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SIMULTANEOUS DETERMINATION OF BIOGENIC AMINES, THEIR PRECURSORS AND METABOLITES IN A SINGLE BRAIN OF THE CRICKET USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION

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SUMMARY

An analytical procedure has been developed for the simultaneous determination of biogenic amines, their precursors and metabolites by high-performance liquid chromatography with amperometric electrochemical detection. Following careful adjustment of various factors involved in the separation efficiency, reversed-phase chromatography with an ion-pairing technique gave simultaneous separation of nineteen biogenic amines and related substances. Peak identification was confirmed by comparison with hydrodynamic voltammograms. The method was sensitive enough to detect each substance in the picomole range. The procedure was applied to quantitate the amount of biogenic amines in a single brain of the cricket.

INTRODUCTION

Biogenic amines, such as dopamine (DA), norepinephrine (NE), epinephrine (E), octopamine (OA) and 5-hydroxytryptamine (5-HT), play an important role in the regulation of the nervous system, in both vertebrates and invertebrates. These amines act as neurotransmitters through chemical synapses.

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Some biogenic amines are also known to modulate synaptic transmission and muscle contraction, acting as neuromodulators [1–7]. In addition, they may perform a hormonal function to regulate physiological processes [8–12].

To understand the mechanism of such control and regulation, neurochemical analysis of biogenic amines, including their precursors and metabolites, should be performed. High-performance liquid chromatography (HPLC) with electrochemical detection (ED) has been widely used to determine biogenic amines in the nervous system (see refs. 13–15 for reviews). However, simultaneous determination of catecholamines, monohydroxyphenolamines and indoleamines, including their precursors and metabolites, has in general been unsuccessful, except for a few studies [16–21], most of which have used HPLC with coulometric ED. This is mainly for two reasons. Firstly, since the separation efficiency of HPLC is influenced by many parameters, such as the composition, the pH and the ionic strength of the mobile phase, the concentrations of the organic modifier and the ion-pairing reagent, the temperature, the flow-rate, and so on, it is difficult to optimize these conditions. Secondly, such parameters also affect the sensitivity and stability of detection. Furthermore, compounds such as OA, tyramine (TA), synephrine (SN) and tryptophan require a higher electrode potential than catecholamines and indoleamines in order to facilitate electro-oxidation [22–24]. A higher electrode potential causes a reduction in the sensitivity and reproducibility of detection.

The aim of this study was to overcome these difficulties and to develop a simultaneous analytical procedure to determine the wide range of substances related to the metabolism of biogenic amines, including precursors and metabolites of catecholamines, monohydroxyphenolamines and indoleamines, by use of amperometric detection. This paper describes a simple procedure in which NE, E, DA, OA and 5-HT and their precursors, 3,4-dihydroxyphenylalanine (DOPA), tyrosine, tryptophan, TA, deoxyepinephrine (epinine, EN), 5-hydroxytryptophan (5-HTP) and their metabolites, SN, 3-methoxytyramine (3-MTA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA), normetanephrine (NMN), metanephrine (MN), 3,4-dihydroxymandelic acid (DOMA), 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, VMA), 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindole-3-acetic acid (5-HIAA) and 6-hydroxymelatonin (6-HM) can be measured. The procedure described here provides a useful method for investigating the synthesis and metabolism of biogenic amines in the central nervous system.

EXPERIMENTAL

Reagents

All standards [L-tyrosine, L-β-3,4-dihydroxyphenylalanine, L-tryptophan, tyramine hydrochloride, 3,4-dihydroxyphenethylamine hydrochloride, 5-hy-

droxy-L-tryptophan, D,L-octopamine hydrochloride, norepinephrine, epinephrine, deoxyepinephrine hydrochloride, 5-hydroxytryptamine creatinine sulphate complex, D,L-synephrine, 3-methoxytyramine hydrochloride, D,L-normetanephrine hydrochloride, D,L-metanephrine hydrochloride, 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid, D,L-3,4-dihydroxymandelic acid, D,L-3-methoxy-4-hydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol, 5-hydroxyindole-3-acetic acid, 6-hydroxymelatonin, 3,4-dihydroxybenzylamine hydrobromide (DHBA) and 5-hydroxyindole (5-HI)] were obtained from Sigma (St. Louis, MO, U.S.A.), as was the disodium salt of ethylenediaminetetraacetic acid (disodium EDTA). Monochloroacetic acid and perchloric acid were obtained from Wako (Osaka, Japan). Sodium-1-octanesulphonic acid (SOS) was obtained from Aldrich (Milwaukee, WI, U.S.A.) and acetonitrile from Nakarai (Kyoto, Japan). All chemicals were analytical-reagent grade.

