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Simultaneous determination of vigabatrin and amino acid neurotransmitters in brain microdialysates by capillary electrophoresis with laser-induced fluorescence detection

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Abstract

Capillary electrophoresis with laser-induced fluorescence detection (CE-LIFD) coupled to in vivo microdialysis sampling was used in order to monitor simultaneously a drug and several neurotransmitters in the brain extracellular fluid. Determination of the antiepileptic drug vigabatrin and the amino acid neurotransmitters glutamate (Glu), L-aspartate (L-Asp) and γ-aminobutyric acid (GABA) was performed on low-concentration samples which were derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) and separated using a pH 9.2 75 mM sodium borate running buffer containing 60 mM sodium dodecyl sulfate (SDS) and 5 mM hydroxypropyl-β-cyclodextrin (HP-β-CD). Glu, L-Asp and vigabatrin derivatized at a concentration of 1.0×10^{-9} M, and GABA derivatized at a concentration of 5.0×10^{-9} M, produced peaks with signal-to-noise ratios of 8:1, 8:1, 4:1 and 5:1, respectively. The nature of the neurotransmitter peaks found in rat brain microdialysates was confirmed by both electrophoretic and pharmacological validations. This method was used for monitoring vigabatrin and amino acid neurotransmitters in microdialysates from the rat striatum during intracerebral infusion of the drug and revealed rapid vigabatrin-induced changes in GABA and Glu levels. This original application of CE-LIFD coupled to microdialysis represents a powerful tool for pharmacokinetic/pharmacodynamic investigations.
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Keywords: Vigabatrin; Amino acid neurotransmitters

1. Introduction

The present work was undertaken to develop an original approach to monitor simultaneously in vivo the antiepileptic drug vigabatrin and several neurotransmitters. Epilepsy is thought to result from an unbalance between inhibitory neurotransmission mediated by γ-aminobutyric acid (GABA) and excitatory neurotransmission mediated by glutamate (Glu) and aspartate (Asp) [1]. Vigabatrin is a currently used antiepileptic drug, which is an inhibitor of the GABA-metabolizing enzyme GABA-transaminase, therefore, inducing an enhancement of brain GABA level [2]. In contrast, its effect on central Glu and Asp levels is poorly documented.

None of the in vivo pharmacological studies describing vigabatrin-induced changes in neurotransmitters mention any correlation between the respective brain concentrations of the drug and its target neurotransmitters. Therefore, a sensitive and selective analytical procedure for the simultaneous assay of vigabatrin, GABA, Glu and Asp is required for further studies of the dose-related side effects and mechanism of action of this antiepileptic drug. Moreover, such a procedure should allow to monitor the extracellular concentrations of these neurotransmitters and the free drug, after sampling with the brain microdialysis technique. This is of special interest since extracellular neurotransmitters represent the fraction actively involved in neurotransmission while only the free drug has generally a pharmacological activity [3,4].

To our knowledge no method for analyzing vigabatrin in microdialysates has been developed, whereas several

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gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) methods have been reported for determining vigabatrin in biological fluids. For example, GC methods with thermoionic detection [5] or with mass spectrometric detection [6] have been reported for the assay of vigabatrin in human plasma or urine. Since vigabatrin does not have a chromophore or fluorophore in its structure, HPLC methods with a UV/Vis detector exhibit poor detection limits [7]. Consequently, *O*-pthaldialdehyde (OPA) [8,9], Dns chloride [10] and 4-chloro-7-nitrobenzofurazan (NBD-Cl) [11] have been used as pre-column derivatizing agents for HPLC with fluorescence detection assays of vigabatrin. Recently, a CE-LIFD method for determining vigabatrin in human plasma after pre-column derivatization with tetramethylrhodamine succinimidyl ester has been published [12].

Amino acid neurotransmitters in microdialysates are conventionally analysed by HPLC with fluorimetric detection [13,14]. However, this technique, which exhibits moderate mass sensitivity and require volumes of samples in the 10–50 μl range, does not easily allow the determination of amino acid neurotransmitters in microdialysates of smaller volumes, collected at a high frequency. In contrast, CE-LIFD has been shown to allow the analysis of amino acid neurotransmitters in low-volume brain microdialysates [15–17]. Recently a CE-LIFD method for the determination of GABA, Glu and L-Asp after pre-column derivatization with naphtalene-2,3-dicarboxaldehyde (NDA) was developed [18].

Thus, due to its high separation efficiency, low-volume sample requirement and highly sensitive mode of detection, CE-LIFD may be the method of choice for monitoring simultaneously vigabatrin, GABA, Glu and Asp in brain microdialysis samples. Since these analytes do not fluoresce, they have to be derivatized to be detected. NDA was chosen for that purpose, since this fluorescent tag which reacts with primary amines in the presence of cyanide to produce highly fluorescent cyano[f]benzoisoindole (CBI) derivatives, has appeared well suited for the high-sensitivity CE assay of amino acids present in samples collected by microdialysis [16,19,20].

