Table 2. White matter DTI measurement in six fibre tracts

		FES ($N = 9$)	pre-onset patients ($N = 10$)	HS ($N = 10$)	F	df	p
LT_ILF	Mean FA	0.40 ± 0.02	0.39 ± 0.02	0.41 ± 0.02	2.75	2	0.082
	Range	0.37–0.44	0.36–0.42	0.38–0.44			
LT_CHP	Mean FA	0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.03	2	0.971
	Range	0.22–0.28	0.22–0.28	0.20–0.27			
LT_SLF	Mean FA	0.40 ± 0.02	0.38 ± 0.04	0.43 ± 0.03	5.78	2	0.008*
	Range	0.36–0.43	0.33–0.45	0.39–0.49			
RT_ILF	Mean FA	0.41 ± 0.02	0.41 ± 0.02	0.42 ± 0.02	1.29	2	0.293
	Range	0.39–0.45	0.38–0.43	0.38–0.45			
RT_CHP	Mean FA	0.26 ± 0.02	0.25 ± 0.03	0.26 ± 0.03	0.35	2	0.711
	Range	0.24–0.32	0.21–0.29	0.20–0.31			
RT_SLF	Mean FA	0.44 ± 0.02	0.44 ± 0.03	0.48 ± 0.02	10.31	2	0.001*
	Range	0.43–0.37	0.38–0.47	0.44–0.50			

Post hoc t-test

		p
LT_SLF		
HS	FES	0.048*
	Pre-onset	0.002*
FES	HS	0.048*
	Pre-onset	0.239
Pre-onset	HS	0.002*
	FES	0.239
RT_SLF		
HS	FES	0.002*
	Pre-onset	<0.001*
FES	HS	0.002*
	Pre-onset	0.430
Pre-onset	HS	<0.001*
	FES	0.430

CHP, cingulum hippocampal part; FES, first-episode schizophrenia; HS, healthy subject.
*$p > 0.05$ (correct).

should make it easier to understand the brain changes that will occur as the disease proceeds.

Some schizophrenia studies have shown left temporal impairment (27–29), while others indicate disruptions of the bilateral temporal area (30–32). In addition, a previous study showed that compared with healthy controls, high-risk subjects for schizophrenia showed lower FA in SLF (19). We observed a volume loss in the bilateral temporal cortices and microstructural disturbance in the bilateral SLF. Consistent with the findings of previous studies that used DTI, 3D-T1 weighted volume data and post-mortem brain study, the present study provides direct *in vivo* evidence of structural anomalies in patient groups. Anomalies of temporal regions have found in patients with schizophrenia, and are associates with delusions and hallucinations (33–36). Previous studies showed that the brain change preceded the episode of clinical symptoms (7–11). Our participants at high risk who did not show the delusion and hallucination may develop the precedent morphological change that would affect on the delusion and hallucination. Some neuroimaging studies focussed on the prodromal state have shown temporal lobe anomalies, but the results on the localisation of disturbance were controversial. Some studies have shown left temporal impairment using DTI and volume data (8,10). However, one study indicated reduction of the bilateral temporal grey matter (11), and some papers denied the temporal change using DTI (7,9). These inconsistencies may result from that they used intake criterion for identifying participants at high risk that included so many psychotic symptoms, such as perceptual disturbance, disorganisation, delusion, hallucination and decrease in mental state or functioning. In this study, we regarded the patients who showed perceptual disturbance revealed by the Rorschach test as the high-risk group that fell under the group 1 of the PACE criteria. By using the simple intake criterion, useful information was obtained. Furthermore, structural and functional imaging studies have revealed that the high-risk group is associated with regional volumetric and functional abnormalities that are qualitatively similar to those in patients with

schizophrenia but are less severe (37). The present observations need to be replicated with a larger study population.

In this study, our participants showed the perceptual impairment. The parietal lobe is known to be an essential part of the sensory integration (38), and it could be expected that there were morphological changes of parietal regions in high risk and schizophrenic patients. However, our results did not show the change of parietal region. Previous study that intended the early-onset schizophrenia showed the parietal abnormality (39), though the other studies unlikely show the parietal change (1,3). Previous childhood-onset schizophrenic study suggested that schizophrenic brain change in parietal lobe was obvious in youth, but the changes appear to be diminished with age (40). Our results that did not show the parietal change may be because the mean age of our participants was in the middle of 20s, and the loss of parietal lobe was attenuated.

Functional, anatomical and histopathological studies provide considerable evidence that the connections between subregions of the cingulate cortex and other brain regions are disturbed in schizophrenia (41,42). Previous neuroimaging studies have shown abnormalities of ACC in schizophrenia (43). In this study, the volume loss in ACC was detected not in the high-risk patient group but in the first-episode schizophrenia group. This may result from the fact that the schizophrenic brain shrinkage progress from posterior to anterior (44). Further follow-up studies that focus on the conversion from the prodromal state into schizophrenia are needed to reveal the pattern of ACC shrinkage.

In this study, we evaluated only a few participants. Further work with the large sample size will be necessary to confirm our results.

In summary, the present study confirms that there are proceeding changes in the brains of schizophrenic patients at the pre-onset state. The findings indicate that brain impairments may be altered in patients at the pre-onset of psychosis, possibly as a result of disrupted developmental mechanisms, and, furthermore, that these pathological changes may be predictive of functional outcome. The present observations remain to be replicated with a larger study population and with follow-up of the high-risk patients.

References

1. Shenton ME, Dickey CC, Frumin M, McCarley RW. A review of MRI findings in schizophrenia. Schizophr Res 2001;**49**:1–52.
2. Kanaan RA, Kim JS, Kaufmann WE, Pearlson GD, Barker GJ, McGuire PK. Diffusion tensor imaging in schizophrenia. Biol Psychiatry 2005;**58**:921–929.
3. Kubicki M, McCarley R, Westin CF, Park HJ, Maier S, Kikinis R. A review of diffusion tensor imaging studies in schizophrenia. J Psychiatr Res 2007;**41**:15–30.
4. Bearden CE, Rosso IM, Hollister JM, Sanchez LE, Hadley T, Cannon TD. A prospective cohort study of childhood behavioral deviance and language abnormalities as predictors of adult schizophrenia. Schizophr Bull 2000;**26**:395–410.
5. Jones P, Rodgers B, Murray R, Marmot M. Child development risk factors for adult schizophrenia in the British 1946 birth cohort. Lancet 1994;**344**:1398–1402.
6. Yung AR, Phillips LJ, McGorry PD et al. Prediction of psychosis: a step towards indicated prevention of schizophrenia. Br J Psychiatry 1998;**172**:14–20.
7. Bloemen OJ, de Koning MB, Schmitz N et al. White-matter markers for psychosis in a prospective ultra-high-risk cohort. Psychol Med 2009;**9**:1–8.
8. Borgwardt SJ, Riecher-Rössler A, Dazzan P et al. Regional gray matter volume abnormalities in the at risk mental state. Biol Psychiatry 2007;**61**:1148–1156.
9. Hoptman MJ, Nierenberg J, Bertisch HC et al. A DTI study of white matter microstructure in individuals at high genetic risk for schizophrenia. Schizophr Res 2008;**106**: 115–124.
10. Jacobson S, Kelleher I, Harley M et al. Structural and functional brain correlates of subclinical psychotic symptoms in 11-13 year old schoolchildren. Neuroimage 2010;**49**:1875–1885.
11. Job DE, Whalley HC, Johnstone EC, Lawrie SM. Grey matter changes over time in high risk subjects developing schizophrenia. Neuroimage 2005;**25**:1023–1030.
12. Exner JE Jr. A primer for Rorschach interpretation. Asheville, NC: Rorschach Workshops; 2000.
13. Exner JE Jr. 2000 alumni newsletter. Asheville, NC: Rorschach Workshops; 2000.
14. Miller TJ, McGlashan TH, Rosen JL et al. Prospective diagnosis of the initial prodrome for schizophrenia based on the structured interview of for prodromal syndromes: preliminary evidence of interrater reliability and predictive validity. Am J Psychiatry 2002;**159**:863–865.
15. Dao TK, Prevatt FA. Psychometric evaluation of the Rorschach comprehensive system's perceptual thinking index. J Pers Assess 2006;**86**:180–189.
16. Ilonen T, Heinimaa M, Korkeila J, Svirskis T, Salokangas RK. Differentiating adolescents at clinical high risk for psychosis from psychotic and non-psychotic patients with the Rorschach. Psychiatry Res 2010;**179**: 151–156.
17. Kimhy D, Corcoran C, Harkavy-Friedman JM, Ritzler B, Javitt DC, Malaspina D. Visual form perception: a comparison of individuals at high risk for psychosis, recent onset schizophrenia and chronic schizophrenia. Schizophr Res 2007;**97**:25–34.
18. Borgwardt SJ, McGuire PK, Aston J et al. Reductions in frontal, temporal and parietal volume associated with the onset of psychosis. Schizophr Res 2008;**106**:108–114.
19. Karlsgodt KH, Niendam TA, Bearden CE, Cannon TD. White matter integrity and prediction of social and role functioning in subjects at ultra-high risk for psychosis. Biol Psychiatry 2009;**66**:562–569.
20. Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S. Fiber tract-based atlas of human white matter anatomy. Radiology 2004;**230**:77–87.

21. Good CD, Johnsrude I, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ. Cerebral asymmetry and the effect of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. Neuroimage 2001;**14**:685–700.
22. Snook L, Plewes C, Beaulieu C. Voxel based versus region of interest analysis in diffusion tensor imaging of neurodevelopment. Neuroimage 2007;**34**:243–252.
23. Huang H, Zhang J, Jiang H, Wakana S, Poetscher L, Miller MI. DTI tractography-based parcellation of white matter: application of the mid-sagittal morphology of corpus callosum. Neuroimage 2005;**26**:295–305.
24. Wakana S, Caprihan A, Panzenboeck MM et al. Reproducibility of quantitative tractography methods applied to cerebral white matter. Neuroimage 2007;**36**:630–644.
25. McGorry PD, Yung AR, Phillips LJ. The "close-in" or ultra high-risk model: a safe and effective strategy for research and clinical intervention in prepsychotic mental disorder. Schizophr Bull 2003;**29**:771–790.
26. Pantelis C, Velakoulis D, McGorry PD et al. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. Lancet 2003;**361**:281–288.
27. Kasai K, Shenton ME, Salisbury DF et al. Progressive decrease of left superior temporal gyrus gray matter volume in patients with first-episode schizophrenia. Am J Psychiatry 2003;**160**:156–164.
28. Szeszko PR, Ardekani BA, Ashtari M et al. White matter abnormalities in first-episode schizophrenia or schizoaffective disorder: a diffusion tensor imaging study. Am J Psychiatry 2005;**162**:602–605.
29. van Haren NE, Hulshoff Pol HE, Schnack HG et al. Focal gray matter changes in schizophrenia across the course of the illness: a 5-year follow-up study. Neuropsychopharmacology 2007;**32**:2057–2066.
30. Andreasen NC, O'Leary DS, Flaum M et al. Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. Lancet 1997;**349**:1730–1734.
31. Ardekani BA, Nierenberg J, Hoptman MJ, Javitt DC, Lim KO. MRI study of white matter diffusion anisotropy in schizophrenia. Neuroreport 2003;**14**:2025–2029.
32. Mathalon DH, Sullivan EV, Lim KO, Pfefferbaum A. Progressive brain volume changes and the clinical course of schizophrenia in men: a longitudinal magnetic resonance imaging study. Arch Gen Psychiatry 2001;**58**:148–157.
33. Chan WY, Yang GL, Chia MY, Lau IY, Sitoh YY, Nowinski WL, Sim K. White matter abnormalities in first-episode schizophrenia: a combined structural MRI and DTI study. Schizophr Res 2010;**119**:52–60.
34. Hubl D, Koenig T, Strik W et al. Pathways that make voices: white matter changes in auditory hallucinations. Arch Gen Psychiatry 2004;**61**:658–668.
35. Shergill SS, Kanaan RA, Chitnis XA et al. A diffusion tensor imaging study of fasciculi in schizophrenia. Am J Psychiatry 2007;**164**:467–473.
36. Yamasaki S, Yamasue H, Abe O et al. Reduced planum temporale volume and delusional behaviour in patients with schizophrenia. Eur Arch Psychiatry Clin Neurosci 2007;**257**:318–324.
37. Benetti S, Mechelli A, Picchioni M, Broome M, Williams S, McGuire P. Functional integration between the posterior hippocampus and prefrontal cortex is impaired in both first episode schizophrenia and the at risk mental state. Brain 2009;**132**:2426–2436.
38. Torrey EF. Schizophrenia and the inferior parietal lobule. Schizophr Res 2007;**97**:215–225.
39. Kyriakopoulos M, Perez-Iglesias R, Woolley JB et al. Effect of age at onset of schizophrenia on white matter abnormalities. Br J Psychiatry 2009;**195**:346–353.
40. Greenstein D, Lerch J, Shaw P et al. Childhood onset schizophrenia: cortical brain abnormalities as young adults. J Child Psychol Psychiatry 2006;**47**:1003–1012.
41. Benes FM. Neurobiological investigations in cingulate cortex of schizophrenic brain. Schizophr Bull 1993;**19**: 537–549.
42. Benes FM. Emerging principles of altered neural circuitry in schizophrenia. Brain Res Rev 2000;**31**:251–269.
43. Glahn DC, Laird AR, Ellison-Wright I et al. Meta-analysis of gray matter anomalies in schizophrenia: application of anatomic likelihood estimation and network analysis. Biol Psychiatry 2008;**64**:774–781.
44. Thompson PM, Vidal C, Giedd JN et al. Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proc Natl Acad Sci U S A 2001;**98**:11650–11655.

Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2012: 1–7
All rights reserved
DOI: 10.1111/j.1600-0447.2012.01853.x

© 2012 John Wiley & Sons A/S
ACTA PSYCHIATRICA SCANDINAVICA

Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation

Ota M, Ishikawa M, Sato N, Hori H, Sasayama D, Hattori K, Teraishi T, Nakata Y, Kunugi H. Glutamatergic changes in the cerebral white matter associated with schizophrenic exacerbation.

