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Simultaneous measurement of serotonin, catecholamines and their metabolites in cat and human plasma by *in vitro* microdialysis–microbore high-performance liquid chromatography with amperometric detection

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ABSTRACT

A method for the simultaneous measurement of norepinephrine, epinephrine, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin and 5-hydroxyindole-3-acetic acid in cat and human plasma by *in vitro* microdialysis–microbore high-performance liquid chromatography with electrochemical detection is described. The detection limit (signal-to-noise ratio = 3) is about 0.05–0.1 pg per injection. The volume of plasma samples required is very small (< 200 μl), hence there is minimal blood loss in repeated blood sampling, especially in experiments using small animals. Within 15 min, a fast isocratic separation of these analytes by using a microbore reversed-phase ODS column is achieved, hence over 90 analyses can be performed in a single working day. As microdialysis *per se* is not destructive to plasma samples, the remaining plasma sample and perfusate can be repeatedly analysed for other substances. This simple, efficient and sensitive method can therefore be used as a routine clinical and basic research technique in the investigation of blood biogenic amines and their metabolites.

INTRODUCTION

The metabolic pathways of catecholamines and serotonin have been established. Therefore, the measurement of these biogenic amines in plasma is a common and important research strategy used in evaluating the etiology of mental [1,2] and neuroendocrinology disorders [3,4] and in studying the role of the autonomic nervous system in various physiological or pathophysiological conditions [5–7]. Many more disease states involve these biogenic amines. For example, patients with haemorrhage shock have higher levels of plasma norepinephrine and epinephrine [8]; patients with idiopathic orthostatic hypotension have lower levels of dopamine than

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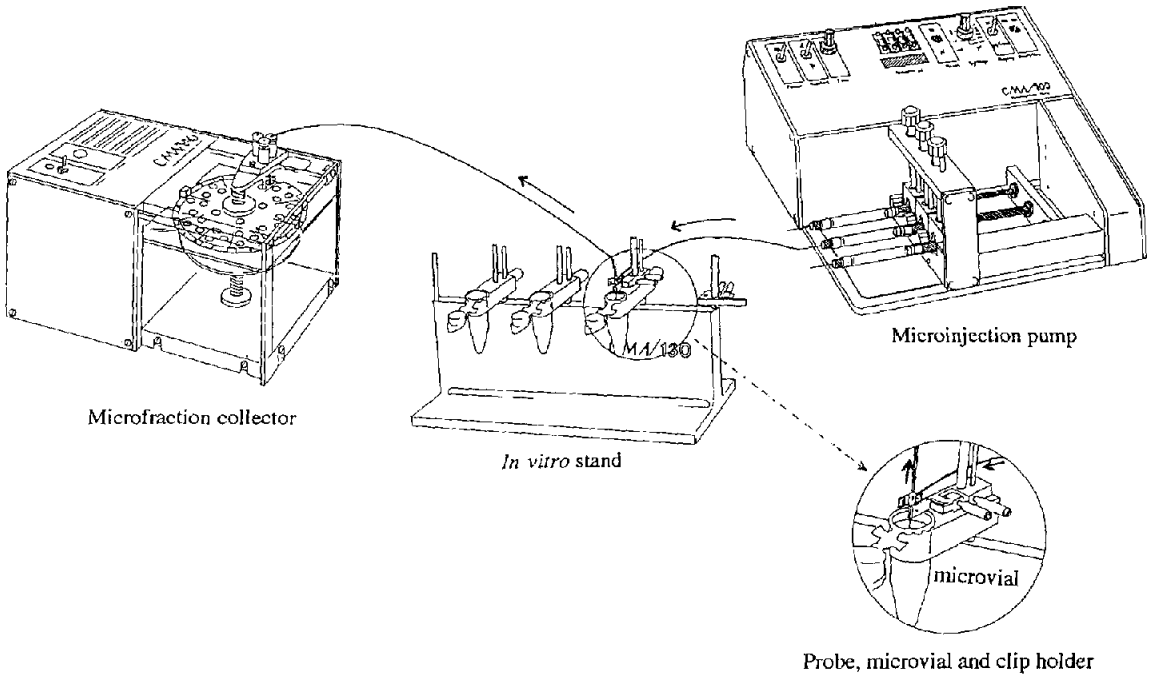

Fig. 1. Diagram of the instruments for the *in vitro* microdialysis experiment: CMA/100 microinjection pump, CMA/20 microdialysis probe with probe clip and holder, *in vitro* stand and CMA/140 microfraction collector. The dead volume between the probe tip and the end of outlet tubing is less than 5 μl.

normal [9]; the diagnosis of pheochromocytoma may be made via plasma catecholamine determination [10]; and a useful monitor for neuroblastoma is the increased secretion of dopamine and norepinephrine [11].

