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Rapid high-throughput assay for the measurement of amino acids from microdialysates and brain tissue using monolithic C_{18}-bonded reversed-phase columns

L.A. Dawson [b], A.J. Organ [a], P. Winter [b], L.P. Lacroix [c], C.S. Shilliam [b], C. Heidbreder [c], A.J. Shah [a,*]

[a] *CASS-Analytical Technologies GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b] *In Vivo Neurochemistry, Neuropharmacology, Psychiatry CEDD, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK*
[c] *Drug Dependency, Psychiatry CEDD, GlaxoSmithKline, Via A. Fleming 4, 37135 Verona, Italy*

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Abstract

A rapid precolumn high-performance liquid chromatography method based on fluorescence detection has been developed for the measurement of multiple amino acids from both ex vivo and in vivo biological samples using monolithic C_{18} columns. A mixture of 18 primary amino acids were derivatised with napthalene-2,3-dicarboxaldehyde (NDA) in the presence of cyanide. The resulting isoindole derivatives were resolved within 10 min using a linear binary gradient elution profile with Rs values in the range 1.2–9.0. The limit of detection (LOD) was found to be between 6.0 and 60 fmol for 5 μl injection with a signal to noise ratio of 3:1. The NDA derivatives were found to be stable for 9 h at 4 °C. This assay has been employed for the rapid analysis of amino acids from brain tissue and microdialysis samples. Examples of application of the method are given.

Keywords: Microdialysis; Derivatisation, LC; Monolithic columns; Amino acids; Neurotransmitters

1. Introduction

Amino acids are known to serve important roles in the central nervous system (CNS). Aspartate (Asp) and glutamate (Glu) are the major excitatory neurotransmitters whereas γ-aminobutyric acid (GABA) acts as the main inhibitory transmitter within the mammalian CNS. Similarly, glycine (Gly) has also been shown to be a neurotransmitter acting as a co-agonist at the *N*-methyl-D-aspartate (NMDA) receptor [1]. Many of these neurotransmitters are of particular interest as they have been implicated in neurodegenerative and neuropsychiatric disorders such as Alzheimers disease [2–5], schizophrenia [6], Down's syndrom [7] and Parkinson's disease [8]. In addition, many of the non-neurotransmitter amino acids such as alanine (Ala), glutamine (Gln), histidine (Hist), lysine (Lys) and serine (Ser) have also been shown to be altered in the brains of patients with disorders such as Huntington's chorea and Parkinson's disease [9]. Thus, measurement of amino acids in samples derived from many neuropsychiatric disorders may provide valuable mechanistic insight into disease cause and progression as well as possibly providing a diagnostic tool (for a recent review, see [10]).

Many of these disease states can be modelled in preclinical species and measurement of amino acids from tissue or samples generated from techniques such as in vivo microdialysis can provide insight into pathological changes that may be mediating disease onset and progression. Furthermore, changes in amino acids can be used to monitor the effects of pharmacotherapies and thus provide insight into the possible mechanism of action of these treatments.

Techniques such as in vivo microdialysis, which allow the sampling of analytes from the extracellular fluid of tissue via the means of a hollow fibre dialysis membrane [11] have proved very useful in elucidating disease mechanisms

* Corresponding author. Tel.: +44-1279-627457; fax: +44-1272-627354.
E-mail address: ajit_j_shah@gsk.com (A.J. Shah).

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and as a tool for the diagnosis of neuropsychiatric disorders. However, the concentration of analytes in microdialysis samples can be very low compared to gross tissue levels, hence, highly sensitive analytical techniques are required for their measurement.

A range of precolumn HPLC [12–16] and CZE [17–22] based assays have been reported for the measurement of amino acids in brain tissue and microdialysis samples. The majority of these are developed for the measurement of the neuroactive amino acids. However, measurement of the extracellular brain level of other amino acids including their precursor and by-products may provide additional insight into neurodegenerative diseases and the evaluation of drugs designed to treat them.

The goal of the present work was to develop a rapid sensitive fluorescent assay for the measurement of amino acids in brain tissue and microdialysates. Several approaches have been adopted to increase throughput in chromatography and these include rapid gradient separation [23], parallel analysis [24] and separations at high flow-rates [25]. Although parallel analysis has been used successfully to reduce analysis time, the cost of implementing this type of system is significantly higher than for a conventional HPLC system. An alternative and simpler approach is to carry out separations at high flow rates with short particulate columns. This approach has been used successfully; however, the separation efficiency of these columns may not be adequate for measurement of analytes at trace levels in biological matrices. Also, the flow rates that are used with these rapid resolution columns result in high backpressure that is not suitable for robust analytical methods.

