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Review

Capillary electrophoresis and microdialysis: current technology and applications

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Abstract

Microdialysis sampling has become an important method for the continuous monitoring from an in vivo environment. This technique has been used to monitor many endogenous molecules, such as neurotransmitters, as well as exogenous species such as drug substances. Microdialysis samples have traditionally been analyzed by liquid chromatographic (LC) methods to gain resolution and quantification of the molecules of interest. However, LC separations have a relatively large injection volume requirement which, as a consequence, increases microdialysis sampling times. Capillary electrophoresis (CE), with its very small sample volume requirements and high resolving power, has therefore gained popularity as an alternative to LC. Reviewed here are many of the technologies currently available for CE and examples of how this technique has been effectively applied to the analysis of microdialysis samples.

Keywords: Reviews; Microdialysis

Contents

1. Introduction

Microdialysis is an in vivo sampling technique which allows monitoring within a physiological environment with a minimum of disturbance to the animal (Fig. 1). This technique has been applied to the sampling of endogenous compounds and drug molecules from major organs, peripheral tissues and systemic fluids. When coupled to a suitable analytical technique it is possible to monitor individual or

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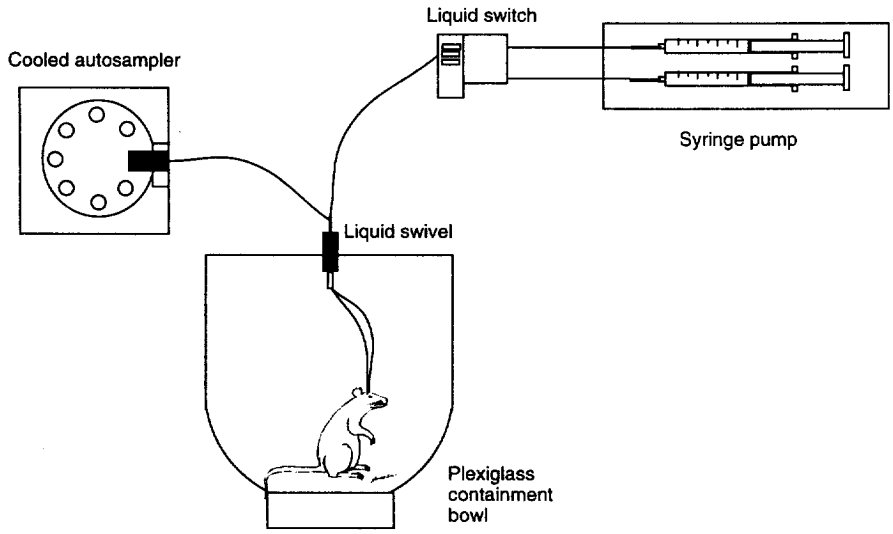

Fig. 1. Diagram showing typical microdialysis sampling apparatus for the freely moving awake animal (diagram adapted with permission from Biotech Instruments, UK and CMA Microdialysis, Boston, USA).

multiple analytes simultaneously. Liquid chromatography (LC) has been the traditional analytical partner of microdialysis. However, capillary electrophoresis (CE), with its ability to resolve complex mixtures efficiently and rapidly, and its low sample volume requirements, is becoming more popular as an alternative to conventional LC.

2. Microdialysis

Microdialysis is generally thought of as a relatively recent technique, however the earliest example of a dialysis probe dates back to 1972 [1]. The development of the U-shaped probe by Zetterstrom et al. in the early 1980s [2] started the evolution and ever increasing use of the technique of microdialysis. The principles of the technique are relatively simple: a fine (0.2–0.5 mm dia.) dialysis probe is implanted into a discrete region of brain, systemic tissue or fluid. Probe construction varies according to the requirements and purpose of the experiment (Fig. 2), but the principles are the same: molecules diffuse down their concentration gradient, across the dialysis membrane in either direction, thus permitting molecules to diffuse into the probe from the extracellular environment. Alternatively, molecules can diffuse from the perfusate out of the probe into the extracellular space (a process known as reverse dialysis), thus providing a means of local administration. Continuous flow through the probe maintains these gradients at the membrane, and recovery across the probe membrane increases with decreased flow-rates. Many different flow-rates can be used, however, 2 μl/min and less is usually the convention. With such low flow-rates it is clear that sample volumes are very low and obviously the shorter the sampling time (i.e., the greater the temporal resolution) the lower these volumes become.

