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

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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.

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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

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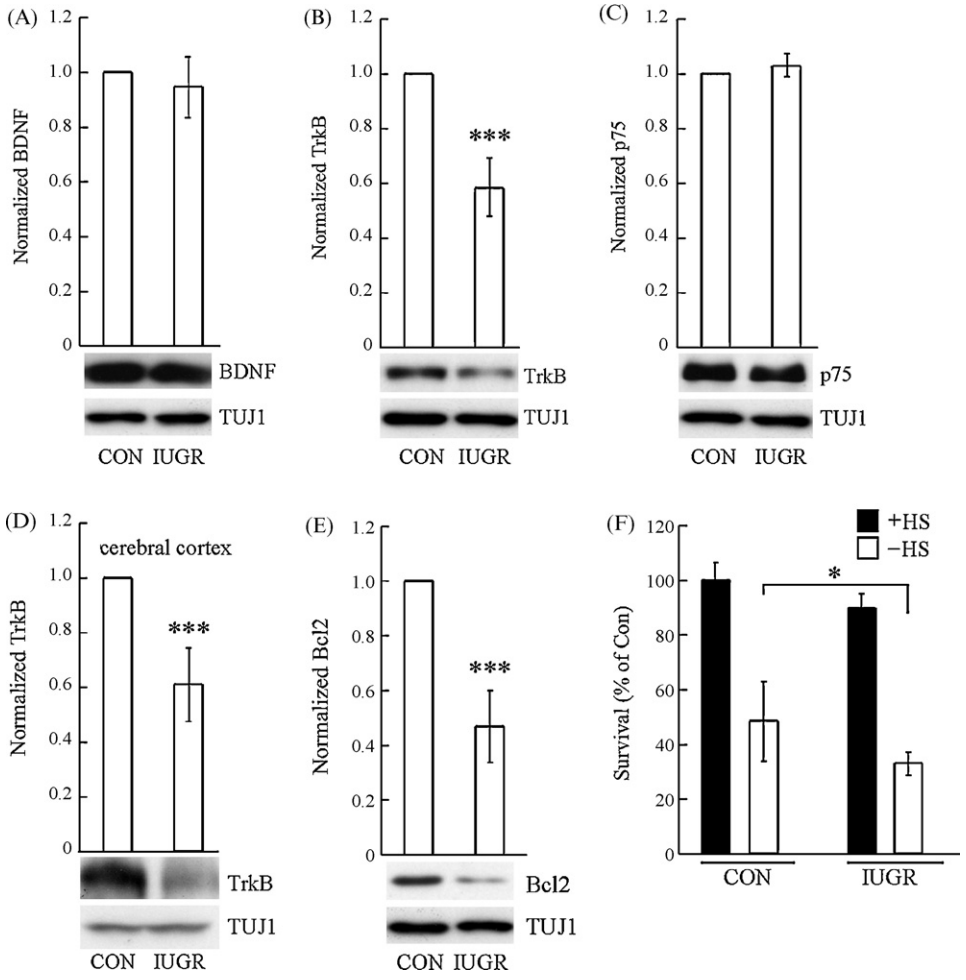

Fig. 1. Reduction in levels of TrkB, Bcl2, and cell viability in cortical cultures prepared from the cerebral cortex of low weight newborn rats with TXA2-induced IUGR. The levels of BDNF (A), TrkB (B), and p75 (C) were examined in 5DIV cortical cultures from IUGR-rats or from control-rats. TrkB was reduced 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 $5 \times 10^5/\text{cm}^2$, 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

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