Objective: Glutamatergic dysfunction in the brain has been implicated in the pathophysiology of schizophrenia. This study was aimed to examine several brain chemical mediators, including Glx (glutamate + glutamine), using ^{1}H magnetic resonance spectroscopy (MRS) in medicated patients with schizophrenia, with and without psychotic exacerbation.
Method: ^{1}H MRS was acquired in 24 patients with schizophrenia, with psychotic exacerbation; 22 patients without exacerbation; and 27 age- and sex-matched healthy volunteers. The levels of metabolites were measured in the left frontal and inferior parietal white matter and compared across the three groups.
Results: The Glx level was significantly elevated in the left inferior parietal white matter in the patients with psychotic exacerbation in comparison with that in the healthy volunteers and the patients without exacerbation ($P < 0.05$). We also detected that there was a significant correlation between Positive and Negative Syndrome Scale-positive scale and Glx level in the left parietal white matter ($r = 0.51$, $P < 0.001$).
Conclusion: Higher than normal Glx levels indicate glutamatergic overactivity in the left inferior parietal white matter with schizophrenic exacerbation, a finding that is in accordance with the glutamatergic hypothesis in schizophrenia. The Glx level measured by ^{1}H MRS could be a biomarker for exacerbation in schizophrenia.

M. Ota[1], M. Ishikawa[2], N. Sato[3], H. Hori[1], D. Sasayama[1], K. Hattori[1], T. Teraishi[1], Y. Nakata[3], H. Kunugi[1]

[1]Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, [2]Department of Psychiatry, National Center of Neurology and Psychiatry and [3]Department of Radiology, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan

Key words: glutamine + glutamate; exacerbation; magnetic resonance spectroscopy; schizophrenia

Miho Ota, Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry 4-1-1, Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan.
E-mail: ota@ncnp.go.jp

Accepted for publication February 17, 2012

Significant outcomes

- The Glx level was significantly elevated in the left inferior parietal white matter in patients with schizophrenia, with psychotic exacerbation.
- The Glx level in the patients with schizophrenia, with psychotic exacerbation was significantly elevated compared with that in the non-exacerbated patients.

Limitations

- The regions of interests (ROIs) were limited to the left frontal white matter and the inferior parietal white matter. Further work with other several ROIs will be necessary to confirm our results.
- The sample size was relatively small. More studies with larger sample sizes are warranted.

Introduction

N-methyl-*D*-aspartate (NMDA) receptor antagonists such as phencyclidine and ketamine induce symptoms closely resembling schizophrenia (1). These findings have suggested the presence of altered NMDA-glutamatergic function in schizophrenia; however, the glutamatergic model of

schizophrenia cannot be fully tested without *in vivo* measures of glutamate metabolism. A previous 1.5-tesla (T) ^{1}H magnetic resonance spectroscopy (MRS) study found higher levels of glutamine and similar levels of *N*-acetylaspartate (NAA) in the left medial prefrontal region of never-treated patients with schizophrenia compared with healthy volunteers (2). Another study using 4.0-T ^{1}H MRS found increased levels of glutamine and normal levels of NAA in the left anterior cingulate and thalamic regions of never-treated, first-episode patients with schizophrenia compared with healthy volunteers (3). In addition, one study showed increased levels of γ-aminobutyric acid and Glx (glutamine + glutamate) in the medial prefrontal cortex of unmedicated patients with schizophrenia compared with controls (4). A review of ^{1}H MRS studies concerning the glutamate in schizophrenia showed an overall increase in glutamine levels in patients with schizophrenia at the early phase of the disease (5). In contrast, one study showed decreased levels of glutamine in the anterior cingulate of chronic schizophrenia patients (6).

Astrocyte is essential for glutamate homeostasis, and its deficit in glutamate uptake might contribute to elevated extracellular glutamate and resultant excitotoxicity to neurons and glia in subjects with schizophrenia. Ultrastructural changes in the cells responsible for myelination, namely oligodendrocytes, have been observed in postmortem and biopsy tissues of patients with schizophrenia. Oligodendrocytes are known to be vulnerable to excitotoxicity induced by hyperglutamatergic states via the overactivation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate receptors (7, 8). The rate of transmission of information in neural networks is critical for the integration of information processing. Dysmyelination causes focal disruption of such networks and dyssynchrony in the reverberating networks that integrate perception, thought and action, which may give rise to psychotic symptoms (9). Degenerative changes have been described in the oligodendroglia and myelinated fibers in postmortem and biopsy tissues of patients with schizophrenia (10). The recent glutamatergic model of schizophrenia has suggested a possible role of glutamate-induced neurodegeneration (11, 12). However, little is known regarding changes in glutamate levels in the white matter in schizophrenia. It is worthwhile to evaluate the levels of metabolites in the left inferior parietal and middle frontal white matter regions that are regarded as the areas associated with psychopathology of schizophrenia (13) and where there are sufficient white matter volumes to put regions of interest (ROI) large enough to get adequate signal-to-noise ratio. We hypothesized that patients with psychotic exacerbation would have higher levels of Glx compared with patients without exacerbation and healthy controls.

Aims of the study

The present study was aimed to examine brain chemical mediators, including Glx, in white matter areas using ^{1}H MRS in schizophrenia patients with and without psychotic exacerbation and healthy controls.

Material and methods

Subjects

The subjects were 24 schizophrenia in-patients with psychotic exacerbation and 22 outpatients without exacerbation. The exacerbation group included one patient with a first episode of psychosis. Consensus diagnosis by at least two psychiatrists was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, (DSM-IV) criteria (14), on the basis of unstructured interviews and information from medical records. Patients who met both of the following two criteria were defined as having exacerbation: i) symptom scores of ≥4 (at least moderate) on at least two of the Positive and Negative Syndrome Scale [PANSS, (15)] items of hostility, excitement, tension, uncooperativeness, and poor impulse control, and a total combined score of ≥17 for these items and ii) psychiatric hospitalization (16, 17). All the in-patients were admitted to an acute psychiatric ward of the National Center of Neurology and Psychiatry Hospital, Japan. Most in-patients underwent an MRS scan within 4 weeks (mean period = 14.5 ± 13.6 days) after their admission to this study. Outpatients were recruited and chosen from those patients who were treated by the authors and those who voluntarily responded to our poster announcement in the Hospital. Twenty-seven age- and sex-matched healthy subjects were recruited from the community through local magazine advertisements and our website announcement. Participants were interviewed for enrollment by research psychiatrists using the Japanese version of the Mini-International Neuropsychiatric Interview (18, 19). Participants were excluded if they had a prior medical history of central nervous system disease or severe head injury or if they met the criteria for substance abuse or dependence. Those who demonstrated no history of psychiatric illness or contact with psychiatric services were enrolled

Table 1. Characteristics of the study sample

	Healthy volunteers	Patients with schizophrenia	
		Exacerbation (+)	Exacerbation (−)
Male/Female	13/14	11/13	12/10
Age	42.8 ± 15.1	45.0 ± 15.4	41.3 ± 11.2
Education	14.0 ± 2.8	13.3 ± 3.1	14.1 ± 2.6
Age at onset		23.5 ± 7.4	25.7 ± 11.8
Duration of illness		20.4 ± 15.6	14.6 ± 8.8
Chlorpromazine equivalent dose of medication (mg)		838.1 ± 472.2*	354.2 ± 366.1*
PANSS positive		17.4 ± 5.2*	11.7 ± 4.7*
PANSS negative		17.5 ± 6.9	15.8 ± 6.5
PANSS general		34.3 ± 9.9	28.7 ± 9.4

PANSS; positive and negative syndrome scale.
*There were significant differences between the schizophrenia groups ($P < 0.001$).

as controls in this study. The characteristics of the participants are shown in Table 1. Daily doses of antipsychotics, including depot antipsychotics, were converted to chlorpromazine equivalents using published guidelines (20, 21).

After the study was explained to the subjects, written informed consent was obtained for participation in the study from every subject. This study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

MRI data acquisition

MR imaging was performed on a Magnetom Symphony 1.5-T (Siemens, Erlangen, Germany). Spectra were acquired with a point-resolved pulse sequence ('PRESS', repetition time = 1500 ms, echo time = 30 ms, 1024 points, 1000 Hz spectral width, 160 averages water-suppressed and 30 averages without water suppression) from a 1.5 × 1.5 × 2.5 cm voxel placed in the left middle frontal white matter region and a 1.5 × 2.5 × 1.5 cm voxel in the left inferior parietal white matter region (see Fig. 1 for illustration of voxel placement) so as to exclude cerebrospinal fluid and gray matter contained in the ROI as much as possible. We visually checked T2 imaging and located ROIs to avoid cerebrospinal fluid. ROIs were positioned by the scanner operator based on anatomical landmarks. The scan took approximately 10 min per region. Global and local shimmings were performed before the ^{1}H-MRS sequence.

Magnetic resonance spectroscopy analysis

Water-suppressed spectra were analyzed using 'LCModel' (20) a fully automated, commercially

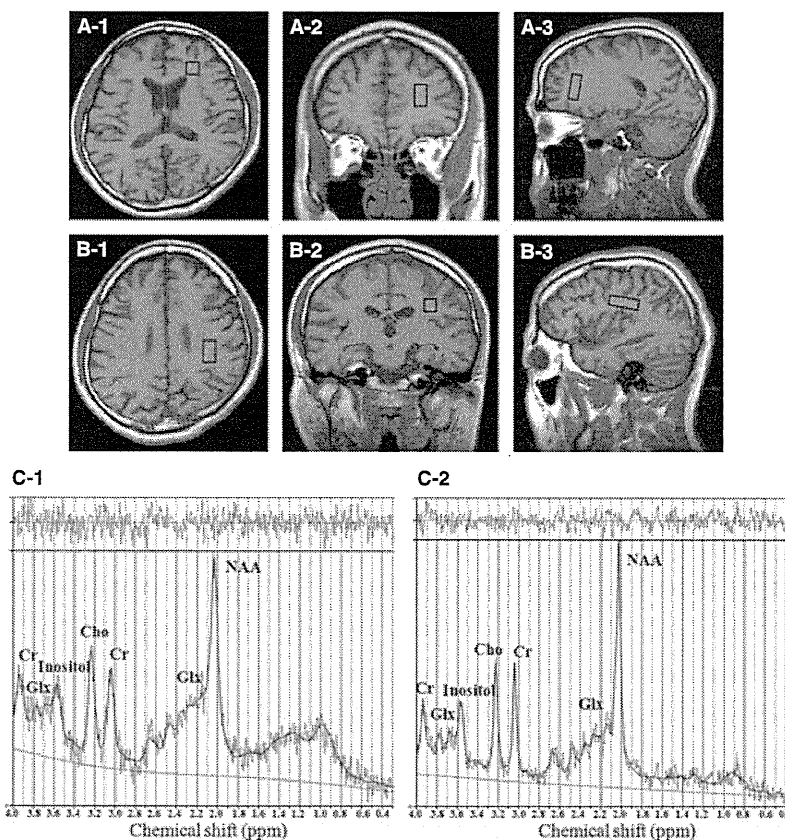


Fig. 1. Regions of interests placement in two regions of interest; left middle frontal white matter (A-1, 2, 3) and left inferior parietal white matter (B-1, 2, 3) and representative spectra of frontal white matter (C-1) and parietal white matter (C-2). Figure 1(C-1 and C-2) shows strong sharp resonances from creatine at 3.0 and 4.0 ppm, Glutamine + Glutamate (Glx) at 2.0–2.5 ppm and 3.8, Glycerophosphocholine + Phosphocholine (Cho) at 3.3 ppm, Inositol at 3.6 ppm and *N*-acetylaspartate at 2.0 ppm.

available curve-fitting software that uses a least squares analysis method for estimating metabolite concentrations in the millimolar range. LCModel was set to 0.2–4.0 ppm to cover the whole spectral region of the metabolites of interest. The quantification model included the following metabolites: aspartate, creatine, inositol, L-alanine, γ-aminobutyric acid, glucose, Glx, glycerophosphocholine + phosphocholine, guanidinoacetate, L-lactate, NAA, *N*-acetylaspartylglutamate, *scyllo*-inositol, and taurine. For each spectrum, the area under each peak was normalized to the unsuppressed water peak [corrected for water T1 relaxation (22)], yielding metabolite concentrations. The fitting quality of each spectrum is shown as percent SD, and those with SD values over 20% were excluded from analysis.

Statistical analysis

The differences in age, sex, and years of education among patients with schizophrenia, with and without exacerbation and healthy subjects were evaluated using analysis of variance (ANOVA). The differences in concentration of creatine, Glx, glycerophosphocholine + phosphocholine, inositol, and NAA, those percent SD were under 20%, in the left frontal and inferior parietal white matter in patients with schizophrenia, with and without exacerbation of psychosis and in healthy subjects were also evaluated using ANOVA. The *post hoc* test was carried out using Bonferroni's correction for multiple comparisons. The differences in age at onset, duration of illness, the chlorpromazine equivalent dose of medication, and the subscales of PANSS between the groups of schizophrenia were evaluated using a two-sample *t*-test.

The statistical correlations between the concentrations of creatine, Glx, glycerophosphocholine + phosphocholine, inositol, and NAA on the one hand and age, chlorpromazine equivalent dose of medication, and the subscales of PANSS on the other in the two patient groups were examined using Pearson's correlation analysis.

Statistical analyses were performed using SPSS Statistics for Windows 17.0 software (SPSS Japan, Tokyo, Japan).

Results

There was no statistically significant difference in age, sex, or education years across the three groups, and no difference in age at onset, duration of illness, or negative or general subscales of PANSS was found between the patient groups with and without exacerbation. The differences in chlorpromazine-equivalent dose of medication ($t = -3.86$; d$f = 44$; $P < 0.001$) and positive PANSS score ($t = -3.91$; d$f = 44$; $P < 0.001$) between the two groups of patients were statistically significant. Six of the 22 patients without psychotic exacerbation and one of the 24 patients with exacerbation were medicated only by typical antipsychotics. The remaining patients were treated with the atypical or both antipsychotics.

