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Fluoxetine coupled with zinc in a chronic mild stress model of depression: Providing a reservoir for optimum zinc signaling and neuronal remodeling

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ABSTRACT

Background: Recently, depression has been envisioned as more than an alteration in neurotransmitters centered around receptor signaling pathways. Consequently, the precise mechanisms of selective serotonin reuptake inhibitor (SSRI) antidepressant drugs such as fluoxetine are being revisited. Zinc is a trace element that has been long implicated in the psychopathology and therapy of depression. Zinc has been found to be sequestered and dispensed during stress and inflammation through a family of proteins called metallothioneins (MTs). In addition, MTs are well known for their antioxidant and therefore cytoprotective action. Changes in MTs, their upstream regulators and downstream effectors in response to fluoxetine have not been yet studied. The aim of the present study is to examine whether depression-induced changes in protein levels and mRNA levels of nuclear factor-erythroid 2-related factor 2 (Nrf2), MTs, antioxidant defensive enzyme heme oxygenase (HO-1), zinc-specific receptor GPR39 and brain derived neurotrophic factor (BDNF) in the hippocampus can be reversed by fluoxetine treatment, zinc supplementation or a combination of the two.

Material and methods: The present study investigated the effect of chronic (4 weeks) combined treatment with zinc hydroaspartate (15 mg/kg) and fluoxetine (10 mg/kg) on a chronic mild stress model (CMS) in male Sprague–Dawley rats.

Results: Hippocampal mRNA and protein levels of Nrf2, HO-1, MTs, GPR39 (protein level only) and BDNF were significantly higher in response to a combined therapy of fluoxetine and zinc than to either monotherapy. Additionally, HO-1 and MTs gene expression was correlated with that of Nrf2 in the FLX-only group.

Conclusion: Fluoxetine therapy activated the expression of MTs and HO-1 through an Nrf2-dependent pathway. When FLX was escorted by zinc, activated MTs had a positive impact on BDNF through the zinc signaling receptor GPR39, resulting in general improvement in neuronal plasticity as well as reduction of neuronal atrophy and neuronal cell loss.

1. Introduction

Depression is a costly psychiatric disorder: with only approximately 30% of adults achieving remission using current therapeutic approaches, it represents a massive social and economic burden (Al-Harbi, 2012). Although the most widely accepted theory of the pathophysiology of depression involves alterations in neurotransmitter and receptor signaling pathways (Taylor et al., 2005), depression has been recently envisioned as an inflammatory disorder (Berk et al., 2013). In this perspective, chronic environmental stress is interpreted as a form of psychological insult, eliciting inflammatory responses that may result in brain damage (Berk et al., 2013). The course of brain damage usually involves a hyperoxidative state; to preserve neuronal cells, this state must be tempered by sufficiently elastic neuronal cells supported by antioxidant enzymes and molecules (Hacioglu et al., 2016). Social withdrawal, fatigue, sadness and other symptoms of depression are part of feeling unwell and not having sufficient brain structural integrity to address external stress insults (Dantzer et al., 2008).

Dissatisfaction with conventional treatments for depression suggested addressing the precise mechanism of potential antidepressant drugs (Jindal et al., 2015). Fluoxetine has been the most prescribed agent for depression in many countries and was the first of a group of antidepressant (AD) agents known as selective serotonin reuptake inhibitors (SSRIs) (Guze and Gitlin, 1994). Surprisingly, fluoxetine has remained the clinically preferred antidepressant due to its superior tolerability and safety; however, great concern has been raised regarding its efficacy due to the high rate of non-response, which adds to social and economic burdens (Magni et al., 2013). Until recently, the

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mechanism of fluoxetine action was thought to simply involve binding with high affinity to the serotonin transporter (5-HTT), thus inhibiting reuptake of serotonin (5-HT) and increasing the availability of this chemical, which contributes to feelings of well-being (Guze and Gitlin, 1994). However, over the past several years experimental and clinical data have suggested additional anti-inflammatory, antioxidant and antiapoptotic actions for fluoxetine (Caiaffo et al., 2016). Previously, catecholamines have been linked to hyperlipidemia and inflammation (hsCRP and fibrinogen) in CVD, explaining the depressive phase of CHF (Hamdy et al., 2011). Thus, a major challenge for successful treatment of depression using fluoxetine is to identify the precise cellular and molecular mechanisms underlying its effects and drawbacks in hope of optimizing treatment for depressed patients.

Zinc has been suggested as a possible adjuvant to fluoxetine based on their close antioxidant and neurotrophic profiles (Gower-Winter and Levenson, 2012). Zinc is sequestered and dispensed during stress and inflammation by a small, cysteine-rich family of proteins called metallothioneins (MTs) (Rice et al., 2016). The present study hypothesized that in a model of depression, fluoxetine would upregulate MTs expression, increasing zinc availability, thus indicating additional benefits to a fluoxetine-coupled zinc treatment strategy. As zinc deficiency studies have reduced expression of BDNF and the zinc G-protein coupled receptor 39 (GPR39) (Młyniec et al., 2014), elevated expression of both was used as an indicator of improved zinc availability and successful antidepressant action. GPR39 is an orphan member of the ghrelin receptor family that is expressed in the hippocampus and it initiates MTs metabotrophic signaling following synaptic zinc release (Młyniec et al., 2014). To address this hypothesis, expression of nuclear factor-erythroid 2-related factor 2 (Nrf2), an upstream transcription factor for MTs metallothioneins (Fujie et al., 2016), was examined together with the expression of heme oxygenase (HO-1), an antioxidant enzyme targeted by Nrf2 (Li et al., 2004). Another factor that is thought to be regulated by Nrf2 is brain derived neurotrophic factor (BDNF) (Mendez-David et al., 2015); thus, BDNF was examined based on its essential roles in neuronal plasticity and network connectivity. While several animal models have been developed to study antidepressant drugs, the present study applied a chronic mild stress model (CMS), which is implemented by exposing rats to a series of chronic mild stressors. CMS better reflects human depression, which is characterized more by daily hassles than traumatic events. The CMS model results in a variety of symptoms that parallel many features of human depression and thereby provide strong face validity to this model (Grønli et al., 2005).

