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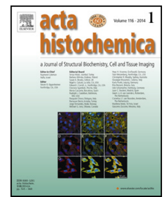

Effect of agomelatine on adult hippocampus apoptosis and neurogenesis using the stress model of rats

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ABSTRACT

Agomelatine (AG) is an agonist of melatonin receptors and an antagonist of the 5-HT_{2C}-receptor subtype. The chronobiotic properties of AG are of significant interest due to the disorganization of internal rhythms, which might play a role in the pathophysiology of depression. The present study was designed to assess the effects of the antidepressant-like activity of AG, a new antidepressant drug, on adult neurogenesis and apoptosis using stress-exposed rat brains. Over the period of 1 week, the rats were exposed to light stress twice a day for 1 h. After a period of 1 week, the rats were given AG treatment at a dose of either 10 mg/kg or 40 mg/kg for 15 days. The animals were then scarified, and the obtained tissue sections were stained with immuno-histochemical anti-BrdU, Caspase-3, and Bcl-2 antibodies. Serum brain-derived neurotrophic factor (BDNF) concentrations were measured biochemically using a BDNF Elisa kit. Biochemical BDNF analysis revealed a high concentration of BDNF in the serum of the stress-exposed group, but the concentrations of BDNF were much lower those of the AG-treated groups. Immuno-histochemical analysis revealed that AG treatment decreased the BrdU-positive and Bcl-2-positive cell densities and increased the Caspase-3-positive cell density in the hippocampus of stress-induced rats as compared to those of the stress group. The results of the study demonstrated that AG treatment ameliorated the hippocampal apoptotic cells and increased hippocampal neurogenesis. These results also strengthen the possible relationship between depression and adult neurogenesis, which must be studied further.

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1. Introduction

Agomelatine (AG) is a clinically effective antidepressant drug with melatonergic (MT1/MT2) agonist and 5-HT_{2C}-receptor antagonist features (Dagyte et al., 2011; Ladurelle et al., 2012). This receptor profile increases the effectiveness of antidepressant and anxiolytic activities (Bertaina-Anglade et al., 2011). In contrast to other antidepressants, AG has no effect on noradrenaline or dopamine. Thus, fewer side effects are observed with the use of AG (Millan et al., 2003; Papp et al., 2003).

Another significant property of AG is its ability to regulate the sleep–wake cycle via its chronobiotic activity. A regular sleep-wake cycle ameliorates synaptic plasticity in the hippocampus and the cerebral cortex. The disruption of synaptic homeostasis, which regulates circadian rhythm, is a symptom that is caused by the pathophysiology of depressive disorders. This emphasizes the importance of circadian rhythm in depression. AG decreases sleep problems and normalizes sleep structure in patients with depression and seasonal mood disorder. These unique features set AG apart from other antidepressants (Bertaina-Anglade et al., 2011; Mairesse et al., 2013; Papp et al., 2003).

Stress is related to many psychiatric conditions, especially depression. Stress models are widely used in animal studies for investigating the neurobiological characterizations of psychiatric disorders and the efficacy of drugs (Dagyte et al., 2011). Various parts of the brain, such as the hypothalamus, frontal cortex, and hippocampus, are affected by stress (Lucca et al., 2009). It has been shown that stress decreases neurogenesis, disrupting

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synaptic plasticity in these regions of the brain. AG improves hippocampal neurogenesis, increasing several cellular signals and the level of several neuroplasticity-associated molecules, such as brain-derived neurotrophic factor (BDNF). Also, AG normalizes stress-induced degeneration in the hippocampus by improving neurogenesis (Dagyte et al., 2011).

Apoptosis and the cell proliferation cycle are vital steps in a cell's life. In experimental studies, apoptotic markers such as Caspase-3 and Bcl-2 antibodies are crucial for detecting the neuro-degeneration. A synthetic analogue of thymidine, 5-bromo-2′-deoxyuridine (BrdU), is also a helpful tool for determining cell-cycle length and evaluating cell proliferation (Matiasova et al., 2014).

The etiology of mood disorders is associated with BDNF, particularly in terms of the neoplastic process and neuronal plasticity related to antidepressant treatment (Calabrese et al., 2011; Martinowich et al., 2003). Especially in the prefrontal cortex, hippocampus, and amygdale (Calabrese et al., 2011, Castren and Rantamaki, 2010), BDNF can be up-regulated with the administration of agomelatine, as in the other groups of antidepressants. However, it has been shown that antidepressant drugs have different influences on the level of BDNF according to specific regions in the brain (Ladurelle et al., 2012). Additionally, BDNF may contribute to circadian rhythms via its receptor, TrkB. In this view, the diurnal influence of agomelatine may be a result of the presence of BDNF (Racagni et al., 2011).

The present study was designed to assess the effects of the antidepressant-like activity of AG on adult neurogenesis and apoptosis based on the stress-induced depression model of the hippocampus of rats.

