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Simultaneous measurement of serotonin, catecholamines and their metabolites in mouse brain homogenates by high-performance liquid chromatography with a microbore column and dual electrochemical detection

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ABSTRACT

A dual electrochemical detector with two working electrodes (anode and cathode) suitable for high-performance liquid chromatography with a microbore octadecylsilica column was applied for the simultaneous measurement of norepinephrine, epinephrine, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxyindoleacetic acid, 3-methoxytyramine and 5-hydroxytryptamine (serotonin) in mouse brain homogenates. Microbore high-performance liquid chromatography provides very good resolution of these analytes and offers selective detection of biogenic amines and their metabolites on the basis of their retention behaviour and electrochemical reversibility. The large early-eluting peak of brain homogenates was eliminated on cathodic detection, thereby providing reliable measurements of early eluates. The detection limit of this method was *ca.* 0.2–0.5 pg per injection for all components, at a signal-to-noise ratio of 3. Owing to the high sensitivity, the brain tissue samples could be kept very small (less than 10 mg). Isocratic separation of these analytes was achieved within 15 min; hence over 90 analyses could be performed in a single working day. This simple, efficient and sensitive method can be used as a basic research tool for the assaying of biogenic amines and their metabolites in brain homogenates.

INTRODUCTION

Measurement of biogenic amines in brain homogenates is an important research strategy commonly used in evaluating the etiology of neuroendocrinological disorders [1,2], and in studying the role of the autonomic nervous system in various physiological or pathophysiological conditions in animal models [3–5]. Many analytical procedures have been devised for these purposes. The quantitative methods for the determination of these compounds employ gas chromatography–mass spectrometry (GC–MS) [6], radioimmunoassay (RIA) [7] and various high-performance liquid chromatography (HPLC) techniques

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with fluorescence and electrochemical detection (ED) or multiple-working electrode detection [8,9]. Recently, HPLC–ED has become a well established and popular assay method for trace levels of biogenic amines in plasma, cerebrospinal fluid, and brain homogenate [10–12]. These biological samples have to be cleaned up prior to HPLC. The catecholamines norepinephrine (NE), epinephrine (E) and dopamine (DA) were usually isolated by retention on alumina [13,14]. The acidic metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were most frequently extracted with ethyl acetate [15,16]. 3-Methoxytyramine (3-MT) and 5-hydroxytryptamine (serotonin, or 5-HT) were generally isolated by ion-exchange chromatography [17,18]. However, most published methods involving the use of conventional extraction procedures appear to have drawbacks, such as the limited number of compounds that can be extracted, low or unstable recoveries caused by difficulties in sample preparation, and the degradation of these analytes owing to time-consuming procedures.

A new trend in the investigation of biogenic amines in brain homogenates is the determination by direct injection of the filtrate of the tissue homogenate into an HPLC system. Advantages of this technique are its speed, minimal sample preparation and low degradation of the compounds analysed. Disadvantages are the incomplete chromatographic separation of all the compounds, resulting in lower selectivity, less reliable quantitative interpretations and longer analysis time. An additional problem is associated with the size of the brain tissue, which is too small, or the concentrations of several constituents in the brain tissue, which are well below the detection limits (4–40 pg per injection) of conventional HPLC–ED assays [19]. Recently, a more sensitive HPLC technique using a microbore packed column with a small-volume flow-cell electrochemical detector [20] has been described for the measurement of biogenic amines. Sub-picogram amounts were detected by HPLC–ED by Huang *et al.* [21] and Cheng *et al.* [22]. In addition, the consumption of mobile phase and sample is much less than that in conventional HPLC. This methodology is routinely applied in many laboratories.

A problem frequently encountered in the analysis of brain homogenates with HPLC–ED is the unreliable identification of sample constituents. Most previous reports on preliminary identification used the comparison of capacity factor (k') (or retention time) of sample constituents and standard compounds [23]. A reliable assignment of peak identity requires the additional determination of other characteristics of a compound. This often involves the collection of fractions eluting from the column, and the confirmation by conventional mass and/or NMR spectra of the fractions. Nevertheless, this verification is difficult if confirmation is needed for several sample constituents, especially at trace levels. Obtaining further information on collected fractions at the picomolar level is all but impossible using the current instrumentation. This can be solved by dual electrode ED (DUED) based on redox properties of the analytes [24,25]. These properties of catecholamines also offer an advantage in solving the large eluting front peak, which always gives rise to small peaks of NE, E and/or DOPAC in the conventional anodic chromatogram of brain homogenate. This front peak can be readily eliminated to obtain well separated peaks of NE, E and DOPAC in the cathodic HPLC chromatogram. Several studies have reported orientations of the dual electrode with respect to the flow axis, which has three configurations: parallel-adjacent, parallel-opposed and series [26]. A series DUED instrument was used in this study. The principle of its selective detection is the choice of potentials for the anode and cathode in the thin-layer flow-cell for oxidation and reduction of the analytes, respectively. Therefore, the reversible, irreversible and quasi-irreversible species can be discriminated from one another on the basis of their reversibility.

