Research article

Nrf2-signaling and BDNF: A new target for the antidepressant-like activity of chronic fluoxetine treatment in a mouse model of anxiety/depression

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HIGHLIGHTS

• Nrf2 signaling is affected in a mouse model of anxiety/depression (the CORT model).
• Chronic fluoxetine treatment restored these levels of expression.
• \textit{Nrf2}\textsuperscript{-/−} mice (KO) display a decrease in BDNF protein levels.
• Fluoxetine increased cortical and hippocampal BDNF in wild type and \textit{Nrf2}\textsuperscript{-/−} mice.
• Fluoxetine-induced BDNF increase is \textit{Nrf2} independent.

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ABSTRACT

Several studies have shown that Nrf2, a major redox-sensitive transcription factor involved in the cellular defense against oxidative stress, increases susceptibility to depressive-like behavior. However, little is known about the influence of antidepressant drugs on Nrf2 signaling and expression of its target genes (GCLC, NQO1, HO-1) in the brain. We found that chronic treatment of a mouse model of anxiety/depression (CORT model) with a selective serotonin reuptake inhibitor (SSRI, fluoxetine, 18 mg/kg/day) reversed CORT-induced anxiety/depression-like behavior in mice. Chronic fluoxetine treatment restored CORT-induced decreases in Nrf2 protein levels and its target genes in the cortex and hippocampus. Furthermore, we found that chronic fluoxetine also increased brain derived neurotrophic factor (BDNF) protein levels in cortex and hippocampus of CORT-treated \textit{Nrf2} knockout mice (KO, \textit{Nrf2}\textsuperscript{-/−}). Taken together, these data suggest that Nrf2 signaling contributes to fluoxetine-induced neuroprotection via an unexpected mechanism involving 5-HT transporter SERT blockade, and not through enhancement of BDNF expression.

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1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine are the most commonly prescribed antidepressant drugs for treatment of major depressive episodes. Unfortunately, SSRIs need 4 to 6 weeks to produce therapeutic responses and are only moderately effective since approximately 30% of depressed patients are resistant to antidepressant drugs [1,2]. Thus, a major challenge in the pharmacological treatment of depression is to identify new biomarkers indicating whether or not antidepressant drugs will be effective. Identification of the precise cellular and molecular mechanisms underlying the efficiency and drawbacks of SSRIs may help to offer better treatments to depressed patients.

A possible relationship between inflammation and depression has received considerable attention in recent years. There is evidence that depression is accompanied by an increase in pro-inflammatory cytokines and a reduction in antioxidant defenses [3,4]. Many antidepressant drugs decrease oxidative stress in chronically stressed animals [5,6]. Chronic SSRI treatment may act by enhancing antioxidant defense mechanisms, but the precise cellular and molecular components of these mechanisms are still controversial [7].

The nuclear factor-erythroid 2-related factor 2 (Nrf2), a basic leucine zipper transcription factor, plays a key role in the...
cellular defense against oxidative stress [8]. Chronic inflammation due to loss of Nrf2 leads to a depressive-like phenotype in Nrf2 knockout mice (Nrf2−/−). Furthermore, this depression-like phenotype in Nrf2−/− mice is associated with reduced tissue levels of monoamines in the prefrontal cortex, but not in the hippocampus [9].

Many Nrf2-dependent genes have been identified by microarray analysis [10]. Among them, glutamate-cysteine ligase catalytic subunit (GCLC), NAD(P)H quinone oxidoreductase 1 (NQO1), and hemoxygenase (HO-1) are directly regulated by Nrf2 [11]. Thus, disruption or loss of Nrf2 signaling enhances susceptibility to cellular oxidative stress and to inflammatory injuries [12]. Although recent studies found little evidence that NQO1/GCLC polymorphisms associate with depression, these Nrf2 signaling components could be associated with the response to antidepressant drugs [13,14].

The aim of the present study is to examine whether changes in protein levels of Nrf2 and antioxidant defensive enzymes (GCLC, NQO1, HO-1) in two brain regions involved in mood disorders, the cortex and hippocampus, can be reversed by chronic fluoxetine treatment in a mouse model of anxiety/depression based on the elevation of glucocorticoids (CORT model) [15,16]. The CORT model, which was previously established and characterized by our lab and by others, mimics the effects of chronic stress and induces an anxiety/depression-like phenotype in several behavioral tests and molecular and cellular readouts.