Many analytical procedures have been devised for these purposes. Quantitative methods for determining these compounds include gas chromatography–mass spectrometry (GC–MS) [12], radioimmunoassay (RIA) [13] and various high-performance liquid chromatographic (HPLC) techniques with fluorescence and electrochemical detection (ED) [14,15]. Inter-laboratory comparisons [16] of many different methods in measuring plasma catecholamines have been performed and no "reference method" was suggested. The disadvantages of GC–MS procedures are that they are not only cumbersome and time-consuming but also require expensive equipment and technical skill. RIA methods are tedious, expensive and hazardous owing to the use of radioactive materials. Therefore, a standard method that identifies as many constituents as possible without tedious pretreatment is seriously needed by various laboratories for the simultaneous measurement of catecholamines, serotonin and their metabolites.

HPLC methods offer advantages of reasonable simplicity, versatility, sensitivity and specificity. Improvements in the sensitivity of HPLC techniques have allowed measurements of catecholamines in small plasma volumes, as would be required for the study of small experimental animals [17]. In comparison with other methods, HPLC–ED is considered to be one of the most popular and highly sensitive methods in the determination of these compounds. However, no simple and ideal HPLC method exists for the simultaneous determination of these compounds in plasma without extensive and time-consuming clean-up procedures. Sample clean-up prior to HPLC–ED is necessary for plasma biogenic amine determinations because many other components that may interfere with the analysis exist

in a plasma sample. These clean-up methods require sample purification by means of ion exchange [18], organic solvents [19] or extraction procedures with alumina [20,21]. The variation of the recovery is a serious problem with all of these methods in inter-laboratory comparisons. In addition, the use of a conventional HPLC column lacks rapidity and simplicity as it requires long retention times for simultaneous measurements of catecholamines, serotonin and their metabolites (40–60 min) with tedious pretreatment or, if a short retention time is required, two columns with different pretreatments must be applied. The other problem is that some of these biogenic amine levels in the plasma are far below the detection limit [22], so that the detection requires at least a 3–5 ml blood sample for a tedious pretreatment.

Microbore HPLC–ED is one of the best methods for the determination of neurotransmitters at trace levels. Microbore columns have advantages of very high sensitivity and a relatively small sample introduction. Microdialysis coupled with microbore HPLC–ED is rapidly becoming a routine research tool dealing with minute amounts of samples in a wide range of applications.

A microbore column (100 mm × 1.0 mm I.D.) in combination with HPLC–ED was successfully applied in simultaneous measurements of catecholamines, serotonin, and their metabolites by Huang *et al.* [23]. This technique markedly reduced the time required for separation to 15 min. Microdialysis is a bioanalytical technique used to sample chemical components from body fluids. This technique usually deals with minute samples, *in vivo* or *in vitro*, and offers several advantages over traditional sampling techniques. The most important one is that because of the closed liquid system of the dialysis probe, local proteins and large molecules are precluded by the membrane. Indeed, the microdialysis technique is one of the fastest developing methods by which biological fluids can be cleaned and applied directly to HPLC analysis [24,25]. A high-efficiency (recovery >60% for most biogenic amines, at very slow perfusion rates) microdialysis probe (CMA/20) also became commercially available recently. Here, we describe a method for the determination of these biogenic amines and their metabolites in plasma using a coupled technique, *in vitro* microdialysis–microbore HPLC–ED, which is very simple and rapid to perform and has very high sensitivity. This method can meet the requirements of routine clinical and basic research laboratories.

