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Melatonin secretion in children with epilepsy ☆

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ABSTRACT

This study examined melatonin (MLT) system in children with epilepsy. Diurnal patterns of salivary MLT, urinary metabolite 6-sulphatoxymelatonin, core body temperature, pulse and blood pressure were measured in 51 children with epilepsy (6.6–17.9 years) and 29 comparison children (5.5–17.3 years). The children with epilepsy preserved MLT and other circadian rhythms. In nine children with epilepsy (17.6%), peak salivary MLT concentrations were very high. There were no associations between MLT secretion/excretion parameters (diurnal profile, peak nocturnal concentrations, area under the time curve, duration of elevated concentrations, acrophase) and seizure characteristics (time, type of seizures, antiepileptic medications). The study observations are important for understanding of the MLT system in epilepsy and for exploring the potential for seizure treatment with melatonin.

1. Introduction

Melatonin (MLT) is a pineal gland hormone that is synthesized from tryptophan and released in circadian rhythm, with peak concentrations at night [1,2]. Its role in epilepsy has been noted since the early 70s [3] and has been linked either to the epileptogenic process directly (γ-aminobutyric acid (GABA) neurotransmission, oxidative stress [4]) or to the exacerbation of seizures via disrupted sleep patterns [5]. Experimental studies have suggested that pinealectomy induces seizures and that exogenous melatonin may have anticonvulsant properties [6–10]. However, it has also been observed that melatonin may increase the frequency and amplitude of epileptiform field potentials in the hippocampus (low Mg^{2+} model, daytime) [11] and, consequently, may increase the risk of seizures.

Examination of the MLT system in children and adults with epilepsy has shown higher nocturnal MLT concentrations than in controls, increase in MLT concentrations during/directly after seizures and shift or loss of MLT circadian rhythm [12–17]. Conversely, a number of studies have demonstrated MLT rhythm and plasma concentrations in epilepsy comparable to those of control populations [18–21]. The results of the evaluation of MLT therapy in epilepsy have also been inconsistent. Initial case descriptions have suggested that melatonin may either reduce seizures [22] or exacerbate the course of epilepsy [23,24]. Recent larger studies of children with epilepsy have shown that exogenous melatonin decreased frequency and duration of seizures and improved quality of sleep in children and adults with intractable epilepsy [22,25–27]. However, clinical studies have had, so far, a number of flaws, i.e., lack of control groups, blinded evaluation, placebo control, as well as comprehensive MLT measurements. Although there may be some potential utility of melatonin in treating epilepsy, the rationale for such therapy is not fully proven, as there is no distinct evidence for the deficit of MLT in children with seizures. Furthermore, the risk for possible seizure propensity in humans has not been fully evaluated.

The main objective of the present study was to characterize the MLT system in children with epilepsy in detail. Diurnal levels of salivary melatonin (N-acetyl-5-methoxytryptamine) and its main urinary metabolite, 6-sulphatoxymelatonin (aMT6s), were compared between children with epilepsy and comparison children matched by age, sex, body weight and stage of puberty. In addition, other circadian rhythms (core body temperature, cardiovascular system) were compared. Possible associations between MLT concentrations and epilepsy phenotypes as well as sleep patterns were evaluated.

2. Subjects and methods

2.1. Study design and subjects

The study was approved by the Bioethics Committee of Lithuania (Registry 2004 12–22 Nr. 69). Participation in the study was voluntary, and the subjects agreed to participate after receiving written and oral information. The written informed consent was obtained

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from the parents and assent from the children, in accordance with the Declaration of Helsinki. The study was carried out in 2005–2007 at the Department of Child Neurology of the Children's Hospital, Vilnius University Hospital.

This was an open exploratory study, comparing two groups of children: epilepsy and comparison group. Children in the comparison group had the following medical conditions: twenty participants had tension-type headache, two adolescents had low back pain, two had simple motor tics, and the other five had the following diagnoses: myotonia, undifferentiated myopathy, inguinal hernia, congenital heart disease, and dyslalia. Routine blood and urine tests were normal. The children were drug naive. They had no family relation to the children in the study group.

The children with active epilepsy (at least one seizure during a year before the study) and on antiepileptic treatment as well as those with newly diagnosed epilepsy were recruited to the study group. Overall duration of epilepsy was from 3 days to 7 years. Magnetic resonance/computed tomography imaging was performed to exclude gross, progressive brain pathology. Sleep and wake electroencephalography (EEG) was registered during the screening period, to confirm the epilepsy diagnosis (10–20 system electrode arrangement, Cadwell Laboratories computer electroencephalograph; Kennewick, WA, USA). The type of epileptic seizures was defined following the International League Against Epilepsy Classification and Terminology [28]. Children with epilepsy had the following seizure types: simple and complex partial seizures (16), partial seizures with secondary generalization (27), generalized tonic–clonic (2), myoclonic (5) and absence seizures (1). The epilepsy syndrome was defined in 35 children: Rolandic epilepsy (21), idiopathic generalized epilepsy (5), temporal lobe epilepsy (7), Lennox–Gastaut syndrome (1) and Landau–Kleffner syndrome (1). Eleven children with epilepsy were medication-free during the study period, 11 were on combined antiepileptic medication (valproate–lamotrigine, valproate–topiramate, oxcarbazepine–topiramate) and 29 children were on monotherapy with valproate (VPA) (16), oxcarbazepine (9), and lamotrigine, sultiam, primidone and topiramate, one each.