This method, which has been developed and validated on both analytical and pharmacological basis, allowed to bring new data on interactions between vigabatrin and both inhibitory and excitatory amino acids.

2. Experimental

2.1. Chemicals

Naphthalene-2,3-dicarboxaldehyde and sodium cyanide (NaCN) were purchased from Fluka (Buchs, Switzerland). D,L-Glutamate, L-aspartate, γ-aminobutyric acid, cysteic acid, boric acid and sodium dodecyl sulfate (SDS) were purchased from Sigma (St. Louis, MO, USA) and hydroxypropyl-β-cyclodextrin (HP-β-CD) was obtained from Aldrich (Steinheim, Germany). Ultra pure water was obtained with a Milli-Q system (Millipore, Bedford, MA, USA) and filtered through a 0.20 μm cellulose acetate Sartorius filter. L-*trans*-Pyrrolidine-2,4-dicarboxylic acid (PDC) and vigabatrin (±4-aminohexenoic acid) were purchased from Tocris Cookson (Bristol, UK), nipecotic acid from Sigma and mercaptopropionic acid from Aldrich.

2.2. Solutions

Sodium borate buffer (500 mM, pH 8.70) was prepared as previously reported [19]. Running buffer was made by dissolving adequate amounts of SDS and HP-β-CD into pH 9.2 75 mM sodium borate buffer. Before to be used, buffers were filtered through a 0.20 μm cellulose acetate Sartorius filter. Artificial cerebrospinal fluid (aCSF) used for probe perfusion had the following composition: NaCl 145.0 mM, KCl 2.7 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.2 mM, NaH_2PO_4 0.45 mM, Na_2HPO_4 1.55 mM (pH 7.4). Each stock solution of 1.0 mM aminoacid (Glu, GABA, L-Asp and cysteic acid) was prepared in 0.1 M HCl and stored at 4 °C. Stock solution of 1.0 mM vigabatrin was prepared in water and stored at −20 °C. On the day of analysis, a working solution containing the four analytes was prepared in aCSF. Mercaptopropionic acid solution was diluted in a 0.9% (w/v) NaCl solution (Cooper, Melun, France). Stock solutions of 1.0 mM nipecotic acid and 20 mM PDC were prepared in water and 20 mM NaOH, respectively and stored at −40°C. The final solutions, 0.1 mM nipecotic acid and 1.0 mM PDC, were prepared through appropriate dilution in aCSF.

2.3. CE-LIF system

CE was performed on a SpectroPhoresis100 module purchased from ThermoSeparation Products (Les Ulis, France) equipped with an external laser-induced fluorescence ZETALIF detector (Picometrics, Toulouse, France). The excitation was performed by an Omnichrome helium–cadmium laser (442 nm, 8 mW, model 4056-30M, Chino, CA, USA). The 490 nm emission intensity was detected by a photomultiplier tube after being filtered by a band pass filter and a notch filter to attenuate background radiations. Data were acquired (100 Hz) with a Borwin data acquisition system (JMBS Developments, Grenoble, France).

2.4. Derivatization procedure

On the same day of analysis, 5 μl of samples or 5 μl of standard solutions were derivatized, at room temperature, by adding 2 μl of a mixture (1:2:1 v/v/v) containing (i) the internal standard (0.1 mM cysteic acid in 0.117 M perchloric acid), (ii) a borate/NaCN solution (mixing solution (100:20 v/v) of 500 mM borate buffer pH 8.7 and 87 mM NaCN in water) and (iii) the NDA solution (2.925 mM in acetonitrile/water, 50:50 v/v). Since the internal standard cysteic

acid reacts less quickly than Glu, L-Asp, GABA and vigabatrin in standard solutions, a period of 1 h at room temperature was found to be necessary to complete the reaction.

2.5. Separation procedure

Separations were carried out with a 60 cm × 50 μm i.d. fused-silica capillary (Composite Metal Services, Worcester, UK) having an effective length of 23 cm. On-column laser-induced fluorescence detection was carried out through a 5 mm wide window opened by removing the polyimide cover of the capillary. A constant voltage was applied, with currents typically less than 35 μA. A 1 s hydrodynamic injection was made from the anodic side of the capillary by applying a 200 mbar depression at the outlet of the capillary. Each day, before analyses began, the capillary was sequentially flushed for 20 min with 1% (w/v) NaOH, then ultra-pure water (7 min) and finally running buffer (7 min).

