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WJBC World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
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doi:10.4331/wjbc.v1.i5.133

World J Biol Chem 2010 May 26; 1(5): 133-143
ISSN 1949-8454 (online)

Functional interactions between steroid hormones and neurotrophin BDNF

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Author contributions: Numakawa T performed the research; Numakawa T, Yokomaku D, Hori H and Adachi N wrote the paper; Numakawa T, Richards M and Kunugi H edited the paper.
Supported by 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 (Kunugi H), and a Grant-in-Aid for Young Scientists (A) (21680034) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Numakawa T)
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Received: April 23, 2010 Revised: May 20, 2010
Accepted: May 24, 2010
Published online: May 26, 2010

Abstract

Brain-derived neurotrophic factor (BDNF), a critical neurotrophin, regulates many neuronal aspects including cell differentiation, cell survival, neurotransmission, and synaptic plasticity in the central nervous system (CNS). Though BDNF has two types of receptors, high affinity tropomyosin-related kinase (Trk)B and low affinity p75 receptors, BDNF positively exerts its biological effects on neurons *via* activation of TrkB and of resultant intracellular signaling cascades including mitogen-activated protein kinase/extracellular signal-regulated protein kinase, phospholipase Cγ, and phosphoinositide 3-kinase pathways. Notably, it is possible that alteration in the expression and/or function of BDNF in the CNS is involved in the pathophysiology of various brain diseases such as stroke, Parkinson's disease, Alzheimer's disease, and mental disorders. On the other hand, glucocorticoids, stress-induced steroid hormones, also putatively contribute to the pathophysiology of depression. Interestingly, in addition to the reduction in BDNF levels due to increased glucocorticoid exposure, current reports demonstrate possible interactions between glucocorticoids and BDNF-mediated neuronal functions. Other steroid hormones, such as estrogen, are involved in not only sexual differentiation in the brain, but also numerous neuronal events including cell survival and synaptic plasticity. Furthermore, it is well known that estrogen plays a role in the pathophysiology of Parkinson's disease, Alzheimer's disease, and mental illness, while serving to regulate BDNF expression and/or function. Here, we present a broad overview of the current knowledge concerning the association between BDNF expression/function and steroid hormones (glucocorticoids and estrogen).

Key words: Brain-derived neurotrophic factor; Steroid hormones; Neurotrophin; Glucocorticoid; Estrogen; Tropomyosin-related kinase; Extracellular signal-regulated protein kinase; Phospholipase Cγ; Phosphoinositide 3-kinase

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Numakawa T, Yokomaku D, Richards M, Hori H, Adachi N, Kunugi H. Functional interactions between steroid hormones and neurotrophin BDNF. *World J Biol Chem* 2010; 1(5): 133-143 Available from: URL: http://www.wjgnet.com/1949-8454/full/v1/i5/133.htm DOI: http://dx.doi.org/10.4331/wjbc.v1.i5.133

INTRODUCTION

Neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5, bind to high-affinity tropomyosin-related kinase (Trk) receptors. It is known that NGF binds to TrkA, BDNF and NT-4/5 bind to TrkB, and NT-3 binds to TrkC (additionally to TrkB, weakly), although there is a common low-affinity p75 receptor for all neurotrophins. Specifically, BDNF and TrkB are broadly and strongly expressed in the mammalian brain and exert beneficial effects on central nervous system (CNS) neurons. Following activation of TrkB, due to binding with BDNF, activation of various intracellular signaling pathways, including mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK), phospholipase Cγ (PLCγ), and phosphoinositide 3-kinase (PI3K) pathways, are triggered[1]. These intracellular signaling cascades have multiple roles in cell differentiation, nerve growth, neuronal survival, and synaptic plasticity in both the developing and mature nervous system[2]. Importantly, dysfunction of BDNF may be involved in the pathophysiology of various brain diseases. A reduction in BDNF levels has also been indicated in various mental disorders[3-5].

Important stress hormones, such as glucocorticoids, are also putatively associated in the pathophysiology of depression[6]. Glucocorticoids play an essential role in coping with stressful conditions, and are well known to regulate the expression of various target genes *via* the glucocorticoid receptor (GR)[7]. In general, the level of blood glucocorticoids is controlled through the hypothalamic-pituitary-adrenal (HPA)-axis[8]. In turn, the sustained increase in glucocorticoids after prolonged exposure to stress may cause extensive damage to the CNS, resulting in the onset of depression[9]. As both BDNF and glucocorticoids may be involved in neuronal function and the pathophysiology of depression, possible crosstalk between BDNF and glucocorticoid function is very interesting. In this review, we provide an overview of the current knowledge, including our studies, concerning the association between BDNF and glucocorticoids.

Estrogen also contributes to numerous neuronal aspects in the CNS. For example, 17β-estradiol (17β-E2), one of the estrogens, promotes cell differentiation and survival in cultured hypothalamic[10], amygdala[11], and neocortical neurons[12]. In cortical cultures, we also reported that 17β-E2 protects neurons from cell death caused by oxidative stress *via* decreasing MAPK/ERK signaling activity[13]. Furthermore, we previously showed that pretreatment of cultured hippocampal neurons with 17β-E2 enhances activity-dependent release of glutamate, the main excitatory neurotransmitter, *via* activation of PI3K and MAPK/ERK pathways. It is important to mention, however, that potentiation by estradiol in the release of the main inhibitory neurotransmitter, GABA, was not observed[14]. Considering that many studies demonstrate that 17β-E2 can stimulate the same signaling pathways as BDNF, we describe relations between estrogen and BDNF in the latter part of this paper.

GLUCOCORTICOIDS AND BDNF

BDNF and intracellular signalings

The *BDNF* gene has at least nine exons. Specifically, exon IX encodes the open reading frame for the entire BDNF protein, while the remaining exons possess their own distinct promoters. Transcription of the *BDNF* gene is initiated from each 5' exon spliced onto the common 3' exon IX in response to the specific stimulus[15] (Figure 1A). The length of the 3' untranslated region of BDNF mRNA influences the dendritic transport of the mRNA in hippocampal neurons[16]. Importantly, neuronal activity also impacts the transcription and secretion of BDNF. Ca^{2+} influx *via* Ca^{2+} channels triggers activation of cAMP-responsive element binding protein (CREB), which regulates transcription of many genes including BDNF[17]. Such mechanisms underlying the production and/or release of BDNF are suggested to be involved in the activity-dependent maturation and modulation of synaptic connections in the adult CNS[18,19]. Recently, it was reported that binding of CREB to promoter IV is necessary for experience-dependent induction of BDNF transcription in addition to facilitating inhibitory synapse development[20].