Microdialysis in the brain has been the most widely used application of this technique with many of the small molecular mass endogenous neurotransmitters (i.e., dopamine [3], serotonin [4], acetylcholine [5], noradrenaline [6], the excitatory amino acids (EAA) [7], γ-aminobutyric acid (GABA) [8] and many of the other amino acids of the brain [9]) being routinely monitored, as well as some of the neuropeptides (e.g., substance P [10] and CCK [11]). Its use within other tissues has been a more recent development, particularly for the study of drug pharmacokinetics [12] and, to a lesser degree, for the monitoring of tissue metabolism [13].

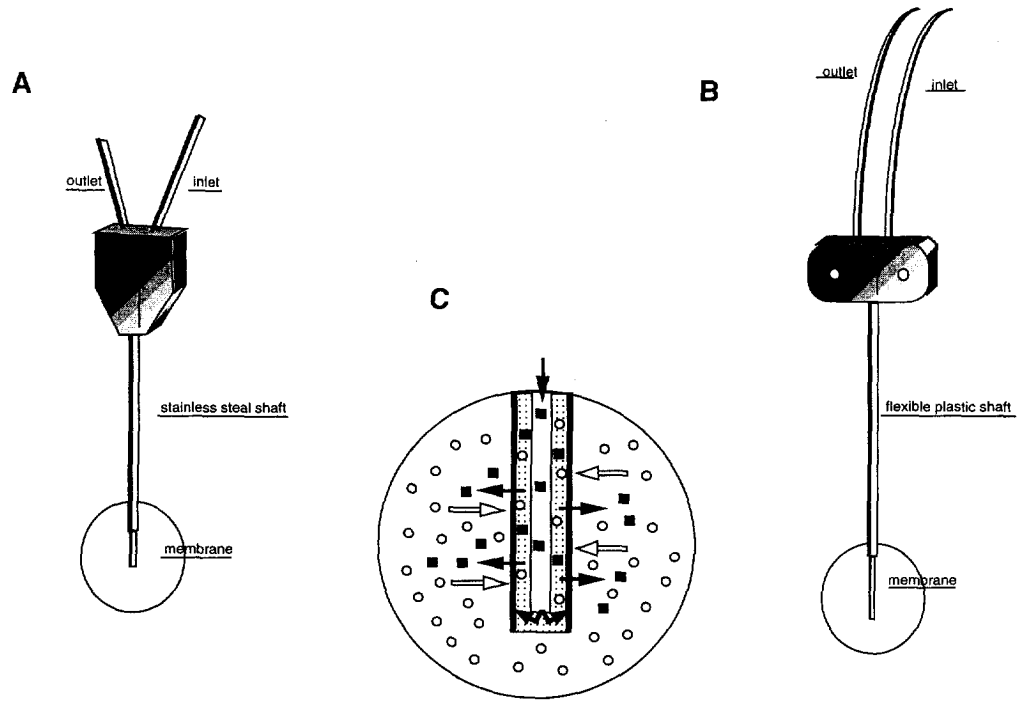

Fig. 2. Microdialysis probe designs: (A) rigid stainless steel shafted design, concentric flow design. These probes are typically used for CNS implantation. (B) Flexible plastic shafted design, again with concentric flow, typically used for peripheral implantation into tissues and blood vessels. Both have variable membrane lengths and are commercially available. (C) Illustrates the membrane and the diffusion of molecules into (○) and out (■) of the probe (reproduced with permission from Biotech Instruments, UK and CMA Microdialysis, Boston, USA).

2.1. Analysis

The analysis of the microdialysis samples has conventionally used HPLC with various detection systems depending on the analyte(s) of interest. For example, the amine neurotransmitters, dopamine, noradrenaline and serotonin, are routinely separated by reversed-phase HPLC with electrochemical detection [3,4,6], a highly sensitive and specific means of measurement. Alternatively, amino acids have been separated via either isocratic [14] and/or gradient reverse phase HPLC [15] with fluorimetric detection following prederivatisation, usually with *o*-phthaldialdehyde (OPA).

These techniques have been used effectively for many years, however, they do have certain disadvantages, namely long separation times and a requirement for relatively large injection volumes (which are necessary to facilitate good detection limits). The requirement for such large volumes has been reduced somewhat by the advent of smaller bore HPLC columns.

3. Capillary electrophoresis

Capillary electrophoresis (CE) has a published history that spans some 40 years. However, the technique has only really been in the modern laboratory since the early 1980s [16] with many commercially available CE units now available. The technique utilizes the same forces as those used in conventional electrophoretic separations, but these are brought to bear within a small bore fused-silica capillary (typically 25–75 μm I.D.). This affords the technique with a number of advantages over its counterpart, liquid chromatography (LC). Firstly, a higher column efficiency allows very high resolution

separation, which in some cases can result in very rapid analysis. Secondly, the technique has a very low sample volume requirement, typically less than 10 nl injection volume, compared to the μl quantities applied to a LC analysis. This low sample volume application is also responsible for CEs' main disadvantage, which is absolute sensitivity; therefore detector selection is of great importance.