2.6. Microdialysis experiments

2.6.1. Preparation of microdialysis probes

Concentric microdialysis probes were constructed in our laboratory from regenerated cellulose dialysis tubing (Spectra/Por hollow fiber, Spectrum Medical Industries; molecular weight cutoff 6000 Da, 0.255 mm o.d., 3 mm active dialysis length) and fused-silica capillary tubing, the body of the probe being made of a 3 cm 26G stainless steel tube [17]. In vitro probe recovery was determined as previously described [17], the probes being perfused at 1 μl/min flow rate, and was found to be (mean ± S.E.M.) equal to 28.0 ± 2.5, 20.9 ± 2.2, 22.9 ± 3.8 and 35.6 ± 5.0% ($n = 3$) for Glu, L-Asp, GABA and vigabatrin, respectively. Since the main purpose of the present study was to determine vigabatrin present in the brain extracellular medium, this drug could not be added to the liquid perfusing the probe collecting the microdialysates. Consequently, a dual-probe microdialysis system [21] was used for allowing simultaneously the in situ application of vigabatrin through one probe and the collection of microdialysates through the other one. The system was constructed by joining two single probes with an interprobe distance of 0.5 mm (Fig. 1).

2.6.2. Animals

Male Sprague–Dawley rats (290–330 g, Harlan, Gannat, France) were housed in a light-, temperature-controlled room, with food and water “ad libitum”. The care and use of laboratory animals were in accordance with the European Communities Council Directives of 24 November 1986 (86/609/EEC). Rats were anesthetized with urethane (1.15 g/kg i.p.) and placed in a stereotaxic frame (David Kopf, Tujunga, CA, USA). Body temperature was maintained close to 37.5 °C using a heated underblanket (Harvard Instruments, Murrieta, CA, USA). The skull was exposed and a dual microdialysis probe was implanted into the right striatum at the following coordinates relative to bregma: anterior 0 mm, lateral 3.5 mm, ventral 6.5 mm below the brain surface according to the atlas of Paxinos and Watson [22]. At the end of each microdialysis experiment, rats were sacrificed in order to verify the placement of the probe. Rats were discarded when a fraction of the active part of the probe was outside the striatum.

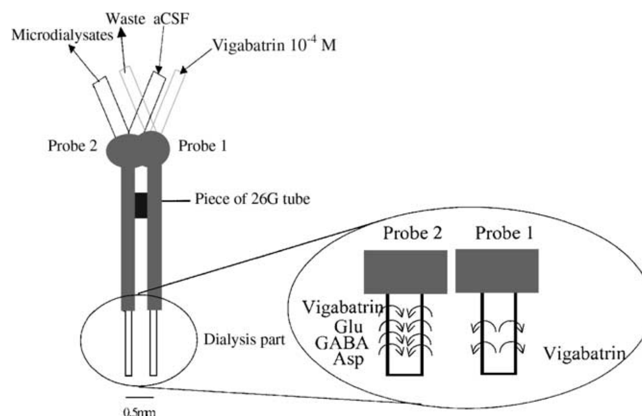

Fig. 1. Dual probe microdialysis system. Two home-made concentric microdialysis probes were glued together being spaced by 0.5 mm through a piece (1 cm long) of 26G stainless steel tube. Vigabatrin was perfused through the delivery probe (probe 1) and diffused into the extracellular space; the effluent of probe 1 was directed towards waste. The probe 2 was continuously infused with aCSF; vigabatrin and neurotransmitters present in the extracellular space diffused into this recovery probe and were present in the collected effluent.

2.6.3. Collection of microdialysates

Before use, probes were flushed at 0.1 μl/min with water during 24 h and afterwards perfused at 1 μl/min with aCSF. After implantation of the probe in the striatum, a 3 h stabilization period was allowed before sample collection. Five-minute microdialysates were collected in PCR tubes (Abgene, Epsom, UK) and immediately stored at −40 °C before derivatization and CE separation. Vigabatrin was administered in situ by reverse dialysis (flow rate 1 μl/min) using the dual-probe microdialysis system (Fig. 1).

2.7. Statistical analysis

Data are given as mean ± S.E.M. expressed as the percent of the values preceding drug administration. Comparisons between treated and control rats were achieved on percentage transformed data using ANOVA and post-hoc comparisons by Tukey–Kramer test. The level of significance was set at $P < 0.05$ for all comparisons.

3. Results and discussion

3.1. Analytical developments

Our starting separation buffer was adapted from the micellar electrokinetic chromatography (MEKC) method

described by Sauvinet et al. [18] for the measurement of GABA, Glu and L-Asp. Under these MEKC conditions (25 kV, pH 9.2, 75 mM borate buffer containing 10 mM HP-β-CD and 70 mM SDS) only GABA, Glu, Asp and the internal standard cysteic acid were separated whereas vigabatrin co-migrated with endogenous molecules contained in the microdialysates.