EXPERIMENTAL

Apparatus and chromatographic conditions

The microdialysis sampling system consisted of a CMA/100 mini-pump, a CMA/140 microfraction collector and CMA/20 microdialysis probes (10 mm in length) from Carnegie Medicin (CMA, Stockholm, Sweden). A diagram of the instrument for the *in vitro* microdialysis experiment is shown in Fig. 1. The microbore HPLC system consisted of a Beckman Model 126 pump, a CMA/200 microautosampler, a BAS-4C amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA), a microbore reversed-phase column (GSK-C_{18}, 5-μm ODS) (150 mm × 1.0 mm I.D.), a Beckman I/O 406 interface and Beckman System Gold data analysis software. The glassy carbon working electrode potential was held at +0.75 V with respect to a silver/silver chloride reference electrode. In order to increase the sensitivity of the microbore HPLC system, a very thin spacer (14 μm) was used instead of a conventional one to create a sub-microlitre thin-layer electrochemical cell and very slow flow-rates (50–60 μl/min) were used to minimize pulse fluctuations.

Chemicals and reagents

Norepinephrine (NE), epinephrine (E), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (serotonin) (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA), ethylenediaminetetraacetic acid (EDTA), diethylamine, sodium 1-octanesulphonate (SOS), sodium dihydrogenphosphate, sodium citrate, isopropylenetryptamine (IPT) (internal standard) and picrotoxin were purchased from Sigma (St. Louis, MO, USA).

HPLC-grade methanol, acetonitrile and tetrahydrofuran (THF) were purchased from Merck. All reagents were of analytical-reagent grade unless stated otherwise.

Sample preparation and assay

Human venous blood samples were obtained between 7.30 and 8.30 a.m. to avoid physical activity and possible diurnal fluctuations in metabolite levels. All subjects had fasted and remained in bed overnight for 12 h before each puncture. Cat plasma samples were obtained under anaesthesia, in a basal condition and in a sympathetic excited state induced by intravenous administration of picrotoxin. Blood samples were collected in pre-chilled polypropylene tubes with heparin as an anticoagulant and centrifuged (10 min, 700 *g* at 4°C) to separate the plasma. Frozen plasma samples were kept at −70°C and thawed in an ice-bath prior to assays.

Stock standard solutions of NE, E, DA, DOPAC, HVA, 5-HT, 5-HIAA and IPT were prepared at a concentration of 0.02 ng/ml in 0.1 *M* perchloric acid and stored at −70°C in the dark and thawed in an ice-bath prior to preparation of a standard mixture. The internal standard IPT and standard mixture were prepared fresh each day from a portion of these stock solutions after appropriate dilution with Ringer solution containing 10^{-7} *M* ascorbic acid in 0.1 *M* perchloric acid.

In a small polypropylene tube, 190 μl of each standard mixture or plasma sample were mixed with 10 μl of Ringer solution containing 10^{-7} *M* ascorbic acid and 0.2 ng of IPT (as an internal standard). In order to prevent carry-over contamination, at the beginning of each perfusion, microdialysis probes were rinsed in a microvial containing Ringer solution for 5 min and then transferred to a small polypropylene tube. Ringer solution was used to perfuse microdialysis probes at 1 μl/min by a high-precision pump (CMA/100). Sequential 10-min perfusates were collected by a microfraction collector (CMA/140) in polyethylene microsample tubes containing 5 μl of 0.1 *M* perchloric acid with 10^{-7} *M* ascorbic acid and 5 μl of the perfusates were analysed for NE, E, DA, DOPAC, HVA, 5-HT and 5-HIAA using a microbore HPLC–ED system. The performance of each microdialysis probe was calibrated by dialysing a known amount of the standard mixture, and recoveries of all analytes were then determined. Plasma concentrations of NE, E, DA, DOPAC, HVA, 5-HT and 5-HIAA were calculated by determining each peak-height ratio relative to the internal standard IPT and also corrected by each probe performance.

