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Short Communication

Detection of octopamine in an insect ganglion by high-performance liquid chromatography–native fluorescence

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ABSTRACT

Biogenic amine levels in the suboesophageal ganglion of the cabbage armyworm larvae, *Mamestra brassicae* (Lepidoptera; Noctuidae), were investigated by high-performance liquid chromatography with native fluorescence detection. The procedure involves direct injection of the sample onto a silica-octadecylsilane high-performance liquid chromatography column with the eluted fractions detected using a dual-fluorescence detector. The suboesophageal ganglion of wandering-stage larvae contained dopa, octopamine, tyramine, tyrosine and related metabolites. Fluorometric detection offers an alternative to laboratories that do not have access to electrochemical detection systems.

INTRODUCTION

High-performance liquid chromatography (HPLC) with dual-channel coulometric electrochemical detection (ED) is now the technique of choice for analysis of phentolamines [1]; however, the technique has not been applied extensively to analysis of the monohydroxy analogues of catecholamines [2]. The infrequent use of HPLC–ED for determination of phentolamines is due in part to the high electrode potentials that are required to effect electro-oxidation of these compounds [3].

On the other hand, HPLC with native dual-fluorescence detection is suitable for simultaneous analysis of both indolealkylamines (λ_{em} 350 nm) and catecholamines (λ_{em} 315 nm) in the same biological sample with excitation at 280 nm.

The biogenic amine octopamine occurs in the suboesophageal ganglion (SG) and has been measured by radioenzymic assay in the larvae of two species of Lepidoptera, the noctuid moth *Spodoptera littoralis* and the sphingid moth *Manduca*

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sexta [4]. This report describes the first use of HPLC–fluorescence detection for analysis of monoamines in Lepidoptera.

EXPERIMENTAL

Insects

The larvae of the cabbage armyworm, *Mamestra brassicae*, were reared at 20°C on an artificial diet [5] under a 16 h light–8 h dark photoperiod. Wandering-stage larvae were used in all experiments.

Sample preparation of suboesophageal ganglia

The SG were excised from female and male larvae ($n=30$) at constant time of the diurnal cycle. They were rinsed well in Ringer solution [240 m*M* sodium chloride, 2.7 m*M* potassiumchloride, 0.4 m*M* calciumchloride and 2.38 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES)] to eliminate contamination from octopamine in the haemolymph. The pH of the solution was adjusted to 7.2 with sodium hydroxide. Tissues were gently homogenized in 0.1 *M* hydrochloric acid solution in a cooled microhomogenizer with a glass pestle, and the resulting homogenate centrifuged at 10 000 *g* for 10 min. The remaining supernatant was then filtered and dried using a rotary evaporator with reduced vacuum pressure. Dry samples were reconstituted with a known volume of 0.1 *M* phosphate buffer solution (pH 2.5) so that a 70-μl aliquot provided an appropriate sample for injection onto the HPLC column. The estimate of recovery of extracted amines was precisely checked. It was found that no loss of sample occured during the extraction, centrifugal filtration (UFC3 OHV, Millipore) and drying process.

HPLC apparatus

The chromatographic system (Japan Spectroscopic, Hachioji, Japan) consisted of two BIP-I liquid chromatography pumps, a system controller (801-SC), an autosampler (850-AS), an 829-01 column selection unit, a two-pen recorder and a two-wavelength fluorimetric detector (FP-540 D). A 15 cm × 6 mm I.D. analytical column packed with silica-octadecylsilane (ODS) (ERC-1161 A, Erma, Japan) was used. Two kinds of HPLC mobile phases (solvent) were used. As external standard, a mixture of reference compounds including 3,4-dihydroxyphenylethyleneglycol (DOPEG), noradrenaline (NA), adrenaline (A), 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine (DA) (100 ng/ml of each) was also prepared.

Chemicals and reagents

Chemicals for analyses were of analytical reagent grade. All amines and related compounds were purchased from Sigma (St. Louis, MO, USA).

RESULTS AND DISCUSSION

Typical chromatograms obtained from standard compounds and from extracted SG are illustrated in Fig. 1A and B. The HPLC with native fluorescence detection procedure demonstrated that dopa (DOPA), octopamine (OA), tyrosine (TYR), tyramine (TYRA) and related metabolites could be measured readily in the SG. The

