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Short communication

Improved method for the measurement of glutamate and aspartate using capillary electrophoresis with laser induced fluorescence detection and its application to brain microdialysis

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Abstract

We have previously published data on the analysis of glutamate in microdialysis samples using a commercially availble CE apparatus. Here we demonstrate further improvements in the analysis of both glutamate and aspartate from very small volume microdialysates. The limit of detection of our system has been increased to 10^{-9} *M* for both glutamate and aspartate. This permits microdialysis sampling time to be reduced to 2 min, thus improving the temporal resolution of microdialysis sampling. Concurrently, migration time has also been reduced such that resolution of both amino acids can be achieved inside 2 min. This new analytical method has been applied to the measurement of the EAA from microdialysis samples from the dentate gyrus of the hippocampus. Extracellular concentrations of both glutamate and aspartate increased to a maximum of 5- and 4.5-fold of preinfusion values, respectively, during infusion of 100 m*M* K^+ through the microdialysis probe. This is consistent with the depolarization-evoked release of both amino acids from this brain region.

Keywords: Glutamate; Aspartate

1. Introduction

The amino acids L-aspartate and L-glutamate are the major excitatory transmitters in the mammalian brain [1]. Elevated extracellular concentrations of these excitatory amino acids (EAA) have been demonstrated in brain following cerebral ischaemia [2] and traumatic brain injury [3]. Most previous studies have used HPLC with fluorescence or electrochemical detection, as the means of analysis, and microdialysis sampling procedures with temporal resolutions of 10–20 min [2,3]. EAAs are responsible for most of the fast excitatory transmission in the mammalian CNS, therefore shorter microdialysis sampling times, hence greater temporal resolution would be advantageous.

We have previously described a CE method coupled to laser induced flourescence for the rapid analysis of glutamate in brain microdialysates [4].

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Reported here is a refined method, with improved sensitivity and analysis time, for the measurement of both L-glutamate and L-aspartate from very small volume microdialysates.

2. Experimental

2.1. Chemicals

All chemicals were analytical grade and purchased from BDH (Poole, UK).

Naphthalene dicarboxaldehyde (NDA) was purchased from Aldrich (Poole, UK).

2.2. Microdialysis

2.2.1. Animals

Male Sprague–Dawley rats (280–350 g, Charles River) were used in all experiments. All animals were group housed in cages with food and water available ad libitum.

2.2.2. Surgical procedure

Following induction of anaesthesia with gaseous administration of halothane (5%) (Fluothane, Zeneca, Cheshire, UK) the animals were secured in a stereotaxic frame with ear and incisor bars. Anaesthesia was maintained by continous administration of halothane (1–2%). A microdialysis probe (O.D. 0.5 mm, length 1 mm) (CMA/Microdialysis, Stockholm, Sweden) was implanted into the dentate gyrus. Coordinates for the dentate were taken according to Paxinos and Watson [5]: RC-3.8, L-1.4, (reference point taken from bregma), V-4.5 from the dura.

2.2.3. Microdialysis procedure

The microdialysis probe was perfused with artificial cerebrospinal fluid (aCSF) (125 m*M* NaCl, 3.0 m*M* KCl, 0.75 m*M* $MgSO_4$ and 1.2 m*M* $CaCl_2$ pH 7.4) at a flow-rate of 0.75 μl/min. The in vitro percentage recovery (i.e. recovery from a solution containing 100 μ*M* glutamate and aspartate) of the probe under these conditions was 7.8±0.4 and 8.6±0.3, and basal microdialysate concentrations were 2.13±0.27 and 2.47±0.47 μ*M* for glutamate and aspartate, respectively. A 30-min period was allowed following probe implantation, in order to allow basal amino acid levels to stabilise, after which time 2-min samples were collected throughout the experimental period. Five preinfusion control samples were collected and all subsequent values were expressed as a percentage of these values. Stimulation of release was achieved by infusion of 100 m*M* K^+ in aCSF (25 m*M* NaCl, 100 m*M* KCl, 0.75 m*M* $MgSO_4$ and 1.2 m*M* $CaCl_2$ pH 7.4) for 20 min.

2.3. Capillary electrophoresis

2.3.1. Apparatus

All analyses were performed on a Beckman P/ACE 2050 with He/Cd laser-induced fluorescence (442 nm) (Omnichrome, Chino, USA). Data was collected and integrated using XChrom (VG Data Systems, Altringham, UK).

