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Lamotrigine reverses masseter overactivity caused by stress maybe via Glu suppression

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HIGHLIGHTS

- Masseter muscle tone increased concurrently with the upregulation of Glu.
- Activities of glutaminase and glutamine synthetase were also altered under stress.
- Lamotrigine at moderate and high doses markedly reversed the masseter hyperactivity.
- Lamotrigine had no effect on dysregulated Glu and Glu-related metabolic enzymes.

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ABSTRACT

Experimental and non-experimental stress significantly increase masseter muscle tone, which has been linked to the symptoms and pathogenesis of several stomatognathic system diseases. Until now, the mechanism underlying this phenomenon has remained unclear. The current study was performed to determine the mechanism of the stress-induced increase in masseter muscle tone and to investigate the effect of lamotrigine on this change. Animals challenged by repeated restraint stress received either saline as a vehicle or lamotrigine in doses of 20, 30 or 40 mg/kg body weight, whereas control animals received saline without stress treatment. Masseter muscle tone was assessed using electromyography. The activity of glutamate-related metabolic enzymes (glutaminase and glutamine synthetase) in the trigeminal motor nucleus was also investigated. Our results showed an interesting phenomenon: masseter muscle activity increased concurrently with the upregulation of the glutamate concentration after stress treatment. The activities of glutaminase and glutamine synthetase in the trigeminal motor nucleus were also upregulated and downregulated, respectively, when the rats were challenged by prolonged stress. The animals treated with lamotrigine at moderate and high doses had significantly decreased masseter muscle tone compared with stressed animals treated with vehicle. These results suggested that increased glutaminase activity and decreased glutamine synthetase activity increased glutamate production and decreased glutamate decomposition, causing an increase in glutamate levels in the trigeminal motor nucleus and eventually increasing masseter muscle tone. The administration of lamotrigine at doses of 30 or 40 mg/kg body weight effectively mitigated the adverse effects of stress on masseter muscle tone via inhibition of glutamate release.

1. Introduction

Numerous studies have suggested that stress induced by negative life events plays a significant role in the onset and progression of several stomatognathic system diseases such as temporomandibular disorders (TMD) and nocturnal bruxism (NB) [1,2]. Prolonged stress results in a series of psychological changes including anxiety and depression and pathologic changes in peripheral serum and masticatory muscles [3,4].

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In addition to these changes, exposure to stress causes a marked increase in masticatory muscle activity in healthy volunteer and dental patients [5,6]. This phenomenon was also observed in animals subjected to experimental stress [7].

Further evidence suggests that masseter hyperactivity closely relates to the symptoms of TMD and NB [1,8]. Furthermore, several studies have reported that the masseter muscle activity, measured through electromyography (EMG), of myofascial pain dysfunction syndrome (MPDS) and TMD patients was higher than that of the healthy subjects during stress conditions [9,10]. These results demonstrated that masseter hyperactivity may be not only a phenomenon induced by stress but also a pathologic process underling the diseases. However, to date, the mechanism of the increased masseter muscle tone (MMT) induced by stress remains enigmatic. Given that the masseter is directly manipulated by the trigeminal nerve, increased electromyographic activity under stress conditions may be attributed to the increase in impulses in the trigeminal motor nucleus (TMN), where the motoneurons of trigeminal nerve are located.

Previous study has shown that endogenous glutamate driven into the TMN contributes to the MMT modification pattern across the normal sleep–wake cycle [11]. As the glutamate concentration changes, corresponding changes in MMT occur consistently. Moreover, exogenous noradrenaline has been found to trigger MMT by amplifying glutamate-driven excitation of motoneurons of the trigeminal nerve [12], further substantiating the important role of glutamate in MMT regulation. Glutamate is an important excitatory neurotransmitter for the central nervous system and is the primary transmitter responsible for controlling motoneuron excitability [13]. In the brain, its production and elimination are regulated by glutaminase (GLS) and glutamine synthetase (GS), respectively [14]. When plasma membrane voltage changes (i.e., action potentials), glutamate synthesized with glutamine by GLS in neurons is released into synaptic cleft and interacts with its receptors continuously until transported into glial cells by excitatory amino acid transporters (EAATs). Subsequently, glutamate is converted into glutamine by GS. Removing of glutamate in synaptic cleft depends on the transport mechanisms while the elimination of glutamate in glial cells relies on the action of GS. Aberrantly upregulated brain glutamate levels have been observed in rats subjected to experimental stress in many papers (This phenomenon was first found by Yamamoto and Moghaddam) [15,16], even though there are no similar reports regarding the TMN region. Based on the above findings, it can be inferred that upregulated glutamate concentrations directly increase in nerve impulses in the TMN, translating the effect of stress into an increase in MMT.

