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AN INTEGRATED SCHEME FOR THE SIMULTANEOUS DETERMINATION OF BIOGENIC AMINES, PRECURSOR AMINO ACIDS, AND RELATED METABOLITES BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

A new method using high-performance liquid chromatography with electrochemical detection (HPLC—ED) for the simultaneous determination of monoamines, their precursor amino acids, and related major metabolites in small samples of brain tissue weighing from 0.5 to 50 mg is described. The method is based on the preliminary isolation of monoamines (dopamine, norepinephrine, epinephrine, and serotonin), their precursor amino acids (tyrosine, 3,4-dihydroxyphenylalanine, tryptophan and 5-hydroxytryptophan), and their major metabolites (3-methoxytyramine, normetanephrine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, vanillylmandelic acid, 3-methoxy-4-hydroxyphenylethyleneglycol, and 5-hydroxyindoleacetic acid) by chromatography on small columns of Amberlite CG-50 and Dowex 50W, and by ethyl acetate extraction. All the compounds in the four isolated fractions were measured by HPLC—ED on a reversed-phase column under four different conditions. The sensitivity was from 0.1 to 40 pmol, depending on the substances analysed. This newly established method was applied to the study of the effects of an aromatic L-amino acid decarboxylase inhibitor (NSD-1015) and a monoamine oxidase inhibitor (pargyline) on the levels of monoamines, their precursor amino acids and their major metabolites in brain regions of mice.

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INTRODUCTION

High-performance liquid chromatography (HPLC) with electrochemical detection (ED) [1—3] has proved to be a sensitive and inexpensive method for measuring catecholamines, indoleamines and their precursor amino acids and related metabolites in tissues and body fluids [4—21]. However, the simultaneous determination of all of the monoamines, their metabolites, and their amino acid precursors has not been reported.

Although HPLC—ED is highly sensitive, it is relatively non-specific and without prior isolation procedures gives rise to complex chromatograms. Therefore, the identification of each peak is sometimes difficult [10, 20, 21]. Most procedures for the HPLC—ED analysis of biogenic amines include a preliminary separation of compounds by alumina adsorption, ion-exchange or Sephadex G-10 columns [2, 6—8] to make the assay more specific.

In this report, we describe a new HPLC—ED assay for the simultaneous determination of all the monoamines, their metabolites and precursor amino acids [dopamine (DA), norepinephrine (NE), epinephrine (E), serotonin (5-HT), tyrosine (TYR), 3,4-dihydroxyphenylalanine (DOPA), tryptophan (TRP), 5-hydroxytryptophan (5-HTP), 3-methoxytyramine (3-MT), normetanephrine (NMN), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), vanillylmandelic acid (VMA), 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG)] based on their prior isolation by Amberlite CG-50 and Dowex 50 columns and by ethyl acetate extraction, using six internal standards [N-methyldopamine (NMDA), α-methyl-DOPA (MDOPA), α-methyl-*p*-tyrosine (MTYR), α-methyltryptophan (MTP), α-methyl-*p*-hydroxytryptophan (MHTP), and 3,4-dihydroxycinnamic acid (DHCA)]. Application of the method to pharmacological studies is also described.

MATERIALS AND METHODS

Materials

The following drugs or chemicals were used: HVA (Calbiochem, La Jolla, CA, U.S.A.); L-TYR, L-DOPA, DL-MDOPA, L-MTYR, L-TRP, MTRP, L-5-HTP, L-NE, L-E bitartrate, DA · HCl, 5-HT creatinine sulphate, NMN · HCl, 3-MT · HCl, NMDA, DOPAC, 5-HIAA dicyclohexylammonium salt, MOPEG piperazine salt, and pargyline, *m*-hydroxybenzylhydrazine (NSD-1015) (Sigma, St. Louis, MO, U.S.A.); and VMA and DHCA (Tokyo Kasei, Tokyo, Japan). MHTP was a kind gift from Dr. J. Daly (NIH, Bethesda, MD, U.S.A.). Other chemicals were of analytical grade.

