Does Electrical Stimulation of the Sciatic Nerve Prevent Suspension-Induced Changes in Rat Hindlimb Bones?

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Abstract: Osteoporosis due to mineral loss is a major health problem resulting from long-term spaceflight. The development of a suitable countermeasure is essential because an advanced decrease in bone density could be irreversible. Therefore the current study was performed to test our hypothesis that the loading of bones by electrical stimulation-induced muscle contraction may prevent the mineral loss caused by gravitational unloading and bone growth will be maintained. During 10 d of hindlimb suspension, electrical stimulation at 1, 50, or 100 Hz was administered through the left sciatic nerve at the gluteal region of rats with approximately 300 g body weight. The dry weight, mineral content, and

mineral density in hindlimb bones were analyzed. The dry weight and mineral content of femur and tibia-fibula in hindlimb-suspended rats tended to be less than in the age-matched cage controls. However, these detrimental effects were prevented by stimulation at 50 and 100 Hz. A positive effect of stimulation was seen even in the nonstimulated limb, although greater effect was induced in the stimulated limb. It is suggested that loading by stimulation-induced muscle contraction at higher frequencies is beneficial for the maintenance of bone growth or the prevention of mineral loss, or both, during hindlimb suspension in rats. [Japanese Journal of Physiology, 48, 33–37, 1998]

Key words: hindlimb suspension, electrical stimulation, bone weight, mineral content, mineral density.

Gravitational unloading causes morphological or mechanical changes, or both, in hindlimb bones of rats [1–10]. Vailas *et al.* [8] reported that ground reaction force is an important factor in maintaining homeostasis in rat hindlimb bone. However, the production of this force is impossible in a weightless environment. Therefore the development of effective countermeasures is required.

It is suggested that the remodeling of bone is regulated in response to mechanical stress caused by muscle contraction [11]. Recently Zerath *et al.* [12] reported that bone formation in hindlimb-suspended rats was enhanced by electrical stimulation at 10 Hz. Although the electromyogram (EMG) activity disappears immediately when the hindlimbs are suspended, it is

gradually recovered and normalized during unloading [13, 14]. However, an atrophy or growth failure is still induced in muscles [15] and bones [6]. Thus the mechanism responsible for the adaptation of bones to gravitational unloading is still unclear. Further, it is not known whether the detrimental effect on bones induced by gravitational unloading can be prevented by electrical stimulation through the sciatic nerve. The current study was therefore performed to test our hypothesis that the loading of bones by muscle contraction induced by an electrical stimulation can prevent the detrimental effects of hindlimb suspension. Stimulation at 1, 50, and 100 Hz was selected to investigate the effects of lower (1 Hz) and higher (50 and 100 Hz) levels of loading.

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METHODS

The animal care and treatment were performed following the institutional guidelines for experimental animals. Forty-five male Wistar rats with body weight of approximately 300 g (9 weeks old) were used in this investigation. The rats were housed in a room where the light:dark cycle (12:12 h) was regulated. The humidity and temperature were maintained at approximately 55% and 23°C. The rats were randomly divided into 5 groups ($n=9$ each): 1) normal cage control, 2) hindlimb-suspended control, 3) hindlimb suspended and stimulated at 1 Hz, 4) hindlimb suspended and stimulated at 50 Hz, and 5) hindlimb suspended and stimulated at 100 Hz. They were pair-fed a powdered diet (20 g) containing vitamin D₃ at 250 IU/100 g (Nihon CLEA, Tokyo, CE-2). The diet also contained 7.1% minerals, including 1.18 g of calcium and 1.08 g of phosphorus per 100 g of dry weight. Tail-cast suspension was performed to unload the hindlimbs in groups 2–5. However, a harness was also placed loosely around the body, as in the previous studies [16, 17], to keep the electrode wires stable. The suspended rats were able to eat food and drink water by using their forelimbs *ad libitum*.

Following anesthesia with I.P. injection of sodium pentobarbital (5 mg/100 g body weight), electrode implantation and hindlimb suspension were performed. As a stimulation apparatus, two stainless steel wires (30 gauge) were fixed along the inside of a half-tygon tubing (6 mm long and 1.5 mm inner diameter). The wires, except for the portion (4 mm) to be contacted with the nerve fibers, were insulated with enamel coating. The wires extended from the tygon tubing were encased in a silastic tubing. This apparatus was placed around the left sciatic nerve fibers at the gluteal region, and the other ends of the wires were connected to an electrical stimulator. The skin was sutured. The right limb was sham-operated in all 5 groups.

