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The effect of dopamine on adult hippocampal neurogenesis

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ABSTRACT

Cumulative studies indicated that adult hippocampal neurogenesis might be involved in the action mechanism of antidepressant drugs and/or the pathophysiology of depression. Dopamine (DA) is involved in the regulation of motivation, volition, interest/pleasure, and attention/concentration, all of which are likely to be impaired in depressed patients. Several previous reports suggest that depression may often be accompanied by a relative hypo-dopaminergic state, and some DA receptor agonists are beneficial effects in the treatment for refractory and bipolar depression. In the present study, to clarify the direct effect of DA on neural progenitor cells, we examined the effect of DA on the proliferation of adult rat dentate gyrus-derived neural precursor cells (ADPs). In addition, we examined the effect of DA receptor agonists on adult rat hippocampal neurogenesis in vivo. Results showed that DA promoted the increase of ADPs via D1-like receptor and D1-like receptor agonist promoted the survival of newborn cells in the adult hippocampus. On the contrary, D2-like receptor agonist did not affect both proliferation and survival. These results suggested that DA might play, at least in part, a role in adult hippocampal neurogenesis via D1-like receptor and the activation of D1-like receptor has a therapeutic potential for depression.

1. Introduction

In the adult mammalian brain, neurogenesis mainly occurs in two regions, subventricular zone (SVZ) situated through the lateral wall of the lateral ventricles and subgranular zone (SGZ) in the dentate gyrus (DG) of hippocampus (Kempermann, 2006). Neural precursor cells (NPCs) reside in the SGZ proliferate, survive, and differentiate into neuron and glia. This differential step of neurogenesis is affected by many factors, including environment, stress, hormone and drugs (Dranovsky and Hen, 2006; Duman et al., 2001). Stress of various kinds is considered to be a risk factor of depression, and decreases the proliferation of NPCs (Gould et al., 1997; Pham et al., 2003). As a clinical therapeutic effect, chronic treatment with different classes of antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), and noradrenalin reuptake inhibitors increases the proliferation (Airan et al., 2007; Malberg et al., 2000; Santarelli et al., 2003). It is also reported that suppression of hippocampal neurogenesis by the irradiation prevents anti-depressive effects induced by fluoxetine or imipramine treatment (Airan et al., 2007; Santarelli et al., 2003). These results suggest that neurogenesis might be involved in the action mechanism of antidepressant drugs and/or the pathophysiology of depression. However, it remains unknown in detail how antidepressant drugs regulate adult hippocampal neurogenesis and how the modified neurogenesis shows anti-depressive effects.

Dopamine (DA) is involved in the regulation of motivation, volition, interest/pleasure, and attention/concentration, all of which are likely to be impaired in depressed patients. Several previous reports suggest that depression may often be accompanied by a relative hypo-dopaminergic state (Papakostas, 2006). Roy et al. (1985) reported lower cerebrospinal fluid of homovanilic acid, a chief metabolite of DA. Several neuroimaging studies showed increased D2/D3-receptor and decreased DA transporter bindings in some brain regions of depressive patients (D'Haenen and Bossuyt, 1994; Meyer et al., 2001; Shah et al., 1997). Therefore, the dopaminergic system may be a target for the pharmacological treatment of depression. In fact, it has been reported that DA receptor agonists such as bromocriptine, pramipexole or the combination therapy of them with antidepressants may be beneficial effects in the treatment for refractory and bipolar depression (Aiken, 2007; Inoue et al., 1996, 2010).

Several studies suggested that DA controls the differential steps of adult hippocampal neurogenesis. SGZ receives dopaminergic projection

Abbreviations: ADP, adult dentate gyrus-derived neural precursor cells; bFGF, basic fibroblast growth factor; BrdU, 5-bromo-2-deoxyuridine; CNTF, ciliary neurotrophic factor; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GCL, granule cell layer; GFAP, glial fibrillary acidic protein; HBSS, hanks balanced salt solution; NPC, neural precursor cells; PBS, phosphate buffered saline; PS, penicillin–streptomycin; SGZ, subgranular zone; SVZ, subventricular zone; SSRI, selective serotonin reuptake inhibitor; Tuj-1, Neuron-specific class III β-tubulin.

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from ventral tegmental area (Baker et al., 2004; Gasbarri et al., 1997; Hoglinger et al., 2004). Experimental depletion of DA in rodents decreased the cell proliferation and survival of NPCs in SGZ (Hoglinger et al., 2004; Khaindrava et al., 2011). Furthermore, postmortem study showed that number of NPCs was reduced in SGZ of patients suffered from Parkinson disease (Borta and Hoglinger, 2007). However, dopaminergic modulation of adult hippocampal neurogenesis has not been investigated well.

NPCs in adult hippocampus are classified into stem/progenitor cells of four types based on stages of neural development (Kempermann, 2006). We have recently established the culture system of adult rat dentate gyrus-derived neural precursor cells (ADPs), which correspond to type 2a early progenitor cells in the adult hippocampus (Boku et al., 2009; Masuda et al., 2012). Encinas et al. (2006) showed that fluoxetine increased the early progenitor cell classified as type 2 in vivo study. In the present study, to clarify the direct effect of DA on NPCs, we examined the effect of DA on the proliferation of ADPs. In addition, we examined the effect of DA receptor agonists on adult rat hippocampal neurogenesis in vivo. These results showed that DA promoted the increase of ADPs via D1-like receptor and D1-like receptor agonist promoted the survival of newborn cells in the adult hippocampus. On the other hand, D2-like receptor agonist did not affect both proliferation and survival.

