Journal of Chromatography, 515 (1990) 265–278
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1910

Detection and identification modes for the highly sensitive and simultaneous determination of various biogenic amines by coulometric high-performance liquid chromatography

HIROSHI TAKEDA*
Department of Pharmacology, Tokyo Medical College, 6–1–1 Shinjuku, Shinjuku-ku, Tokyo 160 (Japan)
TERUHIKO MATSUMIYA
Department of Pharmacology, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259–11 (Japan)
and
TAKESHI SHIBUYA
Department of Pharmacology, Tokyo Medical College, 6–1–1 Shinjuku, Shinjuku-ku, Tokyo 160 (Japan)

ABSTRACT

Detection and identification modes for the rapid, selective, highly sensitive and simultaneous determination of catecholamines, indoleamines and related metabolites by high-performance liquid chromatography (HPLC) with series of coulometric working electrodes (CWE) were investigated. Five detection modes were examined: (1) oxidative single mode using a single CWE, (2) oxidative screen mode using a series of two CWE, (3) redox mode using a series of two CWE, (4) redox–reductive screen mode using a series of three CWE and (5) redox–reductive screen mode using a series of four CWE. For the highly sensitive detection of catechol compounds, oxidative single, redox and redox–reductive screen modes were suitable. Oxidative single and oxidative screen modes were better than the other modes for indole and *o*-methylated catechol compounds. For the selective detection of these compounds, however, the redox–reductive screen mode was best. The specific ratio obtained in HPLC with the redox or redox–reductive screen mode is useful as an index for identification purposes. These findings suggest that HPLC with the redox–reductive screen mode of detection is applicable to neuroscience studies.

INTRODUCTION

Since high-performance liquid chromatography with electrochemical detection (HPLC–ED) for the determination of catecholamine and its metabolites was introduced by Refshauge *et al.*[1] in 1974, this assay method has become widely accepted and applied to measuring neurochemical compounds, such as monoamines[2–5] and their metabolites[3,6,7] and precursors[3,4] in biological samples.

The combination of a thin-layer single amperometric detector with reversed-

0021-9673/90/$03.50

phase HPLC separation techniques is used for the analysis of neurochemical compounds, but it is not necessarily satisfactory with respect to sensitivity, selectivity and reproducibility for the measurement of neurochemical compounds by the direct injection of crude biologial samples into the HPLC–ED apparatus[8,9]. More recently, the use of HPLC with dual or multiple coulometric detectors for the simultaneous determination of catecholamines, indoleamines and their major metabolites and precursors in crude perchloric acid extracts of brain tissues and unprocessed cerebrospinal fluid was reported[10–14].

In this study, suitable detection and identification modes for the rapid, selective, highly sensitive and simultaneous determination of catecholamines (norepinephrine, epinephrine and dopamine), indoleamine (serotonin) and related metabolites (4-hydroxy-3-methoxyphenylglycol, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-methoxytyramine and 5-hydroxyindole-3-acetic acid) by HPLC with coulometric detectors were investigated in detail.

EXPERIMENTAL

Reagents

All reagents were purchased at the highest available purity and used without further purification. 3-Hydroxytyramine hydrochloride (dopamine, DA), 5-hydroxyindole-3-acetic acid (5-HIAA), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), 5-hydroxytryptamine hydrochloride (serotonin, 5-HT), 3-methoxytyramine hydrochloride (3-MT), 4-hydroxy-3-methoxyphenylglycol (MHPG), epinephrine (E), norepinephrine (NE) and deoxyepinephrine hydrochloride (DEP) were purchased from Sigma. 3,4-Dihydroxyphenylacetic acid (DOPAC) and 5-hydroxy-Nω-methyltryptamine oxalate (n-MET) were obtained from Aldrich. Analytical-reagent grade chemicals for sample preparation and chromatography were obtained from Katayama Chemical and Nakarai Chemicals.

Sample preparation

Male Sprague–Dawley rats (Charles River Japan) weighing 270–300 g were killed by decapitation and the brains were rapidly removed. The brains were dissected on a dry, ice-cooled aluminium plate according to the method of Glowinski and Iversen[15]. In this study, striatum tissue samples were used. After dissection, tissue samples were immediately placed in liquid nitrogen and frozen until extraction.

Extraction was based on the procedure of Ikarashi and Maruyama[5]. Dissected tissue was weighed and was initially added to a mixture consisting of 1 ml of 0.1 *M* perchloric acid, 30 μl of 0.1 *M* ethylenediaminetetraacetic acid, 30 μl of 1 *M* sodium hydrogen sulphite and 200 ng each of DEP and n-MET as internal standards. The mixture was then homogenized with an ultrasonic cell disruptor (Sonifier Model 200, Branson) at 13% power output (20 W) at 0°C for 30 s and centrifuged at 20 000 *g* for 15 min at 0°C. The supernatant layer was filtered through a 0.45-μm Millipore filter to separate the insoluble residue. A portion of the supernatant (*ca.* 100 μl) was then carefully transferred to a small tube and further centrifuged at 20 000 *g* for 2 min using a Beckman Microfuge. An aliquot of the supernatant was injected into the HPLC–ED system.

HPLC–ED system

The HPLC–ED system is shown schematically in Fig. 1. The HPLC system consisted of a Model LC-6A solvent delivery system (Shimazu) equipped with an extra damper, a Model 7125 sample injector (Rheodyne) with a 100-μl sample-holding loop, in-line filter unit (0.20-μm graphite filter) (ESA) and a μBondapak C_{18} (10 μm) reversed-phase column (300 mm × 3.9 mm I.D.) (Waters Assoc.). A Model BX-7000A column heater (Ishido) maintained the analytical column at a constant temperature (23°C).