Mobile phase preparation

The mobile phase was prepared by dissolving 80 ml of acetonitrile, 0.42 g of SOS (2.2 m*M*), 2.00 g of sodium dihydrogenphosphate (14.7 m*M*), 8.82 g of sodium citrate (30 m*M*), 10 mg of EDTA (0.027 m*M*) and 1 ml of diethylamine in doubly distilled water. The solution was adjusted to pH 3.5 with concentrated orthophosphoric acid and its final volume was adjusted to 1 l. The mixture was filtered through a 0.22-μm nylon filter under reduced pressure and degassed by purging with helium for 10 min. The flow-rate was 50–60 μl/min, maintaining the column pressure at *ca.* 7.6 MPa.

RESULTS AND DISCUSSION

Quality of the method

A typical chromatogram of a standard mixture of 50 pg of catecholamines, serotonin, their metabolites and IPT (internal standard) is shown in Fig. 2A. All components under study were well resolved. An analysis was completed within 15 min. Fig. 2B and C show typical chromatograms of the second fraction perfusate of plasma obtained from a cat and from a patient with neuroblastoma, respectively. The identities of the peaks were first confirmed by their retention times, standard addition, and then by a superimpose–alignment technique which was provided by the Beckman System Gold data analysis software (version 6.01). All components in Fig. 2B and C were identical with those in Fig. 2A except for some unknown components. Further identification of these unknown components might be of

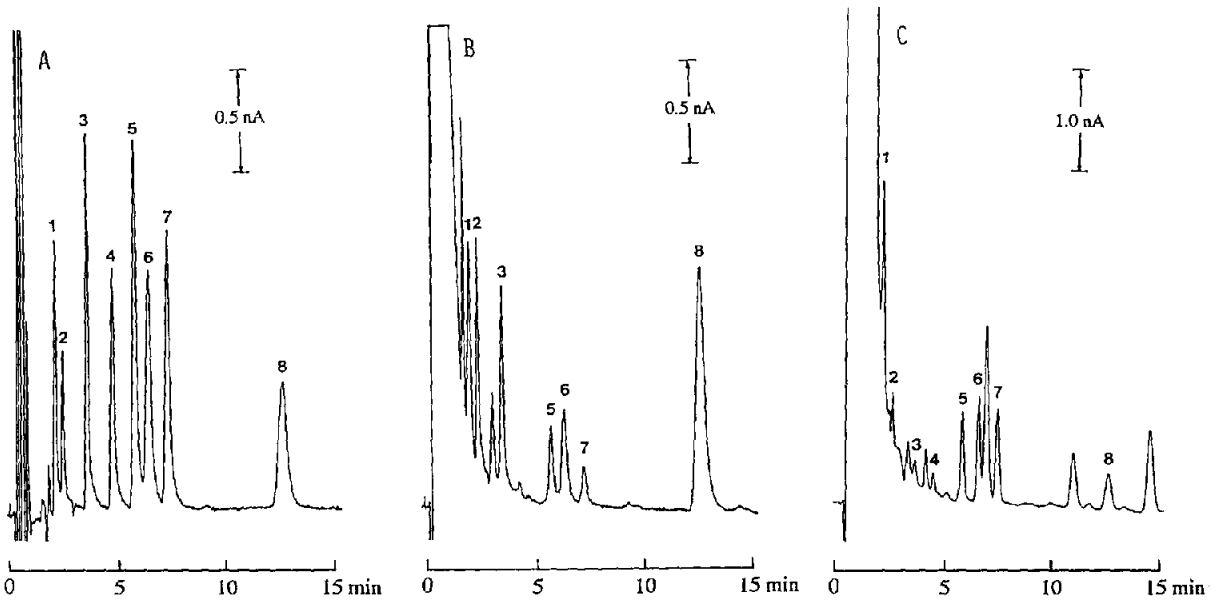

Fig. 2. Typical chromatograms of (A) a standard mixture containing (1) NE (62 pg) (2) E (44 pg), (3) DOPAC (54 pg), (4) DA (46 pg), (5) 5-HIAA (58 pg), (6) IPT (internal standard) (97 pg), (7) HVA (64 pg), (8) 5-HT (47 pg); (B) the second fraction perfusate of the plasma obtained from a cat; (C) the second fraction perfusate of the plasma obtained from a patient with neuroblastoma.

interest in finding new biomarkers for various illnesses. Reliable assignment of peaks is often confirmed by the collection of fractions eluting from a column and obtaining conventional mass spectra. However, confirmation in this way is difficult, especially at trace levels.