One approach that has been used to overcome this issue is to use monolithic columns [26,27]. The porosity of these columns is higher than that of particulate columns [28]. Thus, monolithic columns can be used at high flow rates at backpressures that are significantly lower than those observed with particulate columns. Furthermore, it has been shown that the effective plate height is only marginally reduced as the flow rate is increased [26]. The present work describes the development and application of a rapid precolumn fluorescent assay using a monolithic column for the measurement of amino acid levels from both brain tissue and in vivo microdialysis samples.

2. Experimental

2.1. Reagents

High purity amino acid calibration standard containing a mixture of primary amino acids each at a concentration of 2.5 mM was purchased from Perbio (Tattenhall, UK). Citrulline, glutamine, γ-aminobuytric acid, taurine and sodium cyanide were obtained from Sigma–Aldrich (Poole, UK). Glacial acetic acid of Hipersolv grade and orthoboric acid of Aristar grade were purchased from BDH (Poole, UK). Calcium chloride dihydrate, magnesium chloride hexahydrate, potassium chloride, sodium chloride and sodium dihydrogen orthophosphate of AnalaR grade that were used to prepare artificial cerebrospinal (aCSF) fluid were also from BDH. Acetonitrile 190 far UV and methanol 205 both of gradient quality were obtained from Romil (Cambridge, UK). Ammonium acetate of HPLC grade was purchased from Fisher Chemicals (Loughborough, UK). Napthalene-2,3-dicarboxaldehyde (NDA) was from Tocris (Avonmouth, UK). All aqueous solutions were prepared using deionised water from an Elga maxima system (Elga, High Wycomb, UK).

2.2. HPLC instrumentation and conditions

A Waters 2690 HPLC system (Waters, Elstree, UK) composed of a quaternary gradient pumping system, vacuum degasser, an autosampler and a thermostated column oven was used. Eluates were detected spectrofluorometrically using a Waters 474 fluorescence detector equipped with a 16 μl flow-cell. The excitation and emission wavelengths were set at 420 and 480 nm, respectively. Separations were carried out using a 100 × 4.6 mm i.d. and a 50 × 4.6 mm i.d. Chromolith SpeedRod RP 18e columns (Merck KGaA, Darmstadt, Germany) coupled together using a female type column coupler (Thames Restek, Windsor, UK). Isoindole derivatives of amino acids were separated using a ternary gradient elution profile (Table 1) composed of 50 mM ammonium acetate (pH 5.5), acetonitrile and methanol. The column was thermostated to 30 °C.

2.3. Preparation of derivatising reagents

A 3 mM solution of naphthalene-2,3-dicarboxaldehyde was prepared in methanol. Sodium cyanide (4.9 mg) was dissolved in 1.0 ml of deionised water to give a 10 mM solution. Both reagents were stored at 4 °C when not in use. The reagents were used for 1 week and then discarded.

2.4. Preparation of amino acid standards

A standard stock solution of amino acid was prepared by mixing 10 μl of high purity amino acid calibration standard with an equal volume of citrulline, glutamine, GABA and taurine each at a concentration of 2.5 mM. Standards for calibrations used for in vivo and brain tissue studies were

Table 1
Elution profile used for the separation of isoindole derivatives of amino acids

Time (min)	'A' NH_4Ac (%)	'B' MeOH (%)	'C' MeCN (%)	Flow (ml/min)	Gradient
0	85	10	5	2.5	
10	40	20	40	2.5	Linear
10.1	85	10	5	2.5	Linear
12	85	10	5	4.0	

prepared by diluting this solution with 1 ml of a mixture of aCSF and acetic acid (5:1, v/v) or homogenising buffer, respectively to give a 25 μM solution. The stock solution was stored at 4 °C for up to 1 month and then discarded. Working standards were prepared by diluting the stock solution with a mixture of aCSF and acetic acid (5:1, v/v).