There was a significant effect of group on the Glx concentration in the left inferior parietal white matter ($F_{2,70} = 5.5$; $P = 0.006$). *Post hoc t*-test analyses showed that the Glx level was higher in the patients with exacerbation than in the healthy subjects ($P = 0.009$, corrected) or the patients without exacerbation ($P = 0.033$, corrected) (Table 2).

There was no significant correlation of the concentration of any metabolite with age, education years, chlorpromazine-equivalent dose, or PANSS subscale scores except for a correlation between age and NAA in the left parietal white matter ($r = -0.48$, $P = 0.001$), a correlation between age and inositol in the frontal white matter ($r = 0.45$, $P = 0.002$), and a correlation between PANSS-positive scale and Glx level in the left parietal white matter ($r = 0.51$, $P < 0.001$).

Discussion

To our knowledge, this is the first study that examined the glutamatergic change in relation to the exacerbation of psychotic symptoms. Some previous studies examined glutamatergic changes in first-episode schizophrenia (2, 3, 23), chronic schizophrenia (6, 24–26), and subjects at high risk of schizophrenia (27). However, no study has thus far focused on the possible relationship between the exacerbation of psychotic symptoms and glutamatergic activity.

In our ^{1}H MRS study, a significant increase in the Glx level was observed in the left inferior parietal white matter region in schizophrenia patients with exacerbation compared with healthy subjects and patients without exacerbation. As the majority of physiologically active glutamate is derived from glutamine (28), the higher level of Glx indicates greater glutamatergic activity, which is consistent with the previously described glutamatergic model of schizophrenia (11, 29, 30). Released glutamate is taken up by astrocytes, where it is converted to glutamine, transported back to the presynaptic neuron, and reconverted to glutamate (28). Our findings are in the same direction as those reported in previous studies. A study of first-episode never-medicated

Table 2. Characteristics of the metabolites using magnetic resonance spectroscopy

	Healthy volunteers		Patients with schizophrenia			
			Exacerbation (+)		Exacerbation (−)	
	Lt FW	Lt PW	Lt FW	Lt PW	Lt FW	Lt PW
Creatine* (mean ± SD)	3.4 ± 0.4	3.5 ± 0.4	3.7 ± 0.5	3.7 ± 0.4	3.5 ± 0.6	3.6 ± 0.3
Mean % SD (mean ± SD)	6.0 ± 1.0	5.7 ± 0.8	6.1 ± 1.2	5.4 ± 0.8	6.1 ± 2.0	5.3 ± 0.8
Number of subjects	N = 27	N = 27	N = 22	N = 23	N = 22	N = 22
NAA* (mean ± SD)	5.0 ± 0.7	5.7 ± 0.8	4.9 ± 0.6	5.6 ± 0.6	4.9 ± 0.6	5.7 ± 0.6
Mean % SD (mean ± SD)	6.4 ± 2.1	6.8 ± 2.0	6.7 ± 2.3	6.7 ± 1.3	7.0 ± 2.5	6.8 ± 1.8
Number of subjects	N = 27	N = 27	N = 21	N = 24	N = 21	N = 22
Glutamine + Glutamate* (mean ± SD)	7.1 ± 1.3	6.5 ± 0.9‡	7.2 ± 1.3	7.2 ± 0.8‡ †	7.3 ± 1.0	6.6 ± 0.7†
Mean % SD (mean ± SD)	11.0 ± 2.9	10.4 ± 2.5	12.0 ± 4.0	9.2 ± 2.2	10.0 ± 2.2	9.8 ± 2.2
Number of subjects	N = 26	N = 27	N = 22	N = 24	N = 20	N = 22
Glycerophosphocholine + Phosphocholine* (mean ± SD)	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.1
Mean % SD (mean ± SD)	5.8 ± 1.1	5.5 ± 0.8	5.7 ± 1.0	5.1 ± 0.8	5.9 ± 2.5	5.0 ± 0.7
Number of subjects	N = 27	N = 27	N = 22	N = 24	N = 21	N = 22
Inositol* (mean ± SD)	3.2 ± 0.7	3.2 ± 0.6	3.4 ± 0.7	3.1 ± 0.6	3.6 ± 0.9	3.2 ± 0.6
Mean % SD (mean ± SD)	8.3 ± 2.2	8.1 ± 1.9	8.5 ± 2.9	7.9 ± 2.0	7.4 ± 2.5	7.2 ± 1.5
Number of subjects	N = 27	N = 27	N = 22	N = 24	N = 21	N = 22

Lt FW, left frontal white matter; Lt PW, left inferior parietal white matter; NAA, *N*-acetyl-aspartate; SD, standard deviation.
*Metabolites are presented in institutional values.
Analysis of variance with Bonferroni's correction for *post hoc* test: †There was a significant difference between the schizophrenia groups ($P < 0.05$)
Analysis of variance with Bonferroni's correction for *post hoc* test: ‡There was a significant difference between the inpatients with schizophrenia and healthy volunteers ($P < 0.05$).

acute-phase schizophrenia showed elevated glutamine levels in the anterior cingulate and thalamus (3). In contrast, reduced glutamine levels were observed in the anterior cingulate of chronic stable-phase schizophrenia patients (6). Other previous studies found elevated glutamate or Glx/creatine in the frontal white matter of both medication-naïve and treated patients with schizophrenia (24, 25). Because these first two studies focused on glutamine, whereas the latter two assessed glutamate or Glx/creatine, it is difficult to compare these results directly. However, these results imply a pronounced dysfunction in the glutamate–glutamine homeostasis. Our results are also consistent with a recent report of increased glutamine in healthy participants administered a low dose of ketamine, a drug known to produce schizophrenia-like symptoms (31), and with a report of increased glutamatergic metabolites in adolescents at high risk of developing schizophrenia (27).

Hyperglutamatergic states (and subsequent hypoglutamatergic states) have been suggested to be relevant to the process of schizophrenia (2, 22, 32). Among glial cells, oligodendroglia, which are responsible for myelination, are particularly vulnerable to glutamate-mediated glial cell damage (7). Disruption of the speed of neurotransmission throughout the web of reverberating myelinited networks within the brain may desynchronize and disrupt elements of perception, attention, complex processing of information, and behaviour in schizophrenia. Herein, we provide evidence of changes in such myelin-containing white matter associated with psychosis exacerbation.

Our results showed that there was no difference in the concentration of Glx in the frontal white matter region between the two groups of patients. A previous study showed that during the development of schizophrenia in early adolescent subjects, a dynamic wave of gray matter loss occurred, starting in the parietal association cortices and proceeding frontally to envelop the dorsolateral prefrontal cortex and temporal cortices, including the superior temporal gyri (33). Our results also indicated that disruption of the parietal region with psychotic exacerbation may account for psychotic impairment.

There are several limitations to this study. First, the regions of interest were limited to the left frontal white matter and inferior parietal white matter. Therefore, we obtained no data on metabolic changes in other brain regions that could be related to psychotic exacerbation. In particular, temporal regions were regarded as the areas associated with positive symptoms of schizophrenia (34). We put the relatively large ROIs purely on white matter not to mix the data derived from gray matter and white matter, then it is difficult to put rectangle large ROI in temporal region. Further work with the high magnetic field that adds the high signal-to-noise ratio will be necessary. Secondly, all patients were medicated, and the doses of antipsychotic medication in the patients with exacerbation were significantly higher than those

in the patients without exacerbation. Although using chlorpromazine equivalents to compare dosages of antipsychotic medication is controversial because the conversion of the atypical antipsychotics to chlorpromazine equivalents might not be the same as for the typical antipsychotics (35), one might suspect that the differential dose may account for the difference in Glx between the two groups. However, Theberge et al. (23) showed that glutamine and glutamate levels did not significantly decrease in participants with schizophrenia whose symptoms were stabilized with medication for 10 months, suggesting that medication does not have a major effect on glutamine and glutamate levels. A more recent study by 4T ^{1}H-MRS has also found no changes in NAA, Gln/Glu or any of the other metabolites in any of the regions of interest after antipsychotic treatment in early schizophrenia (36). Moreover, in the present study, chronic patients without exacerbation who received antipsychotic medication showed Glx concentrations similar to those of the controls. Thus, the observed increase in Glx level in the exacerbated group is unlikely to be attributable to the higher dose of antipsychotic medication.

Thirdly, the sample size of the present study was relatively small. Replication studies in larger samples are clearly required.

Our ^{1}H MRS study showed a significantly increased level of Glx in the left inferior parietal white matter region in patients with schizophrenia, with exacerbation compared with healthy subjects and patients without exacerbation. These results suggest that glutamatergic dysfunction in the parietal white matter is involved in psychotic exacerbation. If our results are replicated, the quantification of the brain metabolites using MRS could be used as a good biomarker for exacerbation and could provide valuable hints to the brain pathology underlying the exacerbation of schizophrenia.

Acknowledgements

This study was supported by Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare), Intramural Research Grant (20-3; 21-9) for Neurological and Psychiatric Disorders of NCNP, a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS), and a grant from Core Research of Evolutional Science & Technology (CREST), Japan Science and Technology Agency (JST) (H.K.).

Declaration of interests

None.

References

1. Tsai G, van Kammen DP, Chen S, Kelley ME, Grier A, Coyle JT. Glutamatergic neurotransmission involves structural and clinical deficits of schizophrenia. Biol Psychiatry 1998;**44**:667–674.
2. Bartha R, Williamson PC, Drost DJ et al. Measurement of glutamate and glutamine in the medial prefrontal cortex of never-treated schizophrenic patients and healthy controls using proton magnetic resonance spectroscopy. Arch Gen Psychiatry 1997;**54**:959–965.
3. Theberge J, Bartha R, Drost D et al. Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. Am J Psychiatry 2002;**159**:1944–1946.
4. Kegeles LS, Mao X, Stanford AD et al. Elevated Prefrontal Cortex γ-Aminobutyric Acid and Glutamate-Glutamine Levels in Schizophrenia Measured *In Vivo* With Proton Magnetic Resonance Spectroscopy. Arch Gen Psychiatry 2012; DOI:10.1001/archgenpsychiatry.2011.1519 [Epub ahead of print].
5. Marsman A, van den Heuvel MP, Klomp DW et al. Glutamate in Schizophrenia: A Focused Review and Meta-Analysis of 1H-MRS Studies. Schizophr Bull 2011; DOI: 10.1093/schbul/sbr069 [Epub ahead of print].
6. Theberge J, Al-Semaan Y, Williamson P et al. Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. Am J Psychiatry 2003;**160**:2231–2233.
7. Matute C, Domercq M, Sanchez-Gomez MV. Glutamate-mediated glial injury: mechanisms and clinical importance. Glia 2006;**53**:212–224.
8. McDonald JW, Althomsons SP, Hyre KL, Choi DW, Goldber MP. Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat Med 1998;**4**:291–297.
9. Hyde TM, Ziegler JC, Weinberger DR. Psychiatric disturbance in metachromatic leukodystrophy. Insights into the neurobiology of psychosis. Arch Neurol 1992;**49**:401–406.
10. Christensen J, Holcomb J, Garver DL. State-related changes in cerebral white matter may underlie psychosis exacerbation. Psychiatry Res 2004;**130**:71–78.
11. Olney JW, Farber NB. Glutamate receptor dysfunction in schizophrenia. Arch Gen Psychiatry 1995;**52**:998–1007.
12. Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 2001;**158**:1367–1377.
13. Rowland LM, Spieker EA, Francis A, Barker PB, Carpenter WT, Buchanan RW. White matter alterations in deficit schizophrenia. Neuropsychopharmacology 2009;**34**:1514–1522.
14. American Psychiatric Association. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. 4th edn, Washington, DC: American Psychiatric Press, 1994.
15. Kay SR, Opler LA, Fiszbein A. Positive and Negative Syndrome Scale (PANSS) manual. Schizophr Bull 1987;**13**:261–276.
16. Xiang YQ, Zhang ZJ, Weng YZ et al. Serum concentrations of clozapine and norclozapine in the prediction of relapse of patients with schizophrenia. Schizophr Res 2006;**83**: 201–210.
17. Canuso CM, Dirks B, Carothers J et al. Randomized, double-blind, placebo-controlled study of paliperidone extended-release and quetiapine in inpatients with recently

exacerbated schizophrenia. Am J Psychiatry 2006;**166**:691–701.
18. Sheehan DV, Lecrubier Y, Sheehan KH et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998;**59**:22–57.
19. Otsubo T, Tanaka K, Koda R, Shinoda J, Sano N, Tanaka S. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin Neurosci 2005;**59**:517–526.
20. American Psychiatric Association. Practice Guidelines for the Treatment of Patients with Schizophrenia. Washington, DC: American Psychiatric Press, 1997.
21. Inagaki A, Inada T, Fujii Y, Yagi G. Equivalent Dose of Psychotropics. Tokyo: Seiwa Shoten, 1999.
22. Provencher SW. Estimation of metabolite concentrations from localized *in vivo* proton NMR spectra. Magn Reson Med 1993;**30**:672–679.
23. Theberge J, Williamson KE, Aoyama N et al. Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. Br J Psychiatry 2007;**191**:325–354.
24. Choe BY, Kim KT, Suh TS et al. 1H magnetic resonance spectroscopy characterization of neuronal dysfunction in drug-naive, chronic schizophrenia. Acad Radiol 1994;**1**:211–216.
25. van Elst LT, Valerius G, Buchert M et al. Increased prefrontal and hippocampal glutamate concentration in schizophrenia: evidence from a magnetic resonance spectroscopy study. Biol Psychiatry 2005;**58**:724–730.
26. Pakkenberg B, Scheel-Kruger J, Kristiansen LV. Schizophrenia; from structure to function with special focus on the mediodorsal thalamic prefrontal loop. Acta Psychiatr Scand 2009;**120**:345–354.
27. Tibbo P, Hanstock C, Valiakalayil A, Allen P. 3-T proton MRS investigation of glutamate and glutamine in adolescents at high genetic risk for schizophrenia. Am J Psychiatry 2004;**161**:1116–1118.
28. Rothman DL, Sibson NR, Hyder F. *In vivo* nuclear magnetic resonance spectroscopy studies of the relationship between the glutamate-glutamine neurotransmitter cycle and functional neuroenergetics. Philos Trans R Soc London B Biol Sci 1999;**354**:1165–1167.
29. Carlsson A, Hansson LO, Waters N. A glutamatergic deficiency model of schizophrenia. Br J Psychiatry 1999;**174**:2–6.
30. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991;**148**:1301–1308.
31. Rowland LM, Bustillo JR, Mullins PG et al. Effects of ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. Am J Psychiatry 2005;**162**:394–396.
32. Keshavan MS. Development, disease and degeneration in schizophrenia: a unitary pathophysiological model. J Psychiatr Res 1999;**33**:513–521.
33. Thompson PM, Vidal C, Giedd JN et al. Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. PNAS 2001;**98**:11650–11655.
34. Hubl D, Koenig T, Strik W et al. Pathways that make voices: white matter changes in auditory hallucinations. Arch Gen Psychiatry 2004;**61**:658–668.
35. Nielsen J, Le Quach P, Emborg C et al. 10-year trends in the treatment and outcomes of patients with first-episode schizophrenia. Acta Psychiatr Scand 2010;**122**:356–366.
36. Bustillo JR, Rowland LM, Mullins P et al. 1H-MRS at 4 Tesla in minimally treated early schizophrenia. Mol Psychiatry 2010;**15**:629–636.