BDNF exerts biological effects on the neuronal system following the binding to two types of transmembrane receptors. One transmembrane receptor is a high affinity TrkB receptor, and the other is a low affinity p75 neurotrophin receptor[21]. The binding of BDNF to the extracellular domain of TrkB triggers dimerization of the receptor followed by autophosphorylation (activation) of tyrosin residues located in the intracellular kinase domain. The TrkB phosphorylation induces activation of three intracellular signaling cascades commonly referred to as the MAPK/ERK, PI3K, and PLCγ pathways (Figure 1B). Together, phosphorylation of the tyrosine 515 residue located in the juxtamembrane region and the tyrosine 816 residue in the C-terminus of TrkB accelerate recruitment of the Src homology domain-containing protein (Shc) and PLCγ, respectively[22,23]. Shc phosphorylation leads to activation of the MAPK/ERK pathway, which promotes neuronal differentiation and growth, and of the PI3K/Akt pathway, which is essential for cell survival. PLCγ activation causes production of inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). Increased IP_3 stimulates

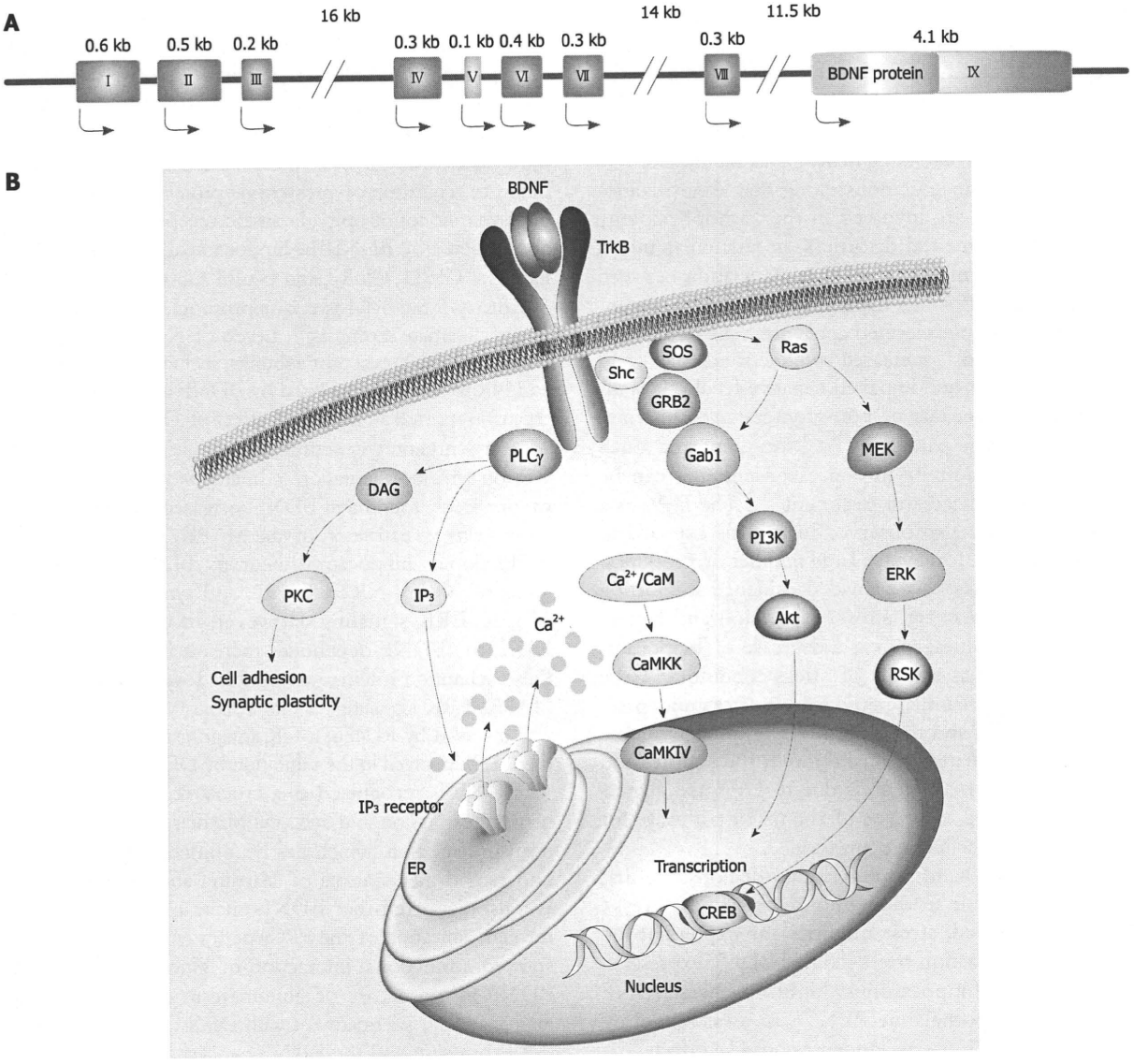

Figure 1 Brain-derived neurotrophic factor (*BDNF*) gene and stimulated intracellular signaling cascades after activation of tropomyosin-related kinase (Trk)B. A: Mouse and rat *BDNF* genes (we referred to the description by Aid *et al*[15]). Each BDNF transcript is comprised of one of eight 5' untranslated exons (exon I -VIII) and the common 3' protein coding exon IX; B: Intracellular signaling after TrkB activation. Following BDNF binding, TrkB dimerization and its phosphorylation at intracellular tyrosine residues occur. Then, the activated TrkB stimulates three main signaling pathways: (1) mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK); (2) phosphatidylinositol 3-kinase (PI3K); and (3) phospholipase Cγ (PLCγ) pathways. MAPK pathway, in which MAPK/ERK kinase (MEK) is involved, plays a role in the neuronal differentiation and outgrowth. PI3K signaling promotes neuronal survival *via* Ras or GRB-associated binder 1 (Gab1). Following PLCγ activation, inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) are both produced. DAG activates protein kinase C (PKC), which is important for regulation of synaptic plasticity. Meanwhile, IP_3 increases intracellular Ca^{2+} concentration *via* IP_3 receptors on the endoplasmic reticulum (ER), resulting in activation of Ca^{2+}/calmodulin (CaM)-dependent protein kinase including CaMKII, CaMKK, and CaMKI. These MAPK/ERK, PI3K, and PLCγ pathways can regulate gene transcription.

