Journal of Chromatography, 415 (1987) 275–288
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3504

SIMULTANEOUS LIQUID CHROMATOGRAPHIC DETERMINATION OF SEVENTEEN OF THE MAJOR MONOAMINE NEUROTRANSMITTERS, PRECURSORS AND METABOLITES

II*. ASSESSMENT OF HUMAN BRAIN AND CEREBROSPINAL FLUID CONCENTRATIONS

PER WESTER*

Umeå Dementia Research Group, Department of Pathology and Geriatric Medicine, University of Umeå, S-901 87 Umeå (Sweden)

JOHAN GOTTFRIES

Department of Neurochemistry, St. Jörgens Hospital. S-422 03 Hisingsbacka (Sweden)

and

BENGT WINBLAD

Umeå Dementia Research Group, Department of Pathology and Geriatric Medicine, University of Umeå, S-901 87 Umeå (Sweden)

(First received August 25th, 1986; revised manuscript received November 13th, 1986)

SUMMARY

The optimized chromatographic method procedure presented in Part I was employed for the assessment of human brain and cerebrospinal fluid neurotransmitters levels. The optimized sample preparation and chromatographic conditions permitted a rapid (<25 min), sensitive and semi-automated high-performance liquid chromatographic analysis which measures all major monoamine neurotransmitters, precursors and metabolites in human brain and cerebrospinal fluid. The brain specimen was deproteinized with perchloric acid (containing Na_2EDTA and sodium sulphite), the internal standard and heparin were added and the samples were sonicated, centrifuged, filtered and injected directly into the chromatographic system. Cerebrospinal fluid was handled in a similar manner except that sonication was excluded. The regional distribution of monoamine neurotransmitter concentrations in human brain and cerebrospinal fluid is presented.

*For Part I, see p. 261.

0378-4347/87/$03.50

INTRODUCTION

This paper is a continuation of Part I [1], which described the optimization of the mobile phase using factorial designs and a computer program to predict chromatograms.

Dopamine (DA), norepinephrine (NE), epinephrine (E) and serotonin (5-HT) have been implicated in several types of behaviour and central nervous system (CNS) functions. In normal ageing and in several neurological and psychiatric disorders there are specific changes in these monoamines that are closely related to these conditions [2]. It is therefore not surprising that numerous methods have been developed for the separation and quantification of different monoamine neurotransmitters in various physiological tissues and fluids. Amongst these, gas chromatography–mass spectrometry (GC–MS) is considered to be the most precise and specific (see e.g., ref. 3) but has the disadvantage that the equipment is expensive and needs derivatization procedures before analyses and is therefore time-consuming. Radioenzymatic methods are probably the most sensitive, but do not allow the simultaneous measurement of monoamine precursors, transmitters and metabolites [4]. The utility of liquid chromatography with electrochemical detection (LC–ED), as pioneered by Adams [5] and Kissinger et al. [6], has become popular in the last decade because it is straightforward and offers good selectivity and sensitivity (for a recent review, see ref. 7). However, most of the methods presented so far measure only a few monoamine compounds simultaneously [8] and few procedures allow the simultaneous determination of monoamine precursors, transmitters and metabolites [8–13]. Amongst these, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), the major noradrenaline metabolite, has been shown to be difficult to separate from early eluting unknown compounds.

The purpose of this study was to employ the optimized HPLC separation described in Part I [1] and develop appropriate conditions for the simultaneous assessment of the monoamine neurotransmitters, precursors and metabolites in human brain and cerebrospinal fluid.

EXPERIMENTAL

Chromatography

The liquid chromatographic system included a Constametric III pump (Laboratory Data Control, Riviera Beach, FL, U.S.A.); a pulse damper consisting of a 16-ml 1-m-PTFE tube double wound with a stainless-steel wire, burst pressure 860 bar (HPLC-Teknik, Robertsfors, Sweden); a stainless-steel presaturator column, 50×8 mm I.D. (HPLC-Teknik), packed with the same packing material as the analytical column, connected between the pump and the injector; a guard coulometric cell (Environmental Sciences Assoc., Bedford, MA, U.S.A.); a Shimadzu SIL-6A autoinjector with an SCL-6A system controller (Shimadzu, Kyoto, Japan), the samples being cooled by a circulating water-bath; a 170×4.6 mm I.D. stainless-steel column (HPLC-Teknik) packed with Nucleosil C_{18} (5 μm) (batch No. 5061) from Macherey-Nagel (Düren, F.R.G.), the column temperature being

controlled by means of a plastic jacket (HPLC-Teknik) coupled to a water thermostat; a Coulochem ESA detector (Environmental Sciences Assoc.) with a Model 7021 serial coupled analytical cell [the first cell consisting of a porous graphite working electrode (coulometric) and the second cell being designed amperometrically in order to achieve an optimal signal-to-noise ratio, which is due to a smaller and thinner porous graphite working electrode and therefore oxidizing approximately 50–60% of oxidizable compounds], both of these electrodes have modified palladium as reference systems; and a Model CI10 integrator (Laboratory Data Control).

Reagents

All reference and internal standards and heparin (p.a. grade) were obtained from Sigma (St. Louis, MO, U.S.A.). Citric acid monohydrate (citrate) (p.a. grade), Titriplex III disodium ethylenediaminetetraacetate (p.a. grade) (Na_2EDTA), anhydrous sodium sulphite (p.a. grade) (Na_2SO_3) and sodium octylhydrogensulphate for surfactant tests (octylsulphate, OSA) were purchased from Merck (Darmstadt, F.R.G.), 60% perchloric acid (reagent grade) from Riedel-de Haën (Seelze-Hannover, F.R.G.), acetonitrile (HPLC grade) from Rathburn (Walkerburn, U.K.) and sodium hydroxide (p.a. grade) from EKA (Bohus, Sweden). All chemicals were used as received.

