Archives of Oral Biology 80 (2017) 10–17

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Mechanical mandible competence in rats with nutritional growth retardation

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ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 5 January 2017
Accepted 12 March 2017

Keywords:
Nutritional growth retardation
Mandible
Bone competence
β-Adrenergic blocker
Rat

ABSTRACT

Objective: In order to provide a better understanding of the sympathetic nervous system as a negative regulator of bone status, the aim of the study was to establish the biomechanical mandible response to different doses of a β-adrenergic antagonist such as propranolol (P) in a stress-induced food restriction model of growth retardation.
Methods: Rats were assigned to eight groups: Control (C), C + P3.5 (CP3.5), C + P7 (CP7), C + P14 (CP14), NGR, NGR + P3.5 (NGRP3.5), NGR + P7 (NGRP7) and NGR + P14 (NGRP14). C, CP3.5, CP7 and CP14 rats were freely fed with the standard diet. NGR, NGRP3.5, NGRP7 and NGRP14 rats received, for 4 weeks (W4), 80% of the amount of controls food consumed. Propranolol 3.5, 7 and 14 mg/kg/day was injected ip 5 days per week in CP3.5 and NGRP3.5, CP7 and NGRP7, CP14 and NGRP14, respectively. At W4, zoometry, mandible morphometry, static histomorphometric and biomechanical competence were performed.
Results: A dose of Propranolol 7 mg/kg/day induced interradicular bone volume accretion reaching a mandible stiffness according to chronological age.
Conclusion: These findings evidenced that sympathetic nervous system activity is a negative regulator of mandible mechanical competence in the nutritional growth retardation model. Propranolol 7 mg/kg/day, under the regimen usage, seems to be appropriate to blockade SNS activity on mandible mechanical performance in NGR rats, probably associated to an effect on bone mechanostat system ability to detect disuse mode as an error.

1. Introduction

Bone mass and mechanical performance are associated to bone modeling and remodeling mechanisms during vertebrate life, both regulated by signalling and nervous mechanisms (Amling, Takeda, & Karsenty, 2000; Elefteriou, 2008).

Previous studies by others reported sympathetic innervation in bone tissue (Mach et al., 2002) and the presence of adrenergic receptors in osteoblasts and osteoclasts (Togari, 2002). Indeed, bone cells effectors express β-2 adrenergic receptor, which appears to be the main adrenergic receptor in bone cells, although β1, α1B, and α2B-adrenergic receptors could also participate in bone cell function (Bonnet, Pierroz, & Ferrari, 2008; Pierroz et al., 2012).

Mechanical properties in axial and appendicular bones in growing rats are mostly affected by nutritional status. Suboptimal chronic energy intake outcome in physiological, metabolic, cellular and behavioral responses considered as survival advantages of the adaptive response, ensuring energy fluxes to tissues that demand constantly energy and reduce morbo-morbility risk (Lifshitz & Moses, 1988; Friedman et al., 2006). Moreover, food restriction affected negatively bone quality. Indeed, previous studies performed in our laboratory showed impaired mechanical femoral and mandible competence, in nutritional growth restricted (NGR) rats. This could be the consequence of altered bone mass and architectural distribution rather than its intrinsic quality (Boyer et al., 2005; Compagnucci et al., 2005).

The nutritional stress model in growing male rats was developed by a 20% restricted balanced diet given for a long time. This model closely resembles the nutritional status in childhood

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http://dx.doi.org/10.1016/j.archoralbio.2017.03.009
0003-9969/

which consume diets with insufficient total energy to sustain normal growth and weight gain (Friedman et al., 2006).

Considering hypothalamus as a centre involved in physiological, metabolic, autonomic, neuroendocrine and behavior functions, malnutrition during high growth rate velocity could outcome in different morpho-functional brain dysfunctions (Soto-Moyano, Belmar, Perez, Ruiz, & Hernandez, 1995); severity, that depends on the time of onset and/or the duration and degree of food restriction. Previously, we demonstrated that global sympathetic hypothalamic activity is enhanced in NGR rats (Olivera et al., 2008). Moreover, corticosterone serum levels were about 300% higher in NGR rats as compared to control rats, suggesting an increase in hypothalamic pituitary adrenal (HPA) axis activity (unpublished data). The impaired biomechanical bone performance observed in NGR rats (Boyer et al., 2005; Compagnucci et al., 2005) could be due, at least in part, to the overall increase of hypothalamic noradrenergic system and HPA axis activities, in response to mild chronic food restriction (Olivera et al., 2008).

Furthermore, β-adrenergic antagonist administration to NGR rats enhanced mechanical effectiveness of a weight-bearing bone like femur, due to an augment in cortical bone mass and its improvement in spatial distribution (Lezón et al., 2009; Lezón, Pintos, Olivera, Bozzini, & Boyer, 2012; Pintos, Lezón, Bozzini, Friedman, & Boyer, 2013; Lezon et al., 2016; Tasat et al., 2014).

The mandible is not a weight-bearing bone as femur (Mavropoulos, Rizzoli, & Ammann, 2007; Van Eijden, 2000). It is considered as a "load-bearing bone" not influenced by body weight but by the mechanical masticatory loading. Indeed, previous studies by us showed different behavior in mandible and femur, in NGR rats (Compagnucci et al., 2005). In congruence, other authors showed similar results in rats under protein deficiency in quantity or quality (Bozzini, Champin, Alippi, & Bozzini, 2011; Alippi, Picasso, Huygens, Bozzini, & Bozzini 2012).