2.5. Derivatisation protocol

Samples were derivatised using a Gilson 231 XL autosampler (Anachem, Luton, UK). For microdialysates, the autosampler was programmed to add sequentially 16 μl of borate buffer (0.1 M, pH 9.2), 4 μl aqueous sodium cyanide and 16 μl of NDA to the sample. A similar programme was used for derivatisation of brain tissue samples except that 90 μl of 0.3 M borate buffer (pH 9.2) was used. The programmes used for derivatisation included steps to mix the sample and reagents and to rinse the syringe between each step with a mixture of acetonitrile and water (50:50, v/v). The derivatised samples were incubated at room temperature for 15 min and then transferred to the Waters 2690 HPLC system. An aliquot (5 μl) was loaded onto the Chromolith SpeedRod columns.

2.6. Microdialysis

2.6.1. Subjects

Male Sprague–Dawley rats (250–300 g; Charles River, UK) were housed in groups of six per cage in a temperature- and humidity-controlled environment with free access to food (restricted to 20 g per day after surgery) and water. Rats were kept on a 12 h light–dark cycle with lights on at 07:00 h. All experimental procedures carried out in the present study were within the guidelines of the Animals (Scientific Procedures) Act 1986.

2.6.2. Surgical procedures

Rats were anaesthetised using medetomidine (0.4 mg/kg s.c.) and fentanyl (450 μg/kg i.p.). Once deep anaesthesia was attained, rats were put in a stereotaxic frame (David Kopf, Topanga, CA, USA). An incision was made to expose the skull. Holes were then drilled for four anchor screws, and another for implantation of an intracerebral guide cannula (CMA 11, Biotech, UK) into the dorsal hippocampus (AP −4.3 mm, ML −2.6 mm, DV −1.7 mm [29]). The guide cannula was implanted and secured using dental cement and the wound sealed. Anaesthesia was reversed using atipamezole (1 mg/kg s.c.) and nalbuphine (2 mg/kg s.c.). The animals were allowed to recover for 1 week before the microdialysis experiment started. On the afternoon prior to microdialysis experiments rats were placed in microdialysis cages to allow habituation to the environment.

2.6.3. Brain microdialysis procedure

On the day of experiments rats were lightly anaesthetised with isofluorane and microdialysis probes (CMA/11, 2 mm active cuprophane membrane, Biotech, UK) were inserted into the guide cannula. Probes were perfused at a flow rate of 1 μl/min with artificial cerebrospinal fluid (aCSF) comprising of 125 mM NaCl, 2.5 mM KCl, 1.18 mM $MgCl_2{\cdot}6H_2O$, 1.26 mM $CaCl_2{\cdot}2H_2O$, and 2.0 mM Na_2HPO_4, adjusted to pH 7.4 with H_3PO_4. Microdialysates were collected every 30 min into vials housed on a cooled autosampler for the whole of the experimental period. Following a two hour equilibration period, three baseline samples were collected prior to compound administration. The Glycine Transporter 1 inhibitor ALX-5407 [30] or vehicle was administered intraperitoneally (i.p.) at 10 mg/kg following baseline sampling; microdialysates were collected for a further 4 h post-administration.

2.7. Ex vivo neurochemistry

2.7.1. Transgenic animals

Wild-type and transgenic mice were prepared and handled as per the methods of Harrison et al. [31].

2.7.2. Preparation of brain tissue samples

Brains were extracted from both transgenic and wild type mice and were frozen and stored at −80 °C until assay. Brain tissue samples were dissected from the dorsal striatum, nucleus accumbens, hippocampus, hypothalamus, cerebellum and frontal cortex (both left and right hemispheres) and snap frozen. Each sample was homogenised in a volume (10 μl/mg wet weight tissue) of homogenising buffer (0.1%, w/v Na metabisulphite; 0.01%, w/v EDTA; 0.1%, w/v L-cysteine; 0.4 M perchloric acid) using a Gallenkamp Soniprep 150 sonicator. A volume of the resultant slurry was centrifuged using a Labofuge 400R (Heraeus Instruments, Langenselbold, Germany) at 5590 × *g* for 10 min at 4 °C. A small volume (16 μl) of the resultant supernatant was treated with NDA and the derivatised sample was analysed using HPLC.

3. Results and discussion

3.1. Derivatisation reaction and conditions

Primary amines react with naphthalene-2,3-dicarboxaldehyde-cyanide at ambient temperature to give the *N*-substituted-1-cyanobenz[f]isoindole (CBI) derivative [32]. The reagent is a modification of *ortho*-phthalaldehyde (OPA) which has been used extensively for amino acid analysis [33]. In comparison to the products formed with OPA derivatisation the CBI derivative formed with NDA are more stable and the optimum excitation and emission maxima are in the visible region of the spectrum [32]. The derivatisation conditions that were used in this work have been reported previously [16].

