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Short Communication

High-performance liquid chromatography of biogenic amines in the corpus cardiacum of the American cockroach, *Periplaneta americana*

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ABSTRACT

The simultaneous determination of biogenic amines in the corpus cardiacum of the American cockroach, *Periplaneta americana*, was carried out using high-performance liquid chromatography with a Neurochem neurochemical analyser. Vanillic acid, dopamine, octopamine and tyramine were detected. Tyrosine and tryptophan were also detected at high levels. Octopamine levels in the corpus cardiacum were increased on injection of an acetone solution. The biological function of the biogenic amines detected is discussed.

INTRODUCTION

In insects, the corpora cardiaca are the principal neurohemal organs. These glands both store and secrete neurosecretory products synthesized in the brain and contain intrinsic cells which produce their own neurosecretory material [1]. In the corpus cardiacum of locusts, biogenic amines have been detected by a radiochemical enzymatic assay [2,3] and by high-performance liquid chromatography (HPLC) [4]. However, there are only two reports on the measurement of biogenic amines in the corpora cardiaca of the cockroach [5,6]. Recently, HPLC with electrochemical detection (ED) using coulometric electrodes has been used successfully to determine catecholamines in some samples from discrete regions of the insect nervous system [7–13].

In this work, we investigated amines in the corpus cardiacum of the American cockroach, *Periplaneta americana*.

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EXPERIMENTAL

Insects

Adult male cockroaches were taken from a colony of *Periplaneta americana* maintained under a 13 h light–11 h dark photoperiod at 26°C and a relative humidity of 65%. They were reared on a pelleted diet and water. Insects were taken between 1 and 5 months after the final moult and divided into two groups of ten 24 h prior to dissection. One group was in the normal state and the other group was subjected to chemical stress.

Injection

As a chemical stressor, 2 μl of acetone were injected into the abdominal haemocoel through the abdominal intersegmental integument between the second and third segments using a microlitre syringe. Thirty minutes after injection, the corpora cardiaca were dissected.

Preparation of corpora cardiaca

The corpora cardiaca of ten male adults were carefully dissected under ice-cold physiological saline [240 m*M* NaCl–2.7 m*M* KCl–3.6 m*M* $CaCl_2$–2.38 m*M* N-2-hydroxyethylpiperazine-N′-2-ethansulphonic acid (HEPES)]. Ten glands from ten animals were placed in 200 μl of ice-cold 0.1 *M* HCl and then homogenized with hand microhomogenizer (glass). Following centrifugation at 10 000 *g* for 10 min, the supernatant was filtered and dried using a rotary evaporator under vacuum. These samples were dissolved in 0.4 *M* perchloric acid (PCA) and aliquots were injected onto the HPLC column. The recovery efficiency was determined by extraction of test samples and the addition of an internal standard followed by analysis. It was found that the recovery was higher than 95% through the extraction, centrifugal filtration (UFC3 OHV, Millipore) and drying process.

HPLC with electrochemical detection (ECD)

The HPLC system used was a Neurochem neurochemical analyser (ESA, Bedford, MA, U.S.A.) consisting of a gradient HPLC system and sixteen high-sensitivity coulometric electrochemical detectors coupled with a compatible computer. The concept and inherent advantages of multi-electrode HPLC systems have been described elsewhere [14,15]. The Neurochem analyser was set to run a mixed linear and step gradient with a reversed-phase C_{18} column, allowing the separation of 24 compounds.

Mobile phase A was 0.1 *M* sodium phosphate containing 10 mg/ml of sodium dodecyl sulphate at pH 3.35 and mobile phase B was methanol–water (1:1, v/v) containing 50 mg/l of sodium dodecyl sulphate at pH 3.45. The sixteen serial electrodes were set in an incremental 60-mV array from 0 to 900 mV. The column and electrodes were maintained at 34°C throughout the run. Data from each electrode were collected on the computer and stored to hard disk for post-run analysis. Each compound would typically be detected on three electrodes, with an average ratio of peak heights between the electrodes of 1:6:1. However, the exact ratio was specific for each compound and could be used to establish the purity of the compound in unknown peaks in the sample eluting from the column at the same time as a known standard.