Blocking increased MMT may reduce the adverse effects caused by stress. However, there is currently no information on suitable drugs or reasonable doses. In the search for solutions to this problem, the antiepileptic drug lamotrigine has emerged as a promising candidate. Lamotrigine is an aromatic medication used primarily in the treatment of seizures and bipolar disorder [17,18]. Lamotrigine stabilizes the presynaptic membrane and inhibits the release of glutamate in several brain area including hippocampus and medial prefrontal cortex [19–21], which is the reason for its use in the present study.

In this trial, to investigate the above hypothesis and determine the effect of lamotrigine on stress-induced MMT increase, the restraint-induced stress model was employed, MMT and glutamate content of metabolic pool in the TMN were assessed using EMG and high performance liquid chromatography (HPLC), respectively, and alterations in the metabolic enzymes of glutamate (GLS and GS) in the TMN were investigated.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by the Committee on the Ethics of Animal Research of the Fourth Military Medical University (Xi'an, China). Eighty male Sprague–Dawley rats weighing 200 to 220 g (approximately 8 weeks old) were housed in 80 cm × 45 cm × 40 cm cages in a temperature-controlled room at 24 °C under a 12 h light/dark cycle and given free access to food and water. The rats were randomly divided into 5 groups: the control group (CON; n = 16), the restraint stress group (RS; n = 16) and three restraint stress with drug treatment groups (DT-1, DT-2 and DT-3; n = 16 each).

2.2. Drug preparation

Lamotrigine (Sigma-Aldrich Inc., Saint Louis, MO, USA) was dissolved in 0.5% carboxymethyl cellulose, 0.4% Tween-80, 0.9% benzylic acid and saline, which served as the control vehicle. To test the effect of lamotrigine on MMT, the rats in the DT-1, DT-2 and DT-3 groups received lamotrigine as a daily intraperitoneal injection of 20, 30 and 40 mg/kg bodyweight, respectively, 30 min before stress application, while the rats in the CON and RS groups received a vehicle injection. The agent dose and preparation was based on previous studies [22,23].

2.3. Surgical preparation

For the EMG recording, sterile surgery was performed on 6 rats in each group under anesthesia induced with intraperitoneal pentobarbital sodium (35 mg/kg bodyweight). The effective depth of anesthesia was determined by the abolition of the pedal withdrawal and blink reflexes. The surgical method is described in a previous study and was performed with minor modifications [11]. For electrode placement in the masseter, the muscle was exposed with a small incision in the skin of the left jaw, and one insulated stainless steel wire EMG electrode (SSM33A70, World Precision Instruments Inc., Sarasota, FL, USA) was implanted into the left deep masseter via a small needle hole. The electrode wire was tunneled subcutaneously through an incision along the dorsal surface of the cranium and held in place by securing it to the skull with dental cement (Rely X, 3M, St. Paul, MN, USA). Then, the incision was stitched with absorbable sutures. An additional reference electrode was also secured on the skull. After surgery, the rats were kept warm until recovery and given soft food for the following 2 days. The rats recovered for at least 7 days before the experimental test. To ensure the accuracy of electrode implantation, we conducted a post-mortem dissection for each animal and used methylene blue to display the location of the electrode head.

2.4. Restraint stress model

The animals in the RS and drug-treated groups were exposed to stress daily as described by Zafir et al. [24] for 4 h (8 am to 12 pm) over a period of 21 days. Restraint stress was accomplished by immobilizing animals in snug body-size cages of wire mesh, which restrain all physical movement without pain. The animals were deprived of food and water during the entire period of exposure to stress. Subsequently, the animals were released from their enclosure and provided free access to water and food.

2.5. Electrophysiological recordings and data analysis

Electrophysiological recordings were collected two times for each rat, the first before the stress intervention began and the second after the end of the 21-day restraint stress treatment (Fig. 1). The recording method, parameter settings and data analysis are described in a previous study and were performed with minor modifications [11]. During the experiment, the animals were housed in a plastic chamber in a sound-attenuated, ventilated and illuminated room. EMG activities were recorded by attaching a lightweight cable to the plug on the rat's head, which was connected to a commutator fixed upon the cage; then, the commutator was linked to the EMG 100c electromyographical amplifier (BioPAC, Goleta, CA, USA) and BioPAC MP150 system (BioPAC,