Table 2. Expression of rat Frz3 and Frz2 in the frontal cortex following antidepressant treatment, as determined by real-time quantitative PCR

		Vehicle	Imipramine	Sertraline
rat Frz3	acute	100.0 ± 9.0	107.3 ± 19.8	72.2 ±10.0*
	chronic	100.0 ± 17.1	49.7 ± 10.1*	62.4 ±16.1*
rat Frz2	acute	100.0 ± 16.5	87.7 ± 12.8	91.3 ±15.5
	chronic	100.0 ± 10.9	95.0 ± 11.2	86.8 ±9.3

Total RNA was extracted from frontal cortices of rats treated with either vehicle or 10 mg/kg of imipramine or sertraline for either 1 day or 21 days. Total RNA used for real-time quantitative PCR is described in the Materials and Methods. Data are percentages of control values (mean ± S.E.M.). Differences were assessed by Student's *t*-test. A value of $*P<0.05$ was regarded as significant.

Table 3. Expression of rat Frz3 and Frz2 in the frontal cortex following ECT, as determined by real-time quantitative PCR

	Control	Single ECT group	Repeated ECT group
rat Frz3	100.0 ± 10.8	59.9 ± 9.7*	54.5 ± 7.2*
rat Frz2	100.0 ± 11.1	138.1 ± 10.3*	153.5 ± 18.0*

Total RNA was extracted from frontal cortices of sham rats or rats receiving either a single dose of ECT (90 mA, 1.0 s) or repeated ECT (90 mA, 1.0 s, every other day for 14 days). Real-time quantitative PCR was performed as described in the Materials and Methods. Data are percentages of control values (mean ± S.E.M.). Differences were assessed by Student's *t*-test. A value of $*P<0.05$ was regarded as significant.

was less than that observed in rats receiving chronic sertraline treatments. Interestingly, single-dose and repeat ECT significantly decreased Frz3 expression in the frontal cortex (Table 3). Although neither chronic nor single-dose administration of antidepressants affected Frz2 expression (Table 2), both single-dose and repeat ECT increased Frz2 expression (Table 3). We did not detect Frz1 in either treatment condition (data not shown), perhaps because Frz1 expression in the frontal cortex is too weak to detect in adult rats (13). To determine the pharmacological specificity of these drugs, we examined the effect of a non-antidepressant antipsychotic drug, haloperidol, on Frz3 expression. Chronic haloperidol treatment failed to induce significant changes in Frz3 expression in the frontal cortex (data not shown), suggesting that the effects of imipramine and sertraline are specific.

Discussion

In the present study, we determined the full-length nucleotide sequence of rat Frz3 cDNA. The pattern of rat Frz3 expression was similar to the patterns of mouse Frz3 and human Frz3 expression (11, 14). In adult rats, we found Frz3 mRNA in various tissues, but in different amounts. In the rat, Frz3 is transcribed into two major transcripts: an 11.0-kb mRNA in brain, kidney, and uterus and a 1.7-kb mRNA in testis (Fig. 3A). In humans, the Frz3 is also transcribed into two major transcripts, a 14.0-kb mRNA in brain and a 1.8-kb mRNA in pancreas (12). They concluded that the 1.8-kb transcript encodes truncated Frz3 protein containing a N-terminal cysteine-rich domain and four transmembrane domains, which permit the alternative polyadenylation of the mRNA at an internal AATAAA signal in the coding region. This internal signal also exists in rat Frz3 and is located in a similar region as that in

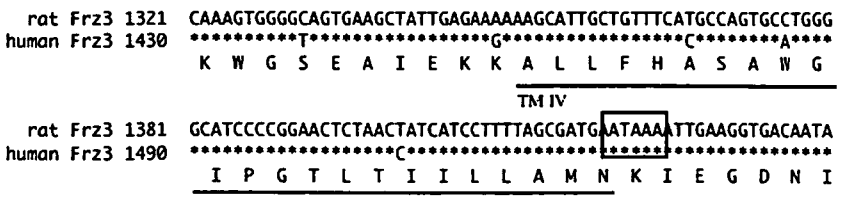

Fig. 4. Alignment of the nucleotide sequence for the fourth transmembrane domain and surrounding region of rat Frz3 and human Frz3. Nucleotide positions are numbered on the left. Asterisks represent identical nucleotides found in rat Frz3. The predicted fourth transmembrane domain is underlined. An alternative polyadenylation site is enclosed within a rectangle.

human Frz3 (Fig. 4). Examination of rat Frz3 expression in brain during development and aging revealed that Frz3 is expressed continually at high levels from late embryogenesis (E20.5) to early postnatal development (postnatal 4 weeks) (Fig. 3B). This expression diminished with age. These findings suggest that Frz3 exerts profound effects on growth and development of the central nervous system.

In adult rat brain, Frz3 mRNA was expressed predominantly in the cerebral cortex and the hypothalamus and moderately in the hippocampus. Using real-time quantitative PCR, we demonstrated that Frz3 expression in rat frontal cortex decreased after chronic treatment with either imipramine or sertraline. Our data indicate that Frz3 would be one of the common functional molecules in the frontal cortex affected by the different types of chronic antidepressant treatments. Although it is possible that the therapeutic action on a single brain region underlies antidepressant treatment, it is also possible that pharmacological effects on multiple brain regions are responsible for the real therapeutic action of antidepressants and ECT. Studies to further characterize the neuronal circuitry of these brain regions will help elucidate the neuroanatomical substrates of antidepressive effects.

Interestingly, we also observed this altered pattern of Frz3 gene expression after both single-dose and repeated ECT. Single-dose treatments with imipramine failed to affect Frz3 expression, whereas those with sertraline decreased expression (Table 2). This decrease, however, was less than that observed in rats receiving chronic sertraline treatments. Together, the decreased Frz3 expression may initiate the adaptive neuronal changes resulting from chronic antidepressant treatment.

On the other hand, the non-antidepressant antipsychotic drug haloperidol, however, did not affect Frz3 expression. Moreover, Frz2 expression failed to decrease after chronic or single-dose administration of antidepressants, and rather, it increased after ECT. These results suggest that therapeutic treatments for depression specifically decrease Frz3 expression. The physiological role of the Frz2 induction after ECT is yet to be elucidated.

Interactions between Frz and certain Wnt ligands mediate specific downstream signals. Activation of the Frz/Wnt pathway leads to inactivation of glycogen synthase kinase (GSK-3β) through the Dishevelled-mediated pathway. GSK-3β normally promotes the turnover of β-catenin, a protein involved in synaptogenesis (15, 16), by direct phosphorylation, marking it for ubiquitylation and degradation in proteasomes. Therefore, the inhibition of GSK-3β leads to accumulation of β-catenin (10). Some researchers propose that β-catenin then enters the nucleus and binds LEF/TCF transcription factors; the β-catenin-LEF/TCF complex then acts as a transcriptional activator of Frz/Wnt target genes (17). In brain, β-catenin inactivation leads to structural malformation and craniofacial development failure (18). Dysfunction in the pathway also affects behavior. Transgenic mice overexpressing GSK-3β exhibit spatial learning deficits (19). It was recently proposed that the Frz/Wnt pathway participates in a complex behavioral phenomenon (20). Moreover, molecules involved in this pathway are encoded by candidate genes thought to be involved in certain neuropsychiatric disorders. Gene-targeting of dishevelled-1, a cytoplasmic signaling protein that regulates β-catenin levels, shows that it influences social behavior and sensorimotor gating in mice (20).