3.2. Optimisation of HPLC conditions

Experiments were carried out to investigate the effect of column length, flow rate, initial and final level of solvents and buffer pH on resolution of CBI derivative of amino acids. A study was carried out to investigate the initial and final levels of acetonitrile and methanol and pH of ammonium acetate buffer on resolution of CBI derivative of amino acids. A 100 × 4.6 mm i.d. Chromolith column and a gradient time of 10 min was used throughout the initial optimisation study. The study was carried out using a standard mixture of 18 amino acids each at a concentration of 2.5 μM. The effect of

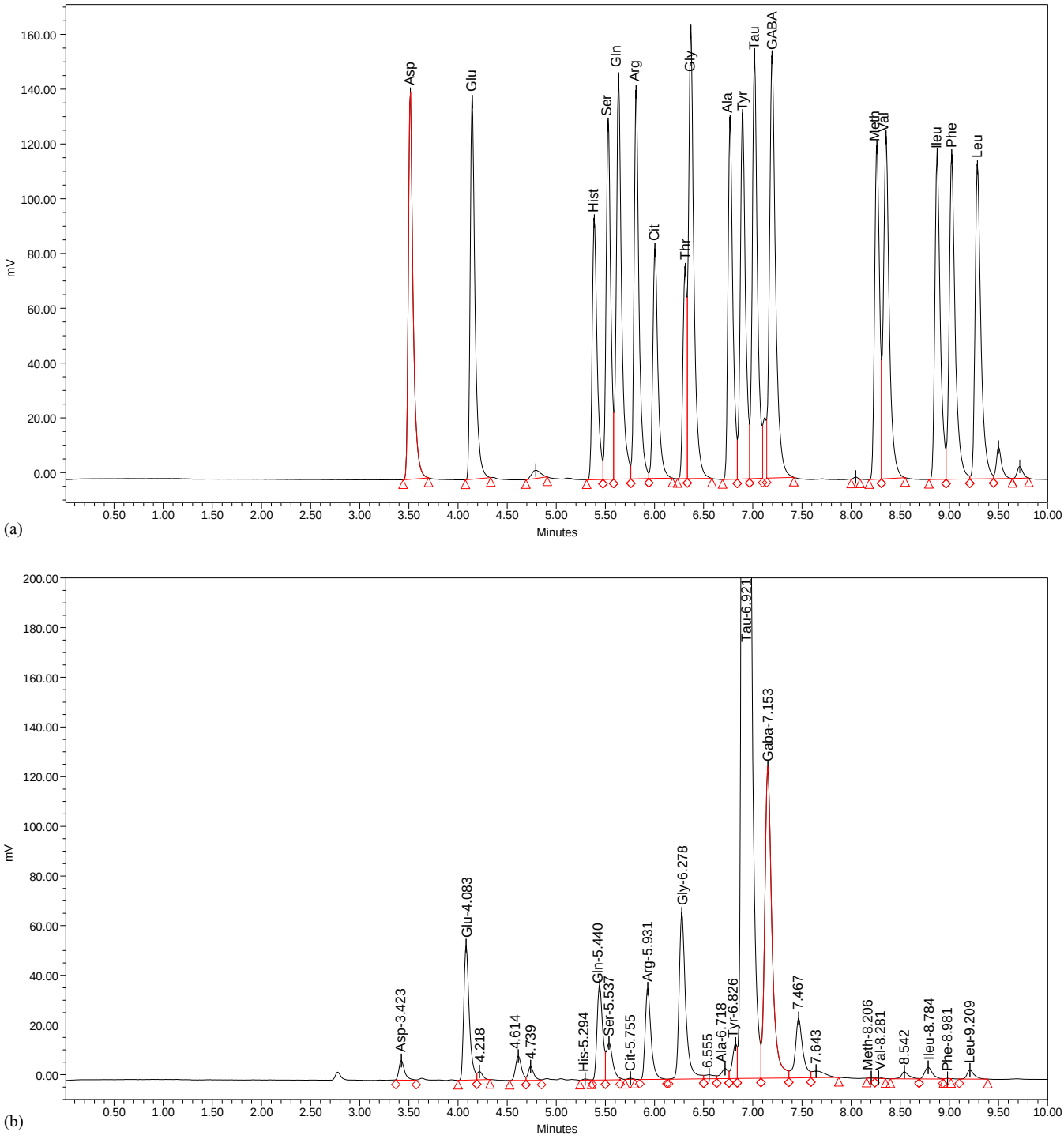

Fig. 1. (a) Chromatogram of mixture of amino acids each at a concentration of 8 μM in borate buffer and (b) frontal cortex tissue sample treated with NDA-CN. The samples were derivatised using the procedure described in Section 2. An aliquot (5 μl) of derivatised sample was loaded onto a 50 × 4.6 mm i.d. Chromolith SpeedRod RP 18e column connected in series to a 100 × 4.6 mm i.d. column containing the same stationary phase.

