Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 7, pp. 899–905, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00
Pergamon Press plc

Short Communication

Simultaneous determination of monoamines and their principal metabolites in brain tissue of the young chick by reversed-phase ion-pair high-performance liquid chromatography

D. NGUR,* C. WAMBEBE and G. OSUIDE

Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Samaru-Zaria, Nigeria

Keywords: *High-performance liquid chromatography; monoamines and their principal metabolites; ion-pair; reversed-phase; brain tissue; chicks.*

Introduction

Estimation of the catecholamine metabolite urinary profile has been shown to provide useful information of neuronal activity and therefore relied upon in the clinical diagnosis related to malfunction of adrenal medulla [1]. Diseases such as phaeochromocytoma give rise to overproduction of catecholamines [2]. Schizophrenia and Parkinsonism may be investigated by clinical estimation of catecholamines. The specificity of electrochemical detection in high-performance liquid chromatography (HPLC) offers obvious advantages in neurochemical research and will obviously lead to a better understanding of the pathophysiology of these diseases and a better management.

Several reports are available in the literature for the analysis of monoamines and their principal metabolites [3, 4]. These methods have not, however, produced good separation of monoamines of interest in our laboratory. In the course of our investigations on the analysis of monoamines and their principal metabolites, a simple, reproducible and sensitive method was developed and has been used successfully in our laboratory for the analysis of these compounds in brain tissue.

Materials and Methods

Chemicals and reagents

All the monoamines, their principal metabolites and disodium ethylenediaminetetracetic acid were obtained from Sigma Chemicals Co. (St. Louis, MO, USA).

*Correspondence should be addressed to D. Ngur, Department of Pharmacology, Midecal College of Ohio, P.O. Box 10008, OH 43699, USA.

Analytical grade methanol obtained from Merck (Darmstadt, FRG) was double-glass distilled to remove all unwanted impurities before use. HPLC grade water was prepared by deionizing and glass distillation of tap water before passing through Waters norganic catridge. Hexane sulphonic acid was obtained from Kodak (Rochester, NY, USA), potassium dihydrogen orthophosphate was obtained from BDH (Poole, UK). Orthophosphoric acid (90%, v/v) was obtained from May and Baker Ltd (Dagenham, UK).

Chromatographic system

The liquid chromatographic system used was a Waters model 204 comprising a Waters M-45 solvent delivery system, a U6K universal injector, an omniscribe strip chart recorder model D5000 (Bausch and Lomb, TX, USA) and an electrochemical detector comprising model LC-17 oxidative electrode and a LC-4B electronic controller (Bioanalytical Systems Inc., West Lafayette, IN, USA). The voltage of the glassy carbon electrode was maintained at +750 mV against the silver–silver chloride reference electrode throughout the experiment. The sensitivity of the LC-4B electronic controller was maintained at 2 AUFS, ampere units (per) full scale (deflection).

The mobile phase comprised 10% methanol/90% water (with 336.2 mg hexane sulphonic acid, 1.3609 g potassium dihydrogen orthophosphate and 67.24 mg dissodium EDTA) in 1 l. The pH of the eluent was adjusted with phosphoric acid to 3.5. The solution was then filtered and degassed under vacuum using a 0.45-mm filter (Millipore, Bedford, MA, USA). The flow rate was maintained at 1 ml min^{-1} at a constant room temperature of 25°C.

Preparation of standards

Stock solutions were obtained by dissolving 1 mg of the different compounds in 100 ml of 0.1 M perchloric acid containing 0.01%, w/v, sodium metabisulphite and 0.01%, w/v, Na_2EDTA. Stock solutions were prepared every week. Standard solutions were obtained by diluting 1 ml of the stock solutions 50 times.

Preparation of samples

Different brain areas such as the hyperstriatum, optic tectum, brainstem and cerebellum were homogenized in 1 ml of 0.1 M perchloric acid solution containing 0.01%, w/v, sodium metasulphite, 0.01%, w/v, Na_2EDTA and the internal standard isoprenaline (200 $\mu g\ l^{-1}$). Brain regions were pooled from two chicks; and five such determinations were made in both control and drug-treated group. After centrifugation at a speed of 30,000*g* and temperature of 2°C, the supernatant was passed through a 0.45-μm, filter (Millipore, Bedford, MA, USA) to remove particulate matter. Five microlitres of the filtrate were injected for the simultaneous determination of the monoamines and their principal metabolites.

Results and Discussion

The separation of a standard mixture of L-dopa, noradrenaline (NA), adrenaline (AD), 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxytryptophan (5-HTP), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic (HVA) and 5-hydroxytryptamine (5-HT), using isoprenaline (ISO) as internal standard, was achieved isocratically.

The calibration data shown in Table 1 indicate a significant correlation between peak height ratio and the doses used.