Internal standard and the quantification

Standard solutions were prepared at a concentration of 1 mg/ml in the mobile phase and stored at −80 °C. Working concentration was achieved by serial dilution in the mobile phase. Deionized and double-distilled water was used throughout. Quantitations, based on peak height of the chromatograms, were obtained by calculating the ratio of the peak height of the substance to the peak height of the internal standard. Concentrations were obtained by the comparison of ratios between the sample and standard chromatograms. In this study, DHBA was used as the internal standard, however, using this procedure, 5-HI could also be used as another internal standard.

Animals

Adult male crickets were taken at one week after the adult eclosion from a colony of *Gryllus bimaculatus* DeGeer reared at 28 ± 0.5 °C under a 14 : 10 light–dark cycle. Insects were isolated 3 h prior to dissection. The supraesophageal ganglion (brain) was quickly removed and immediately frozen in liquid nitrogen and stored at −80 °C before use.

Sample preparation

The brain was homogenized with a micro glass homogenizer (Wheaton, Milluille, NJ, U.S.A.) in 30 μl of ice-cold 0.1 *M* perchloric acid containing the internal standard, DHBA. After stirring for 30 min on ice, the homogenate was centrifuged at 15 000 *g* for 30 min at 4 °C. The supernatant was injected directly onto the HPLC column.

Apparatus

Chromatography was carried out using a solvent-delivery pump (Shimadzu LC-6A, Kyoto, Japan). Samples were injected through an injection valve

(Rheodyne 7125, Cotati, CA, U.S.A.) fitted with a 20-μl sample loop. Separations were performed on a Chemcosorb C_{18} reversed-phase column (250 mm × 4.6 mm I.D., 5 μm average particle size) (Chemco, Osaka, Japan) maintained at 30°C in a column oven (Shimadzu CTO-6A).

An amperometric electrochemical detector with a glassy carbon electrode (Shimadzu L-ECD-6A) was used. The detector potential was usually set at 0.95 or 1.0 V versus an Ag/AgCl reference electrode. In order to obtain hydrodynamic voltammograms, step-by-step reductions of the applied potential were made from 1.0 to 0.3 V. The detector cell was held at a constant temperature of 30°C by placing in the column oven. Signals from the electrochemical detector were recorded and integrated using a data processor (Shimadzu C-R3A).

Chromatography

The mobile phase contained 0.18 *M* monochloroacetic acid, 0.16 *M* sodium hydroxide, 50 μM disodium EDTA, with 1.85 m*M* SOS as the ion-pair reagent and 8.5% (v/v) acetonitrile as the organic modifier. The pH was finally adjusted to 3.6 by the addition of sodium hydroxide. The mobile phase buffer was filtered through a 0.22-μm filter (Millipore, GVWP 04700, Bedford, MA, U.S.A.) and degassed under vacuum. The buffer was changed between runs. The flow-rate was kept constant at 0.7 ml/min.

RESULTS

Chromatographic conditions

The relationship between the retention time and the pH of the mobile phase was examined at pH 2.8–4.2 (data not shown). The pH value of the eluent was held at 3.6 in order to provide an optimal time of analysis, resolution and sensitivity (see Discussion). The effects of the concentration of the ion-pair reagent and the organic modifier on the retention time were examined. The retention times of the amines and their basic metabolites were dependent upon an increase in the concentration of SOS. The concentration of SOS did not markedly affect the retention time of the acidic and neutral metabolites within the tested range (Fig. 1). As for the precursor amino acids, the retention times of tryptophan and 5-HTP increased with increasing concentration of SOS, but those of tyrosine and DOPA were not affected (Fig. 1). The relationship between the retention time and the concentration of SOS reflects the degree of dissociation of the functional groups of the substances. At high concentrations of SOS, the separation between DOPAC and OA between 3-MTA, 5-HT and 6-HM is not satisfactory. In addition, the time of analysis was too long. Therefore, optimal separation was achieved at a concentration of 1.85 m*M* (400 mg/l) SOS.

