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Medial-frontal cortex hypometabolism in chronic phencyclidine exposed rats assessed by high resolution magic angle spin 11.7T proton magnetic resonance spectroscopy

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Abstract

Background—Proton magnetic resonance spectroscopy (¹H-MRS) clinical studies of patients with schizophrenia document prefrontal N-acetylaspartate (NAA) reductions, suggesting an effect of the disease or of antipsychotic medications. We studied in the rat the effect of prolonged exposure to a low-dose of the NMDA glutamate receptor antagonist phencyclidine (PCP) on levels of NAA, glutamate and glutamine in several brain regions where metabolite reductions have been reported in chronically medicated patients with schizophrenia.

Methods—Two groups of ten rats each were treated with PCP (2.58 mg/kg/day) or vehicle and were sacrificed after 1 month treatment. Concentrations of neurochemicals were determined with high resolution magic angle (HR-MAS) ¹H-MRS at 11.7Tesla in *ex-vivo* punch biopsies from the medial frontal and cingulate cortex, striatum, nucleus accumbens, amygdala and ventral hippocampus.

Results—PCP treatment reduced NAA, glutamate, glycine, aspartate, creatine, lactate and GABA in medial frontal cortex. In the nucleus accumbens, PCP reduced levels of NAA, aspartate and glycine; similarly aspartate and glycine were reduced in the striatum. Finally the amygdala and hippocampus had elevations in glutamine and choline, respectively.

Conclusions—Low-dose PCP in rats models prefrontal NAA and glutamate reductions documented in chronically-ill schizophrenia patients. Chronic glutamate NMDA receptor blockade in rats replicates an endophenotype in schizophrenia and may contribute to the prefrontal hypometabolic state in schizophrenia.

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Keywords

phencyclidine; N-acetyl aspartate; glutamate; schizophrenia; ^1H -MRS

1. Introduction

The NMDA hypofunction model of schizophrenia proposes dysfunction of this glutamate receptor in GABAergic interneurons (Moghaddam and Javitt, 2012). This leads to a downstream, unrestrained hyperglutamatergic (excitotoxic) state, which results in neuronal damage in distributed cortical brain areas and may account for the cognitive deficits and poor clinical outcomes. Reductions in NAA, an acetate donor localized to neuronal mitochondria and considered a marker of neuronal viability and density, have been repeatedly found in chronically ill schizophrenia patients, mainly in frontal and mesial temporal regions, using ^1H -MRS (Steen et. al, 2005). While increased glutamine levels, a potential marker of enhanced prefrontal glutamate turnover, have been reported early in the illness, glutamate reductions were described in chronic patients (Abbott and Bustillo, 2006). Consistently, in a pharmacological model of psychosis, acute NMDA receptor blockade with ketamine increased prefrontal glutamine in healthy volunteers (Rowland et al, 2005). Finally, chronic (1 month) NMDA receptor blockade with low dose intermittent PCP in rats lead to reduced temporal NAA measured with HPLC (Reynolds et al, 2005), as well as reductions in GABAergic and overall prefrontal metabolism (Cochran et. al, 2003). Hence, this paradigm is consistent with proposed sequelae of the NMDA hypofunction model of schizophrenia.

The goal of the study was to examine whether chronic NMDA blockade, an influential pathophysiological model of schizophrenia, can induce some of the neuro-metabolic abnormalities reported in the clinical ^1H -MRS literature. Based on previous clinical reports we hypothesized that NAA and glutamate would be reduced in frontal and temporal regions after prolonged exposure to PCP.