Animal experiments

All experiments were carried out on male DDY mice weighing about 20 g (six weeks of age). They were housed five per cage with food and water ad libitum and under a normal dark and light rhythm. Animals were sacrificed between 10:30 a.m. and 12:00 a.m. to avoid possible changes caused by circadian rhythm in the levels of biogenic amines, their metabolites and precursor amino acids, and in their biosynthesis rate. NSD-1015 and pargyline

were dissolved in saline, and all injections were given intraperitoneally (i.p.) in a volume of 1 ml per 100 g body weight.

Mice were killed by cervical dislocation; the brains were removed within 30 sec, and were immediately dropped into ice-cold saline. The brains were dissected on a glass plate over ice into ten regions by the method of Carlsson and Lindqvist [22] with modifications [23]. Tissue samples after dissection were frozen on dry ice and analysed immediately or stored at −80° C until analysis.

Tissue samples (0.5—50 mg) were weighed and homogenized by a tissue sonicator (Ohtake Works, Tokyo, Japan) with 1.0 ml of ice-cold 0.32 *M* sucrose. An aliquot of the homogenate can be used both for the analysis of monoamines, their metabolites and precursor amino acids and for the enzymatic analysis of monoamine-related enzymes. When the latter enzyme analysis was not necessary, homogenization was carried out with 0.8 ml of the perchloric acid solution described below.

Preparation of Amberlite CG-50 and Dowex 50 columns

Amberlite CG-50 (type II, 200—400 mesh) and Dowex 50W (200—400 mesh) were washed with water and then by cycling through sodium and acid forms with 2 *M* sodium hydroxide and 2 *M* hydrochloric acid and finally with distilled water. A column of Amberlite CG-50 (1 cm × 0.4 cm I.D.) was washed with 3 ml of 0.1 *M* sodium phosphate buffer (pH 6.5) containing 0.1% Na_2EDTA and 3 ml of water [24]. A column of Dowex 50 W (H^+) (1.8 cm × 0.4 cm I.D.) was also prepared.

Both Amberlite and Dowex columns can be regenerated after use by washing them with water, 3 ml of 2 *M* NaOH—1% Na_2EDTA, 5 ml of water, 3 ml of 2 *M* hydrochloric acid and 5 ml of water.

Isolation of monoamines, their precursor amino acids, and metabolites

The isolation of monoamines, their metabolites and precursor amino acids on Amberlite CG-50 and Dowex 50 columns was carried out according to the method of Kiss et al. as modified by Oka et al. [23]. Freshly prepared 1.0 ml of ice-cold 0.4 *M* perchloric acid containing 0.25% Na_2EDTA, 0.5% $Na_2S_2O_5$, and internal standards (NMDA, MDOPA, MTYR, MTRP, MHTP, and DHCA) were added to 0.5 ml of the homogenate. After standing for 15 min in an ice bath, samples were centrifuged at 20,000 *g* for 15 min at 4° C. Supernatants were transferred to test tubes containing 0.1 ml of 0.5 *M* sodium phosphate buffer (pH 6.5), and 0.2 ml of 1 *M* potassium carbonate was added to adjust the pH to 6.1—6.3; the mixtures were frozen at −80° C. After thawing, extracts were centrifuged at 1600 *g* for 10 min at 4° C and the supernatants were poured onto Amberlite CG-50 columns (1 cm × 0.4 cm I.D.). After the samples had passed through, the columns were washed successively with 1 ml of 0.01 *M* sodium phosphate buffer (pH 6.5) containing 0.1% Na_2EDTA, 1 ml of water and 0.1 ml of 0.5 *M* hydrochloric acid. Column effluents and washings were collected in tubes containing 0.1 ml of 1 *M* phosphoric acid and 0.05 ml of 2 *M* hydrochloric acid and carefully mixed. Monoamines (NE, E, DA, 3-MT, 5-HT, and NMDA) were eluted with 0.4 ml of 0.5 *M* hydrochloric acid from the Amberlite CG-50 columns, and their HPLC—ED analysis (System 1) was performed immediately. The effluents plus washings from the Amberlite