When the concentration of SDS was raised from 0 to 100 mM, the migration times of the NDA-derivatives glu-cyano[f]benzoisoindole (Glu-CBI) and GABA-cyano[f]benzoisoindole (GABA-CBI) increased noticeably, whereas the migration times of vigabatrin-CBI, Asp-CBI and cysteic acid-CBI were poorly increased. The best separation was obtained with 60 mM SDS and that concentration was finally chosen.

Thereafter, HP-β-CD concentration was optimized to improve resolution. This compound induced a splitting of the peak of Asp-CBI corresponding to a partial resolution of D and L forms. However, since more than 96% of the Asp present in the brain of adult animals correspond to L-Asp [23] and since no D-Asp was detected in our microdialysates, L-Asp, instead of the racemic D,L-amino acid, was chosen as a standard. The effect of the addition of HP-β-CD on the separation of Glu-CBI, L-Asp-CBI, GABA-CBI, vigabatrin-CBI, and cysteic acid-CBI were studied using a 75 mM sodium borate buffer containing 60 mM SDS. When the concentration of HP-β-CD was raised from 0 to 12 mM, the migration time of GABA-CBI and Glu-CBI decreased, whereas L-Asp-CBI, vigabatrin-CBI and cysteic acid-CBI were affected to a lower extend. The best separation in microdialysates was obtained with 5 mM HP-β-CD.

In conclusion, the final composition chosen for the running buffer was: pH 9.2, 75 mM sodium borate containing 60 mM SDS and 5 mM HP-β-CD.

A 1 s hydrodynamic injection of sample, made by applying a 200 mbar depression at the outlet of the capillary, was chosen since it appeared as a good compromise between separation and sensitivity. Indeed, if the time of injection was increased, the width of all peaks increased and the analytes were less separated. Finally an adjustment of the applied voltage was needed. Voltages between 17 and 30 kV were tested and the best separation was obtained at 20 kV (data not shown).

In summary, the separations were carried out using a running voltage of 20 kV in a 60 cm × 50 μm i.d. capillary (effective length: 23 cm). Separations were performed in an air-conditioned room (23 °C). Fig. 2 shows the electropherogram of a derivatized microdialysate from rat brain striatum spiked with vigabatrin.

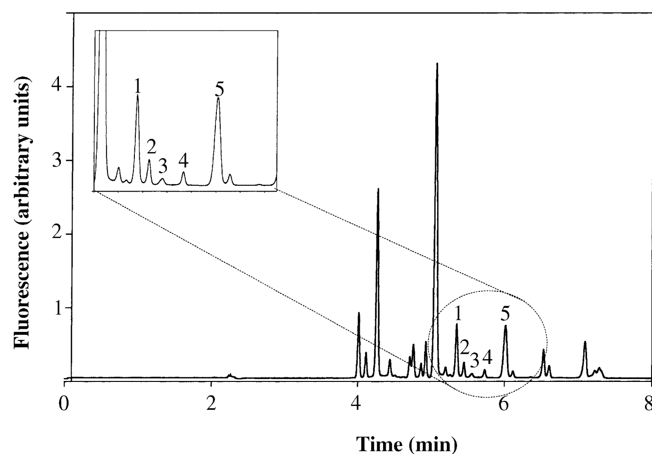

Fig. 2. Typical electropherogram of a derivatized striatum microdialysate spiked with vigabatrin (4) (1.0×10^{-9} M) and cysteic acid (5). The basal concentration of GABA (2), Glu (1) and L-Asp (3) in striatal microdialysates were found to be: 20.0×10^{-9}, 0.98×10^{-6} and 16.0×10^{-9} M. The separation buffer was 75 mM borate buffer (pH 9.2) containing 60 mM SDS and 5 mM HP-β-CD.

3.2. Method validations

At first, identification of the peaks present in microdialysates was made by comparing their migration times to those of standards. Moreover, the heights of the peaks of interest increased when exogeneous Glu-CBI, GABA-CBI and L-Asp-CBI were co-injected with the microdialysate sample, whereas no additional peak appeared.

However, besides the electrophoretic criterion, a pharmacological approach was used to further validate the identification of the analytes of interest in microdialysates. For that purpose, drugs known to increase and/or decrease Glu, Asp and GABA extracellular concentrations were used. Administration by reverse dialysis of 1.0 mM PDC, a Glu/Asp uptake blocker, induced a significant +130% increase of the relative area of the peak present in striatum microdialysates and exhibiting the same migration time as a Glu-CBI standard (Fig. 3). These data suggest that this peak of interest corresponds to endogenous Glu.

The relative area of the peak with the same migration time as L-Asp-CBI standard exhibited changes similar to those of Glu-CBI, since it was enhanced (+1430%) by Glu/Asp uptake blockade (data not shown). This result confirms that this peak corresponds to endogenous L-Asp.

