Unloading Induces Osteoblastic Cell Suppression and Osteoclastic Cell Activation to Lead to Bone Loss via Sympathetic Nervous System*

Received for publication, April 18, 2005
Published, JBC Papers in Press, June 16, 2005, DOI 10.1074/jbc.M504179200

Hisataka Kondo‡‡§§, Akira Nifuji‡, Shu Takeda‡, Yoichi Ezura‡, Susan R. Rittling‡, David T. Denhardt‡, Kazuhisa Nakashima‡, Gerard Karsenty‡, and Masaki Noda‡‡§§***

From the ‡Department of Molecular Pharmacology, the §§21st Century Center of Excellence Program for the Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone, the **ABJS Integrated Action Initiative in JSPS Core to Core Program, and ***Hard Tissue Genome Research Center, Tokyo Medical and Dental University, Tokyo 101-0062, Japan, ‡Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, and ‡‡Rutgers University, Rutgers, New Jersey 08854

Osteoporosis is one of the major health problems in our modern world. Especially, disuse (unloading) osteoporosis occurs commonly in bedridden patients, a population that is rapidly increasing due to aging-associated diseases. However, the mechanisms underlying such unloading-induced pathological bone loss have not yet been fully understood. Since sympathetic nervous system could control bone mass, we examined whether unloading-induced bone loss is controlled by sympathetic nervous tone. Treatment with β-blocker, propranolol, suppressed the unloading-induced reduction in bone mass. Conversely, β-agonist, isoproterenol, reduced bone mass in loaded mice, and under such conditions, unloading no longer further reduced bone mass. Analyses on the cellular bases indicated that unloading-induced reduction in the levels of osteoblastic cell activities, including mineral apposition rate, mineralizing nodule formation. Unloading-induced increase in osteoclast number and surface as well as urinary deoxypyridinoline was all suppressed by the treatment with propranolol and that isoproterenol-induced reduction in the levels of mineralized nodule formation was suppressed by propranolol treatment and that isoproterenol-induced reduction in these levels of bone formation parameters was no longer suppressed by unloading. Unloading-induced reduction in the levels of mineralized nodule formation in bone marrow cell cultures was suppressed by propranolol treatment in vivo. In addition, loss of a half-dosage in the dopamine β-hydroxylase gene suppressed the unloading-induced bone loss and reduction in mineralized nodule formation. Unloading-induced increase in the levels of osteoclastic activities such as osteoclast number and surface as well as urinary deoxypyridinoline was all suppressed by the treatment with propranolol. These observations indicated that sympathetic nervous tone mediates unloading-induced bone loss through suppression of bone formation by osteoblasts and enhancement of resorption by osteoclasts.

Osteoporosis is one of the major age-related diseases in our modern world (1–4). Especially, high fracture risk in osteoporosis patients results in not only loss of quality of life but also loss of life in a certain fraction of aged patients. The number of osteoporosis patients is estimated to be close to 10% of the whole population in many advanced countries. Among this patient population, a significant number of patients have disuse osteoporosis based on bedridden conditions caused by aging-related cardiovascular as well as cerebrovascular diseases (5).

Bone has been known to be lost upon the removal of the mechanical stimuli, and prolonged lack of mechanical stimuli leads to disuse osteoporosis (5–9). However, the mechanisms underlying such disuse osteoporosis are largely unknown (2, 5, 6). Bone mass is determined by the actions of osteoblasts, which make bone, and those of osteoclasts, which resorb bone (2, 12–15). The balance between the two activities is under the control of hormones and cytokines. Usually, simultaneous changes in the two activities are considered to be coupled to compensate bone loss. However, in the case of disuse osteoporosis, bone formation activities are not enhanced even in the presence of enhanced bone resorption. Rather, bone formation is significantly suppressed in disuse osteoporosis (16, 17).

Therefore, disuse osteoporosis is a critical pathological situation where bone mass is continuously lost without having any compensatory activity against the reduction of bone. However, how such a critical reduction in bone formation occurs in unloading-induced pathological bone loss is not yet known.