pH on resolution was investigated using 50 mM ammonium acetate buffer in pH range 4.0–5.5 with fixed proportions of initial and final methanol and acetonitrile. A decrease in k' with an increase in pH of elution buffer in the range investigated was observed for all CBI derivatives. The resolution of the majority of the amino acid derivatives was found to increase with an increase in pH of the separation buffer. A 50 mM ammonium acetate buffer of pH 5.5 was found to give adequate separation of all CBI derivatives. A series of runs was carried out using this buffer with different proportions of initial and final acetonitrile and methanol in 10 min linear gradient elution profiles. It was found that using a mixture of ammonium acetate buffer, acetonitrile and methanol from 85:5:10 (%, v/v) and ramping to 40:40:20 (%, v/v) at a flow rate of 2.0 ml/min gave good resolution of majority of amino acids. Furthermore, it was found that in the absence of methanol and at the same elutropic strength the resolution of pairs CBI–alanine–tyrosine, CBI–tyrosine–taurine, CBI–arginine–glutamine and CBI–methionine–valine deteriorated significantly. A further improvement in resolution of CBI derivatives was obtained by coupling a 10 and 5 cm Chromolith column. This led to an increase in resolution and separation time. The maximum k'-value increased from 3.8 to 5.6 and partial resolution of the critical pair threonine/glycine was obtained. To reduce the analysis time the flow rate was increased from 2.0 to 2.5 ml/min. This resulted in a small decrease in resolution. Fig. 1 shows chromatograms of mixture of standard amino acid and a frontal cortex tissue sample treated with NDA and separated using the optimal conditions.

3.3. Linearity, precision and detection limits

Studies of HPLC-peak area as a function of concentration of amino acid were carried out using standard mixtures of amino acids. For eight concentrations of amino acids in the range 0–5 μM linear correlations were obtained with coefficients in the range 0.9597–0.9708. The repeatability of the precolumn assay was measured using five samples of 500 nM standard mixture of amino acids. The samples were derivatised and three repeat injections were carried out from each sample. The coefficient of variation of HPLC-peak area of NDA derivatised amino acids were found to be between 1.0 and 6.4%. Consecutive chromatograms from this study showed that the retention times for the derivatives had coefficient of variation of 0.1–3.0%.

Detection limits of 0.01–1 μM (6–600 fmol on column) were obtained with signal-to-noise ratio of 3:1 (Table 2). Fig. 1 shows that the HPLC-peak height of amino acids at this concentration is relatively similar from histidine to leucine. This can be attributed to similar quantum efficiencies of NDA-amino acid derivatives and relatively similar dilution of analytes that is observed with rapid gradient separation. However, the limit of detection (LOD) of each analyte was found to be different and this was dependent on it's background level in aCSF, brain homogenising solution, derivatisation reagents and glass ware.

Table 2
Detection limits for amino acids with signal-to-noise ratio of 3 where noise is measured peak-to-peak

Amino acid	Detection limit (nM)
Asp	90
Glu	90
Hist	30
Ser	3
Arg	30
Gln	90
Cit	30
Gly	300
Ala	61
Tyr	90
Tau	30
GABA	3
Met	30
Val	3
Ile	30
Phe	30
Leu	30

3.4. Effects of the GlyT1 reuptake inhibitor ALX-5407 (10 mg/kg i.p.) on extracellular levels of glycine and glutamate

Basal levels of amino acids from the rat dorsal hippocampus were Gly 1.11±0.03 μM and Glu 5.142±0.84 μM. Administration of the selective glycine transporter 1 inhibitor ALX-5407 produced a significant increase in extracellular levels of glycine over time as revealed by the interaction of treatment × time ($F_{[10,90]} = 2.61$; $P < 0.01$) with a maximum peak level of 164 ± 12.48% of preinjection values ($t = 60$ min post-administration; Fig. 2) and a significant