Ca^{2+} release from internal Ca^{2+} stores, resulting in the activation of Ca^{2+}/calmodulin-dependent protein kinases (e.g. CaMKII, CaMKK and CaMKIV). DAG activates protein kinase C[23,24]. Overall, BDNF affects CNS neurons through various intracellular signaling pathways triggered by activation of TrkB[2].

Roles of glucocorticoid and BDNF in stress/depression

Increased glucocorticoid levels coupled with reduced BDNF levels have been implicated in the pathophysiology of depression. In general, many stressors activate the HPA axis through increasing the production and consequent release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus (PVN) of the hypothalamus. Following this, secreted CRH, in concert with AVP, stimulate the pituitary to produce adrenocorticotropic hormone (ACTH), which enters the bloodstream to stimulate the adrenal glands. Finally, the adrenal glands respond by producing and releasing glucocorticoids (cortisol in primates including humans, and corticosterone in rodents). Importantly, glucocorticoids participate in an inhibi-

tory feedback loop with the hypothalamus and pituitary glands in order to prevent excess synthesis and/or secretion of CRH and ACTH, respectively. In addition, the hippocampus exerts an inhibitory action on the HPA-axis. Glucocorticoids function as a master regulator for stress responses by targeting many genes *via* the GR[8].

There is evidence demonstrating that abnormalities in the HPA axis are involved in the pathophysiology of a variety of mental disorders, in particular mood disorders[25]. Specifically, a possible association between depression and HPA axis hyperactivity has been demonstrated. For example, elevated concentrations of CRH in cerebrospinal fluid[26], increased volume of adrenal[27] and pituitary glands[28], and impaired negative feedback as indicated by a higher rate of non-suppression to pharmacological challenge paradigms[9,29,30] were reported. Such HPA-axis hyperactivity in depressed patients can be improved after successful treatment[9,31]. The HPA-axis abnormalities are also observed in animals exposed to chronic stress[32]. Moreover, a large number of preclinical and clinical studies have provided evidence supporting the association between stress/depression and hippocampal abnormalities, such as a decrease of hippocampal neurogenesis as a result of stress conditions[33], the increase of hippocampal neurogenesis after antidepressant treatment[34], and the reduced hippocampal volume in depressed patients[35]. Furthermore, the suppression of hippocampal neurogenesis due to HPA-axis hyperactivity is assumed to be one of the major pathways for mood disorders including depression[36].

On the other hand, several studies demonstrate that BDNF plays a role in the pathophysiology of stress/depression. Indeed, stress modifies the expression of BDNF; immobilization stress reduces BDNF expression throughout the hippocampus[37] and increases BDNF levels in the hypothalamic PVN[38]. In a rat model of depression, BDNF exerts antidepressant-like effects[39,40]. As expected, antidepressant treatment increases BDNF levels in limbic structures, most prominently in the hippocampus[41,42]. In patients with depression, decreased serum BDNF levels[43,44] and improvement in attenuated BDNF levels through antidepressant treatment[45] were observed. Furthermore, increased hippocampal BDNF levels were documented in postmortem brains of subjects treated with antidepressants[46]. Interestingly, evidence concerning the possible involvement of BDNF in HPA axis function was shown. In animals, central administration of exogenous BDNF was shown to modify HPA axis function[47,48]. Both BDNF and glucocorticoids may be involved in the pathophysiology of depression and overall neuronal function in the CNS, though the possible interaction between glucocorticoids and BDNF is poorly understood.

Functional interaction between glucocorticoids and BDNF

Many studies indicate that BDNF is important in the regulation of synaptic proteins. In the release of neurotransmitters, synaptic proteins including synaptic vesicle-associated synaptic proteins (e.g. synapsin I , synaptotagmin and synaptophysin) and plasma membrane-associated synaptic proteins (syntaxin and synaptosomal-associated protein of 25 kDa) are critical[49]. Many studies revealed that BDNF upregulates levels of these presynaptic proteins[50-52]. In addition to regulation of presynaptic proteins, expression of postsynaptic ionotropic glutamate receptors (GluRs) are also affected by BDNF. In hippocampal cultures, BDNF increases GluR1, GluR2, and GluR3 subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type ionotropic glutamate receptors[53]. Levels of N-methyl-D-aspartic acid (NMDA) receptor subunits, including NR1, NR2A and NR2B, are also increased by BDNF application[54]. We recently reported an inhibitory effect of DEX (dexamethasone, a synthetic glucocorticoid, and selective ligand for GR) on synaptic maturation[55]. In cultured cortical neurons, we previously found that BDNF increased levels of synaptic proteins *via* activation of the MAPK/ERK pathway[56]. In developing hippocampal neurons, BDNF upregulated levels of NR2A, NR2B, GluR1, and synapsin I through MAPK/ERK signaling. However, in the presence of DEX, the BDNF-dependent increase in expression of these synaptic proteins was inhibited *via* suppression of MAPK/ERK signaling[55]. The inhibitory action of DEX was reversed by RU486, a GR antagonist, suggesting that the GR is involved in the inhibition by DEX.

BDNF is recognized as a crucial regulator for basal neurotransmission and synaptic plasticity including long-term potentiation, which has been intensively studied to understand mechanisms of learning and memory[2,57-64]. We also reported that BDNF elicits glutamate release through activation of the PLCγ pathway[65-67]. Recently, we showed a functional interaction of glucocorticoids with BDNF in the release of glutamate in cultured cortical neurons. After pretreatment with DEX or corticosterone, GR expression and the BDNF-evoked glutamate release were both diminished[68] (Figure 2A and B). On the other hand, the TrkB levels were intact after exposure to glucocorticoids (Figure 2B). Interestingly, we found that the GR interacts with TrkB, and the TrkB-GR interaction may be important for the regulation of BDNF-evoked glutamate release. Following DEX treatment, the TrkB-GR interaction was reduced due to the decline in GR levels. Similarly, the BDNF-stimulated binding of PLCγ to TrkB was also declined. In contrast, GR overexpression enhanced the TrkB-GR interaction, PLCγ activation, and glutamate release. Therefore, it is possible that the TrkB-GR interaction is critical for glutamate release stimulated by BDNF *via* regulation of PLCγ signaling, and that the decrease in TrkB-GR interaction after chronic glucocorticoid exposure resulted in the dysfunction of the BDNF-dependent neurotransmission[68].