3.1. Detectors

There are a range of detection systems available, indeed most of those currently available for LC have been applied to CE. The most common instrumentation and probably the widest used are the UV absorbance detectors. The sensitivity of these systems are limited and although various modifications, such as the so called bubble capillary and the "Z" cell [17], have been made to increase pathlength and hence improve sensitivity, the application of UV detection (UVD) analysis of microdialysates is somewhat limited. Published data involves, predominantly, the measurement of drugs for pharmacokinetic purposes [18–20]. Direct measurement of endogenous species from microdialysates, by UVD, is very rare.

Mass spectrometry (MS) coupling to CE is a relatively recent development, but already its application to microdialysis has become apparent with the report that endogenous GABA from rat brain can be measured [21] using the electrospray interface. This suggests that CE-MS may be useful for the real time monitoring of many of the neurotransmitters and/or drugs which can be measured by microdialysis.

Probably one of the most extensively used detection methods is laser induced fluorescence (LIF) [22–26]. LIF provides the greatest degree of sensitivity of any detector currently available for CE with detection limits approaching the molecular level [27]. This high sensitivity makes LIF the ideal detection system for CE for the analysis of bioanalytical samples where absolute levels are very small, such as in microdialysis. LIF does however, have a number of disadvantages. Individual lasers are generally limited to one or two very specific emission wavelengths. These wavelengths must then coincide with either a molecules native excitation spectrum or with that of an added fluorogenic "tag". Native fluorescence is relatively rare, particularly for the small endogenous molecules present within a microdialysate. Those which do possess some native activity (e.g., 5-HT with an excitation wavelength of 280 nm) are usually at wavelengths which are not compatible with current laser technology. "Tagging" or addition of a specific fluorogenic label to a molecule (Fig. 3), although imparting the required excitation characteristics, does remove much of the selectivity by labeling all molecules with the same specific functional groups (e.g., primary amines). However, with the high efficiency resolving power of CE, separations can be designed to overcome this selectivity problem and as laser technology develops, many new lasers with differing emission wavelengths will become available for use with LIF detection (LIFD).

Electrochemical detection (ECD) has concentration limits which can rival LIF for certain applications, however, the application of ECD to CE is technically more difficult. Firstly, a means of isolating the small electrochemically-induced changes in current from the relatively large currents generated during separation must be developed. Secondly, a reproducible alignment of the EC cells working electrode within the capillary must be maintained. A number of groups have overcome these problems via the use of various joint arrangements [28–31] which serve both to isolate the electrochemical cell current and to maintain electrode alignment (Fig. 4). These systems, however, are exclusively "homemade" arrangements and a commercially available detector is, as yet, not available.

4. Applications

4.1. Amino acids

CE is ideally suited for the analysis of small molecules such as amino acids due to their differing charge to mass characteristics. Many separation procedures have been devised for selective analysis of individual amino acids or multiple residues, from microdialysates.

Probably some of the most common published analyses are those dealing with the excitatory amino acids (EAA), glutamate and aspartate, as sampled

