneta (data not shown). An increase in haemolymph OA seems to be a common response to a variety of stressful stimuli in cockroaches.3,8) However, the effects of stressors on the TA and SN levels have not been described before and, therefore, their roles have not been studied in detail. While it has been clearly shown that OA acted as a neuromediator in various insect preparations, there have only been suggestions that TA might be a neuromediator in invertebrates.9) The present results suggest the possibility of TA and SN not only being precursors or metabolites in the biosynthetic pathway of OA, but also neuromediators. In order to determine the role of TA and SN in regulating the nervous system for the behaviour and response of insects, more detailed studies are in progress and will be reported elsewhere.

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References