2. Material and methods

2.1. Animals

Male Sprague–Dawley (SD) rats (7 weeks old) were obtained from Japan SLC, Inc. (Shizuoka, Japan). The rats were housed in groups of three or four in a temperature-controlled environment (22 ± 1 °C) with free access to food and water. The subjects were maintained on a 12 h light/dark cycle (light phase; 06:00–18:00). The experiments began after 1 week for an acclimatization period.

All experiments were approved by the Hokkaido University School of Medicine Animal Care and Use Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Dopamine hydrate was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). (±)-SKF38393, (±)-SKF-81297, quinpirole, pramipexole dihydrochloride and R-SCH23390 were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Bromocriptine mesilate was donated by Nihon Ciba-Geigy K.K (Tokyo, Japan). Sulpiride was donated by Fujisawa Pharmaceutical Co. Ltd (Osaka, Japan). Each binding affinity of compounds for D1-like and D2-like receptor of rat striatum was described in Table 1 (Andersen, 1988; Chen et al., 2011; Mottola et al., 1992; Neumeyer et al., 2003).

Table 1
The affinities of drugs for D1-like and D2-like receptor of rat striatum.

DA receptor ligand	Ki (nM)	
	D1-like receptor	D2-like receptor
DA	500	70
(±)-SKF38393	18	9300
(±)-SKF81297	1.9	1270
Quinpirole	>5000	720
Pramipexole	>10,000	39
Bromocriptine	>10,000	8
SCH23390	0.14	895
Sulpiride	>10,000	70

2.3. Isolation and culture of ADP

ADPs were isolated from DG of adult male SD rats (8 weeks) as described in a previous report (Boku et al., 2009). Briefly, rats were deeply anesthetized with sodium pentobarbital (Dainippon Sumitomo Pharma Co. Ltd., Osaka, Japan) and were then decapitated. The brains were removed from 8 rats immediately and were placed in cold Hank's balanced salt solution. The coronal sections with a thickness of 1 mm were cut using brain slicer (Muromachi Kikai Co. Ltd., Tokyo, Japan). The DG region of hippocampus was dissected carefully under a dissecting microscope to exclude all regions containing subependymal tissues. The dissected DG tissue was minced with a scalpel blade and digested using a mixture of 2.5 U/ml papain (Worthington Biochemical Corp., Lakewood, NJ), 1 U/ml dispase II (F. Hoffman La Roche Ltd., Indianapolis, IN) and 250 U/ml Deoxyribonuclease I (Worthington) in a water bath at 37 °C for 30 min. The cell mixture was passed through a 70 μm cell strainer. The fraction containing ADP was separated using Percoll gradient centrifugation (GE Healthcare, Uppsala, Sweden). After separation, the cells were washed and suspended with DMEM/F12 medium (Sigma-Aldrich) containing 10% fetal bovine serum (FBS; Invitrogen Corp., Carlsbad, CA) and 1% penicillin/streptomycin (Invitrogen). The suspension cells were plated onto a non-coated plate. After overnight culture at 37 °C, 5% CO_2, the medium was exchanged for proliferation medium consisting of Neurobasal (Invitrogen), B27 supplement minus vitamin A (Invitrogen), 2 mM L-Glutamine (Invitrogen), 1% penicillin/streptomycin, and 20 ng/ml basic fibroblast growth factor (Invitrogen). Subsequently, half of the medium was replaced by new proliferation medium every two or three days. At the 80–90% confluency, cells were passaged by trypsinization onto poly L-ornithine-laminin-coated dishes. The cell density for plating was approximately 1×10^4 cells/cm^2.

2.4. Cell counting of ADP

Viable cells were quantitated using an Alamar Blue assay, which is a rapid and simple non-radioactive assay. 1×10^4 cells were seeded on laminin-ornithine coated 96-well plates in 100 μl/well of proliferation medium. After overnight incubation, to examine the effect of DA receptor ligands on ADP proliferation, cells were treated with each drug at each concentration for 3 days. To examine the effect of DA receptor antagonists on DA-promoted ADP proliferation, antagonists were applied to culture medium 1 h before 30 μM of DA was added and the cells were cultured for 3 days. Three days after the treatment, 10 μl/well of Alamar Blue solution (Invitrogen) was added to medium, and cells were incubated at 37 °C for 3 h. Then the absorbance was measured using a Varioskan Flash (Thermo Fisher Scientific Inc., Waltham, MA) microplate reader at 570 and 600 nm, and cell number based on percentage reduction of Alamar Blue was calculated as described in the manufacture's protocol. Although the DA concentration in the extracellular fluid of the rat hippocampus is under normal conditions reported as 0.1 nM (Simpson et al., 2001), micromolar concentration range of DA was used in in vitro study evaluating the effect of DA on proliferation of the cell derived from SVZ (Hoglinger et al., 2004; O'Keeffe et al., 2009b). Therefore, we used DA at broad concentrations of 1–100 μM. The ranges of other compounds used in this study were determined by preliminary experiments.

2.5. RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells with RNeasy Extraction kit (Qiagen Inc., Hilden, Germany). Total RNA was converted to cDNA with Quantitect Reverse Transcription kit (Qiagen). Polymerase chain reaction (PCR) was performed with AmpliTaq Gold 360 master mix (Applied biosystems, Foster City, CA) in the GeneAmp PCR system 9700 (Applied biosystems). The conditions of PCR were: 95 °C for