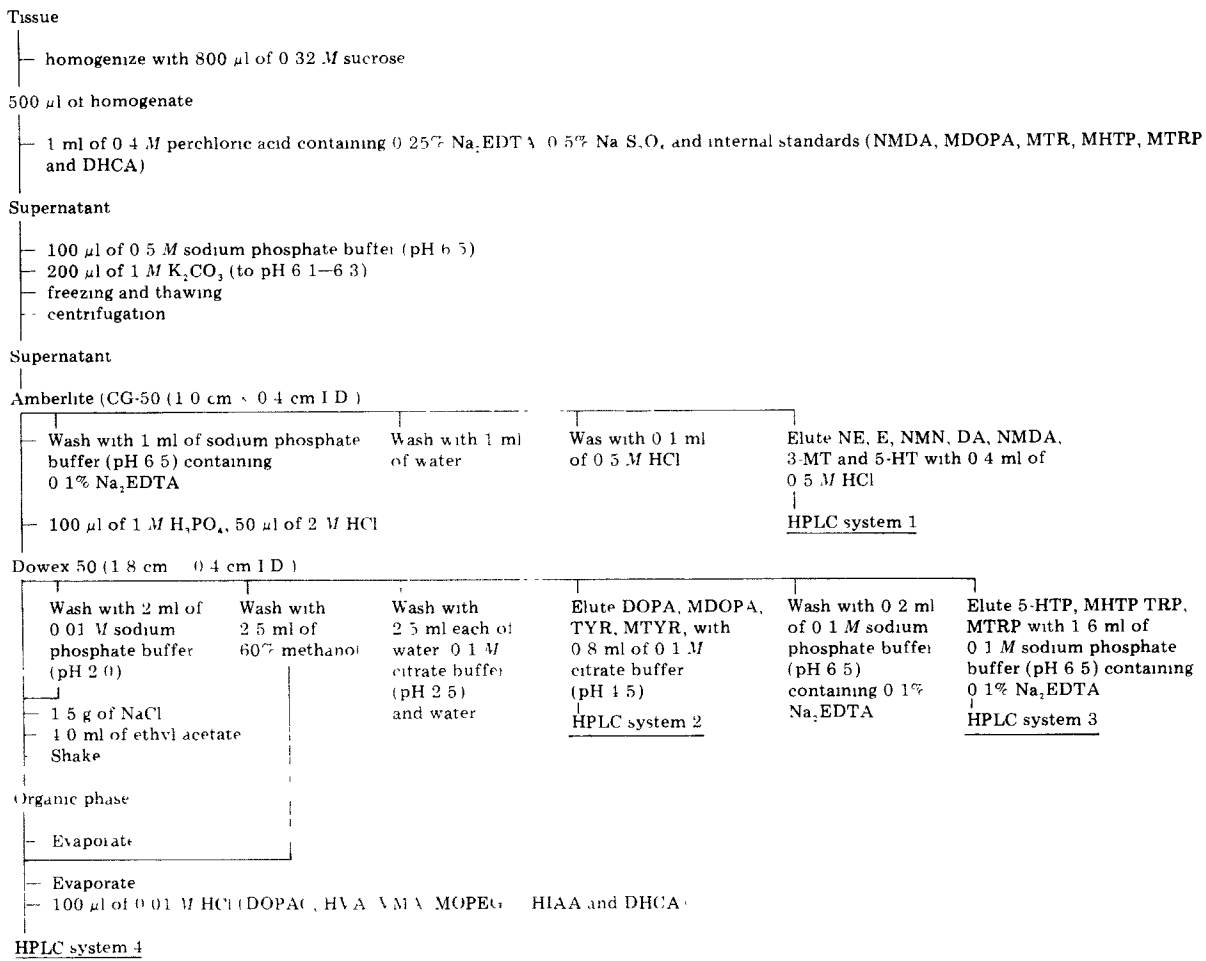

Fig 1 Flow-chart for the preliminary isolation procedure of monoamines, their metabolites and precursor amino acids.

