The effects of agomelatine and melatonin on ECoG activity of absence epilepsy model in WAG/Rij rats

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Abstract: The aim of this study was to evaluate the effect of the melatonergic M1 and M2 receptor agonist and serotonergic 5-HT2C receptor antagonist agomelatine on the spike wave discharges (SWDs) seen in electrocorticographic (ECoG) recordings of WAG/Rij rats with absence epilepsy. Twenty-one WAG/Rij male rats were used in this study. Tripolar electrodes were placed on skulls and control ECoG activities were recorded. Experimental groups received normal saline (Group I: 1 mL, intraperitoneally (i.p)), agomelatine (Group II: 40 mg/kg, i.p), and melatonin (Group III: 40 mg/kg, i.p) injections for 7 days. Following this period, 2-h ECoG recordings were repeated. The number of SWDs and their durations were calculated. The total number and duration of SWDs decreased in both the agomelatine and melatonin groups. The systemic administration of agomelatine and melatonin attenuated the genetic absence epilepsy seizures in WAG/Rij rats. The repressive effect of agomelatine on the absence seizures was similar to that of the melatonin used in this study.

Key words: Absence epilepsy, agomelatine, melatonin, seizure, WAG/Rij rat

1. Introduction

Epilepsy, like many other chronic conditions, has complex interactions with social and psychological functioning (Aydın et al., 2013; Agar, 2015). Idiopathic generalized epilepsy, including absence epilepsy (nonconvulsive), has a genetic basis (Sarkisova and van Luijtelaar, 2011). WAG/Rij rats were originally developed as a well-characterized and validated genetic animal model of absence epilepsy (Coenen and van Luijtelaar, 1987).

Pharmacological, biochemical, and neuroendocrinological studies indicate a relationship between different forms of epilepsy, including absence epilepsy, and depression (Trinka et al., 2006; Mula and Schmitz, 2009; Sarkisova et al., 2010; Sarkisova and van Luijtelaar, 2011). Depressive disorders are the most common type of psychiatric comorbidity in patients with epilepsy with a lifetime prevalence of about 30% (Noe et al., 2011). Treatment of depression usually requires long-term drug treatment; therefore, the choice of antidepressant medication must be made more carefully taking into account the possible adverse effects on seizure threshold. The scientific literature shows that most of the classical antidepressant drugs cause seizures in high doses in humans and animals (Dailey and Naritoku, 1996). Sleep disturbance is also prevalent among patients comorbid for epilepsy and depressive disorder, and can independently affect their quality of life (Bazil, 2003).

Several authors have reported altered circadian rhythms in depressive disorders and there are consistent findings about reduced daily secretion of melatonin playing an important role in this process (Robillard et al., 2013; Barylnik et al., 2014). Most of the studies conducted to date indicate the presence of disturbances in melatonin cycles in depressed patients (Beck-Friis, 1985; Rubin et al., 1992). Although a proconvulsant effect of melatonin has been reported in a few studies (Sandyk et al., 1992; Musshoff and Speckmann, 2003; Stewart and Leung, 2005), the majority of experimental and clinical studies show that melatonin has a protective effect against epilepsy in humans (Banach et al, 2011) and in experimental models of epilepsy (Champney et al., 1996; Borowicz et al., 1999; Moezi et al., 2011; Yıldırım et al., 2013). Intraperitoneal administration of melatonin increased the pentylentetrazol (PTZ)-induced seizure...
and electroconvulsive threshold in mice (Borowicz et al., 1999; Moezi et al., 2011). Chronic treatment with melatonin reduced the incidence and mortality of PTZ-induced seizures, whereas acute treatment with melatonin did not affect them in male gerbils (Champney et al., 1996). Moreover, intracerebroventricular injection of melatonin reduced the mean frequency of penicillin-induced epileptiform activity in rats (Yildirim et al., 2006). Agomelatine is a novel antidepressant agent, which is structurally homologous to melatonin. It is a potent MT₁ and MT₂ melatonin receptor agonist as well as a 5-HT2C serotonin receptor antagonist. It was recently approved as an antidepressant medication with comparable efficacy to classical antidepressant drugs (Demyttenaere, 2011). In addition, agomelatine has been shown to resynchronize altered circadian rhythms both in animals (Fucs et al., 2006) and in humans (Leprout et al., 2005). Agomelatine presented anticonvulsant effects on a variety of experimental epilepsy models (Aguiar, 2012; Dastgheib and Moezi, 2014). However, the anticonvulsant effects of agomelatine and the melatonergic system were only studied on convulsive epilepsy models. There is a complete lack of information about effects of agomelatine on WAG/Rij rats, which show spontaneous absence-like seizures. Therefore, the effects of melatonin and agomelatine were investigated on WAG/Rij rats with absence epilepsy in the present study.