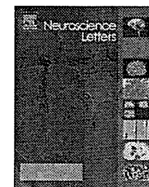
Author's personal copy

Neuroscience Letters 476 (2010) 104–109

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Cortical neurons from intrauterine growth retardation rats exhibit lower response to neurotrophin BDNF

Midori Ninomiya[a,b], Tadahiro Numakawa[b,c,*], Naoki Adachi[b,c], Miyako Furuta[b], Shuichi Chiba[b], Misty Richards[b,d], Shigenobu Shibata[a], Hiroshi Kunugi[b,c]

[a] *Laboratory of Physiology and Pharmacology, School of Advanced Science and Engineering, Waseda University, Shinjuku-ku, Tokyo, Japan*
[b] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo 187-8502, Japan*
[c] *Core Research for Evolutional Science and Technology Program (CREST), Japan Science and Technology Agency (JST), Saitama 332-0012, Japan*
[d] *Albany Medical College, Albany, NY 12208, USA*

ARTICLE INFO

Article history:
Received 21 December 2009
Received in revised form 31 March 2010
Accepted 31 March 2010

Keywords:
BDNF
TrkB
Cell death
Intrauterine growth retardation

ABSTRACT

Intrauterine growth retardation (IUGR) is putatively involved in the pathophysiology of schizophrenia. The animal model of IUGR induced by synthetic thromboxane A2 (TXA2) is useful to clarify the effect of IUGR on pups' brains, however, analysis at the cellular level is still needed. Brain-derived neurotrophic factor (BDNF), which plays a role in neuronal survival and synaptic plasticity in the central nervous system (CNS), may also be associated with schizophrenia. However, the possible relationship between IUGR and BDNF function remains unclear. Here, we examined how IUGR by TXA2 impacts BDNF function by using dissociated cortical neurons. We found that, although BDNF levels in cultured neurons from the cerebral cortex of low birth weight pups with IUGR were unchanged, TrkB (BDNF receptor) was decreased compared with control-rats. BDNF-stimulated MAPK/ERK1/2 and PI3K/Akt pathways, which are downstream intracellular signaling pathways of TrkB, were repressed in IUGR-rat cultures. Expression of glutamate receptors such as GluA1 and GluN2A was also suppressed in IUGR-rat cultures. Furthermore, in IUGR-rat cultures, anti-apoptotic protein Bcl2 was decreased and BDNF failed to prevent neurons from cell death caused by serum-deprivation. Taken together, IUGR resulted in reductions in cell viability and in synaptic function following TrkB down-regulation, which may play a role in schizophrenia-like behaviors.

© 2010 Elsevier Ireland Ltd. All rights reserved.

Schizophrenia is a chronic, severe and disabling brain disease, of which neuropathological basis has remained elusive [18]. Growing evidence has suggested neurodevelopmental impairments in the pathogenesis of schizophrenia [13]. Importantly, obstetric complications play a role in such impairments [8,24,35]. Among various obstetric complications, low birth weight is a strong risk factor for schizophrenia [25].

Intrauterine growth retardation (IUGR) induced by synthetic thromboxane A2 (TXA2) was associated with a delay in postnatal neurological development and learning disabilities in rats in which the neuronal density in the cortical plate was lower than that of control rats [31]. Interestingly, mRNA expression of neurotrophins such as BDNF and NT-3 (neurotrophin-3) was suppressed in the cerebral cortex of TXA2-induced IUGR-rats [14].

BDNF has critical roles in neuronal survival and synaptic plasticity [7,32] through activation of TrkB, and consequent stimulation of downstream signaling including mitogen-activated protein/extracellular signal-regulated kinase (MAPK/ERK), phosphoinositide 3-kinase/Akt (PI3K/Akt) and phospholipase Cγ (PLCγ) pathways. Recently, we reported important regulatory roles of BDNF in synaptic functions via these pathways [23,26,27]. Remarkably, altered serum levels of BDNF and its expression in the postmortem brain of schizophrenia patients have been reported [10,21,33]. Furthermore, forebrain-specific TrkB knockout mice showed schizophrenia-like behaviors, including hyperlocomotion, stereotyped behaviors and cognitive impairments [36].

Though both IUGR and dysfunction of BDNF-TrkB signaling may contribute to the pathogenesis of schizophrenia, the possible change in the BDNF-TrkB signaling in Central Nervous System (CNS) neurons of IUGR has not yet been clarified. Here, we found that cortical neurons from IUGR-rats exhibited lower levels of TrkB, Bcl2, and glutamate receptors. Interestingly, neurons from IUGR-rats showed a decreased response to BDNF when survival was examined.

Female Long-Evans rats (Institute for Animal Reproduction, Ibaraki, Japan) were purchased at 8 days of pregnancy and kept

* Corresponding author at: Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan. Tel.: +81 42 341 2711x5132; fax: +81 42 346 1744.
E-mail address: numakawa@ncnp.go.jp (T. Numakawa).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.03.082

Author's personal copy

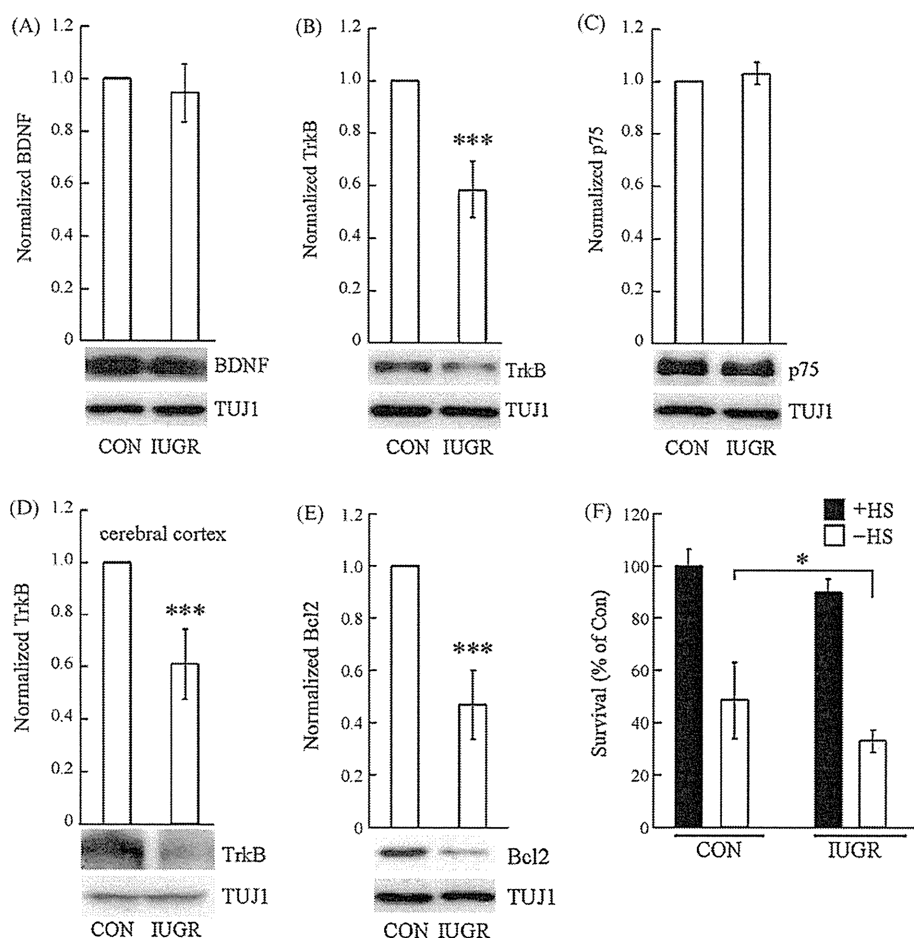


Fig. 1. Reduction in levels of TrkB, Bcl2, and cell viability in cortical cultures prepared from the cerebral cortex of low weight newborn rats with TXA2-induced IUGR. The levels of BDNF (A), TrkB (B), and p75 (C) were examined in 5DIV cortical cultures from IUGR-rats or from control-rats. TrkB was reduced in IUGR-rat neurons. Quantification was carried out after immunoblotting. Normalization to a level in control was performed. Data represent mean ± SD ($n = 6$), $^{***}P < 0.001$. IUGR: intrauterine growth retardation. (D) TrkB down-regulation was observed in homogenates from the cerebral cortex of IUGR-rats, $^{***}P < 0.001$ ($n = 4$). (E) Reduction in Bcl2 expression in cultures from IUGR-rats. Data represent mean ± SD ($n = 7$), $^{***}P < 0.001$. The three independent series of cultures were used for each set of immunoblotting experiments. TUJ1 levels are shown as controls in each representative blot. (F) Decrease in cell viability of cortical neurons from IUGR-rats. To induce neuronal cell death, serum-deprivation was performed. Cell survival was determined by MTT assay. Data represent mean ± SD ($n = 8$, n indicates the number of wells of a plate for each experimental condition), $^{*}P < 0.05$. To confirm reproducibility, the three independent series of cultures were used.

in individual cages under a standard laboratory environment (12L:12D, light on at 15:30; 21–24 °C temperature; free access to food and water). IUGR was induced by TXA2 analog (9,11-dideoxy-9 a, 11a-methanoepoxy-prosta-5Z, 13E-dien-1-oic acid; Cayman Chemical, MI, USA) application on mother rats according to previous studies [20]. Briefly, an osmotic pump (2ML1, Alzet Corp., Palo Alto, CA, USA) containing 2 ml of TXA2 solution (12.5 μg/ml) or PBS for control rats was implanted into the lower portion of the peritoneal cavity under sodium pentobarbital (31.5 mg/kg b.w.) anesthesia on 13 days of pregnancy. Rats were allowed to deliver spontaneously, and pups were fed by their own mothers. Brains of pups were removed at postnatal day 1 (P1) and used for dissociated cultures. To check levels of TrkB in homogenates from the cerebral cortex, the brains were removed from the deeply anesthetized P1 IUGR- or control-rats. All the experiments were approved by the Ethics Review Committee for Animal Experimentation of the National Institute of Neuroscience, Japan.

Cultures were prepared as previously reported [28]. Dissociated cortical neurons were plated on polyethyleneimine-coated culture dishes or 48-well plates (Corning, NY, USA). The cell density was $5 \times 10^5/cm^2$, respectively. Neuronal cultures from cerebral cortex of pups of control or of IUGR were maintained with 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium containing 5% fetal bovine serum and 5% heated-inactivated horse serum for 5 days before the survival assay or collecting samples for immunoblotting. To induce cell death, the culture media was replaced with a serum-free fresh media for 24 h. Then, to determine the cell viability, a mitochondrial-dependent conversion of the tetrazolium salt (MTT) assay was performed [30]. When glial cell contribution was checked, arabinosylcytosine (1.0 μM, SIGMA, MO, USA) was applied at 24 h after cell plating. BDNF (100 ng/ml) was applied 20 min before serum-deprivation. LY294002 (1.0 μM, Calbiochem-Novabiochem, CA, USA) was added 20 min before BDNF application.

MAP2 immunostaining was conducted [27]. Cells were fixed in 4% paraformaldehyde at room temperature for 20 min. After blocking with PBS containing 10% goat serum and 0.2% Triton X-100 for 30 min, anti-MAP2 (1:1000, SIGMA) antibody was incubated overnight at 4 °C. Alexa Fluor 594-conjugated anti-mouse IgG (1:200, Invitrogen, CA, USA) was used as a secondary antibody.