CG-50 columns were applied to Dowex 50 columns (1.8 cm × 0.4 cm I.D.). After the samples had passed through, the columns were washed with 1 ml of 0.01 *M* sodium phosphate buffer (pH 2.0), and with 2.5 ml of 60% methanol. The column effluents plus buffer washings, and 60% methanol washings were collected separately in different tubes. Then the columns were washed successively with 2.5 ml of water, 2.5 ml of 0.1 *M* citrate phosphate buffer (pH 2.5), and 2.5 ml of water. DOPA, MDOPA, and TYR were eluted with 0.8 ml of 0.1 *M* citrate—sodium hydroxide buffer (pH 4.5). The columns were washed with 0.2 ml of 0.1 *M* sodium phosphate buffer (pH 6.5) containing 0.1% Na_2EDTA, and then 5-HTP, MHTP, TRP and MTRP were eluted with 1.6 ml of the same buffer. The effluent plus buffer washing were saturated with 1.5 g of sodium chloride and extracted with 4.0 ml of ethyl acetate with vigorous shaking. The organic phase was collected and evaporated. Previously saved 2.5 ml of 60% methanol washing was added to the same test tube and then evaporated to dryness. This fraction, which contained VMA, DOPAC, MOPEG, 5-HIAA, HVA and DHCA, was dissolved with 0.1 ml of 0.01 *M* hydrochloric acid before HPLC—ED analysis. The isolation procedure is summarized in Fig. 1.

Apparatus

The chromatograph used was a Yanaco L-2000 with a Yanaco VMD-101 electrochemical detector, a two-pen recorder, and a column (25 cm × 0.4 cm I.D.) packed with Nucleosil 7 C_{18} (Macherey-Nagel, Düren, F.R.G.) using a slurry column packing apparatus Model 124 (Chemco Scientific, Osaka, Japan) and a column packer (Umetani Seisakusho, Osaka, Japan). For column packing, 2.5 g of reversed-phase packing material in 3.8 ml of methanol containing 0.1% ammonium acetate, 3.8 ml of acetonitrile, 17.3 ml of carbon tetrachloride and 5.3 ml of slurry solvent C (Macherey-Nagel) were pumped into an empty column, the back-pressure being maintained at 550 kg/cm^2 with methanol containing 0.1% ammonium acetate. The pumping continued with 100 ml of methanol, and then the pressure was reduced during 15 min [25]. The column was washed with 200 ml of 50% methanol, and with water prior to use in order to obtain a low background signal for electrochemical detection.

HPLC analysis

The following analytical systems were used. System 1 for monoamines: 0.1 *M* sodium phosphate buffer (pH 2.2) as the mobile phase with a flow-rate of 0.74 ml/min and the detector potential at 0.65 V against Ag/AgCl electrode. System 2 for DOPA and TYR: 0.1 *M* citrate—sodium hydroxide buffer (pH 3.0) as the mobile phase with a flow-rate of 0.74 ml/min at 0.73 V. System 3 for TRP and 5-HTP: 0.1 *M* sodium phosphate buffer (pH 6.5)—acetonitrile (96:4, v/v) as the mobile phase with a flow-rate of 0.74 ml/min at 0.55 V. System 4 for metabolites: 0.1 *M* sodium acetate buffer (pH 5.2) as the mobile phase with a flow-rate of 1.0 ml/min at 0.6 V.

All mobile phases were freshly prepared from 0.5 *M* stock solution and degassed before use.

Calculations

The concentration of each compound in brain tissue was calculated from the

ratio of peak height (peak height of each compound/peak height of internal standard). The calibration factor was determined from the peak height ratio of each known amount of the standard which was carried through the whole extraction procedure with internal standards.

RESULTS

Fig. 2A shows chromatograms of a mixture of standards of NE, E, NMN, DA, NMDA (internal standard), 3-MT and 5-HT. The separation of monoamine standards on a reversed-phase column was complete. Isolation of monoamines from the midbrain was also complete as shown in Fig. 2B (a saline-treated mouse) and in Fig. 2C (mouse treated with pargyline, a monoamine oxidase inhibitor). Marked increases in the peaks of NE, NMN, DA, 3-MT, and 5-HT were observed in the pargyline-treated mouse. No detectable interfering substances were observed in the chromatograms. The limit of sensitivity for standard monoamines was about 0.1 pmol, and that for NMN and 3-MT was about 0.2 pmol. Since a detector potential of 0.65 V is not maximal for NMN and 3-MT, the sensitivity for NMN and 3-MT depends on the applied potential for electrochemical detection. Linearity was satisfactory up to 5 nmol. NMDA was used as an internal standard for this fraction because its elution profile from an Amberlite CG-50 column was similar to that of each monoamine, and its separation by HPLC was complete.