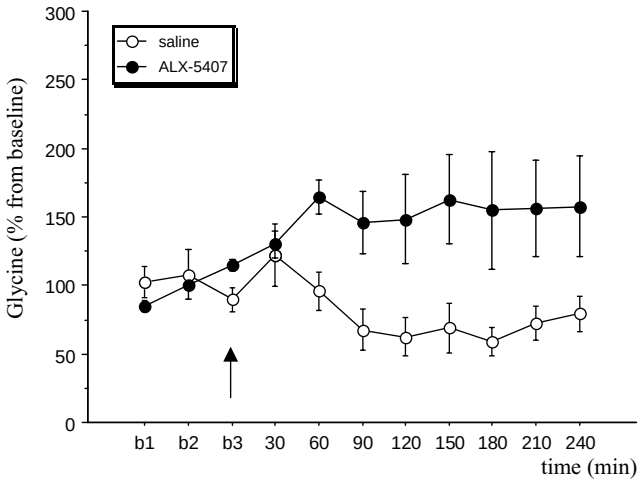

Fig. 2. Effects of ALX-5407 (10 mg/kg i.p.) on extracellular levels of glycine in the dorsal hippocampus of the freely moving rat. Data represent time course after treatment expressed as mean ± S.E.M. ($n = 5$–6 per study group). The arrow indicates the time at which the treatment was administered.

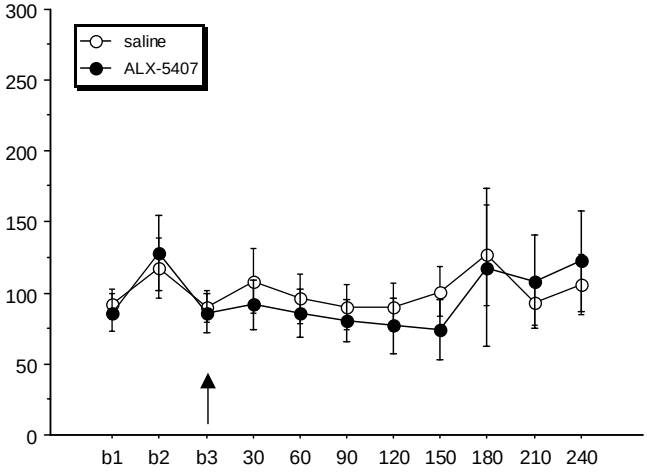

Fig. 3. Effects of ALX-5407 (10 mg/kg i.p.) on extracellular levels of glutamate in the dorsal hippocampus of the freely moving rat. Data represent time course after treatment and area under the curve expressed as mean ± S.E.M. (n = 5–6 per study group). The arrow indicates the time at which the treatment was administered.

increase in total Gly ($F_{[1,9]} = 8.77$; $P < 0.05$). This effect was selective for Gly as no change was observed in extracellular levels of Glu (Fig. 3). These data demonstrate that both Gly and Glu can be measured from in vivo microdialysis samples and provide in vivo data in support of previous in-vitro evidence showing that GlyT1 inhibitors can increase extracellular glycine levels in the hippocampus [30]. Moreover, it would appear that this increase is relatively selective for Gly since other amino acid neurotransmitters measured were not effected by treatment with ALX-5407.

3.5. Neurochemical evaluation of brain tissue levels of amino acids from the novel transgenic lpa_1 mouse

Lpa_1 is a Gi-coupled seven transmembrane receptor with high affinity for the ligand lysophosphatidic acid. A detailed neurological and behavioural analysis of mice homozygous for a targeted deletion at the lpa_1 locus ($lpa1^{(-/-)}$) has recently been reported and demonstrates that the lpa_1 mutation leads to a range of defects resembling those found in schizophrenia. These include craniofacial dysmorphology and a marked deficit in prepulse inhibition (see Harrison et al. [31] for a full characterisation). Since many neurotransmitters, including a number of amino acids, have been implicated in the pathophysiology of schizophrenia we have used the current methodology to examine this novel transgenic animal.