In general, glucocorticoids are believed to display their effects *via* transcriptional regulation of various genes targeted by GR. Remarkably, glucocorticoids acutely activate Trks signaling through the genomic function (*via* transcriptional activity) of the GR. After *in vivo* administration

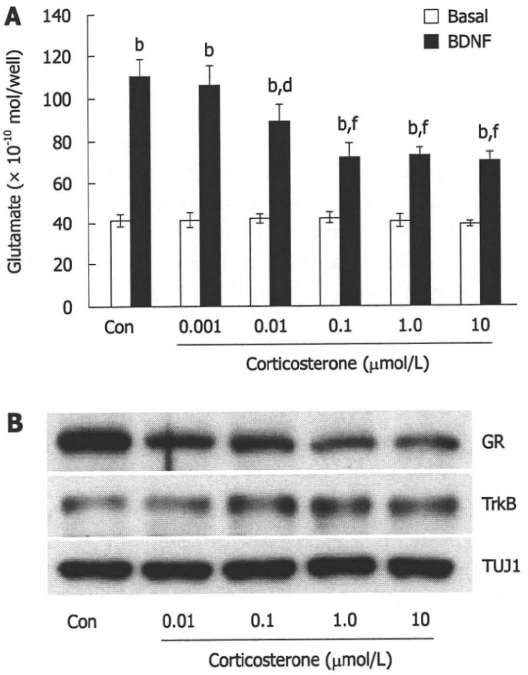

Figure 2 Glucocorticoids depressed BDNF-induced release of glutamate and expression of GR in cultured cortical neurons. A: Dose-dependent inhibitory effect of corticosterone pretreatment on BDNF-induced glutamate release. Corticosterone (0.001-10 μmol/L) was applied at DIV4. Forty-eight hours later, BDNF (100 ng/mL, 1 min) was added and released glutamate was measured by HPLC. Prior to performing the BDNF application, samples were collected without stimulation as the basal release (1 min). Con means no application of corticosterone. Data represent mean ± SD (n = 4). $^{b}P < 0.001$ *vs* basal, $^{d}P < 0.01$, $^{f}P < 0.001$ *vs* BDNF-induced release in Con (t-test); B: Endogenous expression of glucocorticoid receptor (GR) was decreased after corticosterone (0.01-10 μmol/L) was applied at DIV4. Forty-eight hours later, cell lysates were collected for western blotting. Endogenous expression of TrkB was unchanged after exposure to corticosterone. Levels of TUJ1 (class Ⅲ β-tubulin), a neuronal marker, are shown as control.

in the brain and in cultures of hippocampal and cortical neurons, the glucocorticoid-stimulated activation of Trks was induced[69]. In that system, other tyrosine kinase receptors, such as EGF and FGF receptors, were not activated by glucocorticoids. The glucocorticoid-dependent activation of Trks has a neuroprotective role. Accumulating evidence, including our study on BDNF-stimulated glutamate release, demonstrates a nongenomic (not *via* transcriptional activity) function of GR. Löwenberg *et al*[70] reported the functional interaction between the GR and the T-cell receptor (TCR) complex. In T cells, the GR plays an important role in TCR signaling. After the glucocorticoid is bound to the GR, the GR dissociates from the complex, resulting in inhibition of TCR signaling[70]. Rapid action of glucocorticoids may be mediated by the activation of membrane-associated receptors. Some evidence suggests that rapid glucocorticoid actions are stimulated *via* membrane-associated G protein-coupled receptors and activation of downstream intracellular signaling pathways[71]. In rat liver and hepatoma cells, feline McDonough sarcoma-like tyrosine kinase 3 was identified as a GR-interacting protein[72]. It was revealed that Flt3 interacts with both non-liganded and liganded GR, and the DNA-binding domain of GR is sufficient for the interaction. In our cortical cultures, it is possible that the N-terminal region (including DNA binding site) of the GR interacts with TrkB, however, the C-terminal region is also required to reinforce the BDNF-stimulated PLCγ signaling[68]. In the cytoplasm of rat liver cells, GR interaction with 14-3-3 and Raf-1 was identified, implying that the GR directly influences cytosolic signaling[73]. To reveal detailed mechanisms underlying acute functions of GR in the CNS, it may be valuable to study possible interactions between GR and cytosolic signaling mediators.

Using *in vivo* experiments, Gourley *et al*[74] reported a significant decrease in NR2B, GluR2/3, as well as BDNF levels in cortical regions, but not in the dorsal hippocampus, after corticosterone exposure. Moreover, the effect of prenatal DEX treatment in male and female adult rat offspring has been investigated[75]. In this system, DEX male offspring had reduced adrenal gland weight in adult life and demonstrated anxious behavior. By assessing the acoustic startle response as well as the effects of acoustic challenge in the PVN, it was revealed that BDNF and TrkB mRNA were increased after acoustic challenge in the control males and females, but not in the DEX males or females. On the other hand, an enriched environment (EE) can induce changes in stress hormone release and BDNF levels[76]. In general, EE has beneficial neurobiological, physiological and behavioral effects[77]. Bakos *et al*[76] showed that the EE-induced rise in hippocampal BDNF in females was more pronounced than in males. Similar sex-specific changes were confirmed in the hypothalamus. Moreover, a negative association between corticosterone and BDNF levels was observed in both sexes.

Antidepressant drugs and BDNF

As mentioned above, it is possible that upregulation in expression and/or function of BDNF is involved in antidepressant treatment[78]. Antidepressants, including inhibitors of monoamine transporters and metabolism, activate TrkB rapidly in the rodent anterior cingulate cortex and hippocampus *in vivo*[79]. Importantly, acute antidepressant treatments induce activation of PLCγ *via* TrkB, though no alteration in phosphorylation of MAPK or Akt was observed[79]. Using cultured cortical neurons, we also reported that pretreatment with antidepressant drugs, including imipramine and fluvoxamine, enhanced BDNF-induced glutamate release *via* increasing PLCγ activation[80]. In our system, other pathways activated by TrkB (i.e. PI3K/Akt and MAPK/ERK pathways) were not changed after imipramine pretreatment. Importantly, the potentiation of glutamate release by imipramine was inhibited by BD1047, a sigma-1 receptor antagonist, suggesting the possible involvement of sigma-1 receptor function. Recently, we have also shown that SA4503, a sigma-1 receptor agonist, has a neuroprotective effect under oxidative-stress[81]. It is possible that a sigma-1 receptor has multiple functions in the CNS.

