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Research report

Protective effects of phosphodiesterase 2 inhibitor on depression- and anxiety-like behaviors: Involvement of antioxidant and anti-apoptotic mechanisms

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HIGHLIGHTS

- Bay 60-7550 exhibits antidepressant- and anxiolytic-like effects in mouse behaviors.
- Increased cGMP signaling may contribute to the protective effects of Bay 60-7550.
- Bay 60-7550 is able to antagonize the oxidative damage produced by chronic stress.
- Another possible mechanism of Bay 60-7550's function is its anti-apoptotic effects.

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ABSTRACT

Stress occurs in everyday life, but the relationship between stress and the onset or development of depression/anxiety remains unknown. Increasing evidence suggests that the impairment of antioxidant defense and the neuronal cell death are important in the process of emotional disorders. Chronic stress impairs the homeostasis of antioxidants/oxidation, which results in the aberrant stimulation of the cell cycle proteins where cGMP-PKG signaling is thought to have an inhibitory role. Phosphodiesterase 2 (PDE2) is linked to cGMP-PKG signaling and highly expressed in the limbic brain regions including hippocampus and amygdala, which may play important roles in the treatment of depression and anxiety. To address the possible effects of PDE2 inhibitors on depression-/anxiety-like behaviors and the underlying mechanisms, Bay 60-7550 (0.75, 1.5 and 3 mg/kg, i.p.) was administered 30 min before chronic stress. The results suggested that Bay 60-7550 not only restored the behavioral changes but also regulated Cu/Zn superoxide dismutase (SOD) levels differentially in hippocampus and amygdala, which were increased in the hippocampus while decreased in the amygdala. It was also significant that Bay 60-7550 regulated the abnormalities of pro- and anti-apoptotic components, such as Bax, Caspase 3 and Bcl-2, and the indicator of PKG signaling characterized by $pVASP^{ser239}$, in these two brain regions. The results suggested that Bay 60-7550 is able to alleviate oxidative stress and mediate part of the apoptotic machinery in neuronal cells possibly through SOD-cGMP/PKG-anti-apoptosis signaling and that inhibition of PDE2 may represent a novel therapeutic target for psychiatric disorders, such as depression and anxiety.

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1. Introduction

Modern humans experience different stressors from their daily activities, which once become excessive and prolonged, can cause psychiatric disorders. Depression and anxiety are such illnesses that are chronic, recurring and potentially life-threatening, and have been estimated to affect 31% of the US population [1]. Extensive research has been conducted to reveal multiple neural substrates and mechanisms that contribute to the etiology of depression and anxiety, among which the imbalance between oxidation and antioxidant defense system, as well as apoptotic events that occur among neuronal cells have gained attention.

It is well known that chronic stress induces oxidative stress, possibly through activation of HPA axis followed by overproduction of stress hormones such as glucocorticoids and glutamate, and several inflammatory reactions involving TNF-α and IL-1β [2,3]. Among the various organs, the brain is the most susceptible to oxidative stress due to its relatively high consumption of oxygen, high iron content, fatty acids peroxidation, and low antioxidant capacity [2]. Therefore brain oxidative stress is now known as a key mechanism in the pathology of brain disorders such as depression and anxiety. In fact, several studies have shown that chronic restraint stress was able to remarkably induce oxidative damage to the brain, as evidenced by the increased levels of reactive oxygen species (ROS) and lowered levels of antioxidant components [4,5].

Apoptosis has also been proposed to be a mechanism contributing to stress-related mood disorders both in humans and animal models [6]. Cell death often occurs among certain populations of neurons as a result of chronic stress, in which case antidepressants show the ability to oppose the effects and promote neuroprotection. The apoptotic process is generally controlled by proapoptotic (Bax) and antiapoptotic (Bcl-2) proteins [7]. However, the data concerning the level of antiapoptotic protein Bcl-2 so far are contradictory: some reported a decrease [8] while others reported an increase [9] of Bcl-2 expression after chronic stress.