Fig. 2 shows the effect on the retention time of altering the concentration of acetonitrile. Since the decrease of the retention time of the individual sub-

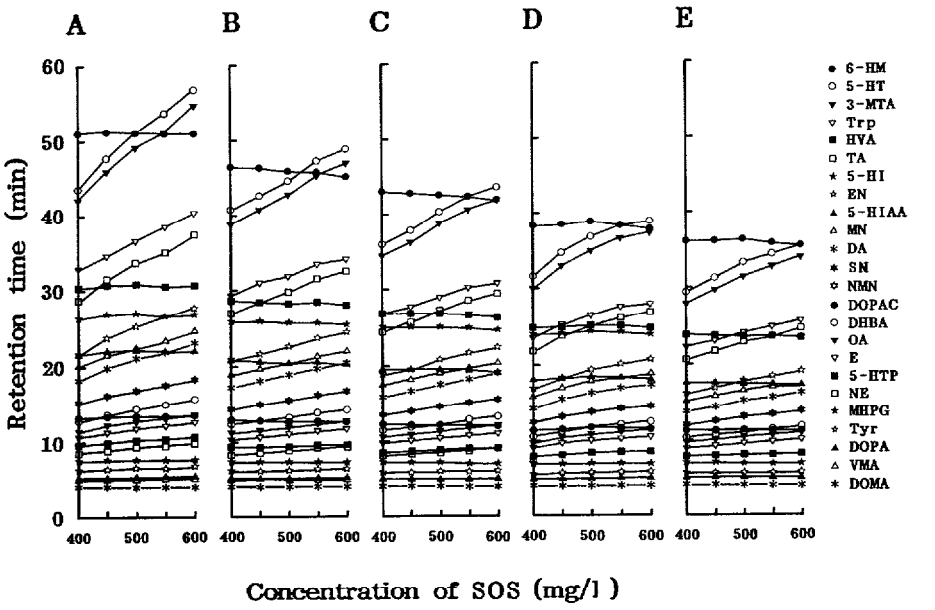

Fig. 1. Effect of the concentration of the ion-pair reagent (SOS) on the retention times of biogenic amines and related substances at various fixed concentrations of acetonitrile. Column, Chemcosorb C_{18} (250 mm×4.6 mm I.D., 5 μm); mobile phase, 0.18 *M* monochloroacetic acid–0.16 *M* sodium hydroxide–50 μM disodium EDTA–SOS–acetonitrile; pH 3.6; temperature, 30.0 °C; flow-rate, 0.7 ml/min; electrode potential, 0.95 V vs. Ag/AgCl reference electrode. Concentrations of acetonitrile: (A) 80 ml/l; (B) 85 ml/l; (C) 90 ml/l; (D) 95 ml/l; (E) 100 ml/l.

stances was not proportional to the concentration of acetonitrile, the order of elution was altered by changing the concentration of acetonitrile. Fine adjustment of the concentration of acetonitrile (8.5%, v/v) was needed to obtain the maximal separation of 5-HI, TA, HVA and tryptophan at a concentration of 1.85 m*M* SOS (Fig. 3). Under these conditions, a flow-rate of 0.7 ml/min was found to result in the best resolution.

