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Review

Measurement of neurotransmitters from extracellular fluid in brain by in vivo microdialysis and chromatography–mass spectrometry

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Abstract

During the last three decades, a great deal of information has been discovered about chemical neurotransmission. However, the most important processes, namely the complex nature of neuronal circuitry, the "cross talk" between multiple neurotransmitter systems, and the varying effects neurochemicals have at different receptors, are still being explored. Techniques such as microdialysis are routinely employed to measure neurotransmitter levels in living tissue systems. Moreover, microdialysis studies have proven to be valuable in the investigation of neurodegenerative and psychiatric disease pathology, as well as in identifying novel drugs to treat such disorders. One particular challenge in performing these experiments is the requirement to couple microdialysis to sophisticated analytical equipment. Recently, considerable attention has been focused on the development of chromatographic–mass spectrometric techniques to provide more sensitive and accurate measurements of neurochemicals collected from in vivo microdialysis experiments. This review will provide a brief overview of the microdialysis technique, as well as how microdialysis and chromatography–mass spectrometry are being used to measure extracellular levels of neurotransmitters. The primary emphasis of this review will be on how these applications are used to measure levels of acetylcholine (ACh), dopamine, norepinephrine and γ-aminobutyric acid (GABA).
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Keywords: Neurochemistry; Acetylcholine; Dopamine; Norepinephrine; GABA; Chromatography–mass spectrometry; In vivo microdialysis

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1. Introduction

Techniques used to monitor brain neurochemistry provide an exciting avenue in which to advance our understanding of neuropsychiatric disease pathology, as well as to aid in the discovery of novel and innovative pharmacotherapies used to treat such illnesses. The technique of microdialysis is routinely used to monitor the chemical constituents of the extracellular space in living tissue systems. In its simplest form, microdialysis provides a “snap shot” of the local environment revealing critical features of what a neurochemical system looks like before, during and after administration of a drug or other physiologically relevant stimuli. The utility of this technique was first described in the field of neuroscience, although today the use of microdialysis is common in pharmacokinetic, pharmacodynamic and, now more recently, in clinical study designs. While several recent reviews focused on the use of microdialysis in these areas of research [1–3], this commentary will focus on how microdialysis applications are being coupled to chromatography–mass spectrometry to measure extracellular neurochemicals such as acetylcholine (ACh), dopamine, norepinephrine and γ-aminobutyric acid (GABA).

2. Principles of microdialysis

2.1. A brief historical perspective

In vivo microdialysis has been used extensively since the late 1960s and early 1970s. Most people working in this research area consider Delgado’s early experiments in 1972 [4] to be the birth of what we know today as in vivo microdialysis. This group initially described a “dialytrode” that was surgically implanted for long-term intracerebral perfusion studies. It was, however, not until Urban Ungerstedt and colleagues [5,6] appropriately refined this technique and coupled microdialysis sample collection to, what was at the time, cutting edge liquid chromatography and electrochemical techniques. This union of in vivo sampling with analytical chemistry quickly expanded the field of microdialysis as well as led to the formation of several microdialysis companies including CMA Microdialysis [7], Applied Neuroscience [8] and BioAnalytical Systems [9]. The commercialisation of microdialysis resulted in numerous research groups employing the technique in a variety of freely moving, unanesthetised animals including mice, rats, guinea pigs, monkeys and humans. Currently, in the 21st century, in vivo microdialysis systems can be fully automated in terms of the collection and injection of dialysis samples. Moreover, the technique of microdialysis has quickly become a common fixture in the pharmaceutical industry to aid in the discovery and development of novel pharmacotherapeutics.

2.2. Microdialysis probes

A principle component of microdialysis is the probe used to capture molecules from the extracellular space [10]. However, regardless of the probe dimensions and specifications [10,11], all microdialysis probes share a fundamental similarity: the semi-permeable membrane. This membrane is where the “dialysis” or the diffusion of molecules between the extracellular fluid and the perfusion fluid takes place. Initially, the microdialysis probe is implanted into a discrete region of interest and a physiological salt solution (e.g., artificial cerebrospinal fluid, aCSF) is slowly and continuously infused through the probe until an equilibrium is achieved between the inside of the probe and the extracellular space. Since the membrane is pervious to endogenous chemicals small enough to pass through holes in the membrane (e.g., 20 kD), molecules diffuse down their concentration gradient into the dialysis probe. After a given amount of time, the perfusion solution inside the probe will contain a representation of the chemicals found in the extracellular space. The fluid inside the probe (also referred to as the dialysate) is collected and subsequently analysed for neurotransmitter content.

2.3. Advantages and disadvantages

In addition to providing critical pieces of information regarding the neurochemical environment, microdialysis studies offer several inherent advantages over other techniques. For instance, by employing stereotaxic surgical techniques, neurochemicals can be measured in discrete areas of the brain that are otherwise inaccessible by other techniques. Moreover, simultaneous sampling from multiple sites is made possible by implanting more than one probe in the same animal. This particular advantage is illustrated in Fig. 1. Another distinct advantage of microdialysis is that the probe can be used to infuse test or known compounds through the probe (known as retrodialysis). Therefore, by using multiple microdialysis probes, the effects of local drug injection on neurochemicals in other brain regions can be monitored. Additionally, when coupled to analytical systems such as mass spectrometry, microdialysis can be used to obtain direct measurements of free drug concentrations in the brain following a systemic injection in vivo [12]. Finally, since it is a sampling technique, microdialysis reduces the overall loss of body fluids.