2.3.2. Separation procedure

Separations were performed in fused-silica capillaries (75 μm I.D., 375 μm O.D., 37 cm) (Composite Metal Services, Hallow, UK) with an applied voltage of 30 kV. A sample (5 nl) was applied to the capillary via a high-pressure injection system. Separations used 30 m*M* boric acid pH 9.5 (pH using 1 *M* NaOH). Total run time was 2 min. The capillary was rinsed with 0.1 *M* NaOH (1.5 min) and running buffer (1.5 min) between analyses.

2.3.3. Derivatization procedure

All samples were prederivatized with naphthalene 2,3-dicarboxaldehyde (NDA) by a modification of the method of Hernandez et al. [6]. Dialysate or standard samples (1.5 μl) were mixed with 50 m*M* boric acid buffer pH 9.5 containing 20 m*M* sodium cyanide (2.9 μl) and 30 m*M* NDA in methanol (0.1 μl). Samples were allowed to react for 3 min at room temperature prior to injection.

2.4. Analysis of data

Results were analysed by ANOVA followed by post hoc analysis where appropriate using the Super ANOVA software application (Abacus Concepts, Berkeley, CA, USA, 1989).

3. Results and discussion

Previous data [4], using the same commercially available CE–LIF system, demonstrated the detection and measurement of glutamate from microdialysis samples. Here we demonstrate the improved detection and measurement of both glutamate and aspartate. The limit of detection for glutamate and aspartate has been improved to 10^{-9} *M*; this is a ten-fold improvement in sensitivity [4]. Standard calibrations revealed a linear relationship between concentration and peak area over the concentration range 10^{-6}–10^{-9} *M* for NDA-derivatized glutamate (slope=0.3 and correlation coefficient=0.99) and aspartate (slope=0.29 and correlation coefficient= 0.99). Concurrently, separation conditions were optimised to produce rapid resolution of both glutamate and aspartate within 2 min, compared to the 10-min separation time previously reported [4]. Reproducibility was good with separations of the EAAs

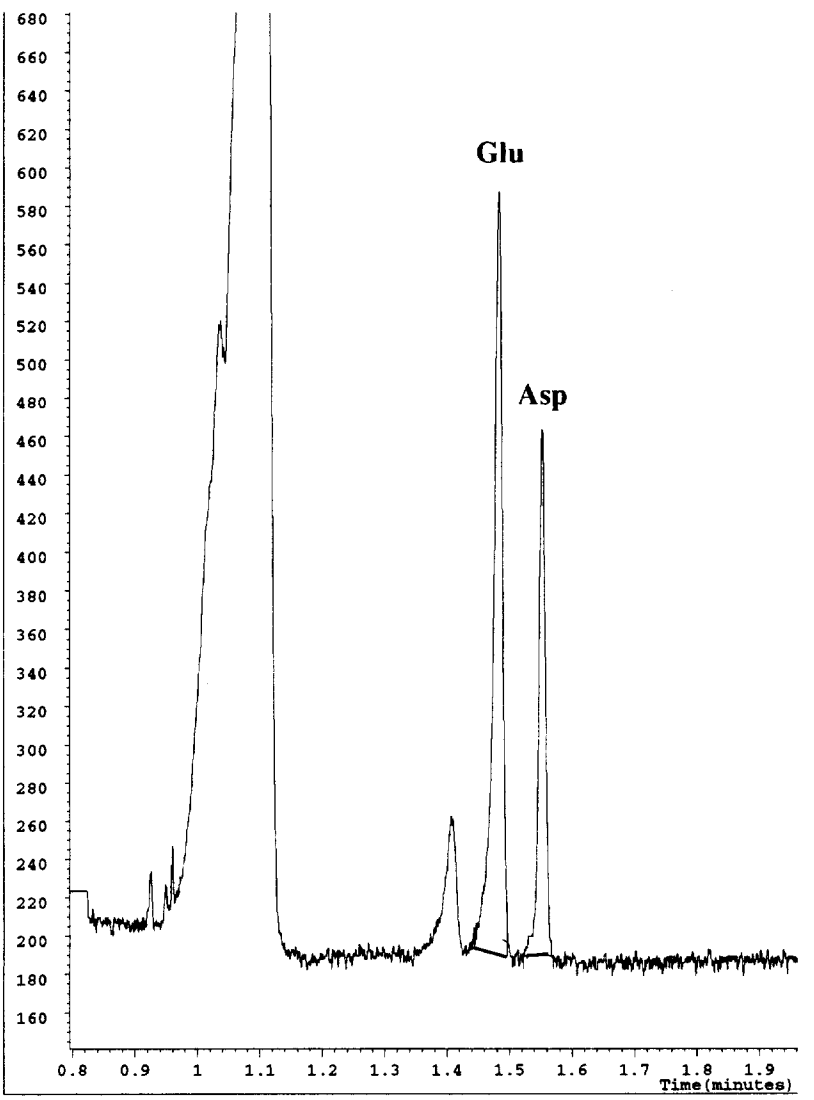

Fig. 1. Electropherograms demonstrating the detection of glutamate and aspartate from a 2-min (1.5 μl) microdialysis sample from the dentate gyrus.

