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Research article

# Rotigotine, a dopamine receptor agonist, increased BDNF protein levels in the rat cortex and hippocampus

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) critically controls the fate and function of the neuronal network and has received much attention as a target of many brain diseases. Dopaminergic system dysfunction has also been implicated in a variety of neuropsychiatric diseases. Rotigotine, a non-ergot dopamine receptor agonist, is used in the treatment of Parkinson's disease and restless legs syndrome. To investigate the effects of rotigotine on neuronal functions both *in vivo* and *in vitro*, rats and primary cortical neurons were administered rotigotine, and the mRNA and protein expression levels of BDNF, its receptor TrkB and downstream signaling molecules, and synaptic proteins were determined. We found that BDNF protein was increased in the cortex and hippocampus of rats after 7 days of rotigotine treatment. In contrast, BDNF mRNAs were reduced 6 h after rotigotine treatment in cultured neurons presumably through the transient suppression of neuronal activity. We identified differential expression of D1, D2, and D3 receptors in the rat brain and cultured neurons. The observed increase in the expression of BDNF protein in the cortex and hippocampus after subchronic administration of rotigotine suggests that it may exert its medical effect in part through improving BDNF function in the brain. In addition, our results highlight the complex relationships between rotigotine and BDNF expression, which depend on the brain region, time course, and dose of the drug.

## 1. Introduction

Brain-derived neurotrophic factor (BDNF) is broadly expressed in the central nervous system (CNS) and plays crucial roles in neuronal maturation and survival, and synaptic functions through intracellular signaling pathways including phosphoinositide 3-kinase/Akt (PI3K/Akt), extracellular signal-regulated kinase (ERK), and phospholipase C-γ (PLC-γ) pathways [1–4]. Because transcription of the *Bdnf* gene is strictly regulated by environmental conditions, it has a complex structure: at least nine 5′ exons (exon I–IX) with a specific promoter and one 3′ exon (exon IX) encoding the entire open reading frame for BDNF protein in both humans and rodents [5–7]. Transcription of *Bdnf* can be initiated at each of the 5′ noncoding exons spliced to the common 3′ exon encoding the pre-proBDNF protein [7]. Extensive research has been devoted to develop new medications for brain diseases under the strategy of improving the reduced BDNF expression/function because BDNF has been suggested to be involved in the pathogenesis of a variety of neurodegenerative and psychiatric diseases [8–11].

Dopamine plays important roles in the mammalian CNS, regulating motor actions and cognitive functions such as learning, memory formation, decision making, and consummatory behaviors controlled by the reward system through activating members of a G protein-coupled receptor (GPCR) family [12,13]. D1 to D5 dopamine receptors have been identified and classified into two groups as D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors, which promote activation or inhibition of adenylyl cyclase (AC), cyclic adenosine monophosphate (cAMP) production, and protein kinase A (PKA), respectively [14,15]. Dopamine receptors are broadly distributed in the CNS: D1 and D2 receptors are the most abundant two subtypes in the brain while D3, D4, and D5 expression is lower and more regionally restricted. Despite extensive research into dopamine signaling in the CNS, actions of dopamine remain elusive because they vary greatly by receptor subtypes, cell types, brain regions, and strength and duration of stimulation [13,16]. On the other hand, dopamine receptor agonists have been clinically used in the treatment of Parkinson's disease patients [17]. Rotigotine is a non-ergot agonist for dopamine receptors and has beneficial effects in the treatment of Parkinson's disease [18–22] and restless legs syndrome [23,24]. It is also of interest that rotigotine exerts an antidepressant effect in rats [25]. Rotigotine's binding affinity for each dopamine receptor subtype has been controversial: Scheller

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et al. reported the highest affinity for dopamine D3 receptor (Ki 0.71 nM), a high affinity for D2, D4 and D5 receptors (Ki 4–15 nM), and a low affinity for D1 receptor (Ki 83 nM) by using an *in vitro* receptor binding assay [26]. In functional studies, however, rotigotine was able to stimulate all dopamine receptor subtypes with similar potency for D1, D2, and D3 receptors. A recent report using radiolabeled rotigotine showed that it was a high-potency agonist for human dopamine D1, D2, and D3 receptors but had low-potency for D4 and D5 receptors [27].

Because D1 and D2 receptor mRNA can be detected in the forebrain regions of human and rodents [28] where altered levels of BDNF have been reported in the patients with neuropsychiatric diseases and their animal models [10,11], we examined whether the expression levels of BDNF and functionally related proteins are changed by rotigotine in rat brain regions, *i.e.*, the prefrontal cortex, hippocampus, and cerebellum and in cultured cortical neurons.

## 2. Materials and methods

### 2.1. Animals and rotigotine administration

All animal experiments were conducted in accordance with the institutional guidelines of the Animal Ethics Committee for the care and use of animals, and all experiments were carried out in accordance with the approved guidelines of the National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan.