Cells were lysed in SDS lysis buffer (1% SDS, 20 mM Tris–HCl (pH 7.4), 5 mM EDTA (pH 8.0), 10 mM NaF, 2 mM Na_3VO_4, 0.5 mM phenylarsine oxide, and 1 mM phenylmethylsulfonyl fluoride). The protein concentration was quantified using a BCA Protein Assay Kit

Author's personal copy

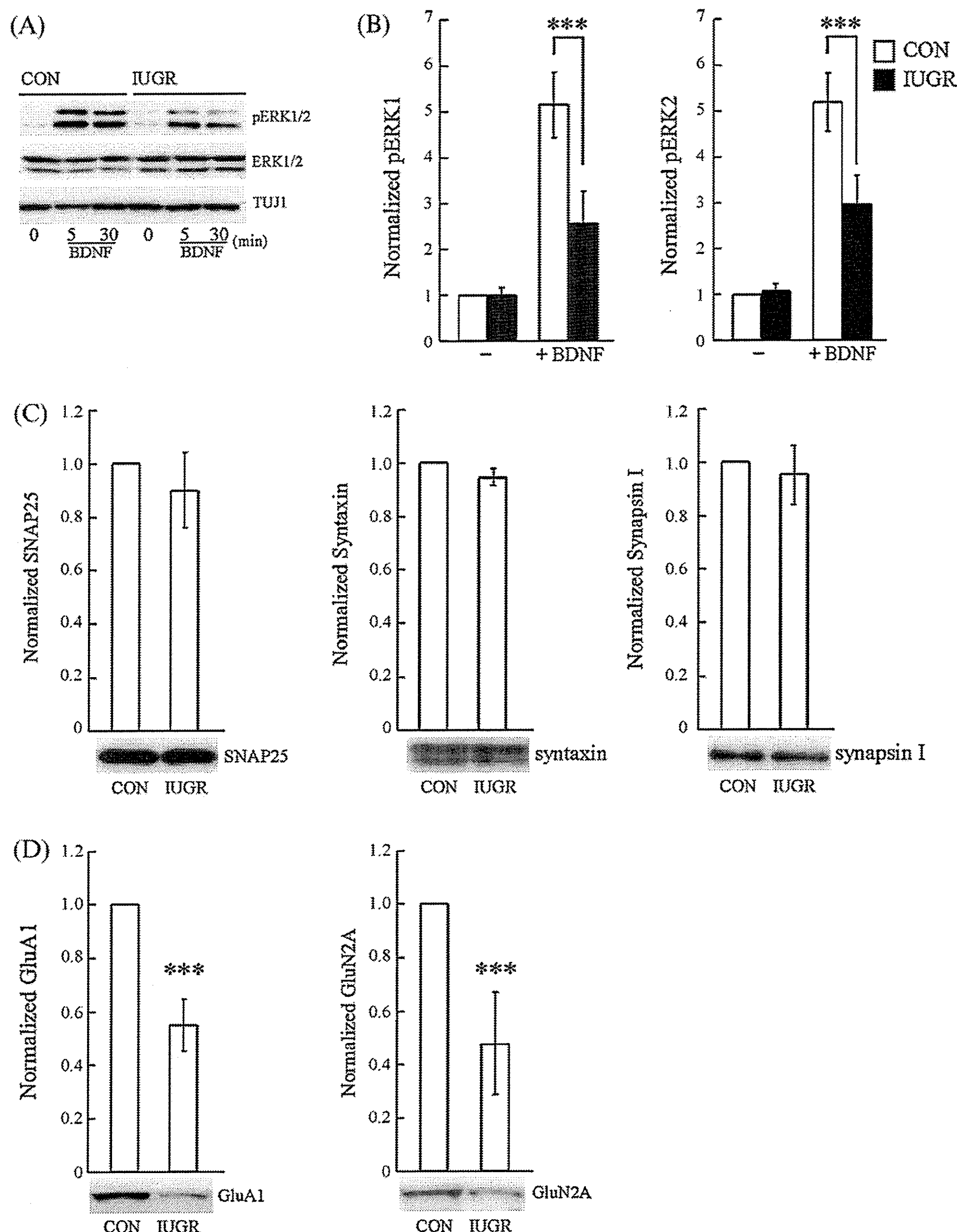


Fig. 2. Decrease in BDNF-stimulated ERK1/2 and in levels of glutamate receptors in IUGR-rat cultures. (A) and (B) Levels of activated ERK1/2 (pERK1/2) in IUGR- or control-rat cultures were examined at 5DIV. Cultured neurons with IUGR- or control-rats were stimulated by BDNF (100 ng/ml) for 0 min, 5 min, or 30 min. pERK1/2 stimulated by BDNF (5 min) was suppressed in IUGR-rat cultures. To quantify the pERK1/2 (BDNF 5 min), normalization to a level in control was performed. Data represent mean ± SD ($n = 5$), $^{***}P < 0.001$. Total ERK1/2 was unchanged. TUJ1 is shown as a control. (C) Presynaptic proteins including SNAP25, syntaxin, and synapsin I were unchanged in IUGR-rat cultures. Data represent mean ± SD (SNAP25, $n = 6$, syntaxin, $n = 6$, synapsin I, $n = 6$). (D) Postsynaptic glutamate receptor (GluA1 and GluN2A) levels in IUGR-rat neurons were reduced. Data represent mean ± SD (GluA1, $n = 6$, GluN2A, $n = 5$). $^{***}P < 0.001$. The four independent series of cultures were used for each set of experiments.

(PIERCE, IL, USA), and equivalent amounts of protein were applied for each immunoblotting. Antibodies were used at the following dilutions: anti-Akt (1:1000, Cell Signaling, MA, USA), anti-pAkt (1:1000, Cell Signaling), anti-ERK (1:1000, Cell Signaling), anti-pERK (1:1000, Cell Signaling), anti-GluN2A (NR2A) (1:500, SIGMA), anti-GluA1 (GluR1) (1:1000, CHEMICON, CA, USA), anti-SNAP25 (1:1000, Synaptic Systems, Gottingen, Germany), anti-syntaxin (1:10000, SIGMA), anti-synapsin I (1:2000, CHEMICON), anti-Bcl2 (1:1000, BD Biosciences, CA, USA), anti-TUJ1 (1:5000, Berkeley Antibody Company, CA, USA), anti-p75 (1:1000, Promega, WI, USA), anti-TrkB (1:1000, BD Biosciences), and anti-BDNF (1:200, Santa Cruz Biotechnology Inc., CA, USA) antibodies. The immunoreactivity was quantified by using Lane & Spot Analyzer software (ATTO Corporation, Tokyo, Japan).

Data shown are presented as mean ± standard deviation (SD). Statistical significance was evaluated using a one-way ANOVA followed by Tukey' s test in SPSS ver11 (SPSS Japan, Tokyo, Japan). Probability values less than 5% were considered statistically significant.

Initially, we examined the possible change in levels of endogenous BDNF and associated receptors in 5 days in vitro (5DIV) neurons prepared from the cerebral cortex of low weight pups with TXA2-induced IUGR (IUGR-rats). Birth weight was decreased by approximately 16% due to IUGR (control rats: 7.76 ± 0.25 g;

Author's personal copy

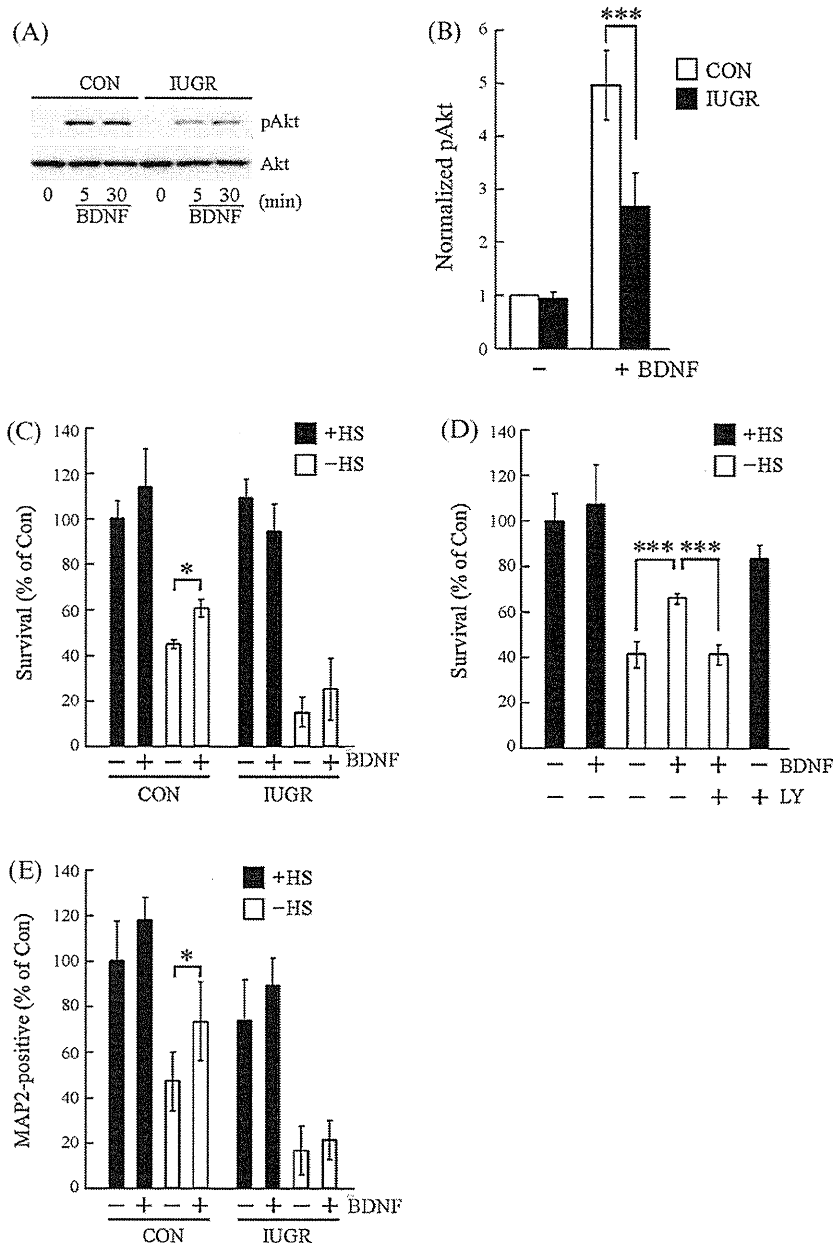


Fig. 3. Cortical neurons from IUGR-rats demonstrated a weakened response to BDNF measured through activation of the PI3K/Akt pathway and neuronal survival. (A) and (B) BDNF-stimulated Akt, a component of the PI3K pathway, was diminished in IUGR-rat cultures at 5DIV. BDNF (100 ng/ml) was applied for the indicated number of minutes. Activated Akt (pAkt, BDNF 5 min) was quantified. Normalization to a level in control was performed. Data represent mean ± SD ($n = 5$), $^{***}P < 0.001$. Three independent series of cultures were used for experiments. (C) The survival-promoting effect of BDNF was lost in IUGR-rat cultures. Cell viability was determined by MTT assay. Data represent mean ± SD ($n = 5$, n indicates the number of wells of a plate for each experimental condition), $^{*}P < 0.05$. To confirm reproducibility, the three independent series of cultures were used. (D) BDNF-dependent survival was blocked by LY294002 (1 μM), an inhibitor for PI3K. Data represent mean ± SD ($n = 6$, n indicates the number of wells of a plate for each experimental condition. MTT assay), $^{***}P < 0.001$. To confirm reproducibility, the three independent series of cultures were used. (E) Neuronal survival was determined by MAP2 immunostaining. The number of MAP2-positive cells was counted. The lower response to BDNF was confirmed in IUGR-rat cultures. Data represent mean ± SD ($n = 11$, n indicates the number of wells of a plate for each experimental condition), $^{*}P < 0.05$.

IUGR-rats: 6.54 ± 0.40 g, $n = 6$ for each). As shown in Fig. 1A, BDNF levels in neurons from IUGR-rats were unchanged compared with those from normally weighed rats (control-rats). In contrast, TrkB, a high affinity receptor for BDNF, was significantly decreased in neurons from IUGR-rats (Fig. 1B). A low affinity common receptor for neurotrophins, p75, was unchanged in cultures from IUGR-rats (Fig. 1C). Such down-regulation of TrkB was confirmed in homogenates from the cerebral cortex of IUGR-rats (Fig. 1D), suggesting that the change observed in TrkB levels in culture is not specific to culture conditions. TUJ1 (class III β-tubulin, a neuronal marker) levels are shown as a control (Fig. 1A–D). As BDNF/TrkB signaling is important for expression of Bcl2 [4], we determined the Bcl2 levels and found marked reduction in IUGR-rat cultures, though TUJ1 was unchanged (Fig. 1E), raising a possibility that IUGR makes cortical neurons vulnerable to death-inducible stimuli. Cell viability of cultures from both IUGR- and control-rats was decreased after serum-deprivation (Fig. 1F). Expectedly, the level of decrease in cell viability of IUGR-rat neurons was larger than that of control-rats. We observed a clear reduction of TrkB and Bcl2 levels in 8DIV cultures (Supplementary Fig.S1), implying that down-

Author's personal copy

regulation of TrkB and Bcl2 proteins due to IUGR is sustained during neuronal maturation. Furthermore, decreased viability of 8DIV neurons from IUGR-rats was confirmed (Supplementary Fig.S1).

Next, activation of intracellular signaling stimulated by BDNF was examined. Activation of ERK1/2 (phosphorylated ERK1/2, pERK1/2) 5 min after BDNF application was reduced in IUGR-rat cultures while total ERK1/2 and TUJ1 levels were unchanged (Fig. 2A and B). In the presence of arabinosylcytosine, which prevents glial cell proliferation, the reduced pERK1/2 in IUGR-rat cultures when BDNF was added was also observed (Supplementary Fig.S2), suggesting that this suppression of ERK1/2 activation is a neuronal, and not glial, response. We previously reported that ERK1/2 regulates synaptic protein expression [23,26]. In the current study, the expression levels of SNAP25, syntaxin, and synapsin I were unchanged in IUGR-rat neurons compared with control (Fig. 2C). Interestingly, ionotropic glutamate receptors (GluA1 and GluN2A) in IUGR-rat cultures were down-regulated (Fig. 2D). It is possible that the decreased activity of the ERK1/2 pathway via IUGR results in down-regulation of glutamate receptors.

