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ORIGINAL ARTICLE

Effect of capsaicin-sensitive sensory neurons on bone architecture and mechanical properties in the rat hindlimb suspension model

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Summary *Background/Objective:* The participation of sensory neural regulation in bone metabolism has been widely studied. However, the physiological role of sensory neural regulation in the functional adaptation to weight bearing is not clear. This study was conducted to investigate the effect of capsaicin-induced sensory neuron lesions on cancellous architecture properties in a hindlimb suspension (HLS) model.
Methods: Thirty-two female rats were randomly assigned to four groups. Groups b and d underwent systemic capsaicin treatment, whereas Groups a and c were treated with vehicle. Then, Groups c and d were subjected to HLS, whereas Groups a and b were allowed hindlimbs full loading. The proximal trabecular and mid-shaft cortical bone structure were evaluated via microcomputed tomography, and the biomechanical properties of the tibial mid-shaft were assessed using the four-point bending test.
Results: The trabecular bone volume was reduced by 40% and 50% in Groups b and c, respectively, and was also reduced significantly in Group d. Trabecular thickness and trabecular separation in Group b were not significantly different from those of Group a. The cortical bone area fraction showed no significant difference among all groups. Compared with Group a, the ultimate strength in Group b decreased by 20.3%, whereas it did not change significantly in Group c.
Conclusion: The results suggest that capsaicin-sensitive sensory neurons play an important role in bone modelling. The effect of capsaicin is similar to HLS. However, HLS has no add-on effect to capsaicin in the reduction of bone density and mechanical properties.

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Translational potential of this article: This study gives clues to the function of sensory neurons in bone modelling.

Introduction

It has been demonstrated that bone tissue is highly sensitive to mechanical stress and can change its shape, structure, and mineral density. The mechanism of bone loss due to immobilisation, paralysis, long-term bed rest, and spaceflight, and the mechanism of bone mass increase owing to regular resistance exercises indicate that bone turnover is sensitive to both external loads arising from gravitational loading and to internal loads generated by muscle activity [1,2]. The ability of bone to sense the mechanical stimuli was considered to be a local interaction between the loading and the affected bone cells [3]. However, the participation of neural regulation has been demonstrated in both local and systemic bone metabolism based on the innervation of the sympathetic and peripheral sensory neurons in bone via osteoblastic and osteoclastic cell guidance [4—6]. Substance P (SP) and calcitonin gene-related peptide (CGRP), which are important neuropeptides, are synthesised in unmyelinated sensory neurons, which are the target of capsaicin, and released from their peripheral terminals [7]. It has been demonstrated that bone integrity was compromised by decreased levels of local neuropeptide in bone in some hereditary sensory neuropathies, such as familial dysautonomia [8,9]. The innervation of the developing mouse femur was guided by nerve growth factor—neurotrophic tyrosine kinase receptor type 1 signalling, in turns, to promote vascular invasion of the ossification centres and osteoprogenitor cell lineage progression [6].

Capsaicin is the major pungent component of hot chili peppers. Transient receptor potential (TRP) vanilloid subfamily member 1 (TRPV1) is identified as the receptor of capsaicin [10]. TRPV1 is expressed in unmyelinated and small-diameter myelinated sensory neurons [11]. The activation of TRPV1 by capsaicin induces Ca^{2+} and Na^{+} influx into the sensory neuron, causing an excitotoxic effect. Large sensory neurons, motor neurons, and sympathetic neurons are affected by lack of vanilloid receptors [12,13]. It has been demonstrated that only the unmyelinated and small-diameter myelinated sensory neurons are destroyed when capsaicin is administered in neonatal rats, whereas the large sensory afferent, motor, and sympathetic fibres are unaffected [14,15]. Capsaicin treatment could deplete SP and CGRP in peripheral nerves, but not in the central nervous system [16,17]. The depletion rate of the unmyelinated fibres in adult rat induced by capsaicin injection has been reported to reach 90—95% [18]. In addition, systemic capsaicin treatment of the adult rat results in the death of at least 50% of the vagal afferent neurons in dorsal root ganglia [19].

Mechanical stimulation influences bone metabolism. However, will there be any difference in the response if the sensory nerve fibres in bone tissue are partially or completely destroyed? The aim of this study is to investigate the role of capsaicin-sensitive sensory neurons in bone modelling in a rat hindlimb suspension (HLS) model. In this study, the unmyelinated sensory fibres were depleted by systemic capsaicin treatment under a functional disuse HLS condition. The bone structure and biomechanical properties of the rat tibia were evaluated to assess the bone response to loading changes after capsaicin treatment.

Materials and methods

Animals

The *in vivo* experiment was approved by the Institutional Animal Care and Use Committee (IACUC), Stony Brook University (Stony Brook, NY, USA). Thirty-two 4-month-old female Sprague—Dawley rats weighing 245 $\pm$ 15 g were used in the study. The animals, which were randomly assigned in equal numbers ($n = 8$) to four groups, received different interventions as follows: Group a, control; Group b, capsaicin only; Group c, HLS only; Group d, combination (HLS after capsaicin treatment). All animals were raised in separate cages in a temperature-controlled room (22°C) with a 12:12-hour light—dark cycle. Standard rodent chow and water were provided *ad libitum* throughout the experiment. All experimental procedures were in accordance with the IACUC guidelines.

Capsaicin treatment

The rats were injected subcutaneously in the back with capsaicin (Sigma, St. Louis, MO, USA). To prevent reflux from the needle tract, the needle was left in the skin for 60 seconds after the injection. The capsaicin treatment protocol consisted of three injections. The initial one was 25 mg/kg, the second one was 50 mg/kg at 6 hours later, and the third one was 50 mg/kg at 24 hours after the first injection [20]. To prevent pulmonary oedema, all rats in the capsaicin treatment groups (Groups b and d) had no water supply 6 hours prior to the capsaicin injection [21]. The protocol was repeated every 2 weeks (Weeks 1, 3, 5, and 7), and all experiments were performed during Weeks 5 to 8. The groups without capsaicin treatment (Groups a and c) underwent the same injection protocol with vehicle (10% Tween 80, 10% ethanol and 80% saline).

Hindlimb suspension

The rats in Groups c and d were hindlimb suspended for 4 weeks during Weeks 5 to 8 following established procedures [22]. Briefly, the animal's tail was cleaned with 70%