HPLC combined with series DUED offers the advantages of high selectivity and sensitivity to overcome the above problems. This paper describes the direct injection of the filtrate of brain homogenates for the simultaneous determination

of biogenic amines and their metabolites. Several aspects of this assay were considered, including the redox characteristics, analytical precision, accuracy and applications. This technique is a suitable tool that meets the requirement of routine brain research laboratories.

EXPERIMENTAL

Apparatus and chromatographic conditions

The HPLC–DUED system comprised a Beckman 126 pump (Beckman Instruments, Taiwan Branch), a CMA-200 microautosampler (CMA/Microdialysis, Stockholm, Sweden), and a microbore reversed-phase column filled with Inertsil ODS-2 (GSK-C_{18}, 5-μm ODS, 150 mm × 1.0 mm I.D., Japan). A Heidolph homogenizer (RZR 2051, Heidolph Elektro, Kelheim, Germany) was used. The dual glassy carbon working electrode potentials were controlled independently by two BAS-4C amperometers (Bioanalytical Systems, West Lafayette, IN, USA) with respect to an Ag/AgCl reference electrode. The anodic and cathodic chromatograms were simultaneously recorded on a Beckman I/O 406 dual-channel interface and analysed via the Beckman System Gold data analysis software (Version 6.01, Beckman Instruments, Taiwan Branch). In order to increase the sensitivity of the microbore HPLC system, a very thin spacer (14 μm) was used instead of a conventional one (51 μm) to create a sub-microliter thin-layer electrochemical cell. Low flow-rates (60–75 μl/min) were used to minimize pulse fluctuation.

Chemicals and reagents

NE, E, DA, DOPAC, HVA, 3-MT, 5-HT, 5-HIAA, ethylenediaminetetraacetic acid (EDTA), diethylamine, sodium 1-octanesulphonate (SOS), monosodium dihydrogenorthophosphate, sodium citrate and isopropylene tryptamine (IPT, internal standard) were purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile and tetrahydrofuran (THF) were purchased from Merck (Merck-Schuchardt, Darmstadt, Germany). Unless otherwise stated, all reagents were of analytical quality.

Sample preparation and assay

Standard stock solutions of NE, E, DA, DOPAC, HVA, 5-HT, 5-HIAA, 3-MT, and IPT were prepared at a concentration of 2 ng/ml in 0.1 *M* perchloric acid. They were stored at −70°C in the dark and thawed in an ice-bath prior to preparation of a standard mixture. The internal standard solution and the standard mixture were prepared every day from a portion of these stock solutions after appropriate dilution with 10^{-7} *M* ascorbic acid in 0.1 *M* hydrochloric acid. ICR mice were obtained from the animal supply centre of Taichung Veterans General Hospital (TCVGH, Taichung, Taiwan). The mice were decapitated and their brain tissue samples were isolated and frozen quickly on dry ice and kept at −70°C until analysis [27]. Brain tissue samples were weighed frozen and were homogenized (500 rpm, 3 min) in ice-cold 0.1 *M* hydrochloric acid containing 10^{-7} *M* ascorbic acid and the internal standard (IPT). The homogenate was centrifuged (4°C, 6000 *g* for 15 min) to remove the precipitated protein and cell debris, then filtered through a Millipore 0.22-μm filter (Ultrafree-MC, Millipore, Bedford, MA, USA). The resulting filtrate (5-μl sample) was directly injected into the HPLC–DUED system.

Concentrations of NE, E, DA, DOPAC, HVA, 5-HIAA, 3-MT and 5-HT in brain homogenates were calculated by determining each peak-area ratio relative to the internal standard IPT in both anodic and cathodic chromatograms. For smaller samples of brain tissue (less than 20 mg), the method was slightly modified. The brain tissue sample punches were expelled into 20–50 μl 0.1 *M* hydrochloric acid containing IPT and ascorbic acid. This suspension was centrifuged and filtered by a Millipore centrifugal-driven Ultrafree-MC filter unit (0.22 μm), then 5 μl of the filtrate were injected into the HPLC–DUED system as previously described.