Bone formation and bone resorption are under the control of the systemic hormones and local cytokines (2, 6). However, none of these factors have been proven to be the major cause of the disuse osteoporosis. In addition to the bedridden patients, astronauts under gravity conditions also lose bone due to the loss of mechanical stress. Analysis of such astronauts returning from space indicated that sympathetic nervous tone is enhanced in their muscle (18, 19). Sympathetic nervous system would regulate bone mass via bone formation by osteoblasts systemically (20). However, nothing has been known about how this system is related to pathophysiology in bone metabolism in the body. Therefore, we examined whether sympathetic nervous tone is involved in reduction of bone mass in a disuse osteoporosis model via osteoblastic and osteoclastic cells using hind limb unloading.

MATERIALS AND METHODS

Animals—Male 129 or C57BL/6J mice (10–14 weeks old) were used for the experiments. Mice were housed for at least 1 week prior to the study.
The mice were subjected to either intraperitoneal injections of propranolol (20 μg/kg of body weight/day) (21) or dopamine β-hydroxylase (DBH)1 gene deletion experiments, heterozygous knockout mice with a C57BL/129sv F2 background and wild type litter mate mice were used (13- and 17-week old females) (24). All of the mice were injected intraperitoneally with calcine at 4 mg/kg at 4 and 2 days before sacrifice. After treatment for 10 or 14 days, mice were anesthetized with trichloroethane at 200 mg/kg and were sacrificed by cervical dislocation.

**Hind Limb Unloading Model—**Hind limb unloading was conducted by applying a tape to the surface of the hind limb to set a metal clip (10, 16). The end of the tape was fixed for an extended bar. The height of the bar was adjusted to maintain the mice at an ~30° head down tilt with the hind limbs elevated above the floor of the cage. The mice were subjected to hind limb unloading for 10 or 14 days. Loaded control mice were also housed individually under the same conditions except for hind limb unloading for the same duration.

**Body Weight—**The body weight of the mice was monitored during the experimental period. There were no significant changes in body weight in any of the groups during the course of the study. This confirmed that stress could be considered minimal in our experiments as previously described (16, 21).

**Two-dimensional Micro-CT Analysis of Bone—**Bone volume/tissue volume (BV/TV) was determined based on two-dimensional micro-CT analyses using a micro-CT apparatus (Musashi, Nittetsu-ELEX Co., Kitakyushu City, Japan). The data were recorded with a calibrated images analysis system (Luzex-F, Nireco). The fractional bone volume (BV/TV) was obtained in an area of 0.47 mm² with its closest and furthest edges at 0.28 and 0.84 mm, respectively, distal to the growth plate of the proximal ends of the tibiae. The threshold level for the measurements was set at 110 for the analyses (16, 21).

**Histomorphometric Analysis of Bone—**At the end of the experiment, the right and left femora of each mouse were removed and fixed in 70% ethanol.

1 The abbreviations used are: DBH, dopamine β-hydroxylase; BV/TV, bone volume/tissue volume; BFR, bone formation rate; TRAP, tartrate-resistant acid phosphatase; MAR, bone mineral apposition rate; MS, mineralizing surface; CT, computed tomography.
FIG. 2. Guanethidine treatment suppressed unloading-induced pathological bone loss. During hind limb unloading of mice (129 strain) for 14 days, guanethidine treatment or vehicle was administered using an osmotic minipump (model 1002). The number of the mice used for the experiments represented in each bar is indicated as N. During the 14-day hind limb unloading of 129 mice, guanethidine treatment was carried out.
Unloading Reduces Bone Mass via Sympathetic Tone