The activation of Akt, a component of the PI3K pathway (well-known as a survival promoting pathway), was also determined. BDNF-stimulated activation of Akt (phosphorylated Akt, pAkt) was reduced in IUGR-rat neurons with or without arabinosylcytosine treatment (Fig. 3A and B, and Supplementary Fig.S2). To test whether the PI3K/Akt pathway is involved in neuronal survival [9], we examined the BDNF-dependent protection of cortical neurons from cell death caused by serum-deprivation. MTT assay revealed that BDNF inhibited the cell death in control-rat cultures, however, the protection by BDNF was not observed in IUGR-rat cultures (Fig. 3C). In control cultures, BDNF upregulated Bcl2, an anti-apoptotic protein (Supplementary Fig.S3). We confirmed that LY294002, a PI3K inhibitor, blocked BDNF-dependent survival in control cultures (Fig. 3D). Furthermore, immunostaining with anti-MAP2 (microtubule-associated protein 2, neuronal marker) antibody revealed that, though the number of MAP2-positive surviving cells was reduced after serum-deprivation in both IUGR- and control cultures, a lower response to BDNF was confirmed in IUGR-rat neurons compared with control (Fig. 3E). Taken together, it is possible that the survival-promoting effect of BDNF was weakened in IUGR-rat neurons.

We found that TrkB (not BDNF) was significantly decreased in cortical cultures from IUGR-rats. Consistent with the reduction of TrkB, BDNF-stimulated MAPK/ERK1/2 and PI3K/Akt pathways were diminished in IUGR-rat cultures compared with control. Bcl2, a survival promoting protein, was also down-regulated in IUGR-rat cultures. We also found a significant decrease in synaptic protein (GluA1 and GluN2A) levels in IUGR-rat cultures. Interestingly, cortical neurons from IUGR-rats showed vulnerability to cell death as well as a weakened response to the survival-promoting effect of BDNF.

Impairment of BDNF and TrkB functions has been implicated in the pathogenesis of schizophrenia [6,11], as well as other neuropsychiatric diseases such as depression [5]. A recent study demonstrated a reduction in BDNF in the dorsolateral prefrontal cortex of schizophrenics [34], suggesting that BDNF down-regulation may affect the functions of intrinsic cortical neurons, afferent neurons, and target neurons. Remarkably, an animal model of IUGR by TXA2 showed decreased BDNF and NT-3 mRNA in the cerebral cortex [14]. In our system, TrkB was decreased by IUGR, although BDNF levels were not altered. In addition to ligand (BDNF), a change in the expression of receptor (TrkB) may contribute to neuronal dysfunction due to IUGR.

Perhaps the down-regulation of TrkB in IUGR-rat neurons occurred as a result of post-transcriptional modifications. Ernst et al. reported that TrkB.T1 (one of the truncated types of TrkB) is down-regulated in the frontal cortex in a subset of suicide victims compared with controls and that this down-regulation is associated with methylation at specific CpG dinucleotides proximal to the coding region [12]. Indeed, several susceptibility genes for schizophrenia are subject to changes in transcriptional activity due to histone modifications and DNA methylation [15]. To date, most studies exploring DNA methylation changes in schizophrenia postmortem brain were focused on the cerebral cortex, primarily its prefrontal areas [3]. Various degrees of aberrant CpG hyper- or hypomethylation have been reported in regulatory sequences of promoters of genes involved in the cortical dysfunction of schizophrenia, including the glycoprotein *REELIN*, *COMT*, and *SOX10* [1,2,3,16,22]. Thus, TrkB reduction via IUGR in our models may be due to DNA methylation, although further studies are required to confirm this possibility.

In IUGR-rat cultures, the expression of postsynaptic proteins, GluA1 and GluN2A, was decreased. In the hippocampus of schizophrenia postmortem brains, reduced expression of subunits for ionotropic glutamate receptors (including NMDA, AMPA, and kainate type receptors) was reported [17]. Recently, we found that intracellular signaling, including the MAPK/ERK pathway, has an important role in the maintenance of synaptic proteins and is involved in schizophrenia [19,23,26,29]. The decrease of TrkB expression may lead to the reduction in postsynaptic proteins as observed in patients with schizophrenia.

TrkB down-regulation may cause reduced activation of the MAPK/ERK and PI3K/Akt pathways in response to BDNF. These pathways are critical for synaptic protein expression and neuronal survival. We confirmed that cortical neurons from IUGR-rats were vulnerable to cell death by serum-deprivation. Bcl2 expression and survival-promoting effects of BDNF were also decreased in IUGR-rat cultures. Increased vulnerability of neurons to neurotoxic damage caused by inadequate neurotrophic support is thought to be involved in the etiology of psychiatric disease [6]. In summary, our results suggest that impairment of BDNF-TrkB signaling caused by IUGR and the resultant decrease in viability of neurons and expression of glutamate receptors may be responsible, at least in part, for the cortical dysfunction observed in schizophrenia-like behaviors. We demonstrated that our in vitro system may offer a useful model for studies to investigate the cellular mechanisms of schizophrenia.

Acknowledgements

We thank Regeneron Pharmaceutical Co., Takeda Chemical Industries, Ltd., and Dainippon Sumitomo Pharma Co. Ltd. for donating the BDNF. This study was supported by the Research Grants for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare, Health and Labor Sciences Research Grants (Research on Psychiatric and Neurological Diseases and Mental Health), Health and Labor Sciences Research Grants, a grant from the Japan Foundation for Neuroscience and Mental Health, the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (H. K.), and a Grant-in-Aid for Young Scientists (A) (21680034) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (T. N.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2010.03.082.

References

[1] H.M. Abdolmaleky, K.H. Cheng, S.V. Faraone, M. Wilcox, S.J. Glatt, F. Gao, C.L. Smith, R. Shafa, B. Aeali, J. Carnevale, H. Pan, P. Papageorgis, J.F. Ponte, V. Sivaraman, M.T. Tsuang, S. Thiagalingam, Hypomethylation of MB-COMT promoter

Author's personal copy

is a major risk factor for schizophrenia and bipolar disorder, Hum. Mol. Genet. 15 (2006) 3132–3145.

[2] H.M. Abdolmaleky, K.H. Cheng, A. Russo, C.L. Smith, S.V. Faraone, M. Wilcox, R. Shafa, S.J. Glatt, G. Nguyen, J.F. Ponte, S. Thiagalingam, M.T. Tsuang, Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report, Am. J. Med. Genet. B: Neuropsychiatr. Genet. 134B (2005) 60–66.

[3] S. Akbarian, The molecular pathology of schizophrenia-Focus on histone and DNA modifications, Brain Res. Bull., in press.

[4] R.D. Almeida, B.J. Manadas, C.V. Melo, J.R. Gomes, C.S. Mendes, M.M. Grãos, R.F. Carvalho, A.P. Carvalho, C.B. Duarte, Neuroprotection by BDNF against glutamate-induced apoptotic cell death is mediated by ERK and PI3-kinase pathways, Cell Death Differ. 12 (2005) 1329–1343.

[5] C.A. Altar, Neurotrophins and depression, Trends Pharmacol. Sci. 20 (1999) 59–61.

[6] F. Angelucci, S. Brenè, A.A. Mathé, BDNF in schizophrenia, depression and corresponding animal models, Mol. Psychiatry 10 (2005) 345–352.

[7] O. Arancio, M.V. Chao, Neurotrophins, synaptic plasticity and dementia, Curr. Opin. Neurobiol. 17 (2007) 325–330.

[8] P. Boksa, Animal models of obstetric complications in relation to schizophrenia, Brain Res. Brain Res. Rev. 45 (2004) 1–17.

[9] H. Dudek, S.R. Datta, T.F. Franke, M.J. Birnbaum, R. Yao, G.M. Cooper, R.A. Segal, D.R. Kaplan, M.E. Greenberg, Regulation of neuronal survival by the serine-threonine protein kinase Akt, Science 275 (1997) 661–665.

[10] N. Durany, T. Michel, R. Zochling, K.W. Boissl, F.F. Cruz-Sanchez, P. Riederer, J. Thome, Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses, Schizophr. Res. 52 (2001) 79–86.

[11] N. Durany, J. Thome, Neurotrophic factors and the pathophysiology of schizophrenic psychoses, Eur. Psychiatry 19 (2004) 326–337.

[12] C. Ernst, V. Deleva, X. Deng, A. Sequeira, A. Pomarenski, T. Klempan, N. Ernst, R. Quirion, A. Gratton, M. Szyf, G. Turecki, Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers, Arch. Gen. Psychiatry 66 (2009) 22–32.

[13] W.G. Frankle, J. Lerma, M. Laruelle, The synaptic hypothesis of schizophrenia, Neuron 39 (2003) 205–216.

[14] E. Fukami, A. Nakayama, J. Sasaki, S. Mimura, N. Mori, K. Watanabe, Underexpression of neural cell adhesion molecule and neurotrophic factors in rat brain following thromboxane A(2)-induced intrauterine growth retardation, Early Hum. Dev. 58 (2000) 101–110.

[15] D.P. Gavin, R.P. Sharma, Histone modifications, DNA methylation, and schizophrenia, Neurosci. Biobehav. Rev. 34 (2010) 882–888.

[16] D.R. Grayson, X. Jia, Y. Chen, R.P. Sharma, C.P. Mitchell, A. Guidotti, E. Costa, Reelin promoter hypermethylation in schizophrenia, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 9341–9346.

[17] P.J. Harrison, A.J. Law, S.L. Eastwood, Glutamate receptors and transporters in the hippocampus in schizophrenia, Ann. N. Y. Acad. Sci. 1003 (2003) 94–101.

[18] P.J. Harrison, D.R. Weinberger, Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence, Mol. Psychiatry 10 (2005) 40–68.

[19] R. Hashimoto, T. Numakawa, T. Ohnishi, E. Kumamaru, Y. Yagasaki, T. Ishimoto, T. Mori, K. Nemoto, N. Adachi, A. Izumi, S. Chiba, H. Noguchi, T. Suzuki, N. Iwata, N. Ozaki, T. Taguchi, A. Kamiya, A. Kosuga, M. Tatsumi, K. Kamijima, D.R. Weinberger, A. Sawa, H. Kunugi, Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling, Hum. Mol. Genet. 15 (2006) 3024–3033.

[20] M. Hayakawa, S. Mimura, J. Sasaki, K. Watanabe, Neuropathological changes in the cerebrum of IUGR rat induced by synthetic thromboxane A2, Early Hum. Dev. 55 (1999) 125–136.

[21] S. Iritani, K. Niizato, H. Nawa, K. Ikeda, P.C. Emson, Immunohistochemical study of brain-derived neurotrophic factor and its receptor, TrkB, in the hippocampal formation of schizophrenic brains, Prog. Neuropsychopharmacol. Biol. Psychiatry 27 (2003) 801–807.

[22] K. Iwamoto, M. Bundo, K. Yamada, H. Takao, Y. Iwayama-Shigeno, T. Yoshikawa, T. Kato, DNA methylation status of SOX10 correlates with its downregulation and oligodendrocyte dysfunction in schizophrenia, J. Neurosci. 25 (2005) 5376–5381.

[23] E. Kumamaru, T. Numakawa, N. Adachi, Y. Yagasaki, A. Izumi, M. Niyaz, M. Kudo, H. Kunugi, Glucocorticoid prevents brain-derived neurotrophic factor-mediated maturation of synaptic function in developing hippocampal neurons through reduction in the activity of mitogen-activated protein kinase, Mol. Endocrinol. 22 (2008) 546–558.

[24] H. Kunugi, Obstetric and delivery complications as risk factors for schizophrenia: neurodevelopmental perspectives, in: A. Grispini (Ed.), Preventive Strategies for Schizophrenic Disorders: Basic Principles, Opportunities and Limits, Giovanni Floriti Editore, 2003, pp. 159–187.

[25] H. Kunugi, S. Nanko, R.M. Murray, Obstetric complications and schizophrenia: prenatal underdevelopment and subsequent neurodevelopmental impairment, Br. J. Psychiatry Suppl. 40 (2001) s25–29.

[26] T. Matsumoto, T. Numakawa, D. Yokomaku, N. Adachi, S. Yamagishi, Y. Numakawa, H. Kunugi, T. Taguchi, Brain-derived neurotrophic factor-induced potentiation of glutamate and GABA release: different dependency on signaling pathways and neuronal activity, Mol. Cell. Neurosci. 31 (2006) 70–84.

[27] T. Numakawa, E. Kumamaru, N. Adachi, Y. Yagasaki, A. Izumi, H. Kunugi, Glucocorticoid receptor interaction with TrkB promotes BDNF-triggered PLC-gamma signaling for glutamate release via a glutamate transporter, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 647–652.

[28] T. Numakawa, S. Yamagishi, N. Adachi, T. Matsumoto, D. Yokomaku, M. Yamada, H. Hatanaka, Brain-derived neurotrophic factor-induced potentiation of Ca(2+) oscillations in developing cortical neurons, J. Biol. Chem. 277 (2002) 6520–6529.

[29] T. Numakawa, Y. Yagasaki, T. Ishimoto, T. Okada, T. Suzuki, N. Iwata, N. Ozaki, T. Taguchi, M. Tatsumi, K. Kamijima, R.E. Straub, D.R. Weinberger, H. Kunugi, R. Hashimoto, Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia, Hum. Mol. Genet. 13 (2004) 2699–2708.

[30] Y. Numakawa, T. Numakawa, T. Matsumoto, Y. Yagasaki, E. Kumamaru, H. Kunugi, T. Taguchi, E. Niki, Vitamin E protected cultured cortical neurons from oxidative stress-induced cell death through the activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase, J. Neurochem. 97 (2006) 1191–1202.

[31] A. Saito, F. Matsui, K. Hayashi, K. Watanabe, Y. Ichinohashi, Y. Sato, M. Hayakawa, S. Kojima, A. Oohira, Behavioral abnormalities of fetal growth retardation model rats with reduced amounts of brain proteoglycans, Exp. Neurol. 219 (2009) 81–92.

[32] R. Stoop, M.M. Poo, Synaptic modulation by neurotrophic factors: differential and synergistic effects of brain-derived neurotrophic factor and ciliary neurotrophic factor, J. Neurosci. 16 (1996) 3256–3264.

[33] K. Toyooka, K. Asama, Y. Watanabe, T. Muratake, M. Takahashi, T. Someya, H. Nawa, Decreased levels of brain-derived neurotrophic factor in serum of chronic schizophrenic patients, Psychiatry Res. 110 (2002) 249–257.

