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Effects of antidepressant drugs on synaptic protein levels and dendritic outgrowth in hippocampal neuronal cultures

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ABSTRACT

The alteration of hippocampal plasticity has been proposed to play a critical role in both the pathophysiology and treatment of depression. In this study, the ability of different classes of antidepressant drugs (escitalopram, fluoxetine, paroxetine, sertraline, imipramine, tranylcypromine, and tianeptine) to mediate the expression of synaptic proteins and dendritic outgrowth in rat hippocampal neurons was investigated under toxic conditions induced by B27 deprivation, which causes hippocampal cell death. Postsynaptic density protein-95 (PSD-95), brain-derived neurotrophic factor (BDNF), and synaptophysin (SYP) levels were evaluated using Western blot analyses. Additionally, dendritic outgrowth was examined to determine whether antidepressant drugs affect the dendritic morphology of hippocampal neurons in B27-deprived cultures. Escitalopram, fluoxetine, paroxetine, sertraline, imipramine, tranylcypromine, and tianeptine significantly prevented B27 deprivation-induced decreases in levels of PSD-95, BDNF, and SYP. Moreover, the independent application of fluoxetine, paroxetine, and sertraline significantly increased levels of BDNF under normal conditions. All antidepressant drugs significantly increased the total outgrowth of hippocampal dendrites under B27 deprivation. Specific inhibitors of calcium/calmodulin kinase II (CaMKII), KN-93, protein kinase A (PKA), H-89, or phosphatidylinositol 3-kinase (PI3K), LY294002, significantly decreased the effects of antidepressant drugs on dendritic outgrowth, whereas this effect was observed only with tianeptine for the PI3K inhibitor. Taken together, these results suggest that certain antidepressant drugs can enhance synaptic protein levels and encourage dendritic outgrowth in hippocampal neurons. Furthermore, effects on dendritic outgrowth likely require CaMKII, PKA, or PI3K signaling pathways. The observed effects may be may be due to chronic treatment with antidepressant drugs.

1. Introduction

Recent studies have determined that depression may be related to impairments in structural and functional plasticity in several brain areas (Pittenger and Duman, 2008; Manji et al., 2001). More specifically, changes in hippocampal structure and function are evident in both depressed patients and animal models of depression (Campbell and Macqueen, 2004; Lucassen et al., 2001; Magariños et al., 1996; Watanabe et al., 1992). These results indicate that depression is associated with dendritic atrophy and the loss of synaptic connections in the hippocampus and that neuritic alteration might underpin hippocampus-related memory and cognitive deficits in depressed patients (Silva, 2003). Therefore, dysfunctional hippocampal plasticity may contribute to the pathophysiology of depression.

The mechanisms underlying the delayed therapeutic effect of antidepressant drugs are still unclear, but it has been suggested that these drugs induce changes involving neuronal plasticity and extensive network reorganization (Ampuero et al., 2010; Pittenger and Duman, 2008). Accumulating evidence indicates that dendritic spine formation and synaptogenesis are among these changes (Ampuero et al., 2010; Chen et al., 2008, 2010; Hajszan et al., 2005; Zheng et al., 2011). Fluoxetine increases the number of spine synapses in the CA1 and CA3 regions of the rat hippocampus (Hajszan

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et al., 2005; Zheng et al., 2011) as well as enhances the expression of synapse-associated proteins in the hippocampi of ovariectomized female rats (O'Leary et al., 2009). However, imipramine does not have an effect on synaptic proteins in rats with learned helplessness, an animal model of depression (Iwata et al., 2006). Treatment with a high dose of desipramine decreases synaptic proteins levels in the rat hippocampus, whereas no change was observed following administration of paroxetine (Martínez-Turrillas et al., 2005). Thus, the effects of antidepressant drugs on synaptic plasticity remain controversial.

Postsynaptic density protein 95 (PSD-95) is preferentially located in dendritic spines and plays a critical role in the regulation of dendritic spine size and shape (Ehrlich et al., 2007; Han and Kim, 2008). Postsynaptic brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain, contributes to axonal branching, dendritic differentiation, and connectivity among neurons (Ji et al., 2005; Lessmann et al., 2003; Poo, 2001). Synaptophysin (SYP), a major integral membrane protein of presynaptic vesicles, is required for vesicle formation and exocytosis (Valtorta et al., 2004). It is widely used as a marker for synapse activity.