Precision and accuracy

The response of the electrochemical detector (y) was found to increase linearly with amounts injected (x) between 0.2 pg and 20 ng for all analytes: the regression equation and correlation coefficient for each analyte were as follows: NE, $y = 352.3x + 15.6$, $r^2 = 0.999$; E, $y = 496.7x + 28.1$, $r^2 = 0.997$; DOPAC, $y = 275.8x + 8.8$, $r^2 = 0.999$; DA, $y = 266.3x + 22.0$, $r^2 = 0.998$; 5-HIAA, $y = 205.1x + 4.9$, $r^2 = 0.992$; HVA, $y = 240.6x + 31.6$, $r^2 = 0.998$; 5-HT, $y = 227.6x + 1.1$, $r^2 = 0.989$. The precision of the assays was tested using a standard mixture in 0.1 M perchloric acid and a Ringer solution containing 10^{-7} M ascorbic acid with or without perchloric acid. The intra-assay variabilities were assessed with 25 replicates (at 1-h intervals) and expressed as coefficients of variation (C.V., %). 5-HIAA (9.1%) and E (6.1%) were relatively unstable whereas the others were stable ($< 5.2\%$) when a standard mixture was prepared in 0.1 M perchloric acid. All components were less stable ($> 5.8\%$) when a standard mixture was prepared in Ringer solution containing 10^{-7} M ascorbic acid. The best matrix gave C.V. $< 3.1\%$ for all components when a standard mixture was prepared in a Ringer solution containing 10^{-7} M ascorbic acid and 0.1 M perchloric acid. This matrix was then chosen for inter-assay evaluation and for further study. The inter-assay variabilities assessed with a standard mixture during ten consecutive working days were less than 7.5% except for DOPAC (10.1%). The intra- and inter-assay stabilities and C.V.s calculated for the standard mixture and pooled cat plasma samples are summarized in Table I. The detection limit (signal-to-noise ratio = 3) of analytes per injection were between 0.05 and 0.1 pg.

Recovery–fraction (from the first to the tenth fraction) histograms for the internal standard IPT using CMA/20 microdialysis probes ($n = 3$)

TABLE I

ANALYTICAL PRECISION AND EFFECTS OF DIFFERENT ANTIOXIDANTS ON THE INTRA-ASSAY (n=25, at 1-h INTERVALS) AND INTER-ASSAY (n=10, IN TEN CONSECUTIVE WORKING DAYS) STABILITIES OF THE MICRO-BORE HPLC-ED SYSTEM

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

Antioxidant	C.V. (%)						
	NE	E	DOPAC	DA	5-HIAA	HVA	5-HT
Intra-assay							
0.1 *N* $HClO_4$	2.8	6.1	3.6	3.8	9.0	4.3	5.2
Ringer + vitamin C	6.8	8.3	9.0	5.8	7.8	8.1	7.7
0.1 *M* $HClO_4$ + Ringer + vitamin C	1.9	3.5	2.0	1.7	3.8	1.8	3.4
Inter-assay							
0.1 *M* $HClO_4$ + Ringer + vitamin C	5.4	6.5	10.1	7.5	6.1	4.9	4.3

are shown in Fig. 3. Although the recoveries of the probes differed from one to another, the internal standard IPT peaks reached plateaux at the second and following fractions. This is predictable, as the total dead volume of a probe was 5.0 μl (the volume from the top of the tip of a probe was 1.4 μl and that of the outlet tubing was 3.6 μl). At a perfusion rate of 1 μl/min, the first 5-min wash-out period should be able to flush out the dead volume of the probe. Then, the following 10-min period (in the collection of the first fraction) should have allowed the system to reach an equilibrium condition. Indeed, the second and following perfusion fractions should have a representable composition of a standard mixture. The second and third fractions were then chosen to evaluate the validities of all the assays in the present experiment unless mentioned otherwise.

