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ORIGINAL ARTICLE

Low urinary 6-sulphatoxymelatonin concentrations in acute migraine

Marcelo Rodrigues Masruha · Domingos Sávio de Souza Vieira · Thais Soares Cianciarullo Minett · José Cipolla-Neto · Eliova Zukerman · Luiz Celso Pereira Vilanova · Mario Fernando Prieto Peres

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Abstract Substantial evidence points to melatonin as playing a role in the regulation of circadian rhythms, sleep, and headache disorders. The objective of the study was to assess 6-sulphatoxymelatonin (aMT6s) levels in a large consecutive series of patients with migraine, comparing with controls. A total of 220 subjects were evaluated—146 had migraine and 74 were control subjects. Urinary samples were collected into the same plastic container since 8:00 p.m. to 8:00 a.m. of the next day (12-h period) and aMT6s was measured with quantitative ELISA technique. Among patients with migraine, 53% presented pain on the day of the urine samples collection. Their urinary aMT6s concentration was significantly lower than in the urine of patients without pain [14.0 ± 7.3 vs. 49.4 ± 19.0; $t(143) = -15.1$; 95% CI $= -40.0$ to -30.8; $P < 0.001$]. There was no significant difference in the aMT6s concentration of patients with migraine without pain on the day of their urine samples collection and controls [49.4 ± 19.0 vs. 42.5 ± 27.9; $t(140) = 1.7$; 95% CI $= -1.2$ to 14.8; $P = 0.094$]. To our knowledge, this is the first study to demonstrate reduction in melatonin levels during attacks in episodic and chronic migraine.

Keywords Circadian rhythm · Pathophysiology · Melatonin · Migraine · Headache

M. R. Masruha · T. S. C. Minett · L. C. P. Vilanova · M. F. P. Peres
Department of Neurology and Neurosurgery, Federal University of São Paulo, São Paulo, Brazil

M. R. Masruha · D. S. de Souza Vieira · E. Zukerman · M. F. P. Peres (✉)
Instituto Israelita de Ensino e Pesquisa Albert Einstein, Hospital Israelita Albert Einstein, Av. Albert Einstein, 627/701, Morumbi, São Paulo 05651-901, Brazil
e-mail: marioperes@yahoo.com

J. Cipolla-Neto
Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Introduction

The hypothalamus is thought to play an important role in migraine pathogenesis [1–4]. It is possible that periodic central disturbance of hypothalamic activity or labile threshold could account for the periodicity of the migraine attack. It also provides a mechanism by which emotional disturbances could be mediated by pathways from the limbic system to the hypothalamus [5]. Almost 60% of patients with migraine report symptoms of elation, irritability, depression, hunger, thirst, or drowsiness during the 24 h preceding headache, which also suggests a hypothalamic site of origin [4].

It has been suggested that if neuronal hyperexcitability and cortical spreading depression during migraine attack are part of the pathophysiology of the disorder, then aura is not the initiating event but rather is initiated by subcortical changes [6]. The suprachiasmatic nucleus (SCN) is a possible subcortical origin of this changes, suggesting that a migraine attack might be an attempt at a drastic resetting of the biological clock in response to its previous decompensation [4].

Compared with the environmental cycle of 24 h, the human biological pacemaker has an intrinsic periodicity of 25.7 hours [7]. The conversion between the intrinsic rhythm and the environmental rhythm is known as "entrainment". The environmental agents that are able to synchronize the endogenous circadian clock are known as

"zeitgebers", among them the most influential is the change in luminosity between day and night [8]. Photic entrainment is mainly mediated by a direct retinal-hypothalamic pathway that conveys to the SCN information about the level of ambient light. Photic information relayed from the SCN to the pineal gland is closely reflected there in the secretion of melatonin, which is probably the strongest biological marker of the circadian rhythm in humans [4].