Electrochemical detection was performed with a coulometric detection system consisting of four coulometric high-efficiency flow-through cells in series: a Model 5010 analytical cell with dual coulometric working electrodes [test electrode 1, T_1 (efficiency 100%); test electrode 2, T_2 (efficiency 100%)] (ESA)[10–14]; a Model 5011 high-sensitivity analytical cell containing dual coulometric working electrodes [test electrode 3, T_3 (efficiency 100%); test electrode 4, T_4 (efficiency 70%)] (ESA)[11–14]; and a Model 5100A control module (ESA). The four coulometric working electrodes (T_1–T_4) were connected in series by installing a Model 5010 analytical cell before a Model 5011 high-sensitivity analytical cell.

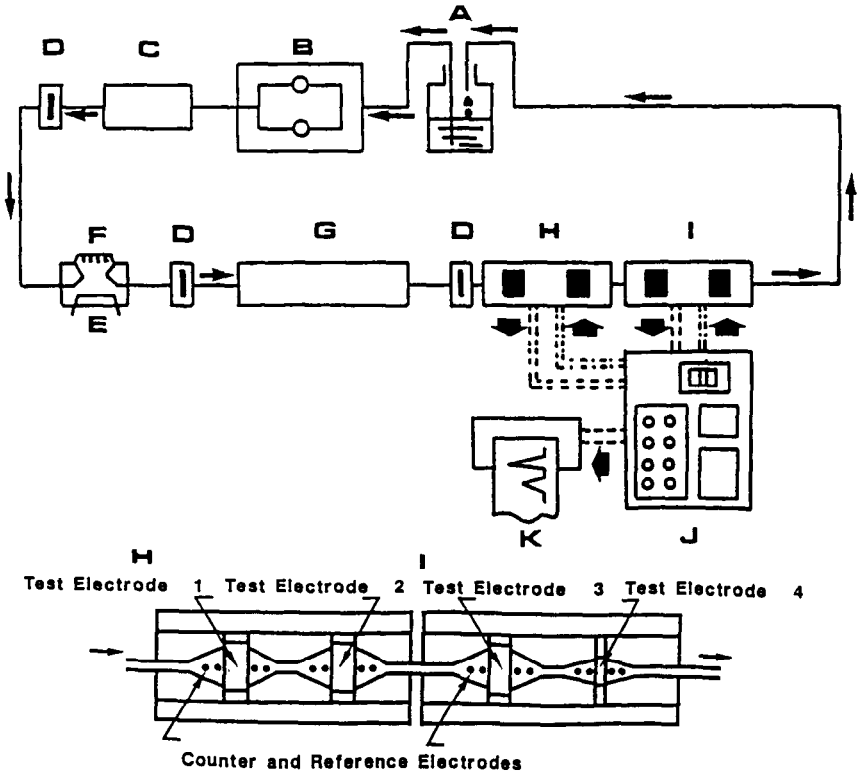

Fig. 1. HPLC–ED system for the simultaneous determination of catecholamines, indoleamines and related metabolites. A = mobile phase; B = pump; C = damper; D = in-line filter; E = injection valve; F = sampling loop; G = analytical column; H = Model 5010 analytical cell (ESA); I = Model 5011 high-sensitivity analytical cell (ESA); J = Model 5100A control module (ESA); K = recorder.

Data analysis was performed with four recorders connected to T_1, T_2, T_3 and T_4. For good separation of catecholamines, indoleamine, related metabolites and internal standards (eleven compounds), a mobile phase consisting of mixed 0.04 *M* phosphate–0.04 *M* citrate buffer (pH 3.0) containing 7.5 m*M* sodium 1-heptanesul-

