Role for parathyroid hormone in mechanical responsiveness of rat bone

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Chow, J. W. M., S. Fox, C. J. Jagger, and T. J. Chambers. Role for parathyroid hormone in mechanical responsiveness of rat bone. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E146–E154, 1998.—We investigated the relationship between parathyroid hormone (PTH) and mechanical stimulation in mechanically induced osteogenesis. In normal rats, mechanical stimulation of the eighth caudal vertebra induced an osteogenic response. This was augmented by a single injection of human PTH-(1–34) 30–45 min before loading. No osteogenic response was seen in thyroparathyroidectomized (TPTX) rats; the osteogenic response was restored by a single injection of PTH before stimulation, suggesting that physiological levels of PTH are necessary for the mechanical responsiveness of bone. c-fos expression was detected only in the osteocytes of those rats that were both mechanically stimulated and given PTH. This suggests that PTH supports mechanically induced osteogenesis by sensitizing either the strain-sensing mechanism itself or early responses of bone to strain-generated signals. The osteogenic response was not augmented by two further daily injections of PTH and was not seen in TPTX rats in which PTH administration was started 3 days after loading. These results reveal a major role for PTH in the mechanical responsiveness of rat bone.

ostecyte; strain; thyroparathyroidectomy; mechanical stimulation

It is likely that bone evolved primarily as a mechanical support. Although much of the information for this function is provided by the genetic program, throughout life bones modify their shape and structure in response to mechanical stimulation so that bone structure remains optimal for the prevailing mechanical environment (22).

Bone also represents a large reservoir of calcium. As such, it is exploited as a target for parathyroid hormone (PTH) in plasma calcium homeostasis. Indeed, the primacy of calcium homeostasis is such that, when the need to maintain calcium homeostasis conflicts with the needs of structure, bone structure can be sacrificed, as can occur during pregnancy and lactation (8). Nevertheless, under normal circumstances, bone efficiently fulfills both its calcium regulatory and mechanical functions. It seems likely that mechanisms have evolved whereby PTH and mechanical responses interact in a way that minimizes interference between the requirements of calcium homeostasis and mechanical adaptation.

Despite the central importance of this interaction to the physiology of bone, there is little information on the effect of PTH on mechanical responses (1, 27). Burkhart and Jowsey (1) found that parathyroidectomy suppressed the loss of bone that occurs with immobilization. This was interpreted as evidence that PTH sustains a drive for bone resorption, which is countered by mechanical usage. Although PTH has been shown to potentiate changes in intracellular signaling pathways induced by mechanical strain of osteoblasts in vitro, consistent with an interaction between PTH and mechanical stimuli, the physiological significance of the experimental conditions used is uncertain, and the relationship between the changes in second messenger systems and bone formation and bone resorption was not elucidated (3, 10).

We recently developed an experimental model in which a brief episode of mechanical stimulation by strains in the lower part of the range to which bones are exposed under physiological circumstances induces a substantial increase in trabecular bone formation over the ensuing days (6, 23). An osteogenic response to strains of physiological magnitude occurs because the distribution of strains engendered by the mechanical stimulus differs from that to which the bone is accustomed (22). The response thus represents the mechanism by which bone adapts its structure to suit new mechanical environments. Because a brief stimulus is sufficient to induce the sequence of events leading to bone formation, this model provides an opportunity to identify interactions between PTH and both the immediate and subsequent phases of the osteogenic response to mechanical stimulation.

MATERIALS AND METHODS

Three experiments were performed.

Assessment of Bone Formation by Fluorochrome Labeling

Animals and experimental procedures. In the first experiment, 13-wk-old female Wistar rats (Tuck and Son, Battlebridge, Essex, UK) were weight matched and divided into three groups of seven animals each (avg wt 235 g). Stainless steel pins were inserted into the seventh and ninth caudal vertebrae of the animals as previously described (4, 6). The animals were subjected to a single episode of loading immediately after insertion of the pins, comprising 30 cycles (1 Hz), using a peak load of 150 N, thereby subjecting the eighth caudal vertebrae to dynamic loading in compression. This load has been found to produce a peak strain magnitude over the diaphyseal cortex of 700 με (4), a strain within the physiological range for mechanical usage in mammalian bone (31). These experimental procedures were performed under general anesthesia (2% halothane in 2 l/min O₂ and 1 l/min N₂O). After the loading session, the pins were immobilized in clamps for the remainder of the experiment. The animals were given a single subcutaneous injection of vehicle (0.001 N HCl in normal saline with 2% heat-inactivated rat serum; Dako, Bucks, UK) or 6 or 60 μg/kg human (h) PTH-(1—34) (Bachem, Torrance, CA) 30–45 min before loading.

