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Research report

Anticonvulsant activity of melatonin, but not melatonin receptor agonists Neu-P11 and Neu-P67, in mice

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HIGHLIGHTS

- Neu-P11 and Neu-P67 have a prolonged duration of action and oral availability, compared to MLT.
- MLT produced potent anticonvulsant effect in 6 Hz, MEST and PTZ tests.
- In contrast, neither Neu-P11 nor Neu-P67 affected the seizure threshold in 6 Hz, MEST and PTZ tests.
- Changes observed in the locomotor activity suggest the interaction of Neu-P11 and Neu-P67 in the CNS that is independent of MT.
- Effects of MLT have yet to be explored; our studies support its potential as an anticonvulsant therapeutic.

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ABSTRACT

The anticonvulsant activity of melatonin (MLT) have been tested in several *in vivo* models and against different convulsive stimuli. Although MLT exerts high affinity towards melatonin receptors (MTs), the potential usefulness in the treatment of epilepsy is limited mainly due to its short half-life. Therefore, the purpose of the present study was to compare the anticonvulsant properties of novel MT agonists Neu-P11 and Neu-P67 with MLT in mice. The anticonvulsant activity of tested compounds was evaluated in pentylenetetrazole-(PTZ) and electrically-induced convulsions. The effect of studied compounds on motor coordination and skeletal muscular strength in mice was assessed in the chimney test and grip test, respectively. The locomotor activity after administration of the tested compounds was also evaluated. In the MEST and 6 Hz tests, only MLT (50 and 100 mg/kg, i.p.) significantly increased the seizure threshold. The i.p. administration of MLT (100 mg/kg) and Neu-P67 (200 mg/kg) resulted in a significantly elevated PTZ seizure threshold for forelimbs tonus. The compounds did not affect muscle strength. No alterations in motor coordination were noted. However, the locomotor activity was significantly decreased after administration of all tested compounds. Our study confirms the anticonvulsant potency of MLT and shows that novel synthetic MT agonists Neu-P11 and Neu-P67 have no effect on epileptic seizures in mice. Our data suggest that the activation of MT can be used in the treatment of seizures, but further pharmacological characterization is needed to understand the anticonvulsant activity of MLT and to design efficient MT-targeting antiepileptic drugs.

Abbreviations: 5-HT, serotonin; ANOVA, analysis of variance; CC_{50}, convulsive current inducing seizure response in 50% of mice; CNS, central nervous system; DMSO, dimethyl sulfoxide; GABA, γ-aminobutyric acid; GI, gastrointestinal; i.p., intraperitoneally; i.v., intravenously; mCPP, m-chlorophenylpiperazine; MEST, maximal electroshock seizure threshold; MLT, melatonin; MT, melatonin receptor; N, newtons; NMDA, *N*-methyl-D-aspartic acid; NO, nitric oxide; PTZ, pentylenetetrazole; SEM, standard error of the mean; TFMPP, trifluoromethyl-phenylpiperazine.

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1. Introduction

Melatonin (MLT) is an indole derivative of tryptophan synthesized primarily by parenchymatous cells in the pineal gland. As a secondary source, MLT is also secreted by the gastrointestinal (GI) tract, skin, retina, bone marrow, salivary glands, platelets, and epithelial hair follicles [11]. MLT displays its physiological function as a regulator of circadian rhythms and sleep-wake cycle in humans by interacting with two types of G protein-coupled receptors, namely melatonin receptor (MT)1 and MT2 [29,31,39,40]. Both receptors are widely distributed in various tissues, such as suprachiasmatic nuclei of the hypothalamus, cerebellum, central dopaminergic pathways, coronary blood vessels and aorta, liver, kidney and gallbladder [9]. Treatment with MLT can improve circadian rhythm sleep disorder and insomnia, seasonal affective disorders, GI diseases (for review see Ref. [10]), cancer and cardiovascular diseases [9]. More recently, MLT has been considered to be effective as an add-on option for epilepsy [1,4].

Epilepsy is a common chronic neurological disease associated with various comorbidities, including sleep disorders, depression and anxiety. MLT concentration in the serum of epileptic or febrile epileptic children is lower compared to control group [18], what suggests that MLT may be involved in the pathophysiology of the disease. However, successful treatment of epilepsy with MLT is not easily achieved; the major obstacle is its rapid elimination from the circulation, as the half-life of MLT ranges from 30 min to 45 min in the blood [7]. Various factors affect MLT bioavailability, including uptake and first-pass hepatic metabolism.