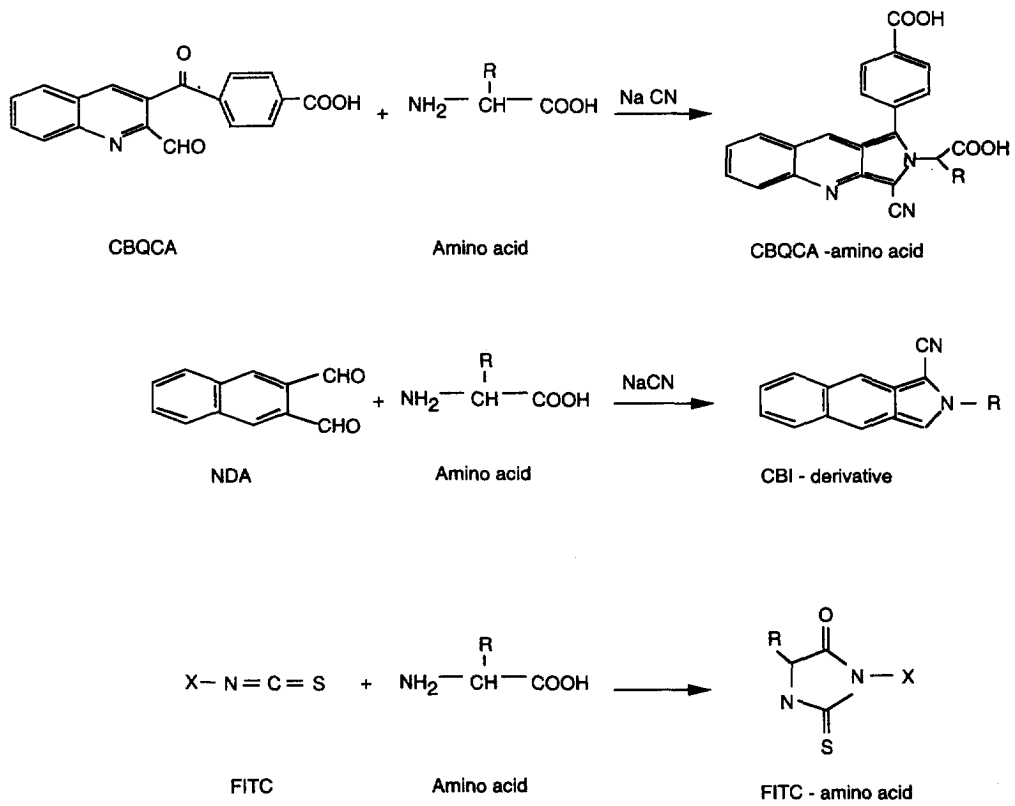

Fig. 3. Derivatisation reactions – represented here are three of the most commonly used fluorescent 'tags' for amino acid analysis by CE. (Top) 3-(4-Carboxybenzoyl)-2-quinolinecarbxaldehyde (CBQCA) reacts in the presence of cyanide ions (at pH 9 and has a reaction time of 2 h) to yield the fluorescent CBQCA-amino acid derivative. This product has an absorption spectrum which is compatable with 488 nm waveband of an argon laser [26]. (Middle) Naphthalene dicarboxyaldehyde (NDA) reacts in the presence of cyanide ions (reaction time 1 min at pH 9.5) to form the the fluorescent CBI-amino acid derivative which has an absorption pattern compatible with the 442 nm band of the He/Cd lasers [23]. (Bottom) Fluorescein isothiocyanate (FITC) reacts (reaction time 18 h at pH 9.5) to form FITC-amino acid products which are compatible with the 488 nm line of the argon lasers [22]. X indicates fluorescein group.

from various brain regions [22–24,32–35]. Glutamate and aspartate have been established as the major excitatory neurotransmitters within the central nervous system (CNS) [36]. They are responsible not only for normal synaptic function, but also play vital roles in many of the dysfunctional conditions of the CNS, as well as being implicated in neurodegeneration [37] due to their ability to induce excitotoxicity under certain circumstances. The physiological control of EAA release is stringently modulated under normal conditions; this makes the temporal resolution of microdialysis sampling very important. Therefore, high temporal resolution sampling regimes are essential for the real time understanding of EAA function and modulation. With this requirement for increased temporal resolution high sensitivity detection is necessary and hence LIFD has been widely utilized for EAA analysis [22–24,33,35]. These articles used a variety of derivatisation and detection methodology to measure glutamate and aspartate: Hernandez and coworkers [22,23] used both fluorescein isothiocyanate (FITC) and naphthalene 2,3-dicarboxyaldehyde (NDA) derivatisations (Fig. 3) to gain temporal resolution of glutamate in 20 and 10 min, respectively. Separation was achieved on a commercially available CE–LIF system using free solution or capillary zone electrophoresis (CZE). This methodology was used to examine the interaction between dopamine and glutamate in rat brain. It was demonstrated that blockade