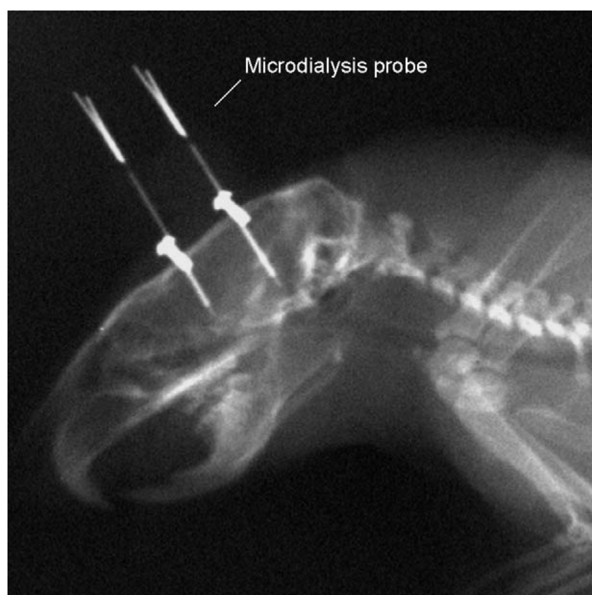


Fig. 1. X-ray showing dual microdialysis probe implantation in a male Sprague–Dawley rat. Probes are secured in their respective brain regions with dental cement and two stainless steel screws.

Despite many distinct advantages of microdialysis, it is a complicated technique with just as many disadvantages. Thus, microdialysis probes must be implanted into a region of interest. In doing so, damage to local tissue/cellular regions could potentially confound experimental results. It should be noted that this damage is considered to be minimal and studies have shown that the blood brain barrier, for example, remains intact following probe implantation [13]. Additionally, studies have shown that local chemical levels and blood flow return to near normal levels approximately 24 h post probe implantation [13]. Furthermore, and simply due to the size of the microdialysis probes, there is no access to the intracellular space. Therefore, subtle changes occurring to molecules residing inside the cell, which are not sufficiently reflected in the extracellular space, cannot be accurately monitored by microdialysis. Microdialysis samples typically have poor or low temporal resolution as samples are usually collected in "buckets" or time intervals greater than 5 min. While this resolution time has been shortened considerably by the advent of highly sensitive analytical techniques, microdialysis samples just simply do not provide "real time" information regarding the neurochemical environment.

3. Analysis of microdialysis samples

3.1. Chromatography

A well-known requirement of microdialysis studies is the need for coupling to sophisticated analytical equipment to analyse the collected samples. Detection of extracellular levels of neurotransmitters remains an analytical and technical challenge due, in large part, to the low levels of neurotransmitters collected (i.e., fmol amounts) and the small microdialysis sample size (i.e., 10–20 μl). In addition, microdialysis samples are complex mixtures containing high concentrations of inorganic salts that require chromatographic separation to allow for the measurements of individual neurotransmitters. For many decades, the most popular separation methods for classical neurotransmitters were carried out using gas chromatography (GC) and high performance liquid chromatography (HPLC) techniques. These methods are discussed briefly below.

A specific requirement of GC is that the substance to be analysed is volatile. Volatilisation of the analytes is achieved by chemical derivitisation to introduce the volatile function group on the molecules. For instance, volatilisation of acetylcholine (ACh) was achieved by demethylation of the quaternary N-atom with sodium benzenethiolate to form the tertiary amine analog [14,15]. GC separation of the neurotransmitter derivatives coupled with mass spectrometry (GC–MS) has been used for quantification of neurotransmitters in plasma, cerebrospinal fluid (CSF) and extracted brain tissue [16–18]. However, the sensitivity of GC–MS assays is inadequate for the detection of extracellular levels of neurotransmitters in brain microdialysate. Other disadvantages of GC–MS assay include time-consuming sample clean-up procedures and inadequate specificity.

By comparison, HPLC provides the advantages of simplicity and stability. While neurotransmitters such as ACh, dopamine, GABA, are polar compounds with low molecular weights, their retentions on standard reversed phase HPLC systems are generally poor. Additional problems that are typically encountered while separating these neurotransmitters using standard reversed phase HPLC systems include spurious HPLC peaks, lengthy column equilibration time, and poor HPLC peak shapes. To circumvent some of these issues, ion-pairing [19] and hydrophobic interaction [20] mechanisms have been used to separate these polar analytes. Other concerns for analysis are the low levels of extracellular neurotransmitters and the small microdialysate sample size. To increase overall sensitivity and to reduce sample dilution, the HPLC methods employed small diameter HPLC columns with pre-columns as well as capillary columns have been developed.

3.2. Mass spectrometry

Methods including electrochemical detection (ECD) [21], fluorescence [22], ultraviolet (UV) [23] and mass spectrometry (MS) [24] have been routinely coupled to HPLC methods for the measurement of neurotransmitter concentrations. However, in many cases, including ECD methods, an indirect measurement of analytes is made. Moreover, co-eluting interferences can affect the overall accuracy of the measurements using these methods. By comparison, MS provides a direct measurement of specific analytes and, depending on the measured analyte (i.e. neuropeptides), can provide a more sensitive platform in which to monitor some neurochemicals.

