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MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY IN A MOUSE MODEL OF SCHIZOPHRENIA

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Abstract

Metabolic brain abnormalities, as demonstrated by ¹H-magnetic resonance spectroscopy techniques, are common occurrences in adult schizophrenia. As mice share important biochemical and genomic similarities with humans, we tested whether brain metabolic abnormalities also occur in a transgenic mouse model of schizophrenia. *In vivo* ¹H-magnetic resonance spectroscopy at 4.7 T of the *chakragati* mouse brain revealed abnormalities in relative levels of choline 3.20 ppm and N-acetylaspartate 2.01 ppm compounds. These results are consistent with a prior proposal that deficits in metabolite ratios may be common features of psychotic disorders. Thus, *chakragati* mice recapitulate certain aspects of the human disease phenotype and further support the utility of this animal model for understanding causal factors underlying uniquely human brain diseases.

Keywords

ckr mice; Choline; Creatine; N-acetylaspartate; Metabolite ratios

INTRODUCTION

The *chakragati* (*ckr*) mouse is a transgenic insertional mutant that models several aspects of the behavioral and molecular pathology expressed in schizophrenia. For instance, the mouse mutant shows behavioral abnormalities, including novelty-driven hyperactivity that is manifested as increased circling activity in the open field (Ratty et al., 1990; Torres et al., 2004; Torres et al., 2005a). Further, *ckr* mice show asymmetric elevations of dopamine (DA) D₂-like receptors in basal ganglia circuitry (Fitzgerald et al., 1993), and high-resolution magnetic resonance imaging (MRI) of these mutants indicates selective enlargement of the lateral ventricles (Torres et al., 2004). As these abnormalities are often diagnosed in patients

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with schizophrenia, genetically mutant *ckr* mice might be relevant for understanding or pinpointing psychoses-causing mechanisms.

Proton (^1H)-magnetic resonance spectroscopy (^1H -MRS) reveals deficits in the concentrations of choline (Cho), creatine (Cr) and N-acetylaspartate (NAA) in certain brain structures of psychotic patients (Renshaw et al., 1995; Yurgelun-Todd et al., 1996; Deicken et al., 1998; Auer et al., 2001). Both Cho and NAA are relevant molecules to cell membrane integrity and neuronal density, respectively (Lyoo and Renshaw, 2002). Cr, in turn, is heavily involved in ATP transduction and thus energy metabolism in nerve cells and glia (Siesjo, 1978). These ^1H -MRS findings suggest tentative brain mechanisms of schizophrenia and support the hypothesis that cell membrane disturbances might be responsible for the cognitive deficits seen in psychotic patients (Horrobin et al., 1994; Fenton et al., 2000). Against this background, we have undertaken an exploratory ^1H -MRS study on homozygous *ckr* mice, their heterozygote kin and age-matched wild-type cohorts to further assess how accurately the *ckr* mutant captures well established deficits of schizophrenia pathology. Results of this study suggest that our mouse mutant indeed recapitulates the neurochemical phenotype it is intended to model.

METHODS

The *ckr* mouse was generated as previously described (Ratty et al., 1990). All transgenic mice used for the experiments reported herein were adult male and female F_2 animals (6–9 months; 30–40 g) of the mixed genetic background of BCF_1 ($\text{C57BL}/10\text{Ros}^{\text{Pd}} \times \text{C3H}/\text{HeRos}$). Mutant, heterozygous and wild-type adult mice were kept in groups of 3–4/cage (same-sex/same-genotype) and maintained on a light:dark cycle of 12:12 hr (lights on at 0700) with free access to food and water. Classification of genotype for both *ckr* and heterozygous mice was conducted by restriction fragment-length polymorphism analysis of biopsied tail DNA taken during the first week of postnatal life (Ratty et al., 1990). All neurochemical procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and with approval from the Roswell Park Cancer Institute IACUC. All efforts were made to minimize animal stress and to reduce the number of mice used for these experiments.