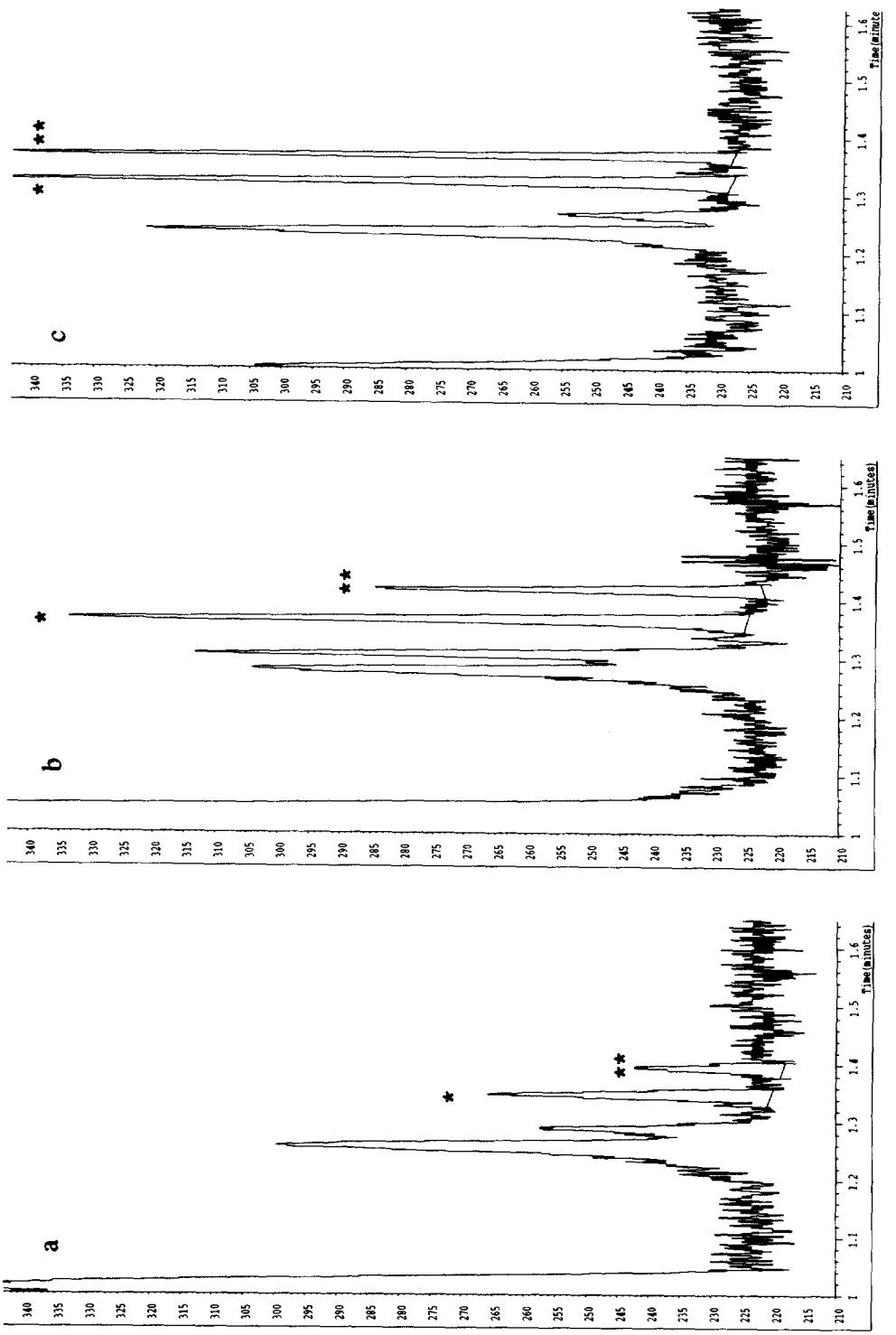

Fig. 2. Electropherograms demonstrating the effects of infusion of aCSF containing 100 m*M* K^+ on the extracellular concentrations of glutamate and aspartate from the dentate gyrus. * – glutamate and ** – aspartate. (a) Preinfusion, (b) 10 min post infusion, (c) 14 min post infusion.

maintained with less than 1% variation in migration time for both neurotransmitters. This improvement in sensitivity now allows the reproducible detection of both glutamate and aspartate from very small volume brain microdialysates (Fig. 1). Linearity of derivatization and reproducibility of separations can be maintained such that detection and quantification of the EAAs within brain microdialysates is now routine.