In the LC–MS assay, an analyte can be identified by both its retention time and molecular weight. Monitoring the selective molecular ions (known as selected ion monitoring, SIM) in the measurement of analytes has been used with a single quadrupole MS to improve the sensitivity of the measurement. Furthermore, a triple quadrupole MS allows specific daughter ions, resulting from dissociation of the parent molecular ion, to be monitored. Monitoring the most abundant daughter ion from dissociation of the molecular ion has been conducted using a multiple reaction monitoring (MRM) scan function (also known as selected reaction monitoring, SRM). The MRM scan function provides an additional dimension of structurally specific filtering for individual analytes. As a result, the signal to noise ratio of an ion chromatographic peak using the MRM scan mode is significantly higher (10–20-fold) than that obtained using the SIM scan mode. Overall, the LC/MS/MS techniques provide a direct, structural-specific measurement of individual components with high sensitivity. In addition, LC/MS/MS systems have minimal baseline drift and can be equilibrated very rapidly.

One critical requirement for the use of the LC/MS or LC/MS/MS systems is that the mobile phase must be volatile. Although ion-exchange reagents, such as alkanesulphonates, are commonly used for cation-exchange chromatography, they are not volatile and therefore not compatible with MS technology. These mobile phase systems could significantly reduce the ionisation efficiency because of ion suppression. Development of separation methods, which could retain and separate the polar analytes and also be suitable for MS, has been a technical challenge for the detection of neurotransmitters. Another concern when using MS techniques is that ion suppressing occurs during electrospray ionisation (ESI) in the presence of salts and other co-eluting analytes. The high ionic strength of microdialysis samples generates high background noise and suppresses the ionisation of analytes resulting in considerable reductions in sensitivity. Therefore, it is essential to resolve the analytes with co-eluting components in the sample matrix to minimise the ion suppression.

4. Measuring extracellular neurotransmitter levels

The rest of this review will focus on the detection methods used to measure specific neurochemicals including ACh, dopamine, norepinephrine and GABA in microdialysate. Table 1 summarises these methods while the major advantages and disadvantages of the techniques are highlighted in the following sections.

4.1. Acetylcholine

The biological role of ACh has been studied since the mid-1920s [25]. Scientists found that ACh is involved in the regulation of the central cholinergic functions, which were related to various CNS diseases including – but not limited to – myasthenia gravis [26], cognitive dysfunction [27,28], Hirschsprung’s disease [29], Tourette’s syndrome, Huntington’s disease, Schizophrenia [30], Parkinson’s disease and Alzheimer’s disease [31–35]. Therefore, the ability to measure brain concentrations of ACh is important in understanding disease pathology as well as in evaluating potential disease-modifying treatments.

The use of in vivo microdialysis to measure ACh was first developed by Damsma et al. [36–40], Westerink et al. [41–43] and Ajima and Kato [44–46]. Reported methods for the measurement of ACh were based upon the use of chemiluminescence [47], electrochemical (ECD) [21], fluorescence [22], MS [24,48] and UV [23] detections. A recent review by Tsai surveyed various analytical techniques that have been adopted for the measurement of ACh [49]. The majorities of these methods relied on detecting ACh indirectly and were insufficient for reliable quantification of ACh in microdialysate. Furthermore, many investigators included acetylcholine esterase (AChE) inhibitors, such as physostigmine or neostigmine, in the perfusate media to enhance basal levels of ACh. The most commonly used approach for the measurement of extracellular ACh was based on brain microdialysis coupled to an HPLC-ECD system with a post-column immobilised reactor (post-reactor). This technique has been shown to measure central ACh release but only in the presence of AChE inhibitors [35,44,50,51]. One fundamental problem with this method was that the use of AChE inhibitors could lead to changes in the physiology of the system. Thus, AChE inhibitors contained in the perfusion fluid could mask small drug related changes in ACh levels as well as alter transmission of other systems within the perfused area [52–57]. For example, De Boer [54] and Acquas [58] reported that perfusion fluid containing neostigmine quantitatively and qualitatively influenced the manner

Table 1
Methods coupled to HPLC to measure neurochemicals from microdialysate

Detector	Neurochemical	Column	Detection limit	Reference
MS (SRM)	ACh	Ion-pair	1.2 fmol	[62]
MS (MRM)	ACh	Cation-exchange	1.0 fmol	[60,61]
MS (MRM)	Dopamine	Ion-pair	200 pmol	[69]
MS (MRM); pre-column derivatisation	GABA	Capillary reversed phase	5 ng/ml	[86]
ECD; post-column IMER	ACh	Cation-exchange	10 fmol	[21,57]
ECD; pre and post-column IMER	ACh	Cation-exchange	10 fmol	[59]
ECD	Dopamine	Cation-exchange	100 pmol	[20]

in which dopaminergic agents regulated ACh overflow in the striatum.

