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Histological and histochemical effects after occlusion alteration in suprahyoid muscles

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ABSTRACT

This study verified the effect of unilateral teeth extraction on the suprahyoid muscles in gerbils (*Meriones unguiculatus*). Ten adult male gerbils weighing about 50 g had induced occlusal alterations by upper molar teeth extraction on the left side while the other ten animals were only subjected to surgical stress, control group. After 60 days, animals of both groups, experimental and control had the suprahyoid muscles removed and processed for histological and histochemical (adenosine triphosphatase (ATPase), nicotine adenine dinucleotide tetrazolium reductase (NADH-TR) and succinate dehydrogenase (SDH)) purposes. The fiber type area was estimated in % according to Weibel method (point-counting method) using a test-system. The myosinic ATPase pH 4.7 activity in the control group of the digastric, milohyoid and geniohyoid muscles presented a small area of type I fiber and a larger area of type IIa fibers; in the experimental group, significant contractile capacity alteration was not observed. Samples of the digastric, milohyoid and geniohyoid muscles, after SDH activity, showed a small area with high metabolic activity fibers, and a large area with intermediary and low metabolic activity fibers in the control group. The milohyoid muscle of the experimental group presented low metabolic fibers in a reduced area, in both sides, however without significant difference. In the experimental group, high metabolic fibers were observed on the left side in a reduced area in the geniohyoid muscle, but without statistical significance. Thus, the geniohyoid muscle did not change the metabolic activity after occlusal alteration. In conclusion, 60 days of unilateral malocclusion induced was able to alter the fibers oxidative activity of the suprahyoid muscles, however, it does not affect the contractile property of the fibers. The digastric muscle has adequate fibers to produce fast contraction and able to resist to fatigue in intermediate degrees, but became more fatigable after unilateral exodontia.

1. Introduction

The significant increase in painful symptoms related to stomatognathic system changes has been contributed to pay attention the researchers regard to basic mechanisms of clinical findings, in which the use of animals in experimental models have been of great value.

Studies about the mechanisms of morphofunctional changes (Gedrange et al., 2003), as well as the structure of muscles in the pathogenesis of occlusal disorders are scarce in the literature, despite knowing that the permanent correction of maxillomandibular malocclusion depends on the mechanisms of muscular adaptation (Thüer et al., 1992), which depends on the adaptive processes in the masticatory neuromuscular system (Ingervall and Bitsanis, 1986).

Gerbil is a docile rodent extensively used as an experimental animal in many studies related to cellular response after drugs and hormone administration, but not so much in studies focusing on the morphofunctional aspect of masticatory muscles and their relation to dental malocclusion.

Some studies related to the adaptive mechanisms in experimental animal models have revealed suppression in the development of masseter muscle (Maeda et al., 1990); morphological, biochemical and ultrastructural changes of muscle fibers after unilateral occlusion was induced are related to many factors

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(Bani et al., 1999; Bani and Bergamini, 2002; Bazan et al., 2008; Iyomasa et al., 2008; Muller et al., 2000; Nishide et al., 2001). It is important to consider that the mandible is suspended in the skull by the temporomandibular joint, especially related to the temporal and masseter muscles, and actively participates in swallowing, chewing, breathing and speech, by the synergic mechanisms of many muscles of the head and neck region (Arellano, 2002). Among the masticatory muscles, suprahyoid muscles (geniohyoid, milohyoid and anterior belly of digastric muscle) are of great interest in this study. Suprahyoid muscles move ahead and upwards the larynx and hyoid bone, while swallowing (Spiro et al., 1994; Jégo et al., 2001). In addition, milohyoid muscles act as diaphragm of the oral cavity (Sehirli and Cavdar, 1996). It has been widely known that the suprahyoid muscles balance the mandible through an intricate muscle, and they also participate in a variety of functions including swallowing, breathing and speech. Thus, the purpose of this study was to verify if these muscles are able to adapt and how they can adapt to occlusal changes promoted by unilateral teeth extraction in gerbil as experimental animal model. The initial hypothesis was to observe if the suprahyoid muscles, specifically, adapt to unilateral teeth extraction, the muscles on the right side should present some characteristics that differ from the left side, ipsilateral side to exodontia. Therefore, the aim of this study was to examine how the unilateral exodontia affects the suprahyoid muscles through routine histological techniques and adenosine triphosphatase (ATPase) activity, NADH and succinate dehydrogenase (SDH) reactions.

