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Agomelatine-induced modulation of brain-derived neurotrophic factor (BDNF) in the rat hippocampus

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that serves as a survival factor for neurons. Agomelatine is a novel antidepressant as well as a potent agonist of melatonin (MT), MT1 and MT2 receptor types and an antagonist of the serotonin (5HT), 5-HT_{2C} receptor. The study herein established whether treatment with agomelatine alters hippocampal BDNF protein expression under chronic unpredictable mild stress (CUMS) condition. Twenty-one day treatment with agomelatine, fluoxetine or vehicle was assessed in 52 Sprague-Dawley rats undergoing CUMS. Ten naïve control rats were also evaluated after 21 days. The behavioral effects of treatments were studied using the open field test (OFT) on day 0, 7 and 21 and sucrose preference test on day 21. Hippocampal BDNF protein expression was measured using immunohistochemistry. The effect of the interventions on hippocampal neurons was histologically examined after H&E staining. Agomelatine mitigated the reduction in rearing behavior by CUMS in the OFT on day 7 as well as sucrose preference on day 21. The mean optical density value of BDNF was significantly higher in the CUMS + agomelatine group than the CUMS and CUMS + fluoxetine groups. The CUMS + agomelatine group had a significantly higher number of BDNF positive cells compared to naïve controls and CUMS group. Histology showed that hippocampal neurons in the CUMS + agomelatine and CUMS + fluoxetine groups were intact and few of them demonstrated karyopyknosis. Agomelatine–a novel antidepressant, but not fluoxetine, increased hippocampal BDNF level and of BDNF positive neurons in rats subject to CUMS.

1. Introduction

Agomelatine is a novel antidepressant with potent agonism of melatonin (MT), MT1 and MT2 receptor types and antagonism of the serotonin (5HT), 5-HT_{2c} receptor types [1]. Agomelatine mediates the chronobiological activity via melatonin receptors in the suprachiasmatic nucleus of the hypothalamus; the endogenous biological clock [2]. Agomelatine has the ability to synchronize circadian rhythms and to exert antidepressant effects in animal and clinical studies [3]. Previous animal studies demonstrated that the antidepressant action of agomelatine is not entirely mediated via identical mechanisms as other conventional antidepressants.

In humans, stress plays an important role in the development of major depressive disorder (MDD) [4]. In animals, the chronic unpredictable mild stress (CUMS) procedure causes generalized disorganization of circadian rhythms which plays an important role in the pathophysiology of depression [4]. Cognitive dysfunction was observed in CUMS-induced depressive rats [5]. Agomelatine demonstrated antidepressant-like activity in CUMS-induced depressive rats by alleviating anhedonic- and anxiety-like behavior [6]. Højgaard et al. suggested that the antidepressant effect of agomelatine is not mainly via attenuating CUMS-induced circadian rhythm disturbances [6]. Instead, the antidepressant property of agomelatine may be a result of a synergistic action of MT1/MT2 agonism and 5-HT(2C) receptor antagonism via multiple mechanisms proposed to be involving neurogenesis and cell survival, activity-regulated cytoskeleton-associated protein (Arc) and

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glutamate secretion, and brain-derived neurotrophic factors (BDNF) [7,8].

In addition to the potential effects on melatonin and serotonergic systems [9], Rossetti et al. reported that chronic agomelatine treatment prevented activation of the interleukin – 1 beta (IL-1β) and metabotropic glutamatergic receptor (Grm2) genes [10]. In an animal model of depression, agomelatine demonstrated significantly lower serum and brain levels of pro-inflammatory cytokines, interleukin (IL)-1β, as well as brain levels of IL-6 [11]. In 30 adult outpatients with MDD, agomelatine treatment for a duration of 12 weeks is associated with reduced levels of C-reactive protein (CRP) [12]. These findings indicate other therapeutic effects of agomelatine in addition to alleviation of depressive symptoms. The modulatory role of agomelatine in inflammatory response may be explained by its inhibition of NF-κB translocation and alteration of microglia activation and enzyme expressions in the kynurenine pathway [13]. Agomelatine is also efficacious in treating other psychiatric disorders such as bipolar depression, anxiety disorder, seasonal affective disorder, alcohol dependence, etc. [14]. Agomelatine's effect on bipolar depression, seasonal affective disorder, and anxiety disorders may be explained by MT1 and MT2 receptors' sleep-promoting and chronobiotic actions and 5-HT2c receptor blockade [14–16]. The strong anxiolytic effect of agomelatine may be because of its 5-HT2c receptor antagonistic property especially in amygdala and hippocampus [17,18] and melatoninergic receptor activation [19]. Agomelatine treatment was found to also protect rat brain from cerebral ischemia and reperfusion injury by suppressing apoptosis [20]. Furthermore, agomelatine offered protection of structural and neurochemical plasticity in the amygdala [21] as well as inhibiting glutamate efflux in rats exposed to repeated restraint stress [22] and foot-shock stress [23]. In addition to the above neurobiological effects, it was of interest to explore the putative involvement of BDNF in the mechanism of action of agomelatine.