MR IMAGE ACQUISITION

MR image acquisition and processing was performed as previously reported (Torres et al., 2005b), and is only briefly described here. To assess cerebral ventricular size *in vivo*, wild-type ($n = 4$), heterozygous ($n = 5$) and homozygous *ckr* mice ($n = 5$) were anesthetized with isoflurane and the brain imaged using a General Electric (GE) CSI 4.7 T/33 cm horizontal bore magnet (GE NMR Instruments, Fremont, CA) with radiofrequency and computer systems incorporating AVANCE digital electronics (Bruker BioSpec platform with ParaVision Version 3.0.1 Operating System, Bruker Medical, Billerica, MA). Anesthesia was maintained with 2–4% isoflurane during the entire imaging experiment and animal respiration was monitored with an MR compatible monitoring and gating system (Model 1025, SA Instruments, Stony Brook, NY). Following acquisition of a series of pilot scans, high-resolution T2-weighted coronal and axial multi-slice scans encompassing the entire brain were acquired. Due to the intrinsically long acquisition times required for T2-weighted imaging, RARE encoding (rapid acquisition with relaxation enhancement) was applied to reduce imaging times. Acquisition parameters for coronal and transverse axial acquisitions consisted of $\text{TE}/\text{TR} = 80/3200$ ms, 20 averages, with an echo train length (ETL) of 8. Axial images were obtained with a 256×192 matrix, and coronal images with a 192×192 matrix. Axial image field of view (FOV) was 4.0×3.2 cm and slice thickness was 0.9 mm; coronal image FOV was 3.2×3.2 cm and slice thickness was 0.9 mm. In addition, coronal images were also obtained from all three genotypes ($n = 4$ –5/genotype) for histological measurements of their ventricular system. For comparisons of ventricular size, sections spanning the entire brain were cut on a microtome at a thickness of

40 μm . Sections (representing a one-in-five sample of the brain) were then mounted onto glass slides and stained with neutral red. Serial sections through the cerebellum were photographed to include the lateral, third and fourth ventricle. The border of each ventricle for each section was then outlined, the cross-sectional ventricular areas were drawn in *Camera lucida*, and estimates of ventricular space area were generated using the NIH IMAGE software package. All quantifications of ventricular size were performed without knowledge of animal genotype.

¹H-MRS ACQUISITION

Spectra of Cho and Cr ratios to NAA in wild-type, heterozygous and homozygous *ckr* mice ($n = 4\text{--}7/\text{genotype}$) were acquired *in vivo* using Point Resolved Spectroscopy (PRESS) with water suppression (Bottomley, 1987). Spectra were acquired within a voxel size of $4.50 \times 3.00 \times 5.50$ mm and localized to the center of the mouse brain. Additional parameters included: echo time (TE) = 100 ms repetition time (TR) = 1500 ms, sweep width = 5 kHz and data points = 4k. The signal was averaged for 2048 excitations, for an MRS acquisition time of 51 min. Prior to scanning, magnetic field homogeneity was optimized for the voxel by manual shimming of first order (x-y-z) shims to the unsuppressed water peak until the spectra exhibited a full-width, half-height (FWHH) of ≤ 15 Hz. To account for eddy current effects and magnetic field drift, reference spectra were acquired prior to and following each acquisition and data were corrected using a manufacturer-supplied algorithm.

MR spectra were processed using MestRec 4.46 (MestReLab Research, A Coruña, Spain). Prior to Fourier transformation (FT) of the data into the frequency domain, the data were zero-filled with 4 k data points to increase digital resolution, and apodized with an exponential window function (LB=3 Hz). Following FT, spectra were phased automatically using a simplex method. Spectra were baseline corrected using a multipoint polynomial baseline correction, and referenced to the NAA peak at 2.01 ppm. Choline and Cr peaks were subsequently defined at ~ 3.20 ppm and 3.02 ppm, respectively. Due to the lack of baseline resolution of the Cr and Cho peaks, peaks were integrated by first fitting each peak with a Gaussian line shapes and the peak height and widths were adjusted to match the corresponding peaks of Cho, Cr, and NAA (see Fig. 3). The relative concentration of each metabolite was obtained by integration of the area under each peak.

STATISTICAL ANALYSIS

Data are expressed as means ($\pm$ SEM), with statistically significance defined at $P \leq 0.05$. Comparison for ventricular size (in mm^2 units) was calculated from serial sections spanning the lateral, third and fourth ventricle of the three genotypes using ANOVAs with a Tukey post-test comparison of the means. Differences in absolute metabolite ratios between wild-type, heterozygous and homozygous *ckr* mice were analyzed using ANOVAs with a Tukey post-test comparison of the means. Metabolite peak areas were adjusted for instrumental transmitter, and corrected for voxel CSF content.

