Quantitation of neurotransmitters and metabolites in microdialysate fluid using advanced gas chromatography-mass spectrometry (GC-MS)

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INTRODUCTION

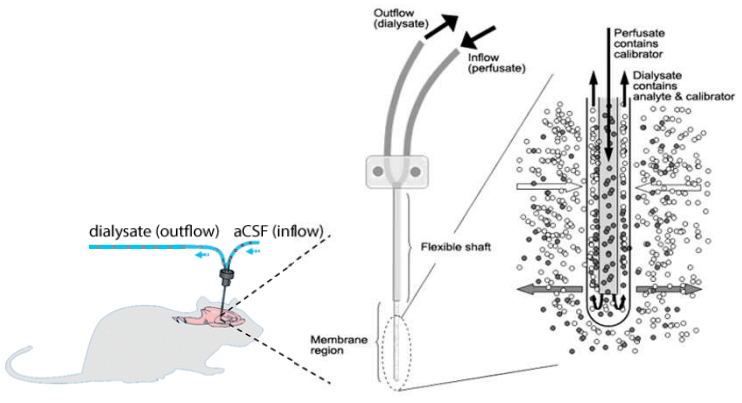
In vivo microdialysis is an invaluable technique for examining the dynamics of metabolic pathways in live animals, but the low sample volumes collected require sensitive and selective analytical detection methods. Accurate and reliable measurement of neurotransmitters and other metabolites in brain is important for examining changes in brain biochemistry and metabolism. Whilst liquid chromatraphy (LC) coupled with electrochemical and fluoresence detector are very sensitive, mass spectrometers (MS) provide enhanced selectivity and specificity as well as high sensitivity. GC-MS/MS with electron capture negative chemical ionisation (ECNCI) is a technique capable of extreme sensitivity, which can be a challenge to LC-MS/MS. Neurotransmitters and metabolites require a suitable derivatisation technique that converts them to volatile derivatives containing electronegative groups.

AIM To develop reliable analytical protocols for measurement of different neurotransmitters and metabolites in cerebral spinal fluid (CSF). The effect of potassium stimulation as a physiological control on extracellular levels of these compounds was also investigated.

METHODS

CSF sampling from Prefrontal Cortex (PFC) and Hippocampus

Microdialysis was setup according to Takeda et al [2]. Stereotaxic implantation of guide cannula in to prefrontal cortex or dorsal hippocampus (CA1) of male C57BL/6 mice (12 weeks old, n=6 per region) was performed under isoflurane anaesthesia. Guide cannula were fixed using anchoring bone screws and dental cement. A microdialysis probe (1 mm length, 15 kDa mol. mass cut off) was inserted after 7 days recovery from surgery, followed by perfusion with artificial CSF (aCSF -flow rate 1 µl/min) and sampling in *freely moving animals* 3h after probe insertion. For biological response experiments, aCSF was enriched with 50 nM K^+ following baseline sample collection.



CSF Sample Preparation

Samples were diluted 1:3 with (methanol:acetonitrile 1:1), centrifuged to remove protein and then derivatised after drying under nitrogen according to Eckstein et al [1]. Polar groups of analytes were converted to volatile fluoro-alkyl derivatives using pentafluoroacetic acid anhydride (PFAA) and pentafluoro-1-propanol PFOH). Excess reagents were evaporated under N_2 and reconstituted in toluene.

GC-MS/MS Analysis

Derivatised samples were analysed by an Agilent 7000B triple quadrupole mass selective detector interfaced with an Agilent 7890A gas chromatograph.
Separations were carried out on a fused silica capillary column (20 m x 0.18 mm i.d.) coated with cross-linked 5% phenylmethylsiloxane (film thickness 0.18 µm), (Restek). Helium was the carrier gas (0.8 ml/min. Selective reaction monitoring (SRM) was performed with argon as collision gas (0.6 ml/min) and reagent gas (1.25 ml/min) using ECNCI mode at 70eV with the ion source and quadrupoles maintained at 150°C. Quantitation was achieved by relating the peak area of the analyte with its corresponding heavy internal standard peak .
Statistical analysis was performed using student's t test after analysis of variance.

RESULTS

The derivatisation technique was successful for a wide class of neurotransmitters and metabolites, producing highly fluorinated derivatives suitable for ECNCI (Fig 1).

