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Research report

# Consequences of long-term treatment with agomelatine on depressive-like behavior and neurobiological abnormalities in pinealectomized rats

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## HIGHLIGHTS

- Agomelatine prevented depressive behavior in pinealectomized rats.
- Agomelatine attenuated enhanced hippocampal 5-HT release in pinealectomized rats.
- Agomelatine restored the negative feedback inhibition of HPA axis.
- Agomelatine exerted a neuroprotection in pinealectomized rats.

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## ABSTRACT

Previous data have shown that the rat model of melatonin deficit can cause a number of neurobiological aberrations. The aim of the present study was to determine whether the antidepressant drug agomelatine, a MT1/MT2 melatoninergic receptor agonist/5-HT2C receptor antagonist is able to prevent some of the behavioral, biochemical and cellular abnormalities induced by pinealectomy. The injection of agomelatine (40 mg/kg, i.p. for 5 weeks)/vehicle started after pinealectomy/sham procedure in Wistar rats. Animals were tested in different behavioral tests for anxiety and depression during the period of agomelatine treatment (chronic effect) and two months later (plastic effect). The effect of agomelatine on KCl-evoked serotonin (5-HT) release from the hippocampus, the activity of the hypothalamic–pituitary–adrenal (HPA) axis and neuronal loss in pinealectomized rats were assessed. Our results showed that agomelatine not only did not prevent the disturbed emotional arousal/anxiety behavior in pinealectomized rats during the treatment but the enhanced motor activity and decreased anxiety state was still observed two months after the discontinuation of treatment. However, the drug corrected a depressive-like behavior (chronic and plastic effect), alleviated the enhanced KCl-evoked 5-HT release in the hippocampus, recovered the suppressed negative feedback inhibition of HPA axis and exerted a neuroprotection in pinealectomized rats. Our findings suggest that pinealectomy can model melancholic depression disorder while the antidepressant action of agomelatine is associated with a correction of 5-HT release in the hippocampus, dysregulated HPA system and neuroprotection in limbic structures.



## 1. Introduction

Although the exact role of melatonin in pathogenesis of depression is uncertain, studies have reported decreased nocturnal and phase-shifted melatonin levels both in animal models and patients with depression [1–4]. A deficiency of melatonin is suggested to underlie the predisposition to melancholic depression characterized by psychopatological and neurobiological disturbances, including agitation, anhedonia, circadian fluctuation of mood, disturbance in sleep physiology, weight loss, increase in plasma cortisol and monoamine oxidase activity [5–7].

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In major depression melatonin per se has no antidepressant action but novel melatoninergic compound agomelatine prescribed as an antidepressant in Europe, possesses potent antidepressant and anxiolytic-like actions both in preclinical models [8,9] and in patients with major depressive disorder (MDD) [10–12]. Unlike melatonin, the underlying mode of action of agomelatine is associated with agonism on melatonergic receptors (MT1 and MT2) and antagonism on 5-HT2C receptor [13,14]. In addition, like melatonin, agomelatine exerts beneficial influence on a disturbed circadian rhythmicity of sleep/wake cycle [15].

It is known that the underlying mechanism of antidepressants such as monoamine oxidase inhibitors, tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs) is mainly associated with an enhancement of synaptic serotonin (5-HT) neurotransmission [16,17]. Although the idea that low serotonin level causes a depressive state [18,19] has been generally accepted, the precise role of 5-HT neurotransmission in depression has not been completely established. Moreover, findings suggesting that 5-HT is elevated in multiple depressive phenotypes require re-examination of the "low serotonin hypothesis" [20,21]. In addition to melatonin system, 5-HTergic afferents from the raphe complex are considered an important modulating component of circadian rhythms [22]. This explains increasing interest in new antidepressants, such as agomelatine and the molecular mechanisms underlying its antidepressant efficacy. Almost all 5-HT in the CNS is synthesized in the dorsal raphe nucleus (DR) [23] from which diffused ascending fibers project to different forebrain regions, including the hippocampus. While melatonin secretion is increased, the release of 5-HT is decreased in the DR at the beginning of the dark phase [24]. Limited data has revealed that exogenously delivered melatonin can influence 5-HT neurotransmission in the DR and other brain areas, including the hippocampus [25,26]. Furthermore, a recent report has shown that the melatonin system can regulate the circadian rhythm of 5-HT secretion [27]. Thus, while exogenous melatonin is dose-dependently able to suppress the 5-HT neurons in the DR during the light phase, pinealectomy can enhance the firing activity of serotoninergic neurons in the second period of the dark phase [27].

