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Effects of Agomelatine and Lacosamide on Kainate-Induced Status Epilepticus, Epileptogenesis and EEG Seizure Activity in Wistar Rat

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The antidepressant Agomelatine (Ago), unlike to classical antidepressants, possesses an unique receptor profile by activation of MT1 and MT2 receptors and antagonism on 5-HT2C receptor. The aim of the present study was to explore the effect of Ago on kainate (KA)-induced status epilepticus (SE) and chronic epilepsy in Wistar rats. Repeated i.p. injection with Ago (40 mg/kg) at the 1st, 6th, 24th, 32th, 48th h after KA neither alleviated the number of paroxysmal events and their duration (electrographic seizures) during SE nor EEG and behavioral spontaneous seizures during the chronic phase of epilepsy. The positive control with lacozamide (LCM) (50 mg/kg) significantly alleviated the SE-induced epileptiform activity. The present results revealed that Ago is unable to prevent SE and is ineffective against EEG registered spontaneous seizures.

Key words: status epilepticus; EEG; agomelatine; lacosamide; BDNF/TrkB

Introduction

The newly developed antidepressant Agomelatine (Ago) is characterized with a unique receptor profile by activation of MT1 and MT2 receptors and antagonism on 5-HT2C receptor that give it an advantage compared to serotonin reuptake inhibitors (SSRIs) to possess chronobiotic activity both in rodents and human [7]. Several previous studies reported that Ago has anticonvulsant effects after acute injection in seizure tests on non-epileptic naïve rodents [1, 4]. However, this antidepressant was able to suppress seizures after single injection but not after chronic treatment due to receptor internalization [4]. Our research team for the first time reported that chronic treatment with Ago after kainate (KA) induced status epilepticus exerts a strong neuroprotection in limbic structures but was ineffective against epileptogenesis and concomitant spontaneous seizures [10]. Moreover, Ago alleviated the comorbid depression through suppression of inflammatory signaling (IL-1β and gliosis) during the chronic epileptic phase in rats.

Lacosamide (LCM), approved by EMEA and FDA, belongs to a new class of antiepileptic drugs (AED) and is used in patients with partial seizures as an adjuvant [2]. This drug possesses different mechanism of action, with sodium channels' modulation, compared to other anticonvulsants such as Lamotrigine, Carbazepine, Phenytoine. The anticonvulsant effects of LCM are confirmed in experimental models of epilepsy [2, 3, 8]. LCM suppressed sound-induced seizures, generalized tonic-clonic seizures, tonic-extension seizures induced by maximal electroshock seizure (MES) test as well as 6-Hz psychomotor seizures in mice and rats [2]. Moreover, LCM is effective in hippocampal kindling model of epilepsy [3] and against SE [8].

In the present study, we aimed further to explore the effect of Ago against SE and spontaneous epileptiform activity recorded during the acute and chronic epileptic phase in KA model of temporal lobe epilepsy. The same protocol of treatment was applied for LCM considered as a positive control.

Materials and Methods

The procedures used in this study are in agreement with the European Communities Council Directives of 24 November 1986 (86/609/EEC). The experimental design was approved by BFSA (contract # D-65/02.05.2017).

Animals

Male Wistar rats (breeding facility of the Institute of Neurobiology, Bulgarian Academy of Sciences, 200-250 g at the beginning of experimental procedure) were housed in groups (4-5 per cage) for habituation a week prior to experiments. After SE, they were housed individually in transparent labeled cages. Standard conditions (20±3°C, 40-50% humidity; 12/12-h light/dark cycle with lights on at 06:00 a.m.) were freely available.

Electrode implantation

Rats were anesthetized with a mix of ketamine (80 mg/kg) and xylazine (20 mg/kg), i.p. and a local anesthesia with procaine 0.5%. Implantation of electrodes was performed as described previously [9]. Electrodes were placed, according to the atlas of Paxinos and Watson, above the frontal (A = +1, L = ±2) and parietal cortex (A = - 4.2, L = ± 3.0), and the reference and the ground electrodes, above the nasal bone with a stainless steel screw attached at the end. The electrode assembly was connected to a six-plug female connector (Plastic One MS363/E363/0) which was fixed to the skull with dental acrylic. The rats were injected intramuscularly (i.m.) with antibiotic to prevent infection and allowed to recover for at least of 7-10 days.