Special compound recognition algorithms are used with the Neurochem analyser which could match standards with unknowns using both retention time and ratios across the electrodes on which the compound was detected. Final concentration data were calculated based on a comparison of the peak height on the dominant electrode of a known standard with that of dominant unknown peaks in the sample.

RESULTS AND DISCUSSION

Dopamine (DA), tyramine (TYRA), vanillic acid (VA) and octopamine (OA) were detected in the corpus cardiacum (Fig. 1A and B). As amino acids, high levels of tyrosine (TYR-4) and tryptophan (TRP) were also detected. Table I gives the biogenic amine levels in the carpus cardiacum. The levels of DA are considerably lower than those reported for the *Locusta* corpus cardiacum [4] (this level is also considerably higher than that reported by Lafon-Cazal [16]). The OA levels in *P. americana* are similar to those in *Locusta* reported by other workers [16,17]. OA appears to be the neurotransmitter mediating the release of the hyperlipaemic hormone from these glandular cells of the locust [17]. Downer *et al.* [5] suggested that DA is present in the corpus cardiacum of the American cockroach, *Periplaneta americana.*

On treatment with acetone, the levels of DA, TYRA and OA were dramatically increased, whereas the VA level decreased (Fig. 1C; Table I). There is no report on the measurement of the biogenic amine levels in the corpus cardiacum following injection of a chemical stressor. OA, one of these amines, has a neurohormonal role in insects. In *Schistocerca gregaria* the concentration of octopamine in the heamolymph increased drastically during the first 10 min of flight [18] and in response to stress

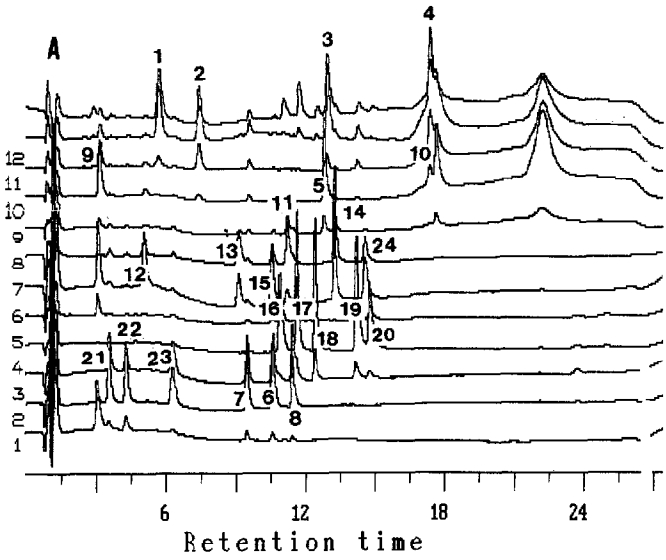

Fig. 1.

(Continued on p. 196)