Detection

The selection of the detector potential is important for the detection sensitivity, stability and the determination of peak purity. Fig. 4 shows the hydrodynamic voltammograms of the standard solution. The substances can be classified according to their electrochemical behaviour into four groups: catechol compounds (dihydroxyphenols), indoles, vanillic compounds and monohydroxyphenols. Catechol compounds, which have two hydroxyl groups on the benzene nucleus, initiated a current at an applied voltage of ca. 300 mV and showed the lowest half-wave potential ($E_{1/2}$) of 380–500 mV. For indoles, a current was initiated at ca. 400 mV and the $E_{1/2}$ was 480–520 mV. Vanillic compounds, which are 3-methoxylated metabolites of catecholamines, re-

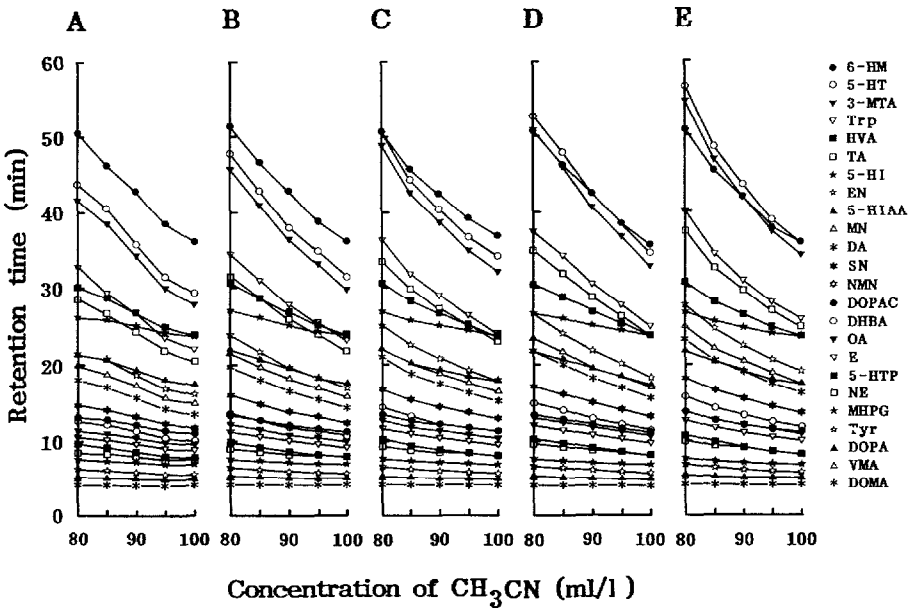

Fig. 2. Effect of the concentration of the organic modifier (acetonitrile) on the retention times of the biogenic amines and related substances at various fixed concentrations of SOS: (A) 400 mg/l; (B) 450 mg/l; (C) 500 mg/l; (D) 550 mg/l; (E) 600 mg/l. Other conditions as in Fig. 1.

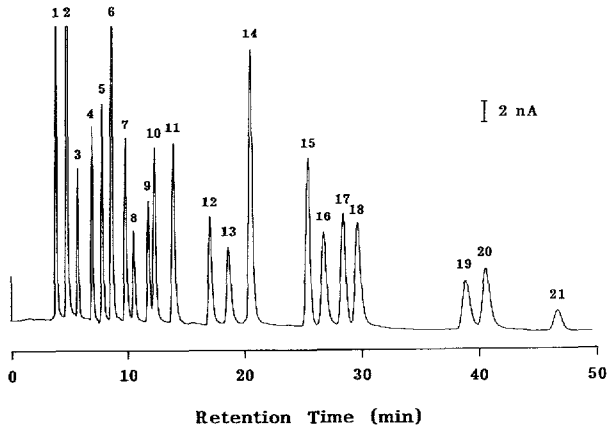

Fig. 3. Typical chromatogram of a standard mixture of 2 ng of each biogenic amine and related substance. Chromatographic conditions, 1.85 m*M* SOS and 8.5% (v/v) acetonitrile; sample volume, 20 μl. Other conditions as in Fig. 1. Peaks: 1=DOMA; 2=VMA, DOPA; 3=tyrosine; 4=MHPG; 5=NE; 6=5-HTP; 7=E; 8=OA; 9=DHBA; 10=DOPAC; 11=NMN, SN; 12=DA; 13=MN; 14=5-HIAA, EN; 15=5-HI; 16=TA; 17=HVA; 18=tryptophan; 19=3-MTA; 20=5-HT; 21=6-HM.