The aim of the present study was to examine whether changes in protein and mRNA levels of Nrf2, MTs metallothioneins, antioxidant defensive enzyme (HO-1), zinc-specific receptor GPR39 and BDNF in the hippocampus, a brain region involved in mood disorders, can be reversed by fluoxetine treatment with or without zinc supplementation in a rat model of chronic mild stress.

2. Materials and methods

2.1. Animals

All animal procedures and care were carried out according to the general guidelines of Research Ethics Committee of the Faculty of Medicine of Ain Shams University, which conformed to the guiding principles of the International Council on Harmonization and the Islamic Organization for Medical Sciences, the United States Office for Human Research Protections and the United States Code of Federal Regulations. This committee operates under Federal Wide Assurance No. FWA00006444. Fifty Adult male Sprague-Dawley rats weighing 180–220 g each were utilized. Rats were obtained from the Laboratory Animal Research Center of the Ain Shams University Faculty of Medicine. Rats were kept under appropriate uniform environmental conditions at a temperature of 25 ± 1 °C with alternating 12 h light and dark cycles. They were provided with their daily dietary requirements in the form of standard diet pellets (El-Nasr Chemical Co., Abu Zaabal, Cairo, Egypt) containing not < 20% protein, 5% fiber, 3.5% fat, 6.5% ash and a vitamin mixture. Water was given ad libitum. Rats were housed in stainless-steel cages (three to four per cage) and kept at the animal house for an acclimation period of one week prior to testing.

2.2. Drugs and chemicals

Fluoxetine (10 mg/kg) (Paget and Barnes, 1946) (Amoun Pharmaceutical Company, Cairo, Egypt) and zinc hydroaspartate (15 mg/kg) (Cieslik et al., 2007) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were dissolved in distilled water and given to animals by gavage daily for four weeks.

2.3. Experimental design

The planned study duration was four weeks. Following an initial measurement of sucrose intake (described below) randomly chosen male rats were divided into the control (n = 10) and stress (n = 40) groups. Control rats received distilled water orally for 28 days. The stress group, which was subjected to the CMS procedure, was further subdivided into 4 subgroups (ten animals each). The first subgroup received distilled water orally for 28 days. The other subgroups were treated with the antidepressant FLX (10 mg/kg/day for 28 days by gavage), zinc (15 mg/kg by gavage) or both FLX (10 mg/kg/day by gavage) and zinc (15 mg/kg by gavage) for 28 days. The administration of drugs was initiated concurrently with the CMS procedure.

In all groups except normal controls, depression was induced by adapting the CMS as described by Grønli et al. (2005) and Willner et al. (1987), and some stressors were included from Moreau et al. (e.g., empty bottle of water, restricted food) (Moreau et al., 1992). After the acclimation period of one week, rats assigned to the CMS groups were exposed to chronic unpredictable mild stressors for four weeks. Each week consisted of tail clamping for 1 min, cold water swimming at 4 °C for 5 min, heat stress at 45 °C for 5 min, paired caging for 2 h, a tilted cage (45°) for 3 h, food deprivation (18 h) immediatelsy followed by 1 h of restricted access to food (five micropellets), water deprivation (18 h) immediately followed by 1 h exposure to an empty bottle, a wet cage (200 ml water in 100 g sawdust bedding) for 21 h, and 24 h of reversed light/dark cycle (7:00 a.m. lights off, 7:00 p.m. lights on).

All animals received the same pattern of stressors. All stressors were applied individually and continuously day and night. Their sequence was random to be completely unpredictable to the animal. Immediately following each stress session, the animals were returned to their home cages and maintained under standard conditions until the next stress session. Control animals were left undisturbed in their room and home cages. They were deprived of food and water for 4 h preceding each sucrose test, but otherwise food and water were freely available in the home cage. Twenty-four hours after the last dose of single or combined treatment, the forced swim test (FST) was performed

2.4. Behavioral experiments

2.4.1. Sucrose intake (CMS-induced anhedonia) and body weight

In the present study, we used a sucrose test to assess anhedonia (loss of interest or pleasure in events that usually would be enjoyed). Sucrose intake (1% sucrose solution) was measured once a week, on separate days (days 0, 7, 14, 21, and 28) during a 1-h window after 4 h of food and water deprivation. Consumption was measured by comparing bottle weight before and after the 1-h window. Baseline was measured 1 week before the start of chronic stress. The food and water deprivation period preceding sucrose intake measurement may be considered as a further stress applied on top of the chronic stress protocol. However, control rats were also exposed to the food and water deprivation as a part of the test (Grønli et al., 2005). Body weights were