Application of 0.1 mM nipecotic acid, a GABA uptake blocker, induced a significant +290% increase of the relative area of the peak exhibiting the same migration time as GABA-CBI standard (Fig. 3). Furthermore, the relative area of the same peak was decreased by 30% as compared to control experiments after systemic injection of 100 mg/kg mercaptopropionic acid, a GABA-synthesis inhibitor (Fig. 3). Consequently, it can be concluded that the microdialysate peak exhibiting the same migration time as standard GABA corresponds to endogenous GABA.

A limited but significant decrease in the relative area of Glu-CBI peak was observed after mercaptopropionic acid injection (Fig. 3). However, this result does not question the identification of Glu in the microdialysates, but rather confirms the tight metabolic link between GABA and Glu [24]. In this respect, mercaptopropionic acid-induced changes in Glu have previously been reported [18,25].

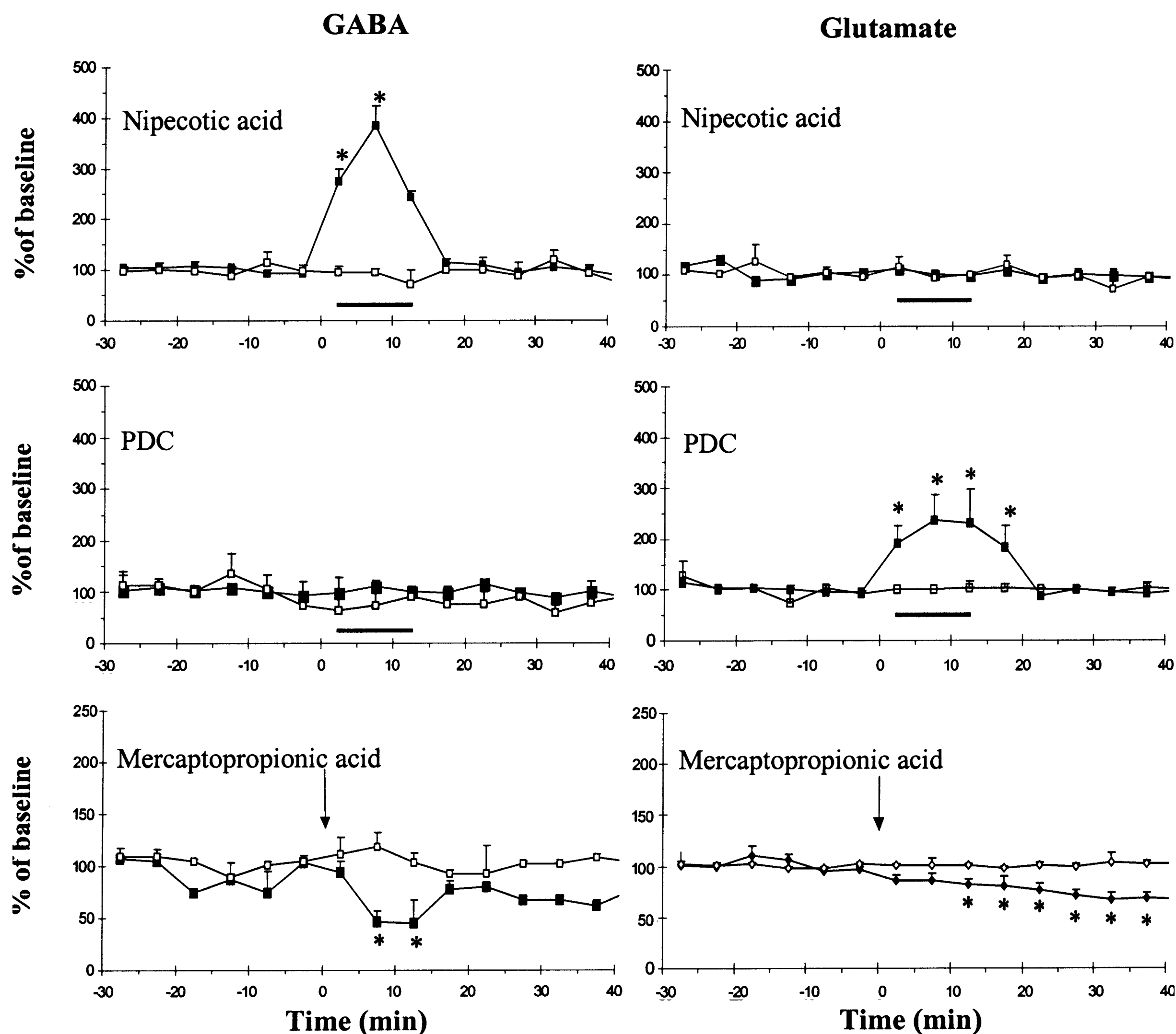

Fig. 3. Pharmacological validation of the identification of GABA (left) and Glu (right). Black squares represent experiments with drug administration ($n = 8$) and white squares, control experiments ($n = 8$). Blockers of GABA uptake (0.1 mM nipecotic acid) and Glu uptake (1.0 mM PDC), were administered by reverse dialysis (black bar). The inhibitor of GABA synthesis (100 mg/kg mercaptopropionic acid) was administered i.p. (black arrow). Microdialysates were collected every 5 min. Data are expressed as percent (mean ± S.E.M.) of the baseline values preceding drug or vehicle administration. $^*P < 0.05$ vs. control animals (ANOVA and post-hoc comparisons by Tukey–Kramer test). The basal concentrations of GABA, Glu and L-Asp in striatal microdialysates were: $(80.3 \pm 11.2) \times 10^{-9}$, $(1.95 \pm 0.11) \times 10^{-6}$ and $(90.5 \pm 18.9) \times 10^{-9}$ M, respectively.

The linearity and repeatability were determined for Glu, L-Asp, GABA and vigabatrin, contained in standard solutions as well as in brain microdialysates (Table 1). The use of an internal standard (cysteic acid) was necessary to improve intra-assay, intra- and inter-day variations. Linear relationships between the concentration of Glu-CBI, L-Asp-CBI, GABA-CBI and vigabatrin-CBI and the peak areas were obtained with regression coefficients shown in Table 1. The limits of detection were determined for analytes in standard solutions (Table 1). Fig. 4 shows that Glu, L-Asp and vigabatrin derivatized at a concentration of 1.0×10^{-9} M, and GABA derivatized at a concentration of 5.0×10^{-9} M, produced peaks with signal-to-noise ratio of 