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Original article

Comparison of platelet serotonin transporter activity in subjects with severe sleep bruxism and control

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ABSTRACT

Purpose: The aim of this study was to evaluate the correlation between sleep bruxism (SB) frequency and serotonin transporter (SERT)-driven serotonin (5-HT)-uptake in platelets.
Methods: Subjects were dental trainee residents and faculty members of Okayama University Hospital who were aware of having severe or no SB. SB frequency was assessed for 3-consecutive nights by a self-contained electromyographic detector/analyzer, which indicated individual SB levels as one of four grades (score 0, 1, 2 and 3). Subjects were classified as normal control (NC) when SB scores indicated only 0 or 1 during the 3 nights, or as severe SB for scores 2 or 3. Those subjects whose scores fluctuated from 0 to 3 during the 3 nights were omitted from further analysis. Fasting peripheral venous blood samples were collected in the morning following the final SB assessment. Amounts of SERTs proteins collected from peripheral platelets were quantified using ELISA, and SERTs transport activity was assessed by uptake assay using [^{3}H]-5-HT.
Results: Thirteen severe SB subjects and 7 NC subjects were eligible. Gender distribution, mean age, 5-HT concentration and total amounts of SERT protein in platelets showed no significant differences between NC and severe SB ($p = 0.85$: *Chi-squared test*; $p = 0.64$, 0.26,

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0.46: *t-test*). However, [^{3}H]-5-HT uptake by platelets was significantly greater in NC compared to severe SB subjects (12.79 ± 1.97, 8.27 ± 1.91 fmol/10^5 platelets/min, $p < 0.001$, *t-test*).
Conclusion: The results of this pilot study suggest a possible correlation between peripheral platelet serotonin transporter uptake ability and SB severity.

1. Introduction

Sleep bruxism (SB) is classified under the subgroup of sleep-related movement disorders, and at sometime in their lives, the majority of people (85–90%) experience mild or moderate symptoms thought to be a direct result of SB [1]. In severe cases, SB can cause dramatic tooth wear, which sometimes is associated with dentin hypersensitivity and tooth mobility, as well as a low prognosis of dental prostheses [2]. However, current management approaches available for SB are largely symptomatic therapies such as oral appliances and contingent counter-stimulation devices and are not considered to be curative. Therefore, a deeper understanding of the molecular mechanisms involved in SB arousals would lead to the development of new therapies for management of SB.

Recently, the central nervous system (CNS) has been hypothesized to play a role in the etiology of SB, since several clinical case reports have shown that SB frequency is alleviated and/or aggravated by administration of medications that regulate monoamine levels at the CNS. Multiple case reports have also assumed that SB events increased after selective serotonin reuptake inhibitors (SSRIs) intake [3,4]. SSRIs are known to bind directly to serotonin transporter (SERT), which regulates pre-synaptic 5-HT levels by re-uptaking and transporting 5-HT. Consequently, SSRIs can indirectly lead to increases in the serotonin (5-HT) concentration at the synaptic cleft. Interestingly, Kishi [5] and Sabuncuoglu et al. [6] reported that administration of a selective 5-HT_{1A} receptor agonist (tandospirone and buspirone, respectively) suppressed SSRI-induced SB activity in human patients. This clinical finding is based on the fact that the 5-HT_{1A} receptors are located pre-synaptically and functions as a 5-HT auto-receptor; therefore, it negatively regulates the 5-HT nerve exocytosis process.

Additionally, 5-HT neurons have been reported to induce excitatory effect on the post-synaptic firing-threshold of motor nerves [7]. Inoue and colleagues examined the mechanisms by which 5-HT altered the post-synaptic firing threshold using the rat trigeminal motor nucleus [8]. They showed that 5-HT induces a dose-dependent attenuation of the medium-duration after-hyperpolarization (mAHP) amplitude through cAMP-dependent activation of protein kinase A (PKA). Based on this fact, they concluded that 5-HT could increase the firing output in jaw-closing motoneurons. Furthermore, the dorsal raphe nucleus, which contains high levels of serotonin receptors, is in close proximity to the trigeminal motor nucleus; thus, there could be a strong potential for 5-HT activation *via* volume transition between these nuclei. We therefore, hypothesized that serotonergic nerve functions could possibly be related to SB frequency; more specifically, that the concentration of 5-HT in the presynaptic terminal could in part regulate diurnal/nocturnal activities of trigeminal motor nerve and muscle hyperactivities (SB).

SERTs are located mainly in peripheral platelets and in the brain [9] and are encoded by a single gene located in the chromosomal region [10], and their amino acid sequences are almost identical except for a difference in post-translational modifications [11]. Nevertheless, direct and accurate investigation of alterations in 5-HT at the synapse is problematic; therefore, we utilized a peripheral platelet model which has been regarded to be comparable to central 5-HT function [12,13].

Based on our hypothesis mentioned above, we examined the peripheral platelet SERT function as a possible indicator of the 5-HT concentration in the peripheral platelets and verified a possible association with SB frequency (high frequent bruxism and normal range subjects) in healthy young adults.

2. Material and methods

2.1. Study subjects

Subjects were recruited among the resident students of Okayama University Hospital in 2010. Those subjects who (1) were receiving orthodontic treatments, (2) had taken psychotropic medication within the past 6 months, (3) used an oral appliance within the past 6 months and (4) presented cutaneous diseases (*i.e.*, atopic dermatitis) were excluded. The initial screening criterion was self-awareness of SB activities by oral interview. In order to compare subjects with high SB levels and normal controls, as a first step, the ambiguity awareness of sever SB or non-SB subjects were rejected to participate this study. In response, eligible candidates were informed about the study objectives and asked to write the informed consent form. Prior to the beginning of the study, approval of the Independent Ethics Committee of Okayama University Graduate school of Medicine, Dentistry and Pharmaceutical Science was obtained (No. 967).

2.2. Measurements of SB frequency

The candidate subjects were then asked to have their SB frequency recorded by means of an electromyography (EMG)-containing detection device (BiteStrip®, Up2dent, Germany), which is composed of electromyography electrodes and an amplifier to acquire masticatory muscle signals, a central processing unit with real-time software that detects and analyzes the EMG patterns. This device has been already validated in comparison with polysomnography [14,15], and it counts the number of masseter muscle hyperactivities as the