Fig 1. Mass Spectrum of glutamate derivative

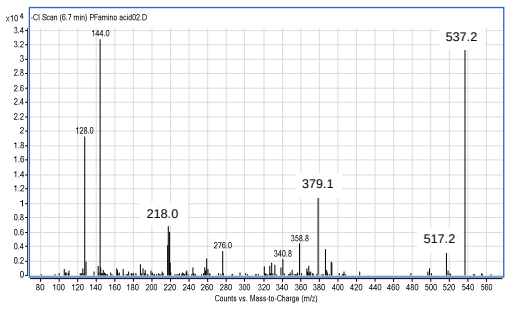
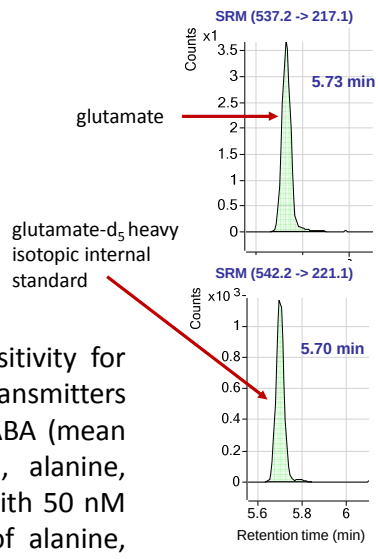
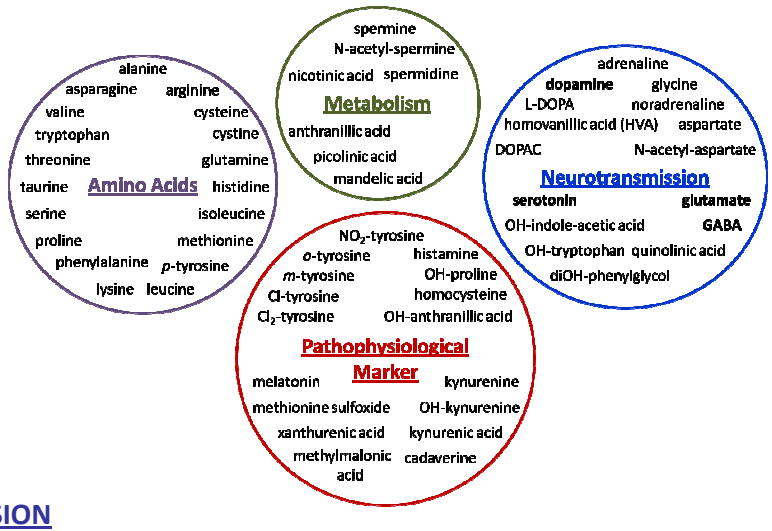


Fig 2. SRM chromatogram of glutamate (10ul control mouse CSF)



10 µl CSF dialysate provided sufficient sensitivity for accurate measurement of several neurotransmitters and several metabolites (Fig 2) including GABA (mean 40 nM ±6 SEM), glutamate (45 nM ±4), alanine, dopamine, glycine and serine. Stimulation with 50 nM K^+ induced a significant 2-3-fold increase of alanine, GABA and glycine ($p < 0.05$).

Fig 3. Example of the wide class of small molecules suitable for GC-MS/MS with ECNCI



DISCUSSION

This technique has recently been used to monitor the kynurenine pathway in rat CSF and brain tissue [3]. Further development of the method for reliable quantitation of additional analytes will establish a comprehensive technique for targeted analysis and profiling of several important neurotransmitter and metabolic pathways in brain (Fig 3). In addition, we are also targeting potential indicators of pathophysiology and oxidative damage to validate biomarkers of neurodegenerative disease that are urgently needed

Conclusion

This GC-MS/MS technique has several significant advantages including:

- Low sample volume required.
- High resolution power of GC.
- Selectivity and specificity of MS/MS detection.
- High sensitivity of ECNCI.

Therefore GC-MS/MS targeted analysis provides a sensitive tool for measuring a large range of neurotransmitters, metabolites and damage biomarkers during a single analysis in small aliquots of CSF. Small amounts of brain, plasma and other tissues can also be analysed. We are currently monitoring metabolic changes in animal models of disease and human post mortem tissue to examine mechanisms of neurodegeneration and identify potential biomarkers.

We are grateful to IHMRI and SRI, NSW for their financial support.

References: [**1]. Takeda, S. et al.** (2011) Novel microdialysis method to assess neuropeptides and large molecules in free-moving mouse." *Neurosci.* 186: 110–119. [**2]. Eckstein, J.A. et al.** (2008) Simultaneous profiling of multiple neurochemical pathways from a single cerebrospinal fluid sample using GC/MS/MS with electron capture detection. J. Mass Spec. 43, (6): 782-790. [**3]. Notarangelo, F.M. et al** (2012) Gas chromatography/tandem mass spectrometry detection of extracellular kynurenine and related metabolites in normal and lesioned rat brain . *Anal. Biochem*. 421,(2), : 573-581

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