Several pharmaceuticals commonly used to treat depression target components of this pathway. The direct inhibition of GSK-3β by lithium, an effective mood stabilizer for the treatment and prophylaxis of manic-depressive illness, leads to β-catenin stabilization both in vivo and in vitro (21, 22). Valproate, another mood stabilizer, regulates GSK-3β-mediated axonal remodeling in developing neurons (22). GSK-3β is inhibited by phosphorylation on serine-9. It was recently demonstrated that serotonergic activity regulates the phosphorylation of GSK-3β in mouse brain. These results raise the possibility that impaired inhibitory control of GSK-3β may occur in conditions where serotonergic activity is dysregulated, such as in mood disorders (23). More recently, it is reported that a selective GSK-3 inhibitor AR-A014418 induces behavioral changes in rats, which are consistent with the effects of antidepressant medications (24). In addition, chronic ECT up-regulates β-catenin and induces β-catenin-associated cell division and differentiation in adult rat hippocampus (25). Detection of changes in the expression levels of molecules participating in the Frz/Wnt cascade may aid the diagnosis of depression or the ability to alter expression levels may contribute to the therapeutic efficacy of drugs used to treat depression.

In conclusion, we demonstrated here that Frz3 is commonly down-regulated following chronic antidepressant treatment or by ECT in rat frontal cortex. It is interesting to investigate the effect of non-monoaminergic candidates for antidepressants, including the non-peptidic selective delta-opioid receptor agonist SNC80 (26), on Frz3 expression. Also, further studies with a specific and sensitive antibody for rat Frz3 will be necessary to clarify the relationship between the Frz3-mediated pathway and the therapeutic efficacy of antidepressant.

Acknowledgments

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Ndrg2 promotes neurite outgrowth of NGF-differentiated PC12 cells

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Abstract

Ndrg2 is a member of the N-myc downstream-regulated genes. Thus far, two different isoforms of rat *Ndrg2* protein, Ndrg2S and Ndrg2L, have been identified. Recently, we have identified rat Ndrg2 as a novel target molecule of antidepressants and ECT. The functional role of Ndrg2 in the central nervous system, however, remains unclear. In the present study, we examined the expression of endogenous Ndrg2, cellular localization of transfected Ndrg2 protein, and morphological changes resulting from overexpression of Ndrg2 in NGF-differentiated PC12 cells. Neurites began to sprout 1–2 days after exposure to NGF; subsequent neurite growth continued for 5 days. During this time, we evaluated *Ndrg2* mRNA expression by real-time quantitative PCR and found that expression significantly increased in a time-dependent manner. Interestingly, V5-conjugated Ndrg2S and Ndrg2L proteins expressed in NGF-differentiated PC12 specifically localized to cell surface membranes and growth cones. Moreover, Ndrg2S and Ndrg2L overexpression promoted neurite elongation in NGF-differentiated PC12 cells. In conclusion, our findings offer novel insights into the physiological roles of Ndrg2 in the central nervous system.

Keywords: N-Myc downstream-regulated genes; Neurites elongation; Differentiation

Ndrg2 is a member of the N-myc downstream-regulated genes, the Ndrg family; thus far, four members of this family, Ndrg1–4, have been identified [12]. Ndrg family members may be intimately involved in cellular differentiation and development. Indeed, Ndrg1 expression is induced by hypoxia and has been implicated in cell growth regulation and Schwann cell signaling for axonal survival [4,8,10,13]. In human leukemia cells, Ndrg1 expression is upregulated by differentiation-related retinoids and Vitamin D3 [8]. Suppression of Ndrg4 expression by Ndrg4 antisense transfection inhibits neurite outgrowth in PC12 cells [6]. Stable expression of human Ndrg2 in glioblastoma cell lines decreases cell growth rates [2]. More recently, *Ndrg2* mRNA and protein has been shown to be upregulated in Alzheimer's disease brains [5].

Although Ndrg members do not possess a clear functional motif, they do share a high level of sequence homology. Phylogenetic analysis of Ndrg1–4 revealed that Ndrg1 and Ndrg3 belong to one subfamily, while Ndrg2 and Ndrg4 belong to another [9]. Interestingly, Ndrg2 is highly related to Ndrg1, which has been linked to stress responses, cell proliferation, and differentiation, although *Ndrg2* itself is not repressed by N-Myc [7]. Thus far, four different isoforms of rat *Ndrg2* have been identified [1]. The 5′-untranslated region (UTR) for *Ndrg2a1/Ndrg2a2* is 87 bp, whereas the 5′-UTR for *Ndrg2b1/Ndrg2b2* is 50 bp. In the translated region, *Ndrg2a1/Ndrg2b1* has an additional 42 bp insertion compared to *Ndrg2a2/Ndrg2b2*. In this study, we denote *Ndrg2a1/Ndrg2b1* and *Ndrg2a2/Ndrg2b2* to represent *Ndrg2L* and *Ndrg2S*, respectively. Comparison and alignment of amino acid sequences indicated that Ndrg2L is longer than Ndrg2S by 14 amino acids and that both isoforms share the characteristic Ndrg family sequence.

Recently, we have identified rat Ndrg2 as a novel target molecule of antidepressants and electroconvulsive treatment (ECT) [11]. However, the functional role of Ndrg2 in the central nervous system remains unclear. In the present study,

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we examined the expression of endogenous Ndrg2, cellular localization of transfected Ndrg2 protein, and morphological changes resulting from overexpression of Ndrg2 in nerve growth factor (NGF)-differentiated neural crest-derived rat phenochromocytoma (PC12) cells. PC12 cells have been used extensively for examining neurite outgrowth in response to NGF treatment [3], and thus provide a good model for assessing Ndrg2 effects in a neural context.

PC12 cells were cultured in Dulbecco's modified eagle's medium (DMEM, Sigma-Aldrich, Inc. MO, USA) supplemented with 10% fetal calf serum, 5% horse serum (Invitrogen, CA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen) at 37 °C in a humidified atmosphere comprised of 5% CO_2. Cells were treated with NGF in uncoated 6 cm dishes. Neuronal differentiation of PC12 cell was generated by culturing the cells in DMEM with or without 50 ng/mL NGF for various lengths of time (0, 6, 24 h and 2, and 5 days). At each of these time points, the cells were assessed with a phase contrast microscope, and then RNA was extracted. As described above, rat Ndrg2 protein consists of two splice variants, Ndrg2S and Ndrg2L. However, we previously demonstrated using conventional reverse transcriptase-polymerase chain reaction (RT-PCR) that transcript processing into long and short forms of *Ndrg2* does not appear to be significantly regulated in NGF-differentiated PC12 cells (data not shown). Therefore, we performed mRNA expression analysis of *Ndrg2* with real-time quantitative polymerase chain reaction (RTQ-PCR) using an ABI PRISM 7000 instrument (Applied Biosystems, Foster City, CA, USA); total levels of *Ndrg2S* and *Ndrg2L* mRNA were examined in the present study. Total RNA was extracted from samples using Isogen reagent (Nippon Gene Co., Tokyo, Japan) according to the manufacturer's instructions. Total RNA samples treated with RNase-free DNase I was used to synthesize the first strand cDNA via reverse transcriptase and oligo-dT primer. PCR primers were designed using Primer Express Software (Applied Biosystems). A quantity of cDNA corresponding to 20 pg of total RNA was amplified by PCR. The following primers were used for rat *Ndrg2* (5′-AACTTTGAGCGAGGTGGTGAGA-3′ and 5′-ATTCCACCACGGCATCTTCA-3′) and β-actin (5′-TCGCTGACAGGATGCAGAAGG-3′ and 5′-GCCAGGATAGAGCCACCAAT-3′). The SYBR® Green PCR Core Reagents Kit (Applied Biosystems) was utilized for fluorescence detection of cDNA. For quantification, we used the Standard Curve Method (User Bulletin, ABI PRISM 7000 Sequence Detection System). Briefly, for rat Ndrg2 and β-actin, an absolute standard curve was obtained by plotting the threshold cycle following PCR amplification of serial dilutions of control cDNA template.