Mobile phase preparation

The mobile phase was prepared by adding 65 ml of acetonitrile, 6 ml of THF, 0.44 g of SOS (2.3 m*M*), 1.86 g of monosodium dihydrogenorthophosphate (13.7 m*M*), 8.82 g of sodium ci-

trate (30 m*M*), 9.29 mg of EDTA (0.025 m*M*) and 1 ml of diethylamine to doubly distilled water. The solution pH was adjusted to 3.1 with concentrated orthophosphoric acid to optimize the separation and resolution. Slight adjustment of the pH for each batch preparation was necessary to increase the resolution. The final volume of the mixture was adjusted to 1 l, and it was filtered through a 0.22-μm nylon filter under reduced pressure and degassed by sparging with helium for 20 min. The flow-rate was 60–75 μl/min at a column pressure of *ca.* 8.3 MPa.

RESULTS AND DISCUSSION

Choice of detection potentials

Hydrodynamic voltammograms of catecholamines were studied by Kissinger *et al.* [28] and Goto *et al.* [29]. The optimal applied potentials for anode and cathode were +0.60 and +0.20 V, respectively. In our previous studies, the applied anode potential in simultaneous measurements of catecholamines, serotonin and their metabolites was set at +0.75 V [22]. In the present experiment, this potential was also applied constantly to the anode while various potentials from −0.05 V to +0.50 V were applied to the cathode to determine the optimal potential for the cathode.

Typical anodic and cathodic chromatograms of a standard mixture of 100 pg of catecholamines, serotonin, their metabolites, and IPT (200 pg, internal standard) are shown in Figs. 1A and B. Fig. 2 shows the redox ratios of all analytes as a function of potentials applied on the downstream (cathodic) electrode under a fixed potential on the upstream (anodic) electrode at +0.75 V. It was found that the redox ratios of NE, E, DOPAC and DA markedly increased as the applied potential of the downstream decreased. These characteristics are similar to those of catechols in dual electrochemical detection. 3-MT and HVA, metabolites of DA, have very similar electrochemical characteristics because of a methoxy group on their aromatic rings. Their cathodic currents reach a plateau at applied potentials below +0.05 V. There were no cathodic currents for 5-HT and 5-HIAA at applied potentials of +0.10 V and above. The cathodic currents for 5-HT and 5-HIAA could be measured at a potential of +0.05 V and below, but not at very low concentrations (less than 10^{-8} *M*). These currents correspond to relatively complicated oxidation reactions of 5-hydroxyindoles, which were intensively studied by Wrona and Dryhurst [30] and Cheng *et al.* [31]. Therefore, an anodic potential of +0.75 V and a cathodic potential of +0.05 V were chosen for use throughout this study.

Quality of the method

Fig. 1A and B show typical anodic and cathodic chromatograms of a standard mixture, and Fig. 3A and B show those of a brain homogenate. The identities of the peaks were confirmed by their retention times, by standard addition and then by a superimposed alignment technique that was provided by Beckman System Gold software (Version 6.01). Each analysis was completed within 15 min. All components in Fig. 3A and B were well resolved and identical with those in Fig. 1A and B except for some unknown components.

Fig. 4A and B are typical chromatograms of a brain homogenate with the addition of all the standard analytes. Each peak was verified by addition of a standard mixture if the addition increased its height but did not change its shape. Although microbore HPLC provided a good resolution of all analytes in the anodic chromatogram in Figs. 3A and 4A, it was still difficult to measure the peak height or peak area of NE and/or E because of their asymmetric peak shapes and proximity to the large early-eluting peaks. This problem could be solved by measuring NE and/or E peak(s) reliably in the cathodic chromatograms in Figs. 3B and 4B, in which the early-eluting peaks were almost eliminated. In addition, the redox ratios of all analytes of brain homogenates can be very reliable in confirming their identities.

Some interfering peaks and the early-eluting peaks in HPLC methods reported by some investigators are attributed to substances that are either present in the brain formed during pretreatment [32]. If these interfering peaks co-elute