The mobile phase was filtered under vacuum through a 0.22-μm GSWP filter (Millipore, Bedford, MA, U.S.A.). Acetonitrile was filtered separately through a 0.22-μm GVWP filter (Millipore).

Standards

Stock solutions of the reference and internal standards were prepared in deionized, distilled water at a concentration of 10^{-3} *M*, aliquoted and stored at -20°C. Working standards (10^{-5}–10^{-8} *M*) were prepared on the day of assay by dilution of the stock solution with 0.1 *M* perchloric acid (containing 0.3 mmol/l Na_2EDTA and 0.5 mmol/l Na_2SO_3).

Sample preparation

Post mortem human brain tissue and cerebrospinal fluid (CSF) were obtained at autopsy from patients without any clinical or neuropathological verified neurological or psychiatric disorders. CSF was taken from the third ventricle. In addition, lumbar CSF was taken from orthopaedic patients immediately prior to spinal anaesthesia. The brain pieces and the CSF samples were placed in airtight plastic vials and immediately frozen and stored at -70°C. For the regional distribution study, six cases (five male and one female) were obtained 7.6 ± 4.2 h (mean $\pm$ S.D.) post mortem from individuals of 62.7 ± 15.4 years, none of whom had taken any neuroactive drugs in the 24 h prior to death. The cause of death was myocardial infarction in all cases.

Frozen brain samples were weighed. To each 100 mg of tissue, 700 μl of 0.1 *M* perchloric acid (containing 0.3 mmol/l Na_2EDTA and 0.5 mmol/l Na_2SO_3), 70 μl of 10^{-5} mol/l isoproterenol (internal standard) and 70 μl of concentrated heparin (25 000 I.E./ml) were added. The tissue was then thoroughly disrupted by

sonication by a Sonifier Cell Disrupter B-30 (Branson, Danbury, U.S.A.) on ice, with continuous pulses set at 3.5 for 30 s, and the suspension was centrifuged at 48 000 *g* for 30 min at 4 °C. The supernatant thus obtained was filtered through a 0.60-μm Millipore membrane filter and then injected directly into the chromatographic system.

To each 100 μl of CSF, 10 μl of 1 *M* perchloric acid (containing 0.3 mmol/l Na_2EDTA and 0.5 mmol/l Na_2SO_3), 10 μl of $5 \cdot 10^{-6}$ *M* isoproterenol (internal standard) and 10 μl of concentrated heparin (25 000 I.E./ml) were added. The CSF was vortexed, centrifuged at 48 000 *g* for 30 min at 4 °C, passed through a 0.60-μm Millipore filter and then injected directly into the chromatographic system.

Dissection method

The brains were placed on an ice-tray immediately after removal from the skull. Dissection was performed macroscopically and fresh from the right hemisphere (midline areas being divided in half). The dissection was performed according to the following procedure and in this order. Hypothalamus: chiasma opticum, the infundibulum and the hypophysis were removed. The area defined with incisions behind and laterally to the corpora mamillaria, anteriorly behind the commisura anterior and the lamina terminalis of the third ventricle. Olfactory tubercle: defined as the cortical grey matter bounded by the olfactory striae anterolaterally and by the diagonal band of Broca posteromedially at a depth of 2 mm. Pons: defined with transverse sections just below the cerebral peduncles and above the medulla oblongata. The corticospinal tract, pontine nuclei and corticopontine tracts were included. Medulla oblongata: defined with sections just below pons and the olives, the whole part being taken. Substantia nigra: transverse sections were made through the cerebral peduncles until exposure of the substantia nigra. Only zona compacta (the dark tissue) was included. Frontal cortex: grey matter was taken from the convexity of the most anterior parts of the frontal lobe (parts of Brodmann areas 10 and 11). Temporal cortex: grey matter was taken from part of Brodmann area 38. Cingulate cortex: grey matter was taken from the cingulate gyrus (part of Brodmann areas 23, 24 and 31). Parietal cortex: grey matter was taken from part of Brodmann area 39. Occipital cortex: grey matter was taken from parts of Brodmann areas 17 and 18. Caudate nucleus: the elongated arched grey nucleus related throughout the floor of the upper part of the side ventricles, defined with an anterior incision at the edge of the head and a posterior incision at the edge of the thin tail. The nucleus was separated with an incision along its full length. Thalamus: defined with a circular, horizontal incision just medial to the caudal part of the caudate nucleus, going around (medially to the pulvinar part) inside the thalamic area and 5 mm inferiorally to the ventricular surface of the top. Hippocampus: defined anterially with an incision at the edge of the head and posterially at the tail where the hippocampal gyrus projects backwards and cranically. The hippocampus was separated with an incision through the hippocampal sulcus along its full length. Amygdala: defined with a transversal incision anterior to the head of hippocampus, the main part of the nuclei was taken. Putamen: the most lateral part of the basal ganglia, between

the external capsule and the lateral medullary lamina of the globus pallidus. Globus pallidus: the most medial part of the lentiform nucleus, medially to the putamen throughout its extent. Both the medial and the lateral parts were included.

Quantitative analysis

The routinely used internal standard technique with isoproterenol as the internal standard was used for the assessment of neurotransmitter concentrations:

$$\text{Concentration}_s = \frac{\text{response factor}_s\ (\text{pmol/mm}) \times \text{peak height}_s\ (\text{mm}) \times \text{amount}_{i.s}\ (\text{pmol}) \times \text{RCF}}{\text{response factor}_{i.s}\ (\text{pmol/mm}) \times \text{peak height}_{i.s}\ (\text{mm}) \times \text{amount}_s\ (\text{mg})}$$

where the subscript s is the neurotransmitter substance and i.s. is the internal standard, and RCF is the recovery compensating factor (see Tables I and II). The response factors were obtained by calibration with standards every fifth sample.

