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Chronic sleep deprivation alters the myosin heavy chain isoforms in the masseter muscle in rats

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Abstract

To investigate the changes in myosin heavy chain (MyHC) isoforms of rat masseter muscle fibres caused by chronic sleep deprivation and a possible link with the pathogenesis of disorders of the temporomandibular joint (TMJ). A total of 180 male rats were randomly divided into three groups ($n = 60$ in each): cage controls, large platform controls, and chronic sleep deprivation group. Each group was further divided into three subgroups with different observation periods (7, 14, and 21 days). We investigated he expression of MyHC isoforms in masseter muscle fibres by real-time quantitative polymerase chain reaction (PCR), Western blotting, and immunohistochemical staining. In rats with chronic sleep deprivation there was increased MyHC-I expression in layers of both shallow and deep muscles at 7 and 21 days compared with the control groups, whereas sleep deprivation was associated with significantly decreased MyHC-II expression. At 21 days, there were no differences in MyHC-I or MyHC-II expression between the groups and there were no differences between the two control groups at any time point. These findings suggest that chronic sleep deprivation alters the expression of MyHC isoforms, which may contribute to the pathogenesis of disorders of the TMJ.

Keywords: Chronic sleep deprivation; Temporomandibular joint disorder; Masseter muscle; Myosin heavy chain; qPCR; Transcriptional change

Introduction

Disorders of the temporomandibular joint (TMJ) are common, and are characterised by such symptoms as dysfunction of the jaw muscles, and restricted movement and structural damage to the jaw, which could seriously affect quality of life.[1] These disorders have many causes, and the role of psychological factors in their pathogenesis has received increasing attention.[2] The modern working environment and fast pace of life have increased the incidence of sleep deprivation, which may be involved in triggering or maintaining the disorders, or both.[3–5] We have previously found that sleep deprivation caused structural alterations to the masseter muscles, although the exact mechanisms that caused the pathological alterations are still not clear.

The myosin heavy chain (MyHC) is an important component of masticatory muscles, which play a vital functional part, and are a potential indicator of structural and functional changes associated with disease. Multiple factors including

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malocclusion and abnormal behaviour of the mandible have been shown to alter the degree of expression and spatial distribution of MyHC. However, the impact of sleep deprivation on it has not previously been reported to our knowledge.

In the present study, we have used the modified multiple platform method to induce sleep deprivation, and compared the composition of fibres and degrees of MyHC expression in masseter muscles between control and sleep-deprived groups to explore the possible pathological mechanisms by which sleep disorders may cause disorders of the TMJ.

Material and methods

Male Wistar rats (8 weeks old and weighing 220 (20) g) were obtained from the Animal Centre of Shan-dong University (Jinan, China) and raised in the Laboratory Animal Centre of Jinan General Military Hospital. Rats were exposed to a 12 hour light/dark cycle and given free access to food and water for 2 weeks before the experiment. The experimental procedures were reviewed and approved by the Ethics Committee of Shan-dong University.

Experimental groups

A total of 180 rats were randomly divided into three groups (60 in each): cage controls, large platform controls, and chronic sleep deprivation group. The three groups were then further divided into subgroups which were separately assessed at 7, 14, or 21 days. Rats in the large platform control and sleep deprivation groups were placed on wide and narrow platforms, respectively, and rats in the cage control group were raised in cages in the same room.

Model for chronic sleep deprivation

According to the modified multiple platform method,[6–8] a chronic sleep deprivation tank was prepared. Fifteen narrow platforms (6.3 cm in diameter) or one wide platform (143.5 cm × 42 cm) were placed in the tank (110 cm × 40 cm × 70 cm). The narrow platforms were 15 cm apart so that rats were able to stand on them only one at a time, without touching each other. During the experiment, water was poured into the tank to a depth 1 cm below the upper surface of the platforms. On the narrow platforms, when the rats reached the paradoxical phase of sleep atonia set in, and they fell into the water and woke up, whereas on the wide platform rats could avoid falling into the water after falling asleep. Rats were placed on the platforms for 18 h each day (from 1600 to 1000 h), the temperature of the tank and the water were maintained at 20 (2) °C and 18 (2) °C, respectively, and rats had free access to food and water.

Collection of tissue

After 7, 14, and 21 days deprived of sleep, animals were anaesthetised with pentobarbital sodium (50 mg/kg body weight), and the deep and shallow masseter muscles were carefully removed and rinsed in cold saline. These were then used for detection of mRNA and analysis of protein expression by real-time quantitative polymerase chain reaction (qPCR), Western blotting, and immunohistochemical staining.

Immunohistochemistry

The isolated deep and shallow masseter muscles were fixed in 10% buffered paraformaldehyde for 24 h, embedded in paraffin, and cut into 5 mm sagittal sections. Endogenous peroxidase activity was inhibited with 3% hydrogen peroxide, and antigens were retrieved by autoclaving at 120 °C for 15 min in 0.01 M citrate buffer, pH 6. Sections were treated overnight with 1/50 anti-fast skeletal myosin antibody (MY-32, Abcam, USA) and 1/80 antislow skeletal myosin heavy chain antibody (NOQ7.5.4D, Abcam, USA) at 4 °C. The secondary biotinylated antimouse IgG antibody was added for 30 min at room temperature, and sections were lightly counterstained with haematoxylin, dehydrated, cleared, and mounted. Five randomly chosen high-power fields (magnification ×400) were examined by two independent clinical pathologists who were unaware of the source of the sample. Digital images were further analysed using Image-Pro Plus (Media Cybernetics, USA).

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated with Trizol Reagent (Invitrogen Life Technologies, USA) and homogenised in a gentle MACS® Dissociator (Miltenyi Biotech, Germany). For RT-PCR, the Ultra SYBR Two Step RT-qPCR kit (CWbiotech, China) was used (with ROX) and we followed the manufacturer's instructions. The following cycle was followed: initial denaturation at 95 °C for 10 min, followed by 40 amplification cycles of 95 °C for 20 s and 55 °C for 60 s. Each RT-PCR assay contained forward and reverse primer 10 μmol as well as cDNA template in a total reaction volume of 25 μl. Data were normalised against GAPDH. Primer sequences were as follows: MyHC-I (forward 5′-ACGGAGGAAGACAGGAAGAACCTAC-3′, reverse 5′-GGGCTTCACAGGCATCCTTAG-3′); MyHC-IIa (forward 5′-TATCCTCAGGCTTCAAGATT-3′, reverse 5′-TAAATAGAATCACATGGGGACA-3′); MyHC-IIb (forward 5′-CTGAGGAACAATCCAACGTC-3′, reverse 5′-TTGTGTGATTTCTTCTGTCACCT-3′); GAPDH (forward 5′-ACCACAGTCCATGCCATCAC-3′, reverse 5′-CCACCACCCTGTTGCTGTA-3′). The $2^{-\Delta\Delta CT}$ method was used to analyse the relative intensity of mRNA expression.[9]

Western blotting

We used the modified Lowry method for western blot analysis. Briefly, deep and shallow masseter muscle tissue was