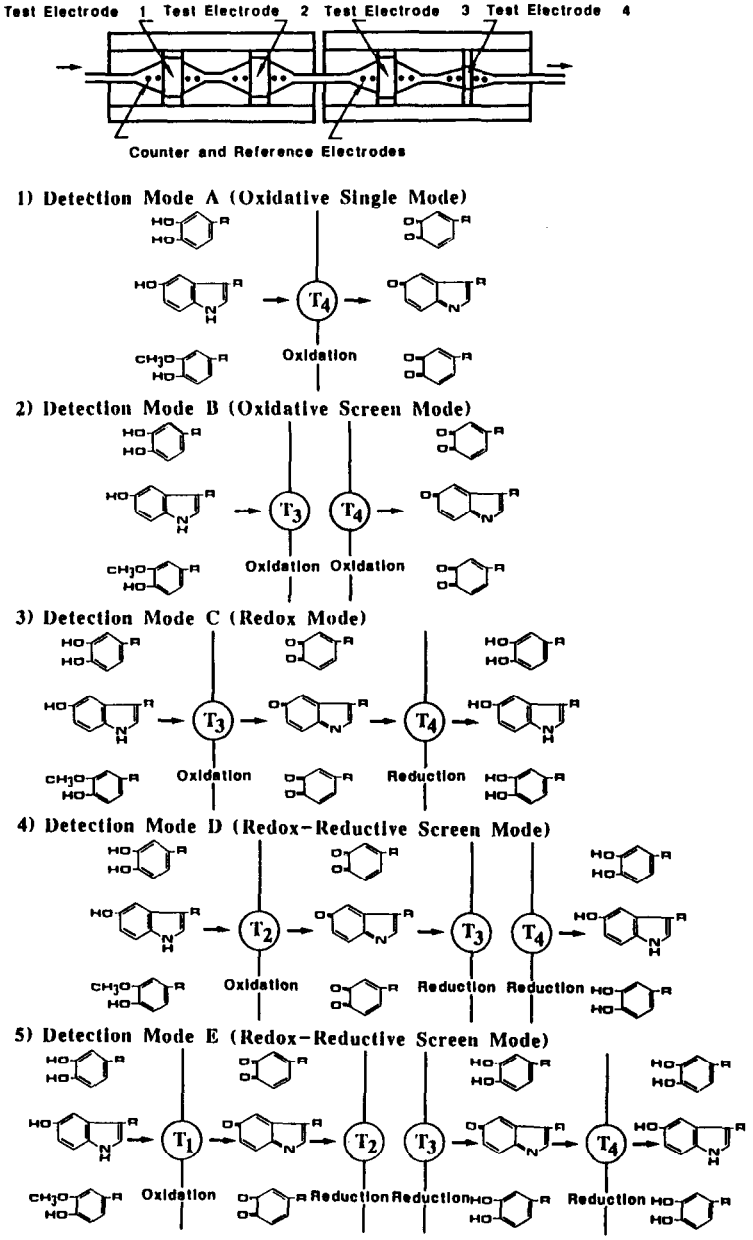

Fig. 2. Detection system and modes for the simultaneous determination of catecholamines, indoleamines and related metabolites. T_1, T_2, T_3 and T_4: test electrodes 1, 2, 3 and 4, respectively.

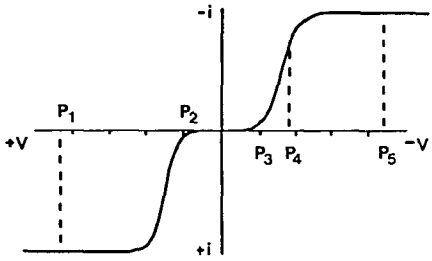

Fig. 3. Oxidation and reduction current–voltage curve. P_1, P_2, P_3, P_4 and P_5 are potentials (see text).

phonate, 0.08 m*M* ethylenediaminetetraacetic acid, 11.7% methanol and 4.7% acetonitrile was used. The mobile phase flow-rate was maintained at 1.0 ml/min. Mobile phase was also circulated in the HPLC–ED system and was exchanged when marked changes in the back-current occurred.

Electrochemical detection modes

The principles of the five electrochemical detection modes examined are illustrated in Fig. 2 and a schematic illustration of the oxidation and reduction current–voltage curve for determination of the applied potentials of each coulometric working electrode used in the five detection modes is shown in Fig. 3.

(1) Detection mode A (oxidative single mode using a single coulometric working electrode). The oxidative single mode was performed using only T_4. The potential of T_4 was set at P_1, corresponding to the top of the oxidation current–voltage curve for the analyte, to measure these compounds.

(2) Detection mode B (oxidation screen mode using a series of two coulometric working electrodes). T_3 and T_4 were used to perform the oxidative screen mode. The first electrode (T_3) was set at P_2, near the low end of the oxidation current–voltage curve for the analyte, to remove compounds having a lower oxidation potential than the analyte. The potential of the second electrode (T_4) was set at P_1, corresponding to the top of the curve for the measurement of the analyte.

(3) Detection mode C (redox mode using a series of two coulometric working electrodes). In the redox mode, T_3 and T_4 were used. The potential of the first electrode (T_3) was set at P_1, corresponding to the top of the oxidation current–voltage curve for the analyte, to oxidize these compounds completely. The second electrode (T_4) was set at P_5, corresponding to the top of the reduction current–voltage curve for the measurement of the analyte.

(4) Detection mode D (redox–reductive screen mode using a series of three coulometric working electrodes). The redox–reductive screen mode was performed using T_2, T_3 and T_4. The first electrode (T_2) was set at P_1, corresponding to the top of the oxidation current–voltage curve for the analyte, to oxidize these compounds completely. The second (T_3) and third electrodes (T_4) were set at different reduction potentials. The potential of T_3 was set at P_3, near the low end of the reduction current–voltage curve for the analyte, to remove compounds having a lower reduction potential than these compounds and the potential of T_4 was set at P_5, corresponding to the top of the curve for actual measurement of the analyte.

(5) Detection mode E (redox–reductive screen mode using a series of four coulometric working electrodes). The redox–reductive screen mode was performed using T_1, T_2, T_3 and T_4. The first electrode (T_1) was set at P_1, corresponding to the top of oxidation current–voltage curve for analyte, to oxidize these compounds completely . The second electrode (T_2) was set at P_3, near the low end of the reduction current–voltage curve for the analyte, to remove compounds having a lower reduction potential than these compounds. For measurement of the analyte, the third (T_3) and fourth (T_4) electrodes were set at different reduction potentials, P_4 and P_5, respectively.

Identification modes

Two identification modes were examined for the selective determination of catecholamines, indolamines and related metabolites by direct injection of crude biological samples into the HPLC–ED apparatus. The principle of the two identification modes is illustrated in Fig. 4.

Identification mode A. In this mode, identification of peaks on the chromatogram was performed on the basis of retention time.