Accumulated preclinical and clinical evidence suggests an important role of over-activated hypothalamic–pituitary–adrenal (HPA) axis as a relevant physiological indicator of depression [28,29]. The dysfunction of HPA axis is manifested as hypercortisoism-associated hyperactivity, a lack of diurnal cortisol secretion and disrupted negative feedback mechanism [30]. Some classical antidepressant drugs can alleviate abolished circadian rhythm of cortisol secretion [31,32]. A recent pilot study considering the close link between cortisol and melatonin rhythm of secretion might represent a useful biomarker for detection and prognosis of treatment outcome in MDD [33] Experimental studies revealed that melatonin deficit modeled in pinealectomy can enhance the activity of HPA axis suggesting that melatonin exerts a tonic negative control on corticosterone secretion [34] possibly through functional MT1 receptors in adrenal gland cortex [35]. Synthesized from serotonin mainly in the pineal gland, melatonin is involved in the circadian regulation of endocrine and neuronal signals via MT1 and MT2 receptors, which are widely expressed in the mammalian brain [12].

By using in vivo and in vitro approaches, the present study aimed to characterize the influence of chronic agomelatine administration as well as the long-term consequences after discontinuation of treatment on behavior that are associated with anxiolysis and depression in a model of melatonin deficit produced by pinealectomy. The impact of agomelatine on KCl-evoked 5-HT release from the hippocampus, activity of HPA axis and neuronal loss in pinealectomised rats was also explored. We hypothesized that beneficial behavioral effects of agomelatine in pinealectomy might be related to its positive influence on a disturbed 5-HT release from the hippocampus, on over-activated HPAand neuroprotection in limbic structures.

## 2. Material and methods

The procedures used in this study were in agreement with the European Communities Council Directive 2010/63/EU. The experimental design was approved by the Institutional Ethics Committee at the Institute of Neurobiology, Bulgarian Academy of Sciences.

### 2.1. Subjects

The experiments were performed on male Wistar rats (sixty-day old) obtained from an animal breeding facility of the Institute of Neurobiology, Bulgarian Academy of Sciences. Following arrival in the laboratory, the animals were housed (3–4 per cage) under standardized conditions (20 ± 3 °C, 40–50% humidity; 12/12 h light/dark cycle with lights on at 07:00 a.m.) and habituated for a week. Food and water were available ad libitum throughout the study except during test procedures.

### 2.2. Experimental groups and agomelatine treatment

The study design with treatment groups and procedures are described in Fig. 1. The rats were randomly distributed in four experimental groups ($n = 16$) as follows: Group I: control sham rats treated with vehicle (Sham-veh); Group II: control rats treated with agomelatine (Sham-ago); Group III: pinealectomized rats treated with vehicle (Pin-veh); Group IV: pinealectomized rats treated with agomelatine (Pin-ago).

Agomelatine treatment (kindly gifted by Servier Company, France), suspended in hydroxyethylcellulose (HEC, 1%), initiated 24 h after the surgery procedure, was performed every day at 17:00 h (i.e. two hours before the onset of the dark phase). The drug was injected intraperitoneally (i.p.) at a dose of 40 mg/kg for a period of 5 weeks. This dose has previously been shown to be effective in behavioral and neurobiological tests [36]. The control groups received HEC/agomelatine in the same conditions.

### 2.3. Surgery

The pinealectomy was performed following the method described by Hoffmann and Reiter [37]. In brief, rats were anesthetized (ketamine 40 mg/kg i.p. and xylazine 20 mg/kg i.p.) and fixed in a stereotaxic apparatus. The skull's skin was incised along the suture lambda and using a pointed dental burr, a piece of bone was removed at the juncture of the lambda and the sagittal suture lines. The venous sinus was exposed and then the pineal gland was briefly grasped with fine forceps and removed. The bone disk was returned to its first position. The skin flaps pulled together. In sham-operated rats, the same procedure was used except for the fact that the pineal gland was not removed.

### 2.4. Behavioral tests

Behavioral tests started 4 weeks after the beginning of agomelatine injection. Time interval among the tests was at least two days in the following order: open field (OF), elevated plus maze (EPM), light–dark (LD), saccharin preference test and forced swimming test (FST). Before the behavioural test, the rats were transferred to a sound-attenuated and well-ventilated room between 09:00 a.m. and 10:00 a.m. For the OF and EPM test the behavior was recorded using an infrared sensitive CCD camera and a video tracking system (SMART PanLab software, Harvard Apparatus, USA).