Twitch or train stimulation was performed in 3 groups (1, 50, and 100 Hz) alternating the anode and cathode for each pulse with 100- μ s duration to avoid polarization at the electrodes. Twitch contraction was induced continuously at 1 Hz for 4 h. The 50 Hz stimulation for 2 s with a 3-s interval was also performed for 4 h. The other group was stimulated at 100 Hz for 1 s with 4-s intervals for 4 consecutive hours. The same patterns of train stimulation were repeated again following 6 h of recovery in the same day. This electrical stimulation for 8 h per day was performed for 10 consecutive days. The stimulus intensity was readjusted in each rat to the maximum at which the rat, ex-

cept the hindlimb muscles, did not respond to stimulation. The intensity was variable daily, depending on the rats.

Rats were sacrificed by I.P. overdose injection of sodium pentobarbital following recovery of approximately 12 h from the last bout of stimulation on the 10th day. The suspended groups were anesthetized when they were still hindlimb-suspended to avoid any effects of acute loading on skeletal muscles and bones. Following the body weight measurement, femur and tibia-fibula were removed from both hindlimbs. The bones were soaked in water for 2–3 weeks so that the remaining tissues could be easily cleaned from the bones. The weight was measured after drying the bones at 64°C for 2 d. The mineral content of whole bone (BMC) was analyzed by dual energy X-ray absorptiometry (Hologic QDR-1000). Bone mineral density (BMD), that is, the bone mineral content per gram dry weight, was also calculated. The statistical significance, examined by ANOVA followed by Scheffe's test or Student's *t*-test for paired comparison between the left and right limbs, was accepted at $p<0.05$.

RESULTS

The mean ($\pm$ SEM) body weights of cage control rats was increased by approximately 3% from 300 ± 12 to 309 ± 15 g during 10 d of experiment ($p<0.01$). However, the weights of the hindlimb-suspended rats with or without electrical stimulation were significantly less than the presuspension levels and those of the age-matched cage controls ($p<0.05$). The weights in the hindlimb-suspended rats without stimulation was 272 ± 13 g. Those in the groups stimulated at 1, 50, and 100 Hz were 221 ± 14 , 290 ± 14 , and 251 ± 16 g, respectively.

No effect of sham operation was observed when the left and right limbs were compared in the cage control and suspended group without stimulation. Both the dry weight and the BMC of femur and tibia-fibula in the freely hindlimb-suspended rats tended to be less than in the cage controls (Figs. 1 and 2). But the detrimental effects caused by suspension unloading were prevented by electrical stimulation. These effects were obvious in both the femur and tibia-fibula when the train stimulation was performed at either 50 or 100 Hz. The femur dry weights in the 1 and 50 Hz groups were not significantly different from the freely suspended group, even though the mean values tended to be greater (Fig. 1). But the suspension-related decrease in the femur dry weight was prevented by 100 Hz stimulation. The effects of stimulation tended to be

Fig. 1. Dry weight, bone mineral content (BMC), and bone mineral density (BMD) in femur. Mean $\pm$ SEM. $^{\dagger}p < 0.05$ vs. free suspension and $*p < 0.05$ vs. nonstimulated right side.

induced even in the nonstimulated side. But the dry weight was greater in the stimulated side than in the contralateral side, especially in the 1 and 100 Hz groups ($p < 0.05$). Similar responses were observed in the BMC. The BMC in the bones stimulated at 50 or 100 Hz was significantly greater than BMC in the freely suspended group ($p < 0.05$). The BMD remained constant in response to suspension unloading and electrical stimulation.

The responses of the dry weight and BMC in the

Fig. 2. Dry weight, bone mineral content (BMC), and bone mineral density (BMD) in tibia and fibula. Mean $\pm$ SEM. $^{\dagger}p < 0.05$ vs. free suspension and $*p < 0.05$ vs. nonstimulated right side.

tibia and fibula were similar to those in the femur (Fig. 2). The dry weights of bones stimulated at 50 and 100 Hz were significantly greater than those in the suspended group without stimulation ($p < 0.05$). As was seen in the femur, stimulation effects tended to be induced in bones from the nonstimulated limb. However, the beneficial effects of stimulation in the 1, 50, and 100 Hz groups were significantly greater than in the control side ($p < 0.05$). Although similar patterns of responses were noted in the BMC, a statistically

significant effect was seen only in the bone stimulated at 50 Hz. The BMC in this group was significantly greater than in the free suspension group and the contralateral side ($p < 0.05$).