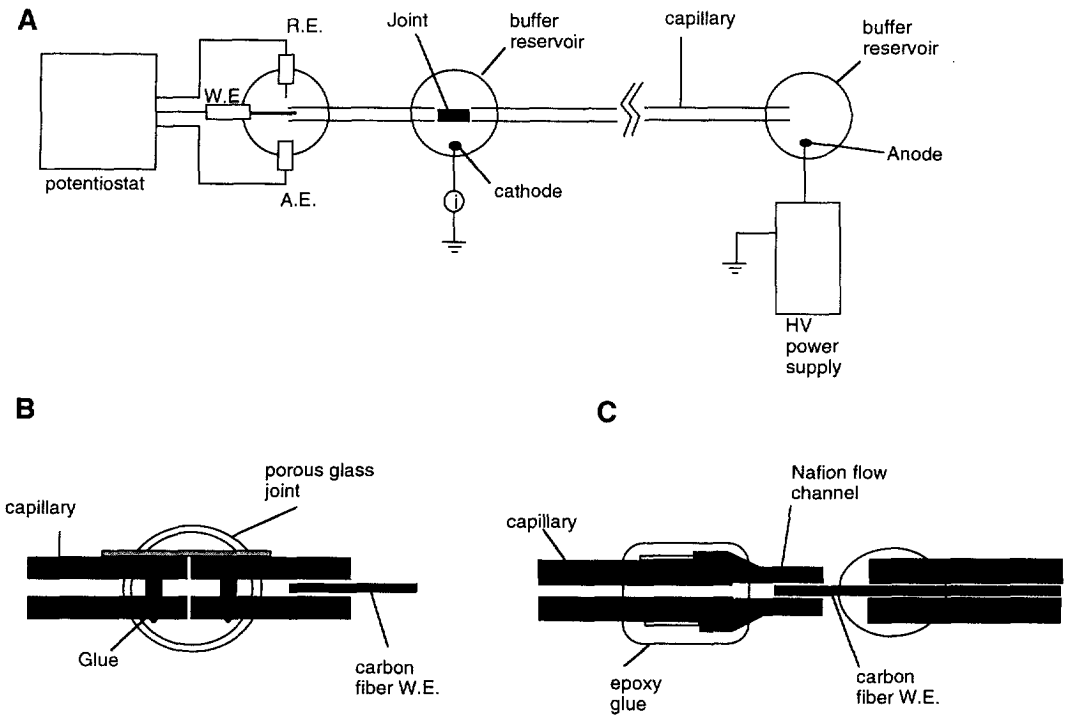

Fig. 4. Electrochemical detection: (A) schematic representation of CE with ECD adapted from [32]. Working electrode (W.E.) is a carbon fiber, Ag/AgCl reference electrode (R.E.) and the auxillary electrode (A.E.) is a platinum wire. (B) Schematic of a porous glass joint assembly [28], (C) schematic of an end-column electrical decoupler [30]. Examples of the decoupling mechanisms which have been developed to isolate the electrochemical cell from the separation current in order to allow electrochemical detection.

of dopamine receptors, by haliperidol, resulted in a decrease in extracellular glutamate concentrations. Dawson et al. [24] again used NDA derivatisation with a commercially available CE to measure glutamate with a temporal resolution of 20 min, however in this case micellar electrokinetic chromatography (MEKC) was used to gain resolution of the amino acid. Pharmacological characterisation of this method employed infusion of tetrodotoxin (a sodium channel blocker) and removal of calcium to demonstrate the neuronal origin of the sampled glutamate. Similarly Berquist et al. [26] and Stiller et al. [38] used MEKC with a commercially available apparatus to resolve not only the EAAs but a number of amino acids with a temporal resolution of 15 min. This method, however, employed 3-(4-carboxybenzoyl)-2-quinolinecarbxaldehyde (CBQCA) derivatisations (Fig. 3). Further increases in temporal resolution of sampling procedures have been achieved using similar methodology as above: Dawson et al. [35] have reduced sampling time to 2 min and have gained resolution of both glutamate and aspartate (Fig. 5). The effect of K^+ stimulation was used to demonstrate that these transmitters can be monitored routinely in response to pharmacological manipulation (Fig. 6). This method, however, still used manual sample handling and derivatisation. For further reductions to near-real-time analysis Zhou et al. [39] have devised on-line derivatisation and direct coupling of CE to microdialysis sampling (Fig. 7). This type of on-line system allows the CE to act as a type of biosensor with temporal resolution now becoming a function of separation time. Again, this system was characterised using K^+ stimulation to demonstrate a temporal resolution of 90 s. It should be noted that a rapidly reacting derivatising agent, such as NDA, is essential for on-line analysis. Although amino acids are relatively stable when compared to the amine transmitters, obviously the derivatisation must be complete before analysis can