The chromatographic separation of the fraction containing DOPA and TYR

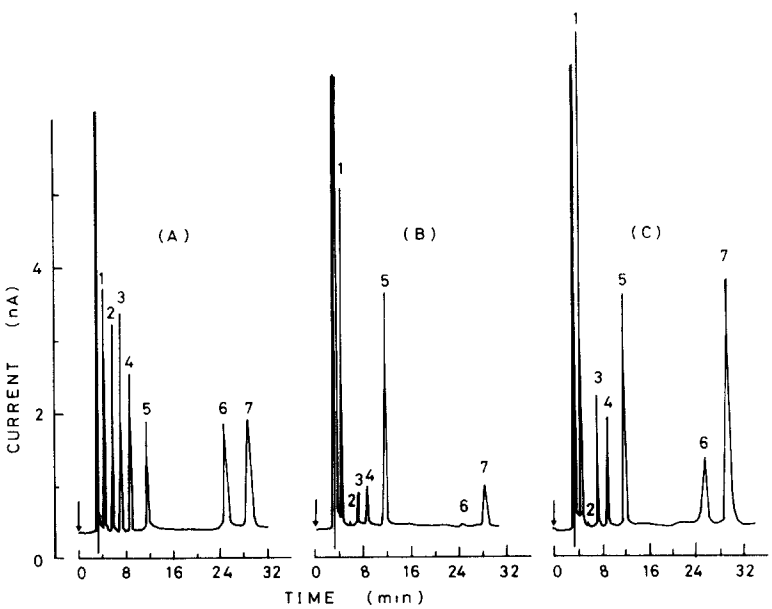

Fig 2. Chromatograms of the monoamine fractions. (A) A mixture of standards· 5 pmol each of NE, E, DA and NMDA, and 10 pmol each of NMN, 3-MT and 5-HT. (B) The extract from 44 mg of midbrain from a saline-treated mouse (i.p., 60 min), and (C) the extract from 36 mg of midbrain from a pargyline-treated mouse (75 mg/kg, i p., 60 min) were subjected to the whole procedure. Peaks: 1 = NE; 2 = E; 3 = NMN; 4 = DA; 5 = NMDA (internal standard); 6 = 3-MT; and 7 = 5-HT. HPLC conditions are described in Materials and methods.

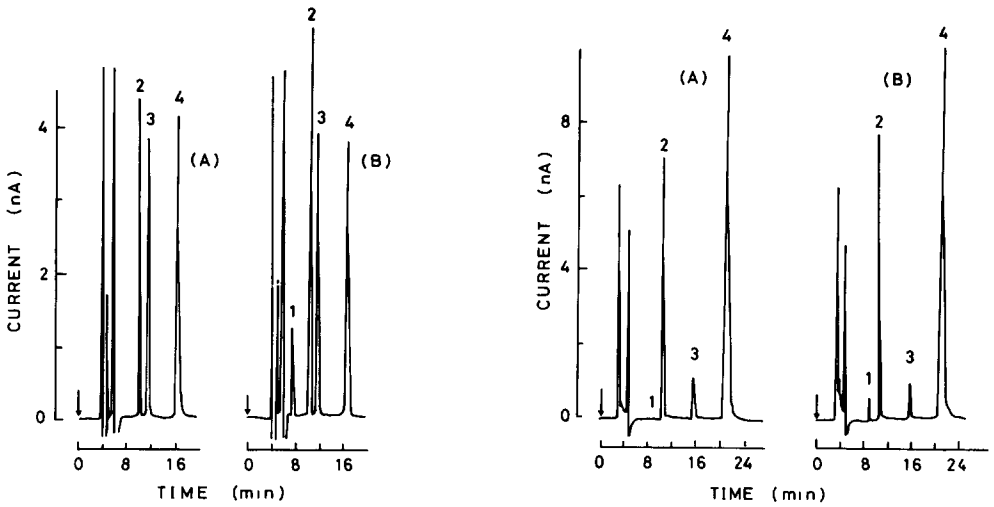

Fig. 3. Chromatograms of DOPA and TYR fractions. The extract from each 39 mg of midbrain of (A) a saline-treated mouse (i.p., 30 min), and of (B) an NSD-1015-treated mouse (100 mg/kg, i.p., 30 min), were subjected to the complete extraction procedure. HPLC conditions are described in Materials and methods. Peaks: 1 = DOPA; 2 = TYR; 3 = MDOPA (internal standard); and 4 = MTYR (internal standard).