Increasing evidence indicates that cyclic adenosine monophosphate (cAMP)- or cyclic guanosine monophosphate (cGMP)-mediated signaling appears to participate in neuronal modulation related to depression and anxiety [10,11]. As an enzyme family that mainly hydrolyzes these cyclic nucleotides, phosphodiesterases (PDEs) have been pointed out in several reviews regarding their possible involvement in stress-related emotional/cognitive disorders [11,12], but detailed mechanisms remain to be investigated. Indeed, chronic stress impairs the homeostasis of antioxidants/oxidation, which results in the aberrant stimulation of the cell cycle proteins on which cAMP/cGMP-dependent signaling is thought to have an inhibitory role. Inhibition of PDEs can increase intracellular cAMP and/or cGMP and affect the downstream signaling [12,13]. Some PDE inhibitors, such as those of PDE4 and PDE5, have been known for their neuroprotective effects against emotion and cognitive disorders, but are also known for their side effects [10,14]. PDE2, as a relatively new player in this field, catalyzes both cGMP and cAMP and is found in brain regions such as hippocampus and amygdala that are essential components of the neural circuitry mediating psychiatric disorders [15]. Moreover, PDE2 is also found in the adrenal glands [16], which is an integral part of the HPA axis, thereby making PDE2 a likely candidate in controlling stress-induced emotionality and depressive behaviors [17].

The present study was designed to evaluate the long-term effects of a specific PDE2 inhibitor Bay 60-7550 on chronic unpredictable stress (CUS)-induced depression- and anxiety-like behaviors. To further examine underlying mechanism, possible antioxidant and anti-apoptotic actions of Bay 60-7550 were also investigated.

2. Materials and methods

2.1. Animals

Male ICR mice, 12–16 weeks of age and weighing 25–30 g were used (Harlan, Indianapolis, IN) for all the experiments. Rodent chow and tap water were freely available. Mice were kept in a temperature-controlled room under standard laboratory conditions, with a 12 h light/12 h dark cycle (lights on at 6:00 a.m.). All experiments were carried out according to the "NIH Guide for the Care and Use of Laboratory Animals" (revised 2011) and were approved by the Institutional Animal Care and Use Committee.

2.2. Drugs and treatments

Bay 60-7550 (97% purity) was purchased from Cayman Chemical Company Inc (Chicago, IL), and dissolved in 10% DMSO (Fisher Scientific, Fair Lawn, NJ). The positive control drugs desipramine and diazepam were purchased from Sigma Aldrich (St. Louis, MO). Mice were given Bay 60-7550 at 0.75, 1.5 and 3 mg/kg, desipramine at 10 mg/kg, or diazepam at 1.5 mg/kg, once daily via intraperitoneal injections (i.p.) in a volume of 10 ml/kg body weight 30 min before stress every day. Selection of Bay 60-7550's working dose was based on our previous studies with minor modifications [17,18]. Control animals received vehicle only. Behavioral testing was done 24 h after the last stress event. Hippocampus and amygdala were dissected from brains immediately after behavioral testing and stored at −80 °C until analyses were carried out.

2.3. Chronic unpredictable stress (CUS) procedure

The CUS paradigm exposed mice to two of eight different stressors daily (forced swim, restraint stress, overnight lights, cage tilting, cold stress, food/water deprivation, etc., as shown in Table 1) for ten consecutive days as described previously [19–21]. This protocol has been shown to cause significant changes characteristic of depressive/anxiogenic behavior, as well as a number of associated cellular and neurochemical changes. Control groups also were handled every day but not subjected to the stressors. 24 h after the last stress, each animal was subjected to two behavioral tests in two consecutive days, one each for depression and anxiety behaviors (as shown in Table 1). Brain samples from each mouse were collected after behavioral tests for mRNA or protein level analyses to achieve n = 10.

2.4. Tail suspension test

The tail-suspension test (TST) for depressive behavior in mice was carried out as described previously [22]. Mice were suspended from a stand arm by a 1.9 cm × 15 cm with masking tape loop attached to the end of their tail, leaving the last 1 cm of tail exposed. Mice were observed over a 6-min duration and the time spent immobile during the last 4-min period was recorded.

2.5. Forced swimming test

The forced swimming test (FST) was carried out similarly to that described elsewhere [23]. Briefly, mice were individually placed in glass cylinders (height: 25 cm; diameter: 10 cm; containing 10 cm depth of water at 24 ± 1 °C) for 6 min. A mouse was determined to be immobile when there were only small movements necessary to keep its head above water. The duration of immobility was recorded during the last 4 min of the 6-min testing period.