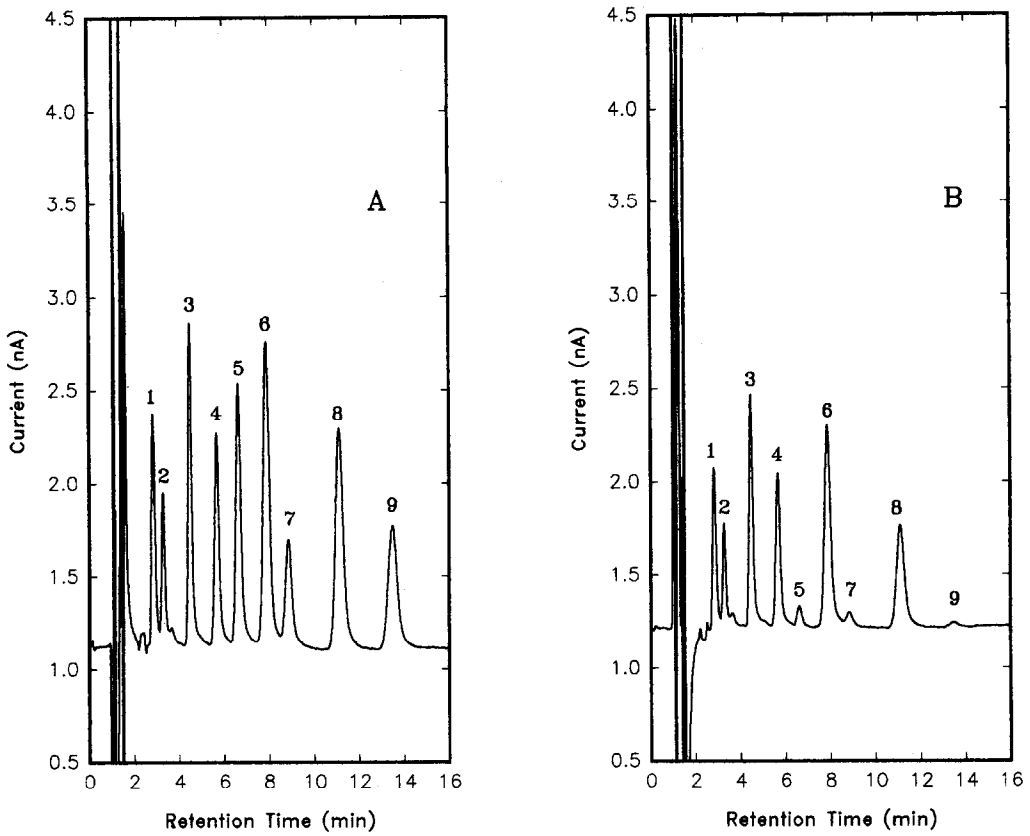

Fig. 1. Typical chromatograms of a standard mixture containing: (1) NE, 151 pg; (2) E, 141 pg; (3) DOPAC, 136 pg; (4) DA, 121 pg; (5) 5-HIAA, 114 pg; (6) IPT (as an internal standard), 117 pg; (7) HVA, 130 pg; (8) 3-MT, 117 pg; and (9) 5-HT, 110 pg. (A) Anodic current; (B) cathodic current. Applied potentials (*vs.* Ag/AgCl): anode, +0.75 V; cathode, +0.05 V.

with those catecholamines in the anodic chromatogram, the cathodic chromatogram can be an alternative way of detecting those analytes precisely.

In using our simple and rapid procedures, we also have had to pay attention to some interfering substances endogenous to the brain homogenates. Unknown substances appeared in chromatograms such as those shown in Fig. 3A and B. Some unknown substances have been proposed and/or identified [32]. However, most unknown substances have not been reported before and might be of importance. Further investigation of these substances would probably yield more detailed physiological information.

Precision and accuracy

The responses of the dual detector increased linearly with the amount injected between 5 pg

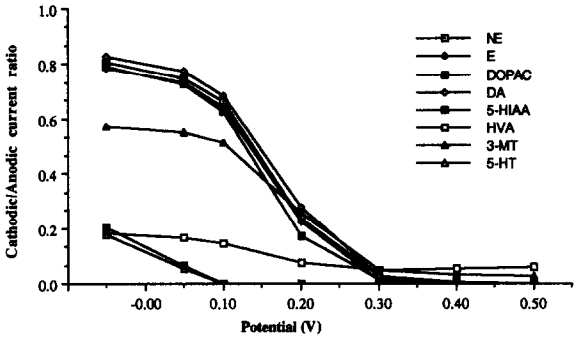

Fig. 2. Hydrodynamic voltammograms of serotonin, catecholamines, and their metabolites in a standard mixture containing 100 pg each using the HPLC–DUED system.