Tissue amino acid levels were analysed from a range of specific brain regions (i.e. dorsal striatum, nucleus accumbens, hippocampus, hypothalamus, cerebellum and frontal cortex) from both wild type (age matched litter controls) and lpa_1 knock out mice [34]. No overt comparative changes were observed in the majority of brain regions examined (data not shown). However, the frontal cortex and hippocampus showed marked deficits in the levels of a number of amino acids examined (Table 3). More specifically, significant differences were seen with arginine ($F_{1,10} = 13.884$; $P = 0.0039$), aspartate ($F_{1,10} = 6.691$; $P = 0.0271$), GABA ($F_{1,10} = 6.039$; $P = 0.0338$), glutamine ($F_{1,10} = 12.019$; $P = 0.0061$), leucine ($F_{1,10} = 4.823$; $P = 0.05$), phenylalanine ($F_{1,10} = 9.386$; $P = 0.012$), taurine ($F_{1,10} = 11.062$; $P = 0.0077$), threonine ($F_{1,10} = 6.096$; $P = 0.0332$), and tyrosine ($F_{1,10} = 9.722$; $P = 0.0109$) (Table 3). This could suggest some level of cellular atrophy in this region although no marked pathology has been observed. A full characterisation of this novel transgenic is underway. However, these data along with the microdialysis demonstrate the utility of this analytical technique for the rapid and high sensitivity measurement of a large range of both metabolic and releasable neurotransmitter amino acids from a variety of biological samples.

Table 3
Ex vivo neurochemical phenotyping of *lpa1* knockout (KO) mice compared with their wild-type (WT) litter mates

Amino acid	FC (KO)	FC (WT)	Hipp (KO)	Hipp (WT)
Brain region				
Arg	53.296 ± 1.655	67.118 ± 6.090	**1.574 ± 0.249** b ↓	3.916 ± 0.577
Asp	**11.406 ± 2.755** b ↓	21.686 ± 1.675	**0.188 ± 0.015** a ↓	0.288 ± 0.035
GABA	30.641 ± 1.438	32.636 ± 1.862	**1.203 ± 0.149** a ↓	2.256 ± 0.402
Gln	46.665 ± 2.403	52.245 ± 4.846	**1.586 ± 0.180** b ↓	3.012 ± 0.370
Glu	124.09 ± 6.518	139.25 ± 8.294	2.946 ± 0.190	3.430 ± 0.180
Leu	2.446 ± 0.342	3.254 ± 0.347	**1.003 ± 0.153** a ↑	0.561 ± 0.131
Meth	0.283 ± 0.021	0.444 ± 0.068	0.051 ± 0.005	0.058 ± 0.004
Phen	2.972 ± 0.391	4.167 ± 0.391	**0.075 ± 0.009** b ↓	0.178 ± 0.032
Ser	**3.225 ± 0.167** a ↓	3.810 ± 0.168	0.099 ± 0.011	0.137 ± 0.016
Tau	308.56 ± 13.295	314.27 ± 18.588	**8.982 ± 1.082** b ↓	21.820 ± 3.705
Thr	13.023 ± 0.510	14.888 ± 0.971	**0.383 ± 0.052** a ↓	0.725 ± 0.128
Tyr	11.528 ± 0.364	13.466 ± 1.228	**0.518 ± 0.067** b ↓	0.985 ± 0.134

Neurochemical indices were assessed in multiple brain regions. Significant effects were selective to the frontal cortex (FC) and hippocampal (Hipp) regions (data presented here). Data are mean ± S.E.M. (n = 6 per study group). (a) $P < 0.05$, (b) $P < 0.010$, (c) $P < 0.001$ denotes significant changes in knockout vs. wild-type mice.

4. Conclusions

A rapid HPLC assay has been developed for the measurement of amino acids from both brain microdialysates and brain tissue samples. The limits of detection achieved are suitable for the routine measurement of low levels of amino acids from either brain tissue or in vivo microdialysate samples from the brain.