To determine the localization of Ndrg2S and Ndrg2L protein in PC12 cells, V5-conjugated Ndrg2S and Ndrg2L were transfected and overexpressed in NGF-differentiated PC12 cells, and V5 was detected immunochemically with an anti-V5 antibody. The coding regions for *Ndrg2S* and *Ndrg2L* were obtained from rat brain via PCR with high fidelity Platinum pfx DNA polymerase (Invitrogen) and the following set of primers: 5′-CTCGAGGCCACCATGGCAGAGC-3′, 5′-GAATTCTCTCAACAGGAGACTTCCATGGT G-3′. These primers contain either *Xho*I or *Eco*RI sites (underlined) to facilitate subcloning. Electrophoresis of agarose gels containing PCR products resulted in two reproducible bands that corresponded to *Ndrg2S* and *Ndrg2L*. Both of the amplified products were ligated into pCR II-TOPO vectors (Invitrogen) and transformed into competent DH5α *Escherichia coli* cells. The resulting plasmid vectors were recombined into pIRES-EGFP (Clontech, CA, USA) and pEF-DEST 51 (V5/His-tagged C-terminal region) vectors via GATEWAY cloning technology (Invitrogen) and adaptive attB1 and attB2 site primers. PC12 cells (2×10^5 cells) were seeded onto collagen-coated 25 mm diameter glass coverslips and transfected with 2 μg of recombinant plasmid in serum-free medium using 2 μL Lipofectamine reagent (Invitrogen). Three hours after transfection, 50 ng/mL of NGF (Sigma-Aldrich) was added to the dishes to initiate cell differentiation. To examine the specificity of transfection, Western blot analysis were performed on protein extracts derived from the transfected PC12 cells. The cells were homogenized in ice-cold Sucrose-Tris buffer, protein concentration was determined, 20 μg fractions of protein were separated by 7.5% SDS-PAGE, then blotted onto nitrocellulose membranes. The membranes were assessed immunochemically for Ndrg2-V5 fusion proteins by incubating them in blocking buffer (5% skim milk), anti-V5 antibody (1:500; Invitrogen) in blocking buffer, followed by anti-mouse IgG (1:20,000; American Qualex). The immunoreactive bands were visualized on film by the ECL system. Three days after transfection, NGF-differentiated PC12 cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) in 0.1 M phosphate buffer for 15 min at room temperature, washed with PBS three times, permeabilized with 0.2% Triton X-100 in PBS for 10 min, and blocked with 1% fetal calf serum prepared in PBS for 30 min. PC12 cells transfected with either V5-conjugated Ndrg2S or Ndrg2L were incubated with anti-V5 antibody (1:200), followed by a fluorescein-labeled anti-mouse IgG (1:100; American Qualex) for 1 h at room temperature. For each dish, 10–20 fluorescent images in the identical viewing field were captured at an excitation wavelength of 488 nm with a Bio-Rad MRC-500 laser scanning confocal attachment (Bio-Rad, MA, USA) mounted on a Nikon Diaphot inverted microscope (Nikon, NY, USA).

To determine whether Ndrg2 promotes neurite elongation, we analyzed morphological changes in PC12 cells that overexpressed either Ndrg2S or Ndrg2L. In this study, we used the pIRES-EGFP vector, which can express green fluorescent protein (GFP) and target molecules separately. Two days after transfection, cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 15 min at room temperature, washed with PBS three times, permeabilized with 0.2% Triton X-100 in PBS for 10 min, and blocked with 1% fetal calf serum for 30 min. PC12 cells transfected with pIRES-EGFP containing either Ndrg2S or Ndrg2L were incubated with Alexa Fluor 568-conjugated phalloidin (Molecular Probes,

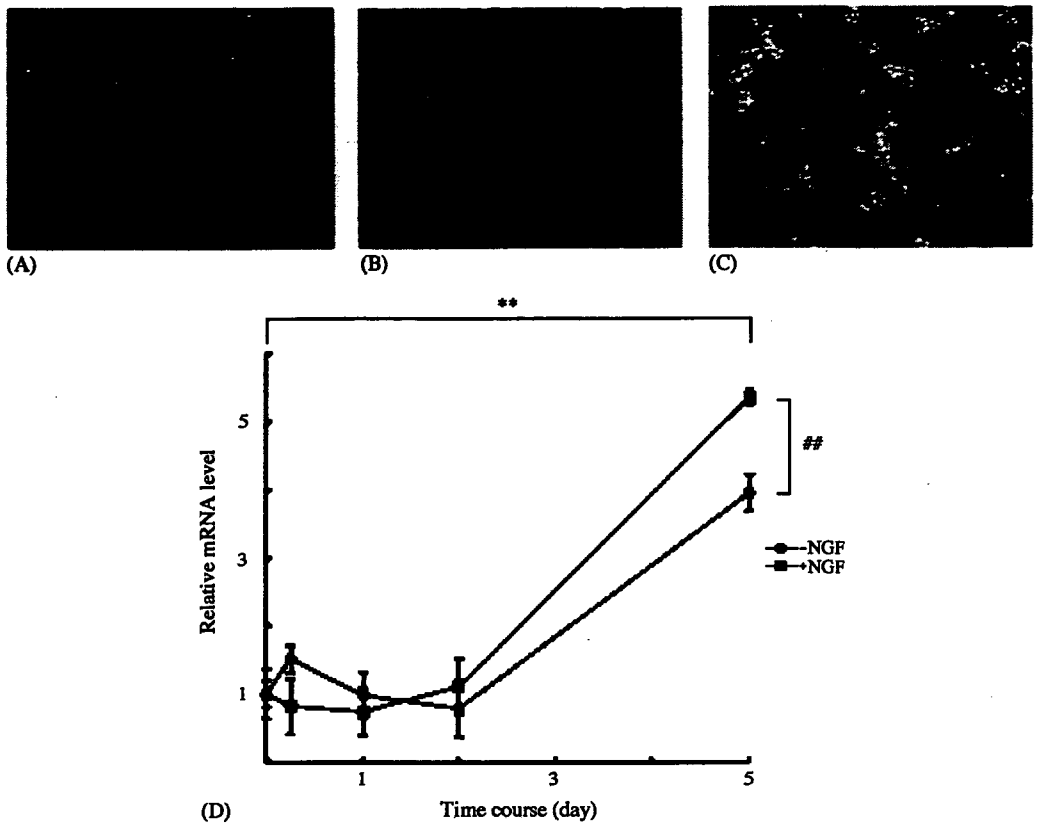

Fig. 1. Messenger RNA expression of *Ndrg2* during varying stages of neurite outgrowth in PC12 cells treated with (square) or without (circle) 50 ng/mL NGF. Images of PC12 cells cultured with NGF for 0 day (A), 2 days (B) and 5 days (C) were obtained from phase-contrast microscopy (scale bars = 200 μm). Neurites began to sprout 1–2 days after exposure to NGF; subsequent neurite growth continued for 5 days. During this time, the expression of *Ndrg2* mRNA was significantly increased in a time-dependent manner (D). Data are expressed as mean ± S.E.M. of three independent experiments. $^{**}p < 0.01$ ANOVA followed by the Dunnett's test, $^{\#\#}p < 0.01$ Student's *t*-test.

Leiden, Netherlands) to visualize F-actin filaments for double staining. For each dish, 20–30 fluorescent images were obtained using a laser scanning confocal microscopy imaging system (Bio-Rad). Only cells with GFP-immunofluorescence were regarded as transfectants. The length and the number of individual neurites from over one hundred cells were analyzed blindly using NIH image software running on an Apple Computer. NIH Image is a public domain program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Neurites sprouting more than 5 μm from the cell body were analyzed.

Data are presented as mean ± S.E.M. for each group. For the RTQ-PCR experiments, statistical differences were assessed using analysis of variance (ANOVA) followed by the Dunnett's test or Student's *t*-test. For the neurite outgrowth experiments, statistical differences were assessed using Bonferroni correction. A value of $p < 0.05$ was regarded as significant.