2. Materials and method

2.1. Animals

Twenty-one male adult WAG/Rij rats (6 months old) with spontaneous absence epilepsy, weighing 250–300 g, were used in this study. All described procedures were approved by the local ethics committee of Gaziosmanpaşa University (2012/009). Animals were housed in groups of 3 or 4 under environmentally controlled conditions (12-h light/dark cycles at room temperature) and permitted free access to food and water.

2.2. Experimental design

Animals were divided into the following experimental groups:

- **Group I** (control group): Normal saline (NS, 0.9% NaCl w/v) was administered intraperitoneally (i.p.) in a volume of 1 mL for 7 days.
- **Group II** (agomelatine group): Agomelatine at a dose of 40 mg/kg i.p. was administered in a volume of 1 mL for 7 days.
- **Group III** (melatonin group): Melatonin at a dose of 40 mg/kg i.p. was administered in a volume of 1 mL for 7 days.

Each experimental group was composed of seven rats.

2.3. Surgical procedure

All animals were equipped with tripolar electrodes (MS 333/2A) for electrocorticographic (ECoG) recordings.

Before the surgery, they were anesthetized and sedated with ketamine (100 mg/kg, i.p) + xylazine (10 mg/kg, i.p). Rectal temperature was maintained at 37.5 °C by a thermostatically controlled heating blanket (Kozan et al., 2006). Animals were placed in a stereotaxic frame; the skin and subcutaneous tissue were lifted off the bone and folded back. Small burr holes were made into the skull with a drill without damaging the dura and permanent bipolar stainless steel electrodes (0.12 mm diam., Plastic One, Roanoke, VA, USA) were unilaterally implanted in the skulls of animals above the somatosensory cortex and the motor cortex, stereotaxically (Čakil et al., 2011; Arslan et al., 2013, 2014). The stereotaxic coordinates using the bregma as a landmark were 2 mm anterior and 3.5 mm lateral for the frontal electrode, and 6 mm posterior and 4 mm lateral for the occipital electrode (Paxinos and Watson, 1998). A reference electrode was implanted over the cerebellum. Electrodes were fixed to the skull with dental cement. For postsurgery analgesia, rats received a single intramuscular injection of 0.1 mg/kg buprenorphine hydrochloride. After the surgery, animals were housed individually.

2.4. Electrocorticographic recordings and analysis

After 5 days of healing, rats were placed individually in a registration cage (25 × 30 cm in width, 35 cm high) and connected to recording leads. One day before the ECoG recording session, rats were moved to Plexiglas recording cages and allowed to habituate to the recording procedure for 2 h. All ECoG recordings were obtained between 0900 and 1200 hours using AcqKnowledge software (version 3.8) and the MP-150 multichannel physiological analysis system (BIOPAC Systems Inc., Goleta, CA, USA) from free-moving animals in a noise-isolated room. Rats were continuously observed throughout the recording. After the drug administration procedure, rats were connected to recording leads again and after treatment. Seizures were assessed with offline analysis of spike wave discharges (SWDs) on the recordings. Total number and cumulative length of SWDs in the recordings were used for evaluating the seizures.

2.5. Drugs and drug administration

Sterile physiological normal saline, ethanol, ketamine hydrochloride (HCl) and xylazine hydrochloride, melatonin, and agomelatine (Sigma Chemical Co., St. Louis, MO, USA) were used. Melatonin and agomelatine were dissolved in 1% ethyl alcohol. The required doses were administered i.p. in a volume of 1 mL for 7 days. The moderate doses of the drugs were determined in accordance with previous studies (Yildirim and Marangoz, 2006; Dastgheib and Moezi, 2014; Demir Özkay et al., 2015).
2.6. Statistical analysis
Statistical analyses of each parameter were performed using SPSS 15.00. Comparison of ECoG recordings of the same groups before and after drug administration were made by paired-sample t-tests. The results are given as mean ± standard error of mean (SEM). For all statistical tests, P < 0.05 was considered statistically significant.