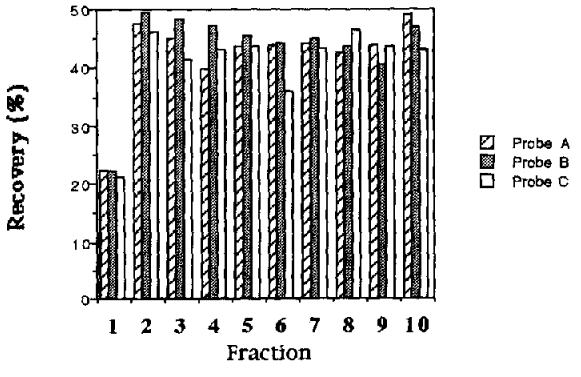

Fig. 3. Recovery–fraction histograms for IPT internal standard (100 pg). The IPT was dialysed by CMA/20 microdialysis probes perfused with Ringer solution at 1 μl/min for 100 min. Ten consecutive fractions were collected at 10-min intervals.

In order to check the reliabilities of the probes, a known mixture of eight analytes (*ca.* 50 pg of each) was dialysed by nine different CMA/20 probes perfused with Ringer solution and six consecutive fractions were collected. The data were calculated and are reported as recoveries relative to IPT in Table II. The recoveries of NE, DA, HVA and 5-HT relative to IPT were very close to 1.0 and that of E was about 1.5 with C.V. between 4 and 15% for the second to the sixth fractions. The recoveries of DOPAC and 5-HIAA to IPT were lower (0.48–0.94) with standard deviations between 12 and 26% for the second to the sixth fractions. Because of these lower recoveries of DOPAC and 5-HIAA, measurements of these two components led to greater standard deviations. This phenomenon can be explained by variations of the diffusion rates of analytes across the membrane. However, these variations between probes or analytes can be

TABLE II

RECOVERIES RELATIVE TO IPT INTERNAL STANDARD (100 pg) OF SEROTONIN, CATECHOLAMINES AND THEIR METABOLITES IN A STANDARD MIXTURE CONTAINING 50 pg OF EACH ANALYTE

Microdialysis probes ($n=9$) were perfused by Ringer solution at 1 μl/min (consecutive fractions were collected at 10-min intervals).

Fraction	Recovery relative to IPT						
	NE	E	DOPAC	DA	5-HIAA	HVA	5-HT
1st	0.99±0.21	1.63±0.18	0.38±0.14	0.86±0.08	0.34±0.14	0.98±0.12	0.71±0.20
2nd	1.04±0.16	1.34±0.19	0.63±0.17	0.97±0.07	0.48±0.11	1.01±0.08	0.96±0.08
3rd	1.06±0.16	1.34±0.19	0.71±0.16	0.99±0.04	0.54±0.08	1.05±0.08	0.98±0.06
4th	1.05±0.16	1.42±0.15	0.81±0.10	1.01±0.05	0.60±0.09	1.05±0.06	0.99±0.05
5th	1.10±0.18	1.48±0.16	0.88±0.12	1.03±0.05	0.68±0.11	1.07±0.07	1.02±0.07
6th	1.12±0.16	1.54±0.17	0.94±0.13	1.04±0.02	0.81±0.15	1.09±0.07	1.06±0.05

carefully corrected by the recovery of the internal standard IPT in any particular event.

In order to check the validity of the assays, pooled cat plasma samples ($n=9$) with and without addition of a known amount of standard mixture (about 50 pg each) were dialysed and analysed. The second and third fractions were collected and evaluated as shown in Table III. The values of these analytes in pooled cat plasma samples, corrected by the IPT recovery at each run, were consistent between two fractions and the C.V. of each pair of fractions was between 3 and 10%. Addition of a standard mixture has two aspects. First, the addition of a known amount of analytes should increase their peak heights but not change their peak shapes. Second, the addition and observed amounts of analytes should be consistent in each pair of fractions

TABLE III

CONCENTRATIONS OF CATECHOLAMINES, SEROTONIN AND THEIR METABOLITES IN POOLED CAT PLASMA WITH AND WITHOUT THE ADDITION OF A KNOWN AMOUNT OF STANDARD MIXTURE

The second and third fractions were collected at 10-min intervals.