Two novel MT agonists, Neu-P11 [piromelatine, *N*-(2-(5-methoxy-1H-indol-3-yl)ethyl)-4-oxo-4H-pyran-2-carboxamide] and Neu-P67 possess high affinity at MTs and exert prolonged duration of action compared to MLT. Neu-P11 is a melatonin and serotonin (5-$HT_{1A/1D}$) receptor agonist, and 5-HT_{2B} receptor antagonist [24]; Neu-P11 displays γ-aminobutyric acid (GABA) enhancing properties, but it does not directly interact with GABA receptors. Neu-P11 exhibits a multimodal effect: antineurodegenerative, antidepressant, anxiolytic [44], antidiabetic [36], antihypertensive [20] and antinociceptive [9]. More recently, Neu-P11 has demonstrated its usefulness in myocardial ischemia-reoxygenation injury in *in vitro* models [50], as well as in improving sleep pattern [37] and insulin sensitivity [36]. At present, Neu-P11 is in Phase II Clinical Trial (https://ClinicalTrials.gov Identifier: NCT02615002) for the treatment of Alzheimer's Disease. Neu-P67, in turn, acts not only at MTs but also exerts an agonistic effect at the 5-HT_{1A} and 5-HT_7 site (unpublished data). Both MTs analogs display long half-life and oral availability.

The present study was conducted to investigate the effect of Neu-P11 and Neu-P67 on the seizure threshold in three acute seizure tests in mice. Moreover, acute side effects of each compound were evaluated in neuromuscular strength, motor coordination and locomotor activity tests. To further verify the anticonvulsant potential of Neu-P11 and Neu-P67, and allow for direct comparisons, MLT was included in the study and tested in comparable manner with both compounds.

2. Materials and methods

2.1. Animals

Experimentally naïve male albino Swiss mice (Laboratory Animals Breeding, Słaboszów, Poland) weighing 22–30 g were used in all experiments. The total number of animals used in the study was 560. The animals were housed in Makrolon cages under controlled laboratory conditions (22–23 °C, relative humidity, 45–55%, 12 h light/dark cycle, lights on at 6:00 a.m.). A nutritionally-balanced rodent chow pellets (Agropol S.J., Motycz, Poland) and tap water were available *ad libitum*. To minimize circadian influences, experiments were performed between 8:00 and 16:00 h, after at least 7 days of acclimatization. Each animal was used only once. The experimental protocol followed the European Communities Council Directive of September 22, 2010 (2010/63/EU) and Polish legislation acts concerning animal experimentations and was approved by the Local Ethics Committee at the Medical University of Lublin (license numbers 37/2013 and 3/2014).

2.2. Drug administration

Neu-P11 and Neu-P67 were obtained from Neurim Pharmaceuticals Ltd., Israel. Melatonin was purchased from Tocris Bioscience (MO, USA). Melatonin, Neu-P11 and Neu-P67 were dissolved in 10% dimethyl sulfoxide (DMSO, ICN Biomedicals, Inc., Aurora, OH, USA) in saline and administered intraperitoneally (i.p.) at the dose ranging from 25 to 200 mg/kg. Neu-P11, Neu-P67 and MLT were given 20 min, 20 min, and 15 min before testing, respectively. Control animals received i.p. injection of 10% DMSO. The vehicle had no effects on the observed parameters. Pentylenetetrazole (PTZ, Sigma-Aldrich, Poznań, Poland) was dissolved in saline and infused intravenously (i.v.). The pretreatment schedules and doses of all drugs were selected based on our preliminary studies and available literature [10,24,44,48].

2.3. The 6 Hz psychomotor seizure threshold test in mice

The psychomotor seizure thresholds were examined using square-wave alternating current stimuli (0.2 ms duration pulses at 6 Hz for 3 s) applied via saline-soaked corneal electrodes using a Grass S48 stimulator coupled with a constant current unit CCU1 (both from Grass Technologies, West Warwick, RI, USA). A drop of ocular anaesthetic (1% solution of tetracaine hydrochloride) was applied on the corneas 1 min before the stimulation. Before testing, the electrodes were soaked in 0.9% of saline for good electrical contact. The seizures induced by 6 Hz stimulation were characterized by immobility or stun posture, which was frequently followed by rearing, forelimb clonus, twitching of the vibrissae and elevated or Straub tail [16,48]. The absence of the features listed above or the renewal of normal exploratory behaviour within 10 s after stimulation were considered as lack of seizures. The 'up-and-down' method described by Kimball et al. [23] was used in order to choose the current intensity. Each animal was stimulated only once at any given current intensity that was lowered or raised by 0.06 log intervals depending on whether the previously stimulated animal did or did not respond with convulsions, respectively. Data obtained in groups of 18–20 animals were used to determine the threshold current causing 6 Hz-induced seizures in 50% of mice (CC_{50} with confidence limits for 95% probability).

2.4. Maximal electroshock seizure threshold (MEST) test in mice

The electroshock seizures were induced by sine-wave alternating current (maximal output voltage 500 V, 50 Hz for 0.2 s) applied via saline-soaked transcorneal electrodes delivered by a rodent shocker (type 221; Hugo Sachs Elektronik, Freiburg, Germany) as described earler [48]. To minimize the pain, an ocular anaesthetic (1% solution of tetracaine hydrochloride, Sigma-Aldrich) was applied into each eye 1 min before stimulation. Transcorneal electrodes were soaked in 0.9% saline to maximize the conductance. During stimulation mice were manually immobilized and immediately after the stimulation placed in a Plexiglas arena (37 cm × 21 cm × 14 cm) for behavioural observation for the presence or absence of seizure activity. Tonic hindlimb extension served as an endpoint. The thresholds for maximal electroconvulsions