Induction of status epilepticus (SE) and EEG/video recording and analysis

After at least of 30 min baseline EEG was obtained, SE was induced by i.p. injection of kainic acid (KA) (10 mg/kg, diluted in sterile saline, Abcam, UK). Seizure severity was evaluated by a modified Racine's scale as described previously [9]. Electrographic activity of SE and chronic epileptic phase was recorded for 12 h. Ictal events were calculated manually off-line through inspections of the files using Acknowledge software ACK100W (BIOPAC Inc., USA). Seizure activity during SE was assessed by counting the number, total duration and percent duration of paroxysmal events that lasted at least 5 s, had a frequency (≥8 Hz) and amplitude ≥two time baseline as described previously [5]. This data were analyzed separately i) before treatment (i.e. 0-1 h after beginning of SE) and ii) after the 1st treatment (2-6 h after SE).

For characterization of EEG activity during the chronic phase, the following indices were quantified: the number and total duration of "spike-trains" (<5 s and >20 s) and paroxysmal events (<20 s) as described previously [8].

A video monitoring system (infrared-sensitive colored cameras S-2016, AVTECH, Taiwan, no. AVC307R connected to a computer with software analyzing video-records) was used for continuous recording (24 hr/day for four months) to detect spontaneous behavioral seizures of class IV-V. Partial seizures of class I and II from video-recording were neglected from final analysis.

Experimental design and drug administration

The rats received i.p. injection of vehicle (veh), agomelatine (Ago) (40 mg/kg, dissolved in hydroxyethyl cellulose (HEC)1%, kindly gifted by Servier Company, France) or lacozamide (LCM) (50 mg/kg) at the 1st, 6th, 24th, 32th, 48th h after injection of KA. The drug doses were chosen on the doses used in previous reports in rats [9, 10, 11].

Statistical analysis

All dataare presented as mean±S.E.M. Statistical analyses were performed using Sigma Stat software. Nonparametric statistics were used in all experiments because the distribution of values was not normal due to small number of animals in groups. Data were analyzed using Kruskal-Wallis test followed by Mann-Whitney post hoc test. Statistical analysis was performed with SigmaStat® 11.0. A $p<0.05$ was accepted as statistically significant.

Results

Effect of agomelatine and lacosamide on the severity of status epilepticus

The electrographic data during SE are presented in Table 1. Following systemic administration of KA at a convulsant dose of 10 mg/kg, rats presented continuous scratching, mastication and staring occurring during the first 10 min, which were not associated with obvious changes in the cortical EEG. The electrographic onset of SE that occurred within the following 30-40 min was associated with behavior of class III and IV. There was no difference among the three groups in the number of paroxysmal events and their duration during the 1st h after KA injection. However, unlike the LCM group that had a lower number and shorter duration of paroxysmal events compared to vehicle group, a tendency for increase in paroxysmal events was observed in the Ago group compared to vehicle group after the 1st injection of Ago without reaching significance (**Table 1**). After the second injection, the LCM group was characterized with significantly lower duration of paroxysmal events than vehicle-treated group while the Ago-treated group showed a tendency of higher number in paroxysmal events.

Effect of repeated treatment with agomelatine and lacosamide on spontaneous epileptiform activity (spontaneous electrographic and behavioral seizures) during the chronic phase of epilepsy

All rats treated with either vehicle or agomelatine during SE developed epilepsy with appearance of electrographic and behavioral spontaneous seizures. No difference in the total number of behavioral motor seizures was detected between KA-veh and KA-Ago group four months after SE **(Fig. 1).** Three out of five rats (60%) treated repeatedly with LCM during SE did not exhibited behavioral motor seizures registered during continu-

ous 24-h video-monitoring until the fourth month after SE while two rats in LCM group exhibited lower number of spontaneous behavioral seizures than KA-veh group. However, no difference between the vehicle and Ago group in the number of paroxysmal events and their duration (electrographic seizures) and behavioral seizures was detected **(Fig. 1, Table 2)**. The EEG of LCM group was characterized with occasional paroxysmal events, with significantly decreased number of spike trains and seizures compared to vehicle group four months after SE **(Fig.1, Table 2)**.