Fluoxetine, which is a widely prescribed medication

for depression, improves neuronal function in the visual system of rats. In the adult rat visual cortex following chronic administration of fluoxetine, BDNF levels were increased. In addition, a similar increase in BDNF levels in the hippocampus was also indicated[82]. Antidepressants, including monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, noradrenaline reuptake inhibitors, and tricyclic, noradrenergic, serotonergic antidepressants, all cause upregulation of BDNF[83]. Russo-Neustadt *et al*[84] reported that reboxetine (for 2 d) caused an increase in BDNF transcription in several hippocampal regions. The same increase was also induced after reboxetine application was combined with voluntary physical activity for 2 wk. On the other hand, citalopram (for 2 d) induced upregulation of BDNF in only the CA2 region of the hippocampus, and when combined with voluntary physical activity, the CA4 and dentate gyrus exhibited increased BDNF levels after 2 wk[84]. Recently, O'Leary *et al*[85] demonstrated that fluoxetine increases Phospho-Synapsin, postsynaptic density 95 (PSD-95), and synaptic GluR1 in the hippocampus of ovariectomized rats. Furthermore, they clarified that fluoxetine caused an increase in PSD-95 levels in ovariectomized wildtype mice but not in ovariectomized TrkB T1 (a truncated form of the TrkB receptor) transgenic mice, suggesting an involvement of TrkB signaling in fluoxetine action[85]. The influence of chronic antidepressant treatment on BDNF expression under stressful conditions has been investigated. After male rats were treated for 21 d with vehicle or with duloxetine and exposed to an acute swim stress (for 5 min) 24 h after the last injection, the chronic duloxetine modulated the rapid transcriptional changes of BDNF isoforms induced by swim stress[86]. In their system, a significant increase of exon VI and exon IX of BDNF was only found in rats that were pretreated with duloxetine, though exon IV was upregulated by stress in both vehicle- and duloxetine-treated rats. As shown, the effect of antidepressants on BDNF expression and function is gradually becoming more clear, though further studies are needed to understand the molecular mechanisms associated with each BDNF exon and their effect on clinical depression.

ESTROGEN AND BDNF

Estrogen, one of the sex steroids, is known to have strong effects on various brain functions including sex differentiation, learning and memory, synaptic plasticity, and neuroprotection[87-90]. In general, estrogen is mainly produced in the ovaries and the corpus luteum, and reaches the brain through blood vessels. Furthermore, it has been recently reported that estrogen is produced *de novo* from cholesterol in the brain[91-93]. Therefore, it is very interesting to know how estrogen production is regulated and how estrogen affects brain function. In this section, we briefly introduce several functions of estrogen in the brain. Specifically, as many studies suggest a link between estrogen and BDNF, we review one hypothesis concerning estrogenic action and potential interactions with BDNF.

Modulation of synaptic plasticity, learning and memory, and neuroprotection by estrogen

Sexual dimorphism in the brain is determined during critical perinatal periods[87,94]. It is well known that the determination is influenced by genetic background and sex steroid exposure. In the male brain during the perinatal stage, testosterone is converted to estrogen by cytochrome P450, and, in turn, the converted estrogen plays a role in brain differentiation. On the other hand, in the female brain, maternal estrogen does not affect sexual dimorphism because the estrogen in the serum binds to an estrogen-specific binding protein called α-fetoprotein. Therefore, the estrogen complex is not able to access the brain. In summary, estrogen converted from testosterone causes differentiation to a male brain, while brains that are not exposed to such steroids become female brains.

In addition to contributing to sex differentiation in the brain, estrogen is associated with brain functions including learning and memory[95-98]. Ovariectomy impairs spatial memory formation, synaptogenesis and LTP in rodents[99,100]. Estrogen administration inversely enhances spatial memory formation, spinogenesis, and LTP in rats[101-103]. Within the *in vitro* system, positive regulation of estrogen on synaptic function is also observed. 17β-E2 treatment enhances spine formation in cultured hippocampal neurons[104], suggesting that postsynaptic modulation by estrogen is occurring. Additionally, we previously reported that 17β-E2 potentiated the depolarization-dependent release of glutamate, the main excitatory neurotransmitter, in cultured hippocampal neurons[14]. In our system, activation of MAPK/ERK and PI3K signaling is required for potentiation by 17β-E2. Importantly, the memory deficit in patients suffering from Alzheimer's disease is recovered by postmenopausal estrogen replacement therapy[105].

Estrogen has a protective effect on neurons, preventing cell death caused by oxidative-stress or excessive glutamate treatment[106-112]. We also found 17β-E2 treatment to be protective[13]. Exposure of cortical neurons to oxidative stress induced overactivation of MAPK/ERK and intracellular Ca^{2+} accumulation, resulting in apoptotic-like cell death. However, pretreatment with 17β-E2 demonstrated an inhibitory effect on MAPK/ERK overactivation, Ca^{2+} accumulation, and cell death. Furthermore, estrogen is a potent neuroprotective agent in animal models of neuronal death[89]. Chen *et al*[113] demonstrated a protective effect of 17β-E2 on CA1 hippocampal cells after ischemia in gerbils. 17β-E2 treatment has been shown to improve neurological outcomes following traumatic injury in male rats, although no effect was seen in intact females. Neuronal loss due to administration of dopaminergic toxins and kainic acid can be attenuated with 17β-E2 treatment[111].