Fig. 3. Isoproterenol treatment suppressed bone mass levels, and no further suppression was observed by unloading. Two-dimensional micro-CT tomography and BV/TV of the tibiae of loaded and unloaded mice treated with β-agonist isoproterenol. During 10-day hind limb unloading of C57BL/6 mice, they were injected intraperitoneally with either isoproterenol or vehicle. The number of mice in each group is indicated as N. a, two-dimensional micro-CT pictures of the midsagittal planes of the proximal regions of the tibiae after 14 days of hind limb unloading (Unload) or loading (Control Load) in vehicle-treated or isoproterenol-treated 129 mice. Micro-CT analyses were conducted as described under “Materials and Methods.” b, fractional trabecular BV/TV was obtained based on the image analysis of micro-CT pictures of the tibiae after 10 days of either hind limb unloading (Unload) or loading (Control Load) in vehicle-treated or isoproterenol-treated mice. Data are expressed as means and S.D. *, statistically significant difference (p < 0.05), #, statistically significant difference between the vehicle group and drug-treated group (p < 0.05) (either control-loaded or unloaded groups).

Fig. 4. Propranolol treatment suppressed unloading-induced reduction in bone formation parameters. Mice were treated with propranolol as described in the legend to Fig. 1. The number of mice in each group is indicated as N. a, calcine double-labeled surfaces of the bones in the metaphyses of the femora were analyses after hind limb unloading (Unload) or loading (Control Load) in vehicle- or propranolol-treated mice. The arrows indicate the lines of calcine labeling (white) used to obtain data shown in b, c, and d. b, c, and d, an unloading-induced reduction in osteoblastic activity in vivo does not occur in propranolol-treated mice. In the undecalcified sections of the distal ends of the femora, MAR (b), MS (c), and BFR (d) at 0.3–0.8 mm distal to the growth plate in the metaphyseal region were measured as described under “Materials and Methods.” The mice were injected intraperitoneally with calcine at 4 mg/kg and 4 days before sacrifice at 10 days. Data are expressed as means and S.D. of five bones from each of the four groups: vehicle- and propranolol-treated mice loaded and unloaded groups, *, statistically significant difference (p < 0.05), #, statistically significant difference between the vehicle group and drug-treated group (p < 0.05) (either control-loaded or unloaded groups), §, statistically significant difference between vehicle-treated unloaded group and drug-treated control-loaded group (p < 0.05).

effects of isoproterenol on bone mass (Fig. 3). These three lines of evidence based on dynamic histomorphometry indicated that bone formation is the target of sympathetic tone in mice subjected to hind limb unloading.

a, two-dimensional micro-CT pictures of the midsagittal planes of the proximal regions of the tibiae after 14 days of hind limb unloading (Unload) or loading (Control Load) in vehicle-treated or guanethidine-treated 129 mice. Two-dimensional micro-CT analyses were conducted as described under “Materials and Methods.” b, fractional trabecular BV/TV was quantified based on the image analysis of two-dimensional micro-CT pictures of the tibiae after 14 days of either hind limb unloading (Unload) or loading (Load) in vehicle-treated or guanethidine-treated mice. Data are expressed as means and S.D. *, statistically significant difference (p < 0.05), #, statistically significant difference between the vehicle group and drug-treated group (p < 0.05) (either control-loaded or unloaded groups), §, statistically significant difference between vehicle-treated unloaded group and drug-treated control-loaded group (p < 0.05). c–e, body weight was taken during the experiments for propranolol (c), guanethidine (d), and isoproterenol (e). No major alteration was observed.
We further examined whether our observations can be detected at cell levels in culture. For this purpose, a nodule formation assay was conducted by using bone marrow cells obtained from the bone of the mice after they were subjected to hind limb unloading or control loading in the presence or the absence of the treatment with pharmacological agents. Hind limb unloading reduced nodule formation in the cultures of cells obtained from the animals (Fig. 7, column 1 versus column 2). Propranolol treatment in vivo suppressed the unloading-induced reduction in the mineralized nodule formation in culture (Fig. 7, column 3 versus column 4). Isoproterenol treatment suppressed the levels of nodule formation in loaded control mice (Fig. 8, column 1 versus column 3), and in the presence of isoproterenol treatment in vivo, hind limb unloading failed to further reduce the levels of nodule formation in bone marrow cells in culture (Fig. 8, column 3 versus column 4). Thus, these in vitro experiments indicated that β-adrenergic sympathetic tone mediates unloading-induced reduction in mineralization of bone marrow cell cultures.