2. Material and methods

2.1. Animals

Twenty gerbils "*Meriones unguiculatus*," male, weighing approximately 55 g at the beginning of the procedures were used and randomly distributed in two groups: control ($n = 10$) and experimental ($n = 10$). These animals, of both groups, were used for histological and histochemical studies. Animals were lodged at animals' house of the Faculty of Dentistry of Ribeirão Preto, University of São Paulo, Brazil, and kept in number of five animals per box of polyethylene, with controlled temperature between 22 and 24 °C and with timer set to 12 h of lighting, daily. The animals received food and water "ad libitum". All procedures of this study were approved by the Local Ethics Committee on the Use of Animals.

2.2. Induction of occlusal alteration in animals

In the experimental group, the malocclusion by upper molar teeth extraction was induced on the left side. Control group, no build ups and served as sham-operated (this group was subjected to the same trauma during jaw opening for teeth extraction). Under aseptic conditions, all animals, control and experimental groups, received intraperitoneal anesthesia—tribromoethanol (0.25 g/kg of body weight). As prophylactic measure, it was administered to the animals a single dose of antibiotic (Pentabio-

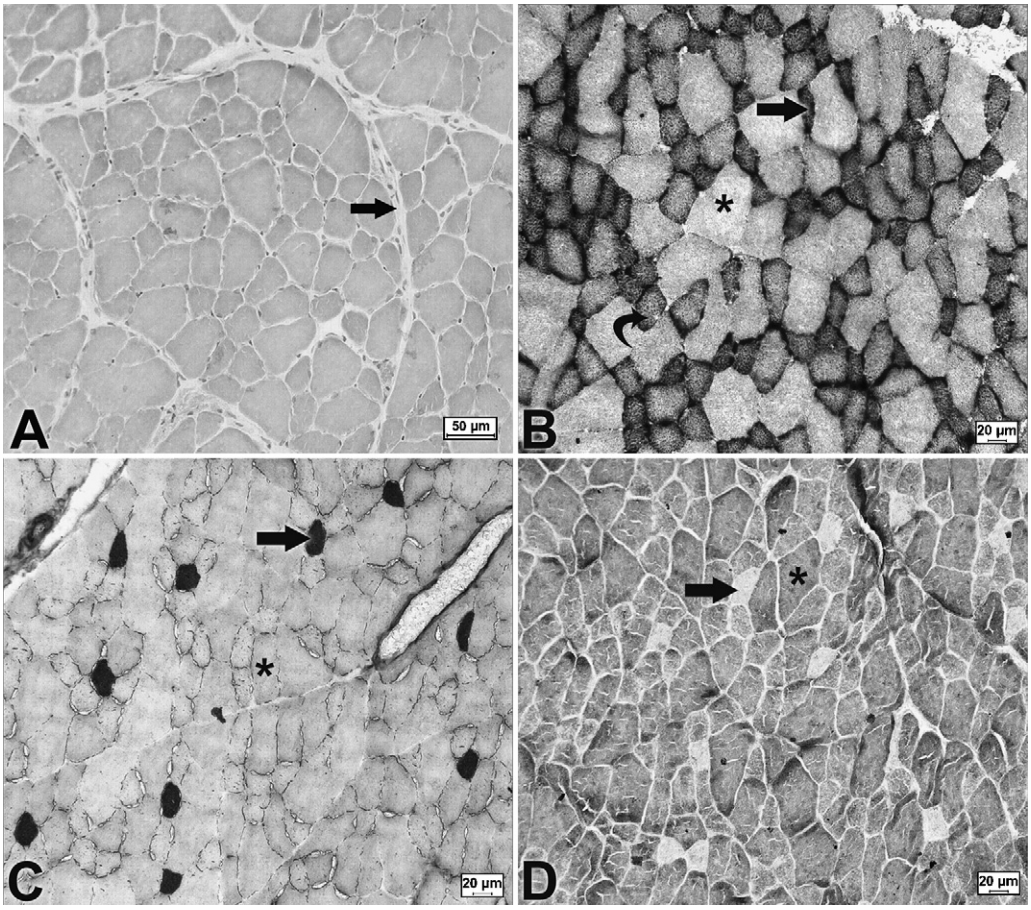

Fig. 1. Anterior belly of digastric muscle in cross-cryosections of the control group. (A) Stained with hematoxilin–eosin shows varied morphology and diameter fibers, and nervous fibers in the perimysium (arrow), bar = 50 μm. (B) Nicotinamida tetrazolium reductase (NADH-TR) activity revealed fiber with: high oxidative capacity (arrow), intermediary oxidative capacity (curve arrow), and low oxidative capacity (*), bar = 20 μm. Scarce type I fibers (arrow) and high proportion of type II fibers (*), after myofibrilar ATPase activity pH 4.3 (C) and pH 10.2 (D), bar = 20 μm.