2. Materials and Methods

2.1. Drug exposure

All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of New Mexico Health Sciences Center and were performed according to the guidelines of the NIH. Male Sprague-Dawley rats weighing about 300 g each were purchased from Harlan (Indianapolis, IN), housed in pairs and allowed to acclimatize for 2 weeks before the start of the study. Rats were housed at the animal facility under a 12:12 light: dark cycle (lights on at 7 am) and received Purina rat chow and tap water *ad libitum*. After a handling period of about one week, PCP (2.58 mg/kg/injection, IP) was administered to rats (n=10) as 14 injections over a period of 26 days. Injections were given daily for the initial 5 days (week 1), then at days 8, 10 and 12 (week 2), days 15, 17 and 19 (week 3) and days 22, 24 and 26 (week 4; as per Reynolds et. al, 2005). Control rats (n=10) received equivalent volumes of saline. Three days after the last injection, rats were exposed briefly to isoflurane then sacrificed by decapitation. Brains were rapidly removed, placed into rat brain matrix and 2 mm coronal slices obtained on an ice-chilled stage. Four slices were selected which approximately corresponded to the following sections of a standard rat brain atlas (+ or – refers to anterior or posterior from Bregma, respectively): *slice 1*, +3.7 to +1.7 mm; *slice 2*, +1.7 to –0.3 mm; *slice 3*, –2.3 to –4.3 mm; and *slice 4* from –4.3 to –6.3. Regions of interest were identified visually in each slice (Bustillo et. al, 2006): MFC (*slice 1*), CG, Str and Nac (*slice 2*); Amy (*slice 3*) and VH (*slice 4*). These regions were selected because of their distinct dopamine projection fields and/or their homologous overlap with areas studied

with clinical ^1H -MRS in chronically treated schizophrenia subjects (Steen et. al, 2005). Two mm circular punches were obtained from appropriate regions and immediately placed in pre-cooled plastic centrifuge tubes, frozen on solid CO_2 , and then stored at -80°C until HR-MAS ^1H -MRS analysis.

2.2. HR-MAS ^1H -MRS

Frozen intact tissue samples were weighed (~3mg) and placed directly into a Bruker zirconium rotor (4 mm diameter, 10 μL capacity) containing 5 μL buffer (pH= 7.4; 100 mM potassium phosphate, 200 mM formate, 1 g/L NaN_3 and 3mM trimethylsilyl-propionate [TSP Sigma; St Louis, MO] diluted with an equal volume of D_2O containing 0.75% TSP). TSP serves as an internal chemical shift reference (0.00 ppm), formate (8.44 ppm) for phase corrections, and D_2O to lock on the center frequency. The rotor (with sample) was placed into a Bruker magic angle spinning probe maintained at 4°C in a vertical wide-bore (8.9 cm) Bruker 11.7 T magnet with an AVANCETM DRX-500 spectrometer (Bruker Biospin Corp., Billerica, MA). Rotors were spun at $4.2 \pm 0.002\text{ kHz}$ at 54.7° relative to the static magnetic field B_0 .

Identical brain region from all animals were randomly analyzed in a single session by an operator blind to drug treatment. Spectra were analyzed with a custom LCModel utilizing a linear combination of 27 individual neurochemical model spectra (basis set) as well as non-specific lipid signals to fit the tissue spectrum and calculate absolute concentration values for neurochemicals with signals between 1.0 – 4.2 ppm. The precision of the LCModel fit to the spectral data was estimated with Cramér-Rao lower bounds which were acceptable under 25% and typically less than 10% for most of the metabolites reported herein. To account for variations in the mass of individual samples, absolute concentrations of MR visible metabolites were corrected for tissue weight and are expressed as nmol/mg of wet tissue weight; variance associated with means suggests a substantial degree of analytical reproducibility (Ghoddoussi et al. 2010).

2.3. Statistical Analyses

Each metabolite level was analyzed with a 2 (treatment group) by 6 (region) Proc Mix ANOVA using SAS software. To protect against Type-I error, only significant treatment by region interactions were followed up with Fisher's least significant difference method of post-hoc comparisons. Data are presented as mean $\pm$ S.D. in all cases.

3. Results

As shown in Figure 1, the following neurochemicals were reliably fitted by the spectral model: alanine, aspartate, choline, GABA, glutamine, glycine, glutamate, glycerophosphorylcholine, glutathione, *myo*-inositol, lactate, NAA, NAAG, phosphorylethanolamine, phosphorylcholine, taurine, succinate, betaine and total creatine.