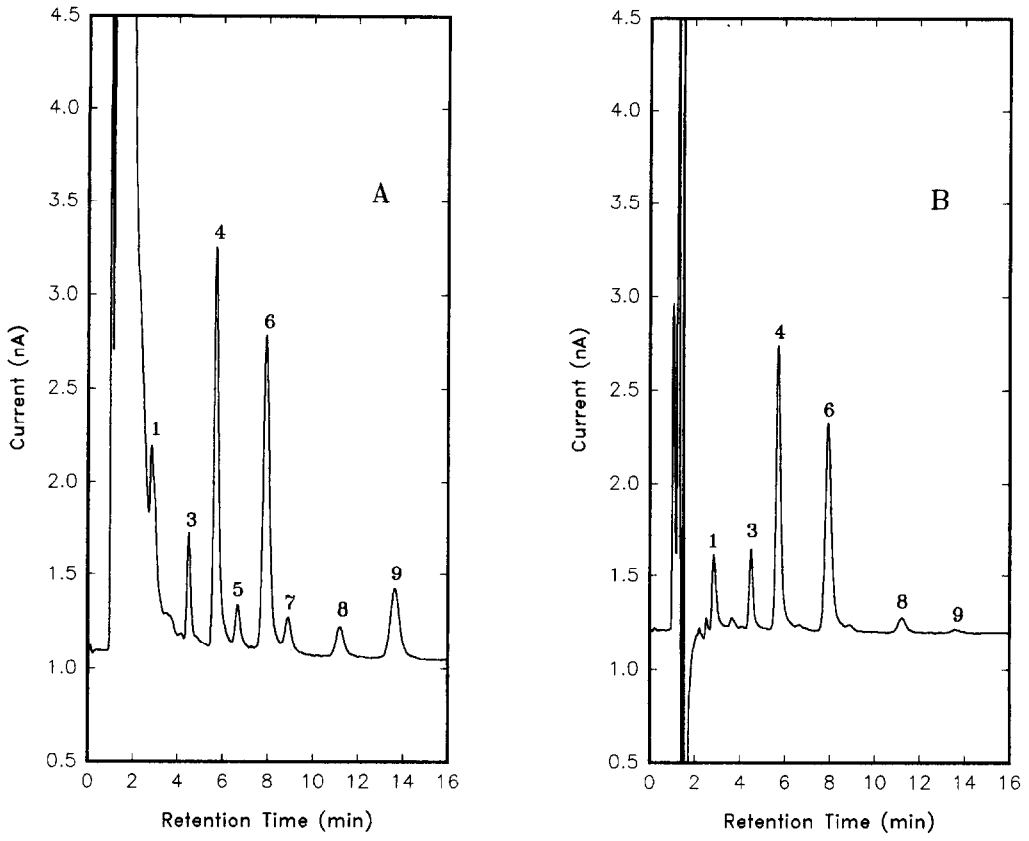

Fig. 3. Typical chromatograms of a brain homogenate containing: (1) NE, (3) DOPAC, (4) DA, (5) 5-HIAA, (6) IPT (as an internal standard), (7) HVA, (8) 3-MT and (9) 5-HT. (A) Anodic current; (B) cathodic current. Applied potentials (*vs.* Ag/AgCl): anode, +0.75 V; cathode, +0.05 V.

and 20 ng for all analytes (Table I). The precision of the assays was tested using a standard mixture and a brain homogenate in 0.1 *M* hydrochloric acid containing 10^{-7} *M* ascorbic acid. The intra- and inter-assay coefficients of variation (C.V.), calculated for the standard mixture and brain homogenate samples, are summarized in Table II. The detection limits per injection were between 0.2–0.5 pg, at a signal-to-noise ratio of 3. The intra-assay variabilities of a standard mixture and a brain homogenate were assessed from 25 replicates (at 1-h intervals) and expressed as C.V. For the anodic responses of a standard mixture in 0.1 *M* hydrochloric acid, the C.V. of 5-HIAA (6.41%) was relatively high (because of its instability) compared with the other values ($\leq$4.09%). Conversely, because the cathodic currents of 5-HT and 5-HIAA were barely detectable, their C.V. were not calculated; this offers an advantage for their positive identification. The C.V. of the cathodic response of HVA was very high (13.01%) because only a very low reduction current could be measured at +0.05 V. Other C.V. values were acceptable ($\leq$4.75%). For the anodic responses of the brain homogenate in 0.1 *M* hydrochloric acid and ascorbic acid, the C.V. values of HVA (8.16%) and 5-HIAA (5.61%) were relatively high, and the others were fairly precise ($\leq$4.67%). E was not detectable in the anodic chromatogram, but it could be detected in the more concentrated brain homogenates. The C.V. values of all analytes measured cathodically (NE, DOPAC, DA, and 3-MT) were fairly low ($\leq$4.97%). The concentrations of HVA, 5-HT,