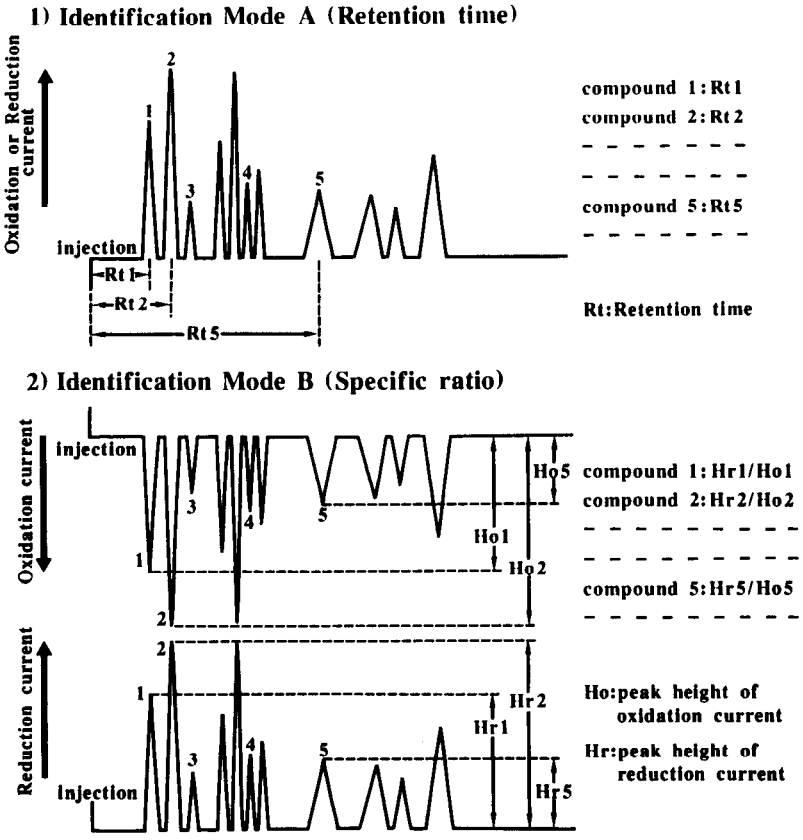

Fig. 4. Identification modes for the selective determination of catecholamines, indoleamines and related metabolites.

Identification mode B. The specific ratio of the reduction current response to the oxidation current response monitored in detection mode C or D was used for the identification of peaks on the chromatogram.

RESULTS

Applied potential

For determination of the optimum potentials for each coulometric working electrode (T_1, T_2, T_3 and T_4) in the five electrochemical detection modes, current–voltage curves for catecholamines, indoleamines and related metabolites were investigated.

Oxidation and reduction current–voltage curves for catecholamines, indoleamines and related metabolites in T_1, T_2 or T_3 (coulometric working electrodes of 100% efficiency) are shown in Figs. 5 and 6. The oxidation current responses for catechol (DA, NE, E, DOPAC, DEP), indole (5-HT, 5-HIAA, n-MET) and *o*-methylated catechol compounds (3-MT, HVA, MHPG) were over +0.00, +0.10 and +0.25 V, respectively. The maximum current responses for catechol and indole compounds were obtained in the range +0.15 to +0.40 V, followed by decreases in

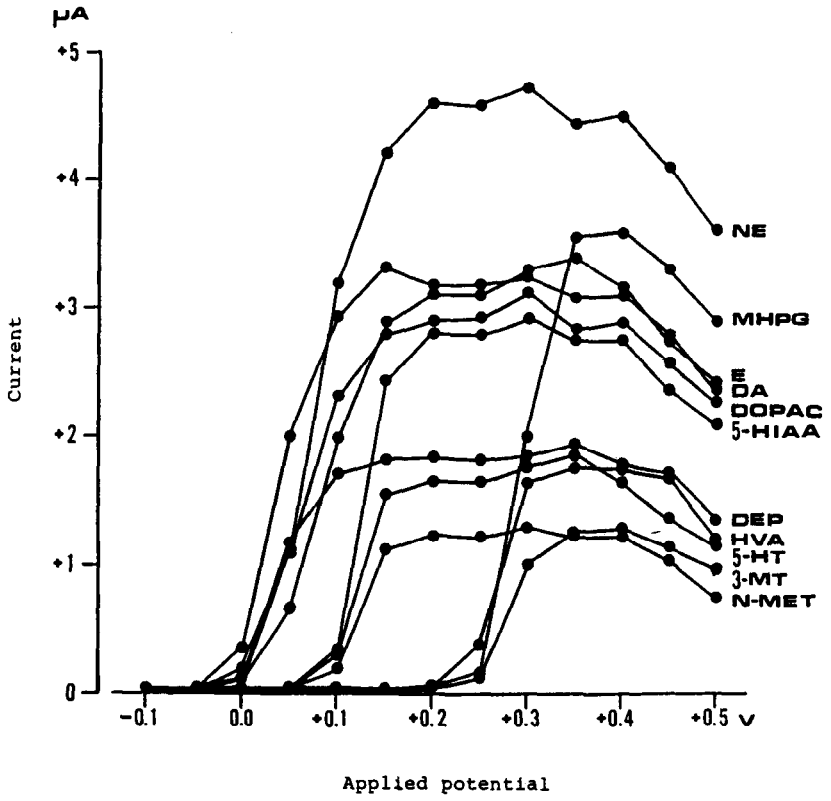

Fig. 5. Relationship between applied oxidative potentials and reaction currents in coulometric working electrode of 100% efficiency.