2. Materials and methods

2.1. Animal housing

Thirty-six female Wistar rats (12 weeks old) weighing 220–250 g were used for this study. The rats were divided randomly into six groups ($n = 6$ in each group): control group (C), stress group (Stress), 10 mg/kg agomelatine-treated group (AG-1), 40 mg/kg agomelatine-treated group (AG-2), 10 mg/kg agomelatine-treated stress group (AG-1 + Stress), and 40 mg/kg agomelatine-treated stress group (AG-2 + Stress). All the procedures were performed in accordance with the protocol approved by the Ethical Committee (for Experimental Animal Care and Use) of the Faculty of Veterinary Sciences at Atatürk University. Throughout the study, pairs of rats were placed in cages in a room with a 12-h daylight/darkness cycle (6 a.m.–6 p.m. light, 6 p.m.–6 a.m. dark) and an ambient temperature of 23 ± 2 °C with 55 ± 10% humidity.

2.1.1. Experimental light stress protocol

During a week-long acclimatization period before the start of the experiment, all rats were weighed and grouped. The light stress that would be applied was brightlight (a 100 W lamp) in aplastic box (10 cm high, 5 cm wide, and 20 cm long, transparent with plastic walls). For adaptation stress, light stress was applied to the rats before the 7 days of AG treatment. After the adaptation period, both light stress and AG treatment were applied to the animals for 1 h twice a day for a period of 1 week. Light stress was applied according to the recommendations of previous studies (Lemaire et al., 2000; Mairesse et al., 2013), though these were slightly altered to reflect the use of adult rats. The stress and experimental protocols are presented in Fig. 1.

2.1.2. AG treatment

After one week of exposure to light stress, the rats began to receive AG treatment at doses of either 10 mg/kg or 40 mg/kg, along

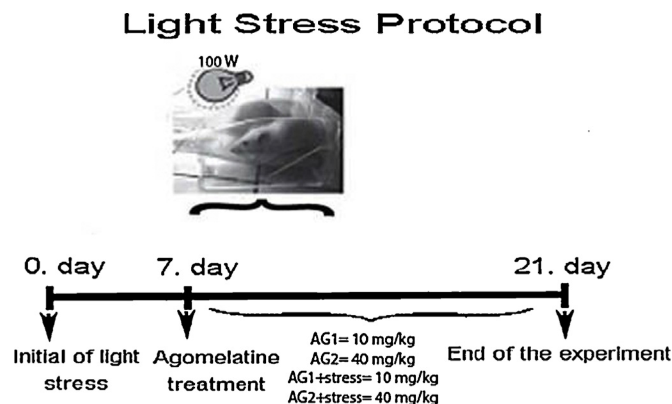

Fig. 1. Light stress protocol of rats.

with light stress in the stress-induced groups, for 15 days. Agomelatine (Sigma Co. USA) dissolved in 1% hydroxylethylcellulose (HEC) was injected intraperitoneally at doses of either 10 mg/kg or 40 mg/kg daily at 6:30 p.m. for 15 days, according to the recommendation of previous studies (Hanoun et al., 2004).

2.1.3. Blood and tissue sampling

Cardiac blood samples were collected from the aortas of all rats after administering anesthesia via an intraperitoneal 10 mg/kg dose of xylazine (Rompun, Bayer, İstanbul, Turkey) and 40 mg/kg dose of ketamine (Ketalar, Pfizer, İstanbul, Turkey). The blood samples were collected into serum tubes and stored at 4 °C after being separated by centrifugation for biochemical BDNF analysis. Afterward, the animals were killed using an injection of 60-mg/kg sodium pentobarbital (Pentothal, Abbott, IL, USA). Brain tissue samples were dissected and removed immediately and fixed in a 10% neutral formaldehyde solution for light microscopic analysis.

2.1.4. Biochemical BDNF analysis

Serum BDNF concentrations were measured using an Elisa device (μ-Quant, BioTek Instruments, Winooski, VT, USA) that was part of a rat-specific Elisa BDNF Immunoassay Kit (Boster Biological Technology, Wuhan, China, Cat no: EK0308). The analyses were performed according to the manufacturer's instructions. The results are expressed as means (pg/ml) ± standard deviation (SD) of the concentration for all groups in Table 1.

2.1.5. Histologic analysis

The fixed tissues were then dehydrated and embedded in paraffin, and serial sections with the thickness of 7 μm were cut using a microtome (*Leica RM2125RT, Leica Microsystems, Wetzlar, Germany*). Subsequently, we obtained a total of 20 serial and 8 randomly selected slides for each animal. For immunohistochemistry, the 8 randomly selected sections were deparaffinized in xylene and dehydrated in descending alcohols, and then antigen retrieval was performed by heating the slides in ethylene diamine tetra acetic acid (EDTA, pH 8.0). Then, for the blocking of endogenous peroxidase activity, the sections were incubated with 3% H_2O_2. Normal bovine serum was used to block nonspecific binding sites of antibodies, and then sections were incubated with anti-caspase-3 (Abcam, England, dilution: 1/50, Cat no: ab4051) and anti-Bcl-2 (Abcam, England, dilution:1/50, Cat no: ab7973) for one hour, respectively. Following this, they were incubated with biotinylated secondary antibody and then streptavidin horseradish peroxidase (Abcam, England, Cat no: ab93677) for 30 min each. To demonstrate the reactions, 3,3′-diaminobenzidine tetrahydrochloride (DAB, Invitrogen, USA, Cat no: 85-9673) was used. Nuclei were stained with Harris's haematoxylin, dehydrated through an ethanol

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