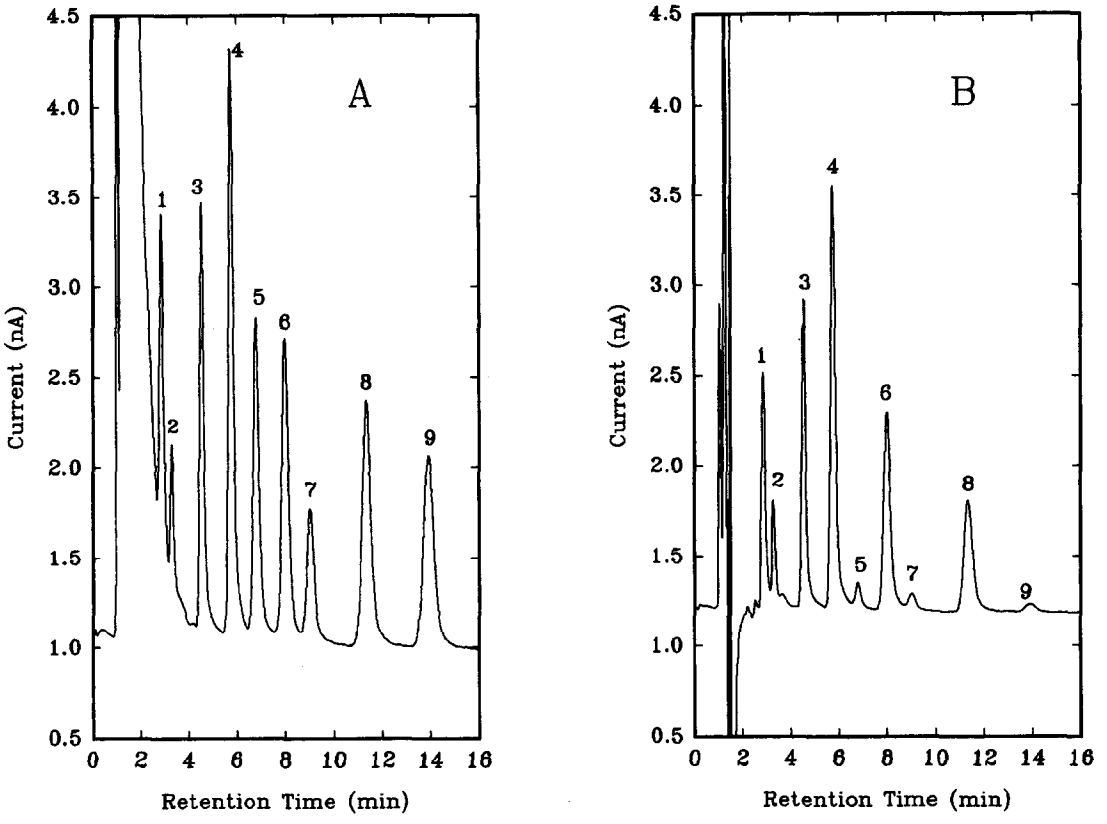

Fig. 4. Typical chromatograms of a brain homogenate spiked with a standard mixture containing (1) NE, (2) E, (3) DOPAC, (4) DA, (5) 5-HIAA, (6) IPT (as an internal standard), (7) HVA, (8) 3-MT and (9) 5-HT. (A) Anodic current; (B) cathodic current. Applied potentials (*vs.* Ag/AgCl): anode, +0.75 V; cathode, +0.05 V.

5-HIAA, and E in this brain homogenate were too low to be detected in the cathodic chromatogram. The inter-assay variabilities assessed with a standard mixture during six consecutive days were less than 8.0% for both the anodic and the cathodic responses.

In order to check the validity of the assays, brain homogenate samples (n = 15) with and without addition of different known amounts of standard mixture were analysed. The amount of standard mixture measured (obtained by measuring the amount of the analytes in spiked brain homogenate with addition of the standard mixture and subtracting the amounts measured in the brain homogenate without the addition) was compared with the known amount of standard mixture added, as shown in Table III. The amount measured and the amount added were very close for both the anodic and the cathodic responses. However, for NE, E, and DA the accuracy was better for the cathodic responses than that for the anodic responses. 5-HIAA, HVA, 3-MT, and 5-HT gave poor cathodic responses, so in these cases the accuracy was better for the anodic responses. DOPAC gave very consistent results for both types of responses. In the applications of HPLC–DUED, the concentrations of DOPAC, 5-HIAA, HVA, 3-MT and 5-HT were determined from the anodic chromatogram and the concentrations of NE, E and DA were determined from the cathodic chromatogram.

Calibration curves were also made with spiked brain homogenate samples, as shown in Table IV. The linearity of these curves is almost identi-

TABLE I

CORRELATION OF ANODIC OR CATHODIC CURRENT WITH THE AMOUNT OF CATECHOLAMINES, SEROTONIN, AND THEIR METABOLITES IN A STANDARD MIXTURE

Applied potentials: anode, +0.75 V; cathode, +0.05 V.