References

[1] J.W. Johnson, P. Ascher, Nature 325 (1987) 529.
[2] G.E. Fagg, A.C. Foster, Neuroscience 9 (1983) 701.
[3] M.N. Rossor, N.J. Garrett, A.L. Johnson, C.Q. Mountjoy, M. Roth, L.L. Iversen, Brain 105 (1982) 313.
[4] 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.
[5] A.W. Procter, A.M. Palmer, P.T. Francis, S.L. Lowe, D. Neary, E. Murphy, R. Doshi, D.M. Bowen, J. Neurochem. 50 (1988) 790.
[6] G. Tsai, L.A. Passani, B.S. Slusher, Arch. Gen. Psychiatry 52 (1995) 829.
[7] G.P. Reynolds, C.E.J. Warner, Neurosci. Lett. 94 (1988) 224.
[8] J.O. Rinne, T. Halonen, P.J. Riekkinen, U.K. Rinne, Neurosci. Lett. 94 (1988) 182.
[9] F. Fonnum, J. Neurochem. 42 (1984) 1.
[10] A.J. Shah, F. Crespi, C.A. Heidbreder, J. Chromatogr. B 781 (2002) 151.
[11] Y.L. Hurd, J. Kehr, U. Ungerstedt, J. Neurochem. 51 (1988) 1314.
[12] J.Y. Chang, P. Martin, R. Bernasconi, D.G. Braun, FEBS Lett. 132 (1981) 117.
[13] K.L. Rogers, R.A. Philibert, A.J. Allen, J. Molitor, E.J. Wilson, G.R. Dutton, J. Neurosci. Methods 22 (1987) 173.
[14] H. Liu, M.C. Sandua-Pena, J.D. Harvey-White, S. Kalra, S.A. Cohen, J. Chromatogr. A 828 (1–2) (1998) 383.
[15] P. Lindroth, K. Mopper, Anal. Chem. 51 (1979) 1667.
[16] A.J. Shah, V. de Biasi, S.G. Taylor, C. Roberts, P. Hemmati, R. Munton, A. West, C. Routledge, P. Camilleri, J. Chromatogr. B 735 (1999) 133.
[17] P.L. Weber, T.J. O'Shea, S.M. Lunte, J. Pharm. Biomed. Anal. 12 (3) (1994) 319.
[18] F. Robert, L. Bert, L. Lambás-Seòas, L. Denoroy, B. Renaud, J. Neurosci. Methods 70 (2) (1996) 153.
[19] L. Bert, F. Robert, L. Denoroy, L. Stoppini, B. Renaud, J. Chromatogr. A 755 (1) (1996) 99.
[20] L.A. Dawson, J.M. Stow, C.T. Dourish, C. Routledge, J. Chromatogr. A 700 (1995) 81.
[21] L.A. Dawson, J.M. Stow, A.M. Palmer, J. Chromatogr. B 694 (1997) 455.
[22] S.Y. Zhou, H. Zuo, J.F. Stobaugh, C.E. Lunte, S.M. Lunte, Anal. Chem. 67 (3) (1995) 594.
[23] T.P. Piepponen, A. Skujins, J. Chromatogr. B 757 (2) 277.
[24] W.A. Korfmacher, J. Veals, K. Dunn-Meynell, X. Zhang, G. Tucker, K.A. Cox, C.C. Lin, Rapid Commun. Mass Spectrom. 13 (20) (1999) 1991.
[25] R.T. Philip, L.A. Romanyshyn, Rapid Commun. Mass Spectrom. 16 (12) (2002) 1225.
[26] J.T. Wu, H. Zeng, Y. Deng, S.E. Unger, Rapid Commun. Mass Spectrom. 15 (13) (2001) 1113.
[27] F. Rabel, K. Cabrera, D. Lubda, Am. Lab. 32 (24) (2000) 20.
[28] B. Bidlingmaier, K.K. Unger, N. Von Doehren, J. Chromatogr. A 832 (1999) 11.
[29] G. Paxinos, C. Watson, in: The Rat Brain Stereotaxic Coordinates, second ed., Plenum Press, New York, 1986.
[30] B.N. Atkinson, S.C. Bell, M. De Vivo, L.R. Kowalski, S.M. Lechner, V.I. Ognyanov, C.S. Tham, C. Tsai, J. Jia, D. Ashton, M.A. Klitenick, Mol. Pharmocol. 60 (6) (2001) 1414.
[31] S.M. Harrison, C. Reavill, G. Brown, J. Brown, J.E. Cluderay, B. Crook, C. Davies, L.A. Dawson, E. Grau, C. Heidbreder, P. Hemmati, G. Hervieu, A. Howarth, Z.A. Hughes, A.J. Hunter, J. Latcham, S. Pickering, P. Pugh, D.C. Rogers, C.S. Shilliam, P.R. Maycox, Mol Cell Neurosci. 24 (2003) 1170.
[32] P. De Montigny, J.F. Stobaugh, R.S. Givens, R.G. Carlson, K. Srinivasachar, L.A. Sternson, T. Higuchi, Anal. Chem. 59 (8) (1987) 1096.
[33] P. Lindroth, K. Mopper, Anal. Chem. 51 (11) (1979) 1667.
[34] R. Bergeron, T.M. Meyer, J.T. Coyle, R.W. Greene, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 15730.