Neurite outgrowth is regulated by several signaling molecules including calcium/calmodulin-dependent protein kinsase II (CaMKII), cAMP-dependent kinase (PKA), phosphatidylinositol 3-kinase (PI3K)/Akt, and mitogen-activated protein kinase (MAPK)/extracellular signaling-regulated kinase (ERK) (Kobayashi et al., 1997; Sánchez et al., 2004; Vaillant et al., 2002). CaMKII is concentrated in the cytoplasm and dendrites and engages in extensive cross talk with PKA and Akt (Colbran and Brown, 2004). The activation of cAMP response element binding protein (CREB) via cAMP/PKA signaling increases the expression of cAMP responsive genes, including BDNF.

A central role of PI3K/Akt signaling in the determination of dendritic morphogenesis has been reported (Kumar et al., 2005). This suggests that this type of signaling is important for the action of antidepressant drugs on neuronal plasticity (Pittenger and Duman, 2008). Taken together, these findings suggest that the effects of antidepressant drugs on these signaling cascades induce morphological changes within hippocampal neurons. Thus, the identification of different classes of antidepressant drugs that affect hippocampal dendritic morphology via CaMKII, PKA, and PI3K is required.

It is proposed here that antidepressant drugs act as key regulators of synaptic proteins and neuronal plasticity. The present study included selective serotonin reuptake inhibitors (SSRIs), escitalopram, fluoxetine, paroxetine, sertraline, a selective serotonin reuptake enhancer (SSRE), tianeptine, the monoamine oxidase inhibitor (MAOI), tranylcypromine, and the tricyclic antidepressant (TCA), imipramine. Furthermore, the current study utilized a model of toxicity, namely the omission of B27 in the culture medium of primary hippocampal cells, which causes cell death (Bastianetto et al., 2006). Growth medium B27 facilitates the optimal growth and long-term survival of rat embryonic hippocampal neurons. It has been reported that a deprivation of growth medium N2, which consists of constituents similar to those in B27, induces hippocampal cell death by activating caspases 3, 8, and 9, which are enzymes that play a pivotal role in apoptosis-associated cell death (Bastianetto et al., 2006). Moreover, our laboratory has demonstrated a decrease in the expression of synaptic proteins following B27 deprivation in rat hippocampal cultures (Park et al., 2013). Within the toxic environment of B27 deprivation, this study assessed the expression of synaptic proteins in rat hippocampal neurons following the application of different classes of antidepressant drugs. Furthermore, the influence of these drugs on dendritic morphology and the involvement of various intracellular signaling pathways on hippocampal dendritic outgrowth were evaluated.

2. Material and methods

2.1. Drugs and reagents

This study utilized neurobasal medium, fetal bovine serum (FBS), horse serum (HS), B27 supplement, L-glutamine, penicillin–streptomycin, and trypsin (Invitrogen; Carlsbad, CA, USA). The antidepressant drugs used were fluoxetine, paroxetine, sertraline, tranylcypromine, tianeptine (Tocris Bioscience; Ellisvile, MO, USA), escitalopram (Lundbeck; Copenhagen, DK), and imipramine (Sigma; St. Louis, MO, USA). Antibodies used for Western blotting included anti-synaptophysin (sc-7568), anti-BDNF (sc-546), anti-goat and anti-rabbit IgG-horseradish–peroxide conjugates (Santa Cruz Biotechnology; Santa Cruz, CA, US), anti-α-tubulin and anti-mouse IgG peroxidase conjugates (Sigma), and anti-PSD95 (AB9634; Millipore; Temecula, CA, US). Antibodies used for immunostaining included anti-MAP-2 (Millipore), Alexa Fluor 568 goat anti-mouse IgG, and Hoechst 33258 (Invitrogen). Specific kinase inhibitors included the PI3K inhibitor LY294002 (Cell Signaling Technology; Beverly, MA, USA), the PKA inhibitor H-89 (Calbiochem; San Diego, CA, USA), and the CaMKII inhibitor KN-93 (Sigma).