3. Results
SWD numbers and durations are shown in Figures 1A–1D. In the control group, the total number of SWDs for a 2-h epoch was 58.43 ± 4.24 with a mean total duration of 348.7 ± 33.94 s (Figure 1A). After 1 week of NS administration, the total number of SWDs was 58.86 ± 4.26 and their mean duration was 344.3 ± 40.75 s (Figure 1B). The administration of NS for 7 days did not alter the total number of SWDs (Figure 2) or the mean duration of SWDs (Figure 3) (P > 0.05).

The total number of SWDs was 59.86 ± 5.77 and 34.57 ± 3.40 and their mean durations were 349.9 ± 44.29 and 154.5 ± 17.31 s before and after 1 week of agomelatine injection, respectively (Figure 1C). The total number of SWDs was 60.57 ± 5.84 and 36.29 ± 3.99 and their mean durations were 345.0 ± 49.71 and 169.5 ± 32.08 s before and after 1 week of melatonin injection, respectively (Figure 1D). Both melatonin (40 mg/kg, i.p.) and agomelatine (40 mg/kg, i.p.) significantly decreased the total number (P < 0.05) (Figure 2) and the mean duration (P < 0.05).

![Figure 1](image_url)

**Figure 1.** (A) The total number and duration of SWDs for 2-h epoch were 58.4 ± 4.2 and 348.7 ± 33.9 s in WAG/Rij rats with genetic absence epilepsy, respectively. (B) Administration of normal saline (1 mL, i.p) for 7 days did not alter the total number and duration of SWDs in WAG/Rij rats with genetic absence epilepsy. (C) Administration of agomelatine (40 mg/kg, i.p) for 7 days significantly decreased the total number and duration of SWDs to 34.5 ± 3.4 and 154.5 ± 17.3 s in WAG/Rij rats with genetic absence epilepsy, respectively. (D) Administration of melatonin (40 mg/kg, i.p) for 7 days significantly decreased the total number and duration of SWDs to 36.2 ± 3.9 and 169.5 ± 32.0 s in WAG/Rij rats with genetic absence epilepsy, respectively.
(Figure 3) of SWDs in the ECoG recordings. There were no significant differences regarding the SWD parameters between the melatonin and agomelatine groups (P > 0.05).

4. Discussion
The results of the present study revealed that subchronic and systemic administration of agomelatine doses showed a considerable antiepileptic effect similar to melatonin on absence seizures in WAG/Rij rats.

Most of the evidence has suggested that melatonin has a prominent role in epilepsy. Significant changes were found in day–night melatonin levels during convulsions in patients with intractable epilepsy (Bazil et al., 2000). The level of melatonin dramatically increased following seizures (Bazil et al., 2000). Melatonin has also been shown to exert an anticonvulsant activity in various animal models of seizures (Costa-Lotufo et al., 2002; Yalyn et al., 2006; Yildirim and Marangoz, 2006) and reduced spiking.
activity and seizure frequency in patients with intractable epilepsy (Antón-Tay, 1974). Furthermore, Tchekalarova (2013) demonstrated that melatonin attenuated seizure frequency and protected against various behavioral alterations, memory deficits, and neuronal damage during the chronic epileptic state in a kainate model of temporal lobe epilepsy. Melatonin, at doses of 40 and 80 mg/kg, showed an anticonvulsant effect on PTZ-induced clonic seizure threshold in mice, whereas the melatonin dose of 20 mg/kg did not alter the threshold of clonic seizure (Moezi et al., 2011). In line with previous studies, the moderate dose of melatonin (40 mg/kg) reduced the total number and duration of SWDs in WAG/Rij rats with absence epilepsy in the present study. In contrast, chronic administration of melatonin, at a dose of 10 mg/kg, for 14 days did not affect the frequency of penicillin-induced epileptiform activity in rats (Yildirim et al., 2013). However, pinealectomy reduced latency to the onset of initial epileptiform discharges and significantly increased the frequency of penicillin-induced epileptiform activity in rats (Yildirim et al., 2013). Moreover, Stewart and Leung (2005) suggested that nocturnal activation of hippocampal Mel(1b) receptors depresses GABA(A) receptor function in the hippocampus, resulting in enhanced seizure susceptibility in male rats.