As expected, NGF induced PC12 cells to differentiate into neuron-like cells, the differentiation of which was manifested by neurite outgrowth (Fig. 1). Neurites began to sprout 1–2 days after exposure to NGF; subsequent neurite growth continued for 5 days (Fig. 1A–C). To determine more specifically the role of Ndrg2 in PC12 cell differentiation, we assessed *Ndrg2* mRNA expression during varying stages of neurite outgrowth in these cells. During this time, Ndrg2 mRNA expression was significantly increased in a time-dependent manner (Fig. 1D). Indeed, the mRNA expression was not changed for 2 days after the treatment with or without NGF. Interestingly, 5 days after the treatment, the mRNA expression was significantly increased and surpassed those found on day-0 by 5.4- and 3.9-fold, respectively (Fig. 1D). In addition, *Ndrg2* mRNA expressions on day-5 were significantly different between the cells treated with or without NGF (Fig. 1D). Although, a causal relationship between Ndrg2 expression and neurite outgrowth is not proved by our observation, our data may suggest that the increased levels of Ndrg2 is somehow related to differentiation of PC12 cells.

Interestingly, V5 immunoreactivity representing Ndrg2 expression localized on cell surface membranes and growth cones (Fig. 2A). Fluorescent signals were not detected in untransfected PC12 cells (data not shown). To verify the specificity of transfection, protein extracts derived from transfected PC12 cells were immunoblotted. As expected, Ndrg2S and Ndrg2L were detected as distinct ~39.3 and 40.8 kDa bands, respectively (Fig. 2B). In the present study,

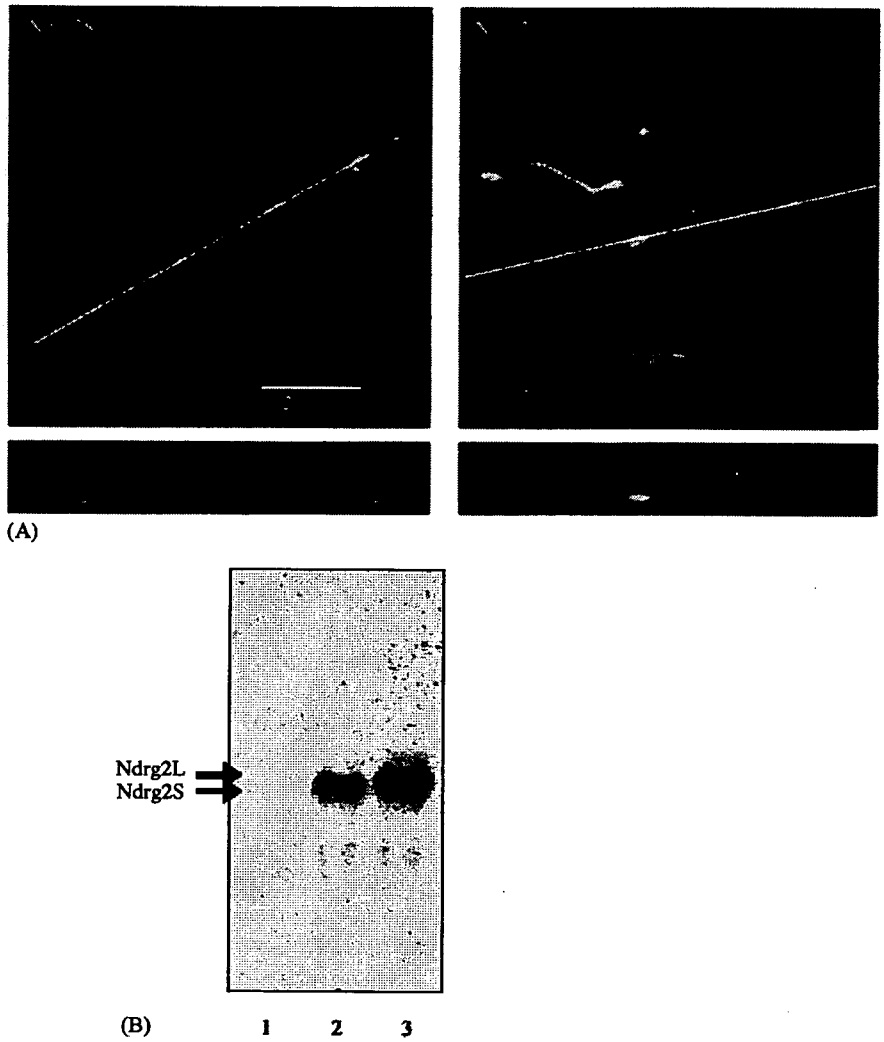

Fig. 2. Immunocytochemical localization of transfected Ndrg2S and Ndrg2L in NGF-differentiated PC12 cells. V5 immunoreactivity for both V5/HIS tagged Ndrg2S (left panel) and Ndrg2L (right panel) proteins localized on cell surface membranes and growth cones (A). Typical longitudinal section (A, up) and cross-section (A, bottom) images of Ndrg2S and Ndrg2L transfected PC12 cells were demonstrated. To verify the specificity of transfection, protein extracts derived from non-vector (lane 1), Ndrg2S (lane 2) or Ndrg2L (lane 3) transfected PC12 cells were immunoblotted (B). As expected, Ndrg2S and Ndrg2L were detected as distinct ~39.3 and 40.8 kDa bands, respectively.

we found Ndrg2S and Ndrg2L to specifically localize to cell surface membranes and growth cones, suggesting that these proteins are transported to the tip of neurites in these cells. Interestingly, human Ndrg2-GFP fusion protein has been localized to the cytosol in transfected COS-7 cells [9]. In addition, Ndrg2-V5 fusion protein has been expressed at the perinuclear region in the oligodendrocytic 6E12 cells [5]. The distribution of Ndrg2, however, may vary depending on the type of cell lines used in different transfection experiments. Together, our results suggest two possibilities: (1) Ndrg2 is growth cone components, and/or (2) Ndrg2 is a mediator of neurite outgrowth.

Indeed, 2 days after transfection, the neurites of transfected cells were clearly longer than those found in control cells (Fig. 3A). The mean length of the longest neurite of each cell was significantly longer in transfected cells (control, $27.1 \pm 1.9\,\mu m$; Ndrg2S transfectant, $34.9 \pm 2.5\,\mu m$; Ndrg2L transfectant, $37.8 \pm 3.2\,\mu m$; Bonferroni correction $p < 0.05$; Fig. 3B). On the other hand, the mean number of neurites per cell did not vary between the groups (control, 2.38 ± 0.13; Ndrg2S transfectant, 2.15 ± 0.11; Ndrg2L transfectant, 2.27 ± 0.10; Fig. 3C). As discussed above, the expression of endogenous Ndrg2 mRNA was not influenced by NGF-differentiation in PC12 cells at this time point. Together, our data demonstrated that overexpression of Ndrg2S and Ndrg2L promoted elongation of neurites in NGF-differentiated PC12 cells, even though the number of neurites remained unchanged.

