Neurochemistry International 63 (2013) 465–475

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Etazolate rescues behavioral deficits in chronic unpredictable mild stress model: Modulation of hypothalamic–pituitary–adrenal axis activity and brain-derived neurotrophic factor level

Ankur Jindal *, Radhakrishnan Mahesh, Shvetank Bhatt

Department of Pharmacy, Birla Institute of Technology & Science, Pilani 333031, Rajasthan, India

ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 28 July 2013
Accepted 15 August 2013
Available online 22 August 2013

Keywords:
Etazolate
Depression
CUMS
HPA axis
BDNF

ABSTRACT

Preliminary study in our laboratory showed that etazolate produced antidepressant- and anxiolytic-like effects in rodent models, however, the ability of etazolate to produce antidepressant- and anxiolytic-like effects and underlying mechanism(s) in chronic unpredictable mild stress (CUMS) model have not been adequately addressed. This study was aimed to investigate the beneficial effects of etazolate on CUMS-induced behavioral deficits (depression- and anxiety-like behaviors). In addition, the possible underlying mechanism(s) of etazolate in CUMS model was also investigated by measuring serum corticosterone (CORT) and brain-derived neurotrophic factor (BDNF) levels. Mice were subjected to a battery of stressors for 28 days. Etazolate (0.5 and 1 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.) were administered during the last 21 days (8–28th) of the CUMS paradigm. The results showed that 4-weeks CUMS produces significant depression-like behavior in tail suspension test (TST) and partial anxiety-like behavior in elevated plus maze (EPM) and open field test (OFT). Stressed mice have also shown a significant high serum CORT and low BDNF level. Chronic treatment with etazolate (0.5 and 1 mg/kg., p.o.) and fluoxetine (20 mg/kg., p.o.) produced significant antidepressant-like behavior in TST (decreased duration of immobility), whereas, partial anxiolytic-like behavior in EPM (increased percentage of open arm entries) and OFT (increased % central ambulation score, total ambulation score and time spent in center zone). In addition, etazolate and fluoxetine treatment significantly ($p < 0.05$) increased the BDNF level and inhibited the hypothalamic–pituitary–adrenocortical (HPA) axis hyperactivity, as evidenced by low serum CORT level in stressed mice. In addition, etazolate and fluoxetine also showed significant antidepressant- and anxiolytic-like effects in normal control mice. In this study no significant changes were observed in locomotor activity in actophotometer test. Moreover, we did not find any effect of etazolate and fluoxetine on CORT and BDNF levels in normal control mice. In conclusion, the results of the present study suggested compelling evidences that etazolate has more marked effect on depression-like behavior in mice, which is atleast in part may be related to their modulating effects on the HPA axis and BDNF level.

1. Introduction

Depression is a recurrent psychiatric disorder often manifested with symptoms at the psychological, behavioral and physiological levels (Nemeroff, 2007; Patten, 2008). World Health Organization has predicted that by the year 2020, major depression disorder will be the second largest contributor to global burden of disease (Manji and Duman, 2001; Nestler et al., 2002; Pitchot et al., 2010), after ischemic heart disease. It is clinically characterized by a depressed mood, anhedonia (reduced interest or pleasure in daily activities), low self-esteem and difficulties in concentrating (American Psychiatric Association, 1994; Hankin, 2006; Perahia et al., 2009). Considerable research has been shown that depression and anxiety are distinct psychiatric disorders with a high comorbidity. Various classes of drugs are currently available for the treatment of depression and anxiety disorders. Selective serotonin reuptake inhibitor (SSRI) class is generally prescribed medication for the treatment of several forms of depression and anxiety disorders (Borsini et al., 2002; Hidalgo and Davidson, 2000). Despite their therapeutic actions, SSRI class agents are associated with a wide variety of side effects such as weight changes, insomnia, drowsiness or sedation, agitation, fatigue, dry mouth, gastrointestinal disturbances, headache and sexual dysfunction (Dording et al., 2002). Hence, the ability of currently available antidepressants to improve daily functioning and productivity is questionable.

