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Anticonvulsant effects of the selective melatonin receptor agonist ramelteon

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ABSTRACT

Objective: The endogenous hormone melatonin has previously been shown to exert anticonvulsant effects in a variety of experimental models. Accordingly, we asked whether ramelteon, a synthetic and selective melatonin receptor agonist, might also possess anticonvulsant and/or antiepileptogenic properties.

Methods: The effects of ramelteon (30 or 100 mg/kg intraperitoneally twice daily for 5 days) were evaluated in two animal models of epilepsy. In the rat rapid kindling model, baseline hippocampal afterdischarge properties, kindling progression, and hippocampal excitability in kindled animals were measured. Anti-ictogenic efficacy was assessed after acute administration in untreated kindled rats. In the spontaneously epileptic *Kcna1*-null mouse model, we determined seizure frequency and periodicity using continuous video/EEG monitoring over 72 hours. Further, circadian rest–activity rhythms in ramelteon-treated animals were studied with actigraphy.

Results: In kindled animals, ramelteon reversed kindling-induced hippocampal excitability; however, it did not modify baseline afterdischarge properties, the progression and establishment of the kindled state in the rapid kindling model. However, in *Kcna1*-null mice, ramelteon (200 mg/kg/day) significantly attenuated seizure periodicity and frequency and improved circadian rest–activity rhythms compared with control animals.

Conclusions: The selective melatonin receptor agonist ramelteon possesses anticonvulsant properties in a chronic epilepsy model. Our findings provide further support for melatonin receptors being potential novel targets for anticonvulsant drug development.

1. Introduction

Epilepsy remains a continuing health concern despite the increasing availability of newer anticonvulsant drugs. Although seizures in two-thirds of patients can be successfully controlled with these medications, seizures in the remaining third remain refractory to medical therapy [1]. Most anticonvulsant drugs target neuronal membrane-bound ion channels, such as voltage-gated sodium and calcium channels, *N*-Methyl-D-aspartate (NMDA) receptors, and γ-aminobutyric acid type A ($GABA_A$) receptors, and there are many other drugs—both currently available and in various stages of development—that have unknown mechanisms of action. In this context, there is a strong need to develop antiepileptic drugs with novel mechanisms of action.

Melatonin is an endogenous neuroactive compound that plays a critical role in regulating circadian rhythms and sleep–wake cycles [2]. Intriguingly, melatonin has been shown to possess anticonvulsant properties in several experimental models of epilepsy. Specifically, melatonin demonstrates anticonvulsant efficacy against pentylenetetrazol (PTZ)-induced seizures [3] and retards amygdala kindling [4]. Further, exogenous melatonin administration prevents the reduction in seizure threshold in the PTZ model caused by alterations in light–dark cycles [5]. Finally, pinealectomized animals have an increased susceptibility to lithium–pilocarpine-induced seizures compared with controls [6], suggesting that melatonin deficiency lowers seizure threshold. Despite these laboratory observations, there is presently a lack of compelling human data supporting the anticonvulsant properties of melatonin.

Ramelteon is a selective melatonin MT-1 and MT-2 receptor agonist approved in the United States for treatment of insomnia. As such, and given the broad experimental literature on the anticonvulsant effects of melatonin, we asked whether ramelteon might also possess anticonvulsant efficacy. Specifically, we hypothesized that ramelteon might be efficacious in either induced and/or spontaneous models of epilepsy. For our studies, we chose the rapid kindling model of epileptogenesis [7–9], wherein both

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antiepileptogenic and anti-ictogenic effects can be accurately assessed [10,11], and also the *Kcna1*-null mouse model of spontaneous recurrent seizures, which closely models a genetic form of refractory partial-onset epilepsy in humans [12–15].

2. Methods

2.1. Rapid kindling model

2.1.1. Animals

Experiments were performed in male Wistar rats (Charles River, Wilmington, MA, USA), aged Postnatal Days 50 to 60 (P50–P60). All procedures were conducted in accordance with the policies of the National Institutes of Health and the UCLA Office for the Protection of Research Subjects.

2.1.2. Surgery

Animals were anesthetized with isoflurane and stereotaxically implanted with a twisted bipolar stimulating electrode (PlasticsOne, Inc., Roanoke, VA, USA) in the left ventral hippocampus (4.8 mm posterior, 5.3 mm lateral from bregma, 6.5 mm deep from the brain surface) and a tripolar recording electrode (PlasticsOne, diameter 0.23 mm) in the neocortex [9,10].

