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Differential regional *N*-acetylaspartate deficits in postmortem brain in schizophrenia, bipolar disorder and major depressive disorder

Lindsay M. Reynolds, Gavin P. Reynolds*

Department of Psychiatry, Queen's University Belfast, Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL, UK

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ABSTRACT

There is substantial evidence for the involvement of the hippocampus and subcortical regions in the neuropathology of schizophrenia. Deficits of *N*-acetylaspartate (NAA) have been found in schizophrenia and bipolar disorder which may reflect neuronal loss and/or dysfunction. *N*-acetylaspartylglutamate (NAAG) is the most abundant peptide transmitter in the mammalian nervous system. It is an agonist at presynaptic metabotropic glutamate receptors mGluR3, inhibiting glutamate release. NAA and NAAG and were measured in hippocampal, striatal, amygdala and cingulate gyrus regions of human postmortem tissue from controls and subjects with schizophrenia, bipolar disorder and major depressive disorder. There are significant deficits in hippocampal NAA concentrations in all patient groups. In the amygdala there are significant NAA deficits in schizophrenia and depression and significant deficits of NAAG in the amygdala in the depression group. The deficits in NAA reported in this study confirm the importance of hippocampal and other subcortical structures in the neuropathology of the major psychiatric disorders.

1. Introduction

There is substantial evidence for the involvement of the hippocampus and subcortical regions in the neuropathology of schizophrenia. There are volumetric reductions in these regions and both neurones and glia are affected (Harrison, 2008).

As it is a non-invasive neurochemical technique, magnetic resonance spectroscopy (MRS) has become an important tool in monitoring disease progression and in therapy evaluation in patients with neurodegenerative and psychiatric disorders. A major signal using this technique is from NAA. NAA, an amino acid present in high concentrations in the CNS, is synthesised in neuronal mitochondria from acetyl-coenzyme A and aspartate by the enzyme NAA transferase. It is considered a neuron-specific metabolite (Moffett et al., 1991) and its reduction a marker of neuronal loss. In addition, a study demonstrating the reversibility of NAA deficits following acute brain injury indicates that NAA may be a marker of both neuronal integrity and function (De Stefano et al., 1995). Although the biological function of NAA is not fully understood it has been shown to reflect glutamate concentrations and thus may provide a marker of glutamatergic neuronal function (Petroff et al., 2002).

In schizophrenia, frontal lobe NAA deficits have been shown to correlate with psychopathology and it may therefore be useful as an indicator of disease severity (Sigmundsson et al., 2003). Of particular interest are the numerous MRS studies that measure brain NAA in discrete brain regions in psychiatric patients. These have provided many reports of changes of NAA concentrations in schizophrenia, some in bipolar disorder and a few in depressions.

Although earlier work proved quite varied, probably due to the wide range of sampling techniques used and the variability in voxel size, recent findings have been more consistent. A meta-analysis of MRS studies on schizophrenia has reported there is a consensus that NAA is reduced by 5% in the hippocampus and in the frontal lobe noting however that most work is inadequately powered to detect deficits of 10% (Steen et al., 2005). A study of the precision and variability of MRS measurements of NAA in the cingulate gyrus and hippocampus using schizophrenic and control subjects, suggests that more than 200 subjects would be needed to detect a 5% difference between patients and controls (Venkatraman et al., 2006). A systematic review of NAA estimated by MRS in bipolar disorder shows reductions in the prefrontal and cingulate cortex, hippocampus and basal ganglia (Yildiz-Yesiloglu and Ankerst, 2006). A relationship between NAA in the dorsolateral prefrontal cortex and depression has been reported (Grachev et al., 2003).

N-acetylaspartylglutamate (NAAG) is the most abundant peptide transmitter in the mammalian nervous system. It is an agonist at presynaptic metabotropic glutamate receptors mGluR3 thus inhibiting glutamate release and may therefore be important

* Corresponding author. Tel.: +44 2890972631; fax: +44 2890975870.
E-mail address: g.reynolds@qub.ac.uk (G.P. Reynolds).

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in the regulation of NMDA receptors (Moghaddam and Adams, 1998). Inhibition of NAAG degrading enzymes are associated with a reduction of schizophrenia-like symptoms in animal models (Olszewski et al., 2008). Despite the potential relevance of this peptide it is not known whether NAAG concentrations in the brains of psychiatric patients are altered; MRS rarely differentiates NAA from NAAG. This postmortem study measuring the two compounds separately allows us to determine whether NAAG changes are apparent in psychiatric illness.

Three postmortem studies of brain tissue have estimated NAA and NAAG. Omori et al. (1997) examined thalamus from schizophrenic subjects by in vitro MRS and found NAA deficits, relative to creatine, although they were not statistically significant; Tsai et al. (1995) determined NAA and NAAG in postmortem tissue from schizophrenic subjects. They reported increased NAAG concentrations and both decreased activity of the enzyme cleaving NAAG, *N*-acetyl-alpha-linked acidic dipeptidase (NAALADase) and decreased glutamate concentrations in the hippocampus. Lastly, previous postmortem work from this laboratory examining frontal and temporal cortex tissue found losses of NAA in temporal cortex from schizophrenic and bipolar subjects and of NAAG in temporal cortex from schizophrenic subjects (Nudmamud et al., 2003). The present study extends this work by looking at hippocampal and subcortical structures employing an improved technique of taking tissue from frozen sections. This enables us to use small quantities of tissue and provides precise dissections.