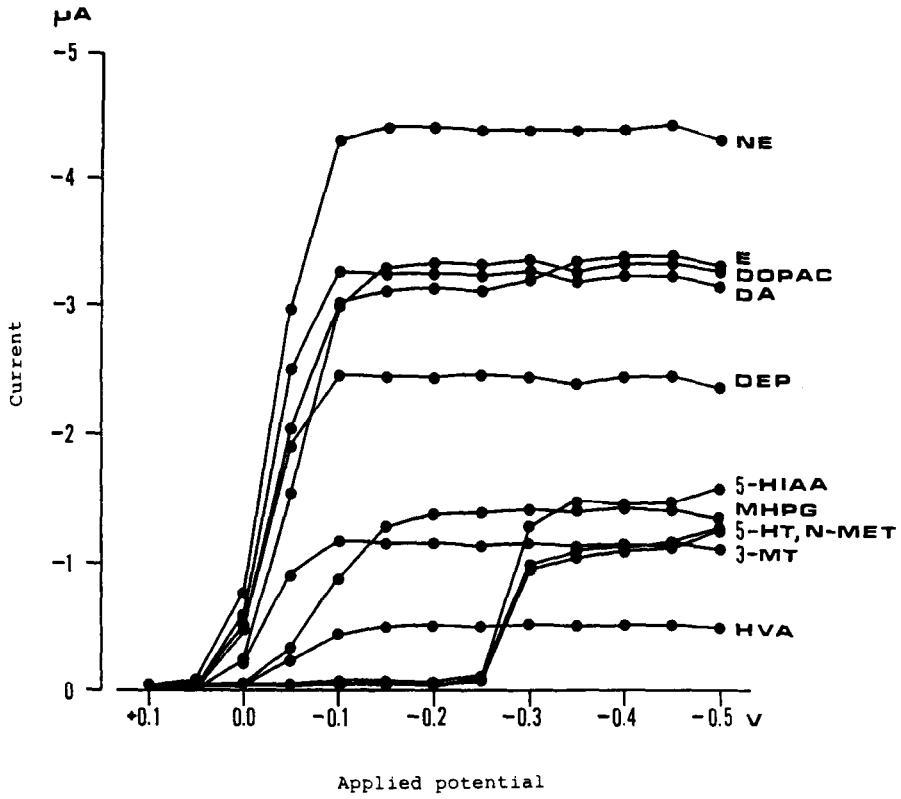

Fig. 6. Relationship between applied reductive potentials and reaction currents in coulometric working electrode of 100% efficiency.

these responses. The oxidation current responses for *o*-methylated catechol compounds were maximum in the range +0.35 to +0.40 V (Fig. 5).

Reduction current responses for catechol and *o*-methylated catechol compounds were detected below −0.00 V, and stable maximum responses were obtained in the range −0.10 to −0.45 V. Current responses for indole compounds were below −0.25 V and these responses were maximum in the range −0.30 to −0.45 V (Fig. 6).

The oxidation and reduction current–voltage curves in T_4 (coulometric working electrode of 70% efficiency) are shown in Figs. 7 and 8. Oxidation current responses for catechol, indole and *o*-methylated catechol compounds were detected over +0.05, +0.15 and +0.30 V, respectively.

Current responses for catechol and indole compounds were maximum in the ranges +0.30 to +0.45 and +0.25 to +0.45 V, respectively, followed by decreases in these responses. Maximum current responses for *o*-methylated catechol compounds were obtained in the range +0.40 to +0.45 V (Fig. 7). Reduction current responses for catechol and *o*-methylated catechol compounds were maximum in the range −0.25 to −0.50 V and those for indole compounds in the range −0.30 to −0.45 V, followed by increases in these responses (Fig. 8).

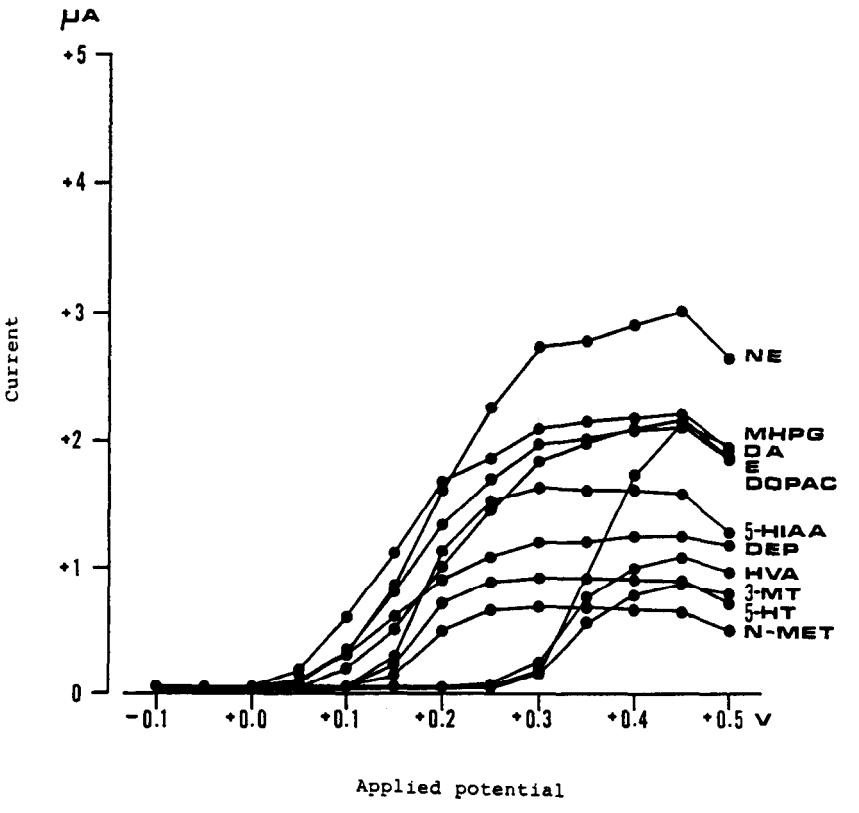

Fig. 7. Relationship between applied oxidative potentials and reaction currents in coulometric working electrode of 70% efficiency.