Melatonin is absent during the day in human beings [9] and its nocturnal secretion is the main biological event signaling to the organism when it is night [10]. Once melatonin is synthesized in the pineal gland, it is quickly released, generating a blood melatonin rhythm reminiscent of that seen in the gland. Being an amphiphilic molecule, melatonin is capable of entering every cell in the organism; additionally, it readily crosses all morphophysiological barriers, including the blood–brain barrier and the placenta. Melatonin is enzymatically-degraded in the liver to 6-hydroxymelatonin and finally excreted in the urine as 6-sulphatoxymelatonin (aMT6s) [10]. Urine analysis is widely used as a measure of melatonin secretion, since it is correlated with the nocturnal profile of plasma melatonin secretion [11]. The advantages of urinary metabolite measurement are substantial, particularly the non-invasive nature of fluid collection, the ability to perform very long-term studies and the feasibility of studies in circumstances where blood sampling is difficult.

Substantial evidence points to melatonin as playing a role in the regulation of circadian rhythms, sleep, and headache [12]. An interictal decrease in the levels of nocturnal plasma melatonin[13] and an alteration of the urinary aMT6s excretion throughout the ovarian cycle have also been described in migraine patients [14, 15]. Furthermore, seasonal variations in daily melatonin secretion have been reported, patients with migraine being more likely to have headache during the bright Arctic summer season [16, 17].

These data prompted us to test the hypothesis of decreased levels of melatonin during migraine attacks, assessing aMT6s levels in a large consecutive series of patients with migraine, comparing with controls.

Materials and methods

The study was performed from February to December 2006, at Albert Einstein Hospital, São Paulo, Brazil. The inclusion criteria were: patients with migraine and controls subjects of both sex, aged 18–65 years, able to understand the consent form and explanations given by the research team, and with satisfactory diuresis. Migraine was defined according to International Classification of Headache Disorders diagnostic criteria, Second edition [18]. The control subjects did not have migraine or any other primary headache. The exclusion criteria were: chronic diseases, instable medical condition, secondary headache, continuous usage of any kind of medication, drug addiction or alcohol abuse. The patients who remained aroused with light exposure during the night of urine collection were also excluded. All subjects provided written consent for the experimental procedure approved by the local ethics committee (Federal University of São Paulo and Albert Einstein Hospital, São Paulo, Brazil).

Subjects were asked to collect urine into a plastic container from 8:00 p.m. to 8:00 a.m. of the next day (12-h period). Female patients were advised to collect the samples at least 5 days away from the beginning or the end of menses. The patients were asked to provide information regarding the presentation or not of a migraine headache on the day of urine collection.

Sample's volumes were recorded and 5-ml aliquots were stored frozen at −20°C until analysis. aMT6s has been found to be extremely stable without preservative for at least 5 days at room temperature and for at least 2 years at −20°C and no special precautions were necessary during sample collection [19]. aMT6s was measured by quantitative ELISA (Bühlmann Laboratories, Allschwil, Switzerland). At a dilution of 1:200, 50 µl of urine sample was assayed directly; where necessary, samples were re-assayed at dilution of 1:400.

Data analysis

The chi-square test (χ^2) (without Yates correction) was used for categorical data comparisons. Mean differences of continuous measurements were tested by the Student's t test (t) followed by the Mann–Whitney test, which without exception did not show any discrepant results (only the parametric tests' results will be reported). The Pearson's product-moment correlation coefficient (r) was used to assess the relationship between two continuous variables. A P value of less than 0.05 was considered to indicate statistical significance; all tests were two-tailed. Ninety-five percent confidence intervals (CI) were calculated for the difference between means. All statistical analyses were performed on a personal computer with the statistical package SPSS 11.5.1 for Windows.

Results

A total of 268 subjects were referred to evaluation. From this total, 48 were excluded: 12 patients did not correctly provide their urine samples, 3 refused to take part in the study, 19 did not give reliable headache information and 17

had at least one exclusion criteria. The remaining 220 subjects were in accordance with inclusion criteria. Among them, 146 had migraine and 74 were control subjects.