Table 2 indicates the retention times and capacity factors for the different monoamines. A peak is usually identified using some measure of retention. Often, retention times can be used as such but a more universal term frequently used is the capacitor factor (K^1). This is given as

$$K^1 = \frac{V_1 - V_0}{V_0},$$

where V_1 and V_0 are measured in terms of same units, e.g. time, volume or distance for the analyte (V_1) and the void retention (V_0). The shortest retention time was that of L-dopa (2.8 min) compared with a column hold up time (V_0) of 1.4 min. The longest retention was that of 5-HT (22.6 min). This separation was achieved using a Novak Pak 5 mm column. ISO, the internal standard used has a K^1 value of 10 which obviously does

Table 1
Peak height ratio regression statistics for HPLC calibration data of monamines and their major metabolites using ISO as internal standard

Compound	Range 250 pg–4 ng (2 AUFS)		
	m	*c*	*r*
L-Dopa	0.114	−0.304	0.999
NA	0.052	1.713	0.979
MHPG	0.043	1.425	0.979
AD	0.041	1.175	0.982
5-HTP	0.115	0.013	0.999
DA	0.046	−0.025	0.999
5-HIAA	0.07	0.078	0.998
HVA*	0.029	−0.369	0.999
5-HT*	0.017	−0.774	0.997

AUFS, ampere units (per) full scale (deflection).
m, Gradient.
c, Intercept.
r, Correlation coefficient ($n = 5$).
*Range 1–16 ng (2 AUFS).

Table 2
Retention times and capacity factors for monoamines and their major metabolites studied

Compound	Retention time (min)*	Capacity factor (K^1)
L-Dopa	2.8	1.00
NA	3.4	1.43
MHPG	3.9	1.79
AD	4.8	2.43
5-HTP	5.6	3.0
DA	8.0	4.71
5-HIAA	13.4	8.57
ISO	15.4	10.00
HVA	21.2	14.14
5-HT	22.6	15.14

*Column hold-up time was 1.4 min.

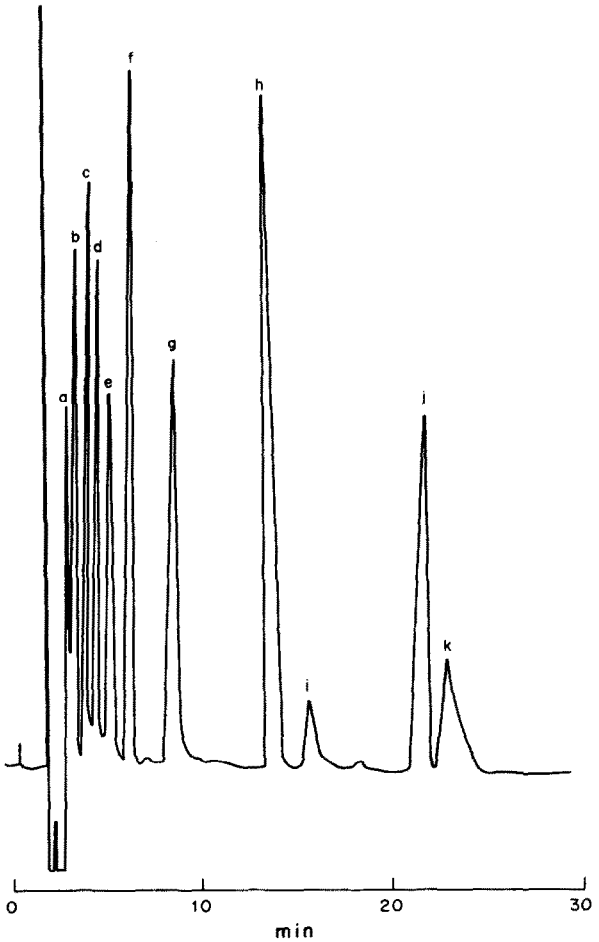

Figure 1
Chromatogram of standards injected. a, Unidentified peak; b, L-dopa (1.0 ng); c, NA (1.0 ng); d, MHPG (1.0 ng); e, AD (1.0 ng); f, 5-HTP (1.0 ng); g, DA (1.0 ng); h, 5-HIAA (1.0 ng); i, ISO (1.0 ng); j, HVA (5.0 ng); k, 5-HT (5.0 ng). Sensitivity, 2 AUFS.

Table 3
Recoveries of the monoamines, and the principal metabolites from brain tissue

Compound	Recovery ± SEM
L-Dopa	87.70 ± 1.02
NA	95.23 ± 1.10
MHPG	80.00 ± 1.70
AD	86.96 ± 1.60
5-HTP	90.50 ± 0.50
DA	92.80 ± 2.30
5-HIAA	90.00 ± 0.90
HVA	95.45 ± 0.50
5-HT	85.58 ± 2.10

SEM, standard error of the mean (n = 5).
Recovery is expressed as spiked brain tissues relative to injection of the standard.

not interfere with the other peaks. A characteristic negative peak appears after the solvent front, but does not interfere with the analysis (see Fig. 1). The appearance of an unidentified peak in the chromatograph is attributed to the impurities in one of the chemicals used in preparing the standards as this peak appears in the blank. High purity chemicals will hopefully remove this interference. The recoveries (Table 3) were obtained as spiked brain samples relative to direct injection of standards. The highest recovery was obtained with HVA and the least with 5-HT. The low recovery level of 5-HT has been attributed to adsorption on surfaces [4].