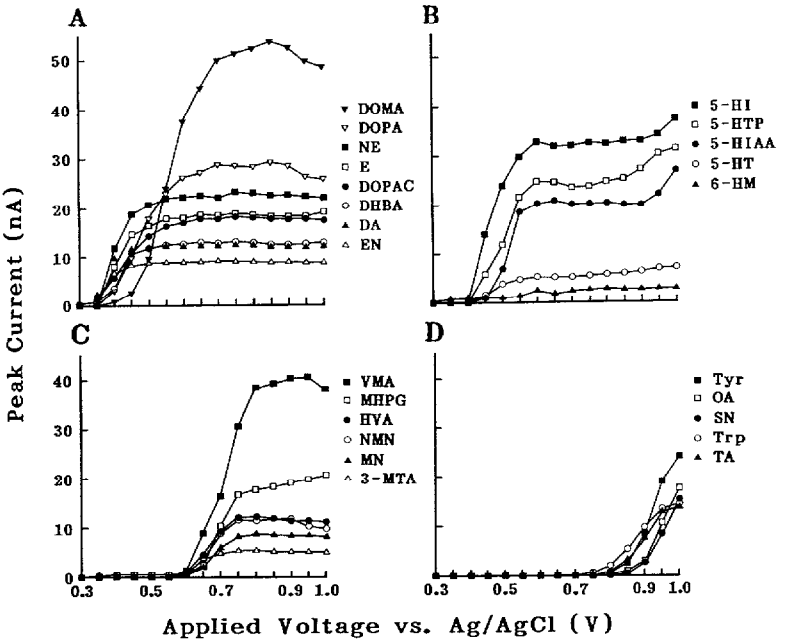

Fig. 4. Hydrodynamic voltammograms for standard substances obtained under the conditions described in the legend of Fig. 3. (A) Catechol compounds; (B) indoles; (C) vanillic compounds; (D) monohydroxyphenols and tryptophan.

quired higher applied voltages and showed higher $E_{1/2}$ values of 550 and 640–680 mV, respectively. Monohydroxyphenols and tryptophan required the highest applied voltage and showed the highest $E_{1/2}$ of 700 mV and more than 870 mV, respectively. Based on the results of hydrodynamic voltammograms, the detector potential for oxidative analysis was normally maintained at 0.95 or 1.0 V versus an Ag/AgCl reference electrode.

Linearity, selectivity and stability of the electrochemical detector

The linearity of the detector response was checked for the injected amount of standards. The response was linear for all substances in the tested range (0.2–40 ng) (data not shown).

It has been shown that the detection sensitivity increases with the potential applied to the electrode, until the response reaches the plateau region (Fig. 4); however, a higher applied potential results in an increase in the background current. In order to maintain good selectivity, sensitivity and stability of detection, the detector should be worked at the lowest oxidation potential. In particular, the background currents should always be monitored and used as an indicator of the stability of the electrode. When equilibration was first achieved at high sensitivity using a new electrode, the background current was

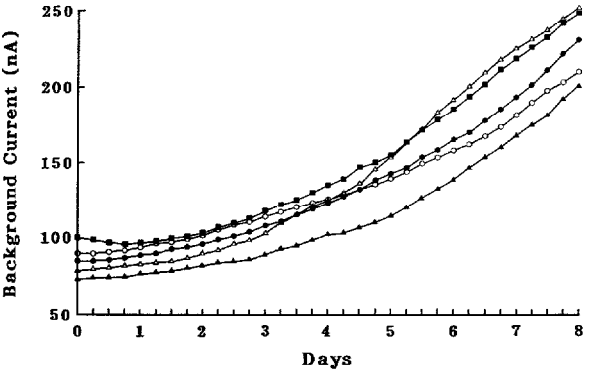

Fig. 5. Time course of the background current for five new electrodes. Data are shown by different symbols. Although measurements of the background current were taken every hour, data are only shown for every 6 h for clarity. Conditions as in legend to Fig. 3.

in the range 80–100 nA. The background current increased gradually with time (Fig. 5). A reduction of selectivity and sensitivity occurred at a background current of ca. 200 nA. The electrode was exchanged (the glassy carbon electrode of L-ECD-6A is disposable) when the background current increased above this value. In many cases, polishing the electrode with a fine aluminium oxide powder restored the sensitivity. The electrode was thoroughly sonicated in distilled water to wash out the aluminium oxide powder, which may cause catalysis of oxidation. When the equilibration of oxidation was achieved at the potential of 0.95 V using a polished electrode, the application of a few reduction potentials of 0.95 V and of 1–2 min duration resulted in an improved sensitivity and selectivity. The HPLC–ED system used in this study was working continuously without a significant loss of sensitivity for at least a week (ca. twenty injections per day) at an applied potential of 0.95–1.0 V.