RESULTS AND DISCUSSION

Chromatographic conditions

A satisfactory chromatographic system was achieved on a chromatographic column with a new batch of Nucleosil C_{18} (5 μm) packing material when compared with the packing material that was used for computerized optimization of the mobile phase [1]. This was achieved by starting with the same mobile phase system as in Part I [1], and then slightly modifying the mobile phase using the β values (i.e., values showing the effects of different variables in the mobile phase and their interactions) in the factorial design. This indicates that the β values for a certain packing material are general, when using the same mobile phase parameters; the reproducibility shows the feasability of using factorial designs when optimizing an HPLC separation [1]. This is of major importance when starting up a new HPLC system but also when changing batches of packing material from the same commercial support supplier. In Fig. 1A, a standard chromatogram is shown with satisfactory peak resolution and with analysis time of less than 25 min. Fig. 1B shows the chromatogram of a human hypothalamus sample. Most of the monoamine neurotransmitters, their precursors and metabolites are present in detectable concentrations. Fig. 1C shows the chromatogram of a human lumbar CSF sample. In addition to the major monoamine metabolites MHPG, HVA and 5-HIAA which have recently been separated and detected simultaneously [9,14–16], quantifiable amounts of the transmitters NE, and DA, the precursor 5-HTP and the metabolite 5-HTOL were observed. The detection level may be further improved by injecting larger volumes than the 50 μl used here. The large peak in the chromatogram of the CSF sample at 6.17 min has not been identified. However, it does not interfere with any major compound of interest. Tyrosine and tryptophan were not detected in any of the tissue or fluid chromatograms. However, by increasing the potential (see below) this is possible, although the retention time of tryptophan in this system is about 40 min.

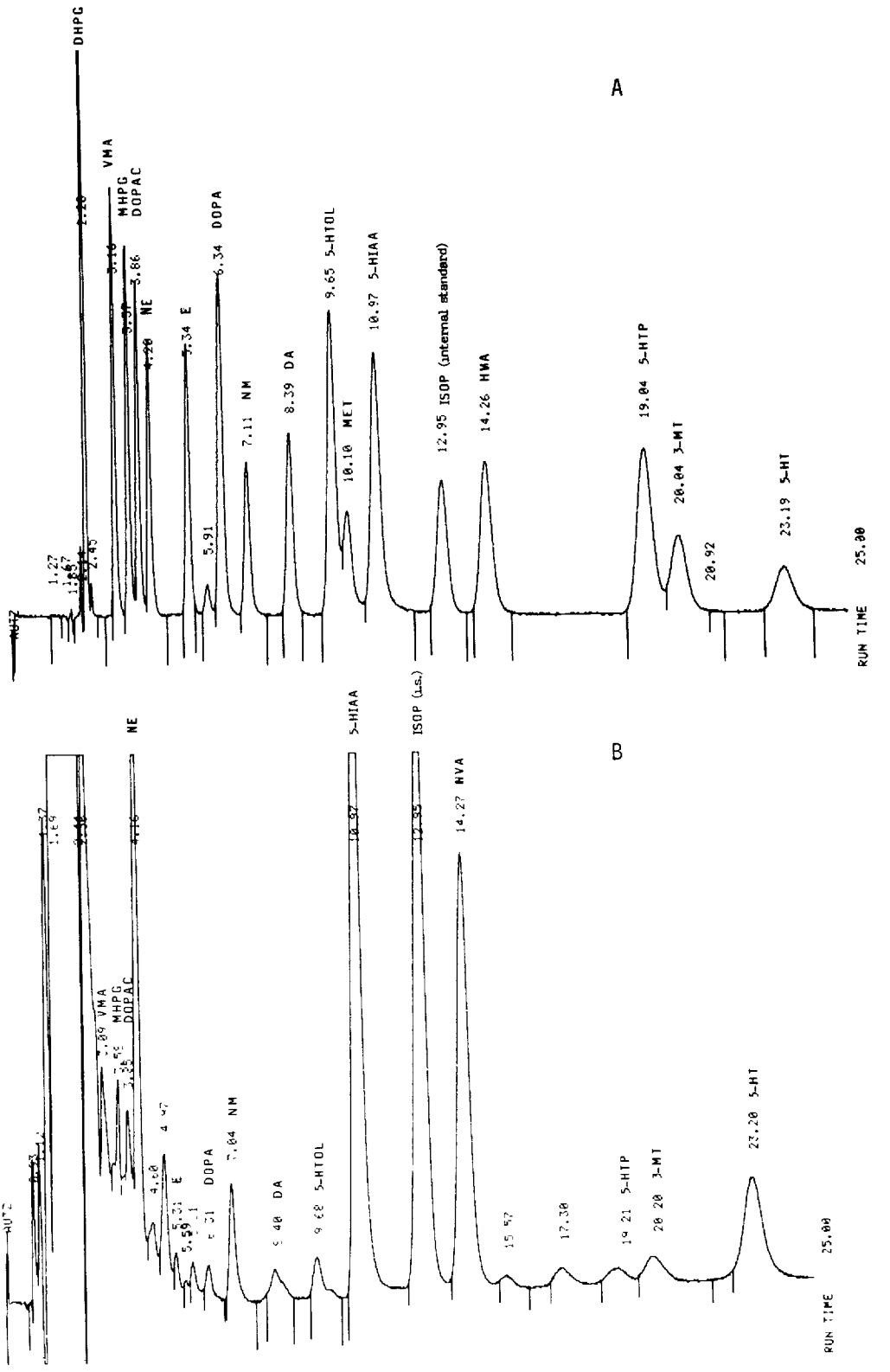
A
DHPG
VMA
MHPG
DOPAC
NE
E
DOPA
NM
DA
5-HTOL
MET
5-HIAA
ISOP (internal standard)
HVA
5-HTP
3-MT
5-HT
RUN TIME 25.00
B
NE
5-HIAA
ISOP (i.s.)
HVA
VMA
MHPG
DOPAC
E
DOPA
NM
DA
5-HTOL
5-HTP
3-MT
5-HT
RUN TIME 25.00

Fig. 1.