The clinical descriptions of children in both groups contained information on gestation age, height, weight, body mass index (BMI, weight/height^2) and Tanner pubertal stage [29,30]. At recruitment, the parents filled in the standardized questionnaire for sleep, Sleep Disturbance Scale for Children (SDSC) [31], which evaluated the sleep quality over the past six months. The SDSC comprises questions describing such sleep disorders as difficulty in initiating and maintaining sleep (DIMS), sleep breathing disorders (SBD), disorders of arousal (DA), sleep-wake transition disorders (SWDT), disorders of excessive somnolence (DOES) and sleep hyperhidrosis (SHY). The SDSC also served as inclusion criteria for the control children; children with total score below 50 (T-value $\leq$ 70‰) and no circadian rhythm or sleep disorders were included in the study. In the group of children with epilepsy, 25 children (49%) had a sleep disorder (total SDSC score T-value > 70‰).

2.2. Protocol and laboratory analyses

The study was carried out during September–March. The light in the corridors and wards was measured with lux meter JU-166 (Vibrator; Russia). During data sampling, fluorescent room lighting provided relatively constant illumination throughout the day (100–200 lx) and with intensities at night, with curtains closed, not exceeding 10 lx (a dim light), so as to prevent suppression of melatonin secretion by bright light [32].

The samples for melatonin were collected on the third day of hospitalization, after children became synchronized to the hospital routines and were asleep from 22:00 to 07:00. Children and their parents were explained the sampling procedures and were instructed to drink 125–250 ml fluids (water, juice) every 3 h during the day, to rinse the mouth 15 min before a saliva sampling during the day, and quickly rinse the mouth and drink a gulp of water at the sample collection during a night period. On the day of sample collection, bananas, chocolate products, coffee, tea, cacao and other caffeine-containing products were excluded.

The sample collection included probes at 3-h intervals, starting at 15:00 and finishing at 12:00 the next day. Saliva samples were collected in the saliva collection device (Buhlman, Allschwil, Switzerland) by asking subjects to chew a polystyrol plug for 1 min. The urine samples were collected into a plastic beaker. All samples were stored at 2–8 °C until the last sample and, then, were taken to the laboratory where they were centrifuged for 5 min (saliva at 1000 $\times g$, urine at 2000 $\times g$); supernatant was transferred to fresh microtubes and was frozen and stored at −20 °C until assay.

At the same time points, all the participants were subjected to measurements of core body temperature, pulse and blood pressure. Body temperature was measured using a Braun thermometer (Germany). Blood pressure and pulse were measured 3 times using an electronic blood pressure meter (Digital Blood Pressure Monitor UA-702, A&D, Ltd., UK), and the mean value was taken.

Laboratory analysis of MLT concentrations was conducted at the Division of Immunology, State Research Institute Center for Innovative Medicine. The concentrations were measured in duplicate, using competitive enzyme-linked immunosorbent assay (ELISA) commercial kits (Buhlman Laboratories, Allschwil, Switzerland). For detailed description of the laboratory analyses, see [33]. Data processing was performed by BioTek's Gen5 Data Analysis Software. The standard curve was calculated by using a four-parameter algorithm.

2.3. Quantifications of melatonin parameters

The melatonin system was evaluated by using parameters that describe volume of MLT and circadian phase. Individual diurnal profiles were inspected visually, and the peak (maximum) values of salivary MLT and urinary aMT6s were identified for each subject. The area under the concentration-time curve (AUC) was calculated using the trapezoidal method and expressed as pg/ml·h for salivary MLT and ng/ml·h for urinary aMT6s. Total AUC and AUC for daytime and nighttime (21:00–9:00) periods were calculated. Circadian phase variables were acrophase, onset-offset time and duration of elevated melatonin (time above threshold). A threshold to determine onset-offset values was calculated as the mean of consecutive daytime values plus twice the standard deviation of these points [34].

2.4. Statistical analysis

Prior to statistical analyses, all data were inspected for outliers, skewness, and homogeneity of variance to ensure their appropriateness for parametric statistical tests. Both parametric and non-parametric tests were used, depending on the variables analyzed. Differences between the groups in demographic characteristics were assessed using the t-test for independent samples (two-tailed). Comparison of circadian rhythms between the study groups was performed using the analysis of variance. Potential associations between the clinical data and laboratory measurements were assessed by Pearson's product moment correlation (r). In all the analyses, the statistical significance was set at $p<0.05$. Statistical analysis was performed with software STATISTICA, v10, StatSoft, Inc., Tulsa, OK, USA.

3. Results

There were 83 children recruited in the study. In three cases, data sets were not complete due to technical failures in sample collections. The final study group consisted of 80 subjects, 29 comparison group