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Functional interaction between BDNF and mGluR II *in vitro*: BDNF down-regulated *mGluR II* gene expression and an mGluR II agonist enhanced BDNF-induced *BDNF* gene expression in rat cerebral cortical neurons

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ABSTRACT

Accumulating evidence suggests functional interaction between brain-derived neurotrophic factor (BDNF) and metabotropic glutamate receptor (mGluR) signaling pathways in the central nervous system (CNS). To date, eight subtypes of mGluRs, mGluR1–8, have been identified, and a previous study suggested that BDNF leads to down-regulation of GluR2 mRNA in rat cerebral cortical cultures. However, precise transcriptomic effects of BDNF on other mGluRs and their cellular significance on the BDNF signaling pathway remain largely unknown. In this study, we assessed the transcriptomic effects of BDNF on mGluR1–8 in primary cultures of rat cerebral cortical neurons, and transcriptomic impacts of mGluR(s) whose expression is regulated by BDNF, on BDNF target genes. Real-time quantitative PCR (RT-qPCR) revealed that stimulation of the cultures with 100 ng/mL BDNF led to marked reductions not only in the gene expression levels of mGluR2, but also in those of mGluR3, both of which belong to group II mGluRs (mGluR II). There were, on the other hand, no changes in the amounts of mGluR I (mGluR1 and 5) and III (mGluR4, 6, 7, and 8) mRNA. Further, 10 ng/mL of BDNF, which mainly activates the high-affinity BDNF receptor, TrkB, but not the low-affinity receptor, $p75^{NTR}$, was able to induce down-regulation of mGluR II mRNA. The BDNF-induced suppression of mGluR II was not significantly attenuated in the presence of tetrodotoxin (TTX), a blocker for voltage-gated sodium channels. In addition, on stimulation with BDNF (100 ng/mL), no significant down-regulation of mGluR II mRNA was seen in cultured astrocytes, which only express the truncated form of TrkB. Finally, we assessed the transcriptomic effect of mGluR II on the expressions of BDNF target genes, *BDNF* and activity-regulated cytoskeleton-associated protein (*Arc*). LY404039, an mGluR II agonist, enhanced the BDNF-induced up-regulation of *BDNF*, but not *Arc*. On the other hand, LY341495, an mGluR II antagonist, down-regulated BDNF mRNA levels. Collectively, these observations demonstrated the detailed functional interaction between BDNF and mGluR II: Activation of mGluR II positively regulates self-induced BDNF expression, and, in turn, BDNF negatively regulates

Abbreviations: ANOVA, analysis of variance; AraC, cytosine arabinoside; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DAPI, 4′,6-diamidino-2-phenylindole; DIV, days *in vitro*; GFAP, glial fibrillary acidic protein; EGF, epidermal growth factor; 5-HT, 5-hydroxytryptamine; FBS, fetal bovine serum; MEM, minimum essential medium; KO, knockout; LTD, long-term depression; MAP-2, microtubule-associated protein 2; mGluR, metabotropic glutamate receptor; O4, oligodendrocyte marker O4; $p75^{NTR}$, p75 neurotrophin receptor; RT, room temperature; RT-qPCR, real-time quantitative polymerase chain reaction; TrkB, tropomyosin receptor kinase B; TTX, tetrodotoxin.

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the gene expression of *mGluR II* in a neuronal activity-independent manner, in cortical neurons, but not in astrocytes.

1. Introduction

Metabotropic glutamate receptors (mGluRs) are a family of G-protein-coupled receptors. In the central nervous system (CNS), neurons and glia, including astrocytes and oligodendrocytes [1], express mGluRs, and to date, eight subtypes of mGluRs (mGluR1–8) have been identified and subdivided into three groups. Group I mGluRs (mGluR I: mGluR1 and 5) stimulate inositol phosphate metabolism and mobilization of intracellular Ca^{2+}. Group II mGluRs (mGluR II: mGluR2 and 3) and group III mGluRs (mGluR III: mGluR4, 6, 7, and 8) negatively regulate the catalytic activity of adenylyl cyclase, which catalyzes the production of 3′,5′-cyclic adenosine monophosphate (cAMP) [2,3].

A 14 kDa secretory molecule, brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family. BDNF is known to play essential roles in neuronal differentiation, development, survival, and plasticity [4–6] through high- and low-affinity binding to tropomyosin receptor kinase B (TrkB) and p75 neurotrophin receptor ($p75^{NTR}$), respectively [4,7] in the CNS. BDNF expression is positively autoregulated by BDNF itself, which is involved in neuronal functions, such as synaptic maturation and memory consolidation [8,9]. BDNF-induced up-regulation of BDNF is mediated by glutamate receptors, including NMDA and AMPA receptors [10,11], but whether mGluRs are involved in BDNF's autoregulation of its expression remains unclear. However, it has been reported that the mGluR signaling pathways may regulate *BDNF* gene expression. Activation of mGluR I leads to increased BDNF expression in rat C6 glioma cells [12]. mGluR I-dependent long-term depression (LTD) induces expression of a precursor form of BDNF (proBDNF) in rat hippocampus slice cultures [13]. mGluR II was shown to modulate 5-hydroxytryptamine (5-HT) (2A/2B/2C) agonist-induced increases in cortical BDNF expression [14]. Activation of mGluR II causes growth arrest and DNA damage via binding of 45-β (Gadd45-β), a key regulator of active DNA demethylation, to specific promoter regions of *BDNF* [15]. LY341495, an mGluR II antagonist, induces antidepressant-like effects in rodents, which is blocked by K252a, an inhibitor for Trk tyrosine kinases [16].

