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Short communication

Circadian profile of salivary melatonin secretion and its concentration after epileptic seizure in patients with drug-resistant epilepsy – Preliminary report

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ABSTRACT

Background: The results of a few previous studies assessing melatonin concentration in epileptic patient are ambiguous. This study aimed at: (1) comparing the circadian profile of salivary melatonin excretion in epileptic patients with that in healthy subjects and with circadian frequency profile of seizures and (2) assessing the effect of epileptic seizure upon salivary melatonin concentration.
Methods: The study included thirty patients suffering from drug-resistant epilepsy aged from 22 to 45 years (mean age 37.17, SD ± 10.25). All subjects had their saliva taken in order to determine melatonin concentration and its circadian excretion profile performed every 4 h. Additionally, saliva samples were collected in order to assess concentration of melatonin directly after epileptic seizure and 2 h later.
Results: The circadian profile of melatonin secretion in epileptic patients did not differ significantly from a profile in healthy subjects. Epileptic women showed statistically higher average salivary melatonin concentration at 2 a.m., 6 a.m. and 10 a.m., compared to epileptic men; this may be related to lower age average of women as well as to their different hormonal profile.
Conclusion: The significantly higher salivary melatonin concentration at 6 a.m. in patients with diurnal seizures (occurring mainly in the morning) may suggest proconvulsive effect of this hormone. Epileptic seizure did not lead to significantly elevated salivary melatonin concentration. Epileptogenic effect of melatonin might be corroborated by significantly elevated salivary melatonin levels directly after nocturnal tonic–clonic seizure which affected patients with highest concentration of this hormone at 2 a.m. These observations would need confirmation based on studies of larger groups of epileptic patients.

Introduction

Melatonin, a naturally occurring hormone, is important in the regulation of several biological processes. Its first recognized function was the regulation and facilitation of sleep. Melatonin has been used for many years in treating disorders resulting from rapid travel across time zones. Melatonin exerts immunostimulatory effects and regulates gonadal function; its ability to scavenge free radicals has implications for treating neoplastic and degenerative diseases as well as atherosclerosis [1].

In the central nervous system melatonin exerts neuroprotective effects also via its antioxidant action. As a free radical scavenger, it reduces oxidative stress caused by epileptic seizure which results in decreased neuronal damage [2–4].

Melatonin secretion is characterized by a circadian rhythm which is regulated by light. There is a large individual variability in the amount of melatonin secretion [5]. Serum concentration of this hormone is low during daytime (10–20 pg/ml) and elevated several-fold at night (80–150 pg/ml) with peak values between midnight and 3 a.m. The melatonin secretion rhythm develops around the 6th month of life and its average concentration peaks

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between the ages of 4 and 7. A distinct decrease in melatonin level occurs around sexual maturation age. Values reached at that period progressively decrease until the age of 40–50 and after further drop by the age of 65–70 when the circadian rhythm of melatonin secretion almost totally disappears [6].

There have been suggestions that melatonin effects depend upon changes in neurotransmission of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the mammalian brain. Melatonin effects depend also on the presence of striatal dopamine receptors D1 and D2 [7]. Some data suggest attenuated influx of calcium ions into the cell as an effect of melatonin activity and it seems that melatonin acts in this way as an antagonist of calcium channels. Low melatonin levels raise concentration of hypothalamic and cortical GABA which inhibits paroxysmal excitability. Increased melatonin concentration, in turn, lowers GABA concentration. At higher concentration melatonin can most likely induce seizures by lowering GABA concentration in the brain [7].

Some authors have suggested that endogenous melatonin contributes to increased paroxysmal activity by its inhibitory effect upon dopaminergic system, the latter being a natural regulator which decreases paroxysmal activity [8,9]. In the brain melatonin is metabolized to kynurenine. This compound, together with its metabolite, kynurenic acid, also seems to influence paroxysmal activity. Experiments involving rats have shown that kynurenine has proconvulsive effect whereas kynurenic acid inhibits paroxysmal activity [7]. Anticonvulsant effect of melatonin was demonstrated in experimental animal models [2–4].

Serum and salivary melatonin, primarily in epileptic children, has been assessed in only a few clinical studies so far published. The majority of these reports have corroborated anticonvulsant effects of melatonin [1,10–14]. The results of studies involving humans suggest though that endogenous melatonin contributes to increased paroxysmal activity [1,8,9,15].

Melatonin concentration measured at various time points after epileptic seizure was rarely analyzed.