Methods for determining basal levels of acetylcholine in the central nervous system, without the use of AChE inhibitors, are an invaluable tool. Using improved HPLC-ECD techniques Huang et al. [21] and Kato et al. [59] reported that AChE inhibitors were not necessary in the detection of basal ACh in brain microdialysate. Huang et al. described a peroxidase-redox polymer modified glassy carbon electrode operating at +100 mM versus Ag/AgCl to detect the reduction of hydrogen peroxide. With this method, a detection limit of 10 fmol was obtained. Kato et al. [59] reported detection of basal ACh in dialysate from rat frontal cortex by HPLC using a horseradish peroxidase-osmium redox polymer electrode with pre-enzyme reactor (HRP-GCE). The authors stated detection limit of 10 fmol for ACh levels in the dialysate.

The most sensitive assay that has been reported in the literature for the analysis of ACh is coupling in vivo microdialysis with liquid chromatography-tandem MS (LC/MS/MS) [60–62]. In these studies, microdialysis probes were surgically implanted into specific brain regions of the rat. The probes were perfused at a flow rate of 1–2 μl/min with aCSF. The microdialysates were collected every 20–30 min and samples were directly analysed off-line by LC/MS/MS. The separation of ACh was based on cation-exchange [60,61] or ion-pair chromatography with volatile reagents [62,63]. The analytes were detected by tandem MS in the positive ESI mode. In order to achieve the best sensitivity and structure specificity, a specific daughter ion from dissociation of the molecular ion of ACh was monitored using MRM scan mode on triple quadrupole MS [60,61] or SRM scan mode on ion trap MS [62]. The detection limit for ACh was found to be 1.0 and 1.2 fmol by using triple quadrupole MS [60,61] and ion trap MS [62], respectively. One of the major advantages of LC/MS/MS techniques over HPLC detectors is that ACh is identified by both its retention time and its specific parent–daughter ion transition (MRM or SRM). LC/MS/MS techniques provide a direct, sensitive and structure-specific measurement of ACh. Additionally, LC/MS/MS techniques result in minimal baseline drift and rapid equilibration [60].

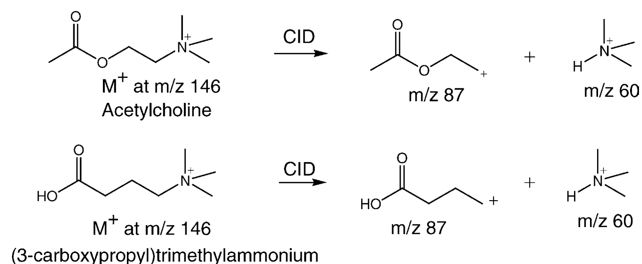
The selection criteria of HPLC columns and mobile phase systems are important for detection of ACh using LC/MS/MS techniques. Liberato et al. reported a LC/MS assay in which a C18 microbore column with a flow rate of 10 μl/min was used to separate ACh and choline. For these studies, octanesulfonic acid was used as the ion-pairing agent while the molecular ions of analytes were detected by MS under ESI with SIM scan mode [64]. The detection limit for ACh was 10 pmol, which was insufficient to detect extracellular ACh in the microdialysate. Zhu et al. employed heptafluorobutyric acid in the mobile phase as the ion-pairing reagent to separate ACh and choline in their study [62]. The authors claimed that heptafluorobutyric acid showed good separation and had shorter equilibration time of less than 5 min. They reported a detection limit of 1.2 fmol on column using the mobile phase containing 20 mM ammonium acetate and 20 mM heptafluorobutyric acid at pH 3.2. However, the authors also indicated that the method could not be used for routine measurement of ACh. When attempting to reproduce the findings of Zhu et al., our research group observed a significant signal drop within a short period of time (<1 h) by using Zhu's mobile phase system [65]. This observation suggested that the relatively high viscosity of the mobile phase with 20 mM heptafluorobutyric acid and 20 mM ammonium acetate at pH 3.2 could result in serious contamination of the MS ion source, resulting low sensitivity. Hows et al. [60] developed a cation-exchange chromatography with elution buffer, consisting of a mixture of ammonium acetate, ammonium formate and acetonitrile. The detection limit of this method was 1 fmol. This system was more robust than the previous systems and the authors reported that the assay has been used routinely for the measurement of ACh from brain microdialysis samples.

Electrospray ionisation utilises high voltage to ionise molecules, which have an ionisable group, to form protonated or deprotonated molecular ions in positive or negative electrospray ionisation, respectively. The quaternary amine function group of ACh has a positive charge at acidic conditions. There is no need to ionise the molecules of ACh in acidic conditions, resulting in good sensitivity. The molecular ion (M^+) at *m/z* 146 of ACh is the predominant ion formed in the ESI source at a low pH (e.g., pH 4) of the mobile phase. One important consideration is the potential ion suppression from the components in the dialysates. In vivo microdialysates contain high concentrations of salts including NaCl, KCl and $MgCl_2$. It is essential to resolve the analytes from these components in the sample matrix to minimise ion suppression. Hows and colleagues reported that the detection sensitivity of ACh using the cation-exchange columns was poor when the pH of an elution buffer was greater than 4.0. This was likely due to the interference from a co-eluting HPLC peak [60]. The sensitivity was dramatically improved when the pH of elution buffer was low. Zhang et al. used a gradient method that resulted in early elution of these inorganic salts [61]. This approach reduced ion suppression that is typically observed during the ESI process.