The incidences of stressful life events play an important role in the pathophysiology of both depression and anxiety disorders

* Corresponding author. Mobile: +91 8890657646; fax: +91 01596 244183.
E-mail address: kumarjindal26@gmail.com (A. Jindal).

0197-0186/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuint.2013.08.005

(Kendler et al., 1998, 1999). CUMS model of depression has been widely used in antidepressants screening for investigating the pathophysiology of depression and the associated therapeutic interventions (Katz et al., 1981; Garcia et al., 2009). This model was developed in an attempt to resemble a variety of behavioral, neurochemical and neuroimmune alterations, resemble to the human depressive disorder (Holsboer, 2000; McEwen, 2005). Furthermore, recent studies reported that chronic stress also induced anxiety-like behaviors in rodents (Haenisch et al., 2009; Hageman et al., 2009; Regenthal et al., 2009; Huynh et al., 2011).

It is reported that pathophysiology of depression and neurobiology of stress is associated with hyperactivation of HPA axis and alteration in BDNF level (de Kloet et al., 2005; McEwen, 2008). The hyperactivation of HPA axis and alteration of BDNF level implicate in neuronal degeneration, which is a common feature of stress-related psychiatric diseases such as depression and anxiety (Duman et al., 2000; de Kloet et al., 2005; McEwen, 2008; Goshen et al., 2008). The hyperactivation of the HPA axis is characterized by increase level of circulating glucocorticoids (Sapolsky et al., 2000; Murray et al., 2008). High glucocorticoids level leads hippocampal neurodegeneration (Sapolsky et al., 2000; Murray et al., 2008) and induces depressive-like behaviors in rodents and these behaviors, however, is significantly reversed by antidepressant treatments (Johnson et al., 2006; Murray et al., 2008).

Preclinical and clinical studies have indicated that alteration in level/functioning of BDNF is also related to the pathophysiology of depression disorder (Murakami et al., 2005; Castrén et al., 2007) and plays an important role in the actions of antidepressants treatment (Duman and Monteggia, 2006; Calabrese et al., 2009; Kunugi et al., 2010). BDNF, a member of the nerve growth factor family is highly expressed in brain regions such as hippocampus and cortex (Murakami et al., 2005). Post-mortem analyses of brain tissue samples from depression patients showed a decrease BDNF level (Castrén et al., 2007), whereas infusion of BDNF in brain produced antidepressant-like effect in rodents (Siuciak et al., 1997). Moreover, clinical studies reported that depressive patients have a low serum BDNF level as compared to control subjects (Karege et al., 2002, 2005; Aydemir et al., 2006, 2007) and treatment with antidepressants restore the normal level/functioning of BDNF (Shimizu et al., 2003; Başterzi et al., 2009). The functioning of HPA axis and BDNF level therefore, plays an important role in the pathogenesis of stress-related depression and anxiety disorders. As such, they can serve as hormonal/biochemical parameters to evaluate the therapeutic intervention for stress-related disorder.

Phosphodiesterase-4 (PDE4) enzyme hydrolyzes cAMP and implicates in the regulation of mood disorder. PDE4 enzyme inhibitors increase intracellular availability of cAMP and influence the cAMP/CREB/BDNF signaling cascade (Manji and Duman, 2001). Moreover, PDE4 inhibitors implicates in neuronal survival and synaptic plasticity by regulating CREB/BDNF transduction cascade (Houslay, 2001; O'Donnell and Zhang, 2004). Thus, considering the findings mentioned above etazolate, a PDE4 inhibitor (Chasin et al., 1972; Nicholson et al., 1991; Drott et al., 2010), may produce antidepressant- and anxiolytic-like effects in CUMS model. Although, several previous literatures shown that apart from its effect on PDE4 enzyme, etazolate is also an inhibitor of adenosine receptors and modulator of GABA-A receptor (Chasin et al., 1972; Daly et al., 1988; Marcade et al., 2008).