In addition to an anxiety/depression-like phenotype, Nrf2 knockout mice (KO, Nrf2−/−) also display a reduction in brain-derived neurotrophic factor (BDNF) expression in the hippocampus [9]. A potential link between Nrf2 and neurotrophins (such as BDNF) is of interest considering the essential roles of neurotrophins in neuronal plasticity and network connectivity and in mood disorders and their treatments [17]. Thus, we also use Nrf2−/− mice to evaluate whether chronic fluoxetine-induced increases in cortical and hippocampal BDNF levels require the Nrf2-signalling pathway.

2. Materials and Methods

2.1. Animals

Adult male C57BL/6Ntac mice were purchased from Taconic Farms (Lille Skensved, Denmark). All mice were 7–8 weeks old, weighed 23–25 g at the beginning of the treatment, and were maintained on a 12 L:12 D schedule (lights on at 06:00). They were housed in groups of five. Food and water were provided ad libitum. All testing was conducted in compliance with the laboratory animal care guidelines and with protocols approved by the Institutional Animal Care and Use Committee (Council directive # 87-848, October 19, 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permis-87-848, October 19, 1987, Ministère de l’Agriculture et de la Forêt, France). Fluoxetine hydrochloride (160 mg/mL, equivalent to 18 mg/kg/day; Anawa Trading, Zurich, Switzerland) was dissolved in 0.45% hydroxypropyl-β-cyclodextrin, (β-CD), Sigma–Aldrich Saint-Quentin Fallavier, France). Fluoxetine hydrochloride (160 mg/mL, equivalent to 18 mg/kg/day; Anawa Trading, Zurich, Switzerland) was dissolved in 0.45% β-CD/corticosterone solution. Chronic fluoxetine treatment began after 4 weeks of chronic corticosterone administration.

A blunted response of the hypothalamic-pituitary-adrenal axis (HPA) is found in the mouse model of elevated glucocorticoids (CORT model) [15]. The CORT model also displays hallmark characteristics of anxiety/depression. The dose and duration of corticosterone treatment was selected based on previous studies [15,19].

2.3. Behavioral Testing

Each cohort of animals was tested in four different behavioral models of anxiety/depression. Each animal, was successively tested in the open field (OF), elevated plus maze (EPM), novelty suppressed feeding (NSF), and splash test (ST) over the course of one week (see Supplemental material). Behavioral testing occurred during the light phase between 07:00 and 19:00. Behavioral testing was performed after 4 weeks of drug treatment (Supplemental Fig. S1A and S1B).

To address behavioral variability and obtain comprehensive and integrated measures in each group, emotionality-related data were normalized using a Z-score methodology as previously described [20]. In this methodology, behavioral measures are first averaged within each test, then across tests to ensure equal weighting of the four tests in the composition of the final Z-score.

2.4. Sample Preparation

For western blot analysis, mice were sacrificed by cervical dislocation. Cortex and hippocampus were then rapidly dissected. The proteins were extracted in a lysis buffer containing 20 mM Tris (pH 7.4), 137 mM NaCl, 2 mM EDTA (pH 7.4), 0.1% Triton, 25 mM β-glycerophosphate, 1 mM Na3VO4, 2 mM sodium pyrophosphate, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. The homogenates were then centrifuged at 15,000 rpm for 20 min at 4°C.

2.5. Immunoblotting

Equal amounts of denatured proteins were loaded and run on a 10% or 12.5% SDS-PAGE gel and were then transferred onto a PVDF membrane (Amersham Biosciences, Les Ulis, France). Membranes were then incubated with antibodies raised against BDNF at 1:1000 (Santa Cruz, Heidelberg, Germany); Nrf2 at 1:1000 (Santa Cruz, Heidelberg, Germany), GCLC at 1:1000 (Abcam, Cambridge, UK), NQO1 at 1:1000 (Abcam, Cambridge, UK), HO-1 at 1:1000 (Abcam, Cambridge, UK), β-Actin (Santa Cruz Biotechnology, Heidelberg, Germany) was used as a loading control. Immunoreactive bands were detected using appropriate peroxide-conjugated secondary antibodies and a chemiluminescent reagent kit (Pierce Biotechnology) using a ChemiDoc XRS System (Bio-rad, Marnes-La-Coquette, France). Bands were quantified using Image Lab software. The densitometry values were normalized against the β-actin values as described previously [21]. Control conditions were considered to be 100% and experimental variables were normalized with respect to this value.

2.6. Statistical Analysis

All experimental results are presented as the mean ± SEM. Data are analyzed using Prism 6 software (GraphPad La Jolla, USA). Comparisons of behavioral data between groups (Supplemental Fig. S1) were performed using one-way ANOVA followed by Fisher’s PLSD.
Fig. 1. Chronic fluoxetine treatment restores cortical and hippocampal Nrf2 signaling in chronic corticosterone treated mice.