Substance[a]	Anode		Cathode	
	Standard curve equation[b]	r^2	Standard curve equation[b]	r^2
NE	$y = 3.9704x + 21.111$	0.996	$y = 2.4990x - 0.29901$	0.997
E	$y = 2.8188x - 3.5080$	0.999	$y = 1.5064x - 1.3705$	0.993
DOPAC	$y = 4.9065x - 28.865$	0.996	$y = 2.9316x - 14.863$	0.996
DA	$y = 5.3300x + 2.3575$	0.997	$y = 3.3732x + 1.8985$	0.998
5-HIAA	$y = 6.3533x + 88.510$	0.994	$y = 0.46168x - 5.9300$	0.913
HVA	$y = 2.1725x - 0.86659$	0.994	$y = 0.32239x + 12.558$	0.981
3-MT	$y = 7.8718x - 3.9835$	0.999	$y = 4.4400x - 2.4845$	0.998
5-HT	$y = 8.0183x - 50.845$	0.997		

[a] Amounts ranging from 5 pg to 20 ng.
[b] y = peak-area measurement; x = amount of analytes added in pg.

TABLE II

ANALYTICAL PRECISION AND THE EFFECTS OF DIFFERENT ANTIOXIDANTS ON THE STABILITIES OF INTRA-ASSAY AND INTER-ASSAY

Standard mixtures consist of 50 pg each of the catecholamines, serotonin, and their metabolites.

Antioxidant	Coefficient of variation (%)							
	NE	E	DOPAC	DA	5-HIAA	HVA	3-MT	5-HT
Intra-assay[a]								
Standard mixture in 0.1 *M* HCl								
Anodic	1.69	2.12	2.58	3.22	6.41	4.09	3.07	2.82
Cathodic	2.14	4.75	3.08	4.58	N.D.[b]	13.01	2.84	N.D.
Brain homogenate in 0.1 *M* HCl								
Anodic	2.25	N.D.	3.36	1.28	5.61	8.16	4.03	4.67
Cathodic	2.81	N.D.	4.97	1.73	N.D.	N.D.	3.30	N.D.
Inter-assay[c]								
Standard mixture in 0.1 *M* HCl								
Anodic	4.29	5.47	5.17	4.20	5.31	5.57	3.10	2.55
Cathodic	3.41	2.54	5.26	3.19	N.D.	7.56	6.93	N.D.

[a] n = 25, at intervals of 1 h.
[b] N.D. = not detectable.
[c] n = 6, on six consecutive days.

TABLE III

COMPARISON OF THE AMOUNTS OF STANDARD MIXTURE ADDED TO AND MEASURED IN BRAIN HOMOGENATES

The measured value was obtained by subtracting the amount measured in brain homogenates without addition from that measured in spiked brain homogenates. Anodic and cathodic potentials were +0.75 V and +0.05 V, respectively. A relatively close agreement between the two values indicates accurate assay of the analytes of brain homogenates. Values are in picograms.

NE		E		DOPAC		DA		5-HIAA		HVA		3-MT		5-HT	
Added	Measured	Added	Measured	Added	Measured	Added	Measured	Added	Measured	Added	Measured	Added	Measured	Added	Measured
Anode (n = 3)															
37.8	31.5	35.4	27.9	34.1	34.1	30.3	29.8	28.5	29.7	32.6	31.9	29.3	28.5	27.6	29.4
75.7	62.2	70.7	55.0	68.3	67.2	60.5	59.3	57.0	61.9	65.2	62.7	58.7	56.0	55.2	56.3
113.6	91.1	106.1	81.4	102.4	94.0	90.8	85.6	85.5	88.7	97.9	85.8	88.1	80.2	82.8	82.3
151.5	121.0	141.5	112.1	136.5	116.0	121.0	106.5	114.0	107.0	130.5	109.6	117.5	104.4	110.5	97.5
Cathode (n = 3)															
37.8	33.24	35.4	31.0	34.1	38.7	30.25	30.7	28.5	35.4	32.6	25.27	29.3	29.1	27.6	N.D.[a]
75.7	67.5	70.7	64.1	68.3	71.5	60.5	62.1	57.0	65.5	65.2	64.0	58.7	55.1	55.2	N.D.
113.6	101.4	106.1	95.1	102.4	101.9	90.8	88.3	85.5	95.8	97.9	78.9	88.1	77.6	82.8	N.D.
151.5	134.1	141.5	126.8	136.5	130.3	121.0	110.0	114.0	110.0	130.5	104.7	117.5	101.1	110.5	N.D.

[a] N.D. = not detectable.