Trimble [16] noticed in 1978 for the first time that a generalized tonic-clonic seizure sometimes causes a several-fold increase in serum prolactin [16]. This observation led other investigators to study the impact of epileptic seizures on the endocrine system. Epileptic seizures, mainly generalized tonic–clonic or complex partial and sometimes simple partial ones cause the so-called hormonal storm. Epileptic seizures can stimulate hypothalamus directly or indirectly *via* a neurotransmitter system or by releasing other substances, *e.g.* endogenous opioids, in response to seizure-related stress. Elevated serum concentrations of prolactin, cortisol, corticotropin, somatotropin, thyreotropin, triiodothyronin, thyroxin, lutropin and folitropin have been found directly following epileptic seizure [17–22]. These changes can persist for up to 2 h after seizure and that involving prolactin for even up to 24 h.

Objective

The results of a few studies previously assessing melatonin concentration in patients suffering from epilepsy are ambiguous. So far, circadian rhythm of epileptic seizures was not compared to melatonin circadian profile and melatonin concentration after epileptic seizure was rarely analyzed. Consequently, the study aimed at:

(1) comparing the circadian profile of salivary melatonin excretion in epileptic patients with that in healthy subjects.
(2) assessing the relationship between circadian fluctuations of salivary melatonin concentration changes in epileptic patients with circadian frequency profile of seizures.
(3) assessing the effect of tonic–clonic seizure, as well as of complex partial seizure, upon salivary melatonin concentration.

Methods

The study included thirty patients of an outpatient epilepsy clinic (15 females and 15 males) suffering from drug-resistant epilepsy. The study subjects ranged from 22 to 45 years of age (median 37.17, SD ±10.25). Median age of male patients was 42.07, SD ±9.52, whereas that of female patients was 32.27, SD ±8.68. Average span of disease duration was 21.33, SD ±11.91 years; complex partial seizures with secondary generalization were dominating (60% of patients). Complex partial seizures without secondary generalization occurred in one third of patients and two of patients had primary generalized tonic–clonic seizures. Seizures occurred in all patients with a frequency of several per month. Written informed consent was obtained from all patients included in the study. None of the subjects included in the study suffered from progressive organic brain disease or other illness except for epilepsy. None suffered from sleep disturbances.

The epileptic patients were divided into three groups: group A with diurnal seizures (between 6 a.m. and 10 p.m.), group B with nocturnal seizures (between 10 p.m. and 6 a.m.), and group C with seizures at various times of day and night. These groups did not differ significantly in terms of gender, clinical picture of epilepsy or treatment applied.

All subjects had their saliva taken in order to determine melatonin concentration and its circadian excretion profile during at least a 24-h-long seizure-free period, with collection performed every 4 h (at 2, 6 and 10 a.m., as well as at 2, 6 and 10 p.m.). Additionally, saliva samples were collected from each patient in order to assess concentration of melatonin directly after epileptic seizure (tonic–clonic or complex partial), as well as after 2 h later.

The control group consisted of twenty healthy volunteers (10 females and 10 males) in the same age range as the examined patients. Circadian profile of melatonin secretion in the subjects from control group was examined by collecting saliva samples six times during day and night, similarly as from the studied epilepsy patients. Saliva samples were collected also during the same period of the year (fall) in the stable weather conditions.

All subjects participating in the study were normally active during daytime and resting between 10 p.m. and 6 a.m. with light switched off. All of them slept alone. None of them wore eyeglasses or contact lenses. None of subjects suffered from personality disorders. In addition they did not eat, drink or brush their teeth within 30 min prior to taking saliva samples. Care was exercised to examine the circadian profile of melatonin excretion in saliva in women during the follicular phase of the menstrual cycle.

During collection of saliva samples at nighttime light was not turned on; alternatively, red light was turned on. Saliva samples were taken using cotton swabs (Salivettes, Sarstedt) placed by the subject under his/her tongue.

The saliva samples were kept in the refrigerator (ca. 2 °C) until delivered in vacuum flask to the laboratory. Subsequently, the samples were centrifuged (450 × *g*/10 min.), frozen and stored at −75 °C until determination of melatonin concentration. Samples were first methanol-extracted using C_{18} columns (DRG MedTek, Germany, 100 mg, 1.0 ml) and then melatonin was assessed with a radioimmunological method (RIA kit from DRG, USA) at a sensitivity of 2 pg/ml.

Data concerning weather conditions were obtained from the Institute of Meteorology and Water Management (Katowice, Poland). Weather conditions were considered stable when fluctuation in barometric pressure was no greater than 10 hPa,