(A and E) Effects of chronic fluoxetine treatment in CORT-treated mice on cortical and hippocampal Nrf2 expression. For each condition, a representative western blot is shown (VV: vehicle/vehicle; CV: corticosterone/vehicle; CF: corticosterone/fluoxetine). Values plotted are mean ± SEM (n = 7–11/group). *p < 0.05, #p < 0.05 versus vehicle/vehicle group and corticosterone/vehicle group, respectively.

(B and F) Effects of chronic fluoxetine treatment in CORT-treated mice on cortical and hippocampal glutamate-cysteine ligase catalytic subunit (GCLC) expression. For each condition, a representative western blot is shown (VV: vehicle/vehicle; CV: corticosterone/vehicle; CF: corticosterone/fluoxetine). Values plotted are mean ± SEM (n = 7–11/group). *p < 0.05, **p < 0.01, #p < 0.05 versus vehicle/vehicle group and corticosterone/vehicle group, respectively.

(C and G) Effects of chronic fluoxetine treatment in CORT-treated mice on cortical and hippocampal G NAD(P)H dehydrogenase quinone1 (NQO1) expression. For each condition, a representative western blot is shown (VV: vehicle/vehicle; CV: corticosterone/vehicle; CF: corticosterone/fluoxetine). Values plotted are mean ± SEM (n = 5–12/group). *p < 0.05, #p < 0.05, ##p < 0.01 versus vehicle/vehicle group and corticosterone/vehicle group, respectively.

(D and H) Effects of chronic fluoxetine treatment in CORT-treated mice on cortical and hippocampal hemoxygenase (HO-1) expression. For each condition, a representative western blot is shown (VV: vehicle/vehicle; CV: corticosterone/vehicle; CF: corticosterone/fluoxetine). Values plotted are mean ± SEM (n = 9–11/group). *p < 0.05, **p < 0.01, #p < 0.05 versus vehicle/vehicle group and corticosterone/vehicle group, respectively.
post hoc analysis. Protein expression levels were analyzed using two subsequent unpaired t tests, assessing first the efficiency of the chronic CORT treatment, then the effect of fluoxetine treatment in animals already treated with chronic CORT. Statistical significance was set at \( P < 0.05 \).

3. Results

3.1. Effects of chronic fluoxetine treatment on cortical and hippocampal Nrf2 signaling in mice chronically treated with corticosterone

Prior to examining the Nrf2 pathway in the CORT model, we confirmed that chronic fluoxetine treatment reversed the corticosterone-induced anxiety-depression-like phenotype in mice (Supplemental Fig. S1). In these animals, we next examined whether changes in cortical and hippocampal protein levels of Nrf2 and antioxidant defensive enzymes (GCLC, NQO1 and HO-1) are affected by chronic corticosterone and/or fluoxetine treatment.

In the cortex, chronic fluoxetine treatment (\( t = 2.76, \ P < 0.05 \)) significantly reversed corticosterone-induced decreases in Nrf2 (29.3%, \( t = 3.46, \ P < 0.05 \); Fig. 1A), GCLC (−22.5%, \( t = 3.43, \ P < 0.05 \); Fig. 1B), NQO1 (−29.3%, \( t = 2.72, \ P < 0.05 \); Fig. 1C), and HO-1 (−42%, \( t = 2.69, \ P < 0.05 \); Fig. 1D) protein expression levels (###\( P < 0.05 \); and ###\( P < 0.01 \) for GCLC, NQO1 and HO-1 in Fig. 1B–D, respectively).

In the hippocampus, Nrf2 protein expression was not decreased by chronic corticosterone treatment (Fig. 1E). However, the protein expression levels of the other components of the Nrf2 signaling pathway were altered in the chronic CORT model (GCLC: 43.4%, \( t = 4.31, **P < 0.01 \); Fig. 1F; NQO1: −62.3%, \( t = 2.95, \ P < 0.05 \); Fig. 1G and HO-1: −43.3%, \( t = 5.37, \ P < 0.05 \); Fig. 1H). Interestingly, chronic fluoxetine treatment corrected chronic corticosterone-induced alterations of all components except for HO-1 (Nrf2: \( t = 3.02, \ P < 0.05 \); Fig. 1E; GCLC: not significant; Fig. 1F; NQO1: \( t = 3.31, ##P < 0.05 \); Fig. 1G; HO-1: \( P > 0.05 \); Fig. 1H).