2.1.3. Studies of antiepileptogenic effects

Three to five days after surgery, the animals were connected to a DS8000 electrical stimulator (World Precision Instruments, Sarasota, FL, USA) and to an MP100/EEG100B acquisition system (BIOPAC, Santa Barbara, CA, USA). Afterdischarge threshold (ADT) and afterdischarge duration (ADD) were measured by applying electrical stimuli to and recording from the hippocampal electrode. Stimulation parameters were: 10-s train, 50-ms peak interval, 1-ms pulse duration, square-wave biphasic waveform, starting at 0.1 mA with 0.05-mA increments delivered every 10 minutes [9,10]. On detection of ADT, animals were injected intraperitoneally with either ramelteon (30 or 100 mg/kg dissolved in dimethylsulfoxide [DMSO]) or DMSO as control treatment. Twenty minutes later, afterdischarge properties were studied again, and the animals were subjected to rapid kindling: 60 stimulations delivered every 5 minutes at the parameters described above, but at 0.05 mA above ADT, as determined after ramelteon or DMSO injection [9,10]. During kindling, electrical activity was recorded from the cortical electrode; animal behavior was recorded using a digital video camera. Twenty-four hours after the last kindling stimulation, hippocampal afterdischarge properties were examined again. ADT and ADD were analyzed before kindling; the stimulations required to reach the first stage 1 seizure [16], the stimulations required to reach the first stage 4 seizure [16], and all stage 4 seizures were counted during kindling; and ADT and ADD were measured after kindling.

2.1.4. Studies of anti-ictogenic effects

These experiments were performed using previously described methods [9,10]. Animals were subjected to rapid kindling as described above, but in the absence of ramelteon treatment; 24 hours after the last kindling stimulation, afterdischarge properties were examined and the animals were injected with ramelteon (30 or 100 mg/kg IP) or DMSO (control). Twenty to thirty minutes after the injections, afterdischarge properties were recorded again. The following parameters were analyzed: ADT, ADD, and behavioral seizures in response to the threshold stimulation.

2.1.5. Analysis

Data were analyzed using Prism 4 Software (GraphPad, San Diego, CA, USA), with repeated-measures ANOVA + Bonferroni post hoc test.

2.2. Kcna1-null mouse model

2.2.1. Animals

Kcna1-null mice were bred at the Barrow Neurological Institute (BNI) vivarium, reared in a quiet, temperature-controlled room, and entrained to a 12-hour light/dark cycle, with lights on at Zeitgeber Time (ZT) 00:00. Tail clips were taken by P7 and sent to Transnetyx Inc. (Cordova, TN, USA) for genotyping. All protocols were conducted in accordance with NIH guidelines and approved by Barrow Institutional Animal Care and Use Committee.

2.2.2. Monitoring and treatment

Mice were individually placed in an $8 \times 8 \times 16$-in. transparent Plexiglas arena (with bedding; food and water provided ad libitum) and allowed to habituate for 3–4 hours. Electroclinical seizures (in *Kcna1*-null mice) and rest–activity patterns (of *Kcna1*-null and wild-type [WT] mice) were monitored for 3–5 days prior to treatment and for 5 days during treatment. During treatment, mice were injected twice daily with ramelteon (30 or 100 mg/kg in DMSO) or DMSO as the control treatment at approximately zeitgeber (ZT)00:00 and ZT10:00.

2.2.3. EEG electrode implantation surgery and seizure scoring

Electrical and behavioral seizures were recorded using Stellate video/EEG technology and Harmonie software (Stellate, Quebec, Canada). Animals were anesthetized with isoflurane (5% induction, 2% maintenance) prior to transmitter implantation. A wireless, PhysioTel telemetry transmitter (Data Sciences International, St. Paul, MN, USA) was implanted in a subcutaneous pocket along the dorsal flank. The biopotential leads were implanted bilaterally on the dura, 2 mm lateral of the midsagittal suture and 1 mm caudal of bregma, with the ground implanted in the occipital bone (the transmitter contained an internal reference electrode). Animals were allowed to recover 3 days prior to seizure and rest–activity monitoring. Behavioral seizures were scored on a modified Racine scale [16] and correlated with EEG interictal and ictal activity. Generalized tonic–clonic seizures typically began with tonic arching and tail extension, followed by forelimb clonus, or rearing and forelimb clonus, then generalized synchronous forelimb and hindlimb clonus, after which there was postictal depression. Time of onset and severity were recorded for each seizure. Number of seizures observed during each hour was subsequently collapsed into 4-hour ZT time bins—ZT 00:00; ZT 04:00; ZT 08:00; ZT 12:00; ZT 16:00; ZT 20:00—for each animal.

2.2.4. Actigraphy

Actigraphy is a noninvasive method of monitoring human sleep–wake and animal rest–activity cycles. Behavioral rest–activity cycles were assessed using the Vital View data acquisition system, which integrates radio telemetry technology and switch-closure activity monitoring (Mini Mitter Company, Inc; Bend, OR, USA). The activity was monitored in 3-minute epochs and scored on an activity scale of 0–50. Data were analyzed with ActiView Biological Rhythm Analysis software (Mini Mitter Co., Inc.). The time of peak activity was determined by the maximum value of a fitted cosine function. A χ^2 periodogram method was used to determine the length of the rest–activity (nocturnal) period (length of the average rest–activity cycle or oscillation in hours) in all groups of animals.