From these results, the optimum applied potentials for each coulometric working electrode in the five electrochemical detection modes were as follows: (1) the potential of T_4 in detection mode A was +0.35 V; (2) the potentials of T_3 and T_4 in detection mode B were +0.00 and +0.35 V, respectively; (3) the potentials of T_3 and T_4 in detection mode C were +0.35 and −0.35 V, respectively; (4) the potentials of T_2, T_3 and T_4 in detection mode D were +0.35, +0.05 and −0.35 V, respectively; and (5) the potentials of T_1, T_2, T_3 and T_4 in detection mode E were +0.35, +0.05, −0.15 and −0.35 V, respectively.

Detection modes

The characteristics of the five electrochemical detection modes examined are shown in Table I. In all detection modes, a linear and close correlation between the current responses of catecholamines, indoleamines and related metabolites and their amounts in the range 0.5–5000 pg was observed. Also, assay limits for the simultaneous determination of these compounds were in the high femtogram to low picogram range. The current responses of catechol compounds obtained in detection modes A, C, D and E were larger than in detection mode B. On the other hand, the

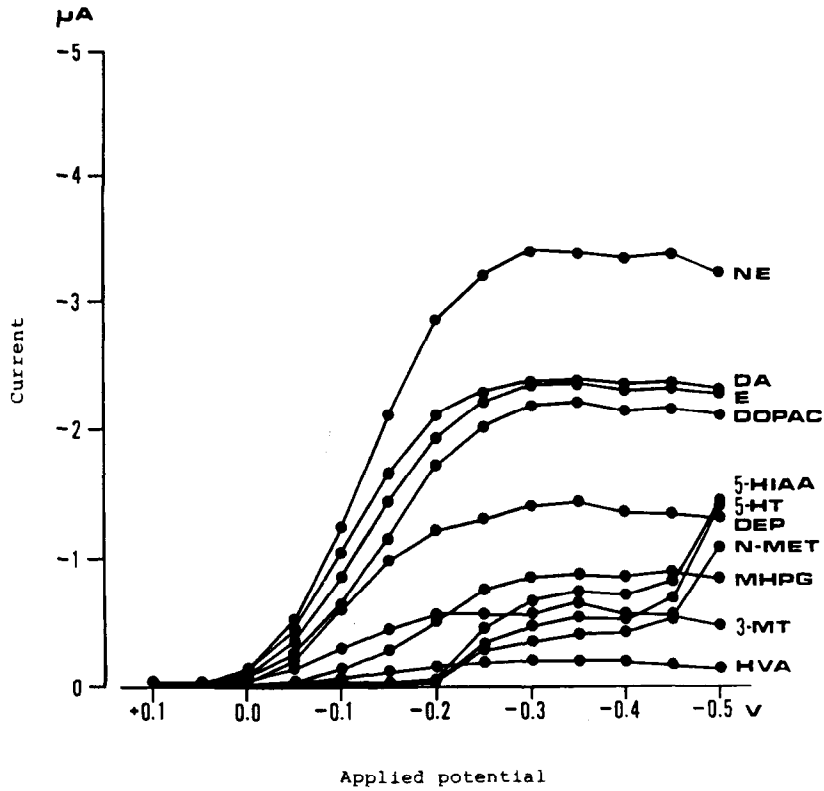

Fig. 8. Relationship between applied reductive potentials and reaction currents in coulometric working electrode of 70% efficiency.

current responses of indole and *o*-methylated catechol compounds in detection modes C, D and E were small compared with those in detection mode A or B.

Identification modes

The retention times of catecholamines, indoleamines and related metabolites under the HPLC–ED conditions employed were as follows: MHPG, 4.32 ± 0.16 (mean ± S.D., $n=5$); NE, 4.94 ± 0.26; E, 5.58 ± 0.30; DOPAC, 7.56 ± 0.33; DA, 8.98 ± 0.55; DEP, 10.10 ± 0.64; 5-HIAA, 11.40 ± 0.85; HVA, 15.05 ± 0.99; 3-MT, 17.59 ± 1.14; 5-HT, 20.40 ± 1.45; and n-MET, 23.32 ± 1.42 min.

The specific ratios (all $\times 10^{-2}$) of the reduction current response to the oxidation current response of each analyte monitored in detection mode C were 23.9 ± 2.0 MHPG) (mean ± S.D., $n=5$), 75.8 ± 2.6 (NE), 74.2 ± 0.7 (E), 70.4 ± 0.3 (DOPAC), 73.1 ± 0.3 (DA), 73.5 ± 1.5 (DEP), 24.8 ± 0.8 (5-HIAA), 9.6 ± 0.4 (HVA), 41.9 ± 1.8 (3-MT), 29.6 ± 1.7 (5-HT) and 28.5 ± 0.4 (n-MET). In detection mode D, these ratios (all $\times 10^{-2}$) were 49.3 ± 0.8 (MHPG), 63.8 ± 0.2 (NE), 64.3 ± 0.4 (E), 62.2 ± 0.7 (DOPAC), 67.5 ± 1.2 (DA), 66.0 ± 0.7 (DEP), 41.9 ± 0.3 (5-HIAA), 18.1 ± 0.4 (HVA), 51.4 ± 0.6 (3-MT), 38.1 ± 1.1 (5-HT) and 37.8 ± 0.9 (n-MET).