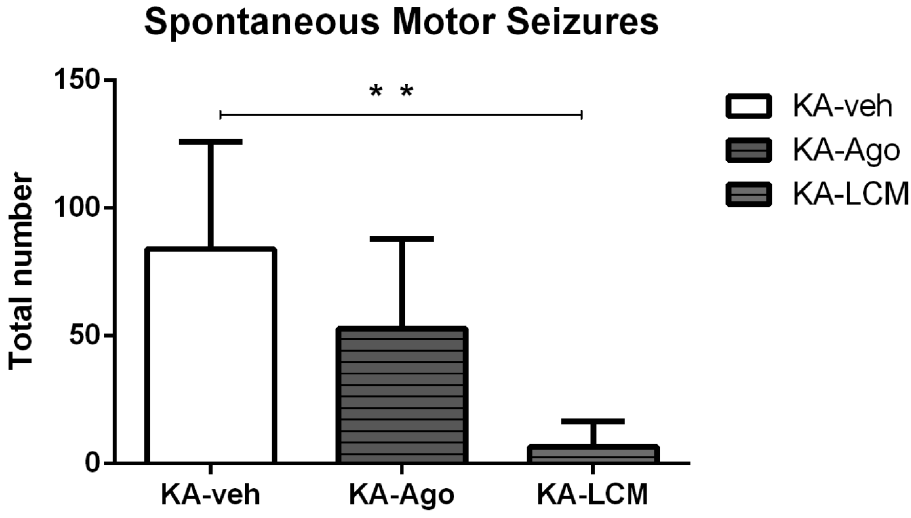

Fig. 1. Vertical bar chart shows the total number of behavioral motor seizures between KA-veh (KA group treated with vehicle), KA-Ago (KA group treated with agomelatine) and KA-LCM (KA group treated with lacosamide) group four months after SE.Data are presented as mean±S.E.M.**p<0.01 compared to the vehicle group(Mann-Whitney test).

Table 1. Characteristics of status epilepticus in different treatment groups. Data are presented as mean±S.E.M. Number of animals is given in parenthesis. *p<0.05 compared to the vehicle group (Normality test).

Group	No. of paroxysmal events		Duration of paroxysmal events (sec)	
	Before treatment (0-1 h)	After the 1st treatment (2-6 h)	Before treatment (0-1 h)	After the 1st treatment (2-6 h)
Vehicle (n=5)	39.3± 7.5	56.8±10.0	3079.3±815.9	6728.5±131.7
Agomelatine (n=5)	36.5±13.5	98.8±15.8	2047.8±306.0	7666.8±275.9
Lacosamide (n=5)	33.0± 8.5	26.5±2.4*	1541.5±388.0	180.0±478.0*

Table 2. Cortical paroxysmal activity in different treatment groups four months after kainate-induced status epilepticus. Data are presented as mean±S.E.M. Number of animals is given in parenthesis. *p<0.05 compared to the vehicle group (Mann-Whitney test).

Group/Rat	Chronic phase			
	No. of paroxysmal events (sec)	No. of spike-trains	No. of seizures	Duration of paroxysmal events (sec)
Vehicle (n=5)	155±27	36.0±10	112±9.7	7503±1154
Agomelatine (n=5)	119±17	88.7±17	110±6.7	6773± 154
Lacosamide (n=5)	81±13*	14.5± 3*	75±17	6344±1583

Discussion

The results of this study are in accordance with our previous work revealing that chronic Ago treatment after KA-induced SE is unable to mitigate the development of epileptogenesis and onset of spontaneous seizures (EEG and video recorded) in the same model of TLE in rats. Moreover, recently we have found that Ago exacerbate the beginning of the chronic phase through decrease of the latent seizure-free period and increase of the frequency of behavioral seizures in the first month after SE. In the present study, we applied another protocol with repeated treatment of this drug in short intervals simultaneously with appearance of SE. The positive control LCZ confirmed results from other experimental studies in rodents [11] confirming that this drug is effective against SE and epileptiform activity during the chronic phase of epilepsy. Numerous preclinical studies, including our results suggest that melatonin is effective against seizure activity in both seizure tests and models of epilepsy [10]. The role of MT1 melatonin receptor in the anticonvulsant effect of melatonin is proposed. Experimental data focused on the role of 5-HT2C receptors in seizure susceptibility are ambiguous [6]. Therefore, it can be speculated that the effects of Ago in the present study might be mediated by antagonism on 5-HT2Creceptors.

Conclusion

To summarize, in the KA-induced SE model Ago is ineffective against development of SE and concomitant spontaneous epileptiform activity compared to the positive control with LCM detected by continuous EEG and video recording. Future studies are required to elucidate the role of MT and 5-HT2Creceptors in its effect.

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