In order to examine the effects of the sympathetic nervous tone on unloading-induced reduction in bone mass and bone formation in a genetic model rather than pharmacological modulation, DBH (dopamine β-hydroxylase) knockout mice were subjected to hind limb unloading. Hind limb unloading reduced bone mass by about 68.6% in wild type littermate mice (Fig. 9, column 1 versus column 2). Heterozygous loss for the dopamine β-hydroxylase gene attenuated the reduction in bone loss by about 29.7% after hind limb unloading (Fig. 9, column 3).
versus column 4). The rate of bone loss due to unloading (calculated as (control load − unload)/(control load × 100%)) was significantly reduced from 68 ± 7% in DBH+/+ (wild type) to 30 ± 24% in DBH+/− (Fig. 8c) (p < 0.05). The nodule formation in cultures of bone marrow cells of the wild type littermate was reduced by unloading (Fig. 9b, column 1 versus column 2). The marrow cells obtained from DBH gene heterozygous knockout mice indicated suppression of hind limb unloading-induced reduction in nodule formation (Fig. 9b, column 3 versus column 4).

During the course of unloading-induced bone loss, bone resorption also occurs as critical events to reduce bone mass. Unloading in tail-suspended mice caused an increase in osteoclast number (Oc.N/BS) and osteoclast surface (Oc.S/BS) based on histomorphometry in vivo as reported previously (Fig. 10, b–g, column 1 versus column 2). In contrast, inhibition of sympathetic tone by treatment with propranolol or guanethidine, suppressed such unloading-induced increase in osteoclast number (Oc.N/BS) (Fig. 10, a and 5 for propranolol, d for guanethidine; column 3 versus column 4) and osteoclast surface (Oc.S/BS) (Fig. 10, c for propranolol, e for guanethidine; column 3 versus column 4). The levels of Oc.N/BS and Oc.S/BS were enhanced by either unloading or isoproterenol treatment alone to similar levels (Fig. 10, f and g, column 2 versus column 3). The simultaneous presence of unloading conditions and isoproterenol treatment resulted in similar levels in the increase in osteoclast number (Oc.N/BS) and surface (Oc.S/BS) to those in mice subjected to either one of the two conditions alone (Fig. 10, f and g, column 4 versus columns 2 and 3). Furthermore, unloading-induced increase in deoxypyridinoline excretion into urine (Fig. 11, column 1 versus column 2) was also suppressed by guanethidine treatment (Fig. 11, column 3 versus column 4).
DISCUSSION

Our data reveal that sympathetic nervous tone is mediating unloading-induced bone loss via reduction in osteoblastic cell activity as well as enhancement in osteoclastic cell activity. This is the first report that sympathetic control of the bone mass is involved in the unloading-induced bone loss by control-

FIG. 10. Pharmacological inhibition or activation of sympathetic tone suppresses or enhances unloading-induced increase in bone resorption, respectively. Mice were subjected to hind limb unloading as described under “Materials and Methods.” To inhibit sympathetic tone, some of these mice were treated with propranolol or guanethidine. For activation of sympathetic tone, some of these mice were treated with isoproterenol. After unloading for 10 or 14 days, decalcified sections of the hind limb bones were prepared to determine osteoclast number and osteoclast surface. The number of mice in each group is indicated as N. a, histological sections showing the osteoclasts stained for TRAP (red). b–g, sympathetic tone inhibition by treatment with propranolol (b and c) or guanethidine (d and e) suppressed unloading-induced increase in osteoclast number (b and d) and osteoclast surface (c and e). Sympathetic tone activation by the treatment with isoproterenol (f and g) enhanced unloading-induced increase in osteoclast number (f) and osteoclast surface (g). *, statistically significant difference (p < 0.05). Each group consisted of five bones from five mice. #, statistically significant difference between the vehicle group and drug-treated group (p < 0.05) (either control-loaded (column 1 versus column 3) or unloaded groups (column 2 versus column 4). §, statistically significant difference between vehicle-treated unloaded group (column 2) and drug-treated control-loaded group (column 3) (p < 0.05).
Unloading Reduces Bone Mass via Sympathetic Tone