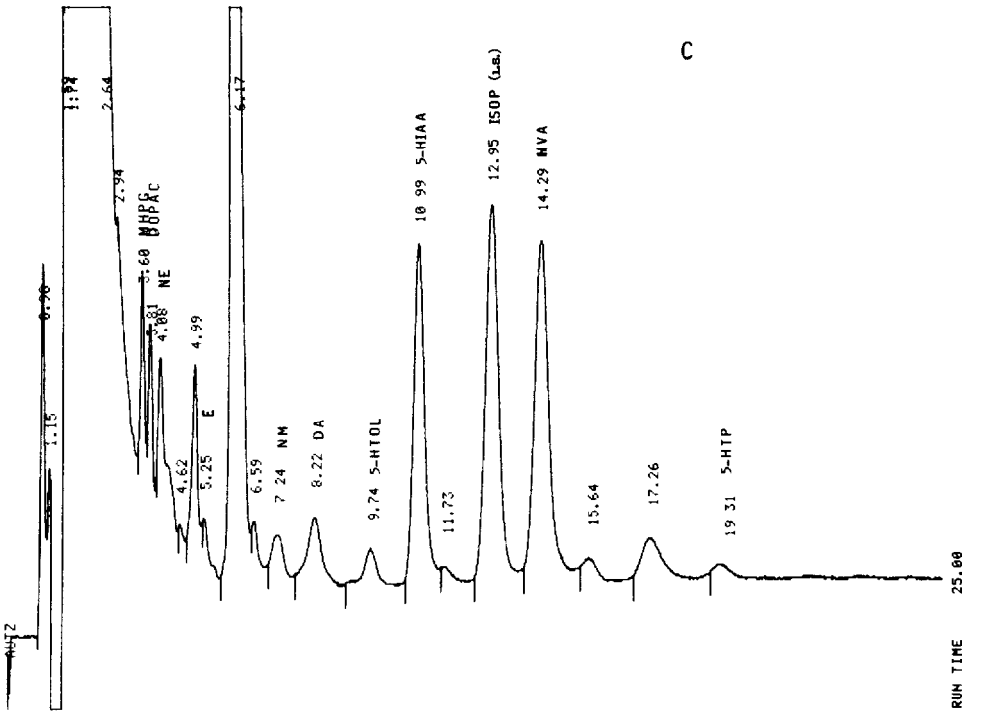

Fig. 1. (A) Standard chromatogram; 5 pmol of each substance were injected. The mobile phase consisted of 100 m*M* citrate buffer including 0.3 m*M* Na_2EDTA, 5.5% (v/v) acetonitrile and 0.334 mmol/l octylsulphate at a pH of 2.35. The guard cell was set at 1.05 V, the first detector cell at 0.15 V and the second detector cell (coupled to the integrator) at +0.75 V. The flow-rate was 1.2 ml/min. (B). Chromatogram of a tissue extract of human hypothalamus; 20 μl were injected. The tissue was prepared as described under Experimental. Chromatographic conditions as in A. (C) Chromatogram of a sample of human lumbar cerebrospinal fluid; 50 μl were injected. The sample was prepared as described under Experimental. Chromatographic conditions as in A. Peaks: DHPG = dihydroxyphenylethylene glycol; VMA = vanillylmandelic acid; MHPG = 3-methoxy-4-hydroxyphenylethylene glycol; DOPAC = dihydroxyphenylacetic acid; NE = norepinephrine; E = epinephrine; DOPA = 3,4-dihydroxyphenylalanine; NM = normetanephrine; DA = dopamine; 5-HTOL = 5-hydroxytryptophol; MET = metanephrine; 5-HIAA = 5-hydroxyindoleacetic acid; ISOP = isoproterenol (internal standard); HVA = homovanillic acid; 5-HTP = 5-hydroxytryptophan; 3-MT = 3-methoxytyramine; 5-HT = 5-hydroxytryptamine (serotonin).

Sample preparation

In initial experiments different methods of protein precipitation were tested. Thus, addition of ten volumes of (A) mobile phase, (B) acetonitrile and (C) mobile phase–acetonitrile (50:50, v/v) to brain tissue was compared with the standard method with 0.1 *M* perchloric acid (PCA) (D). Protein precipitation by adding an acidic mobile phase has been favoured by some workers [12,13,17], whereas others have chosen ethanol [18,19] or acetonitrile [16] as precipitating agents. However, in the present system, perchloric acid gave the best chromatographic performance while A, B and C gave asymmetric peaks with shoulders on the slopes of the ascending part. The best chromatographic performance was achieved by adding 700 μl of 0.1 *M* PCA to every 100 mg of brain tissue and 10 μl

TABLE I

RECOVERY IN BRAIN

A piece of cerebellum was gently homogenized in 0.1 mol/l perchloric acid (containing 0.3 mmol/l Na_2EDTA and 0.5 mmol/l Na_2SO_3). The homogenate was aliquoted, weighed and known amounts of authentic standards were added (1–327 pmol). The replicates were then carried through the sample preparation procedure (see Experimental). In addition, the same range of standards were added to 0.1 mol/l perchloric acid aliquots without any tissue. The recoveries represent the slope obtained from the linear regression plots of the compound in the presence of tissue ($r > 0.996$) divided by the slope obtained in the absence of tissue ($r > 0.997$). Five different standard concentrations were added in triplicate to the tissue. The recoveries were then compared with that of ISOP (internal standard).