In order to demonstrate the application of this CE–LIF method for microdialysis, a 2-min sampling regime was employed for the monitoring of the effects of K^+ stimulation on the extracellular concentrations of glutamate and aspartate in the dentate gyrus of the anaesthetised rat. A 20-min application of 100 m*M* K^+ through the microdialysis probe evoked a significant ($p<0.05$) increase in extracellular concentrations of both glutamate and aspartate (Fig. 2). These increases showed a dose dependency reaching a maximum of 527±125% (Fig. 3) and 450±162% (Fig. 4) of preinfusion values for glutamate and aspartate, respectively, after 24 min (14 min post infusion). The observed dose dependent increases are consistent with the K^+ evoked-release of these amino acids. However, from the fall off in extracellular concentrations of glutamate and aspartate, after maximum values were attained, it would appear that some depletion of neurotransmitter may have occurred due to over stimulation of nerve terminals.

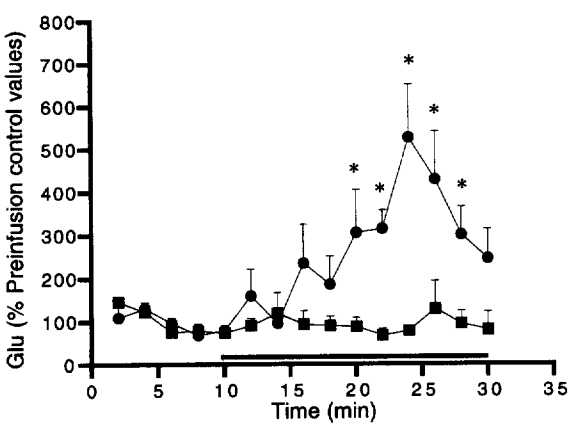

Fig. 3. Effects of infusion of aCSF containing 100 m*M* K^+ on the extracellular concentrations of glutamate in the dentate gyrus: (■) vehicle controls, (●) 100 m*M* K^+ treatment. * demonstrates statistical significance ($p<0.05$) from vehicle controls. Data expressed as mean±S.E.M., $n=6$ per study group.

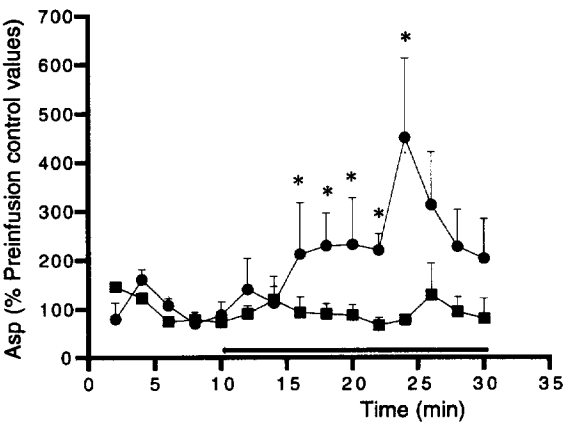

Fig. 4. Effects of infusion of aCSF containing 100 m*M* K^+ on the extracellular concentrations of aspartate in the dentate gyrus: (■) vehicle controls, (●) 100 m*M* K^+ treatment. * demonstrates statistical significance ($p<0.05$) from vehicle controls. Data expressed as mean±S.E.M., $n=6$ per study group.

These improvements now allow 2-min sampling to be performed on a routine basis, thus improving the temporal resolution of monitoring the effects of physical and/or pharmacological manipulations of EAAs from the brain. Using current technology there is sufficient sensitivity for further improvement, however decrease in sampling below 1 min is constrained by sample-handling limitations. On-line systems or more automated sample handling regimes would be required to further increase the time resolution of microdialysis sampling on a routine basis. However, it should be noted that although on-line systems, such as those used by Zhou et al. [7], remove sample handling limitations, temporal resolution then becomes a function of separation time; so if this separation procedure was applied to an on-line injection system the temporal resolution would still be 2 min.

Although temporal resolution of microdialysis sampling has been reduced 10-fold, this is still by no means a measure of real time physiological changes. However, these improvements do serve to decrease the dilution factor of any increase/decrease that may occur within any given sample and improves the probability of detecting changes more accurately.

In summary, using a refined CE–LIF method for

the analysis and measurement of glutamate and aspartate has facilitated the improved temporal resolution of microdialysis sampling for these neurotransmitters.

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