Reasonable chromatographic separation in ACh measurements is important to minimise potential chemical interferences. Both Zhou [62] and Zhang [61] observed another neurotransmitter, (3-carboxylpropyl)-trimethylammonium (iso-ACh), in the microdialysate from rat brains. Iso-ACh is an isobar of ACh and it produces the same MRM transition as that of ACh (Scheme 1). Differentiation of iso-ACh and ACh was determined by their HPLC retention times. Therefore, baseline separation of these two components is essential for the accurate quantification of ACh (Fig. 2) [61].

4.2. Dopamine and norepinephrine

Dopamine, norepinephrine and epinephrine are catecholamines. Dopamine is the most recently discovered catecholamine transmitter in the mammalian brain [66–68]. Heidbreder et al. developed an analytical method by using



Scheme 1. Fragment ions of the molecular ions of ACh and iso-ACh observed in their product ion mass spectra.

ion-exchange chromatography with ECD to improve selectivity and minimise artifacts for measurement of dopamine and norepinephrine in brain dialysates [20]. However, this system required a long equilibration time, resulting in low throughput of analysis. Furthermore, as with most HPLC based methods, the analytes can only be identified by matching retention times which may result in false positives introduced by co-eluting chemical interferences. To circumvent these issues, Hows et al. developed a reversed phase HPLC-tandem MS assay for simultaneous measurement of dopamine and norepinephrine in rat microdialysates [69]. Using this approach, separation of dopamine and norepinephrine was carried out using either a Discovery C18 HS or a Synergi hydro RP column and a mobile phase system containing water and acetonitrile with 0.1% of formic acid. The analytes were detected using tandem MS in the positive ESI mode. The detection limits for dopamine and norepinephrin were 200 and 1000 pM, respectively. This latter method was used to evaluate the effect of acute systemic administration of cocaine on dopamine and norepinephrine in the shell subregion of the nucleus accumbens. An interesting note from the authors was that the basal level of dopamine in the microdialysates was 0.47 nM using LC/MS/MS, which was much lower than the basal levels of 5.5 nM detected using HPLC-ECD in the same laboratory using the same samples. When the flow rate of microdialysis studies was taken into account, the basal level of 0.47 nM from LC/MS/MS analysis was consistent with published concentrations [70,71]. They found that the HPLC peak detected using LC-ECD could not be attributed solely to dopamine and was most probably contributed by co-eluting chemicals. This result demonstrated that LC/MS/MS provided a means of unequivocally identifying analytes and could be used to

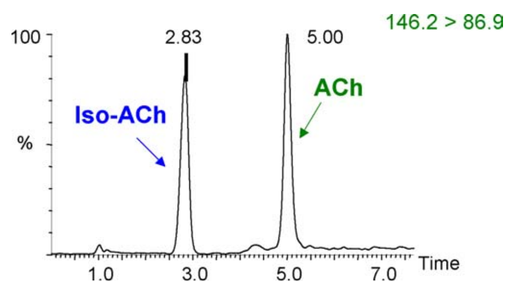


Fig. 2. The MRM chromatograms of ACh and (3-carboxylpropyl)-trimethyl-ammonium in microdialysates from rat hippocampus [62].

discriminate between HPLC peaks assigned to artifacts or analytes.

Unfortunately, this LC/MS/MS method was not sensitive to determine basal levels of norepinephrine. The β-substituted aliphatic hydroxyl group to the primary amine moiety of norepinephrine further reduced the stability of the protonated molecular ion by forming $[MH–H_2O]^+$ fragment ions. Therefore, the detection limit of norepinephrine was five-fold higher than that of dopamine. Maintaining good HPLC separation and simultaneously obtaining high ESI ionisation efficiency for the biogenic amines dopamine and norepinephrine remain to be a technical challenge. ESI of the biogenic amines utilises the primary amine moiety to form protonated molecular ions ($[M+H]^+$). The protonated molecular ions with a charged primary amine moiety were generally unstable, and they rapidly dissociated to form a predominant $[MH–NH_3]^+$ fragment ion in the ESI source. Since the protonated molecular ion was not the predominant ion in their mass spectra, monitoring the protonated molecular ions might result in reduced sensitivity.

4.3. GABA

GABA is a ubiquitous, non-protein amino acid produced through the α-decarboxylation of L-glutamic acid. GABA is the predominant inhibitory neurotransmitter in mammalian central nervous systems [72]. Numerous studies indicate that abnormal GABA levels in physiological fluids are correlated with various neurological disorders. Thus, it has been shown that elevated GABA levels were observed in the CSF of meningitis patients [73], while low levels of GABA were measured in the CSF of patients with Alzheimer's disease [74].

The direct monitoring of GABA is very difficult because it is insensitive to electrochemical and UV–vis spectroscopic methods. It is also difficult to detect GABA in combination with an enzymatic reaction because neither oxidase nor hydrogenase can be found, unlike other neurotransmitters such as L-glutamate and ACh. Various methods have been developed for the detection of GABA. These methods were based on techniques including HPLC [75–78], GC–MS [79,80], CE–MS [81], CE-laser induced fluorescence detection [82], electrochemical sensor [83], spectrophotometry [84] and HPLC–MS [85]. Many of these techniques, particularly the GC–MS based method, require time-consuming sample pretreatments such as liquid–liquid or solid phase extraction. The intention of the CE–MS method was to determine levels of GABA in the rat brain using in vivo microdialysis. However, these methods were shown to be inadequate as the signal was too weak for quantification analysis [81].

