Evidence for neuronal dysfunction in the anterior cingulate of patients with schizophrenia: A proton magnetic resonance spectroscopy study at 3 T

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Abstract

The anterior cingulate region is thought to be dysfunctional in schizophrenia, but whether this is the result of reduced neuronal integrity or changes in neurotransmitter systems remains an issue of debate. Fifteen male patients with schizophrenia and 14 male controls were assessed using proton magnetic resonance spectroscopy, with regions of interest placed in the right and left dorsal and rostral cingulate. The metabolites of interest were N-acetylaspartate (NAA), a putative neuronal marker, and glutamate + glutamine (Glx), which may index synapse number. Schizophrenia patients had lower NAA concentrations throughout the dorsal and rostral portions of the anterior cingulate and in both hemispheres, but showed no changes in Glx. Anterior cingulate involvement in schizophrenia is likely to be a result of neuronal loss or dysfunction.

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1. Introduction

Dysfunction of the anterior cingulate cortex (ACC) is thought to play a key role in the pathophysiology of schizophrenia (Todtenkopf et al., 2005), although the exact mechanism of its involvement remains unclear. The mechanism can be examined using magnetic resonance spectroscopy (MRS), which measures the concentration of various brain metabolites in vivo.

One frequently studied metabolite is N-acetylaspartate (NAA), which is reduced in conditions where there is persistent or reversible loss of functional neuronal integrity (Urenjak et al., 1993). In schizophrenia, NAA appears to be reduced by 4–5% in the ACC (Steen et al., 2005). However, more recent studies at high magnetic-field strengths (>1.5T) have failed to replicate this and instead report alterations in the glutamate–glutamine
system (e.g. Théberge et al., 2003). This discrepancy may relate, in part, to the limited scope and variability of prior studies, where ACC voxels have been placed in one hemisphere alone (or across the midline), and/or use a large region of interest incorporating extra-cingulate regions (e.g. Jessen et al., 2006). This is problematic because of the known lateralized ACC alterations in schizophrenia (e.g. Yücel et al., 2002) and apparent functional differences between dorsal and rostral ACC subregions (Bush et al., 2000).

Discerning whether NAA or glutamate–glutamine (or both) are affected in schizophrenia is important since reductions in the former would imply neuronal loss or dysfunction, whereas reduction in the latter might be more specific to reductions in the number of glutamatergic synapses (Théberge et al., 2003; Hassel and Dingledine, 2006). Therefore, we used high-magnetic-field-strength MRS to intensively study ACC neurometabolites in schizophrenia using four voxel placements; the left and right dorsal and rostral ACC. Based on previous work, we hypothesised that there would be a significant reduction in NAA.

2. Methods and materials

2.1. Subjects

We recruited 15 male outpatients with a current diagnosis of schizophrenia (mean age=31.5±7.5 years, median illness duration=9 years, range 1.1–26.8 years) and 14 healthy male control subjects (mean age=33.5±8.5 years). Healthy subjects were recruited from the community and patients were recruited through the North-Western Mental Health Care Network, Melbourne. No participant had a history of major medical or neurological illness, and no healthy subject had a history of psychiatric illness using the SCID-IV. Thirteen patients were receiving fixed antipsychotic doses (4=clozapine, 1=risperidone; 4=quetiapine, 1=olanzapine, 2=aripiprazole, 1=zuclopenthixol); the remaining two were medication-free. All participants provided written informed consent to the study’s protocol, which was approved by the local research and ethics committee.

2.2. Clinical assessment

Patients were administered the Positive and Negative Symptom Scale (Kay et al., 1987) to assess general psychopathology. Rather than use the total or subscale scores, we used a five-factor model (Negative Syndrome, Activation, Delusions and Hallucinations, Autistic Preoccupation, and Affective Syndrome) that best approximates the underlying factor structure of the instrument.

2.3. 1H MRS imaging acquisition

MRI data was acquired using a 3 T GE Signa Horizon LX whole body scanner (GE Healthcare, Milwaukee). Volume-localized 1H MRS was recorded using a standard short-echo point resolved spectroscopy sequence (TR=3000 ms, TE=30 ms, with a nominal voxel size ~6.5 cm³). Sixty-four transients were acquired from the dorsal ACC, whereas 128 transients were acquired from the rostral ACC due to the increased susceptibility artefacts from the ethmoid sinuses, orbits, and local vasculature. Two MRS voxels were placed in each hemisphere encompassing the dorsal and rostral ACC (Fig. 1). For the former, the posterior boundary of the voxel was ~10 mm posterior of a vertical line from the anterior commissure. The inferior border was ~5 mm superior to the corpus callosum. For the latter, the posterior boundary was the front of the genu of the corpus callosum while the inferior border was the line connecting the anterior to posterior commissure. For both regions the medial border was 1–2 slices lateral to the parasagittal slice. Absolute levels of N-acetyl compounds (NAA) and glutamate/glutamine (Glx) were determined using LCModel (Provencher, 1993) with a basis set recorded onsite and calibrated using the tissue water signal as an internal standard. Results are presented in institutional units approximating millimolar concentration.

Spectral quality for both control and patient groups was good, with a signal-to-noise ratio (averaged across all regions and hemispheres) of 14.5±3.65 and 14.1±3.15, and an FWHM of 0.067±0.02 ppm and 0.074±0.02 ppm respectively. The LCModel output includes Cramer–Rao
lower bounds (CRLB), which are a measure of reliability. Typically, values <30% are considered a good fit. The mean CRLB for NAA and Glx were 6.1 and 10.5 respectively for controls, and 7.0 and 9.9 respectively for patients.