RESULTS

T2-weighted axial images of brain sections oriented to the lateral ventricles revealed an enlargement of the anterior ventricular system for both adult heterozygous and homozygous *ckr* mice (Fig. 1); a brain lesion index previously reported by our group in adolescent mice (Torres et al., 2005b). Further, this ventricular aberration was only detected in the lateral, as opposed to the third or fourth, ventricular system. One way-ANOVA with genotype as the independent variable and lateral ventricular size as the dependent variable indicated a significant effect of genotype ($P \leq 0.05$). These results confirm structural abnormalities in the adult *ckr* mutant and suggest that the structural changes may be progressive.

We next examined whether *ckr* mice differ in brain metabolite (i.e., Cho, Cr and NAA) ratios as localized by ^1H -MRS at 4.7 T. Figure 2 shows *in vivo* spectra from representative MRI voxels in brains of wild-type, heterozygous and homozygous *ckr* animals. Resonance spectra acquired *in vivo* from all three genotypes were typically of high quality and featured prominent peaks for Cho, Cr and NAA (Fig. 3). Table 1 lists the mean $\pm$ SEM concentrations of spectroscopic metabolite ratios for all three genotypes. The Cho/NAA ratio ranged from 0.618 ± 0.027 to 1.140 ± 0.120 whereas the Cr/NAA ratio ranged from 0.759 ± 0.094 to 0.925 ± 0.082 . These ranges are in agreement with those previously reported by other ^1H -MRS studies (Lindquist et al., 2000; Mathew et al., 2003; O'Neill et al., 2004). The Cho/NAA ratio was significantly different ($P \leq 0.05$) between homozygous *ckr* mice and wild-type cohorts. Similarly, the Cho/NAA ratio of heterozygous and wild-type mice was found to be statistically significant ($P \leq 0.05$). The Cr/NAA ratio, on the other hand, did not differ at all among any of the three mouse genotypes ($P \geq 0.05$).

DISCUSSION

The current study extends and further supports the hypothesis that the *ckr* mutant is an appropriate model for understanding the neurochemical basis of certain clinical manifestations of schizophrenia (Torres et al., 2004; Torres et al., 2005a; 2005b). Indeed, this and other genetic mouse models of psychoses have been successful in providing insights into potential neural mechanisms that underlie the disease (Gainetdinov et al., 2001; Tremolizzo et al., 2002; Miyakawa et al., 2003; Karayiorgou and Gogos, 2004; Torres et al., 2004). This is good news as mice share important biochemical and genomic similarities with humans. But, genetically engineered mouse models for schizophrenia often raise doubts as to how such mutants can accurately translate psychotic syndromes (e.g., hallucinations and incongruity of affect) into testable measures. The most direct and simple way to develop and establish valid animal models of schizophrenia is to pick apart components of the human disease, particularly those that have a mouse counterpart (Chen et al., 2006; O'Tuathaigh et al., 2007). For example, abnormal behavioral responses to the open field, deficits in social interaction, pre-pulse inhibition abnormalities and alterations in DA transmission are schizophrenia-related syndromes that can be modeled relatively accurately in mice. It is encouraging, therefore, to think that by dissecting the complexity of schizophrenia into more tractable components in transgenic animals, such efforts might reveal mechanisms critical to schizophrenia pathogenesis.

The *ckr* mouse highlights the utility of studying certain aspects of schizophrenia syndromes to ultimately pinpoint disease-causing mechanisms. This particular mouse was serendipitously generated by microinjections of a 24-kb genomic fragment containing the mouse Ren-2^d renin gene into BCF (C57BL/10Ros^{pd} $\times$ C3H/HeRos) fertilized oocytes (Ratty et al., 1990). Genetic and physical analyses of this insertion revealed that 2.5 copies of the transgene, comprising 65–70 kb, had integrated, duplicated and inverted portions in a vicinity of D16Ros 1 within chromosome 16 of the mouse genome. This loss-of-function disorder is expressed only when bred to homozygosity and mating studies indicate an autosomal recessive mode of inheritance (Ratty et al., 1990).

Using ^1H -MRS, we have detected *in vivo* metabolic abnormalities in homozygous *ckr* and their heterozygote kin. More specifically, a significant decrease of Cho/NAA ratios was observed in brains of *ckr* and heterozygous mice relative to those of wild-type cohorts. These findings are consistent with previous studies reporting decreases of Cho and NAA ratios in some schizophrenia patients, an effect also seen in brains of individuals diagnosed with bipolar depression (Tsai et al., 1995; Winberg et al., 2000). Further, the finding of decreased Cho and NAA ratios in the *ckr* mouse brain are strikingly similar to models of stressful early rearing in non-human primates (Mathew et al., 2003), and in adolescent humans with post-traumatic stress syndrome (De Bellis et al., 2000). Thus, there is neurochemical commonality between