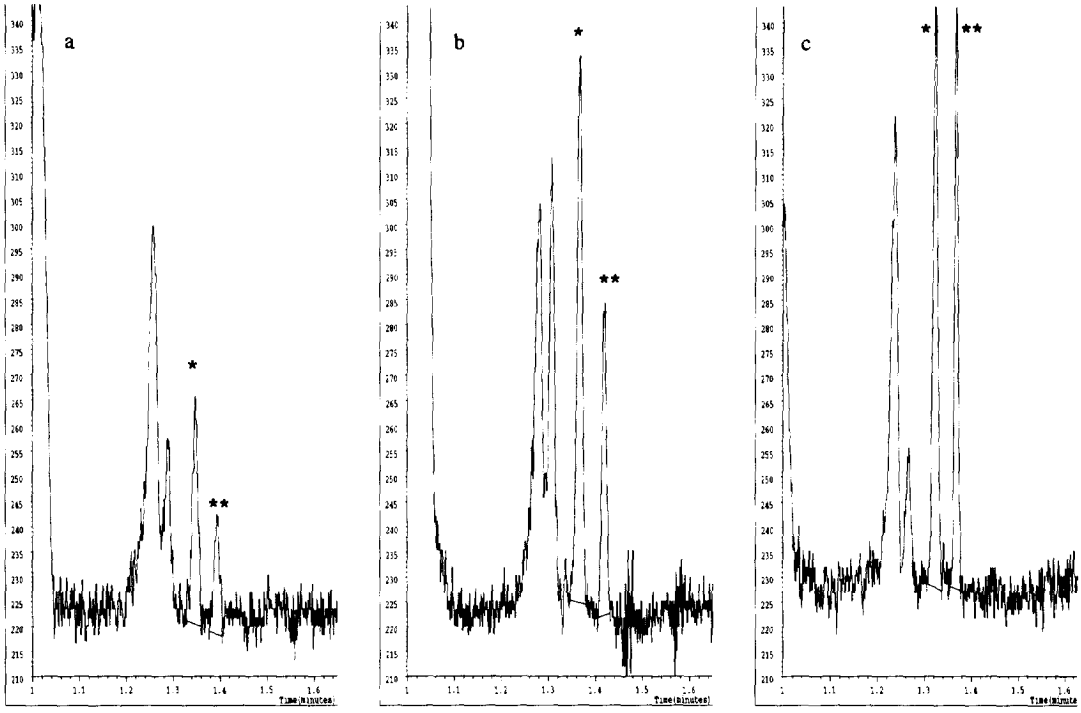

Fig. 5. Electropherograms demonstrating the effects of infusion of aCSF containing 100 m*M* K^+ on the extracellular concentrations of glutamate and aspartate from the dentate gyrus of the rat. (*) Glutamate and (**) aspartate. Separation was achieved on a fused-silica capillary (75 μm×37 cm) using 30 m*M* borate buffer pH 9.5 [35]. (a) Preinfusion, (b) 10 min post infusion, (c) 14 min post infusion.

occur. This is not necessary for the off-line analysis of amino acids as demonstrated by the use of the slower derivatisation reactions of FITC [22] and CBQCA [26,38].

The major alternative for the LIFD of the low levels of amino acids recovered from microdialysates is ECD [34,40,41]. Derivatisation is still necessary to allow electrochemical detection; NDA/CN can again be used as it also renders the product with electrochemical properties conducive to ECD. Using the electrochemical cell construction, detailed in Fig. 4, it has been demonstrated that ECD can be used to measure EAAs [34] or multiple amino acid residues [40,41] with comparable temporal resolution to that attainable by LIFD.

Recent preliminary data by Takada et al. [21] has demonstrated the use of mass spectrometry detection for CE and its application to microdialysis. This was used to identify endogenous GABA from rat striatum and although detection was possible, accurate quantification was not. However, this demonstrates the feasibility of CE–MS for the measurement of underivatised amino acids.

4.2. Catacholamines/monoamines

Monoamines and catacholamines such as serotonin, dopamine and noradrenaline are some of the best characterized of all neurotransmitters. Microdialysis sampling of these small molecules has been routinely performed since the outset of the technique and analysis has also not changed, to any great degree, since this time (conventionally using HPLC with ECD). The application of CE to the analysis of these amines has not become as popular as for the amino acid transmitters. A number of published articles have examined the measurement of the "classic" neurotransmitters by electrochemical methods [42,43], UV absorbance detection [44] and LIF [45] but analysis of amine transmitters from microdialsates has not been reported. However, recently Bert et al. [46,47] have published data