Interaction between estrogen and BDNF-in vitro studies

As described above, estrogen has multiple functions in the brain. Some reports suggest involvement of BDNF in modulating estrogen actions[114]. Sohrabji *et al*[115] showed that estrogen can regulate the expression of BDNF *via*

the estrogen response element on the *BDNF* gene. They searched motifs resembling the canonical ERE (GGTCANNNTGACC) in the *BDNF* gene by using a computerized gene homology program. One ERE-like motif was confirmed in the currently known sequence for the *BDNF* gene, which consisted of a set of pentameric sequences with near perfect nucleotide homology (1-bp mismatch). The motif lies at the 5' end of exon Ⅸ (was exon V) that codes for the BDNF protein. They also showed that estrogen receptor-ligand complexes bind to and protect the BDNF ERE-like motif from DNase cleavage. Therefore, it is possible that BDNF levels are regulated by estrogen. In dissociated hippocampal cultures, 17β-E2 downregulates the expression of BDNF in GABAergic neurons to 40% of control within 24 h of exposure, and the downregulation returns to basal levels within 48 h[116]. This GABAergic dysfunction results in an increase in excitatory tone in pyramidal neurons, and leads to a 2-fold increase in dendritic spine density. Interestingly, exogenous BDNF blocks the effects of 17β-E2 on spine formation, and BDNF depletion with a selective antisense oligonucleotide mimics the effects of 17β-E2. This group demonstrated that 17β-E2 increases spine density *via* changing the degree of excitation/inhibition balance to favor excitation. Recently, it was reported that 17β-E2 increases protein levels of BDNF in hippocampal slice cultures[117]. In contrast, another group reported that 17β-E2 does not change the expression of BDNF in cultured hippocampal neurons[118]. In hypothalamic slice cultures, levels of BDNF mRNA were not changed by either acute or chronic treatment of 17β-E2[119]. In midbrain cultures, 17β-E2 increased BDNF protein levels[120]. Remarkably, 17β-E2 induces the release of BDNF in dentate gyrus granule cells in hippocampal slice cultures, and 17β-E2-dependent synaptogenesis was induced *via* the secreted BDNF[118].

Estrogen has been found to produce acute effects in which specific membrane receptor actions may be involved[121-125]. As mentioned above briefly, estrogen activates MAPK/ERK, PI3K, and CREB pathways[14,126]. Interestingly, BDNF also stimulates the same intracellular signaling pathways. These signaling cascades induced by estrogen are recognized as an acute cellular response, inferring that upregulation of BDNF may not be involved[114].

Interaction between estrogen and BDNF-in vivo studies

Most studies demonstrate that estrogen upregulates mRNA and/or protein expression of BDNF throughout the brain, though some groups have shown that estrogen downregulates or has no influence on BDNF levels in some brain regions[127,128]. Importantly, it was reported that 17β-E2 administration in ovariectomized female rats increased BDNF expression in the hippocampus by reverse transcriptase-polymerase chain reaction (RT-PCR)[129], in the cerebral cortex by RT-PCR[115], in the olfactory bulb by RT-PCR[115] and by Western blotting[130] and in the septum by RT-PCR[129]. Meanwhile, in some reports, estrogen has no effect on BDNF expression in the hippocampus by *in situ* hybridization[128,131] and by ELISA[129], in the cerebral cortex by *in situ* hibridization[128,131], RT-PCR[132] and ELISA[129] and in the olfactory bulb by RT-PCR[129] and ELISA[129]. Some groups report that exogenous estrogen application decreases BDNF levels in the cerebral cortex by ELISA[133]. In addition, BDNF mRNA levels in the hippocampus and cerebral cortex have been shown to fluctuate by estrous cycles in female rats[128,131]. Although there are many studies addressing the relationship between estrogen and BDNF expression levels, future studies should clarify the detailed interactions between estrogen and BDNF-mediated neuronal function in addition to elucidating the molecular mechanisms underlying estrogen-controlled BDNF expression.

Interaction between other sex steroids and BDNF

Progesterone and testosterone also regulate BDNF expression. Recently, Aguirre *et al*[117] reported that, in hippocampal slice cultures, progesterone upregulates BDNF proteins. 17β-E2 was also shown to protect hippocampal neurons from NMDA induced cell death. In their report, long-term progesterone treatment following 17β-E2 application attenuates 17β-E2-induced neuroprotection in hippocampal slice cultures. Moreover, Kaur *et al*[134] demonstrated that progesterone upregulates both BDNF mRNA and protein levels in cerebral cortical explants. In their system, K252a, an inhibitor for TrkB, inhibits progesterone-induced protection against glutamate toxicity, suggesting that BDNF upregulation is required for the progesterone action in neuroprotection. Interestingly, this progesterone-dependent protection is mediated *via* MAPK/ERK and PI3K pathways. In contrast, two independent groups provided evidence that progesterone-dependent neuroprotection is not through BDNF in rodents[135-137]. Collectively, the evidence concerning the interaction between progesterone and BDNF remains mixed, warranting further study. On the other hand, testosterone administration was shown to increase BDNF protein levels in castrated male rats[138]. Another group also indicated that BDNF mediates the effects of testosterone on neuronal survival[139]. It is also possible that BDNF contributes to testosterone function in the brain.

CONCLUSION

In addition to BDNF, steroid hormones such as glucocorticoids and estrogen regulate cell survival and neuronal function in the CNS. Several studies demonstrate that glucocorticoids and estrogen regulate the expression levels of BDNF in many brain regions. As upregulation of BDNF is putatively involved in the beneficial effects of several antidepressants, further investigation concerning the detailed mechanisms underlying such hormone-dependent production of BDNF is critical. Furthermore, it is well known that production and secretion of BDNF is affected by neuronal activity, though the detailed mechanisms concerning hormone-stimulated intracellular signaling and how this regulates BDNF dynamics remains to

be elucidated. Considering that neuronal activity and/or Ca^{2+} signaling regulate BDNF expression, it is possible that decreases in BDNF-stimulated intracellular signaling and neuronal function occur before reduction in BDNF levels in patients with depression is confirmed. Further studies concerning how these factors (steroid hormones and BDNF) influence each other and consequent intracellular signaling is required. Recently, the neuronal roles of microRNAs (miRs), that regulate diverse gene expression *via* targeting mRNAs to cleavage or to inhibit translation, have been proposed in BDNF function. For example, miR-132 is increased by BDNF and has a role in neuronal outgrowth[140]. We currently found that glucocorticoid reduced BDNF-dependent upregulation of glutamate receptors *via* decreasing of levels of the miR-132[141]. As a possible crosstalk point of steroid hormones and BDNF, the regulation of brain-specific miRs may be interesting.

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S- Editor Cheng JX **L- Editor** Lutze M **E- Editor** Zheng XM

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

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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.