[34] C.S. Weickert, T.M. Hyde, B.K. Lipska, M.M. Herman, D.R. Weinberger, J.E. Kleinman, Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia, Mol. Psychiatry 8 (2003) 592–610.

[35] G.L. Zornberg, S.L. Buka, M.T. Tsuang, Hypoxic-ischemia-related fetal/neonatal complications and risk of schizophrenia and other nonaffective psychoses: a 19-year longitudinal study, Am. J. Psychiatry 157 (2000) 196–202.

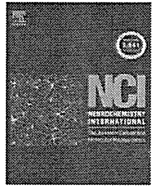
[36] B. Zorner, D.P. Wolfer, D. Brandis, O. Kretz, C. Zacher, R. Madani, I. Grunwald, H.P. Lipp, R. Klein, F.A. Henn, P. Gass, Forebrain-specific trkB-receptor knockout mice: behaviorally more hyperactive than "depressive", Biol. Psychiatry 54 (2003) 972–982.

Neurochemistry International 59 (2011) 551–558

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci



Review

MicroRNA function and neurotrophin BDNF

Tadahiro Numakawa [a,b,*], Misty Richards [c], Naoki Adachi [a,b], Soichiro Kishi [d], Hiroshi Kunugi [a,b], Kazuo Hashido [d]

[a] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo 187-8502, Japan
[b] Core Research for Evolutional Science and Technology Program (CREST), Japan Science and Technology Agency (JST), Saitama 332-0012, Japan
[c] Albany Medical College, Albany, NY 12208, USA
[d] Administrative Section of Radiation Protection, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

ARTICLE INFO

Article history:
Received 20 April 2011
Received in revised form 15 June 2011
Accepted 16 June 2011
Available online 24 June 2011

Keywords:
MicroRNA
BDNF
Synaptic plasticity

ABSTRACT

MicroRNAs (miRs), endogenous small RNAs, regulate gene expression through repression of translational activity after binding to target mRNAs. miRs are involved in various cellular processes including differentiation, metabolism, and apoptosis. Furthermore, possible involvement of miRs in neuronal function have been proposed. For example, miR-132 is closely related to neuronal outgrowth while miR-134 plays a role in postsynaptic regulation, suggesting that brain-specific miRs are critical for synaptic plasticity. On the other hand, numerous studies indicate that BDNF (brain-derived neurotrophic factor), one of the neurotrophins, is essential for a variety of neuronal aspects such as cell differentiation, survival, and synaptic plasticity in the central nervous system (CNS). Interestingly, recent studies, including ours, suggest that BDNF exerts its beneficial effects on CNS neurons via up-regulation of miR-132. Here, we present a broad overview of the current knowledge concerning the association between neurotrophins and various miRs.
© 2011 Elsevier B.V. All rights reserved.

1. Introduction

miRs regulate the expression of target genes and ultimately affect translation. Through this process, multiple mechanisms are impacted, including differentiation, cell death, and cell metabolism in plants and animals (Bartel, 2004; Chapman and Carrington, 2007; Pillai et al., 2007). Recent evidence suggests that brain-specific miRs may be involved in the regulation of neuronal function. For instance, miR-132 is important for neurite outgrowth, while miR-134 negatively regulates the spine size in neurons (Vo et al., 2005; Schratt et al., 2006). Furthermore, change in expression and/or function of some miRs may be associated with several brain diseases such as schizophrenia (Mellios et al., 2009), panic disorder (Muiños-Gimeno et al., 2010), and Rett syndrome (Wu et al., 2010), etc. Additionally, regulation of neurodegeneration-related toxic molecules by miRs has been suggested (Eacker et al., 2009). Taking these findings together, an extensive examination of the basic functioning of miRs and their relationship with brain disease pathophysiology is very important.

Many studies discuss the involvement of BDNF, a critical neurotrophic factor, in brain function. On a cellular level, it is well-established that BDNF influences proliferation, maturation, survival, and synaptic function via activation of intracellular signaling cascades including mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK), phospholipase Cγ (PLCγ), and phosphatidylinositol 3-kinase (PI3K) pathways (Huang and Reichardt, 2003; Minichiello, 2009; Russo et al., 2009; Numakawa et al., 2010a). As expected, alteration in expression/function of BDNF is involved in the pathophysiology of several brain diseases including neurodegenerative diseases and mental disorders (Karege et al., 2005; Numakawa et al., 2011). As both miRs and BDNF affect neuronal processes, the crosstalk between these molecules is very interesting. In the present review, we focus on the current relationship between miRs and BDNF in neurons, in addition to giving a broad overview of the basic functioning of miRs.

2. Biogenesis of microRNA and its basic function

The first miR, *lin-4*, was initially discovered as a direct target when researchers were performing genetic analysis of defects in post-embryonic development of *Caenorhabditis elegans* (Chalfie et al., 1981; Ambros, 1989). *Lin-4* encodes a 22-nucleotide non-coding RNA that binds to the 3′UTR of *lin-14* mRNA, which serves to regulate protein translation (Wightman et al., 1991; Lee et al., 1993). The second miR to be identified was *let-7* (Reinhart et al., 2000). The *let-7* miR encodes a 21-nucleotide RNA that binds to the 3′UTR of *lin-41* and *hbl-1* (*lin-57*) in order to inhibit translation (Slack et al., 2000; Abrahante et al., 2003; Lin et al., 2003; Vella

* Corresponding author at: Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), 4-1-1, Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan. Tel.: +81 42 341 2711x5132; fax: +81 42 346 1744.
E-mail address: numakawa@ncnp.go.jp (T. Numakawa).

0197-0186/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.neuint.2011.06.009

et al., 2004). Both *let-7* and its target *lin-41* are evolutionarily conserved throughout a wide range of animal species, including vertebrate, ascidian, hemichordate, mollusc, annelid and arthropod, suggesting a general role of small RNA molecules in developmental regulation. As a result of the above findings, extensive analysis and identification of new miRs were triggered (Pasquinelli et al., 2000). Since this time, several hundred miRs have been identified in various organisms such as viruses, worms, flies, fish, frogs, mammals and plants (Lagos-Quintana et al., 2001, 2003; Lau et al., 2001; Lee and Ambros, 2001; Llave et al., 2002; Reinhart et al., 2002; Lim et al., 2003; Pfeffer et al., 2005; Watanabe et al., 2005). The miR database now lists over 15,000 miR gene loci in over 140 species, and over 17,000 distinct mature miR sequences (Kozomara and Griffiths-Jones, 2011).

Generally, miR genes are transcribed by RNA polymerase II as primary miRs (pri-miRs are hundreds to thousands of nucleotide-length transcripts) containing cap structures and poly (A) tails (Cai et al., 2004; Lee et al., 2004; Parizotto et al., 2004, and see

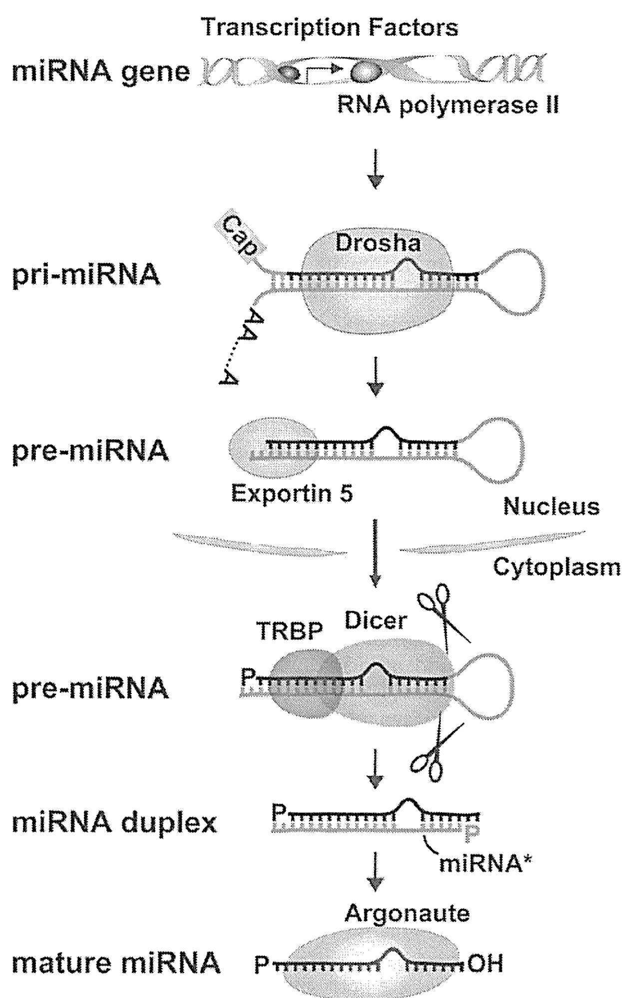


Fig. 1. MicroRNA biogenesis. An miRNA (miR) gene is generally transcribed by RNA polymerase II as primary miR (pri-miR). At this point, some transcription factors engage in the transcriptional control of miR genes. In the nucleus, the RNase III endonuclease Drosha cleaves the pri-miR to produce an approximately 70 nucleotide stem loop structure as precursor miR (pre-miR). Exportin5 transports the pre-miR into the cytoplasm, where it is cleaved by another RNase III endonuclease, Dicer, together with the trans activator RNA (TAR)-binding protein (TRBP), generating approximately 22 nucleotide miRNA/miRNA* duplex. One strand of the duplex remains Argonaute as a mature miR, while the other strand (miR*) is degraded.

Fig. 1). At this point, known transcription factors engage in the transcriptional control of miR genes. For instance, the myogenic transcription factors, such as Myogenin and MyoD, bind to regions upstream of miR-1 and miR-133 loci and are likely to regulate their expression during myogenesis (Rao et al., 2006). HBL-1, a protein product of *hbl-1* and one of *let-7*'s targets, is responsible for inhibiting the transcription of *let-7* temporarily in seam cells, vulval precursor cells, and the hypodermal syncytium 7. This negative feedback mechanism between *let-7* and HBL-1 has been identified and characterized by researchers (Roush and Slack, 2009). Epigenetic control also contributes to miR gene expression. For example, the miR-203 gene locus is hypermethylated in several hematopoietic tumors, including chronic myelogenous leukemia and some acute lymphoblastic leukemias (Bueno et al., 2008).

Following this, Drosha, an RNase III enzyme, cleaves the pri-miRs into approximately 70 nucleotide stem-loop structures as precursor miRs (pre-miRs) (Lee et al., 2003, and see Fig. 1). In plants, instead of Drosha protein, Dicer-like protein 1 (DCL1) acts to convert pri-miRs to pre-miR duplexes (Papp et al., 2003; Xie et al., 2004). Then, the excised approximately 70 nucleotide fold-back double-strand RNA (dsRNA) precursors are exported from the nucleus to the cytoplasm by the protein Exportin5 (Yi et al., 2003). In the cytoplasm, the pre-miRs are cleaved by the RNase III enzyme, Dicer, generating approximately 22 nucleotide siRNA-like complexes (miR/miR*) loaded onto Argonaute (Ago) protein (Hutvágner et al., 2001; Maniataki and Mourelatos, 2005, and see Fig. 1). Specifically, the stem of pre-miR has an imperfectly double-stranded structure, and the region of RNA duplex is essentially the same as siRNA. The mature miR is partially paired to the miRNA* and the small RNA resides on the opposite side of the pre-miR stem. One strand of the duplex remains in Ago as a mature miR, while the other strand (miR*) is degraded (Schwarz et al., 2003; Du and Zamore, 2005, and see Fig. 1).

Following Dicer cleavage, the resultant approximately 22 nucleotide RNA duplex is loaded onto Ago protein so as to generate an effecter complex of the RNA-induced silencing complex (RISC) recruited by trans activator RNA (TAR)-binding protein (TRBP) and PACT (Protein activator of PKR; dsRNA activated protein kinase) (Chendrimada et al., 2005; Haase et al., 2005; Lee et al., 2006). GW182 proteins are also crucial for the miR-mediated gene silencing in animal cells. GW182 family proteins interact directly with Ago. The middle and C-terminal regions act as an autonomous domain in repressive function and the function is independent of both the interaction with Ago and of P-body (Processing body) localization (Ding et al., 2005; Eulalio et al., 2009). Translationally repressed mRNAs are collected in discrete cytoplasmic foci as P-bodies (Pillai et al., 2005; Eulalio et al., 2007; Parker and Sheth, 2007).