Fig. 4. Chromatograms of 5-HTP and TRP fractions. Each 6 mg of hypothalamus from (A) a saline-treated mouse (i.p., 30 min) and from (B) an NSD-1015-treated mouse (100 mg/kg, i.p., 30 min) were carried through the whole extraction procedure. HPLC conditions are described in Materials and methods. Peaks: 1 = 5-HTP; 2 = MHTP (internal standard); 3 = TRP; and 4 = MTRP (internal standard).

from the midbrain is shown in Fig. 3. The fraction from saline-treated animals did not show a detectable peak of DOPA (Fig. 3A), but the extract from the midbrain of a mouse treated with an aromatic L-amino acid decarboxylase inhibitor (NSD-1015) showed a marked DOPA accumulation as indicated in Fig. 3B. We used 0.73 V as an applied oxidation potential. At higher oxidation potential the TYR peak was increased but interfered with the peak of MDOPA, because of high TYR concentration in tissues. At the oxidation potential of 0.73 V, the sensitivity of DOPA was 1 pmol, that of TYR 10 pmol.

Fig. 4 shows the chromatographic separation of the fraction containing 5-HTP and TRP. The chromatogram of the extract from the hypothalamus of a saline-treated animal (Fig. 4A) shows a trace peak of 5-HTP. Fig. 4B is the chromatogram of the extract from the hypothalamus of a mouse treated with a DOPA decarboxylase inhibitor (NSD-1015), and the peak of 5-HTP was observed. Both 5-HTP and TRP could be measured accurately based on two different internal standards, MHTP and MTRP, because MHTP and MTRP had similar chemical properties on a Dowex 50 column and similar oxidation potentials to 5-HTP and TRP, respectively. The sensitivities for 5-HTP and TRP were 1 pmol and 40 pmol, respectively.

Fig. 5A illustrates the separation of a mixture of standards of VMA, DOPAC, MOPEG, 5-HIAA, HVA and DHCA (internal standard). The sensitivity for DHCA was lower than that for other metabolites. However, DHCA proved to

be a suitable internal standard because the separation from the other compounds on a reversed-phase column was complete. Fig. 5B shows the chromatogram of the extract from the midbrain of saline-treated mouse. Clear peaks of DOPAC, MOPEG, 5-HIAA and HVA were observed, but the peak of VMA was not detected. The sensitivity of each metabolite was about 1 pmol.

In order to demonstrate the specificity and the utility of this assay, we investigated the levels of all monoamines and related compounds that can be measured by our newly established method, after pharmacological manipulations, i.e. administration of an aromatic L-amino acid decarboxylase inhibitor (NSD-1015) and of a monoamine oxidase inhibitor (pargyline). The effects on the levels of biogenic amines, their precursor amino acids and major metabolites in the striatum, hypothalamus, and pons—medulla oblongata are shown in Table I. Increases of NE, NMN, DA, 3-MT, 5-HT and decreases of DOPAC, MOPEG, 5-HIAA and HVA were observed in the tissues from monoamine oxidase inhibitor (pargyline) treated animals. As indicated in Table I, NSD-1015 inhibited aromatic L-amino acid decarboxylase and caused DOPA and 5-HTP accumulations in each brain region which give the in vivo activity of tyrosine and tryptophan hydroxylases. However, NSD-1015 also had an inhibitory effect on monoamine oxidase, and incrased monoamine levels and decreased their metabolite levels.

TABLE I

EFFECTS OF INHIBITORS OF AROMATIC L-AMINO ACID DECARBOXYLASE (NSD-1015) AND MONOAMINE OXIDASE (PARGYLINE) ON THE LEVELS OF BIOGENIC AMINES, PRECURSOR AMINO ACIDS AND THEIR METABOLITES IN DIFFERENT BRAIN REGIONS OF MICE

Values are expressed in nmol/g wet tissue (mean ± S.E.M. from five animals). Treatments: saline, i p., 60 min; NSD-1015, 100 mg/kg, i.p., 30 min, and pargyline, 75 mg/kg, i p , 60 min.