our spectroscopic results and psychiatric disorders marked by early adverse experiences. It should be noted that we also identified metabolic abnormalities in heterozygous mice with the *ckr* mutation. These findings indicate that insertional mutagenesis in heterozygous animals has resulted in a well-characterized metabolic abnormality as well. Indeed, heterozygous mice also show enlargement of the lateral ventricles, an abnormality reported as a structural aberration in schizophrenia patients and their unaffected kin (Torres et al., 2005b). In this regard, it is thought that the key to unlocking the etiology of schizophrenia may lie not in the patients themselves but in their unaffected relatives. The homozygous *ckr* mouse with its heterozygous kin offers the opportunity to test further this hypothesis.

Although Cho, Cr and NAA molecules serve important function to nerve cells ranging from energy metabolism to membrane integrity, the clinical significance of these compounds is unclear (Arnold et al., 2001; Barker, 2001; Sullivan et al., 2001). Further, pharmacological treatment (e.g., olanzapine) and sedation during MR acquisition represent major confounding variables in schizophrenia. Our spectroscopic finding, viewed as an exploratory study, also has several limitations. For instance, due to the limits of sensitivity in small animal MRI, we were unable to identify which exact brain regions showed differences in Cho/NAA ratios. This information is critical if we are going to link abnormalities in neurochemical ratios and brain sites of suspected pathology. Bearing its limitations, the present study indicates that alterations of the D16Ros1 locus within chromosome 16 of the *ckr* genome have led to a phenotype which shows certain similarities with that of schizophrenia.

Of interest, (micro) deletions of the 22q11 locus in humans are also associated with an increased risk to develop schizophrenia; the first association between a chromosomal defect and schizophrenia onset (Karayiorgou and Gogos, 2004). Micro-deletion syndromes may therefore be the genetic contributions to the high risk for schizophrenia in humans and perhaps in the occurrence of schizophrenia-related phenotypes in *ckr* mutants. This association is further supported by the fact that the syntenic region of the human 22q11 locus lies on mouse chromosome 16. This chromosomal synteny might be coincidental or might suggest that loci at these chromosomes harbor genes that alone, or in combination with other genes, are causally implicated in schizophrenia pathogenesis. Studies addressing these issues are currently ongoing projects in our laboratories.

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Fig. 1.

T2-weighted axial brain sections of adult mouse genotypes selected for ^1H -MRS. Note enlargement of the lateral ventricles (yellow banding) in heterozygous and homozygous *ckr* mice relative to a wild-type cohort. Ventricular area ranges from $\sim 0.150 \text{ mm}^2$ (in wild-type mice) to ventricular areas of $\sim 0.900 \text{ mm}^2$ (in heterozygous and homozygous mice) as measured in neutral-red coronal sections taken from Bregma 1.10 mm to Bregma 0.20 mm of Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129/Sv Mouse Brains (Hof et al., 2000).

Fig. 2.

Representative *in vivo* spectra from mouse brains featuring major peaks for Cho, Cr and NAA. ^1H -MRS voxel was localized to the center of the mouse brain for all three genotypes (see Table 1 for voxel placement). Parts per million (ppm).

Fig. 3. Example of line fitting procedure for peak integration. Panel A: Original spectrum (black). Panel B: Gaussian line shapes used to fit Cho, Cr and NAA peaks (red). Panel C: Synthetic spectrum (blue) created from sum of Gaussian line-shapes. Residuals (green) represent the difference between the synthetic spectrum (blue) minus the actual spectrum (black). Very low residual values below the peaks of interest indicate good fit of synthetic spectra relative to actual spectra. Cho = 3.21 ppm; Cr = 3.02 ppm; NAA = 2.01 ppm. Parts per million (ppm).

Table 1**Metabolic Ratios in Mouse Brains**

Metabolic Ratio	Wild-Type	Heterozygous	Homozygous
Cho/NAA	$0.618 \pm 0.027^*$	1.140 ± 0.120	1.082 ± 0.115
Cre/NAA	0.759 ± 0.094	0.925 ± 0.082	0.885 ± 0.039

Axial proton-density-weighted MRI sections of a male wild-type mouse brain showing location (red box) of single ^1H MRSI voxel sampled between the rostral corpus callosum and posterior thalamus (top).

* denotes $P \leq 0.05$ ($F = 5.5$; $df = 2, 13$) when metabolic ratios from wild-type mice ($n = 4$) are compared with heterozygous ($n = 5$) and homozygous mice ($n = 7$).