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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doi:10.1016/j.neulet.2010.03.082

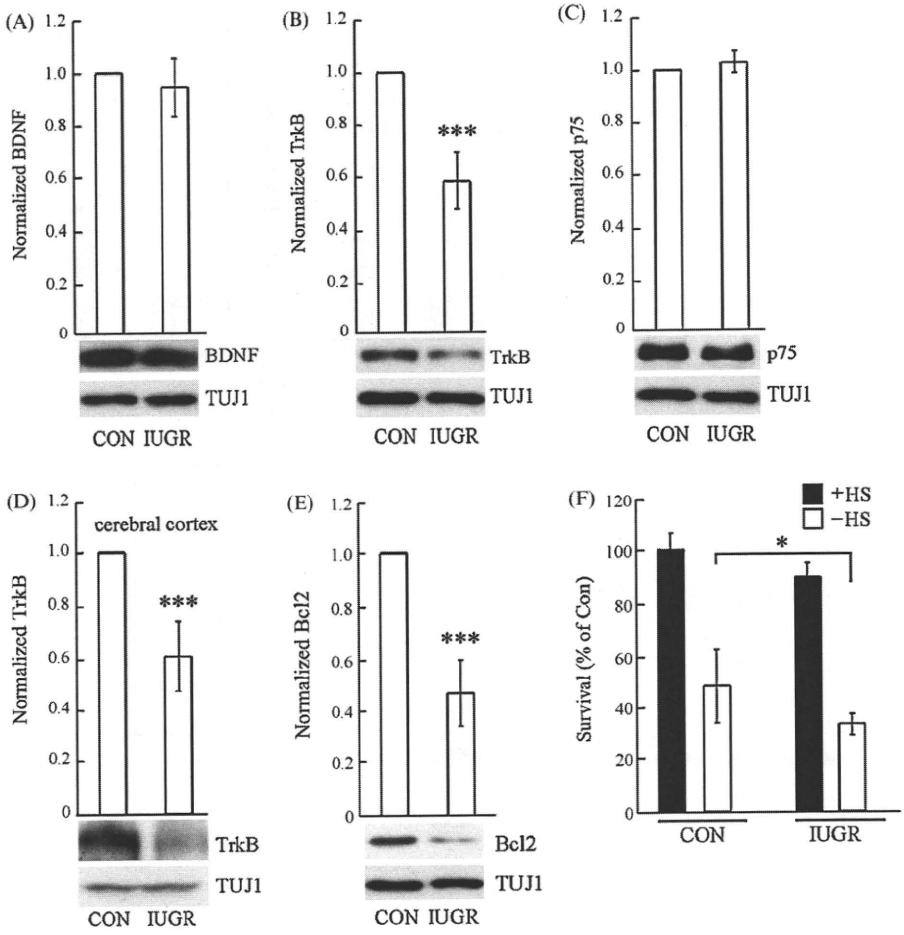

Fig. 1. Reduction in levels of TrkB, Bcl2, and cell viability in cortical cultures prepared from the cerebral cortex of low weight newborn rats with TXA2-induced IUGR. The levels of BDNF (A), TrkB (B), and p75 (C) were examined in 5DIV cortical cultures from IUGR-rats or from control-rats. TrkB was reduced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

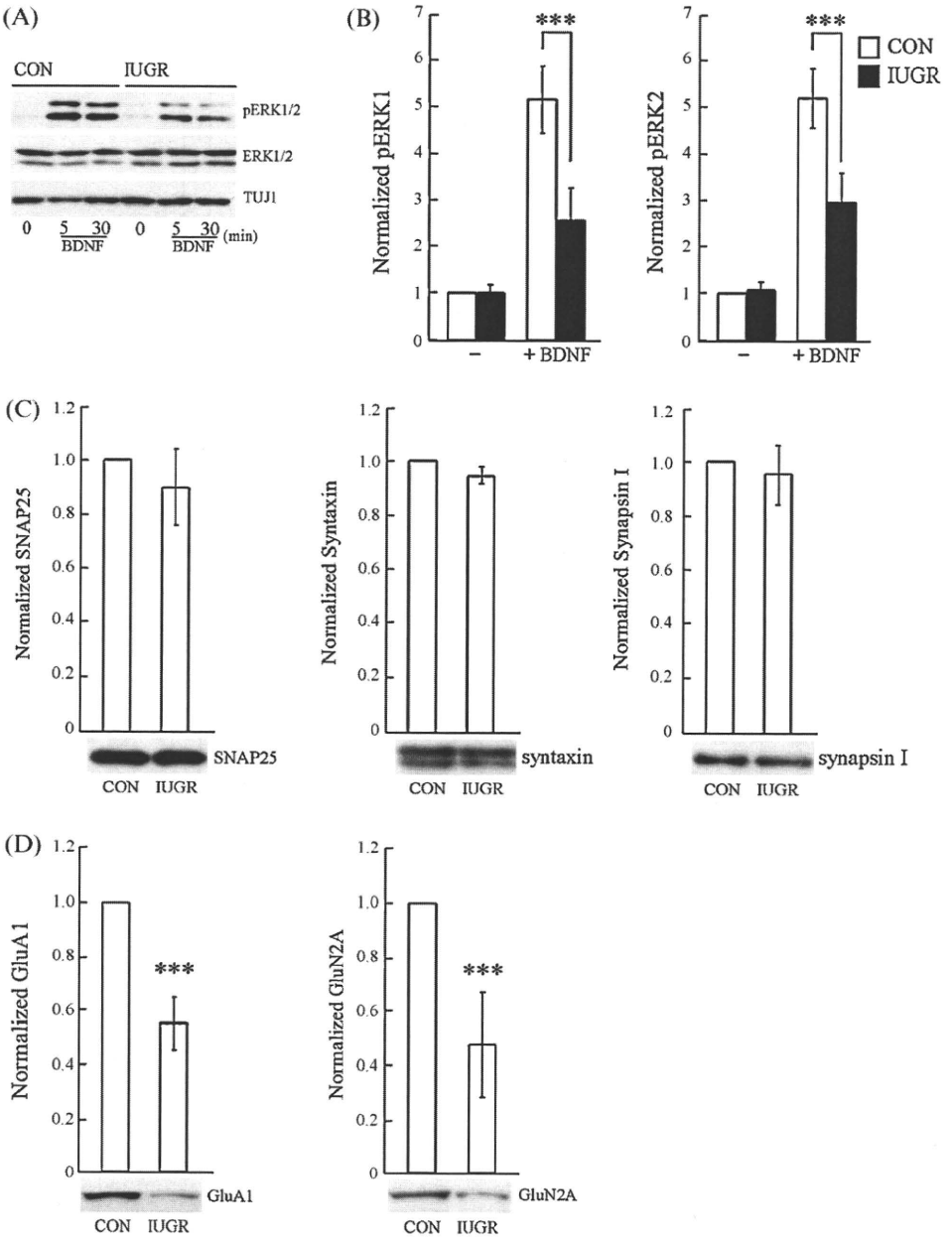

Fig. 2. Decrease in BDNF-stimulated ERK1/2 and in levels of glutamate receptors in IUGR-rat cultures. (A) and (B) Levels of activated ERK1/2 (pERK1/2) in IUGR- or control-rat cultures were examined at 5DIV. Cultured neurons with IUGR- or control-rats were stimulated by BDNF (100 ng/ml) for 0 min, 5 min, 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;