Compound	Striatum			Hypothalamus
	Saline	NSD-1015	Pargyline	Saline
NE	1.16 ± 0.13	0.96 ± 0.04	1.82 ± 0.23	5.47 ± 0.51
E	0.11 ± 0 01	0.11 ± 0.01	0.13 ± 0.01	0 42 ± 0.06
NMN	0.15 ± 0.03	0.22 ± 0.03	0.69 ± 0.04 §	0.58 ± 0.04
DA	29.4 ± 2.79	27 5 ± 2.21	36.5 ± 1.76 §	0.62 ± 0.05
3-MT	2.42 ± 0.21	3.45 ± 0.26**	6 55 ± 0 27 §	n d. § §
5-HT	1.21 ± 0.12	0.94 ± 0.16	2 30 ± 0 49**	n d.
DOPA	n.d.	4.03 ± 0.23 §	n d	n d
TYR	68.3 ± 4.0	103 ± 2.2 §	57 8 ± 1.7	76 7 + 4.5
5-HTP	0.06 ± 0.00	0.61 ± 0.03 §	n.d	n.d
TRP	31.1 ± 2.8	28.0 ± 2 7	27.8 ± 2.0	27.3 ± 2.6
VMA	n.d.	n.d.	n.d	n.d.
DOPAC	14.8 ± 1.68	7.54 ± 0 36***	2.59 ± 0.34 §	1.96 ± 0.15
MOPEG	0.23 ± 0.01	n d	n.d	0.46 ± 0.06
5-HIAA	1.58 ± 0.18	0.91 ± 0.05**	0.54 ± 0.04*	1.27 ± 0.19
HVA	5.71 ± 0.83	4.52 ± 0 21	1.59 ± 0 26 §	1.04 ± 0 09

*$P < 0.05$.
**$P < 0.02$
***$P < 0.01$
§$P < 0.001$
§§n d = not detectable

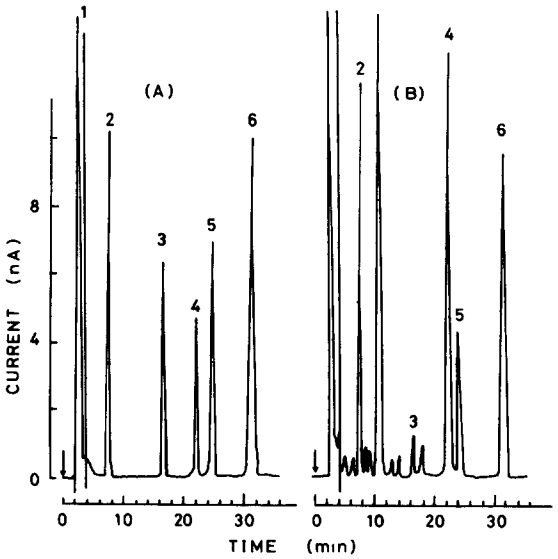

Fig. 5. Chromatograms of the fractions of monoamine metabolites. (A) A mixture of standards (20 pmol of VMA, 40 pmol each of DOPAC, MOPEG, and 5-HIAA, and 200 pmol of DHCA). (B) A tissue sample of 42 mg of midbrain from saline-treated mouse (i.p., 60 min) was subjected to the whole extraction procedure as described in Materials and methods. HPLC conditions are described in Materials and methods. Peaks: 1 = VMA; 2 = DOPAC; 3 = MOPEG; 4 = 5-HIAA; 5 = HVA; and 6 = DHCA (internal standard).