In the second experiment, animals of similar age were thyroparathyroidectomized (TPTX) or sham TPTX, and ve-
nous blood was obtained by tail vein bleeding 5 days after the operation. TPTX animals in which the operation was considered to be successful (serum calcium <1.8 mM) were then weight matched (avg wt 235 g) and divided into 11 groups of 8 animals each. The day after the tail vein bleeding, animals were pinned and subjected to the loading regimen described above. The groups of TPTX animals were given vehicle or 6, 60, or 600 µg/kg hPTH-(1—34) as a single subcutaneous injection 30–45 min before loading, taken as day 1, or once daily on 3 consecutive days, commencing 30–45 min before loading, or on 3 consecutive days, commencing 3 days after loading. A group of sham-operated animals was subjected to 30 cycles of loading and injected with vehicle 30–45 min before mechanical stimulation. Thus the groups comprised 1) sham TPTX, vehicle; 2) TPTX, vehicle; 3) TPTX, 6 µg/kg hPTH on day 1; 4) TPTX, 60 µg/kg hPTH on day 1; 5) TPTX, 600 µg/kg hPTH on day 1; 6) TPTX, 6 µg/kg hPTH on days 1–3; 7) TPTX, 60 µg/kg hPTH on days 1–3; 8) TPTX, 600 µg/kg hPTH on days 1–3; 9) TPTX, 6 µg/kg hPTH on days 4–6; 10) TPTX, 60 µg/kg hPTH on days 4–6; and 11) TPTX, 600 µg/kg hPTH on days 4–6. All the above procedures and injections were performed between 0800 and 1200.

During the experiment, the animals were housed in hanging cages in groups of three or four at 21°C with a 12:12 h light-dark cycle. Food (rat-mouse diet 1, SDS, Witham, UK; calcium content 1.3%) and water were given ad libitum to the animals. In experiment 1, the animals received intraperitoneal administration of fluorochromes, tetracycline (30 mg/kg, (Sigma, Poole, Dorset, UK), and calcine (25 mg/kg; Lederle Laboratories, Gosport, UK) 2 and 7 days after loading, respectively. In experiment 2, the animals received fluorochromes 2 and 6 days after loading. Animals were killed by CO₂ inhalation 24 h after the last fluorochrome injection. The procedures were performed under institutional guidelines and have been approved by the UK Home Office.

Bone histomorphometry. The sixth (C_6) and eighth caudal vertebrae (C_8) were removed, freed of soft tissue, fixed in 70% alcohol for 48 h, dehydrated through graded alcohols, and embedded undecalcified in London resin (London Resin, Basingstoke, UK). Sections (15 µm) obtained longitudinally through the center of axis of the vertebra were prepared for fluorochrome microscopy. For static parameters, 7-µm sections were prepared and stained with toluidine blue. We have found, in previous experiments (7; unpublished observations), that there is no significant difference in bone formation between the sixth and eighth vertebrae of pinned but non-loaded animals and that bone formation in the nonloaded sixth vertebra is not affected by mechanical stimulation of the eighth vertebra. Therefore, in the present experiments, we used the nonloaded sixth vertebra as an internal control for the effect of mechanical stimulation on the eighth vertebra.

The volume of the cancellous bone was measured in a standard area (1.8 mm²) situated at least 0.75 mm from the growth plate to exclude the primary spongiosa and trabeculae connected to the cortical bone. Dynamic parameters were measured, at an objective magnification of ×20, in the same area as that used for assessing bone volume. Measurements were made by tracing features of interest on a digitizer pad viewed in the computer with a dedicated bone software (Osteomeasure, Osteometrics, Atlanta, GA). Three sections of bone containing at least 4 cm of total bone surface (BS) were measured per vertebra. These measurements included double-labeled surface (dLS/BS) and interlabel distance. The mineral apposition rate (MAR; µm/day) was obtained by dividing the distance between the two labels on the trabeculae by the label period. The bone formation rate (surface referent; BFR/BS) was obtained from a product of MAR and dLS/BS. Similarly, the static parameters, osteoblast surface (ObS/BS) and eroded surface (ES/BS), were measured on three sections and stained with toluidine blue in an interactive fashion at an objective magnification of ×20. All measurements were performed without prior knowledge of treatment of the experimental group.

Assessment of Gene Expression by mRNA In Situ Hybridization

Experimental procedures and preparation of tissue sections. We performed a third experiment for assessment of gene expression. Female Wistar rats (13 wk old) were divided...
into four groups of five. The seventh and ninth caudal vertebrae of two groups were pinned and left nonloaded. The other two groups were subjected to 30 cycles of loading as described above. One each of the pinned, nonloaded, and loaded groups was given a single subcutaneous injection of PTH (80 µg/kg) or vehicle 30–45 min before pinning or mechanical loading. The animals were killed 1 h after pinning or loading after intracardiac perfusion-fixation with 4% paraformaldehyde, under general anesthesia. The sixth (nonloaded) and eighth (loaded) caudal vertebrae were removed, postfixed in 4% paraformaldehyde at 4°C for a further 24 h, and decalcified in 10% EDTA (Sigma) before embedding in paraffin wax.