Fraction	Concentration (ng/ml)						
	NE	E	DOPAC	DA	5-HIAA	HVA	5-HT
Pooled plasma							
2nd	12.4±2.0	6.1±1.2	19.8±2.8	4.1±0.8	13.9±1.8	14.3±1.6	69.9±0.7
3rd	12.0±1.8	6.2±0.5	17.3±1.8	4.4±0.3	10.5±0.8	11.4±0.8	66.4±2.3
Mean	12.2	6.2	18.6	4.3	12.2	12.9	68.2
Pooled plasma + standard mixture							
2nd	21.4±3.3	14.5±2.6	26.7±1.4	11.7±0.9	21.6±1.3	23.4±1.3	76.9±3.0
3rd	21.0±2.5	13.2±0.6	27.2±1.6	11.4±0.7	20.2±0.9	23.9±1.1	75.6±2.2
Mean	21.2	13.9	27.0	11.6	20.9	23.7	76.3
Amount added	9.2	6.6	8.0	6.9	8.6	9.6	7.1
Amount measured[a]	10.0	7.7	8.4	7.3	8.7	10.8	8.1

[a] Amounts of analytes measured were calculated by subtracting the mean values of the 2nd and 3rd pooled plasma from the mean values of the 2nd and 3rd pooled plasma plus standard mixture.

TABLE IV

CONCENTRATIONS OF SEROTONIN, CATECHOLAMINES AND THEIR METABOLITES IN CAT AND HUMAN PLASMA SAMPLES DETERMINED BY THE *IN VITRO* MICRODIALYSIS–MICROBORE HPLC-ED METHOD

Sample	*n*	Concentration (ng/ml)						
		NE	E	DOPAC	DA	5-HIAA	HVA	5-HT
Neuroblastoma	4	0.25 ± 0.07	1.63 ± 0.21	2.72 ± 1.71	0.23 ± 0.32	5.11 ± 0.96	7.25 ± 1.91	4.18 ± 1.76
Cat plasma	5	4.17 ± 5.12	6.59 ± 4.45	2.66 ± 1.22	N.D.[a]	8.86 ± 3.20	4.88 ± 1.65	59.83 ± 46.29
Picrotoxin-injected cat plasma	5	45.46 ± 32.62	132.36 ± 123.27	2.70 ± 1.05	N.D.[a]	10.96 ± 5.86	5.65 ± 1.90	31.20 ± 12.16

[a] Not detectable.

(the second *versus* the third fraction) after careful correction. The amount measured by our assay in the perfusate of each pooled plasma with addition of a standard mixture, minus the value of these components originally in the plasma, was compared with the known amount added. The amounts of analytes measured and added were very close. These results indicate that both the second and third fractions are adequate for the determination of these analytes. Results of studies in plasma samples obtained from patients with neuroblastoma and from cats are shown in Table IV. These data are very close to those obtained using a deproteination technique and alumina extraction methods in a previous study. Although our quantitative results correlate well, these data were not interpreted clinically.

Many problems arise with reproducibilities of conventional extraction methods, particularly with varied batch-to-batch and extensive pretreatment before use. The method described in this paper can be an alternative procedure to simplify pretreatment. The automated HPLC method is advantageous when many samples have to be analysed in a short period of time. In this study, good recoveries of catecholamines, serotonin and their metabolites (about 50%) in microdialysis sampling were found and only microdialysis probes should be standardized based on the internal standard IPT.

Interfering peaks in HPLC methods reported by some investigators are attributed to some substances present in the plasma or created after pretreatment [26]. Although the microdialysis procedure is simple, rapid and creates no substances, we should pay attention to interfering substances originally existing in the plasma. Some interfering peaks of unknown substances did occur in the chromatograms using a microdialysis probe in sampling plasma shown in Fig. 2B and C. These unknown substances have not been reported before and might be of importance. Further investigation of these substances will probably lead to more detailed physiological information.

A great advantage of the described method is a very low detection limit (typically 0.1–0.05 pg per injection). No further purification was necessary to obtain a complete separation within 15 min. Hence over 90 analyses can be done in one working day with the automatic microbore HPLC system. The required volume of plasma samples is very small ($< 200\ \mu l$). Measurements of plasma contents in very small volumes have great analytical potential in the fields of routine paediatric examination, repeated blood samplings and small experimental animal studies. As microdialysis *per se* is not destructive to plasma samples, the remaining same plasma sample, in addition to the perfusate, can be repeatedly analysed for other substances.