Unloading-induced bone loss was suppressed by the treatment of the animals with propranolol, a beta-adrenergic receptor antagonist, suggesting that beta-adrenergic receptors in the sympathetic nervous system are the target of unloading-induced bone loss. The peripheral sympathetic nervous targets receive signals from the proximal upper nervous ending, which releases noradrenalin as a neurotransmitter into the synaptic gap. We observed that unloading-induced bone loss was again suppressed by the treatment of the animals with guanethidine.

These data indicate that the depletion of noradrenalin in the proximal ending of the synaptic gap could suppress the unloading-induced osteoporosis. Furthermore, unloading failed to further suppress the bone volume that was reduced by a beta-adrenergic agonist, isoproterenol. These three series of observations further indicate that sympathetic nervous tone is involved in unloading-induced pathological bone loss.

We also examined the effects of heterozygous deletion of the DBH gene. DBH is required for the sympathetic nervous tone. Therefore, we subjected the heterozygous knockout mice to hind limb unloading. Unloading-induced bone loss was attenuated by the absence of the half-dosage of dopamine-beta-hydroxylase gene, indicating that the presence of a full dosage of DBH gene in the animals is necessary for the complete effects of the unloading-induced bone loss. Furthermore, DBH data excluded the possibility that pharmacological experiments might be influenced by possible artifacts due to the systemic drug administration. Thus, both pharmacological and genetic interventions of sympathetic signals supported the idea that the sympathetic nervous system causes pathological loss of bone in unloading-induced osteopenia.

Since bone formation is the critical activity to determine the levels of bone loss due to unloading, it is the major target to elucidate the mechanisms required for unloading-induced loss of bone mass. Dynamic histomorphometric analyses on osteoblastic cell activity in vivo revealed that the reduction in bone formation activity in vivo due to unloading was suppressed by a series of pharmacological agents including propranolol and guanethidine. Furthermore, bone cell culture experiments using the bone marrow cells taken from the animals subjected to either pharmacological or genetic interventions of the sympathetic nervous tone indicate that these interventions suppressed unloading-induced reduction in mineralized nodule formation. The interpretation of the reduction in the formation of osteoblastic bone nodules after 3 weeks in culture would be that progenitor cell populations for osteoblastic cell lineage could be reduced at the point of harvesting the cells from animals at the end of unloading. This suppression was blocked by the treatment with propranolol. We also carried out 3-week culture experiments to form bone nodules in the presence or absence of isoproterenol or propranolol and guanethidine in culture using bone marrow cells that were taken from wild type (untreated) C57Bl6 mice. There was no effect of these agents in culture to modulate the nodule formation in the bone marrow cells from untreated mice (data not shown). These data support the idea that the progenitor population in bone marrow in vivo would be reduced by the unloading condition or by treatment with drugs such as propranolol, guanethidine, and isoproterenol during the periods of the tail suspension experiments. Therefore, the action of sympathetic nervous tone during unloading-induced bone loss renders cell level effects on osteoblastic cell activity.

It is known that propranolol at doses of 10 μg/g body weight could reduce blood pressure in mice. It is also known that such blood pressure change could be enhanced by the treatment with isoproterenol. However, there has yet been no clear evidence in clinical or experimental settings in terms of a direct relationship between blood pressure and bone mass. However, at this point, we cannot fully exclude the possibility that propranolol or isoproterenol used in our experiments may have affected bone mass via regulation of blood pressure, and these points have to be elucidated in the future.