TABLE IV

CORRELATIONS OF THE ANODIC OR CATHODIC CURRENT WITH THE AMOUNTS OF STANDARD MIXTURE MEASURED IN A BRAIN HOMOGENATE, AS DESCRIBED IN TABLE III

Substance	Anode		Cathode	
	Standard curve equation[a]	r^2	Standard curve equation[a]	r^2
NE	$y = 3.3580x + 9.3750$	1.000	$y = 2.2634x - 0.14700$	1.000
E	$y = 2.2423x - 1.8350$	0.999	$y = 1.3785x - 0.93700$	1.000
DOPAC	$y = 4.0606x + 36.199$	0.996	$y = 2.6364x + 27.611$	0.999
DA	$y = 4.7331x + 35.500$	0.994	$y = 3.4824x + 27.304$	0.993
5-HIAA	$y = 6.9638x + 72.450$	0.974	$y = 0.38093x + 8.9350$	0.985
HVA	$y = 1.8686x + 23.576$	0.990	$y = 0.36779x - 1.5692$	0.944
3-MT	$y = 7.0734x + 35.715$	0.999	$y = 3.9305x + 29.640$	0.999
5-HT	$y = 6.3633x + 85.750$	0.972		

[a] y = peak-area measured; x = amount of analytes measured in pg.

cal with that of curves obtained with the standard mixture. Values for whole brain samples, as well as for regional brain tissues (cortex, hippocampus, substantia nigra, striatum, hypothalamus, raphe, cerebellum and locus coeruleus) are listed in Table V. Our data are in agreement with those reported by others [33,34].

Many problems arise with the reproducibilities of conventional extraction methods, owing particularly to batch-to-batch variations and extensive pre-treatment prior to HPLC. The filtration method described in this paper can be an alternative procedure to simplify pre-treatment. The automatic HPLC method is a great advantage when many samples have to be analysed in a short time.

CONCLUSION

HPLC–DUED is a powerful analytical tool for the selective and sensitive detection of catecholamines, serotonin, and their metabolites in brain tissue homogenates. A dual electrochemical detector with two working electrodes (anode and cathode) suitable for microbore HPLC has been developed for the simultaneous measurement of NE, E, DA, DOPAC, HVA, 5-HIAA, 3-MT and 5-HT. This dual electrochemical detector has been successfully used for the measurement of these compounds in homogenates of nuclei, whole brain and other local brain tissue. Chromatographic peaks were characterized on the basis of their retention behaviour and the ratio of responses in the redox cycle. The described method offers sensitive and selective detection of all these biogenic amines and their metabolites. In addition, the large early-eluting peaks of brain homogenate were also eliminated on the cathodic electrode. The detection limit was *ca.* 0.2–0.5 pg per injection for all analytes. Owing to great sensitivity of HPLC–DUED, the brain tissue samples can be very small (less than 10 mg). Isocratic separation is achieved within 15 min, and over 90 analyses can be performed in a single working day. This simple, efficient and sensitive method can be used as a basic research tool for the assaying brain neurotransmitters and their metabolites.

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TABLE V

LEVELS OF SEROTONIN, CATECHOLAMINES, AND THEIR METABOLITES IN MICE WHOLE BRAIN, AND VARIOUS BRAIN REGIONS

Sample	*n*	Concentration (ng/ml)							
		NE	E	DOPAC	DA	5-HIAA	HVA	3-MT	5-HT
Whole brain	8	603 ± 42	N.D.[a]	166 ± 15	1604 ± 112	316 ± 29	187 ± 23	153 ± 33	783 ± 57
Cortex	4	1273 ± 185	61 ± 22	69 ± 13	104 ± 26	680 ± 72	74 ± 7	N.D.	1193 ± 153
Hippocampus	4	581 ± 53	N.D.	17 ± 1	54 ± 9	495 ± 28	38 ± 4	N.D.	855 ± 83
Substantia nigra	4	723 ± 104	65 ± 36	200 ± 50	533 ± 94	915 ± 96	307 ± 9	58 ± 32	1949 ± 445
Striatum	4	215 ± 40	N.D.	779 ± 126	10 756 ± 240	509 ± 25	1133 ± 102	773 ± 81	763 ± 33
Hypothalamus	4	2031 ± 394	N.D.	153 ± 48	415 ± 57	552 ± 47	193 ± 28	N.D.	1651 ± 168
Locus coeruleus	4	1273 ± 185	61 ± 22	69 ± 13	104 ± 26	680 ± 72	74 ± 7	N.D.	1193 ± 153
Raphe	4	943 ± 263	100 ± 30	96 ± 42	142 ± 46	960 ± 174	174 ± 52	N.D.	1067 ± 181
Cerebellum	4	464 ± 98	N.D.	33 ± 2	44 ± 3	90 ± 10	N.D.	N.D.	212 ± 40

[a] N.D. = not detectable.

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