Ma et al. reported an HPLC–MS method for simultaneous determination of GABA and glutamate in rat brain tissue [84]. The separation of these amino acids was carried out using an Inertsil ODS-2 packed column (150 nm × 4.6 nm i.d.) with a mobile phase system composed of acetonitrile and ammonium acetate. Ionisation occurred using atmospheric pressure

chemical ionisation (APCI) and detected using the SIM mode. The method had a detection limit of 2.5 ± 0.3 μg/ml, which was insufficient to detect the basal GABA concentrations in plasma or microdialysates. The low sensitivity of the method was likely due to multiple factors. First, the sample dilution by mobile phase, when using a 4.6 nm ID column, reduced the actual sample concentrations in the ESI source. Second, the protonated molecular ions of GABA with a charged primary amine moiety were unstable and were not the dominant ions in the ESI mass spectra. Monitoring the protonated molecular ions could result in reduced sensitivity. Third, GABA, which is a very polar molecule with an acid and a primary amine moiety, eluted at very early retention time under the study conditions described in the paper. The co-eluted chemical interferences could cause serious ion suppression, resulting in low ionisation efficiency. Finally, the detection sensitivity when using SIM scan mode on single triple quadrupole MS was much lower than that observed using MRM scan mode on triple quadrupole MS [84].

In order to achieve better detection sensitivity, capillary HPLC–MS hyphenation has been gaining research interest. Song et al. reported a capillary liquid chromatographic/tandem MS method for quantification of GABA in human plasma and CFS [86]. GABA is highly hydrophilic, and it only remains on a reversed phase column for a very short period of time. Pre-column derivatisation of GABA with 7-fluro-4-nitrobenzoxadiazole (NBD-F) was deployed to increase its hydrophobilicity and molecular size, thus strengthening its retention on the column. An extraction column (10 mm × 0.25 mm, 7 μM, C18) was used for preconcentration and for stacking the samples. Separation was carried out using C18 column (50 mm × 0.25 mm, 5 μM) with a detection limit of 5 ng/ml. This method was used for quantitation of GABA in human CSF and revealed basal concentrations of GABA to be 44.3 ± 10.0 ng/ml. Due to this level of sensitivity, this method could potentially be used for determination of extracellular levels of GABA in brain.

5. Conclusions

The technique of in vivo microdialysis coupled with chromatography–MS provides accurate and structurally specific measurements of extracellular neurotransmitter levels. As discussed in this review, the measurements of basal neurotransmitter levels in microdialysate continue to be both an analytical and technical challenge. Furthermore, enhancements in the detection sensitivity are highly sought after to provide more detailed information regarding the neurochemical environment. In most cases, the detection sensitivity with tandem MS was higher than that reported with various HPLC detectors (see Table 1). In addition, LC/MS/MS systems rapidly equilibrate, resulting in a dramatic increase in the assay throughput. This approach has been successfully used for measuring basal levels of ACh, dopamine and GABA from extracellular fluid in brain. The advent of such analytical approaches will greatly benefit our understanding of chemical neurotransmission in vivo and, undoubtedly, aid in the exploration of neurodegenerative and psychiatric disease pathology, as well as in identifying novel therapies to treat such disorders.