Compound	Recovery (%)	Recovery compensating factor
ISOP	88	1.00
VMA	86	1.02
MHPG	92	0.96
DOPAC	93	0.95
NE	88	1.00
E	93	0.95
L-DOPA	81	1.09
NM	92	0.96
DA	74	1.18
5-HTOL	95	0.93
MET	86	1.02
5-HIAA	87	1.01
HVA	82	1.07
5-HTP	52	1.69
3-MT	78	1.13
5-HT	66	1.33

of 1 *M* PCA to every 100 μl of CSF. Addition of Na_2EDTA and Na_2SO_3 to the PCA has been shown to stabilize the indoles 5-HT and 5-HIAA at room temperature [20], which has previously been a great problem, especially when using autoinjection of samples. Addition of heparin to the samples has been shown to give a cleaner chromatogram with less interference of peaks, especially in the early part of the chromatogram [21], probably owing to precipitation of lipoprotein complexes [22].

This minimal sample preparation has been used for several hundred sample injections without any notable loss of chromatographic performance. Our experience is that when trying to repack guard columns, it is very difficult to achieve the required chromatographic efficiency. Therefore, used columns are emptied and this packing material is used in the presaturator column placed before the autoinjector. This column functions to protect the Nucleosil C_{18} material from citrate, which reportedly destroys the coupling of the C_{18} chains to the silica particles [23,24].

Linearity between amount injected and peak height; detection limits; reproducibility

A linear response was achieved for all substances in the range 1–256 pmol ($r > 0.9994$). The detection limit, defined as a ratio of peak height to noise greater

TABLE II

RECOVERY IN CEREBROSPINAL FLUID

CSF was deproteinized in 1 *M* perchloric acid (containing 0.3 mmol/l Na_2EDTA and 0.5 mmol/l Na_2SO_3). CSF aliquots were weighed and known amounts of authentic standards added (1–328 pmol). The replicates were then carried through the sample procedure (see Experimental). In addition, the same range of standards were added to 0.1 mol/l perchloric acid aliquots without any CSF. The recoveries represent the slope obtained from linear regression analysis of the compound in the presence of CSF ($r > 0.996$) divided by the slope in the absence of CSF ($r > 0.997$). Five different standard concentrations were added in triplicate to the CSF. The recoveries were then compared with that of ISOP (internal standard).

Compound	Recovery (%)	Recovery compensating factor
ISOP	100	1.00
VMA	91	1.10
MHPG	95	1.05
DOPAC	93	1.08
NE	88	1.14
E	97	1.03
L-DOPA	101	0.99
NM	98	1.02
DA	96	1.04
5-HTOL	92	1.09
MET	94	1.06
5-HIAA	99	1.01
HVA	94	1.06
5-HTP	92	1.09
3-MT	89	1.12
5-HT	86	1.16

than 2, varied between 6 fmol (NE) and 40 fmol (5-HT), which is well within the range of thin-layer amperometric detectors (e.g., refs. 8 and 17) and almost within the range of the highly sensitive microbore HPLC system with amperometric detection [25].

In order to test the precision of the chromatographic system, ten consecutive samples of standards, human olfactory tubercle extracts and human CSF samples (from the third ventricle) were injected. The coefficient of variation (C.V.) was 0.2–5.2% with an average of 1.8% for the standards, 0.3–8.5% with an average of 2.8% for the human brain samples and 1.0–4.4% with an average of 2.1% for the cerebrospinal fluid samples.

Voltammetric response; peak purity verification

The guard cell (placed before the autoinjector) was set at +1.05 V to minimize the background current from the mobile phase. The coulometric detector cell 1 was set at +0.15 V, which was the highest potential that did not oxidize any of the transmitter compounds, apart from a small proportion of DOPAC (<10%). This was done in order to avoid a negative deflection in the baseline, which occurred when using only one detector cell. The amperometric detector cell 2 was set at +0.75V; this was the lowest potential required to oxidize totally all trans-