Many studies indicate that miRs repress post-transcriptional activity. In *C. elegans*, *lin-4* negatively regulates *lin-14* via repressing its translation without inhibiting *lin-14* mRNA biogenesis and translation initiation (Olsen and Ambros, 1999). Bantam binds to the 3′UTR of the pro-apoptotic gene *hid* to exert a negative effect on its translation in *Drosophila* (Brennecke et al., 2003). Most animal miRs inhibit the translation of target mRNAs (Fig. 2). In plants, most miRs mediate the stabilization of target mRNAs (Hake, 2003, and see Fig. 2). Plant miRs pair with mRNA almost perfectly and their complementary sites are located throughout the transcribed region of the target, not limited to the 3′UTR region. The Epstein-Barr virus (human herpesvirus) has been shown to encode miRs, though viral miRs do not have close homologs in other viral or host genomes. Viral miRs are expressed individually or in clusters from either polymerase or promoters, and regulate both viral and host genes (Pfeffer et al., 2004). In the fly and animal, miRs pair with their targets through limited 2–7 nucleotides of its 5′ region (called the seed region), to prohibit translation and direct degradation of

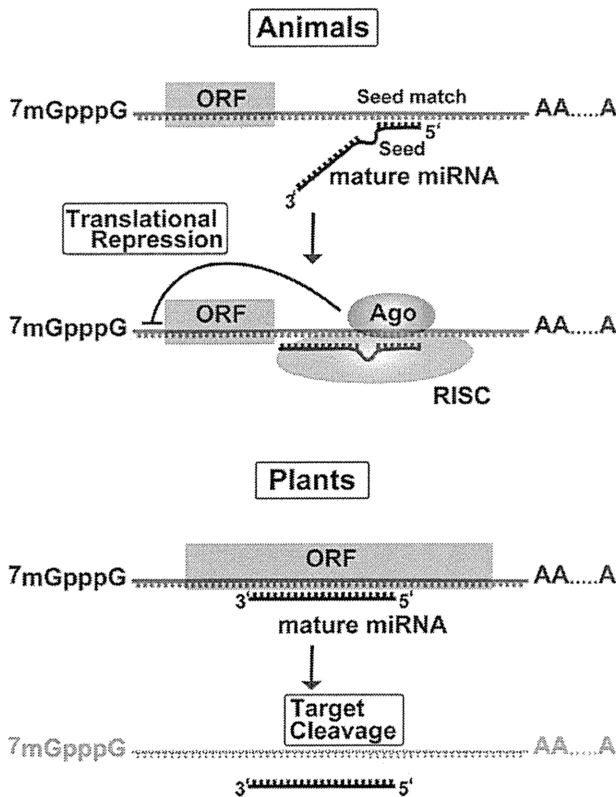


Fig. 2. MiR targeting in animal- and plant-cells. Most animal miRs recognize partially complementary binding sites, which are generally located in 3′UTRs. In the fly and animal, miRs pair with their targets through limited 2–7 nucleotides of its 5′ region (called the seed region), to prohibit translation and direct degradation of target mRNAs. Plant miRs pair with target mRNA almost perfectly and their complementary sites are located through the transcribed region of the target mRNA, not limited to the 3′UTR region, and direct endonucleolytic mRNA cleavage within the base-paired region.

target mRNAs (Fig. 2). Considering that the seed region is so short, miRs are predicted to regulate a huge number of genes. In fact, human miRs may regulate as many as one-third of all protein-coding genes (Lewis et al., 2003, 2005; Xie et al., 2005).

3. miR expression in brain

miRs play a role in various cellular processes, including apoptosis and metabolism in both plants and animals. Recently, miRs that affect neuronal function in the brain have been reported. Characterization of the expression profiles of 119 miRs in mouse and human organs indicate that these miRs are phylogenetically conserved, organ-enriched and organ-specific. Interestingly, 13 of these miRs are enriched in the brain. Moreover, eight brain miRs are induced during neuronal differentiation of human and mouse EC cells after retinoic acid application, suggesting that there may be a conserved role in mammalian neuronal development (Sempere et al., 2004). Indeed, several studies indicate a relationship between miR and neuronal development. For example, miR-124a, which is perfectly conserved at the nucleotide level from worms to humans, is expressed throughout the embryonic and adult CNS (Lagos-Quintana et al., 2002). miR-124 plays an important role in neuronal differentiation and function, as overexpression of miR-124 in differentiating mouse P19 cells promotes neurite outgrowth, while blockade of miR-124 function decreases neurite outgrowth and levels of acetylated α-tubulin (Yu et al., 2008). Interestingly, miR-18 and -124a reduce glucocorticoid receptor (GR)-mediated events in addition to decreasing GR protein levels by binding to the 3′ untranslated region of GR (Vreugdenhil et al., 2009). Glucocorticoid is a stress hormone and putatively involved in the pathophysiology of mental disorders such as depression (see Sections below). Furthermore, expression of miR-9 is also found in the CNS and the knockdown of the miR results in inhibition of neurogenesis along the anterior–posterior axis (Bonev et al., 2011). miR-134 is also localized in the dendritic compartment of hippocampal neurons (Schratt et al., 2006). In midbrain dopamine neurons, miR-133b specifically expresses and regulates neural maturation and function via a negative feedback circuit that includes Pitx3, a paired-like homeodomain transcription factor (Kim et al., 2007). Recently, it was shown that overexpression of miR-137 promotes the proliferation of adult neuronal stem cells, whereas a reduction of miR-137 results in enhancement in differentiation (Szulwach et al., 2010). Taken together, all these studies indicate that brain-specific miRs have multiple roles in neuronal function. In the following section, we focus specifically on the relationship between miR function and the neurotrophin BDNF, which is well known as a critical mediator in synaptic plasticity.

4. miRs and BDNF

4.1. BDNF and synaptic function

In addition to BDNF, a member of the neurotrophin family, nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4/5 are found mainly in mammals. As high-affinity tropomyosin-related kinase (Trk) receptors, TrkA (for NGF), TrkB, and TrkC (for NT-3) are essential for proper neurotrophin functioning. In the brain, it is well known that expression of both BDNF and its receptor TrkB is very strong, as this ligand–receptor complex is responsible for multiple neuronal functions. Via stimulation of TrkB, BDNF initiates activation of various intracellular signaling cascades such as MAPK/ERK, PLCγ, and PI3K pathways. BDNF/TrkB signaling is essential for cell differentiation and survival, neurite outgrowth, and synaptic function (Huang and Reichardt, 2003; Reichardt, 2006; Minichiello, 2009; Russo et al., 2009; Numakawa et al., 2010a). BDNF is critical in glutamatergic functioning, contributing to the maintenance of both NMDA and AMPA receptors as well as glutamate-mediated synaptic plasticity (Caldeira et al., 2007a, 2007b; Yoshii and Constantine-Paton, 2010). We also demonstrated a BDNF-dependent increase in levels of glutamate receptor subunits including NR2A, NR2B, and GluR1, in addition to presynaptic synapsin I and synaptotagmin (Matsumoto et al., 2006; Kumamaru et al., 2008, and see Fig. 3). In the upregulation of synaptic proteins, activation of MAPK/ERK signaling was required (Matsumoto et al., 2006; Kumamaru et al., 2008). A recent study showed that unsupervised learning (*in vivo*) and theta burst stimulation (with hippocampal slices) increases the number of postsynaptic densities co-localized with activated TrkB in the hippocampus. The potentiation of the co-localization was inhibited by a scavenging extracellular BDNF or NMDA receptor antagonist (Chen et al., 2010), suggesting that ligand BDNF and stimulation of glutamate receptors are both involved in the learning process. In general, it is believed that an increase in intracellular Ca^{2+} concentration contributes to the activity-dependent synaptic secretion of BDNF. An increase in Ca^{2+} via voltage-gated Ca^{2+} channels (VDCC), internal Ca^{2+} stores, and NMDA receptors was, in fact, demonstrated (Hartmann et al., 2001; Kuczewski et al., 2009; Lessmann and Brigadski, 2009). Taken together, an intimate relationship exists between BDNF and synaptic function. A detailed investigation of the mechanisms underlying BDNF-mediated changes in synaptic plasticity is paramount, and the possible

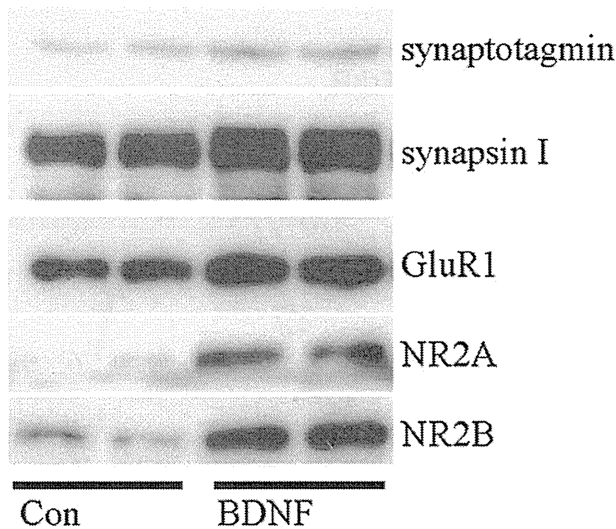


Fig. 3. BDNF increased expression of synaptic proteins including synaptotagmin, synapsin I, GluR1, NR2A, and NR2B. Cultured cortical neurons were prepared from postnatal 2 day-old rats. Six-hours after cell plating, BDNF (100 ng/ml) was added. After an additional 5-day culture, the cells were lysed for immunoblotting.

association of these mechanisms with brain-specific miRs is a topic of increased interest.

4.2. Neuronal function of miRs and BDNF

Brain-specific miRs play a role in synaptic function. In addition, BDNF is also a key player in synaptic plasticity in CNS neurons. Indeed, the interaction between BDNF and brain-specific miRs is an area of recent focus. Remarkably, after the loss of brain-specific miRs, enhanced learning and memory is observed in mice (Konopka et al., 2010). After deletion of the Dicer1 gene in mouse forebrain, a whole set of brain miRs (including miR-124, -132, -137, -138, -29a, and -29c) was significantly decreased. In Dicer1 mutant mice, the efficacy at hippocampal synapses and translation of BDNF was higher than in controls (Konopka et al., 2010). As mentioned above, Schratt et al. reported involvement of miR-134 in BDNF-regulated dendritic spine size in hippocampal neurons (Schratt et al., 2006). The miR-134 is localized in dendrites, and negatively regulates the spine size via repressing the translation of Limk1 mRNA (LIM kinase 1), which is known to regulate dendritic structures (Bamburg, 1999; Meng et al., 2004). They demonstrate that BDNF relieves suppression of Limk1 translation caused by miR-134 (Schratt et al., 2006). The current study shows that miR-134 regulates memory and neuronal plasticity (Gao et al., 2010). It is possible that the mammalian Sir2 homolog SIRT1 promotes synaptic plasticity via mediating expression of CREB by miR-134, as mutant mice lacking SIRT1 catalytic activity showed reduction in both CREB and BDNF proteins and upregulation of miR-134. A knockdown of miR-134 reversed the hippocampal long-term potentiation in SIRT1 mutant mice (Gao et al., 2010). In the cell differentiation of SH-SY5Y cells, a human neuroblastoma cell line, possible involvement of miR-125b has been reported (Le et al., 2009). miR-125b was significantly upregulated after all-trans-retinoic acid and BDNF application, and ectopic expression of miR increased differentiated SH-SY5Y cells with neurite outgrowth (Le et al., 2009). miR-375 also plays a role in regulation of neurite differentiation. Though exposure to BDNF increased neurite outgrowth in human neuroblastoma BE(2)-M17 cells, the miR-375 overexpression diminished BDNF action. Interestingly, BDNF-promoted neurite outgrowth was inhibited by silencing HuD (neuronal RNA-binding protein) or overexpressing miR-375, suggesting that miR suppresses neurite outgrowth via decreasing the HuD levels. Indeed, reporter analysis revealed that miR-375 decreased HuD expression through targeting the HuD 3′UTR (Abdelmohsen et al., 2010).

miR-132 contributes to the action of BDNF. In cultured cortical neurons, BDNF induces upregulation of miR-132 (Vo et al., 2005). Interestingly, overexpression of miR-132 increases the outgrowth of primary neurites, and transfection of an antisense RNA for miR-132 results in a substantial decrease in neurite outgrowth (Vo et al., 2005). Recently, we also reported that BDNF induced a marked increase in miR-132 levels in cultured cortical neurons (Kawashima et al., 2010, and see Fig. 4). miR-132 upregulation may be a direct result of the BDNF in neurons, as the other miRs such as miR-9, -124, -128a, -128b, -134, -138, and -16 (as a control) were unchanged. While BDNF increased the expression of synaptic proteins including NR2A, NR2B and GluR1 (Fig. 3), transfection of double strand-miR-132 also upregulated these glutamate receptors (Kawashima et al., 2010, and see Fig. 4). Antisense RNA to inhibit endogenous miR-132 function decreased the number of BDNF-increased glutamate receptors. Interestingly, we found that blockade of the MAPK/ERK pathway suppressed BDNF-increased miR-132 levels (Fig. 4). As described above, we previously showed an important role of ERK signaling in the maintenance of glutamate receptor expression (Matsumoto et al., 2006; Kumamaru et al., 2008; Tuerxun et al., 2010). Taken together, it is possible that an increase of miR-132 via ERK activation is required for upregulation of glutamate receptors after BDNF stimulation. Furthermore, Remenyi et al. reported upregulation of miR-212/132 by BDNF stimulation in cultured cortical neurons (Remenyi et al., 2010). In their system, miRs were regulated by the ERK pathway, specifically via downstream MSK1 (mitogen- and stress-activated kinase 1) and CREB signaling.

Importantly, we found that the BDNF-increased ERK1/2 activation, miR-132 expression, and postsynaptic proteins were all diminished following chronic treatment with glucocorticoids, suggesting that treatment with chronic glucocorticoids caused suppression in BDNF-dependent neuronal function via reducing miR-132 expression (Kawashima et al., 2010, and see Fig. 5). Interestingly, increased blood levels of glucocorticoids (cortisol in humans, and corticosterone in rodents), which are stress hormones released from the adrenal glands, are putatively associated with the pathophysiology of mental disorders including depression (McEwen, 2005; Kunugi et al., 2010). Prolonged exposure to glucocorticoids as a result of stressful conditions may cause depression via damaging vital neuronal functions, as many studies suggest that downregulation of expression/function of BDNF is also closely related to the onset of depression (Altar, 1999; Nestler et al., 2002; Knable et al., 2004; Gervasoni et al., 2005; Karege et al., 2005; Numakawa et al., 2010b). We recently found a functional interaction of glucocorticoids with BDNF in cortical neurons. In cortical cultures, GR expression and an interaction between the GR and TrkB were decreased after exposure to glucocorticoids. The BDNF-evoked release of glutamate was also reduced because the GR-TrkB interaction is important for glutamate release induced by BDNF (Numakawa et al., 2009). Considering that BDNF affects levels of miRs, and glucocorticoids impact the level/function of BDNF negatively, further investigation into the relationship between glucocorticoids and miRs expression is warranted. It was reported that miR-18 and -124a decreased GR-mediated events in addition to reducing GR expression levels (Vreugdenhil et al., 2009). Both miRs suppressed levels of glucocorticoid-induced leucine zipper expression induced by DEX, a GR-selective glucocorticoid, and the reporter assays indeed revealed that miR-124a putatively bound to the 3′UTR of GR (Vreugdenhil et al., 2009). As the expression of miR-124a is restricted to the brain (Lagos-Quintana et al., 2002), further study of the neuronal role of miR-124a is needed.