Molecules localized within growth cones have been shown to function in synaptic transduction and in neurite sprouting, extension, and guidance. Thus, since Ndrg2 mainly localized to cell surface membranes and growth cones of PC12 cells, Ndrg2 may also play a role in the mechanisms underlying such synaptic events. Previously, we found that chronic

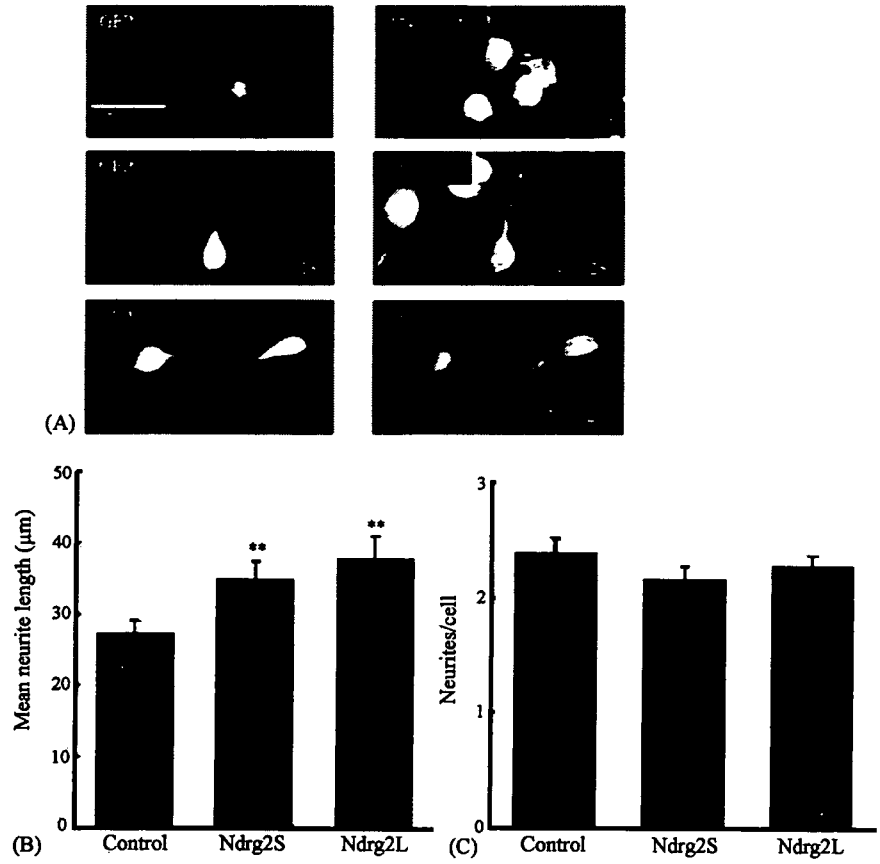

Fig. 3. Neurite outgrowth of NGF-differentiated PC12 cells after Ndrg2S and Ndrg2L transfection. The images of GFP-expressing cells were collected using confocal microscopy (A, left). F-actin was visualized in the identical field with Alexa Fluor 568-conjugated phalloidin (A, right). Two days after transfection, the mean length of the longest neurite of each cell was significantly longer in transfected cells (B). On the other hand, the mean number of neurites per cell did not vary between the groups (C). Data are expressed as mean ± S.E.M. of 130–150 cells analyzed. $^{**}p < 0.01$, Bonferroni correction.

antidepressant treatment or ECT decreased Ndrg2 expression [11]. Our findings suggest that gene expression-dependent alterations of synaptic function, which includes Ndrg2, may be an important component of the pharmacological action of antidepressants and ECT. In conclusion, our findings offer novel insights into the physiological roles of Ndrg2 in the central nervous system.

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Repetitive transcranial magnetic stimulation induces kf-1 expression in the rat brain

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Abstract

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive approach used for stimulating the brain, and has proven effective in the treatment of depression, however the mechanism of its antidepressant action is unknown. Recently, we have reported the induction of kf-1 in rat frontal cortex and hippocampus after chronic antidepressant treatment and repeated electroconvulsive treatment (ECT). In this study, we demonstrated the induction of kf-1 after rTMS in the rat frontal cortex and hippocampus, but not in hypothalamus. Our data suggest that kf-1 may be a common functional molecule that is increased after antidepressant treatment, ECT and rTMS. In conclusion, it is proposed that induction of kf-1 may be associated with the treatment induced adaptive neural plasticity in the brain, which is a long-term target for their antidepressant action.

Keywords: Depression; Antidepressant; Microarray

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Introduction

Antidepressants are widely used in the treatment of depression. On the other hand, electroconvulsive treatment (ECT) is also an important therapy that is believed to have a rapid onset of antidepressant activity. In addition, newer findings regarding the mechanisms of action of ECT have led to novel developments in treatment technique to further improve this highly effective treatment for major depression. These new approaches include new methods for inducing more targeted seizures (eg, transcranial magnetic stimulation, TMS). TMS was introduced by Barker et al in 1985 as a new method for noninvasive and almost painless stimulation of the central nervous system (George et al., 2002). The development of stimulators capable of delivering stimulation frequencies of up to 60 Hz (repetitive TMS, rTMS) increased the potential clinical applications of rTMS. It is reported that rTMS over the prefrontal cortex is as effective as ECT in the treatment of nondelusional major depressive disorder (Grunhaus et al., 2003). Despite some differences in the physical properties of magnetic and electrical stimulation, rTMS shares many of the behavioral and biochemical actions of ECT and other antidepressant treatments. For example, rTMS reduces immobility in the Porsolt swim task and enhances apomorphine-induced stereotypy, as does ECT (Lisanby et al., 2000). However, the mechanism of the antidepressant action of rTMS is unknown.

By inducing electric currents in brain tissue via a time-varying strong magnetic field, rTMS has the potential to either directly or trans-synaptically modulate neuronal circuits thought to be dysfunctional in depression. Recent animal studies have broadened our understanding of how rTMS affects brain functioning. There is compelling evidence that rTMS causes changes in neuronal circuits as reflected by behavioural changes (Post and Keck, 2001). These alterations suggest regional changes in neurotransmitter release, transsynaptic efficiency, signaling pathways and in gene transcription. Indeed, it is demonstrated that rTMS stimulates subcortical dopamine release, modulates cortical beta-adrenergic receptors, reduces frontal cortex 5-HT_2 receptors, increases 5-HT_{1A} receptors in frontal cortex and cingulate, and increases N-methyl-D-aspartate receptors in the ventromedial hypothalamus, basolateral amygdala, and parietal cortex (Lisanby et al., 2000; Padberg and Moller, 2003). However, in order to optimize rTMS for therapeutic use, it is necessary to understand the neurobiological mechanisms involved, particularly the nature of the changes induced and the brain regions affected. Previously, we investigated neuronal response to rTMS and ECT in terms of c-Fos expression (Doi et al., 2001). In rats rTMS sessions induced widespread nuclear c-Fos-like immunoreactivity in frontal cortex, lateral orbital cortex, striatum, lateral septal nucleus, piriform cortex, dentate gyrus, Ammon's horn, cingulate cortex, parietal cortex, thalamus, occipital cortex, and amygdala; this reactivity was greater than with control rats, which were treated as the rTMS-treated rats but without magnetic stimulation. ECT produced even stronger c-Fos expression than rTMS in all regions except thalamus (no difference) and striatum (stronger with rTMS). Thus, functional modification of neuroanatomical substrates as demonstrated by c-Fos expression may partially differ between rTMS and ECT (Doi et al., 2001). Studies to further characterize the neuronal circuitry of these brain regions will help elucidate the neuroanatomical substrates of antidepressive effects by rTMS.

An increasingly popular working hypothesis is that chronic treatment with drugs of various classes, ECT or rTMS have common antidepressant effects because they regulate transcription of the same set of downstream genes. Indeed, antidepressants and ECT have been shown to affect the expression of immediate early genes and transcription factors, including c-Fos, FosB, NGF1-A and CREB (see review by Yamada and Higuchi, 2002). These proteins activate or repress genes that encode specific proteins by binding to DNA regulatory elements, and they may be involved in critical steps that mediate treatment-

induced neural plasticity. Interestingly, we recently demonstrated the induction of kf-1 in rat frontal cortex and hippocampus after chronic antidepressant treatment and repeated ECT (Yamada et al., 2000, Nishioka et al., 2003). Therefore, in the present study, to identify a novel candidate target molecule of rTMS, we have examined the expression of kf-1 after rTMS in the rat brain, and compared the effects to those of chronic antidepressant treatment and repeated ECT. Here, we first demonstrated the induction of kf-1 after rTMS in rat frontal cortex and hippocampus.