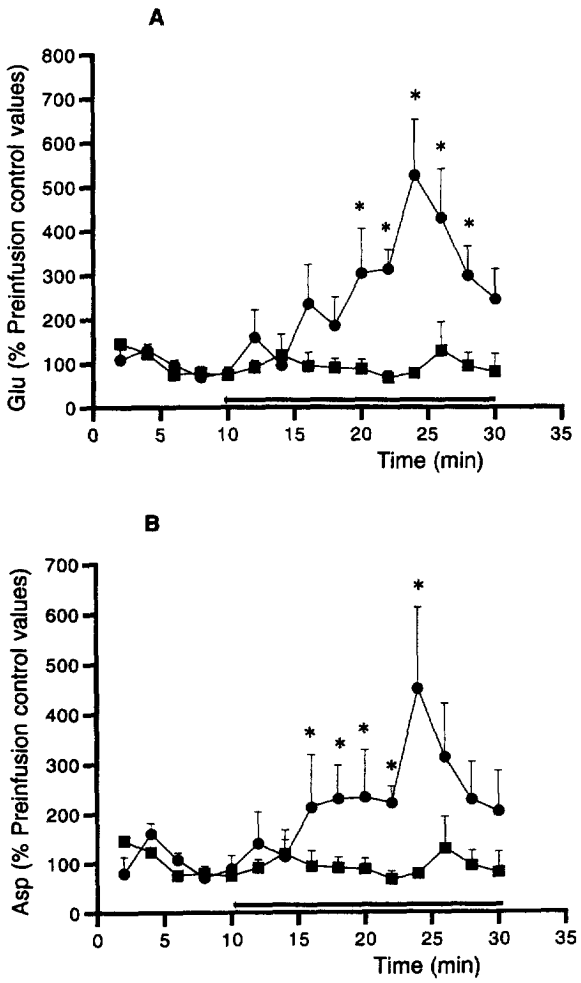

Fig. 6. Effects of infusion of a CSF containing 100 m*M* K^+ on the extracellular concentrations of glutamate (A) and aspartate (B) in the dentate gyrus (■) vehicle controls, (●) 100 m*M* K^+ treatment. * Demonstrates statistical significance ($p<0.05$) from vehicle controls. Data expressed as mean±S.E.M, $n=6$ per study group. Solid bar indicates infusion period [35].

demonstrating the analysis of both dopamine and noradrenaline by CE–LIF. These report the simultaneous monitoring of dopamine, noradrenaline and the EAAs from the same very low volume (500 nl) sample. On-line derivatisation (NDA was used due to its rapid reaction and hence stabilization of dopamine and noradrenaline) was followed by two separate analyses, one to resolve EAAs and another for the amines. These data demonstrate the sampling and monitoring of rapid fluctuations in catecholamines from an in vitro environment, however, in vivo evaluation is currently underway (personal communication).

4.3. Peptides

Peptides are extremely important biological molecules with huge diversity in function and structure. Many peptides can and have been measured by microdialysis. The application of CE, with various modes of separation and detection, to peptide analysis is an ever expanding field [48–50]; however, the combination of these two techniques has as yet not been extensively utilized. Advis et al. [51] demonstrated the resolution and measurement of luteinizing hormone-releasing hormone (LHRH) from the hypothalamus of the sheep using microdialysis and CE–UVD analysis. This method required the concentration of pooled samples and relatively large (20 nl) injection volumes, suggesting that sensitivity may have been an issue for LHRH analysis. Detection limits could be increased by the use of a more sensitive detector such as LIF or EC, however, peptides with electrochemical activity are rare and effective derivatisation of a complex molecule can be difficult, particularly at the low concentrations present within a microdialysate.

4.4. Drugs – pharmacokinetic study

Pharmacokinetic study is probably the second largest use of microdialysis next to the measurement of endogenous brain neurotransmitters. Pharmacokinetics is the study of the absorption, distribution, metabolism and elimination of any given drug introduced into a system. Microdialysis provides a means of sampling free (i.e., non protein bound) drug from any given system (e.g., blood, brain extracellular fluids, systemic tissues and the various organs) with the minimum of disturbance. Once again CE can be utilized for the analysis and measurement of both original administered substances and also subsequent metabolic products. The coupling of these two techniques has been achieved by many groups and utilized for the pharmacokinetic analysis of a variety of drug molecules. Tellez et al. [19] used microdialysis and CE–UVD analysis to determine the distribution of phenobarbital between