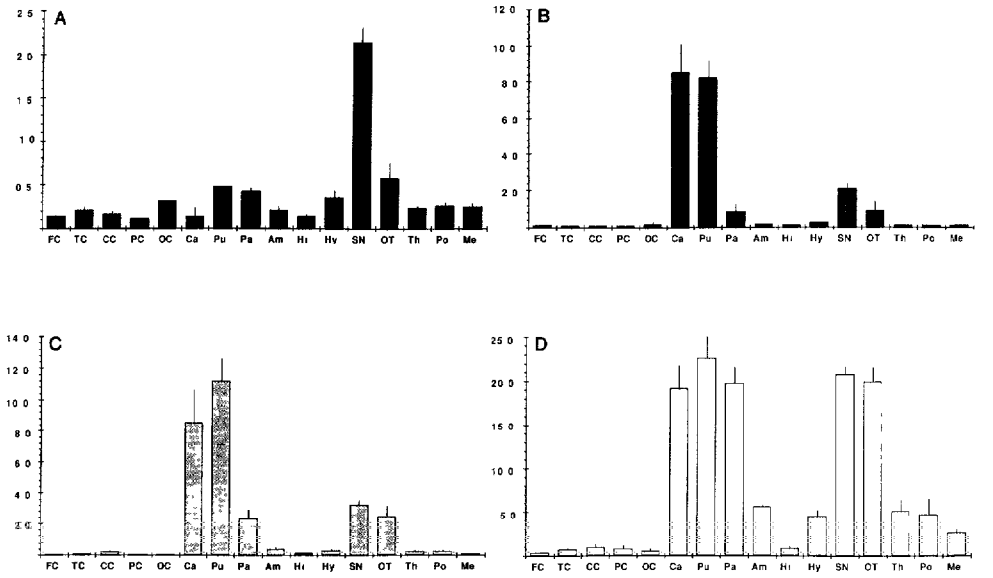

Fig. 2. Regional distribution of (A) DOPA, (B) DA, (C) 3-MT and (D) HVA in nmol/g wet weight. Results are given as means ± S.E. ($n = 6$). The tissue was prepared as described under Experimental. Chromatographic conditions as in Fig. 1A. Abbreviations: FC = frontal cortex; TC = temporal cortex; CC = cingulate cortex; PC = parietal cortex; OC = occipital cortex; Ca = caudate nucleus; Pu = putamen; Pa = globus pallidus; Am = amygdala; Hi = hippocampus; Hy = hypothalamus; SN = substantia nigra; OT = olfactory tubercle; Th = thalamus; Po = pons; Me = medulla oblongata.

mitter substances of interest. In order to verify the purity of the transmitter peaks in the brain and the CSF samples, the voltammetric response for standards and samples was compared. A similar pattern was observed in both instances, indicating purity of peaks in the tissue and fluid chromatograms (data not shown). In addition, a good correlation was found when comparing levels of the CSF monoamine metabolites measured with a similar HPLC–ED technique and a GC–MS system [26].

Recovery

In brain tissue, a high recovery (78–95%) was obtained for all substances except 5-HT and its precursor 5-HTP 66 and 52%, respectively (Table I). In addition, the recovery of ISOP was similar to that of the other substances which means that recovery compensation will be of minor importance for most of the substances. This high recovery is in agreement with the literature values reported for the same type of tissue handling [8,9,18,21,27,28], and may be due to the minimal preparation of the samples prior to injection.

In cerebrospinal fluid, an almost complete recovery was seen for all substances (Table II), which was not unexpected as almost no protein precipitation would occur when the samples were deproteinized with 1 *M* perchloric acid. This complete recovery is in agreement with previous studies [9,26].

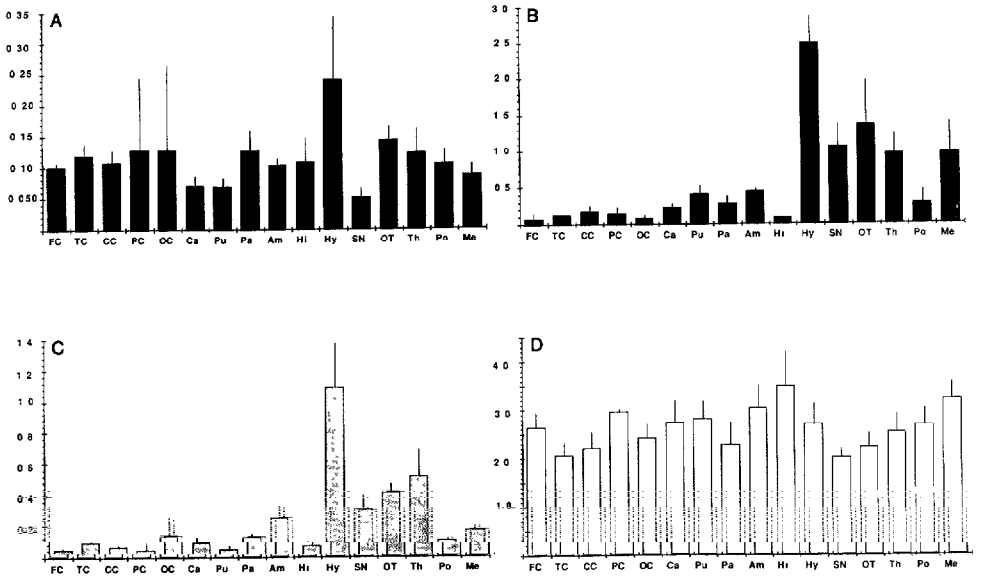

Fig. 3. Regional distribution of (A) E, (B) NE, (C) NM and (D) MHPG in nmol/g wet weight. Results, preparation, conditions and abbreviations as in Fig. 2.

Applications

The regional distribution of free monoamine concentrations in different regions of human brain are shown in Figs. 2–4. The distribution of dopamine (Fig. 2B) corresponded to that of 3-MT (Fig. 2C), showing the highest concentrations in the caudate nucleus and putamen, lower in the substantia nigra, olfactory tubercle and globus pallidus, while all other regions showed levels below 0.4 nmol/g. The distribution of HVA (Fig. 2D) corresponded in part to that of DA and 3-MT. However, the levels in the globus pallidus, substantia nigra and olfactory tubercle were as high as those in the caudate nucleus and putamen with lower levels in the amygdala, hypothalamus, thalamus, pons and medulla oblongata, whereas the lowest values were seen in the cortical regions and in the hippocampus. Notably, the catecholamine precursor DOPA (Fig. 2A) was also detected in measurable amounts in all regions, with the highest concentration in the substantia nigra, while this precursor was evenly distributed throughout other regions examined.

For E (Fig. 3A), the highest concentrations were found in the hypothalamus, lower in the cortical regions, globus pallidus, amygdala, hippocampus, olfactory tubercle, pons and medulla oblongata and the lowest levels in the caudate nucleus, putamen and substantia nigra.