Analysis of biogenic amines and their related substances in the central nervous system of the cricket

Typical chromatograms of a single brain of the cricket are shown in Fig. 6. Each peak was identified by comparison of both chromatographic and electrochemical behaviour with those of the standards; spiking the samples with standards provided additional proof. Furthermore, samples were rerun under different pH, SOS and acetonitrile conditions to confirm the peak identification. Samples and standards were injected alternately for identification and quantitative analysis. The contents of biogenic amines and their related substances in the brain are given in Table I. Three biogenic amines (OA, DA and 5-HT), two precursors (TA and tryptophan) and two metabolites (SN and 5-HIAA) could be detected. Results showed that 5-HT occurred in the largest amount followed by OA, and there was about two times as much OA as DA.

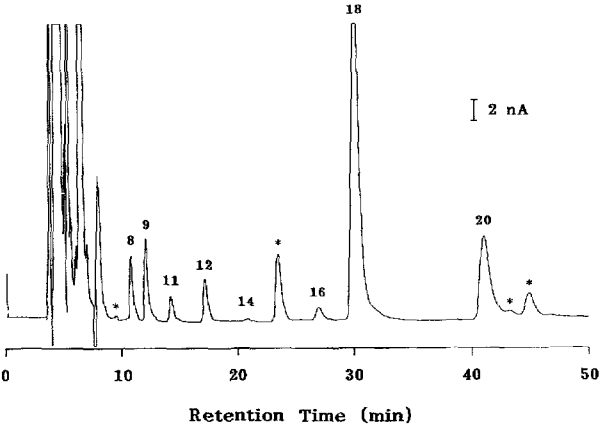

Fig. 6. Typical chromatogram of the extract from a cricket brain. Conditions and peak identification as in legend to Fig. 3. The peaks marked with asterisks could not be identified.

TABLE I

CONCENTRATIONS OF BIOGENIC AMINES AND RELATED SUBSTANCES IN THE CRICKET BRAIN, ESTIMATED BY HPLC–ED

Data are mean ± S.D. of five brains. Mean tissue weight, 1.03 ± 0.10 mg. N.D. = not detectable.

Compound	Concentration (pmol per brain)
Precursors	
Tyramine	1.09 ± 0.11
Tryptophan	43.16 ± 7.47
Amines	
Octopamine	13.59 ± 2.09
Dopamine	7.12 ± 0.57
Epinephrine	N.D.
5-HT	14.28 ± 3.22
Metabolites	
Synephrine	2.92 ± 0.25
Homovanillic acid	N.D.
Metanephrine	N.D.
5-HIAA	0.13 ± 0.02

DISCUSSION

Simultaneous determination of biogenic amines at high sensitivity

Quantitative analyses of biogenic amines, including their precursors and metabolites, in the nervous tissue and body fluids provides important information that can be used to elucidate the neuronal and hormonal functions of biogenic amines. The techniques for the quantitative analysis of biogenic amines have

improved rapidly over the past decade (see ref. 14 for review), resulting in the development of fluorometric, gas chromatographic–mass spectrometric (GC–MS) and radioenzymatic techniques. Biogenic amines are distributed throughout the nervous tissue and body fluids, but they occur in extremely small amounts. In order to obtain a good detection sensitivity of substances in a small volume of nervous tissue, it is necessary to detect these substances in at least the nanogram range. However, each of the methods mentioned above has both merits and demerits for the quantitative analysis of biogenic amines. Since the substances have different excitation spectra except for derivatised compounds, the fluorometric detection is not suitable for simultaneous determination. Although the GC–MS method offers a high sensitivity and specificity [25–27], it is not suitable for routine analysis since it is time-consuming and requires sample derivatization before analysis. Radioenzymatic assay techniques are also extremely sensitive [28–30], but they can determine only one compound at a time. Simultaneous determination of biogenic amines, including their precursors and metabolites, is essential to elucidate their physiological function. Reversed-phase HPLC cannot directly analyse highly polar and ionic compounds since they elute near the void volume. However, the application of the ion-pairing technique allows high-resolution separation of a wide range of ionizable substances, such as amines and organic acids [31–33]. The recent development of ED affords high sensitivity without elaborate sample pretreatment and time-consuming operation. Therefore, the combination of ion-pair reversed-phase HPLC and ED is ideally suited for the simultaneous determination of biogenic amines and related substances. In particular, simultaneous determination at high sensitivity is essential for the analysis of the functional role of biogenic amines in the microdissected region of the nervous tissue or in the small nervous tissue of insects.