Materials and methods

Experimental animals

Male Sprague-Dawley rats (age 7–10 weeks, Sankyo Labo Service Co. Tokyo, Japan) were housed in a temperature-controlled environment with 12 h light/ 12 h dark cycle with free access to food and water. Although a sexual difference of kf-1 expression in the brain is not clear, only the male rats were used to minimize the possible variability naturally found in the female rats. Rats were randomly separated into control and treated groups. It is reported that rTMS reduces immobility time in the Forced Swim Test model of depression, suggesting an antidepressant effect, which is evident at a range (1–25 Hz) of frequencies (Sachdev et al., 2002). With repeated administration, it is suggested that the antidepressant effect is likely to be sustained. In this study, rTMS was administered with the figure-eight magnetic coil in contact with the head pointed to the bregma, 1.9 tesla, 20 Hz/train, 3.5 sec, 70 pulses, once daily for 10 days (Magstim 200 rapid stimulator, Magstim Company, Whitland, UK). Control rats were treated as the rTMS-treated rats but without magnetic stimulation. Experimental animals for chronic haloperidol treatment (antipsychotic agent, as non-antidepressant control drug) received vehicle, 0.5 mg/kg or 2.0 mg/kg of haloperidol (Sigma Chemical Co., St. Louis, MO, USA) dissolved in saline, by daily intraperitoneal injection for 21 days. Three or four rats were used for each treatment group.

Twenty-four hours after the final rTMS or haloperidol injection, animals were euthanized by decapitation. The brain was quickly removed, dissected, and immediately frozen in liquid nitrogen and stored at −80°C until use. All animal studies were carried out in accordance with protocols approved by the Showa University Ethic Committee in line with the OPRR Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Northern blot analysis

Complimentary DNA fragment of kf-1 was cut out from PCR II-TOPO vector and labeled with [α-^{32}P]dCTP, and then used as a probe for northern blot analysis. Rat multiple tissue Northern blot nylon membrane (Clontech, Palo Alto, CA, USA) was used for the experiment. Hybridization procedure was carried out following the manufacture's instructions. After the hybridization, the membrane was exposed to X-ray firm for 24 h.

Fabrication of cDNA Microarray and fluorescence image analysis

Fabrication of cDNA microarray was described by our group previously (Yamada et al., 2000). Briefly, cDNA inserts were amplified by vector primers and spotted in duplicated on the glass slide

using GMS417 Arrayer (Affymetrix, Inc., CA, USA). To make the fluorescence-labeled probe for hybridization, total RNA samples obtained from rat hippocampus from control or sertraline group was extracted by Isogen reagent (Nippon gene Co., Tokyo, Japan) following the manufacture's instruction. Then, three independent total RNA samples from each group were pooled and used for the next procedure. Poly A^+ RNA was then purified from pooled total RNA with oligo-dT columns (Takara, Tokyo, Japan). One μg of poly A^+ RNA from control or rTMS samples was converted to cDNA in the presence of Cy-5 or Cy-3-dUTP respectively to make fluorescence-labeled probes. Hybridization of probes to microarray was done competitively. The probes were mixed and placed on an array, overlaid with coverslip, and hybridized for 16.5 h at 65°C. After hybridization and washing procedure, each slide was scanned with GMS418 Array Scanner (Affymetrix, Inc., CA, USA). Then, gene expression levels were quantified and analyzed using ImaGene software (Bio-Discovery Ltd. Swansea, UK).

Messenger RNA expression analysis with RT-PCR

The first strand cDNA was synthesized with reverse transcriptase and 1 μM of oligo-dT primer, from 2 μg of total RNA samples treated with RNase-free DNase I, and diluted to a final volume of 100 μL. One μL of each cDNA sample was added to 24 μL of PCR reaction mixture containing 0.5 μM of a pair of primers for kf-1, 5′ -GGAATACGGACAGGACTTTC-3′ and 5′ -TCCGA-GAAGCTGCATGGGC-3′ (Amersham Pharmacia Biotech, Tokyo, Japan). A pair of primers for ß-actin, 5′ -TCCTGACCCTGAAGTACCCCATTG-3′ , 5′ -GGAACCGCTCCATTGCCGATAGT-3′ was also used for normalization. To ensure the fidelity of this analysis, we assayed several cycles of PCR to determine the liner range for amplification of PCR product in each region of the brain. Amplification of kf-1 was performed as follows: 3 min at 94°C for initial denaturation, an appropriate cycles of 94°C denaturing for 30 sec, 55°C annealing for 30 sec, and 72°C extension for 1 min, followed by a final extension at 72°C for 7 min. Typically, 36, 24, or 30 cycles were used for samples obtained from frontal cortex, hippocampus or hypothalamus, respectively. Amplification of ß-actin for normalization was performed as follows: 3 min at 94°C for initial denaturation, 18–20 cycles of 94°C denaturing for 30 sec, 55°C annealing for 30 sec, and 72°C extension for 1 min, followed by a final extension at 72°C for 7 min. The PCR products were electrophoresed in a 1% agarose gel containing SYBR green, a nucleic acid gel stain reagent GelStar (Takara, Tokyo, Japan). The optical density of the digitized image was quantified using a fluorescence image analyzer, FM-bio II (Hitachi, Tokyo, Japan).

Results

Northern blot analysis

Previously, we have demonstrated the presence of a single transcript of about 3.5 kb in size for mRNA prepared from several rat tissue regions, which hybridized to the [^{32}P]-labeled kf-1 probe. These regions included brain, lung and kidney, liver and heart, but at much lower levels in spleen and muscle (Yamada et al., 2000). In the present study, northern blot analysis also demonstrated the presence of a single transcript of about 3.5 kb in size for mRNA prepared from several rat brain tissues (1: olfactory, 2:

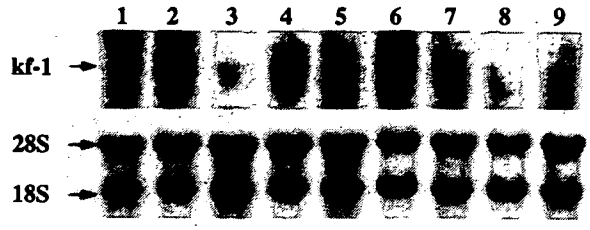

Fig. 1. Expression of kf-1 in rat brain tissues. Complimentary DNA fragment of kf-1 obtained from EST analysis was labeled with [^{32}P]dCTP and used as a probe. Rat brain tissues (1 olfactory, 2 cerebral cortex, 3 hippocampus, 4 thalamus, 5 hypothalamus, 6 midbrain, 7 cerebellum, 8 pons with m. oblongata, 9 spinal cord) were analyzed by Northern blot analysis. Photograph of the gel demonstrated that the total RNA samples used in this study contained intact and similar levels of 28S and 18S ribosomal RNA bands as determined by denaturing gel electrophoresis. Northern blot analysis demonstrated the presence of a single transcript of about 3.5 kb in size for mRNA prepared from several rat brain tissues, which hybridized to the kf-1 probe. Note: The print, made for the review process, was taken directly from the high-quality image file, which will be used in preparing the publication, if the manuscript is found acceptable.

cerebral cortex, 3: hippocampus, 4: thalamus, 5: hypothalamus, 6: midbrain, 7: cerebellum, 8: pons with m. oblongata, 9: spinal cord), which hybridized to the [^{32}P]-labeled kf-1 probe. As shown in Fig. 1, the total RNA samples used in this study contained intact and similar levels of 28S and 18S ribosomal RNA bands as determined by denaturing gel electrophoresis. The expression of kf-1 was at higher levels in the midbrain, thalamus and cerebral cortex, but at relatively lower levels in the olfactory, cerebellum, pons with m. oblongata and spinal cord (Fig. 1).

Messenger RNA expression analysis with ADRG microarray

As expected, we obtained low background and consistent results in duplicated experiments. After normalization with the signals for both negative and positive controls, several spots of our interest on the ADRG microarray showed increased or decreased fluorescence intensities after rTMS (data not shown). The expression of kf-1 (ADRG34) in ADRG microarray was 2.39 times increased in rTMS group when compared to controls. While, the expression of kf-1 in ADRG microarray was 2.17 times in antidepressant treatment group (Yamada et al., 2000) and 1.97 times in ECT group (Nishioka et al., 2003).