DISCUSSION

Cyclic stretching of tendons and bending of bones during locomotion stimulate bone remodeling [18]. Beneficial effects on bones by external load application or bending were also reported [19–22]. Electrical stimulation of leg muscles (8 h/d for 19 d) at 10 Hz caused an improved trabecular bone formation in tibial metaphysis of hindlimb-suspended rats, although suspension-induced bone loss was not prevented effectively [12]. It is suggested that frequencies of loading between 5 and 20 Hz are more potent osteogenic stimuli than lower frequencies are [19, 22]. Better effects were seen in response to 50 or 100 Hz stimulation in the current study. These beneficial effects in response to higher frequencies may be related to a greater load application to the bones as a result of fusion of muscular tension development.

The bone weight and BMC in the 1 Hz group normalized by body weight at the end of experiments tended to be similar to those in the 50 and 100 Hz groups, since the body weight was less (data not shown). But those levels in the 1 Hz group relative to the body weight before suspension were lower than in the other stimulated groups, and the mean values were insignificantly greater than in the free suspension group. Therefore it is also suggested that the suspension-induced loss of bone mineral can be inhibited to some degree by stimulation at 1 Hz.

Positive effects of stimulation were also induced even in the nonstimulated side. This phenomenon may be caused by an increased loading suggested by an enhanced EMG activity in the nonstimulated side, when the sciatic nerve in the contralateral side was stimulated. However, the magnitude of EMG was less than in the stimulated limb. Significantly less dry weight and BMC in the nonstimulated limb than in the stimulated side could be related to the loading at a lower level.

The effects of electrical stimulation, relative to the freely suspended bones, tended to be greater in the femur than in the tibia and fibula ($p > 0.05$). The magnitude of EMG in the vastus lateralis was greater than in tibialis anterior and gastrocnemius. Thus more strain may be applied to the femur, since the mass of thigh muscles is also obviously greater.

The bone weight and BMC in the 50 and 100 Hz groups tended to be even greater than those in the

age-matched cage controls ($p > 0.05$). In general, agonists and antagonists do not contract simultaneously during normal locomotion. But the electrical stimulation through the left sciatic nerve in the current study induced a simultaneous contraction, detected by EMG, of almost all muscles in the left limb. Thus it is speculated that the load applied to the bones in the 50 and 100 Hz groups might be greater than in the cage controls, although we did not measure the loads induced by the various types of stimulation or locomotion. As far as we know, the load applied to the bone due to muscle contraction has not been quantified yet. The hypothesis of the present study that the loading of hindlimb bones by muscle contraction induced by electrical stimulation can prevent the detrimental effects of hindlimb suspension was approved. However, such stimulation at a given constant frequency may not be physiological for muscles, since the frequency characteristics are different for fast- and slow-twitch muscle fibers.

It is possible that the suspended rats spilled food in some amount because of the strange posture, even though all rats were pair-fed. Decreased food consumption may influence the growth and mineral content of bones. However, it is also reported that rats gained less bone weight per gram of food consumed during spaceflight than in ground controls [3]. Thus the differences seen in the bones between the control and experimental groups in the current study may not be directly caused by inanition as a result of reduced food consumption. Furthermore, beneficial effects were observed in bones of stimulated groups even though their body weights were less than the age-matched controls and presuspension levels (~ 300 g), may be partly due to stress.

Generally, osteoporosis is associated with decreases in BMC and BMD. However, the BMD in the hindlimb-suspended group was normal in the current study, even though the BMC was reduced. A significant increase in body weight (3%, $p < 0.01$) was seen in the cage control rats during 10 d of experiments. This growth may affect the bone weight and mineral content. Since the parameters in bones were analyzed only at the end of experiments in the current study, it is not clear how the growth itself affected the data. However, the bone growth may be inhibited during suspension, because the growth of ankle dorsiflexor tibialis anterior and extensor digitorum longus, which do not atrophy, is inhibited by hindlimb suspension of rats [23]. Therefore the results seen in the bones of hindlimb-suspended rats might be caused by both growth retardation and mineral loss. It is suggested that these detrimental effects of gravitational unload-

ing on bones are prevented by loading caused by electrical stimulation, especially at higher frequencies.

In conclusion, the effects of electrical stimulation on hindlimb bones during 10 d of suspension were studied in adult rats. Electrical stimulation at 1, 50, and 100 Hz was administered through the left sciatic nerve at the gluteal region. The dry weight and mineral content of femur and tibia-fibula in hindlimb-suspended rats tended to be less than in the age-matched cage controls. However, these detrimental effects were prevented by stimulation at 50 and 100 Hz. A positive effect of stimulation was seen even in the nonstimulated limb, although greater effect was induced in the stimulated limb. It is suggested that loading by stimulation-induced muscle contraction at higher frequencies is beneficial for the prevention of suspension-induced mineral loss in rat hindlimb bones.

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