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In vivo antioxidant status: A putative target of antidepressant action

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ABSTRACT

Oxidative stress is a critical route of damage in various psychological stress-induced disorders, such as depression. Antidepressants are widely prescribed to treat these conditions; however, few animal studies have investigated the effect of these drugs on endogenous antioxidant status in the brain. The present study employed a 21-day chronic regimen of random exposure to restraint stress to induce oxidative stress in brain, and behavioural aberrations, in rodents. The forced swimming (FST) and sucrose preference tests were used to identify depression-like phenotypes, and reversal in these indices indicated the effectiveness of treatment with fluoxetine (FLU; 20 mg/kg/day, p.o.; selective serotonin reuptake inhibitor), imipramine (IMI; 10 mg/kg/day, p.o.; tricyclic antidepressant) and venlafaxine (VEN; 10 mg/kg/day, p.o.; dual serotonin/norepinephrine reuptake inhibitor) following restraint stress. The antioxidant status was investigated in the brain of these animals. The results evidenced a significant recovery in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), glutathione reductase (GR) and glutathione (GSH) levels by antidepressant treatments following a restraint stress-induced decline of these parameters. The severely accumulated lipid peroxidation product malondialdehyde (MDA) and protein carbonyl contents in stressed animals were significantly normalized by antidepressant treatments. The altered oxidative status is implicated in various aspects of cellular function affecting the brain. Thus, it is possible that augmentation of *in vivo* antioxidant defenses could serve as a convergence point for multiple classes of antidepressants as an important mechanism underlying the neuroprotective pharmacological effects of these drugs observed clinically in the treatment of various stress disorders. Consequently, pharmacological modulation of stress-induced oxidative damage as a possible stress-management approach should be an important avenue of further research.

1. Introduction

Chronic exposure to stress can induce several psychiatric conditions, including depression (De Kloet et al., 2005). Alterations in oxidative biology are increasingly being recognized as a critical route of damage toward the pathophysiology of stress-induced psychiatric disorders (Berk, 2007). Increased oxidative stress occurs in major depression, as evidenced by defective plasma antioxidant defenses in conjunction with enhanced lipid peroxidation in these patients (Bilici et al., 2001; Khanzode et al., 2003; Ozcan et al., 2004). Oxidative imbalance, in terms of significantly increased nitric oxide (NO) with a significantly decreased SOD activity has also been reported in bipolar depressive episode, even though NO activity was normalized, SOD remained still decreased than the control value, showing incapacity of coping with oxidative stress (Selek et al., 2008). In stress disorders, oxidative stress triggers or exacerbates several routes of damage such as mitochondrial dysfunction, dysregulation of calcium homeostasis (Amoroso et al., 2000), disruption of energy pathways (Papadopoulos et al., 1997), damage to neuronal precursors, impairment of neurogenesis (Kroemer, 1997) and induction of signalling events in apoptotic cell death (Cregan et al., 2002). These events make a significant contribution towards the resultant disease pathophysiology, as evidenced by atrophy/morphological changes in the brain characteristic in stress-induced depression (Sheline et al., 1996; Cotter et al., 2002) and other stress-related disorders (Koenen et al., 2001). In spite of the vital association of oxidative stress with depression pathophysiology, the role of endogenous antioxidant status in the therapeutic actions of chronic antidepressant treatments has been relatively understudied, although they are widely prescribed for the treatment of stress and stress-related depression and anxiety (Diamond and Rose, 1997).

Some antidepressant drugs have been demonstrated to up-regulate gene expression and activity of the important neuroprotective antioxidant enzyme superoxide dismutase (SOD) (Li et al., 2000; Kolla et al., 2005). However, their effect on important enzymes which function in conjunction with SOD, such as catalase (CAT), was not determined in any of these studies. On the basis of these findings, it is

Abbreviations: CAT, catalase; FLU, fluoxetine; FST, forced swimming test; GSH, glutathione; GR, glutathione reductase; GST, glutathione S-transferase; IMI, imipramine; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; VEN, venlafaxine.

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reasonable to expect protective effects of antidepressants on other antioxidant defenses as well, because several antioxidant mechanisms, alongside SOD, serve to counterbalance the potential deleterious effects of reactive oxygen species (ROS). In support of this hypothesis, we have previously demonstrated that treatment with fluoxetine (selective serotonin reuptake inhibitor) can reverse and prevent psychological stress-induced oxidative damage, as evidenced by the elevation of not only SOD activity, but a wide range of key components of the endogenous antioxidant system following the stress paradigm (Zafir and Banu, 2007). The involvement of major cellular antioxidants such as catalase (CAT), glutathione (GSH) and glutathione *S*-transferase (GST) as a possible target in the action of other antidepressants has not been investigated in details in animal models of stress and depression that can be extrapolated to the human situation. Therefore, the present study was designed to study the pharmacological modulation of oxidative stress by imipramine (IMI; tricyclic antidepressant) and venlafaxine (VEN; dual 5HT/norepinephrine reuptake inhibitor), in comparison to fluoxetine (FLU). The chronic restraint stress model employed in the present study, incorporates both physical and psychological components of stress, and is widely used to induce oxidative and neurotoxic damage. The experimental application of such long-term stress allows investigators to model risk factors for human brain (neuropsychiatric) disorders, particularly the features of depression, for which stress is clinically both a predisposing and contributing factor (De Kloet et al., 2005).