2. Methods

Brain tissue, as frozen sections, from patients with schizophrenia, bipolar disorder and depression and from controls were provided by the Stanley Foundation Neuropathology Consortium and stored at −70 °C. Demographic details are provided in Table 1. This series of 60 matched and well-characterised subjects (15 per group) has been fully described by Torrey et al. (2000). There were no significant differences between groups with regard to age, sex, postmortem delay, brain pH, brain weight or storage time.

Frozen brain sections (10 μm) were rapidly thawed. Two sections were combined for each analysis. The relevant regions of grey matter were scraped off the glass slide with a razor blade and collected in a plastic tube. The sections were provided in the region groups striatum, hippocampus and amygdala. The striatal sections were subdissected; caudate nucleus, putamen and nucleus accumbens were identified and scraped off separately. The hippocampus sections provided hippocampus and parahippocampal gyrus samples. The amygdala tissue was identified by reference to a dissection guide (Heimer, 1983).

Table 1
Demographic and clinical information of schizophrenic, bipolar disorder, major depression, and control groups.

	Group			
	Control	Schizophrenia	Bipolar disorder	Major depression
Demographic variable	$n \pm 15$	$n \pm 15$	$n \pm 15$	$n \pm 15$
Age (*y*, mean ± SD)	48.1 ± 10.7	44.2 ± 13.1	42.3 ± 11.7	46.4 ± 9.3
Gender (male, female)	9M, 6F	9M, 6F	9M, 6F	9M, 6F
Postmortem interval (hr, mean ± SD)	23.7 ± 9.9	33.7 ± 14. 6	32.5 ± 16.1	27.5 ± 10.7
Brain hemisphere used (Right:Left)	7:8	6:9	8:7	6:9
Age of onset (*y*, mean ± SD)		23.2 ± 8.0	21.5 ± 8.3	33.9 ± 13.3
Duration of illness (*y*, mean ± SD)		21.3 ± 11.4	20.1 ± 9.7	12.7 ± 11.1

The method used to isolate and quantify the NAA and NAAG has been used previously (Nudmamud et al., 2003; Reynolds et al., 2005; Harte et al., 2004, 2005). 0.1 M perchloric acid was added to the tissue and after mixing, the tube was left on ice for approximately 10 min. The precipitated protein was removed by centrifugation at 12,000×*g* for 5 min 50 μl of this solution was mixed with 30 μl 0.2 M sodium carbonate and made up to 1 ml with 50 mM phosphate buffer (pH 6). The NAA and NAAG were extracted from the solution using SAX anion exchange columns. The columns were conditioned with 1 ml methanol, 2 × 1 ml 5.88 ml/l phosphoric acid (85%) and 5 × 1 ml 1 M phosphate buffer (pH 6.00). The sample was added, the column washed with 2 × 1 ml 2 mM phosphate buffer (pH 6.00) and 1 ml ultra pure water. NAA and NAAG were eluted with 3 × 0.5 ml 5.88 ml/l phosphoric acid (85%) phosphoric acid. 50 μl of the eluate was injected onto reversed phase octadecyl column (C18) particle size 4 μm (4.6 × 150 mm) using a mobile phase of 1.18 ml/l phosphoric acid (85%) and a flow rate of 0.5 ml/min. The detector was set at 215 nm. The NAA and NAAG in the samples were identified by their retention times when compared to standards and the compounds were quantified using peak height measurements. The protein precipitate was dissolved in 500 μl 1.5 M sodium hydroxide and the protein estimated by the Coomassie blue method. The NAA and NAAG concentrations were reported in nmol/mg protein.

All statistics were performed using SPSS for Windows v.10 (SPSS Inc, Chicago IL). A Repeated Measures Analysis of Variance (ANOVA) was used to analyse the data, with the repeated measures factor being brain region, followed by pairwise planned comparisons on the predicted least squares means. A stepwise regression analysis was performed to determine any relationship between the variables pH, brain weight, age, storage time, postmortem interval, total drug exposure and NAA and NAAG in each region. There were significant correlations between NAA in cingulate gyrus and age, between NAA in amygdala and storage time and between NAAG in hippocampal gyrus and storage time. The variables age and storage time were used as covariates in the pairwise comparisons of regional NAA and storage time was used as a covariate in the NAAG pairwise comparisons.

Within the disease groups correlations were assessed with Pearson's correlation coefficient to determine any relationship between NAA and NAAG and the variables pH, brain weight, age, storage time, postmortem interval and total drug exposure, onset of illness, duration of illness and lifetime antipsychotic dose (schizophrenia and depression) in each region.

Student's *t* test was used to test the NAA and NAAG means in the following; the under and over 18 years onset groups (schizophrenia and bipolar only); patients with and without psychotic symptoms (bipolar only); patients receiving lithium treatment or not (depression and bipolar only).

3. Results

For NAA there was a significant overall difference between the diagnosis groups (F (3, 56) = 3.138, P = 0.032), and there was a significant difference between the brain regions (F (6, 336) = 30.492, $P < 0.001$). For NAAG there was no overall difference between the diagnosis groups (F (3, 56) = 1.658, P = 0.186), but a significant difference between the brain regions (F (6, 336) = 24.612, $P < 0.001$).

The means and SEM together with the significant results generated by the pairwise comparisons are shown in Table 2. It can be seen that there are decreases in NAA concentrations in all patient groups in all regions studied. These are significant in the hippocampus for all patient groups; in the parahippocampal gyrus for schizophrenia; in the amygdala for schizophrenia and