8:1, 8:1, 4:1 and 5:1, respectively, with no interference with the blank. Moreover, Fig. 4 shows that the differences between the limit of detection of Glu-CBI, L-Ap-CBI, GABA-CBI and vigabatrin-CBI are not linked to any interfering contaminant. They should rather be due to differences in quantum fluorescence between these NDA-derivatives. No precise mechanism is available to explain such differences; it can only be hypothesized that these variations in fluorescence may be related to the respective structure of each NDA-derivatives or that the background electrolyte affects differently the fluorescence of each NDA-derivatives. For instance, such differences in fluorescence between NDA derivatives have already be reported for amino acids and catecholamines in several previous papers [26–28].

In the present work, the limits of detection of GABA, Glu and L-Asp were 10, 1000 and 15 times, respectively, lower

Table 1
Quantitative parameters for the analysis of GABA, vigabatrin, Glu and L-Asp in standard solutions and in microdialysates

Standards	Glu	GABA	Vigabatrin	L-Asp
Calibration range (M)	1.0×10^{-9} to 1.0×10^{-6}	1.0×10^{-8} to 1.0×10^{-5}	1.0×10^{-9} to 1.0×10^{-6}	1.0×10^{-9} to 1.0×10^{-6}
Regression coefficient for calibration (r^2)[a]	0.998	0.995	0.997	0.996
Intra-assay repeatability (CV %)[b]	3.5	10.0	7.0	3.9
Intra-day repeatability (CV %)[c]	2.8	9.8	7.6	5.4
Inter-day repeatability (CV %)[d]	2.0	10.3	8.3	3.1
Accuracy (%)[e]	1.2	1.2	1.6	1.5
Detection limit (M)[f]	0.4×10^{-9}	3×10^{-9}	0.8×10^{-9}	0.4×10^{-9}
Microdialysates				
Calibration range of standard solutions added to microdialysates (M)	1.0×10^{-9} to 1.0×10^{-6}	1.0×10^{-8} to 1.0×10^{-5}	1.0×10^{-9} to 1.0×10^{-6}	1.0×10^{-9} to 1.0×10^{-6}
Regression coefficient of calibration (r^2)[a]	0.992	0.990	0.992	0.992
Intra-assay repeatability (CV %)[b]	3.9	4.6	4.5	4.0
Intra-day repeatability (CV %)[c]	3.0	8.9	7.9	6.9
Accuracy (%)[e]	1.2	3.3	5.5	2.3

Volume of sample: 5 μl.
[a] Number of concentrations tested = 7; three replicates for each concentration.
[b] 10 injections from the same vial. Tested standard concentrations: 5×10^{-7} M for Glu, L-Asp and vigabatrin; 5×10^{-6} M for GABA.
[c] 10 replicates. Tested standard concentrations: 5×10^{-7} M for Glu, L-Asp and vigabatrin; 5×10^{-6} M for GABA.
[d] Three replicates daily over 10 days. Tested standard concentrations: 5×10^{-7} M for Glu, L-Asp and vigabatrin; 5×10^{-6} M for GABA.
[e] Three replicates, spiked concentrations: 5×10^{-7} M for Glu, L-Asp and vigabatrin; 5×10^{-6} M for GABA.
[f] Extrapolations (signal-to-noise = 3) based on calibration curve.