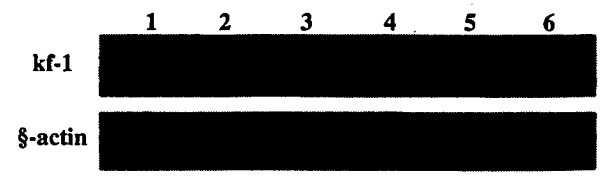

Fig. 2. A typical image of gel electrophoresis after RT-PCR. RNA was extracted from rat frontal cortex obtained from control rats (lane 1–3), or rTMS-treated rats (lane 4–6), and used for RT-PCR analysis. Control rats were treated as the rTMS-treated rats but without magnetic stimulation. The PCR products were electrophoresed in a 1% agarose gel. The reproducible single band corresponding to rat kf-1 (upper) or β-actin (under) was observed on a gel, respectively. Note: The print, made for the review process, was taken directly from the high-quality image file, which will be used in preparing the publication, if the manuscript is found acceptable.

Table 1
The mRNA expression of kf-1 after rTMS and repeated ECT

	Frontal Cortex	Hippocampus	Hypothalamus
Repetitive transcranial magnetic stimulation, rTMS			
Control	100 ± 5.7	100 ± 16.9	100 ± 12.2
rTMS	142.4 ± 8.4*	156.7 ± 15.8*	101.5 ± 9.7^{ns}
Repeated electroconvulsive treatment, ECT[1]			
Control	100 ± 8.2	100 ± 14.1	100 ± 6.1
ECT	161.7 ± 11.5*	172.8 ± 22.3*	96.3 ± 3.1^{ns}

[1]Data for repeated ECT were reported by our group previously (Nishioka et al., 2003). Briefly, rats for ECT were anesthetized and received a 90 mA, 1.0 sec electric shock via ear-clip electrodes every other day for 14 days. Data are expressed as % of the control data (means ± s.e.m.) of three independent experiments. $^{ns}p > 0.05$ and $*p < 0.05$, Student's t-test.

Messenger RNA expression analysis with RT-PCR

The induction of kf-1 after rTMS was also confirmed by RT-PCR analysis. The reproducible band corresponding to kf-1 at the size of 199 bp existed on a gel. A typical image of gel electrophoresis after RT-PCR is shown in Fig. 2. As shown in Table 1, the mRNA levels of kf-1 after rTMS were significantly increased in the frontal cortex (100 ± 5.7% for control and 142.4 ± 8.4% for rTMS samples, respectively) and hippocampus (100 ± 16.9% for control and 156.7 ± 15.8% for rTMS samples, respectively), but not in the hypothalamus (100 ± 12.2% for control and 101.5 ± 9.7% for rTMS samples, respectively) after normalization by ß-actin expression.

On the other hand, to determine the pharmacological specificity of this antidepressant action, the effect of non-antidepressant antipsychotic drug, haloperidol, on rat kf-1 expression was investigated. Interestingly, the mRNA level of kf-1 after chronic haloperidol treatment was not changed in the frontal cortex, hippocampus and hypothalamus (Table 2).

Table 2
The mRNA expression of kf-1 after chronic treatments with antidepressants and antipsychotic agent, haloperidole

	Frontal Cortex	Hippocampus	Hypothalamus
Chronic haloperidole treatment			
Control	100 ± 6.1	100 ± 19.9	100 ± 8.3
0.5 mg/kg	96.1 ± 9.8^{ns}	108.9 ± 2.8^{ns}	98.8 ± 11.8^{ns}
2.0 mg/kg	94.3 ± 5.9^{ns}	120.2 ± 19.2^{ns}	102.1 ± 13.7^{ns}
Chronic antidepressant treatment[1]			
Control	100 ± 9.3	100 ± 3.7	100 ± 18.8
Imipramine	165.6 ± 9.9*	204.0 ± 20.8*	112.9 ± 8.9^{ns}
Sertraline	182.2 ± 8.8*	173.4 ± 13.6*	129.2 ± 14.6^{ns}

[1]Data for chronic antidepressant treatments were reported by our group previously (Yamada et al., 2000). Briefly, rats for chronic antidepressant treatment received either vehicle, 10 mg/kg of imipramine or sertraline by daily intraperitoneal injection for 21 days. Data are expressed as % of the control data (means ± s.e.m.) of three independent experiments. $^{ns}p > 0.05$ and $*p < 0.05$, Student's t-test.

Discussion

Although the therapeutic action of antidepressants most likely involves the regulation of serotonergic and noradrenergic signal transduction, to date, no consensus has been reached concerning their precise molecular and cellular mechanism(s) of action. With expressed-sequence tag (EST) analysis, we had been continuing our effort to elucidate the involvement of some common biochemical changes induced after chronic treatment with two different classes of antidepressants, imipramine (a tricyclic antidepressant) or sertraline (a serotonin selective reuptake inhibitor, SSRI). Until now, we have molecularly cloned several cDNA fragments as ESTs, which we named them antidepressant related genes, ADRGs (Nishioka et al., 2003; Yamada et al., 1999; Yamada et al., 2002; Yamada et al., 2001; Yamada et al., 2000). Previously, we developed our original cDNA microarray (ADRG microarray) using ADRG genes. By gene expression analysis using ADRG microarray and fluorescence-labeled probes, we identified several interesting candidate genes and ESTs (Yamada and Higuchi, 2002). One of the spots, ADRG34, was significantly increased in sertraline treated rat hippocampus on the ADRG microarray. Moreover, we have determined the nucleotide sequence of the full-length cDNA for ADRG34 (Yamada et al., 2000). This cDNA encoded 685 amino acid residues yielding a mass of 79 kDa, containing a RING-H2 finger motif at the carboxy-terminus. Homology analysis with the EMBL/GeneBank database indicated that ADRG34 is a putative rat homologue of mouse and human kf-1 gene (Yasojima et al., 1997). Kf-1 was originally identified as the gene whose expression has been augmented in the cerebral cortex of a sporadic Alzheimer's disease patient (Yasojima et al., 1997). The RING-H2 finger motif of rat kf-1 was identical to those of mouse and human kf-1 (Yamada et al., 2000). RING finger proteins were assessed for their ability to facilitate E2-dependent ubiquitination and ubiquitinqtion was observed (Lorick et al., 1999). The abnormality of Ub-proteasome system may induce various pathological conditions. A common induction of kf-1 after chronic antidepressant treatment, repeated ECT and rTMS might indicate that the relationship between ubiquitin system and mechanism of the alleviation of depression is significantly important.

In the present study, northern blot analysis demonstrated the presence of a single transcript of about 3.5 kb in size for mRNA prepared from several rat brain tissues (Fig. 1). Interestingly, the expression of kf-1 was at higher levels in the midbrain, thalamus and cerebral cortex, but at relatively lower levels in the olfactory, cerebellum, pons with m. oblongata and spinal cord. Gene expression analysis using ADRG microarray demonstrated that the expression of kf-1 was 2.39 times increased in rat frontal cortex treated with rTMS, when compared to controls. While, the expression of kf-1 in ADRG microarray was 2.17 times in antidepressant treatment group (Yamada et al., 2000) and 1.97 times in ECT group (Nishioka et al., 2003). The induction of kf-1 after rTMS was also confirmed by RT-PCR analysis. As shown in Table 1, the mRNA levels of kf-1 after rTMS were significantly increased in the frontal cortex and hippocampus after normalization by ß-actin expression. Interestingly, we previously demonstrated the induction of kf-1 in rat frontal cortex and hippocampus after chronic antidepressant treatment and repeated ECT (Nishioka et al., 2003). On the other hand, the mRNA level of kf-1 after chronic haloperidol treatment was not changed in the frontal cortex, hippocampus and hypothalamus (Table 2). Thus, it is proposed that the induction of kf-1 is specific to antidepressive treatments.