Figure 2 is a chromatograph showing the hyperstriatum of the young chick. Table 4 shows the estimated concentrations of the different amines and their principal metabolites after treatment with 6-OHDA, 12.5 mg kg^{-1}. 6-OHDA is a neurotoxin that causes a preferential neurodegeneration of noradrenergic nerves. However at 10 min it showed a significant increase in the level of NA in the hyperstriatum ($P < 0.004$). Other monoamines and metabolites were, however, not affected by this acute treatment.

Analysis of discrete brain regions of the chick (1 g) by simple homogenization of brain tissue, centrifugation and direct injection gave a good separation without interference from the brain tissue. The estimated concentrations in the different brain regions of the chick are consistent with previous reports [5].

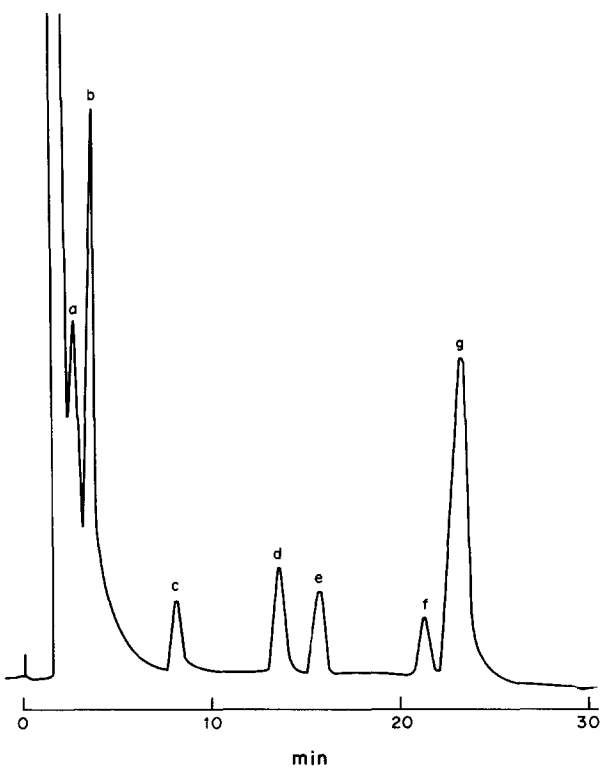

Figure 2
Chromatogram of 1 g of tissue of 6-OHDA (12.5 mg kg^{-1}) treated (10 min) hyperstriatum of the young chick. a, Unidentified peak; b, NA; c, DA; d, 5-HIAA; e, ISO; f, HVA; g, 5-HT. Sensitivity, 2 AUFS.

Table 4
The effect of 6-OHDA (12.5 mg kg^{-1}) on the levels of monoamines (in $\mu g\ g^{-1}$ wet tissue) of the young chicks brain

Brain region		NA	DA	5-HIAA	HVA	5-HT
Hyperstriatum	(Control)	0.06 ± 0.004	0.17 ± 0.006	0.03 ± 0.005	0.06 ± 0.006	0.40 ± 0.003
	(Treated)	0.24 ± 0.03*	0.17 ± 0.01	0.02 ± 0.007	0.07 ± 0.01	0.45 ± 0.01
Brainstem	(Control)	0.19 ± 0.02	0.06 ± 0.007	0.05 ± 0.001	0.12 ± 0.007	0.26 ± 0.003
	(Treated)	0.22 ± 0.009	0.07 ± 0.02	0.06 ± 0.01	0.16 ± 0.005	0.23 ± 0.06
Optic tectum	(Control)	0.08 ± 0.005	—	0.04 ± 0.001	0.07 ± 0.004	0.15 ± 0.009
	(Treated)	0.14 ± 0.003	—	0.03 ± 0.002	0.12 ± 0.005*	0.18 ± 0.01
Cerebellum	(Control)	0.08 ± 0.005	—	0.06 ± 0.004	—	0.20 ± 0.02
	(Treated)	0.09 ± 0.002	—	0.06 ± 0.002	—	0.14 ± 0.02

* $P < 0.004$.
Monoamine levels are expressed as means ± SEM (standard error of mean), $n = 5$.
Statistical analysis is by one-way ANOVA.
6-OHDA treated 12.5 mg kg^{-1} i.p. pre-treatment time, 10 min.

References

[1] A. M. Krstulovic, *J. Chromatogr.* **229**, 1–34 (1982).
[2] A. M. Krstulovic, S. W. Dziedzic, I. Bertani-Dziedzic and D. E. Di Rico, *J. Chromatogr.* **217**, 523–537 (1981).
[3] B. H. C. Westerink, *J. Neurochem.* **42**, 934–940 (1984).
[4] J. Wagner, T. Vitali, M. G. Palfreyman, M. Zraika and S. Huot, *J. Neurochem.* **38**, 1241–1254 (1982).
[5] D. D. Johnson, A. T. Jaju, L. Ness, J. S. Richardson and R. D. Cramford, *Can. J. Phys. Pharmac.* **59**, 144–149 (1981).

[*Received for review 7 April 1988*]