The distribution of NE (Fig. 3B) corresponded to that of NM (Fig. 3C), showing the highest levels in the hypothalamus, lower in the substantia nigra, olfactory tubercle, thalamus and medulla oblongata and the lowest levels in the other regions. MHPG (Fig. 3D), the main noradrenergic metabolite in human brain,

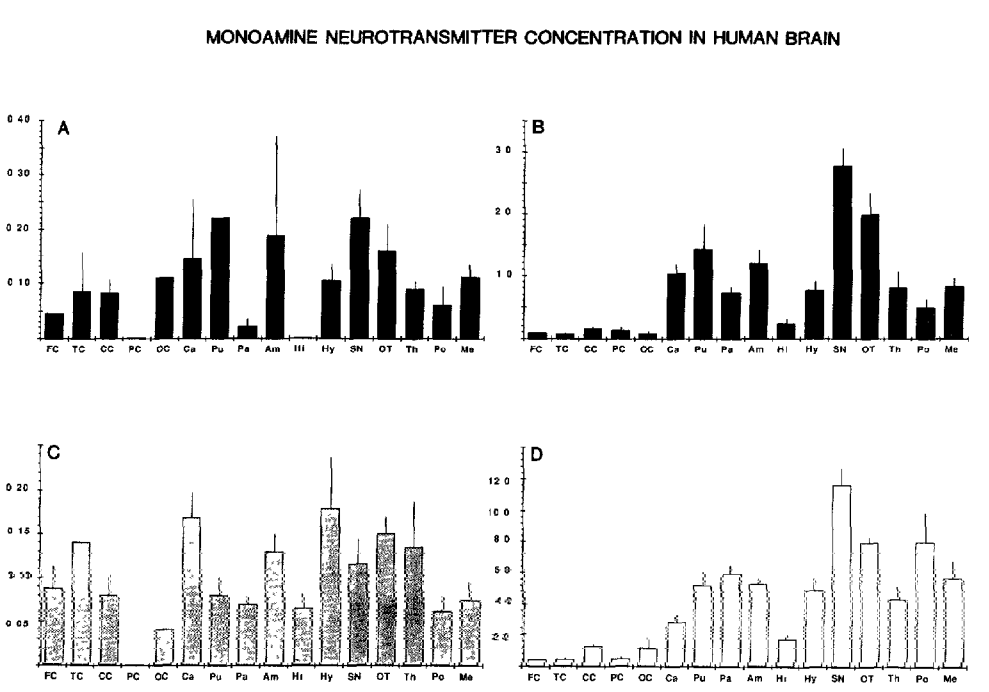

Fig. 4. Regional distribution of (A) 5-HTP, (B) 5-HT, (C) 5-HTOL and (D) 5-HIAA in nmol/g wet weight. Results, preparation, conditions and abbreviations as in Fig. 2.

showed a distribution that did not correlate with that of NA, NM or A, showing a fairly even distribution with levels from 2.02 to 3.48 nmol/g.

The distribution of serotonin (Fig. 4B) corresponded fairly well to that of its

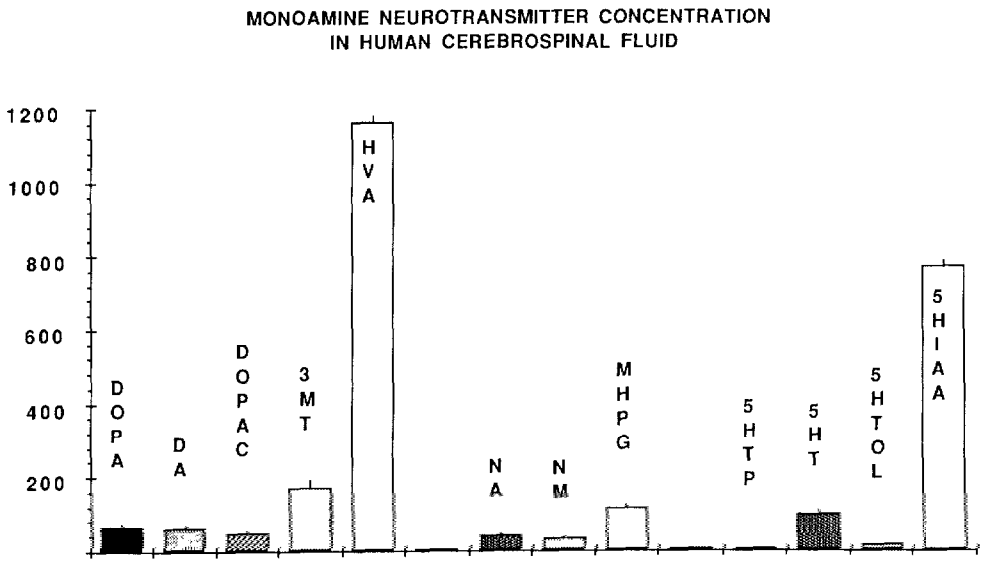

Fig. 5. Distribution of monoamine neurotransmitters in human CSF obtained from the third ventricle in nmol/l CSF. Results, preparation, conditions and abbreviations as in Fig. 2.

precursor 5-HTP (Fig. 4A) and its metabolites 5-HTOL (Fig. 4C) and 5-HIAA (Fig. 4D), showing the highest concentrations in the substantia nigra and lower in the olfactory tubercle, caudate nucleus, putamen, globus pallidus, thalamus, pons and medulla oblongata. The concentrations and the distribution of the monoamine neurotransmitters are, in general, consistent with those published previously [29–32].

The distribution of CSF obtained from the third ventricle is shown in Fig. 5. As would be expected, ca. two- to ten-fold greater concentrations were found compared with those found in lumbar human CSF (see ref. 25 for a summary of lumbar CSF levels), which is probably due to rostocaudal gradients of these substances [33].