To our knowledge, studies examining an association between the pro-oxidant effects of chronic stress and stress-induced behavioral aberrations are lacking. The forced swimming test (FST) is a well-validated model for experimental depression (Porsolt et al., 1977), widely employed to predict antidepressant efficacy and to determine depression-like behavior in animals after exposure to other stressors (Naitoh et al., 1992; Detke et al., 1997, Takeda et al., 2006). Exposure to restraint stress also produces a marked diminished interest in rewarding stimuli, evidenced by reduced preference for a palatable sucrose solution (Papp et al., 1991; Zurita and Molina, 1999; Rademacher and Hillard, 2007). This represents a disturbance in the ability to experience pleasure, an effect suggested to model human anhedonia, a core symptom of major depression episodes according to DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; American Psychiatric Association, 1994; Willner, 2005). Repeated antidepressant treatments antagonize stress-induced anhedonia (Zurita et al., 1996) and also stress-induced behavioural passivity in the FST (Brotto et al., 2001). If oxidative stress plays any significant role in stress-induced depressive illness, it may be expected to be reversed by chronic antidepressant administration, perhaps in parallel with normalization of behavioural parameters of depression. Therefore, to examine whether induction of experimental depression could be associated with oxidative stress in the central nervous system, and to further test the hypothesis that ascribes augmentation of cellular antioxidant status to antidepressant action, we investigated the reversal of restraint-induced oxidative stress by FLU, IMI and VEN, within the context of a simultaneous restoration of restraint-induced behavioural deficits.

2. Methods

2.1. Animals

Swiss Albino rats weighing 100–125 g were housed in standard laboratory conditions (25±5 °C; 55% humidity) and natural light/dark cycle with free access to standard pellet chow (Ashirwad Industries, Chandigarh, India) and drinking water *ad libitum*. All experimental procedures were carried out within the light period of the light/dark cycle. The experimental protocol was in strict accordance with regulations and prescribed animal ethical procedures outlined by the Institutional Research Committee.

2.2. Experimental protocol

All animals were handled daily for seven days preceding the 21-day treatment period to minimize non-specific stress on experimental days. Animals were randomly assigned to five weight-matched groups (defined below) of six animals each, receiving restraint stress and/or drug treatments, while unstressed, untreated animals constituted the control group and were accustomed to daily handling. Baseline body weights and preference to a 1% sucrose solution were determined immediately prior to experimental manipulations. Subsequently, body weights and sucrose preference (as a hedonic measure) were monitored at weekly intervals throughout the duration of the experiment. Upon termination of the 21-day period, all animals were studied in the forced swimming test. The animals were grouped as follows:

Group I Control
Group II FLU (20 mg/kg/day, p.o., for 21 days)
Group III IMI (10 mg/kg/day, p.o., for 21 days)
Group IV VEN (10 mg/kg/day, p.o., for 21 days)
Group V Restraint stress (4 h daily, for 21 days)
Group VI Restraint stress (4 h/day), followed by FLU (20 mg/kg/day, p.o., for 21 days)
Group VII Restraint stress (4 h/day), followed by IMI (10 mg/kg/day, p.o., for 21 days)
Group VIII Restraint stress (4 h/day), followed by VEN (10 mg/kg/day, p.o., for 21 days)

2.3. Restraint stress

Animals were submitted to stress for 4 h each day at random times during the light period of the light/dark cycle to avoid habituation. Restraint stress was accomplished by immobilizing animals in wire mesh restrainers fitted closely to body size, as described elsewhere (Zafir and Banu, 2007). Animals were deprived of food and water during the entire period of exposure to stress. Subsequently they were released from their enclosure and provided access to water. 30 min post-release the animals received either food or the treatment under study, according to the experimental protocol.

2.4. Drugs

Fluoxetine hydrochloride (20 mg/kg/day; Cadila, India), imipramine hydrochloride (10 mg/kg/day; Nicholas Piramal India Ltd., India) and venlafaxine (10 mg/kg/day; Ranbaxy Laboratories Ltd., India) were dissolved in 0.9% physiological saline and administered via oral route following the daily stress regimen, for 21 consecutive days. Drug solutions were freshly prepared and all doses were selected based on preliminary pilot studies and reported findings (Kulkarni and Dhir, 2007; Zafir and Banu, 2007).

2.5. Forced swimming test

This test included modifications of the Porsolt's procedure (Porsolt et al., 1977; Porsolt et al., 1978). Animals were dropped individually into a glass cylindrical tank (height 40 cm, diameter 20 cm) containing fresh water (25±2 °C) up to a depth of 30 cm and forced to swim for 10 min. This depth of water prevented subjects from supporting themselves by touching the base of the swim tank with their hind paws. After each swim session the tank was thoroughly rinsed in order to remove the presence of any potential alarm substances and the water changed. After testing, each animal was towel-dried and returned to its home cage. Active components of forced-swim behaviour were assessed based on reported descriptions (Lucki, 1997). The behavioural measures scored according to these criteria