It is intriguing to consider the differences in the time courses of the beta-adrenergic receptor modulators. Propranolol has been known to reduce blood pressure in days, whereas isoproterenol and guanethidine alter blood pressure immediately. It is not known whether such time course differences seen in their effects on blood pressure may also affect their modulation of the bone mass. However, both propranolol and guanethidine blocked unloading-induced bone loss similarly in our experiments. This may partially be due to the nature of bone where the biological read out (i.e. bone mass) could be detected in a relatively slow manner compared with blood pressure.

If these types of drugs could be proven to be effective in the treatment of unloading-induced osteoporosis, it has to be considered that side effects might occur in the treatment of the patients. In the future, we may have to identify certain windows of the dosages by which bone effects may be obtained without affecting the blood pressure, or these drugs may be used only for those patients whose side effects could be predicted to be less based on the individual genomic data.

Our data indicated that sympathetic tones regulate unloading-induced enhancement in bone resorption. This was evidenced by the observations that inhibition of sympathetic tone by propranolol and guanethidine, suppressed unloading-induced bone resorption, and this leads to suppression in bone loss. Histomorphometric analyses indicated that unloading activates bone resorption through the increase in osteoclast number and osteoclast surface. These increases in the bone resorption parameters due to unloading were suppressed by treatment with propranolol and guanethidine. Such observations regarding the inhibitors for sympathetic tone effects on unloading-induced bone resorption were not limited to particular bone, since suppression of sympathetic tone by the treatment with guanethidine also suppressed unloading-induced
Unloading reduces bone mass via sympathetic tone

Increase in deoxypyridinoline excretion into urine, which is a systemic bone resorption maker. These data revealed that sympathetic tone regulates unloading-induced bone resorption as well.

Involvement of sympathetic tone in the induction of bone resorption after unloading was further supported by the analysis on the mice subjected to simultaneous unloading and isoproterenol treatment. Either isoproterenol treatment or unloading alone could cause an equivalent increase in the levels of bone loss as well as an increase in the levels of bone resorption parameters (osteoclast number and osteoclast surface). The simultaneous presence of unloading conditions and isoproterenol treatment resulted in an increase in bone loss as well as an increase in osteoclast number and osteoclast surface to levels equivalent to those in the cases of either of the two conditions alone. These data further support the notion that sympathetic nervous tone and the unloading condition would share signaling pathways.

Unloading induces rapid bone loss and increases fracture risk significantly, especially in elderly bedridden patients. In fact, deoxypyridinoline excretion into urine was reported to increase in astronauts within a few hours after they are exposed to microgravity conditions in space. Such rapid bone loss is caused by an immediate response of osteoclastic activity when the body is subjected to unloading conditions. Although this phenomenon is so clearly observed in human (bedridden patients and astronauts) and various animal models, the mechanism for such a response of bone resorbing activity has not been identified. Since the nervous system could elicit signals at a relatively fast speed, our identification of the sympathetic tone as responsible for the unloading-induced increase in bone resorption and loss of bone mass would explain the rapid response of osteoclasts to unloading.

Although we have used DBH knockout mice to see the effects of such a deletion of the gene on the bone loss due to tail suspension, this enzyme is also required to produce epinephrine in the adrenals as well as norepinephrine. The enzyme required to produce epinephrine from norepinephrine, phenylethanolamine-N-methyltransferase, has been suggested in several reports to be subjected to induction by immobilization-induced stress. As a result, the DBH heterozygous knock-out mice results may not represent conclusive proof of a prominent role for the sympathetic nervous system here. However, our data on the effects of unloading on bone in DBH knockout mice is at least in part in accordance with the idea that sympathetic tone is involved in the bone loss due to unloading.

In conclusion, our data indicate that sympathetic tone is in charge of the pathological reduction in bone mass upon unloading by suppressing osteoblastic cell actions and enhancing osteoclastic actions. These data predict that identification of the involvement of systemic modulation in the bone loss in unloading conditions could give a clue to appropriate measures to treat patients with disuse osteoporosis (9).