The results on etazolate safety and tolerance profile are encouraging (Vellas et al., 2011). Preclinical studies as well as pharmacokinetic and safety profiles in clinical studies (Phase I and Phase IIb) have established that etazolate is a well-tolerated drug and devoid of major side effects (Drott et al., 2010; Vellas et al., 2011). There were no specific sedative, gastrointestinal, cardiovascular or biological adverse effects observed in the treated groups (Vellas et al., 2011). Moreover, no emesis effect was observed clinically with etazolate treatment, which is main advantage of it over other PDE4 inhibitors. Although, dosing in elderly volunteers had minor adverse effects such as feeling abnormal and balance disorder (see details at:http://www.alzforum.org/dis/tre/drc/detail.asp?id=122).

Several clinical studies have shown that etazolate could be a lead candidate for the treatment of Alzheimer's disease (Drott et al., 2010; Vellas et al., 2011). Etazolate shows neuroprotective properties against beta amyloid (Aβ) peptide toxicity by increasing the release/production of the soluble form of the amyloid precursor protein (sAPPα), an endogenous neuroprotector (Maillet et al., 2003; Marcade et al., 2008). Previous data reported that PDE4 inhibitors like rolipram and others show beneficial effect at counteracting (Aβ)-induced memory alterations by stimulating the cAMP level (Gong et al., 2004; Shrestha et al., 2006). Moreover, cAMP functionally modulates GABA-A receptor (Moss et al., 1992) and has been linked to release/production of the sAPPα (Maillet et al., 2003; Marcade et al., 2008). Recently, we have investigated that etazolate produces antidepressant-like effects in rodent models of depression (Jindal et al., 2012, 2013a). In addition, we have also showed the anxiolytic-like effect of etazolate in animal models, which is in accord with previous studies (Beer et al., 1972; Horovitz et al., 1972; Jindal et al., 2013b). However, the potential beneficial effects of etazolate in CUMS model and underlying mechanism(s) are yet to be explored. Hence, the present study was designed to examine the effect of etazolate on the CUMS-induced behavioral deficits in mice. The possible underlying mechanism(s) of etazolate in CUMS models was also investigated by measuring serum CORT and BDNF levels.

2. Material and methods

2.1. Animals

Behavioral based experiments were carried out using Swiss Albino mice (20–25 g), procured from Hissar Agricultural University, Haryana, India. Animals were kept in polypropylene boxes under standard laboratory conditions (temperature 23 ± 2 °C and room humidity 60 ± 5%) and maintained on 12:12 h light/dark cycle. Standard diet and filtered water were given ad libitum. Experiments on animals were carried out in accordance with the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/14/03).

2.2. Experimental design

Fifty-six mice (eight mice in each group) were divided into seven groups. (1) Non-stressed + vehicle; (2) non-stressed + etazolate (1 mg/kg, p.o.); (3) non-stressed + fluoxetine (20 mg/kg, p.o.); (4) stressed + vehicle; (5) stressed + etazolate (0.5 mg/kg, p.o.); (6) stressed + etazolate (1 mg/kg, p.o.) and (7) stressed + fluoxetine (20 mg/kg, p.o.).

2.3. Schedule for drugs administration and behavioral tests

Etazolate hydrochloride was purchased from the Tocris bioscience, UK. Fluoxetine was procured as a generous gift sample from Glenmark Pharmaceuticals Ltd, India. Etazolate and fluoxetine were freshly prepared before administration. The drugs were administered (between 9:30 and 10:30 a.m) by per oral route once a day during the last 21 days (8–28th days) of the CUMS procedure. The behavioral testing was done at least 15–18 h after the last dose in order to avoid the acute effects of drug treatment. The stressed mice were subjected to behavioral tests on day 29 (SLA), 30 (TST), 31 (EPM) and 32 (OFT) (Supplementary Fig. 1). The doses and duration of etazolate (Jindal et al., 2012, 2013a,b) and fluoxetine (Mao