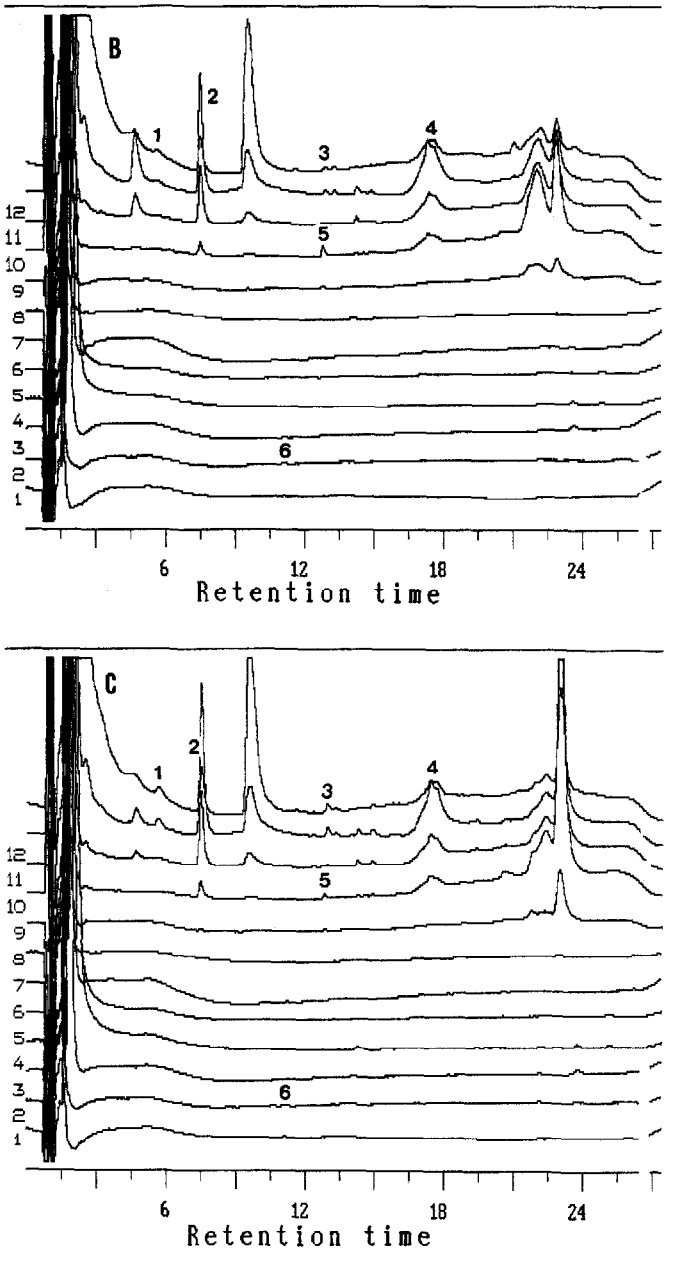

Fig. 1. Chromatograms of (A) standard, (B) sample of the normal carpora cardiaca and (C) sample of carpora cardiaca following injection of 2 μl of acetone solution. Peaks: 1 = octopamine (OA); 2 = tyrosine (TYR-4); 3 = tyramine (TYRA); 4 = tryptophan (TRP); 5 = vanillic acid (VA); 6 = dopamine (DA); 7 = 3,4-dihydroxyphenylacetic acid (DOPAC); 8 = epinine (EPIN); 9 = 4-hydroxy-3-methoxymandelic acid; 10 = melatonin; 11 = metanephrine; 12 = methoxyhydroxyphenyl glycol; 13 = normetanephrine; 14 = homovanillic acid; 15 = methyldopa; 16 = hydroxytyptophan; 17 = hydroxyindoleacetic acid; 18 = acetylserotonin; 19 = serotonin; 20 = methylserotonin; 21 = norepinephrine; 22 = dopa; 23 = epinephrine; 24 = methoxytyramine. Retention time in min.

TABLE I

BIOGENIC AMINE LEVELS IN THE CORPUS CARDIACUM OF *PERIPLANETA AMERICANA*

Treatment	Amount found in corpus cardiacum (pg)							
	TYR-4	DA	TYRA	DOPAC	VA	TRP	EPIN	OA
Untreated	2031.000 ± 22.20	8.100 ± 1.90	35.700 ± 10.70	N.D.[a]	113.400 ± 0.20	147.600 ± 0.00	N.D.	43.100 ± 15.50
Acetone	2655.300 ± 92.10	25.200 ±0.00	121.800 ± 0.00	N.D.	49.200 ± 1.20	N.D.	N.D.	151.100 ± 0.90

[a] N.D. = Not detected.

[19,20]. Under these conditions, circulating OA may act as a sympathetic or stress hormone, increasing the availability of carbohydrate and lipid and stimulating the oxidation of these substances [21]. However, our finding is the first report that the OA, DA and TYRA contents per single corpus cardiacum increased on treatment with acetone solution in comparison with that of a control. As OA in the ganglion is synthesized via TYRA from TYR-4 in the central nervous system of *Manduca sexta* [22]. OA-involved enzyme activity in the ganglion under the influence of a chemical stressor such as acetone may be high. It would be interesting to investigate the induction of OA-involved enzyme activity by chemical stressors.

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