CONCLUSION

Although compromises between economy and performance often have to be made, we conclude that the method presented here is a good alterna-

tive to some rather complicated methods, such as alumina or cation-exchange extractions currently used for the determination of serotonin, catecholamines and their metabolites in plasma. It is suggested that this method can be considered as a "reference method" in inter-laboratory comparisons of the determination of plasma biogenic amines and their metabolites. We would recommend this method as a suitable tool for use in basic research and routine clinical applications.

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REFERENCES

1 G. Curzon, in W. B. Essman (Editor), *Serotonin in Health and Disease, Vol. 3, The Central Nervous System*, Spectrum Publications, New York, 1978, p. 403.
2 M. Pines, *Science*, 83 (1983) 55.
3 H. Yao, K. Fukiyama, Y. Takada, M. Fujishima and T. Omae, *Jpn. Heart J.*, 26 (1985) 593.
4 I. J. Kopin, *Pharmacol. Rev.*, 37 (1985) 333.
5 C. Y. Chai, A. M. Y. Lin, C. K. Su, S. R. Hu, L. S. Kao, J. S. Kuo and D. S. Goldstein, *J. Auton. Nerv. Syst.*, 33 (1991) 35.
6 W. H. Kaye, H. E. Gwirtsman, D. T. George, M. H. Ebert, D. C. Jimerson, T. P. Tomai, G. P. Chrousos and P. W. Gold, *J. Clin. Endocrinol. Metab.*, 64 (1987) 203.
7 D. S. Goldstein, C. R. Lake, B. Chernow, M. G. Ziegler, M. D. Coleman, A. A. Taylor, J. R. Mitchell, I. J. Kopin and H. R. Keiser, *Hypertension*, 5 (1983) 100.
8 W. Raum and R. Swerdloff, *Life Sci.*, 28 (1981) 43.
9 M. G. Ziegler, C. R. Lake and I. J. Kopin, *N. Engl. J. Med.*, 296 (1977) 293.
10 M. Misukoshi, T. Hano, M. Kuchii, I. Nishio and Y. Masuyama, *Jpn. Circ. J.*, 49 (1985) 1035.
11 L. Helson, G. A. Johnson and R. Smith, *Med. Pediatr. Oncol.*, 8 (1980) 317.
12 J. Roboz, in A. M. Krstulovic (Editor), *Quantitative Analysis of Catecholamines and Related Compounds*, Ellis Horwood, Chichester, 1986, p. 46.
13 P. Hjemdahl, *Acta Physiol. Scand., Suppl.*, 527 (1984) 43.
14 A. M. Krstulovic and A. M. Powell, *J. Chromatogr.*, 171 (1979) 345.
15 P. T. Kissinger, C. Refshauge, R. Dreiling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.
16 B. Kagedal and D. Doldstein, *J. Chromatogr.*, 429 (1988) 177.
17 F. Ehrenstrom, *Life Sci.*, 43 (1988) 615.
18 J. Odink, H. Sandman and W. H. P. Schreurs, *J. Chromatogr.*, 377 (1986) 145.
19 A. Yoshida, M. Yoshioka, T. Sakai and Z. Tamura, *J. Chromatogr.*, 227 (1982) 162.
20 A. H. Anton and D. F. Sayre, *J. Pharmacol. Exp. Ther.*, 138 (1962) 360.
21 G. Eisenhofer, D. S. Goldstein, R. Stull, H. R. Keiser, T. Sunderland, D. L. Murphy and I. J. Kopin, *Clin. Chem.*, 32 (1986) 2030.
22 I. N. Mefford, *Methods Biochem. Anal.*, 31 (1985) 221.
23 T. Huang, R. Shoup and P. Kissinger, *Curr. Sep.*, 9 (1990) 139.
24 U. Ungerstedt, in C. A. Marsden (Editor), *Measurement of Neurotransmitter Release In Vivo*, Wiley, New York, 1984, p. 81.
25 P. Lonnroth and U. Smith, *J. Int. Med.*, 227 (1990) 295.
26 G. Eisenhofer, *J. Chromatogr.*, 317 (1986) 328.