In conclusion, a rapid, sensitive and semi-automated technique for the simultaneous separation and detection of the major monoamine neurotransmitters, precursors and metabolites found in human brain and cerebrospinal fluid has been developed. The sample procedure included minimal tissue handling with a high recovery in the tissue and the fluid.

Because of the good reproducibility between different batches of the Nucleosil C_{18} (5 μm) column packing material and the use of the previously presented factorial design [1], it should be possible to set up this HPLC–ED method with batches of Nucleosil C_{18} (5 μm) other than that employed in this study.

ACKNOWLEDGEMENTS

The authors thank Kenneth Johansson for practical advice and Dr. John Hardy, Dr. Anne-Marie O'Carroll, Dr. Cora O'Neill and Dr. Olle Magnusson for help with the manuscript. This work was supported by the Swedish Medical Research Council (Grant No. 12X-5664), Centrala försöksdjursnämnden in Sweden, Nordiska Samfundet mot Plågsamma djurförsök and Stiftelsen för Gamla Tjänarinnor, Sweden.

REFERENCES

1 P. Wester, J. Gottfries, K. Johansson, F. Klintebäck and B. Winblad, J. Chromatogr., 415 (1987) 261.
2 J.A. Hardy, R. Adolfsson, I. Alafuzoff, G. Bucht, J. Marcusson, P. Nyberg, E. Perdahl, P. Wester and B. Winblad, Neurochem. Int., 7 (1985) 545.
3 B.A. Davis, D.A. Durden and A.A. Boulton, J Chromatogr., 374 (1986) 227.
4 M. DaPrada, Trends Pharmacol. Sci., 1 (1980) 157.
5 R.N. Adams, J. Pharm. Sci., 58 (1969) 1171.
6 P.T. Kissinger, C. Refshauge, R. Dreiling and R.N. Adams, Anal. Lett., 6 (1973) 465.
7 I.N Mefford, Methods Biochem. Anal., 31 (1985) 221.
8 S.M. Lasley, I.A. Michaelson, R.D. Greenland and P.M. McGinnis, J. Chromatogr., 305 (1984) 27.
9 J. Wagner, P. Vitali, M.G. Palfreyman, M. Zraika and S. Hout, J. Neurochem., 38 (1982) 1241.
10 O. Magnuson, L.B. Nilsson and D. Westerlund, J. Chromatogr., 221 (1980) 237.
11 K. Ishikawa and J.L. McGaugh, J. Chromatogr., 229 (1982) 35.
12 C.F. Saller and A.I. Salama, J. Chromatogr., 309 (1984) 287.
13 I.C. Kilpatrick, M.W. Jones and O.T. Phillipson, J Neurochem., 46 (1986) 1865.

14 M. Scheinin, W.-H. Chang, K.L. Kirk and M. Linnoila, Anal. Biochem., 131 (1983) 246.
15 M. van Bockstaele, L. Dillen, M. Claeys and W.P. de Potter, J. Chromatogr., 275 (1983) 11.
16 M.A. Javors, C.L. Bowden and J.W. Maas, J. Chromatogr., 336 (1984) 259.
17 C.D. Kilts, G.R. Breese and R.B. Mailman, J. Chromatogr., 225 (1981) 347.
18 J.J. Warsh, A. Chiu and D.D. Godse, J. Chromatogr., 228 (1982) 131.
19 V.M. Gregory, B. Larsen and B. Benson, J. Chromatogr., 345 (1985) 140.
20 B. Mohringe, O. Magnusson, G. Thorell and C.J. Fowler, J. Chromatogr., 361 (1986) 291.
21 R.J. Martin, B.A. Bailey and R.G.H. Downer, J. Chromatogr., 278 (1983) 265.
22 M. Burnstein, H.R. Scholnick and R. Morfin, J. Lipid Res., 11 (1970) 583.
23 K.K. Unger, Porous Silica–Its Properties and Use as Support in Column Liquid Chromatography, Elsevier, Amsterdam, 1979.
24 μBondapak, Column Care and Use Manual, Waters Assoc., Milford, MA.
25 T.A. Durkin, E.J. Caliguri, I.N. Mefford, D.M. Lake, I.A. Macdonald, E. Sundström and G. Jonsson, Life Sci., 37 (1985) 1803.
26 L. Parnetti, J. Gottfries, I. Karlsson, G. Långström, C.-G. Gottfries and L. Svennerholm, Acta Neurol. Scand., (1987) in press.
27 C. Kim, C. Campanelli and J.M. Khanna, J. Chromatogr., 282 (1983) 151.
28 C. van Valkenburg, U. Tjaden and J. Van der Krogt, B. Van der Leden, J. Neurochem., 39 (1982) 990.
29 A. Carlsson, R. Adolfsson, S.-M. Aquilonius, C.-G. Gottfries, L. Oreland, L. Svennerholm and B. Winblad, in M. Goldstein et al. (Editors), Ergot Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspect, Raven Press, New York, 1980, p. 295.
30 R. Adolfsson, C.G. Gottfries, B.E. Roos and B. Winblad, J. Neural Transm., 45 (1979) 81.
31 J.A. Hardy, P. Wester, I. Bäckström, J. Gottfries, L. Oreland, A. Stenström and B. Winblad, Neurochem. Int., (1987) in press.
32 P. Wester, J.A. Hardy, J. Marcusson, P. Nyberg and B. Winblad, Neurobiol. Aging, 5 (1984) 199.
33 R. Sjöström, J. Ekstedt and E. Änggård, J. Neurol. Neurosurg. Psychiat., 38 (1975) 666.