Factors determining the efficiency of separation

The most critical factor necessary to develop a procedure for the simultaneous determination of a wide range of substances is the composition and condition of the mobile phase. In order to separate a mixture of basic, neutral and acidic substances, a fine balance between the pH, the ionic strength, the concentration of the ion-pairing reagent and the content of the organic solvent must be made.

Since the degree of protonation of a functional group depends on its pK_a value [34,35], the selection of the eluent pH is critical for ensuring complete ionization of the substances to be measured and for maximal ion-pairing. The retention of basic compounds, such as catecholamines, increased with increasing pH of the eluent under no ion-pairing conditions. However, basic compounds are unstable at higher pH values and the acidic metabolites cannot be efficiently separated [34].

Lowering the pH tends to protonate both the amines and their correspond-

ing acidic metabolites. Since the amino groups of the substances are completely protonated at lower pH values (2.4–3.8), the retention times of the amines are independent of the pH of the eluent. However, under ion-pairing conditions the ion-pair formation between the charged amino groups and the ion-pairing reagent increases the retention time [13,18,36]. On the other hand, the dissociation of the carboyxlic groups is gradually suppressed by a decrease in the pH of the eluent. The suppression of dissociation causes an interaction between the solute molecule and the hydrocarbonaceous phase. Thus, the acidic substances are retained longer at lower pH values. In the case of the dissociation of both the amino group and carboxylic group, the repulsion between the negatively charged carboxylic groups and the negatively charged anionic detergent suppresses ion-pair formation and thus results in a decrease of the retention time [34,36]. Therefore, in order to develop a procedure for simultaneous determination, the eluent pH should be lowered.

The application of the ion-pair technique to reversed-phase HPLC allows for the selective manipulation of the retention characteristics of either biogenic amines or their acidic metabolites. Addition of acetonitrile to the mobile phase as an organic modifier decreases the retention times of all substances to avoid a lengthy period of separation. The effects of the concentration of SOS and acetonitrile on the retention proved that the fine adjustment between the ion-pair reagent and the organic modifier is necessary for optimal resolution. These results can serve as a guide for the selection of the optimum concentration of SOS and acetonitrile for the isocratic separation of a particular mixture of biogenic amines and their related substances.

The selection of the column is also important for the best resolution. We examined six kinds of column with the same type of microparticular packing (C_{18}, 5 μm) supplied from different manufacturers, but they gave slightly different separations. The fine adjustment of the pH and the concentration of SOS and acetonitrile is found to be crucial for optimal separation using a different column.

Detection potential: hydrodynamic voltammogram

Since each electrochemically active compound has a characteristic oxidation potential, the electrochemical activity depends on the applied electrode potential. The substances are oxidized at distinguishable potentials when the applied potential is increased stepwise. Hydrodynamic voltammetry demonstrated the characteristic electrochemical behaviour of each substance. A comparison of the electrochemical behaviour of the sample and that of the standard provides considerable information concerning the peak identity [37]. Catechol compounds are oxidized to the corresponding orthoquinones, while indoles, which have phenolic hydroxy groups, are converted into quinone-imine. In each of these oxidation processes two electrons are lost to the electrode. The final product of the oxidation of vanillic compounds is the same as that of

catechol compounds. However, soon after the two-electron oxidative process, the positively charged methoxy group is hydrolysed. This requires a higher energy of activation and therefore a higher electrochemical potential [38]. Monohydroxyphenols lose one electron in the oxidation process. Although tryptophan, the precursor amino acid of indoleamine, has a phenolic hydroxy group, it showed similar electrochemical behaviour to monohydroxyphenol. The higher applied voltage required for the electrochemical reaction of tryptophan presumably indicates the conversion of indole into oxyindole [23,33]. These results suggested that the electrode reaction depends on the number and the position of the hydroxy groups attached to the phenyl group. In order to detect the biogenic amines and related substances, including monohydroxyphenols and tryptophan, at a sensitivity of 100 pg, a minimum electrode potential of 950 mV should be applied.