References

[1] M.J. Cano-Cebrian, T. Zornoza, A. Polache, L. Granero, Curr. Drug Metab. 6 (2005) 83–90.
[2] O. Rooyackers, A. Thorell, J. Nygren, O. Ljungqvist, Curr. Opin. Clin. Nutr. Metab. Care 7 (2004) 513–521.
[3] L. Hillered, P.M. Vespa, D.A. Hovda, J. Neurotrauma 22 (2005) 3–41.
[4] J.M.R. Delgado, F.V. de Feudis, R.H. Roth, D.K. Ryugo, B.M. Mitruka, Arch. Int. Pharmacodyn. 10 (1972) 9–21.
[5] U. Ungerstedt, C. Pycock, Bull. Schweiz. Akad. Med. Wiss. 1278 (1974) 1–13.
[6] U. Ungerstedt, M. Herrera-Marschitz, U. Jungnelius, L. Ståhle, U. Tossman, T. Zetterström, Adv. Biosci. 37 (1982) 219–231.
[7] http://www.microdialysis.se/.
[8] http://www.appliedneuroscience.co.uk/.
[9] http://www.bioanalytical.com/.
[10] M. Orlowska-Majdak, Acta Neurobiol. Exp. 64 (2004) 177–188.
[11] S.H. Khan, A. Shuaib, Methods 23 (2001) 3–9.
[12] O. Langer, M. Muller, Curr. Drug Metab. 5 (2004) 463–481.
[13] H. Benveniste, J. Neurochem. 52 (1989) 1667–1679.
[14] W.B. Stavinoha, S.T. Weintraub, Anal. Chem. 46 (1974) 757–760.
[15] S.J. Fidone, S.T. Weintraub, W.B. Stavinoha, J. Neurochem. 26 (1976) 1047–1049.
[16] L.R. Drewes, A.K. Singh, J. Neurochem. 50 (1988) 868–875.
[17] A.K. Singh, L.R. Drewes, J. Chromatogr. 339 (1985) 170–174.
[18] E.A. Pomfret, K.A. daCosta, L.L. Schurman, S.H. Zeisel, Anal. Biochem. 180 (1989) 85–90.
[19] C.S. Chaurasia, C.E. Chen, A. Ashby, J. Pharm. Biomed. Anal. 19 (1999) 413–422.
[20] C.A. Heidbreder, L. Lacroix, A.R. Atkins, A.J. Organ, S. Murray, A. West, A.J. Shah, J. Neurosci. Methods 112 (2001) 135–144.
[21] T. Huang, L. Yang, J. Gitzen, P.T. Kissinger, M. Vreeke, A. Heller, J. Chromatogr. B 670 (1995) 323–327.
[22] R.C. Macdonald, J. Neurosci. Methods 29 (1989) 73–76.
[23] G. Ulin, K. Gustavii, B.A. Persson, J. Pharm. Pharmacol. 28 (1976) 672–675.
[24] J.K. Khandelwal, P.I. Szilagyi, L.A. Barker, J.P. Green, Eur. J. Pharmacol. 76 (1981) 145–156.
[25] H.H. Dale, H.W. Dudley, J. Physiol. 68 (1929) 97–123.
[26] K. Boonyapisit, H.J. Kaminski, R.L. Ruff, Am. J. Med. 106 (1999) 97–113.
[27] M. Testa, L.M. Biasucci, M. Cacciola, A. Lombardo, F. Pennestri, T.H. Lejemtel, F. Loperfido, Am. J. Cardiol. 77 (1996) 149–153.
[28] M. Sarter, J.P. Bruno, Brain Res. Rev. 27 (1998) 143–156.
[29] H. Ikawa, J. Yokoyama, Y. Morikawa, A. Hayashi, K. Katsumata, J. Pediatr. Surg. 15 (1980) 48–52.
[30] K.E. White, J.L. Cummings, Compr. Psychiat. 37 (1996) 188–195.
[31] O. Felician, T.A. Sandson, J. Neuropsych. Clin. Neurosci. 11 (1999) 19–31.
[32] P. Davies, A.H. Verth, Brain Res. 138 (1977) 385–392.
[33] J.T. Coyle, D.L. Price, M.R. Delong, Science 219 (1983) 1184–1190.
[34] W.W. Beatty, N. Butters, D.S. Janowsky, Behav. Neural. Bio. 45 (1986) 196–211.
[35] M. Xu, Y. Nakamura, T. Yamamoto, K. Natori, T. Irie, H. Utsumi, T. Kato, Neurosci. Lett. 123 (1991) 179–182.
[36] G. Damsma, B.H.C. Westerink, A.S. Horn, J. Neurochem. 45 (1985) 1649–1652.