3.2. Effects of chronic fluoxetine treatment on cortical and hippocampal brain derived neurotrophic factor (BDNF) expression in chronic corticosterone treated wild-type and Nrf2−/− mice

These results indicate that Nrf2 signaling components in the cortex and hippocampus may be involved in the pathophysiology and treatment of anxiety/depression. Since BDNF is a key factor in the pathophysiology and treatment of major depressive episodes [17], we next investigated whether the Nrf2 pathway regulated this neurotrophic factor during the antidepressant response.

First, we found that chronic corticosterone modulated BDNF protein expression in a region-dependent manner in wild-type mice [+21.9%, \( t = 2.35, \ P < 0.05 \) versus Veh/Veh; −33.7%, \( t = 2.01, \ P < 0.05 \) versus Veh/Veh for the cortex (Fig. 2A) and for the hippocampus (Fig. 2B), respectively]. Moreover, chronic fluoxetine treatment further increased cortical BDNF levels (+48.5%, \( t = 2.24, \ #P < 0.05 \) versus CORT/Veh; Fig. 2A), and reversed the chronic corticosterone-induced decrease in hippocampal BDNF levels (+26.2%, \( t = 1.87, \ #P < 0.05 \) versus CORT/Veh; Fig. 2B).

Interestingly, using a mouse model with constitutive Nrf2 deficiency (Nrf2−/− mice), we found that Nrf2 is a regulator of BDNF expression. Cortical and hippocampal BDNF levels were decreased in Nrf2−/− mice [−42.6%, \( t = 3.34, **P < 0.01 \) versus Nrf2 WT/Veh; −23.3%, \( t = 1.76, \ P < 0.05 \) versus Nrf2 WT/Veh for the cortex (Fig. 2C) and hippocampus (Fig. 2D), respectively]. Surprisingly, chronic fluoxetine treatment increased BDNF expression in the cortex (+187.4%, \( t = 8.07, ###P < 0.01 \) versus Nrf2−/−/CORT/Veh; Fig. 2C) and hippocampus (+59.8%, \( t = 4.11, ###P < 0.01 \) versus Nrf2−/−/CORT/Veh; Fig. 2D). These data suggest that chronic fluoxetine treatment of a mouse model of anxiety/depression increases brain BDNF protein expression through an Nrf2-independent signaling pathway.

4. Discussion

Several phenomena have been investigated to better understand the pathophysiology of depression, including aberrations in neurotrophic factors, alterations in neurotransmitter and receptor signaling pathways, disturbances in the HPA axis, inflammation, immune dysfunctions, and imbalances between oxidative stress and antioxidant defenses.

There is now evidence that depression encompasses an inflammatory response associated with cell-mediated immune activation [22,23]. Oxidative stresses contribute to the pathogenesis of depressive disorders and one hypothesis suggests that various antioxidants could have clinical implications as future targets for depression treatments [7]. Nrf2, a transcription factor involved in the cellular defense against oxidative stress, is one potential target of interest for the treatment of major depressive episodes [24]. Recently, a clinical study discovered upregulation of redox-sensitive transcriptional factors (Nrf2 and NF-κB) in peripheral blood mononuclear cells of depressed patients, which is indicative of a pro-oxidative state [25]. However, very few studies have assessed whether Nrf2 activators have antidepressant-like effects in animal models of anxiety/depression [26]. Fluoxetine reportedly acts on neurons and astrocytes in the dentate gyrus of the hippocampus to reverse the expression of genes involved in oxidative stress in a rat model of anxiety/depression, the chronic mild stress (CMS) paradigm [27]. These data suggest that the antidepressant response may involve neuroprotection against the neurotoxic effects of chronic stress. The present findings show that, in a mouse model of anxiety/depression induced by chronic CORT treatment, Nrf2 protein expression levels were decreased in CORT-treated mice and were normalized by chronic fluoxetine administration in two brain regions involved in mood disorders, the cortex and hippocampus. Moreover, we also found changes in levels of cytoprotective proteins related to cellular stress response, such as GCLC, NQO1 and HO-1, following chronic CORT treatment. The levels of these cytoprotective proteins were then increased by chronic fluoxetine administration. For HO-1, chronic fluoxetine reversed CORT-induced decreases in HO-1 levels in the cortex, but not in the hippocampus. These data suggest that HO-1 may be regulated in the hippocampus by transcription factors other than Nrf2 [28]. We also found that HO-1 is expressed in the cortex and hippocampus of Nrf2−/− mice, and that chronic fluoxetine treatment increases HO-1 protein expression in both the cortex and hippocampus of Nrf2−/− mice (data not shown). These results confirm that HO-1 is only partially regulated by Nrf2 [29].