REFERENCES

1. Riggs, B. L., and Hartmann, L. C. (2002) N. Engl. J. Med. 348, 618–629
2. Raisz, L. G., and Rodan, G. A. (2003) Endocrinol. Metab. Clin. N. Am. 32, 15–24
3. Riggs, B. L. (2004) J. Cell. Biochem. 88, 209–215
4. Riggs, B. L. (2003) J. Cell. Biochem. 88, 209–215
5. Ehrlich, P. J., and Lanyon, L. E. (2002) Osteoporosis Int. 13, 688–700
6. Lanyon, L., and Skerry, T. (2001) J. Bone Miner. Res. 16, 1937–1947
7. Bilek, D. D., Sakata, T., and Halloran, B. P. (2003) Gravit. Space Biol. Bull. 16, 45–54
8. Bilek, D. D., and Halloran, B. P. (1991) J. Bone Miner. Res. 6, 527–530
9. Serhan, C. N. (2004) N. Engl. J. Med. 350, 1902–1903
10. Ishijima, M., Truji, K., Ritting, S. R., Yamashita, T., and Kurosawa, H. (2002) J. Bone Miner. Res. 17, 661–667
11. Parfitt, A. M., Drezer, M. K., Glories, F. H., Kanis, J. A., Malluche, H., Meunier, P. J., Ott, S. M., and Becker, R. R. (1987) J. Bone Miner. Res. 2, 585–610
12. Augat, P., Simon, U., Liebert, A., and Claes, L. (2005) Osteoporosis Int. 16, Suppl. 2, S36–S43
13. Strewler, G. J. (2004) N. Engl. J. Med. 350, 1172–1174
14. Mohamed, A. M. (2002) N. Engl. J. Med. 349, 1671
15. Fuller, K. E. (2004) N. Engl. J. Med. 350, 189–192
16. Ishijima, M., Tsuji, K., Ritting, S. R., Yamashita, T., Kurosawa, H., Denhardt, D. T., Nifujii, A., and Noda, M. (2001) J. Exp. Med. 195, 399–404
17. Harada, S., and Rodan, G. A. (2003) Nature 423, 349–355
18. Fu, Q, Levine, B. D., Paweleczyk, J. A., Erlt, A. C., Diedrich, A., Cox, J. F., Zuckerman, J. H., Ray, C. A., Smith, M. L., Iwase, S., Saito, M., Sugiyama, Y., Mano, T., Zhang, K., Iwasyk, K., Lane, L. D., Buckey, J. C., Jr., Cooke, W. H., Robertson, R. M., Baisch, F. J., Blomqvist, C. G., Eckberg, D. L., Robertson, D., and Biaggioni, I. (2002) J. Physiol. 544, 653–664
19. Cox, J. F., Tahvanainen, K. U., Krusela, T. A., Levine, B. D., Cooke, W. H., Mano, T., Iwase, S., Saito, M., Sugiyama, Y., Erlt, A. C., Biaggioni, I., Diedrich, A., Robertson, R. M., Zuckerman, J. H., Lane, L. D., Ray, C. A., White, R. J., Paweleczyk, J. A., Buckey, J. C., Jr., Baisch, F. J., Blomqvist, C. G., Robertson, D., and Eckberg, D. L. (2002) J. Physiol. 538, 309–320
20. Takeda, S., Elefteriou, F., Levesaur, R., Liu, X., Zhao, L., Parker, K. L., Armstrong, D., Dury, P., and Karsenty, G. (2002) Cell 111, 305–317
21. Sprague, B. E., and Chole, R. A. (2000) J. Bone Miner. Res. 15, 1354–1360
22. Sherman, B. E., and Chole, R. A. (2000) J. Bone Miner. Res. 15, 1354–1360
23. Takeda, S., Elefteriou, F., Levesaur, R., Liu, X., Zhao, L., Parker, K. L., Armstrong, D., Dury, P., and Karsenty, G. (2002) Cell 111, 305–317
24. Thomas, S. A., Matsumoto, A. M., and Palmiter, R. D. (1995) Nature 374, 643–646