TABLE I

CHARACTERISTICS OF DETECTION MODES

Parameter	*Detection mode*					
	A (oxidative single mode)	*B (oxidative screen mode)*	*C (redox mode)*	*D (redox–reductive screen mode)*	*E (redox–reductive screen mode)*	
Assay limit (pg)	0.5–2.0 pg	0.5–2.5 pg	0.5–6.0 pg	0.5–5.0 pg	1.0–8.0 pg	
Linearity (γ) (0.5–5000 pg)	0.9987–0.9998	0.9992–0.9997	0.9997–0.9999	0.9994–0.9999	0.9996–0.9999	
Compound (500 pg)	*Response (current, μA)*					
	T_4	T_4	T_4	T_4	T_3	T_4
MHPG	0.11	0.12	−0.04	−0.06	−0.06	−0.004
NE	0.22	0.09	−0.22	−0.17	−0.14	−0.004
E	0.17	0.08	−0.17	−0.15	−0.11	−0.03
DOPAC	0.16	0.05	−0.17	−0.13	−0.12	−0.003
DA	0.15	0.01	−0.16	−0.15	−0.11	−0.003
DEP	0.12	0.01	−0.12	−0.11	−0.08	−0.004
5-HIAA	0.09	0.09	−0.04	−0.02	–	−0.02
HVA	0.07	0.07	−0.02	−0.02	−0.03	−0.001
3-MT	0.06	0.07	−0.04	−0.05	−0.04	−0.001
5-HT	0.06	0.07	−0.03	−0.04	–	−0.03
n-MET	0.06	0.06	−0.03	−0.04	–	−0.03

The values of the specific ratios were different depending on the detection mode employed, but these values were stable compared with the retention times.

Chromatograms

Excellent chromatograms of catecholamines, indoleamines and related metabolites were obtained within 25 min in all HPLC–ED systems with detection mode A, B, C, D or E. In the HPLC–ED system with detection modes other than A, the large "void volume" signal on the chromatogram which was typically observed with direct injection of perchloric acid extracts was markedly reduced. A representative chromatogram obtained by direct injection of a perchloric acid extract of rat striatum tissue into the HPLC–ED system with detection mode D is shown in Fig. 9. The retention times for each peak on this chromatogram were as follows: 4.29 (MHPG), 4.88 (NE), 5.31 (E), 7.49 (DOPAC), 8.68 (DA), 10.02 (DEP), 11.28 (5-HIAA), 14.91 (HVA),

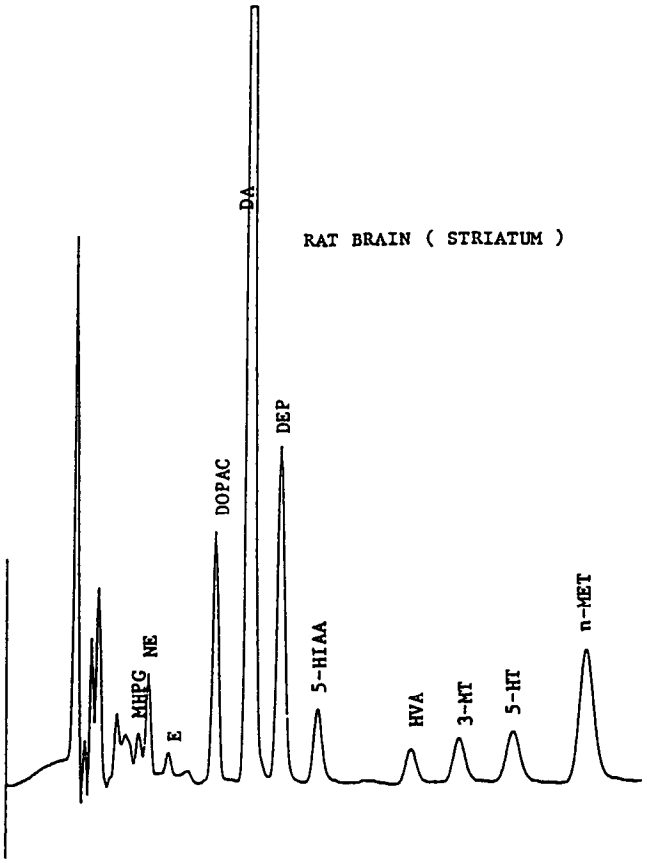

Fig. 9. Representative chromatogram obtained by direct injection of perchloric acid extract of rat striatum tissue into the HPLC–ED system with detection mode D. Mobile phase, mixed 0.04 *M* phosphate–0.04 *M* citrate buffer, (pH 3.0) containing 7.5 m*M* sodium 1-heptanesulphonate, 0.08 m*M* ethylenediaminetetraacetic acid, 11.7% methanol and 4.7% acetonitrile; flow-rate, 1.0 ml/min; column, μBondapak C_{18} (300 mm × 3.9 mm I.D.). Applied potential: test electrode 2, +0.35 V; test electrode 3, +0.05 V; test electrode 4, −0.35 V.

16.43 (3-MT), 19.87 (5-HT) and 22.82 min (n-MET). The specific ratios (all $\times 10^{-2}$) were as follows: 50.7 (MHPG), 64.5 (NE), 64.2 (E), 62.1 (DOPAC),67.3 (DA), 66.1 (DEP), 41.7 (5-HIAA), 17.9 (HVA), 50.8 (3-MT), 37.9 (5-HT) and 37.6 (n-MET). There were no significant differences between these retention times and specific ratios of the sample peaks on the chromatogram shown in Fig. 9 and those obtained with authentic standards.