		Pons—medulla oblongata		
NSD-1015	Pargyline	Saline	NSD-1015	Pargyline
9.68 ± 0.35 §	13.2 ± 0.98 §	4.00 ± 0.14	4.24 ± 0.18	5.02 ± 0.34**
0.41 ± 0.04	0.57 ± 0.05	0.18 ± 0.02	0.10 ± 0.01 §	0.10 ± 0.01 §
1.13 ± 0.14***	2.76 ± 0.15 §	0.80 ± 0.09	1.16 ± 0.16	1.60 ± 0.18***
0.86 ± 0.09*	2.10 ± 0.21 §	0.11 ± 0.01	0.06 ± 0.01**	0.21 ± 0.01 §
n.d.	0.82 ± 0.05	n.d.	n.d.	0.15 ± 0.04
n.d.	2.65 ± 0.47	n.d.	0.61 ± 0.08	0.80 ± 0.22
1.55 ± 0.20 §	n.d.	n.d.	0.56 ± 0.03 §	n.d.
124 ± 6.2 §	94.4 ± 7.1	58.7 ± 4.9	95.8 ± 4.9 §	44.1 ± 1.5*
2.89 ± 0.24 §	n.d.	n.d.	0.88 ± 0.03 §	n.d.
42.9 ± 2.6***	40.3 ± 3.5**	27.7 ± 4.0	38.7 ± 2.1*	26.7 ± 2.6
n.d.	n.d.	n.d.	n.d.	n.d.
2.48 ± 0.13***	1.28 ± 0.18*	0.91 ± 0.06	1.53 ± 0.14***	0.83 ± 0.10
0.49 ± 0.07	n.d.	0.32 ± 0.02	0.26 ± 0.03	n.d.
0.48 ± 0.15*	n.d.	0.83 ± 0.05	0.15 ± 0.02 §	n.d.
0.80 ± 0.04	n.d.	0.16 ± 0.02	0.12 ± 0.01	n.d.

DISCUSSION

This new assay for monoamines and their precursor amino acids and metabolites by HPLC—ED has several advantages.

First, the method permits the simultaneous assay of almost all the monoamines and related compounds. Many methods for the assay of monoamines and related compounds [4—21] have been reported. The method by Mayer and Shoup [19] measured eight compounds (DA, NE, 5-HT, HVA, DOPAC, MOPEG, 5-HIAA and TRP) without any prelimary isolation. The method of Ishikawa and McGaugh [15] uses primary butanol extraction before HPLC—ED and measured twelve compounds (DA, NE, 5-HT, MTYR, NMN, DOPAC, HVA, MOPEG, 5-HIAA, TYR, DOPA, and TRP). Kempf and Mandel [7] used alumina adsorption and diethyl ether extraction to measure nine compounds (DA, NE, 5-HT, MTYR, NMN, DOPAC, HVA, MOPEG, and 5-HIAA). However, our method can measure simultaneously all fourteen monoamines, and their metabolites and precursor amino acids from a single, small tissue sample.

Secondly, this method is highly specific. Wagner et al. [21] simultaneously determined DOPA, 5-HTP, DA, 3-MT, NE, DOPAC, HVA, 5-HT and 5-HIAA in brain tissues by direct injection of perchloric acid extract into the HPLC—ED system. Although direct injection without prior isolation is simple and rapid, the complex chromatogram causes difficulties in identification of compounds of interest and also low sensitivity. Since we used Amberlite CG-50 and Dowex 50 columns and ethyl acetate extraction as a preliminary isolation procedure before HPLC—ED, we obtained simple and clean chromatograms of each substance, and the identification of each compound is easy. The specificity of the method is further increased both by HPLC and by specific detection depending on the applied oxidation potential with ED.

Thirdly, this method is accurate, since proper internal standards are used. We corrected the recovery of each compound during the isolation by using six internal standards.

Fourthly, since the baseline is very stable due to preliminary cleaning of the tissue samples before HPLC—ED, this method is highly sensitive. Sensitivities for the monoamines, their precursor amino acids, and their derivatives are approximately 0.1 pmol, 10 pmol, and 1 pmol, respectively.

We also demonstrated that the present newly established HPLC—ED assay of monoamines and their related compounds is useful in pharmacological studies. We homogenized the tissues with sucrose or perchloric acid, and we could not observe significant differences between the two homogenizing media. A sucrose homogenate has advantages, because, as reported previously [23], the monoamines and their related compounds and the activities of monoamine-related enzymes can be measured simultaneously. In addition, it is also possible to compare in vivo enzyme activities in animals treated with an aromatic L-amino acid decarboxylase inhibitor, measured for example by the accumulation of DOPA (tyrosine hydroxylase activity) and 5-HTP (tryptophan hydroxylase activity), with the in vitro enzyme activities. This is important for the study of the regulation of tyrosine hydroxylase and tryptophan hydroxylase.

In conclusion, this new simultaneous HPLC—ED assay for biogenic mono-

amines and related compounds holds great promise for biochemical, physiological, and pharmacological studies.

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