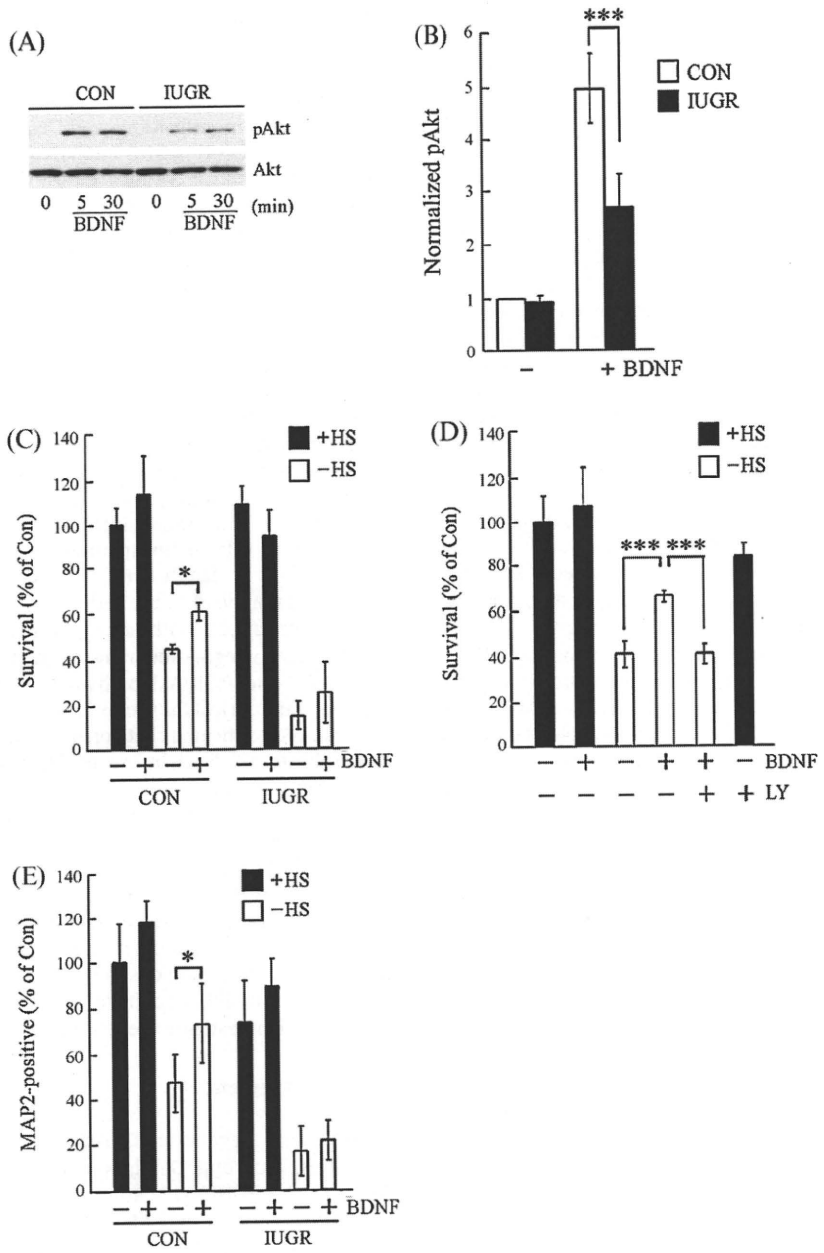

Fig. 3. Cortical neurons from IUGR-rats demonstrated a weakened response to BDNF measured through activation of the PI3K/Akt pathway and neuronal survival. (A) and (B) BDNF-stimulated Akt, a component of the PI3K pathway, was diminished in IUGR-rat cultures at 5DIV. BDNF (100 ng/ml) was applied for the 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-

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.

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Schizophrenia Research 124 (2010) 216–222

Contents lists available at ScienceDirect

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Gene-wide association study between the methylenetetrahydrofolate reductase gene (*MTHFR*) and schizophrenia in the Japanese population, with an updated meta-analysis on currently available data

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ARTICLE INFO

Article history:
Received 9 March 2010
Accepted 14 July 2010
Available online 7 August 2010

Keywords:
Gene-wide association
Japanese population
Meta-analysis
Methylenetetrahydrofolate reductase
Schizophrenia

ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) is a critical molecule for single-carbon transfer reactions. Recent evidence suggests that polymorphisms of MTHFR are related to neural tube deficits and the pathogenesis of schizophrenia. While several studies have demonstrated associations between the gene encoding the MTHFR (*MTHFR*) polymorphisms and schizophrenia, these studies lack consistency. Therefore, we conducted a gene-wide association study (patients with schizophrenia = 696, control subjects = 747) and performed imputation analysis. Additionally, we performed meta-analysis on currently available data from 18 studies for two common functional polymorphisms (rs1801131 and rs1801133).

There were no significant associations with schizophrenia in the single marker analysis for the seven tagging SNPs of *MTHFR*. In the haplotypic analysis, a nominally significant association was observed between the haplotypes, which included four SNPs (rs1801133, rs17421511, rs17037396, and rs9651118) and the schizophrenic patients. Additionally, the imputation analysis demonstrated there were several associated markers on the *MTHFR* chromosomal region. However, confirmatory analyses of three tagging SNPs (rs1801133, rs17037396, and rs9651118) and the top SNP (rs17421511) for the imputation results (patients with schizophrenia = 797, control subjects = 1025) failed to replicate the haplotypic analysis and the imputation results. These findings suggest that *MTHFR* polymorphisms are unlikely to be related to the development of schizophrenia in the Japanese population. However, since our meta-analysis results demonstrated strong support for association of rs1801133 with schizophrenia, further replication studies based on a gene-wide approach need to be considered.

1. Introduction

Schizophrenia is a chronic and disabling mental disorder with a lifetime prevalence of approximately 1% in the global population (Freedman, 2003). Accumulating evidence suggests that both genetic and environmental factors contribute to the

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doi:10.1016/j.schres.2010.07.011