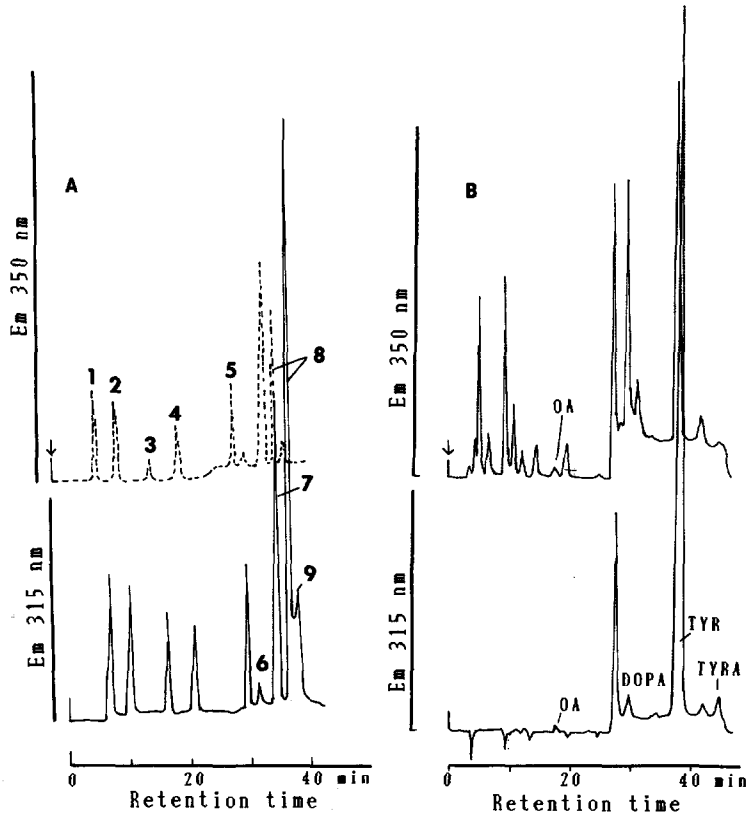

Fig. 1. Representative chromatograms of HPLC with native fluorescence detection. Analytical solvent elution time for stepwise gradient was 20 min (pump A) and 40 min (pump B), sequentially. The mobile phase 1 (pump A) consisted of 120 mg 1-octanesulphonic acid sodium salt and 50 mg disodium EDTA in 0.1 *M* phosphate buffer (pH 2.5), and solvent 2 (pump B) was prepared by adding 37 ml of acetonitrile to 1 l of solvent 1. The flow-rate was 0.8 ml/min, and injection volumes solvent were 100 μl. (A) Chromatogram of standard compounds. Peaks: 1 = DOPEG, 5 ng; 2 = NA, 5 ng; 3 = OA, 5 ng; 4 = A, 5 ng; 5 = DOPA, 5 ng; 6 = DOPAC, 15 ng; 7 = DA, 10 ng; 8 = TYR, 1000 ng; 9 = TYRA, 5 ng. (B) Typical chromatogram of the suboesophageal ganglion (n = 30).

response at 315 nm for the octopamine standard is better than at 350 nm (Fig. 1A), whereas in Fig. 1B an octopamine peak is identified at 350 nm and there is a weak corresponding peak at 315 nm. Accordingly, it is suggested that the octopamine peak in Fig. 1B contains octopamine and other substance(s) which responded better at λ_{em} = 315 nm than at λ_{em} = 350 nm, and shows the same retention time as octopamine. To clarify this possibility, we can check this peak at different wavelengths. The HPLC–fluorescence detection system may be of use in a plot of octopamine fluorescence at different wavelengths.

Octopamine has been found by Martin *et al.* [5,6] and Bailey *et al.* [2], who also detected dopamine, 5-hydroxytryptamine and tyramine in the nerve cord of the American cockroach, *Periplaneta americana*, using the HPLC–ED procedure. Similarly, Robertson [7] has determined the octopamine, dopamine and noradrenaline content of the brain of the locust, *Schistocerca gregaria*, using a sensitive radiochem-

ical enzymic assay. Octopamine and dopamine were present in high concentration, but the noradrenaline content was only 1/25 that of octopamine. The octopamine content of SG from 6th-instar *Spodoptera littoralis* was 0.97 pg per tissue (measured by radioenzymic assay) [4]. The octopamine values are similar to those in the SG of *Mamestra* SG (0.497 pg) using HPLC with native fluorescence detection. Radioenzymic assay for octopamine has been criticized for lack of specificity [8], and the levels using HPLC are lower than those using radioenzymic techniques. Therefore, direct comparisons are difficult because of the variety of analytical methods.

In the present experiments, a high level of tyrosine was found in the SG (Fig. 1B). Vaughan and Neuhoff [9] reported the presence of tyrosine in the cerebral and thoracic ganglia of *Schistocerca gregaria*. Tyrosine is well known as a precusor of two pathways, tyramine–octopamine and L-dopa–dopamine metabolites, but the biological function in the ganglion is not clear. Octopamine is synthesized from tyrosine in the thoracic ganglia of *Manduca sexta* [10]. Octopamine in the brains of the bertha armyworm, *Mamestra configurata*, is also synthesized from L-tyrosine [11].

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