Neurotrophins are a class of proteins that serve as survival factors for neurons [24]. The BDNF belongs to the neurotrophic factor family and plays a crucial role in spatial learning, memory and long-term potentiation [25] as well as synaptic plasticity. Stress reduces the expression of BDNF expression primarily in the hippocampus [26]. The effects of conventional antidepressant, fluoxetine on hippocampal neurogenesis are mediated through 5-HT_{1A} receptors [27] but agomelatine does not act on the 5-HT_{1A} receptors [28]. As a result, the mechanism of agomelatine on hippocampal neurogenesis remains unknown. A previous clinical study showed that two weeks of agomelatine treatment increased the serum levels of BDNF in patients with MDD, but the effect of agomelatine on brain BDNF levels remain to be clarified [24]. A previous animal study using intraperitoneal administration of 40 mg/kg agomelatine demonstrated pro-BDNF expression in the rat brains [26,29]. Further investigation is required to assess the effect of oral agomelatine treatment on BDNF levels in the rat hippocampus at doses for which antidepressant effects of agomelatine has been demonstrated in clinical settings. Molteni et al. demonstrated that acute agomelatine treatment increased expression of BDNF in the rat prefrontal cortex but not the hippocampus after acute (i.e. 16 h) agomelatine treatment [26]. The effect of antidepressant on neurogenesis might depend on the duration of antidepressant treatment. Similarly, acute agomelatine treatment could not achieve resynchronization of circadian rhythm, which is an important property of agomelatine [30]. Based on the above findings, it is important to determine hippocampal BDNF levels in rats exposed to chronic unpredictable mild stress (CUMS) which is a validated animal model of depression. In mice model, the BDNF gene expression levels were downregulated by CUMS but reversed by chronic agomelatine treatment [4]. It is important to study the effects of chronic agomelatine treatment on the hippocampal BDNF levels in the rat model as the rat model has advantages over other murine models largely due to its close physiological similarity to humans, e.g., behavioral and cognitive characteristics [31].

These considerations provided the basis for us to evaluate the effect of agomelatine, fluoxetine, and vehicle in modulating hippocampal BDNF expression under CUMS. Fluoxetine, which acts through the 5-HT1A receptor [32], was used as a positive control of antidepressant. The vehicle group was used to validate the experimental condition. Therefore, we investigated the effect of agomelatine, fluoxetine, and vehicle on depressive-like behaviors in 6-week-old male rats over 21 days. We hypothesized that there would be a significant difference in the hippocampal BDNF levels and the performance of behavioral tasks among rats that received agomelatine, fluoxetine, and vehicle.

2. Experimental procedures

2.1. Animals and study design

A total of 59 healthy male Sprague-Dawley (SD) rats (weighing 200–250 g, 6-week old) were provided from the Animal Experimental Center of Zhejiang University. Animals were adapted to the environment for one week, with a room temperature of 20 ± 3 °C, light/dark cycle of 12 h/12 h and humidity of 40%–70%. Subsequently, the open-field test (OFT) was performed to assess general locomotor activity and depression-like behavior [33]. Seven rats with too high or low baseline stress level (a total score of horizontal and vertical motion lower than 30 points or higher than 120 points) were excluded. The remaining 52 rats were randomized into four groups: the naive control group, the chronic unpredictable mild stress (CUMS) group, the CUMS + fluoxetine group, and the CUMS + agomelatine group. Animals were housed two per ventilated cage with free access to pelleted rodent diet and water ad libitum. All experiments were approved and performed according to the Institutional Guidelines for Animal Care and Use Committee of Zhejiang University.

From Day 1 to Day 21, the 10 rats in the naive control group were raised without CUMS. They were given intragastric gavage of 4.0 ml/kg saline every day. The 14 rats in the CUMS group were subjected to CUMS and intragastric administration of 4.0 ml/kg saline daily. Rats in the CUMS + fluoxetine group (n = 14) and the CUMS + agomelatine group (n = 14) were subjected to CUMS and intragastric administration of fluoxetine (14.4 mg/kg fluoxetine dissolved in 4.0 ml/kg saline) and agomelatine (18.0 mg/kg agomelatine dissolved in 4.0 ml/kg saline) respectively, half an hour before daily stress. The doses of fluoxetine and agomelatine were based on previous studies [11,34]. The antidepressant-like activity of agomelatine in the rat CUMS model of depression is independent of the time of drug administration [1,35]. The design of this study is summarized in Fig. 1.

2.2. The chronic unpredictable mild stress (CUMS) model

The CUMS model was based on the Katz method [36], with slight modifications. Rats in the CUMS, CUMS + fluoxetine, and CUMS + agomelatine groups were exposed to a series of CUMS for 21 consecutive days, including water deprivation for 24 h, fasting for 24 h, tail clamp for 1 min, day-night reversal (12 h/12 h), noise (1500 Hz, 92 dB, 1 h) and physical restraint in a plastic box (25 cm × 7 cm) for 1 h. The CUMS is a validated animal model of depression [37].

2.3. Assessment of animal behaviors

The OFT measures general locomotor activity and anxiety behaviors of animals [38]. Rats were placed in the open field apparatus and freely explored the environment for 5 min. The movements of rats were recorded by the infrared video cameras in open field apparatus and analyzed by the ANY-maze software (Stoelting Co., Wood Dale, IL, U.S.A.). Rearing referred to the behavior when a rat stood on its hind legs in the field and indicated the level of exploratory behavior. Grooming referred to the behavior when a rat licked or scratched itself while it remained stationary in the open field. The OFT was performed on day 0, day 7 and day 21 to dynamically assess the impact of CUMS