2.4. Statistical analyses

Age, education, and premorbid IQ were compared across groups using χ²-analysis and independent sample t-tests in Statistical Package for the Social Sciences (SPSS) version 11.0. Where there was a correlation greater than 0.2 (Spearman’s rank-order correlation coefficients, two-tailed) between the amount of grey matter in the voxel and either metabolite, we corrected for the influence of grey matter using regression. These corrected values were used for subsequent analyses.

Metabolites were analysed individually in a four-way ANOVA with three repeated measures (metabolite [NAA and Glx], hemisphere [left and right] and position [dorsal and rostral ACC]). Spearman’s rank-order correlation coefficients were used to examine the associations between ¹H MRS metabolite levels from each of the regions of interest and clinical measures.

3. Results

The two groups did not significantly differ on age, years of education or premorbid IQ.

Significant main effects were identified for metabolite (Glx>NAA; F₁,27=357.4, p<0.001), hemisphere (left>right; F₁,27=10.9, p=0.003) and voxel (dorsal>rostral; F₁,27=20.1, p<0.001), but not for group (F₁,27=1.68, p=0.206). However, there was a significant metabolite x group interaction (F₁,27=6.63, p=0.016). Post-hoc testing revealed that patients with schizophrenia had significantly lower NAA concentrations in all voxels when compared to controls (Table 1). No significant differences were found for Glx.

### Table 1
Mean metabolite concentrations (SD) for each region-of-interest, in institutional units that approximate millimolar concentration

<table>
<thead>
<tr>
<th></th>
<th>NAA</th>
<th>Glx</th>
</tr>
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<tbody>
<tr>
<td>CTRL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>Dorsal</td>
<td>7.72 (0.68)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>6.54 (1.25)</td>
</tr>
<tr>
<td>Right</td>
<td>Dorsal</td>
<td>7.35 (0.63)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>5.64 (1.43)</td>
</tr>
<tr>
<td>SZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>Dorsal</td>
<td>8.64 (0.74)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>7.34 (1.68)</td>
</tr>
<tr>
<td>Right</td>
<td>Dorsal</td>
<td>8.10 (0.98)</td>
</tr>
<tr>
<td></td>
<td>Rostral</td>
<td>6.22 (1.57)</td>
</tr>
</tbody>
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3.1. Correlational analyses

Correlational analyses between the five PANSS factors and all metabolite concentrations revealed significant positive correlations between right dorsal NAA and the Activation factor (r=0.57, p=0.028), and between right rostral NAA and the Negative Syndrome factor (r=0.68, p=0.005). The only significant correlation between metabolite concentrations and illness duration was a negative association with right dorsal Glx (r=-0.52, p=0.046). None of these correlations survived correction for multiple comparisons.

4. Discussion

This is the first spectroscopic study to intensively study the ACC in schizophrenia. We demonstrated that NAA concentration is reduced in both the rostral and dorsal subregions and in both hemispheres. This reduction, between 9 and 11% of the control mean, is slightly more than double the average of previous studies (Steen et al., 2005). Contrary to previous reports (Théberge et al., 2003), we found no evidence for altered Glx in our patient sample, implying neuronal rather than synaptic loss and/or dysfunction. This is consistent with post-mortem reports of reduced cell density but normal concentrations of synaptic proteins in the ACC of schizophrenia patients (Eastwood and Harrison, 2001; Todtenkopf et al., 2005). Furthermore, the lack of robust correlations between NAA and either symptoms or illness duration suggest that this abnormality may represent a stable trait feature of schizophrenia, consistent with the work of Jessen et al. (Jessen et al., 2006) in a clinical high-risk sample.

While reduced NAA may reflect reduced cell density, it may also be related to reduced metabolism in the ACC. The rate of NAA production appears tightly coupled to the rate of glucose metabolism (Moreno et al., 2001), and PET studies of the ACC have shown it to be hypometabolic in schizophrenia (Haznedar et al., 2004).

Our failure to replicate previous reports of altered Glx is likely to be due to the specificity of our regions-of-interest, but could conceivably be due to treatment differences in our patient sample — all but three of our patients were treated with an atypical antipsychotic, whereas Théberge and colleagues (Théberge et al., 2003) included a majority of patients treated with conventional medications. There is some suggestion that Glx levels may increase following a switch from typical to atypical medication (Goff et al., 2002), supporting this line of reasoning.

The study was limited by the small sample size, although similar numbers have been investigated in previous reports (see Steen et al., 2005). Another limitation
is the chronic medication status of our patient group, although duration of illness (a proxy measure for duration of treatment) did not correlate significantly with any measure and cingulate NAA levels have previously been shown to be relatively unaffected by antipsychotic exposure (Pae et al., 2004).

In summary, we have shown a significant and widespread reduction in NAA in the anterior cingulate of patients with schizophrenia, without evidence of an alteration in the glutamate–glutamine system. It seems likely that this represents a neuropathological insult of pre-illness origin.

Role of the Funding Source
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Contributors
Drs Wood, Yücel and Pantelis designed the study. Drs Wood and Yücel undertook the statistical analyses and Dr Wood wrote the first draft. Ms Clarke recruited and interviewed the participants and Dr Wellard managed the acquisition and quality of the spectroscopy data. All authors contributed to and have approved the final manuscript.

References