[37] G. Damsma, B.H.C. Westerink, A. Imperato, H. Rollema, J.B. De Vries, A.S. Horn, Life Sci. 41 (1987) 873–876.
[38] G. Damsma, B.H.C. Westerink, P. de Boer, J.B. de Vries, A.S. Horn, Life Sci. 43 (1988) 1161–1168.
[39] G. Damsma, P.T.M. Biessels, B.H.C. Westerink, J.B. de Vries, A.S. Horn, Eur. J. Pharmacol. 145 (1988) 15–20.
[40] G. Damsma, P. de Boer, B.H.C. Westerink, H.C. Fibiger, Naunyn-Schmiedeberg's Arch. Pharmacol. 342 (1990) 523–527.
[41] B.H.C. Westerink, G. Damsma, Trend Pharmacol. Sci. 10 (1989) 262–263.
[42] B.H.C. Westerink, P. De Boer, Neurosci. Lett. 112 (1990) 297–301.
[43] E. Moor, E. Schirm, J. Jacso, B.H. Westerink, Neuroscience 82 (1998) 819–825.
[44] A. Ajima, T. Kato, Neurosci. Lett. 81 (1987) 129–132.
[45] A. Ajima, T. Kato, Biogenic Amines 5 (1988) 461–464.
[46] A. Ajima, T. Nakagawa, T. Kato, J. Chromatogr. 494 (1989) 297–302.
[47] J.P. Trnaux, M.C. Chamoin, J. Biochem. Chemilumin. 9 (1994) 65–72.
[48] Y. Ikarashi, K. Itoh, Y. Maruyama, Biol. Mass Spctrom. 20 (1991) 21–25.
[49] T.H. Tsai, J. Chromatogr. B 747 (2000) 111–122.
[50] S. Consolo, C.F. Wu, F. Fiorentini, H. Ladinsky, A. Vezzani, J. Neurochem. 48 (1987) 1459–1465.
[51] G. Damsma, B.H.C. Westerink, J.B. de Vries, C.J. Van den Berg, A.S. Horn, J. Neurochem. 48 (1987) 1523–1528.
[52] G. Cuadra, K. Summers, E. Giacobini, J. Pharmacol. Exp. Ther. 270 (1994) 277–284.
[53] J.K. Liu, T. Kato, Neurochem. Int. 24 (1994) 589–596.
[54] P. De Boer, B.H.C. Westerinck, A.S. Horn, Neurosci. Lett. 116 (1990) 357–360.
[55] T.H. Tsai, C.F. Chen, Neurosci. Lett. 166 (1994) 175–177.
[56] T.R. Tsai, T.M. Cham, K.C. Chen, C.F. Chen, T.H. Tsai, J. Chromatogr. B 678 (1996) 151–155.
[57] J. Kehr, P. Dechent, T. Kato, S.O. Ogren, J. Neurosci. Methods 83 (1998) 143–150.
[58] E. Acquas, H.C. Fibiger, J. Neurochem. 70 (1998) 1088–1093.
[59] T. Kato, J.K. Liu, K. Yamamoto, P.G. Osborne, O. Niwa, J. Chromatogr. B 682 (1996) 162–166.
[60] M.E.P. Hows, J.O. Andrew, S. Murray, L.A. Dawson, R. Foxton, C. Heidbreder, Z.A. Hughes, L. Lacroix, A.J. Shah, J. Neurosci. Methods 121 (2002) 33–39.
[61] M.Y. Zhang, P. Li, Q. Lin, E. Kerns, L.E. Schechter, C.E. Beyer, 52nd ASMS, Nashville, TN, 24–27 May 2004.
[62] Y. Zhu, P.S.H. Wong, M. Cregor, J.F. Gitzen, L.A. Coury, P.T. Kissnger, Rapid Commun. Mass Spectrom. 14 (2000) 1695–1700.
[63] A. Guerrieri, F. Palmisano, Anal. Chem. 73 (2001) 2875–2882.
[64] D.J. Liberato, A.L. Yergey, S.T. Weintraub, Biomed. Environ. Mass Spectrom. 13 (1986) 171–174.
[65] In reproducing Zhu's experimental condition in our lab, we observed serious contamination of ion source by using heptafluorobutyric acid in the mobile phase.
[66] J.R. Cooper, F.R.E. Bloom, R.H. Toth, The Biochemical Basis of Neuropharmacology, eighth ed., Oxford University Press, 2003.
[67] J.D. Chi, J. Odontidas, M. Frankli, J. Chortatogr. B 731 (1999) 361–371.
[68] P. Moleman, J.J.M. Borstrok, Biogenic Amines 3 (1985) 33–71.
[69] M.E.P. Hows, L. Lacroix, C. Heidbreder, A.J. Organ, A.J. Shah, J. Neurosci. Methods 138 (2004) 123–132.
[70] H.O. Pettit, H.T. Pan, L.H. Persons, J.B. Justice, J. Neurochem. 55 (1990) 798–804.
[71] L.H. Parsons, J.B. Justice, J. Neurochem. 58 (1992) 212–218.
[72] V.M. Pickel, E.J. Van Bockstaele, J. Chan, D.M. Cestari, J. Neurosci. Res. 44 (1996) 446–458.
[73] M.Y. Khuhawar, A.D. Rajper, J. Chromatogr. B 788 (2003) 413–418.
[74] F.J. Jimenez-Jimenez, J.A. Molina, P. Gomez, C. Vargas, F. de Bustos, J. Benito-Leon, A. Tallon-Barranco, M. Orti-Pareja, T. Gasalla, J. Arenas, J. Neural. Transm. 105 (1998) 269–277.
[75] Y.H. Kuo, P. Rozan, F. Lambein, J. Ffias, C. Vidal-Valverde, Food Chem. 86 (2004) 537–545.
[76] C.A. Galindo, M. Sitges, Neurochem. Res. 29 (2004) 659–669.
[77] L.F. Hernandez, G. Segovia, F. Mora, Neurochem. Res. 28 (2003) 1819–1827.
[78] T. Kodama, Y.Y. Lai, J.M. Siegel, J. Neurosci. 23 (2003) 1548–1554.
[79] D. Dhossche, H. Applegate, A. Abraham, P. Maertens, L. Bland, A. Benecsath, J. Martinez, Med. Sci. Monit. 8 (2002) 1–R6.
[80] P.L. Pearl, K.M. Gibson, M.T. Acosta, L.G. Vezina, W.H. Theodore, M.A. Rogawski, E.J. Novotny, A. Gropman, J.A. Corny, G.T. Berry, M. Tuchman, Neurology 60 (2003) 1413–1417.
[81] Y. Takada, M. Yoshida, M. Sakairi, H. Koizumi, Rapid Commun. Mass Spectrom. 9 (1995) 895–896.
[82] C. Rocher, L. Bert, F. Robert, J.H. Trouvin, B. Renaud, C. Jacquot, A.M. Gardier, Brain Res. 737 (1996) 221–2130.
[83] O. Niwa, R. Kurita, T. Horiuchi, K. Tormitsu, Anal. Chem. 70 (1998) 89–93.
[84] G. Zhang, A.W. Bown, Phytochemistry 44 (1997) 1007–1009.
[85] D. Ma, J. Zhang, K. Sugahara, T. Ageta, K. Nakayama, H. Kodama, J. Chromatogr. B 726 (1999) 285–290.
[86] Y. Song, M. Shenwu, D.M. Dhossche, Y.M. Liu, J. Chromatogr. B 814 (2005) 295–302.