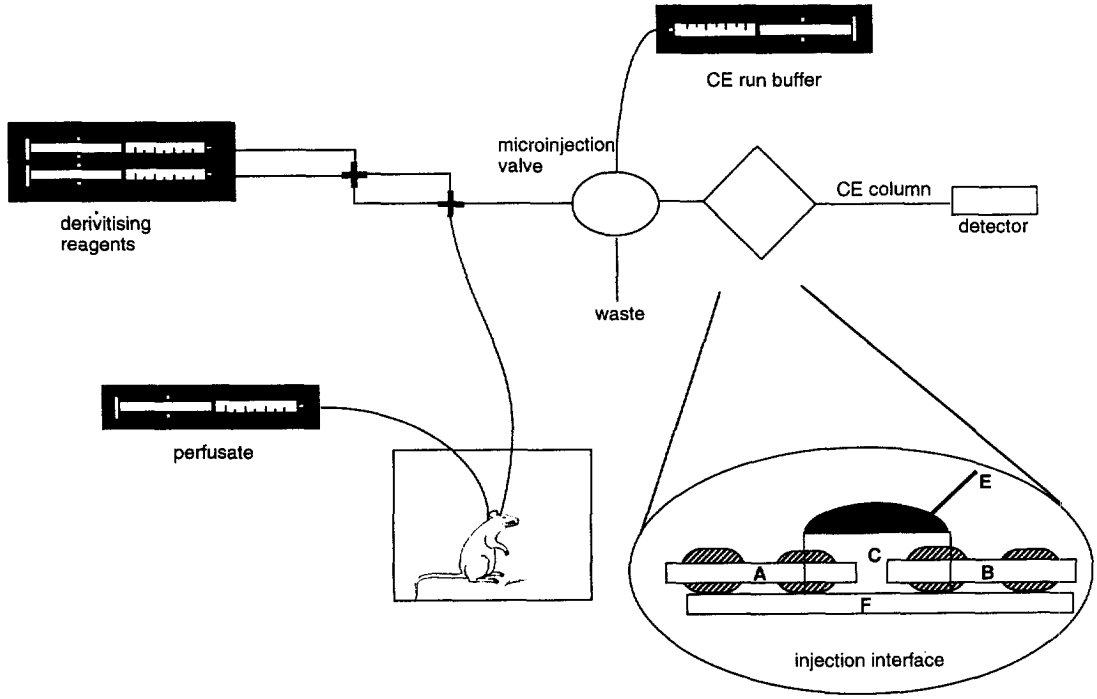

Fig. 7. Schematic representation of microdialysis coupling to CE with on-line prederivatisation adapted from [25,38]. Components of the derivatising cocktail are mixed with the perfusate direct from the in situ microdialysis probe producing fluorescent/electrochemically active products. The microinjection valve converts the μl dialysate to a 60-nl plug. These products are carried to the injection interface [components are: (A) transfer capillary, (B) CE capillary, (C) CE buffer reservoir, (D) CE run buffer, (E) ground eletrode, (F) microscope slide] where they are drawn into the separation capillary by eletroosmotic flow.

the blood and brain by the simultaneous implantation of multiple probes into both brain and the circulatory system of the rat. A 10-min sampling regime was used as increased temporal resolution would require a more sensitive mode of detection. This study demonstrates the importance of accurately evaluating the individual probes recovery, particularly if direct comparisons are made between different pharmacokinetic compartments. Hernandez et al. [18] again used CE–UVD and reverse brain microdialysis to simultaneously demonstrate how infusion of anaesthetics, such as cocaine, procaine and lidocaine, affect the dopaminergic systems within the brain. The administration of drugs was monitored by measuring the amount of anaesthetic lost from the initial infusion concentration by CE–UVD. Dopamine and its metabolites were evaluated by HPLC–ECD. Hu et al. [20] used CE–UVD for the analysis of α-difluoromethylornithine (DFMO, an anticancer agent) sampled from the blood of awake freely moving rats with a temporal resolution of 16 min. Derivatisation of DFMO with NDA was necessary since this molecule lacked a chromophore, however, this produced interference from endogenous amines which also react with the label and had to be separated from the drug. Using UVD for this type of analysis clearly limits the sensitivity for drugs that are present in lower circulating concentrations and/or are rapidly cleared/metabolised. Alternatively, CE-ECD has been employed for these types of study demonstrated by the analysis of the drug L-DOPA, the dopamine precursor used in the treatment of Parkinsons disease, and its metabolites sampled using intravenous microdialysis [52]. This method of detection affords the technique with increased sensitivity, which now allows the simultaneous measurement of metabolites (if metabolites maintain their electrochemical activity) as well as the parent

compound and increased temporal resolution. Also selectivity is increased as only electrochemically active molecules can be detected. Similarly, using ECD with cyclodextrin modified CE, Hadwiger et al. [53] demonstrated that different enantiomers of the chiral catecholamine, isoproterenol, can also be pharmacokinetically evaluated. Sensitivity was further increased in this study by on-column concentration of sample.

Using LIFD, Paez et al. [54] demonstrated that amphetamine (prederivatised with FITC) can be monitored in the brain up to 150 min after administration with a temporal resolution of 1 min. Finally, by a similar method to that applied to EAA evaluation, microdialysis has been directly coupled to CE with LIFD for the continuous on-line analysis of pharmacokinetic evaluation of an antineoplastic drug, SR 4233 [55]. This method has a temporal resolution of 90 s. Again, as for EAA analysis, this is a function of the separation time required for the resolution of SR 4233 and major metabolite, demonstrating that off-line analysis may be preferable under certain circumstances to fully optimize sampling times.

5. Conclusion

The application of CE for the analysis of a variety of molecules sampled by microdialysis has been reviewed here, demonstrating the partnership between the two techniques. There are still a number of microdialysis applications where CE lacks the sensitivity to allow effective development of routine analyses to compete with HPLC, however, as detector technology develops these limits will be extended and the applications which can benefit from the resolving power of CE will dramatically increase.

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