The molecular mechanisms underlying antiepileptic activity of melatonin were suggested as enhancement of norepinephrine secretion through adrenergic receptors (Reiter et al., 2007), reduction of striatal dopaminergic activity through dopaminergic D1 and D2 receptors (Sweis, 2005), downregulation of glutamate secretion through blockage of nitric oxide generation (Munoz Hoyos et al., 1998), and upregulation of GABA release in hippocampus (Stewart and Leung, 2005). However, none of these studies explained the antiepileptic activity of melatonin in absence epilepsy, since absence epilepsy is related to a predominance of inhibitory activity in contrast to convulsive seizures, where an excess of excitatory activity is present (Tolmacheva and van Luijtelaar, 2007; Ngomba et al., 2011; D’Amore et al., 2013; Kovacs et al., 2015). Generation of absence seizures is thought to be associated with excessive thalamic oscillations, due to abnormal intrinsic neuronal properties under the control of an inhibitory GABAergic mechanism (Steriade et al., 1993; Manning et al., 2003; Kovacs et al., 2015). On the other hand, melatonin shows anticonvulsant activity due to its antioxidant, antiexcitotoxic, and free radical-scavenging properties in various experimental epilepsy models and human studies (Florenani et al., 1999; Srivastava et al., 2002; Gupta et al., 2004; Lima et al., 2011; Naziroğlu et al., 2013; Yürük et al., 2015). However, absence seizures do not cause serious excitotoxic damages as seen in convulsive seizures, because of less free radical generation. Interestingly, melatonin was also able to reduce the total number and duration of SWDs of absence epilepsy in this study. Further studies are needed to assess possible molecular mechanisms for these findings.

Subchronic administration of agomelatine between doses of 20 and 75 mg/kg showed antiepileptic effects in different experimental epilepsy models (Aguir et al., 2012; Dastgheib et al., 2014). Agomelatine showed a significant increase in latency to convulsion (at doses of 25 or 50 mg/kg) and also significantly increased the time until death (at doses of 50 or 75 mg/kg) in PTZ-induced and picrotoxin-induced epilepsy, whereas agomelatine caused no significant alterations in latency to convulsions or time until death in the strychnine-, electroshock-, and picrotoxin-induced seizure models (Aguir et al., 2012). Moreover, a single dose of agomelatine (50 or 75 mg/kg) had an anticonvulsant effect on PTZ-induced seizures (Dastgheib and Moezi, 2014). Moderate doses of agomelatine were used (40 mg/kg) in the present study. Agomelatine attenuated both the total number and total duration of SWDs in absence epilepsy of WAG/Rij rats. Although agomelatine is not only a potent melatonin receptor agonist but also acts as a 5-HT2C antagonist, the affinity of agomelatine for the 5-HT2C receptor is in the micromolar range and about 100-fold less than its affinity for melatonin receptors. The results of the present study provide evidence that 5-HT2C antagonism did not provide a synergistic antiepileptic action to the melatonergic system in an absence model of epilepsy, since there was no significant difference between the effects of melatonin and agomelatine used in this study, suggesting the anticonvulsant effect of both substances in the absence model of epilepsy.

Agomelatine is the first antidepressant that was developed based on a hypothesis relating circadian rhythms and depression (Bodinet al., 2010). Absence seizures are also reported to be closely related to mood and especially to sleep time and quality. Though they can only be identified in awake patients, seizure discharges were shown to occur more frequently in sleep (Drinkenburg et al., 2001). Neurotransmitter levels that mediate the epileptic activity are also known to be influenced by circadian cycles (Yehuda and Mostofsky, 1993). A circadian effect of melatonin is attributed to the MT1 and MT2 subtypes of human melatonin receptors, on which agomelatine has agonist effects. Agomelatine also shows a longer half-life and greater affinity for MT1 and MT2 melatonin receptors in different brain areas (Delagrange and Boutin, 2006). Considering the data mentioned above, agomelatine may have a therapeutic effect on sleep disorders induced by absence seizures and may also alleviate epileptic discharges indirectly by improving sleep quality and circadian rhythm.
In conclusion, these data for the first time illustrate that the systemic administration of agomelatine and melatonin attenuated the number and duration of SWDs seen in the ECoG recordings of genetic absence epilepsy seizures in WAG/Rij rats. The repressive effect of agomelatine on the absence seizures was found to be similar to that of melatonin. Agomelatine seems to be recommendable as a potential drug for absence epilepsy and many other complications such as depression and sleep disorders associated with epilepsy.

References