than the basal concentrations of these neurotransmitters in striatal microdialysates. The limit of detection of vigabatrin was 100 times lower than the one achieved with liquid chromatography methods with fluorescence derivatization using OPA [8] or NDB-Cl [11], and 30 times lower than with the CE-LIFD protocol previously reported for the determination of vigabatrin in human plasma [12].

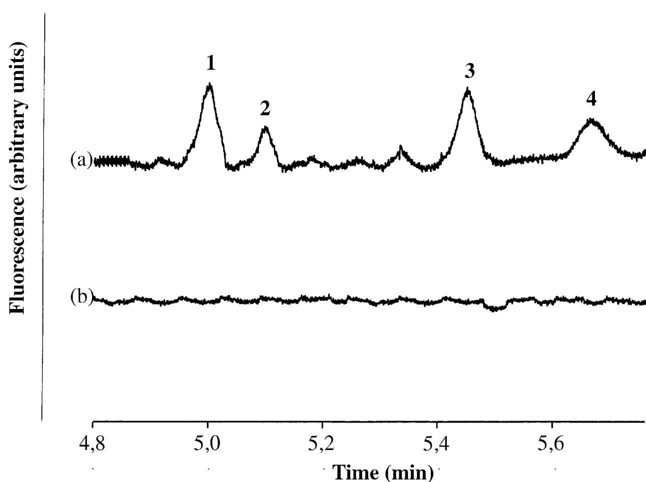

Fig. 4. Electropherograms of a solution containing analytes at concentrations near the limits of detection. (a) Glu (1), L-Asp (3), vigabatrin (4) were derivatized at 1.0×10^{-9} M each and GABA (2) was derivatized at 5.0×10^{-9} M. (b) Blank, i.e. derivatized aCSF. Conditions: see Section 2. Note that the signal-to-noise ratios were 8:1, 8:1, 5:1 and 4:1 for NDA-labeled Glu, L-Asp, GABA and vigabatrin, respectively.

In conclusion, the results obtained on linearity and detection limits show that the present method can be used for the determination of very low-concentrations of Glu, L-Asp, GABA and vigabatrin in microvolumes (i.e. microliter range) of biological samples.

3.3. In vivo microdialysis experiments

This new CE-LIFD separation method allowed us to study vigabatrin-induced changes in amino acid neurotransmitters simultaneously with the concentration of the drug in the brain. For that purpose, 0.1×10^{-3} M vigabatrin was administered for 30 min into the striatum by reverse dialysis using a dual-probe microdialysis system. During the first 5 min of drug infusion, the mean concentration of vigabatrin in microdialysates was 5.2×10^{-6} M (Fig. 5); thereafter, it rose to a mean of 18.0×10^{-6} M between the 5th and the 10th minute of drug infusion. After an unexplained transient decrease between the 10th and the 15th minute, microdialysate vigabatrin concentration was stable around 15.0×10^{-6} M until the end of drug administration. When drug infusion was discontinued, microdialysate concentrations of vigabatrin fell within 10 min to zero value. At the beginning of the vigabatrin infusion, microdialysate GABA concentration exhibited a +262% increase, while Glu concentration exhibited a +37% increase, as compared with their respective baseline levels; in contrast, L-Asp concentration was unchanged (Fig. 5). Between the 5th and the 15th minute of drug infusion, GABA concentration rose to +353% as compared to baseline while the enhancement of Glu remained stable