In order to statistically protect for multiple comparisons, only neuro-metabolites with a significant group by region interaction ($p < 0.05$) were further examined and are presented below.

The following main neurochemicals of interest, based on the clinical schizophrenia literature, were examined. For NAA, there were reductions in the PCP group in MFC [$t(1, 14.9) = -3.05$, $p=0.008$] and Nac [$t(1, 15.2) = -2.57$, $p=0.02$; figure 2]. For glutamate [$t(1, 14.8) = -2.67$, $p=0.02$; figure 2] and GABA [$t(1, 14.5) = -2.76$, $p=0.01$], there were reductions in MFC. For glutamine, there was an increase in the PCP group in Amy [$t(1, 10.4) = 2.4$, $p=0.04$].

The following neurochemical analyses were pursued because they are often reported in the clinical literature, but for which we made no specific predictions. For choline there was an increase in the PCP group in VH [$t(1,17.2)=2.39$, $p=0.03$]. For creatine there were reductions in the PCP group in MFC [$t(1, 14.9)= -3.30$, $p=0.01$] and Nac [$t(1,15.2)= -3.55$, $p=0.03$].

Other neurochemicals of interest, based on the NMDA model, were also analyzed. For glycine, there were reductions in the PCP group in MFC [$t(1,15)= -3.13$, $p=0.007$], Nac [$t(1,15.3)= -2.64$, $p=0.02$] and Str [$t(1, 17.7)= -2.14$, $p=0.05$]. For aspartate, there were reductions in the PCP group in MFC [$t(1,15)= -3.01$, $p=0.009$], Nac [$t(1, 15)= -2.56$, $p=0.02$] and Str [$t(1, 17.9)= -2.13$, $p=0.05$]. For lactate, there was a reduction in the PCP group in MFC [$t(1,13.8)= -2.85$, $p=0.01$]. For the remaining neurochemicals (NAAG, myo-inositol, alanine, glutathione and taurine), there were no group differences.

4. Discussion

Compared to vehicle-treated animals, a low dose chronic PCP exposure decreased levels of neurochemicals associated with neurotransmission (glutamate, GABA, glycine and aspartate) as well as those that may reflect glial energy status (lactate) or neuronal density (NAA) in the rat MFC, a region thought to be equivalent to the prefrontal cortex in humans. In the nucleus accumbens, a region innervated by the prefrontal cortex and ventral tegmental area, repeated exposure to PCP reduced levels of NAA, aspartate and glycine; similarly aspartate and glycine were reduced in the striatum. Finally the amygdala and hippocampus had elevations in glycine and choline, respectively. We specifically focused on neurochemicals that 1) can be measured with $^1\text{H-MRS}$ *in-vivo* in schizophrenia populations or 2) are involved with neurotransmission in the prefrontal cortex (i.e.: NAA, glutamate, glutamine, GABA and glycine) in order to mechanistically inform clinical $^1\text{H-MRS}$ studies.

Our findings are consistent with the two other studies that used the same PCP exposure paradigm but focused on different brain markers. Reynolds et. al. (2005), used HPLC to measure NAA and NAAG in larger sections of MFC, temporal, hippocampal and striatal structures. Only in temporal cortex was there a significant NAA reduction, but the effect size in MFC was large, with reductions in the PCP group, that failed significance (with a sample $n=5$). The same group (Cochran et. al, 2003) reported reductions in glucose metabolism and parvalbumin staining in MFC and behavioral deficits consistent with the cognitive impairments in schizophrenia (Pratt et. al, 2008). Both studies are consistent with a hypometabolic prefrontal state with prolonged NMDA blockade, a useful animal model of certain endophenotypes of schizophrenia (Hajszan et. al. 2006). What mechanism could account for the MFC hypometabolism? PCP is an NMDA receptor antagonist that at low dosages may more sensitively block the tonic glutamatergic stimulation of GABAergic interneurons (Moghaddam and Javitt, 2012). This excitatory deficit presumably leads to disinhibition of cortical pyramidal cells and increased cortical glutamate release in the prefrontal cortex (Moghaddam et. al, 1992). Indeed, high dose PCP can clearly lead to cortical neuronal death after a single dose due to excitotoxicity. Glutamate function is also modified in the absence of excitotoxicity. For example lower, subchronic doses (1 week, 5 mg/kg IP, bid) of PCP markedly reduced MFC dendritic spines and increased astroglia process density, measured with electron microscopy stereology, without evidence of neuronal death (Hajszan et. al, 2006). These findings are consistent with postmortem ultrastructural reports of reduced dendritic spine density in prefrontal cortex in schizophrenia subjects (Glantz and Lewis, 2000), with no neuronal death but increased neuronal density (Selemon et. al, 1995).