Electrode stability

The sensitivity and reproducibility of the electrochemical detector depends on the stability of the electrode. As shown in the hydrodynamic voltammograms, electrochemical activity increased with increasing detector potential. However, the use of a glassy carbon electrode at high potentials results in an increase of the background current, owing to the oxidation of water and mobile phase constituents. This often results in the rapid decay of performance. Thus, the use of an amperometric detector at high potentials has been avoided. On the other hand, simultaneous determination of biogenic amines and their related substances has been mainly developed by use of a coulometric detector [17,19–21]. However, the amperometric detector has many practical advantages over the coulometric one: simple structure of the detection cell, ease of electrode maintenance, rapid response time, ease of background current compliment, low cost, and so on. As described in Results, the sensitivity of the glassy carbon electrodes could be restored by polishing them with fine aluminium oxide powder. Thus the inherent problem of an amperometric detector at high potential can be easily overcome by the periodic maintenance of the working electrode.

Since high background currents decrease the signal-to-noise ratio and reduce the sensitivity and reproducibility of detection, high-purity water and chemicals must be used. Since the temperature affects not only the retention, but also the detector background current and noise [39], use of a column oven to maintain both the column and the detection cell at a constant temperature is recommended. Previous studies have demonstrated that the performance of polished glassy carbon electrodes can be improved by electrochemical pretreatment [40–42]. The pretreatment of polished electrodes by applying reduction potentials helped to increase their sensitivity and selectivity, supposedly by cleaning the surface of contaminants introduced during polishing.

The electrochemical behaviour of biogenic amines and related substances is

affected by the pH of the eluent, since their oxidation involves proton transfer. Since oxidation is suppressed by a decrease in the pH [37], the detector potential must be increased to obtain an enhanced electrochemical reaction. However, increasing the electrode potential also produces an increase in the background current. On the other hand, the best separation of biogenic amines and related substances is obtained at a lower pH value, as described above. For simultaneous determination using HPLC–ED, some compromises may be necessary to achieve a short analysis time and good resolution and detection sensitivity. In the present study, the separation efficiency was given the highest priority rather than the detection sensitivity.

Biogenic amines in the insect central nervous system

Our study has achieved a simultaneous determination of the biogenic amines, including their precursors and metabolites, in a single insect brain. The results showed that OA, DA and 5-HT are substantially distributed in the brain of the cricket. Fluorescence-based and radioenzymatic assay techniques have revealed that these three biogenic amines are major substances in the brain of the locust [28,30,43,44] and the cockroach [28,29,45,46]. The high concentration of OA, DA and 5-HT in the brains of insects suggest that these amines may play an important common role in the control of physiological processes in insects. In particular, OA and 5-HT are present in substantial amounts compared with their metabolites, synephrine and 5-HIAA. No epinephrine was detected in the brain of the cricket, as is the case in many other insect species (see ref. 47 for review).

Previous studies [29,43,44,48,49] have demonstrated that small amounts of NE are present in the brain of many insect species. However, our procedure cannot provide an analysis of NE in the brain of the cricket, since the peak of NE was eluted in the void volume. For such an analysis, the procedure must be further developed by using either a suitable clean-up step or an optimization scheme that slows NE away from the early-eluted volume.

The versatility of the present procedure serves as a useful method for the analysis of biosynthesis and metabolism of biogenic amines in the central nervous system. We have shown that it is possible to detect biogenic amines and related substances using tissue from a single insect at a time. Therefore, this procedure is also useful for the study of the neurochemical basis of behaviour. This analysis can provide important information to elucidate the functional role of biogenic amines in the central nervous system.

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