There were no statistically significant differences between controls and patients with migraine regarding the mean age [38.4 ± 9.0 vs. 38.5 ± 11.2, $t(218) = -0.7$, 95% CI = −3.0 to 2.8, $P = 0.946$] and sex [78 vs. 81% of women, $\chi^2(1) = 0.18$; $P = 0.669$].

Among patients with migraine, 53% presented pain on the day of the urine samples collection. Their urinary aMT6s concentration was significantly lower than in the urine of patients without pain [14.0 ± 7.3 vs. 49.4 ± 19.0; $t(143) = -15.1$; 95% CI = −40.0 to −30.8; $P < 0.001$]. However, there was no significant difference in the aMT6s concentration of patients with migraine without pain on the day of their urine samples collection and controls [49.4 ± 19.0 vs. 42.5 ± 27.9; $t(140) = 1.7$; 95% CI = −1.2 to 14.8; $P = 0.094$] (Fig. 1).

Migraine with aura was observed in 24% of the patients. There was no significant difference in the aMT6s concentration of patients with migraine with and without aura [32.3 ± 21.5 vs. 30.5 ± 22.9; $t(144) = -0.52$; 95% CI = −10.9 to 6.4; $P = 0.604$].

Discussion

Claustrat et al. [13] were the first to demonstrate lower plasma melatonin levels in samples from migraine patients compared with controls. Migraine patients without depression had lower levels than controls, but migraineurs with superimposed depression exhibited the greatest melatonin deficiency. Murialdo et al. [15] also found nocturnal urinary melatonin to be significantly decreased throughout the ovarian cycle of migraine patients without aura compared with controls. During the luteal phase, when melatonin levels should normally increase, migraine patients showed a less pronounced change when compared with controls. Melatonin excretion was further decreased when patients suffered a migraine attack.

Brun et al. [14] studied urinary melatonin in women with migraine without aura attacks associated with menses and controls. Melatonin levels throughout the cycle were significantly lower in the migraine patients than in controls. In the control group, melatonin excretion increased significantly from the follicular to the luteal phase, whereas no difference was observed in the migraine group.

Peres et al. [20] studied plasma melatonin nocturnal profile in chronic migraine patients and controls. Lowered melatonin levels in patients with insomnia were observed compared with those without insomnia, and a phase delay in the melatonin peak in patients versus controls.

To our knowledge, our study is the first to demonstrate reduction in melatonin levels during migraine attacks, not depending on the migraine type. However, patients with migraine that did not present headache in the day of the urine sample collection did not have lower aMT6s levels when compared with controls. In our opinion, two possible explanations could be made to this fact: first, the hypothalamic disturbance may occur only during attacks, mainly in the prodromal phase, so patients in the interictal period will not have reduction of melatonin levels. The other possible explanation is that all patients with migraine could have lower levels of aMT6s, but patients in the interictal phase could have a compensatory increase in melatonin levels. These results support hypothalamic involvement in migraine pathophysiology.

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Conflict of interest None.

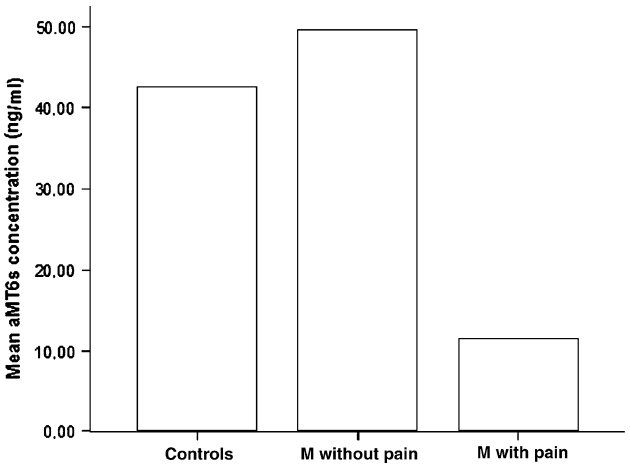

Fig. 1 Mean aMT6 s concentration according to the diagnosis

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