All animals were kept under standard laboratory conditions (22 °C, 40–60% humidity, 8:00a.m light on, 8:00p.m light off) and were housed in transparent plastic cages. Food and water were available *ad libitum*. Rotigotine (Sigma-Aldrich, MO, USA) and haloperidol (Sigma-Aldrich) dissolved in 50% DMSO were administered continuously through subcutaneous osmotic pump implants (Alzet 2ML1, Cupertino, CA, USA) for 7 days. The dose of rotigotine (5 mg/kg/day) was selected based on a previous report [25], and haloperidol which has a 10-fold affinity for D2 receptor compared with rotigotine [26,27,29], was administered at a dose of 0.5 mg/kg/day. Eight-week old male Wistar rats were divided into the rotigotine and the control groups, each of which contained eight animals. For implantation, rats were anesthetized with mixture of medetomidine and midazolam, concomitantly with butorphanol (analgesic). The pump was subcutaneously implanted in the space between the scapulae. The prefrontal cortex, hippocampus, and cerebellum were collected 7 days after the implantation. The PFC was defined as an area > 2.7 mm from bregma, excluding the olfactory bulb. The tissues were quickly frozen in liquid nitrogen after the removal and stored at −80 °C. The data obtained by two independent series of drug administration each of which contained 8 animals (4 rats as control and rotigotine group, respectively) were included in the result presented here, except the experiment with haloperidol (3 rats as control and rotigotine group, respectively).

### 2.2. Rat primary cortical cultures and rotigotine treatment

Cortical neurons were prepared from the cortex of 1- or 2-day-old Wistar rats (SLC, Shizuoka, Japan) as described previously [30]. Dissociated cells were plated at a final density of $5 \times 10^5/cm^2$ on polyethyleneimine-coated culture dishes. Culture medium used in this study contained 5% fetal bovine serum, 5% heated-inactivated horse serum, and 90% of a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium. Cortical neurons were matured for 10–11 days before rotigotine hydrochloride (Sigma-Aldrich) was treated, followed by incubation for 6 or 24 h before sampling.

### 2.3. RNA isolation and real-time quantitative PCR (qPCR)

Total RNAs of cultured neurons and brain tissues were extracted using RNeasy mini kit (Qiagen, CA, USA) according to the manufacturer's protocol. Five hundred ng of total RNA was then used to produce complementary DNAs (cDNAs) by reverse transcription with SuperScript® VIROTM cDNA synthesis kit (Life Technologies Inc., CA, USA). The obtained cDNAs were determined by the quantitative PCR method with StepOnePlus Real Time PCR System (Applied Biosystems, MA, USA). The relative expression levels were calculated using the ΔΔCt method and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as internal control. The primers and probe sets (TaqMan Gene Expression Assays from Applied Biosystems) were as follows: total BDNF: Rn02531967_s1, BDNF exon I: Rn01484924_m1, BDNF exon IV: Rn01484927_m1, BDNF exon VI: Rn01484928_m1, Dopamine receptor D1: Rn03062203_s1, Dopamine receptor D2: Rn00561126_m1, Dopamine receptor D3: Rn00567568_m1, GAPDH: 4352338E and Rn01775763_g1.

### 2.4. Immunoblotting

SDS lysis buffer, containing 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, was used to lyse rat brain tissues and cultured neurons. Protein concentration was quantified with a BCA Protein Assay Kit (Pierce Biotechnology Inc., IL), and equivalent amounts of protein were assayed in each immunoblotting. Primary antibodies used in this study were follows: anti-BDNF (1:500, Santa Cruz Biotechnology Inc., CA, USA), anti-TrkB (1:1000, BD Biosciences, NJ, USA), anti-NR2A (1:500, Sigma-Aldrich), anti-NR2B (1:500, Sigma-Aldrich), anti-GluR1 (1:500, Millipore, MA, USA), anti-ERK (1:1000, Cell Signaling Technology, MA, USA), anti-phospho-ERK (1:1000, Cell Signaling Technology), anti-Akt (1:1000, Cell Signaling Technology), anti-phospho-Akt (1:1000, Cell Signaling Technology), anti-SNAP25 (1:1000, Synaptic Systems, Goettingen, Germany), anti-synaptotagmin (1:1000, Millipore), anti-syntaxin I (1:1000, Sigma-Aldrich), and βactin (1:5000, Sigma-Aldrich). Immunoreactivity of each band was quantified by Lane & Spot Analyzer software (ATTO Corporation, Tokyo, Japan). At least 2 independent series of cultures were used for each set of experiments.

### 2.5. Statistical analysis

Data are expressed as mean ± standard error (S.E.) and statistical significance was calculated using Student's *t*-test or one-way ANOVA followed by Bonferroni's *post-hoc* test by using SPSS ver.18 (SPSS Japan, Tokyo, Japan). The probability values of less than 5% were considered significant.

## 3. Results

### 3.1. Subchronic rotigotine treatment increased BDNF protein levels in the rat cortex and hippocampus

We first determined rotigotine's effect on BDNF and functionally related proteins *in vivo*. Interestingly, BDNF protein levels were significantly increased in the cortex (1.55 ± 0.13, normalized to the control, $p = 0.0018$, Student's *t*-test) and hippocampus (1.31 ± 0.15, $p = 0.0081$) after 7 days of rotigotine administration (Fig. 1A and B). However, BDNF receptor TrkB, glutamate receptor subunits, synaptic proteins, and phospho-ERK and – Akt levels were not significantly changed by rotigotine in any of the three brain regions tested (Fig. 1A, see Supplementary Figs. S1–S3 in the online version at DOI:10.1016/j.neulet.2017.10.006). We next tested whether the effect of rotigotine on BDNF protein levels occurred through dopamine receptors. When haloperidol, a dopamine receptor antagonist, was administered with rotigotine, there was a tendency for attenuation in the effect of rotigotine on BDNF up-regulation without affecting TrkB receptor both in the cortex and hippocampus {(BDNF in the cortex: con, 1.00 ± 0.23; rotigotine, 1.44 ± 0.21; rotigotine + haloperidol, 0.92 ± 0.17), (BDNF in the hippocampus: con, 1.00 ± 0.25; rotigotine, 1.53 ± 0.15;