In turn, the precise transcriptomic effects of BDNF on other mGluR mechanisms also remain unclear. A previous study reported that treatment of rat cortical cultures with 25 ng/mL BDNF for 24 h led to decreased levels of mGluR2 mRNA [17]. With that level of BDNF administration, BDNF can signal via both TrkB and $p75^{NTR}$ [18,19], and therefore the responsible pathway is unclear. In this study, we investigated *in vitro* the BDNF-dependent regulation of *mGluRs* gene expression and transcriptomic effects of mGluR on the BDNF signal pathway in rat cerebral cortical neurons.

2. Materials and methods

2.1. Materials

Recombinant BDNF was kindly provided by Sumitomo Pharmaceuticals (Osaka, Japan). Wistar rats were from NIPPON SLC (Hamamatsu, Japan). We used anti-phospho-Trk (Y490; 1:1000; Cell Signaling Technology, Beverly, MA) and anti-TrkB (1:1000, BD Transduction Laboratories) for Western blot analysis. Anti-mouse IgG and anti-rabbit IgG secondary antibodies conjugated to horseradish peroxidase (1:1000) were purchased from Jackson ImmunoResearch (West Grove, PA). We used anti-microtubule-associated protein-2 (anti-MAP-2; 1:250; Sigma-Aldrich, St. Louis, MO) and anti-glial fibrillary acidic protein (GFAP; 1:500; Merck Millipore, Billerica, MA) for immunocytochemistry. Anti-rabbit and anti-mouse IgG secondary antibodies conjugated to Alexa Fluor 488 (1:200) or 546 (1:2000) were purchased from Molecular Probes (Carlsbad, CA). All animal experiments were conducted in strict accordance with the protocols approved by the Institutional Animal Care and Use Committee of Kagawa University. All efforts were made to minimize animal suffering and the number of animals used for the studies.

2.2. Cell cultures

Primary cultures of dissociated cerebral cortical neurons were prepared from embryonic day 20 (E20) rats (Wistar, both sexes), as described previously [20]. In brief, the neurons were cultured in serum-free neurobasal medium (Gibco, Rockville, MD) containing B27 supplement (Gibco), at a final density of 2×10^6 cells on 6-well plates coated with polyethyleneimine for RT-qPCR assay. Neurons were cultured in medium containing 1 μM cytosine arabinoside (AraC) to suppress glial growth after days *in vitro* (DIV) 3. BDNF was applied from DIV 6 or 7 for 3 days.

Astrocyte pure cultures were prepared as previously described [21]. Briefly, primary cultures of astrocytes were prepared from the cerebral cortex of neonatal Wistar rats. Astrocytes were maintained in 75 cm^2 flasks in Minimum Essential Medium (MEM)-based growth medium containing 100 μg/L epidermal growth factor (EGF) (Gibco), 20 mM glucose, 25 mM $NaHCO_3$, 5% Fetal bovine serum (FBS), and 0.5 mM glutamine. After the astrocytes became confluent (10-14 days), cells were harvested and re-plated on 35-mm dishes. When 70–80% confluency was reached, the medium was replaced with fresh medium without EGF for 48 h before BDNF treatment.

2.3. Quantification of mRNA levels

RT-qPCR was carried out for amplification and quantitative comparison of mGluR mRNA. Total RNA was purified from neurons using an RNeasy mini kit (QIAGEN, Valencia, CA) and amplified with a primer set for mGluR1 (Grm1F; 5-GCCACCACACCACCTCTG-3′ and Grm1R; 5′-TGACGGAATCAGCCAGGAAC-3′) [22], mGluR2 (Grm2F; 5′-TTGTGCGTGCCTCACTCAG-3′ and Grm2R; 5′-CTGTAGGAGCA-TCACTGTGG-3′) [22], mGluR3 (Grm3F; 5′-ATGGTGTCCGTGTGGCT-TATC-3′ and Grm3R; 5′-TGACTGTTTCCCGCTTCTCTG-3′) [22], mGluR4 (Grm4F; 5′-GCCATCACCTTCATCCTCAT-3′ and Grm4R; 5′-GTGTCCGTTGGTCCTGGAA-3′) [23], mGluR5 (Grm5F; 5-AGCTCA-ACTCCATGATGTTGT-3′ and Grm5R; 5′-ATCTCTGCGAAGGT-CGTCAT-3′) [23], mGluR6 (Grm6F; 5′-CCTGCTGTTGGCACTGTGA-3′ and Grm6R; 5′-GACGGCAACCAGTGTGGTT-3′) [23], mGluR7 (Grm7F; 5′-ACAACTGGCGATCACTTCCA-3′ and Grm7R; 5′-GTTCATGGTCTTATGCTCATC-3′) [23], and mGluR8 (Grm8F; 5′-TCCAGCTCCTCGGAGTGTT-3′ and Grm8R; 5′-CTGTTCGCCATAGTCAATGAT-3′) [23] using the ABI Step One Plus RT-PCR (Applied Biosystems, Foster, CA) and One Step SYBR PrimScript RT-PCR Kit II (TAKARA BIO INC., Otsu, Shiga, Japan). Glyceraldehyde-3-phosphate dehydrogenase was also amplified using a primer set (GAPDHF; 5′-TGCACCACCAACTGCTTAG-3′ and