Multiple genetic polymorphisms involved in the etiology of schizophrenia, may functionally converge in disrupting NMDA function (Harrison and Weinberger, 2005). Hence a pharmacological model of persistent NMDA blockade may be relevant to mechanistically inform some of the essentially descriptive neuroimaging and postmortem findings in the disease. Since dendritic spines are the principal inputs of thalamo- and cortico-cortical glutamatergic axons, we speculate that pyramidal neurons may protect themselves from potential glutamate excitotoxicity, by spine disconnection and gradual retraction of the dendritic trees. This would also result in tissue reductions of excitatory and inhibitory neurotransmitters (i.e. : glutamate, GABA, glycine and aspartate), metabolism (lactate and creatine) and overall neuronal tissue density (NAA), consistent with the hypofrontal state described in schizophrenia (Minzberg et. al, 2009).

Several limitations of the present study should be considered when interpreting our results. First, animals were exposed to a fixed low dose of PCP. A higher dose may induce more distinct metabolic and regional effects. Second, the brain regions studied were rather large and a putative PCP effect may be limited to more discrete areas or different regions. However we specifically selected regions in which NAA reductions have been repeatedly described in chronically treated patients (frontal, cingulate, and hippocampus; Steen et. al, 2005). Third, we do not demonstrate NMDA hypofunction, but our results suggest that this is a plausible mechanism to account for the reduced NAA, glutamate and GABA (Yoon et. al, 2010), described in chronically-ill schizophrenia patients. Fourth, lower NAA may not reflect reduced neuronal viability or density, but could be secondary to reductions in aspartate. Finally, our design was cross-sectional and a longitudinal study may be more powerful to detect within subject changes. Future animal studies with repeated *in-vivo* ¹H-MRS measures over several months would address this limitation.

5. Conclusion

We found that chronic NMDA blockade with low dose PCP in rodents, resulted in a neurometabolic profile similar to observations in chronically-ill schizophrenia patients: reduced prefrontal NAA, glutamate and GABA. In addition several other metabolites related to the glutamatergic system (aspartate, glycine and lactate) were also reduced in this region and should be investigated in schizophrenia.

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Abbreviations

NAA	N-acetylaspartate
NAAG	N-acetyl-aspartylglutamate
GABA	gamma amino butyric acid
MFC	medial frontal cortex
CG	anterior cingulate cortex

Str	anterior dorsal striatum
Nac	nucleus accumbens
Amy	amygdala
VH	ventral hippocampus
HPLC	high pressure liquid chromatography

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Figure 1.

Representative ¹H-MRS spectrum from an intact 4 mg sample of rat cingulate showing high spectral resolution acquired with magic angle spinning at 11.7 Tesla. Abbreviations: NAA is N-acetylaspartate, INS is *myo*-inositol, GABA is gamma amino butyric acid; GSH is glutathione; P-EA is phosphorylethanolamine and ASP is aspartate.

Figure 2. Mean (± SD; nmol/mg of wet tissue weight) differences in NAA (A) and glutamate (B) for animals treated with phencyclidine (PCP) and vehicle across various brain regions [amygdala (Amy), Cingulate (CG), medial frontal cortex (MFC), nucleus accumbens (Nac), striatum (Str) and ventral hippocampus (VH)].