DISCUSSION

Assessment of the neurochemical functions of biogenic amines requires a quantitative assay with high sensitivity, selectivity, reproducibility and reliability for the determination of these compounds and their related metabolites and precursors in a single sample. HPLC–ED has become a widely accepted technique for this purpose[1–14]. Several single amperometric electrode detection methods are currently using the quantitative HPLC of the monoamines and their metabolites and precursors in urine, blood, cerebrospinal fluid and brain. However, through experience with developing and applying these methods, certain limitations and areas for improvement have become apparent[8,9]. Major shortcomings and problems are related to detection limits in the high picogram to low nanogram range and the inability to detect the presence of co-eluting substances adequately.

Recently, a system was reported using HPLC with a series of coulometric detectors for the simultaneous determination of catecholamines, indoleamines and their major metabolites and precursors in unprocessed cerebrospinal fluid and crude perchloric acid extracts of brain tissues[10–14]. This greatly enhanced the sensitivity, selectivity and reproducibility of these analyses.

In this study, we investigated the suitable detection and identification modes for the rapid, selective, highly sensitive and simultaneous determination of catecholamines, indoleamines and related metabolites by direct injection of crude perchloric acid extracts of brain tissues into the HPLC system with coulometric detectors. The results obtained suggest that the detection of these compounds is possible by using all the detection modes examined. In particular, for the highly sensitive detection of catechol compounds (NE, E, DA, DOPAC, DEP), the oxidative single, redox and redox–reductive screen modes are suitable. The oxidative single and oxidative screen modes are better than other modes for indole (5-HT, 5-HIAA, n-MET) and *o*-methylated catechol compounds (MHPG, HVA, 3-MT). For the selective and simultaneous detection of these compounds in crude perchloric acid extracts of brain tissues, however, the redox–reductive screen mode using a series of three coulometric working electrodes is the best of the five detection modes examined.

In this detection mode, the first high-efficiency coulometric working electrode is set at +0.35 V to oxidize most of the analyte eluting from the column. This improves the selectivity by eliminating the detection of non-reversible compounds at subsequent electrodes operated in the reductive mode. That is, all compounds that can be completely oxidized at +0.35 V and cannot be subsequently reduced do not appear in the analysis with the recording electrode set at −0.35 V. Second, oxidation of the column effluent greatly reduced baseline drift and virtually eliminates the large void currents routinely observed with amperometric detectors following direct injections of crude samples into an HPLC apparatus. The second coulometric working electrode is

set at +0.05 V to eliminate the detection at subsequent recording electrodes of reversible compounds having a lower reduction potential than the analyte. The third electrode is set at −0.35 V for actual measurement of the analyte.

The results obtained suggest that both retention time and specific ratios are suitable as indices for the identification of each peak in a chromatogram. However, the retention time changes depending on the chromatographic conditions and the specific ratio differs depending on the detection mode employed. Also, the results for specific ratios suggest that they depend on the redox property of each catecholamine, indoleamine or related metabolite. The values may therefore become an important factor, yielding information about peak composition and purity. Careful investigations should be made of the significance of these specific ratios. It is possible to identify peaks by comparing the specific ratios of sample peaks with those obtained with authentic standards.

In conclusion, HPLC with the redox–reductive screen detection mode using a series of three coulometric working electrodes is applicable to the highly sensitive, selective and simultaneous determination of catecholamines, indoleamines and related metabolites by direct injection of crude biological samples.

REFERENCES

1 C. Refshauge, P. T. Kissinger, R. Dreiling, L. Blank, R. Freeman and R. N. Adams, *Life Sci.*, 14 (1974) 311.
2 S. Sasa and C. Blank, *Anal. Chim. Acta*, 104 (1979) 29.
3 I. N. Mefford and J. D. Barchas, *J. Chromatogr.*, 181 (1980) 187.
4 J.Wagner, P. Vitali, M. G. Palhreyman, M. Zraika and S. Huot, *J. Neurochem.*, 38 (1982) 1241.
5 Y. Ikarashi and Y. Maruyama, *Biogenic Amines*, 2 (1985) 101.
6 F. Hefti, *Life Sci.*, 25 (1979) 775.
7 O. Magnusson, L.B. Nilsson and D. Westerlund, *J. Chromatogr.*, 221 (1980) 237.
8 P. J. Langlais, W. J. McEntee and E. D. Bird, *Clin. Chem.*, 26 (1980) 786.
9 P. J. Langlais, E. D. Bird and W. J. McEntee, *Ann. Neurol.*, 12 (1982) 48.
10 J. Dutrieu and Y. A. Delmotte, *Fresenius Z. Anal. Chem.*, 314 (1983) 416.
11 P. J. Langlais, E. D. Bird and W. R. Matson, *Clin. Chem.*, 30 (1984) 1046.
12 W. R. Matson, P. J. Langlais, L. Volicer, P. H. Gamache, E. D. Bird and K. A. Mark, *Clin. Chem.*, 30 (1984) 1477.
13 I. C. Kilpatrick, M. W. Jones and O. T. Phillipson, *J. Neurochem.*, 46 (1986) 1865.
14 H. Sugiyama, T. Fujita, Y. Shin, H. Takeda, K. Sakamoto and R. Ikeda, *Jpn. J. Pharmacol.*, 46 (1988) 252p.
15 J. Glowinski and L. L. Iversen, *J. Neurochem.*, 13 (1966) 655.