2.2. Primary hippocampal cell cultures

All animal manipulations were performed in accordance with the animal care guidelines of the US National Institutes of Health (NIH publication no. 23–85, revised 1996). This experiment was approved by the Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board of Inje Medical (approval no. 2011-036).

Primary cultures of hippocampal neurons were prepared from fetal brains (embryonic day 17; E17) obtained from Sprague–Dawley rats (Orient Bio; Gyeonggi-Do, Korea) in a manner similar to that developed by Kaech and Banker (2006). Briefly, the brains were exposed, the hippocampi were carefully removed, and the samples were dispersed in a neurobasal medium containing 0.03% trypsin for 20 min at 37 °C (5% CO_2). Cells were suspended in the neurobasal medium supplemented with 1% FBS, 1% HS, 2% serum-free growth medium B27 (components: biotin, α-tocopherol acetate, α-tocopherol, vitamin A, bovine serum albumin, catalase, insulin, transferrin, superoxide dismutase, corticosterone, galactose, ethanolamine, glutathione, carnitine, linoleic acid, linolenic acid, progesterone, putrescine, selenium, and triodo-L-thyronine), 0.25% L-glutamine, and 50 U/mL penicillin–streptomycin; this was considered the control condition. In cultures using the neurobasal medium, glial cell growth was reduced to less than 0.5% to create a nearly pure neuronal population (Brewer et al., 1993). During the Western blotting procedure, neurons were plated in 6-well dishes coated with poly-L-lysine at a density of 2×10^5 per well. For the neurite assay, neurons were plated in 12-well dishes at a density of 2×10^4 per well. They were grown under control conditions for either 7 days (neurite assay) or 10 days (Western blotting). A preliminary experiment conducted by our laboratory found that hippocampal cells are reduced by approximately 32% under B27-deprived conditions (data not shown). Following incubation for either 7 or 10 days, the cells were treated with antidepressant drugs in the presence or absence of B27 for either 4 days (Western blotting) or 5 days (neurite assay) before being harvested for further analysis. The culture media and drugs were changed every 2 days.

2.3. Drug treatment

Antidepressant drugs (10 mM) were dissolved in dimethyl sulfoxide (DMSO) and diluted to various concentrations (final concentration of 1% DMSO) with neurobasal medium before use. For the purposes of Western blotting and the neurite assay, cells were cultured for 4 days and 5 days, respectively, with escitalopram (1, 10, and 50 μM), fluoxetine (0.1, 1, and 10 μM), paroxetine (0.1, 1, and 10 μM), sertraline (0.05, 0.1, and 1 μM), imipramine (0.1, 1, and 10 μM), tranylcypromine (1, 10, and 50 μM) or tianeptine (10, 50, and 100 μM) in the presence or absence of B27. Control cells were cultured without antidepressant drugs under control conditions. The concentrations of the drugs used in these experiments were based on observation that lower concentrations of drugs had no effect on the key proteins Akt or ERK responsible for the activation of signaling pathways that regulate neurite outgrowth and neuronal differentiation and the higher concentrations reduce the viability of hippocampal cells (Table 1).

2.4. Western blot analysis

The cells were washed twice with ice-cold phosphate-buffered saline (PBS). Lysis buffer [20 mM Tris–HCl, 137 mM NaCl, 10% glycerol, 1% NonidetP-40, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 2 mM EDTA, and one tablet of complete protease inhibitor (Roche, Laval, Quebec, Canada)] was then added, and the lysates were centrifuged (1000 × g, 15 min, 4 °C) after which the supernatants were boiled in lysis buffer. Equal amounts of protein (20 μg) derived from the cell extracts under each treatment condition were separated on SDS-polyacrylamide gels and transferred electrophoretically onto polyvinylidene fluoride membranes. The blots were blocked by incubation in 5% (w/v) non-fat milk in Tris-buffered saline (TBS) with 0.15% Tween 20 (TBS-T) for 1 h. After incubation with a primary antibody (anti-PSD-95, 1:1000; anti-BDNF, 1:1000; anti-synaptophysin, 1:1000; or anti-α-tubulin, 1:2000) in TBS-T at 4 °C overnight, the membranes were washed three times