The frontal cortex is one of the several brain regions that is implicated in the pathophysiology of depression (Drevets et al., 1992). The hippocampus is another brain region that would be involved in the endocrine, emotional, cognitive, and vegetative abnormalities found in depressed patients. It is reported that repeated ECT and SSRI administration have equivalent effects on hippocampal synaptic plasticity

(Stewart and Reid, 2000). Previous study by other group indicated that TMS has a long-lasting effect on neuronal excitability in the hippocampus (Levkovitz et al., 2001). They compared the effects of chronic TMS with those of the antidepressant drugs desipramine and mianserin. Interestingly, these treatments enhanced the expression of long-term potentiation in the perforant path synapse in the dentate gyrus. Thus, TMS, mianserin, and desipramine are likely to affect the same neuronal populations, which may be relevant to their antidepressant action. Stress-induced atrophy, and, in extreme cases, cell death, may contribute to the loss of hippocampal control of the hypothalamus-pituitary-adrenal (HPA) axis and hypercortisolism often exhibited in depression. Hippocampus is involved in feedback regulation of the HPA axis, and depression is associated with dysfunction of this neuroendocrine axis (Young et al., 1991). There is compelling evidence that rTMS causes changes in neuronal circuits as reflected by decreases in the activity of the HPA axis. However, mRNA level of kf-1 was not changed in rat hypothalamus after rTMS (Table 2). Interestingly, the mRNA level of kf-1 after chronic antidepressant treatment and repeated ECT was not changed in this region of the brain (Nishioka et al., 2003; Yamada et al., 2000).

In conclusion, we have identified kf-1 as a novel candidate target molecule of antidepressants, ECT and rTMS. Although mouse, rat and human kf-1 gene had been molecularly cloned, the physiological function of kf-1 protein the central nervous system is still not clear. Our findings suggest that induction of kf-1 may be associated with the treatment induced adaptive neural plasticity in the brain, which is a long-term target for their antidepressant action. Further characterization of kf-1 in the central nervous system is needed to test our hypothesis.

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Review article

Antidepressant-elicited changes in gene expression Remodeling of neuronal circuits as a new hypothesis for drug efficacy

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Abstract

Although antidepressants have been used clinically for more than 50 years, no consensus has been reached concerning their precise molecular mechanism of action. Pharmacogenomics is a powerful tool that can be used to identify genes affected by antidepressants or by other effective therapeutic manipulations. Using this tool, others and we have identified as candidate molecular targets several genes or expressed sequence tags (ESTs) that are induced by chronic antidepressant treatment. In this article, we review antidepressant-elicited changes in gene expression, focusing especially on the remodeling of neuronal circuits that results. This refocusing motivates our hypothesis that this plasticity represents the mechanism for drug efficacy, and thus a causal event for clinical improvement. Defining the roles of these molecules in drug-induced neural plasticity is likely to transform the course of research on the biological basis of antidepressants. Such detailed knowledge will have profound effects on the diagnosis, prevention, and treatment of depression. Consideration of novel biological approaches beyond the "monoamine hypothesis" of depression is expected to evoke paradigm shifts in the future of antidepressant research.

Keywords: Antidepressant; Depression; Microarray; Neural plasticity; Pharmacogenomics

Contents

Abbreviations: ADRG, antidepressant-related gene; BDNF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element binding protein; CSP, cysteine string protein; ECT, electroconvulsive therapy; EST, expressed sequence tag; GAP-43, growth-associated-protein 43; HPA, hypothalamus–pituitary–adrenocortical; LAL, long attack latency; Mss4, mutation suppressor for the Sec4–8 yeast; NGF, nerve growth factor; RDA, representational difference analysis; SAGE, serial analysis of gene expression; SAL, short attack latenc; SNARE, soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; TOGA, total gene expression analysis.

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1. Introduction

Depression is one of the major psychiatric diseases and is estimated to affect 12–17% of the population at some point during the lifetime of an individual (Wittchen et al., 1994). Therefore, there is a pressing need to develop new and better antidepressants.

Antidepressants are very effective agents for preventing and treating depression and have been used clinically for more than 50 years. Although the therapeutic action of antidepressants most likely involves the regulation of serotonergic and noradrenergic signal transduction pathways, to date, no consensus has been reached concerning the precise molecular and cellular mechanism of action of these drugs. Many antidepressants acutely regulate monoaminergic signal transduction, resulting in a significant increase in synaptic concentrations of the monoamine noradrenaline or serotonin within a few hours of initial treatment. But at the same time, the onset of the clinical effect of these drugs lags by several weeks. A satisfying explanation for the discrepancy in the acute increase of synaptic monoamines and delayed clinical effect remains elusive. Theories that postulate long-term changes in receptor sensitivity have unsuccessfully tried to bridge this gap (Siever and Davis, 1985). Consequently, the monoamine hypothesis does not fully explain this clear discrepancy. This delayed therapeutic action of antidepressants could result from either the indirect regulation of other neuronal signal transduction systems or the regulation of gene transcription following chronic treatment. An increasingly popular working hypothesis is that drugs of various classes have common antidepressant effects after chronic use because they regulate transcription of the same set of downstream genes.

In this article, we review antidepressant-elicited changes in gene expression, especially focusing on the remodeling of neuronal circuits as a new hypothesis for drug efficacy.

2. Antidepressant-elicited changes in gene expression

Hyman and Nestler (1996) proposed an "initiation and adaptation" model to describe the drug-induced neural plasticity associated with the long-term actions of antidepressants in the brain. However, the detailed mechanisms underlying such drug-induced adaptive neuronal changes are as of yet unknown.

2.1. "Reverse" pharmacological approaches

As shown in Fig. 1, three research directions (i.e., brain system and circuits, neural transduction, and transcriptional regulation) need to be considered to understand biochemical, neurophysiological, and/or morphological changes that underlie the long-term actions of antidepressants. Functional genomics is one powerful tool that can be used to reveal the basic molecular constituents of neurons at transcription level and can help explain how these constituents work together in the brain. Therefore, so-called "reverse" pharmacological approaches, armed with functional genomics/proteomics techniques, are thought to be very effective strategies for researching the mode of actions of antidepressants. Understanding the functional role of these molecules will shed light on the pathogenic mechanisms underlying the development of depression. Such detailed and fundamental knowledge about the brain will have profound effects on the way depression is diagnosed, prevented, and treated. Therefore, it is very

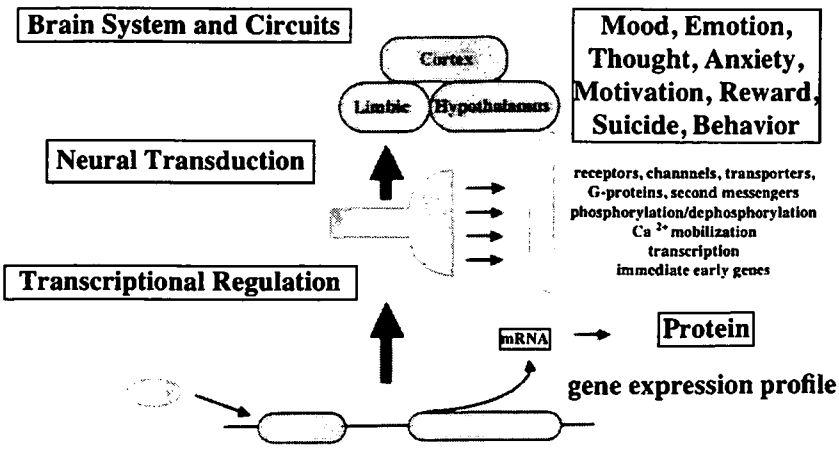

Fig. 1. Three research directions necessary to understand the neural plasticity underlying the long-term actions of antidepressants.