In order to provide a better understanding of the sympathetic nervous system (SNS) as a negative regulator of bone status, the aim of the study was to establish the biomechanical mandible response to different doses of a β-adrenergic antagonist such as propranolol (P) in a stress-induced food restriction model of growth retardation.

2. Materials and methods

2.1. Animals

Weanling male Wistar rats (mean initial body weight: 48.90 ± 1.60 g) were housed and kept under 12 h light–12 h dark cycles and maintained at 21 ± 1 °C with 50-60% humidity. The experiment was conducted in accordance with the principles and procedures outlined in the National Institute of Health Guide Lines for the Care and Use of Laboratory Animals (National Institute of Health, 1985, revised 1990National Institute of Health, 1985National Institute of Health, 1985, revised 1990) and approved by the University of Buenos Aires Ethic Committee.

2.2. Diet

Animals were fed with a standard diet (Purina chow, Gilardoni SA, Buenos Aires, Argentina) of the following composition (g/100 g): protein, 22.7; lipids, 7.09; fiber, 6.0; Ca, 1.3; P, 0.8; ashes, 6.50; water, 7.60; dextrin, balance.

2.3. Experimental design

Rats were randomly assigned to eight groups: Control (C), C + P3.5 (CP3.5), C + P7 (CP7), C + P14 (CP14), NGR, NGR + P3.5 (NGRP3.5), NGR + P7 (NGRP7) and NGR + P14 (NGRP14). C, CP3.5, CP7 and CP14 rats were fed freely with the standard diet. NGR, NGRP3.5, NGRP7 and NGRP14 rats received, for 4 weeks (W4), 80% of the amount of food consumed the previous day by their respectively control groups, corrected by body weight. All rats had free access to water. Propranolol (Richmond Laboratory, Buenos Aires, Argentina) 3.5, 7 and 14 mg/kg/day was injected ip 5 days per week, for four weeks in CP3.5 and NGRP3.5, CP7 and NGRP7, CP14 and NGRP14, respectively. C and NGR received saline injections at an identical dosage regimen. Propranolol regimen administration and doses concentration were chosen according previous studies (Takeda et al., 2002). C and NGR received saline injections at an identical dosage regimen. Body weight and dietary intake were recorded daily, and body length, every 4 days. A Mettler PC 4000 scale (Zurich, Switzerland) was used to measure body weight with an accuracy of ±1 mg. For length measurements, animals were anaesthetized light anesthesia (a mixture of 2% xylazine hydrochloride (0.5 mg/100 g i.p. Konig Laboratories, Buenos Aires, Argentina)) and 5% ketamine hydrochloride (5 mg/100 g i.p. Holliday-Scott SA. Buenos Aires, Argentina). Body length was determined with a scaled ruler in mm from the nose tip to the last hairs of the tail base. Food consumption was measured by using special feeders, which allowed the recovery of spilled food. Food intake was weighed daily with a Mettler scale (accuracy ±1 mg). Animals were euthanized under anesthesia: 0.1 ml of ketamin hydrochloride (100 mg/ml, Holliday Lab., Buenos Aires, Argentina)/100 g body weight was mixed with 0.02 ml of xylazine (100 mg/ml, Konig Lab, Buenos Aires, Argentina)/100 g body weight by intramuscular injection, after 4 weeks of experimental period (W4). The hemimandibles from each animal were then dissected, cleaned of adhering soft tissue, weighed in a Mettler scale and stored at −20 °C wrapped in gauze soaked with Ringer's solution in sealed plastic bags, in accordance with Turner and Burr (1993). Each bone was thawed at room temperature before analysis. Mandibular growth was estimated directly on the right hemimandible by taking measurements (to the nearest 0.05 mm) by the use of digital callipers according to Eratalay, Simmonds, Mofty, Rosenberg, and Nelson (1981) with some modifications (Alippi, Meta, Boyer, & Bozzini, 1999). A Mettler PE 600 scale (Zurich, Switzerland) was used to measure mandible weight expressed in g. Bones were used for mechanical and for histomorphometric studies.

2.4. Mandibular morphometric properties

Mandibular growth as a whole was estimated directly from mandibular weight and area on the right mandible (to the nearest 0.05) by using a digital calliper according to Eratalay et al. (1981) and modifications (Alippi et al., 1999) (Fig. 1) as follows: (1) mandibular area was calculated from a triangle formed between three stable points: the most superior posterior point of the coronoid process (B), the most posterior point of the angular process (C), and the most anterior inferior bone point of the interdental spine (O); (2) mandibular length was measured from the most anterior inferior bone point of the interdental spine to the furthest point on the articular surface of condyle (length OA); (3) the length of the base of the jaw was estimated by the distance OC; (4) length of incisor alveolar process: distance from the most anterior superior bone point of the interdental spine (i) to the point immediately anterior to the anterior surface of the first molar (K) (5) length of alveolar process: measured from K to the alveolar process immediately posterior to the posterior root of the third molar (L). The mandibular length was divided into anterior (KL + Ki) and posterior (OA-anterior part) parts by a vertical line drawn perpendicular to the oclusal plane of the molars immediately posterior to the posterior surface of the third molar. These specific measurements were chosen because they give information