Our findings indicate that fluoxetine reverses the CORT-induced anxiety/depression-like phenotype in mice through a mechanism involving activation of Nrf2 signaling in both the cortex and hippocampus. These results suggest that Nrf2 restores cellular homeostasis and rebalances redox equilibrium, thereby preventing oxidative-stress-related diseases including anxiety/depression [30]. Interestingly, changes in depressive-like behavior were recently described in Nrf2−/− mice, and a subchronic anti-inflammatory treatment (rofecoxib daily for 7 days) reversed the depressive-like behavior [9].

In the present study, we showed that Nrf2, a transcription factor involved in brain inflammation, is regulated by fluoxetine responses in the cortex and hippocampus. Basal cortical and hippocampal expression of BDNF, a neurotrophic factor involved in pathophysiology and treatment of major depressive episodes,
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Fig. 2. Nrf2 regulates basal BDNF expression levels, but not fluoxetine-induced BDNF expression levels. 

(A and B) Effects of chronic fluoxetine treatment in CORT-treated mice on cortical and hippocampal BDNF expression in wild-type control mice. For each condition, a representative western blot is shown (VV: vehicle/vehicle; CV: corticosterone/vehicle; CF: corticosterone/fluoxetine). Values plotted are mean ± SEM (n = 7–8/group). *p < 0.05, #p < 0.05 versus vehicle/vehicle group and corticosterone/vehicle group, respectively. 

(C and D) Effects of chronic fluoxetine treatment in CORT-treated mice on cortical and hippocampal BDNF expression in constitutive Nrf2−/− mice under chronic corticosterone or not. For each condition, a representative western blot is shown (WT/VV: Wild type/vehicle/vehicle; KO/VV: KO Nrf2/vehicle/vehicle; KCV: corticosterone/vehicle; CF: corticosterone/fluoxetine). Values plotted are mean ± SEM (n = 5–14/group). *p < 0.05, **p < 0.01, $$$p < 0.01, $$p < 0.01, ##p < 0.01, versus WT/vehicle/vehicle group, KO/vehicle group and KO/corticosterone/vehicle group, respectively. 

Also regulated by Nrf2. These data suggest that there may be a key relationship between inflammation and depression [3]. 

Decreased BDNF signaling is known to be associated with the pathophysiology of depression, and increased BDNF signaling is associated with the mechanisms underlying the actions of antidepressant drugs [17]. Decreased serum BDNF levels in patients with Major Depressive Disorder are corrected in patients that respond to antidepressant treatment [31,32]. However, the relationship between Nrf2 and BDNF in the brain is not well-characterized. A recent study found a trend for reduced hippocampal BDNF levels in constitutive Nrf2−/− mice [9]. In addition, in the CORT model of anxiety/depression, we found that fluoxetine-induced increases in BDNF levels in the cortex and hippocampus do not require Nrf2. These data suggest that fluoxetine regulates BDNF in these brain regions via an Nrf2-independent mechanism. 

Here, we found a decrease in basal BDNF expression in the cortex and hippocampus in Nrf2−/− mice, supporting the hypothesis that Nrf2 regulates BDNF levels. These findings may reconcile the neurogenic and inflammation hypotheses of depression. These results are also in accordance with recent evidence indicating that Nrf2 regulates adult hippocampal neurogenesis [33]. For instance, Nrf2−/− mice treated with chronic CORT display a robust BDNF
response to fluoxetine in the cortex and hippocampus, suggesting that antidepressant drugs may be effective in Nrf2−/− mice. It also suggests that BDNF regulation in the CORT model is mainly regulated through a Nrf2-independent pathway. We hypothesize that fluoxetine might increase BDNF levels in the cortex through HO-1 in the ischemic rat brain are mediated through activation of the BDNF-TrkB-Pi3K/Akt signaling pathway [34].

In conclusion, these findings based on protein levels analyses in cortex and hippocampus of a mouse model of anxiety/depression suggest that Nrf2 signaling contributes to fluoxetine-induced neuroprotection via a relationship with the 5-HT transporter SERT (inhibited by fluoxetine). However, because fluoxetine increases BDNF expression levels in the cortex and hippocampus of Nrf2−/− mice, there are also Nrf2-independent effects of fluoxetine. Future studies are required to determine whether or not Nrf2 signaling activation increases the efficacy of antidepressants and if Nrf2 is a useful target for future monotherapies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.plantsci.2004.08.011.

References