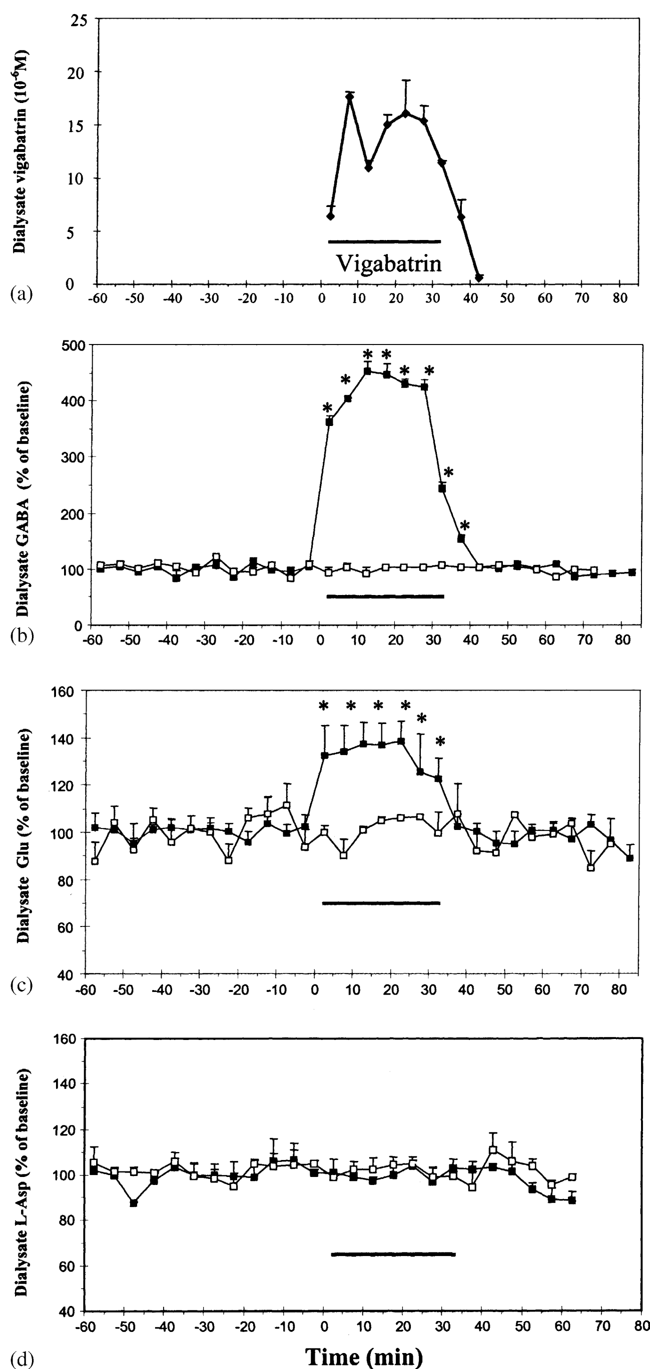

Fig. 5. Simultaneous monitoring of vigabatrin (a), GABA (b), Glu (c) and L-Asp (d) in microdialysates from striatum of anesthetized rats. Vigabatrin (0.1×10^{-3} M) was administered in situ by reverse dialysis (black bar). Black squares represent experiments with drug administration ($n = 5$) and white squares, control experiments ($n = 5$). Data are expressed as percent (mean ± S.E.M.) of the baseline values preceding drug or vehicle administration. $^{*}P < 0.05$ vs. control animals (ANOVA and post-hoc comparisons by Tukey–Kramer test). The basal concentrations of GABA, Glu and L-Asp in striatal microdialysates were found to be: $(58.8 \pm 0.7) \times 10^{-9}$, $(3.48 \pm 0.50) \times 10^{-6}$ and $(52.50 \pm 0.03) \times 10^{-9}$ M, respectively.

(+37%). GABA and Glu levels remained elevated until the end of vigabatrin infusion and normalized rapidly thereafter. Thus, these data show that vigabatrin induces (i) an expected increase in extracellular GABA concentration in relation with the drug concentration at the site of the biochemical changes and (ii) an unexpected increase in extracellular Glu. Since the GABA-transaminase activity gives rise to Glu and semi-succinic aldehyde, one may expect that the inhibition of that enzyme would lead to a decrease in microdialysate Glu concentration [29]. In contrast, the present data showed a limited, but clear-cut, increase in Glu when vigabatrin was present in the striatal extracellular fluid. Although the mechanism of such an effect remains unexplained, the enhanced extracellular Glu level may increase neuronal excitability and may counter, at least temporarily, the antiepileptic action of vigabatrin. In this respect, vigabatrin was reported to exert a biphasic effect, a single systemic dose of vigabatrin inducing an early proconvulsant and, later, anticonvulsant effects in rats [30]. Thus, this method allows to obtain new information on the effect of vigabatrin on neurotransmitters on a short time scale.

4. Conclusion

The high resolving power and large peak capacity of CE provide a reliable separation between amino acid neurotransmitters and a structurally-related drug, the GABA analog vigabatrin. The present microdialysis/CE-LIFD method allows to monitor simultaneously the extracellular concentrations of drug and the resulting biochemical changes. These data on vigabatrin-induced alterations in neurotransmitters in relation with the concentration of the drug at the site of biochemical changes may lead to a better understanding of the mechanism of action of this antiepileptic drug.

The present method represents a new approach for pharmacokinetic/pharmacodynamic investigations, which can be easily set-up in any pharmacology laboratory, since a commercially available CE instrument is used. Besides, obtaining data in this manner can reduce the number of laboratory animals required to obtain both pharmacokinetic and pharmacodynamic
information.

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