Probe preparation and mRNA in situ hybridization. This was performed as previously described (24). Briefly, a rat c-fos cDNA (7) was subcloned into pSP72 (Promega, Southampton, UK) using standard procedures. The vector was linearized with BamH I and transcribed with T7 polymerase to generate a 2-kilobase antisense strand. The sense probe was generated by linearizing with EcoR V and transcribing with Sp6. Linearized vector (1 µg) was labeled with [35S]UTP using a Sp6-T7 transcription kit (Boehringer Mannheim, Mannheim, Germany).

Midfrontal sections (6 µm thick) of vertebrae were deparaffinized, dehydrated, washed, and then fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). After proteinase K treatment [1 µg/ml in 10 mM tris(hydroxymethyl) aminomethane with 2 mM CaCl₂], they were incubated in glycine (2 mg/ml), washed in PBS, and postfixed in 4% paraformaldehyde before acetylation with 0.25% acetic anhydride in 0.1 M triethanolamine. After incubation in a prehybridization buffer, the sections were hybridized with labeled riboprobe at 50°C for 16 h using the probe at a specific activity of 5 × 10⁶ counts·min⁻¹·µl⁻¹ hybridization solution in a container humidified with 50% formamide. The sections were then subjected to stringent washes, dehydrated in graded alcohols containing 0.3 M ammonium acetate, and air dried. Control sections were hybridized with the labeled sense probe. The sixth caudal vertebra of loaded animals treated with vehicle also served as controls. All reagents were obtained from Sigma.

Hybridization was visualized autoradiographically by coating sections with nuclear emulsion (K6, Ilford, UK) before exposure for 14 days at 4°C. The slides were then developed, counterstained with hematoxylin and eosin, and mounted.

Assessment of mRNA in situ hybridization. Osteocytes within the area bounded by a 1.5-mm length of cortical bone at the middiaphysis were assessed for hybridization for mRNA. The percentage of osteocytes exhibiting hybridization was calculated from among a minimum of 1,000 cortical osteocytes in two sections of each vertebra.

Statistical Analysis

All measurements were performed without prior knowledge of the experimental group. Results for each group of animals are expressed as means ± SE. Statistical analysis was performed using Fisher's least significant difference method for multiple comparisons in a one-way analysis of variance. To test the interaction between PTH and mechanical stimulation, the analysis of variance was performed on the difference in increases in bone formation parameters of mechanically loaded C₈ vertebra compared with its own C₆ vertebra, which is subjected to systemic hormonal effects, but is not mechanically stimulated. In experiment 2, separate analyses were performed for groups of TPTX animals, according to dosing regime of PTH. For the second experiment, linear regression analysis was also performed to assess dose responsiveness of parameters of bone formation (BFR/BS, dLS/BS, MAR) to PTH administration. Statistical analysis of c-fos expression in osteocytes was also performed using Fisher's least significant difference method for multiple comparisons in a one-way analysis of variance.

RESULTS

The final body weights of groups of animals did not differ significantly in experiments 1 and 2. The final body weight of the TPTX groups of animals given vehicle or PTH (240 ± 9 to 252 ± 9 g) did not differ significantly from that of the sham TPTX animals.
(249 ± 6 g). In pilot experiments, serum calcium levels of sham TPTX animals on a standard diet (calcium content 1.3%) was 2.48 ± 0.04 mM. This did not differ significantly from animals after overnight starvation (2.53 ± 0.03 mM). The success of TPTX could only be confirmed (serum calcium 1.57 ± 0.07 mM) after overnight starvation, because nonfasted TPTX animals on the standard diet, with a relatively high calcium content, were normocalcemic (serum calcium 2.46 ± 0.06 mM).

As previously noted (6), we found that mechanical stimulation was associated with an increase in BFR/BS in the loaded vs. nonloaded vertebra (Fig. 1), due to a significant increase in both dLS/BS and MAR. The single dose of PTH did not by itself cause a significant change in the indexes of bone formation in the nonloaded sixth caudal vertebra but augmented the mechanical response, so that BFR of the eighth caudal vertebra in animals administered PTH was significantly greater after mechanical stimulation than that of non-PTH-treated rats (Fig. 1). The percent increase in bone formation of vertebrae of rats subjected to both mechanical stimulation and PTH was significantly greater than that of mechanically stimulated vertebrae of rats treated with vehicle. There was no change in bone volume in these short-term experiments (data not shown).

To further test the effect of PTH on mechanical stimulation, we used TPTX rats. Consistent with previous reports (17, 18, 27, 33), BFR was reduced in these animals. In addition, we found that, although a fourfold increase in BFR was observed after mechanical stimulation in sham-operated animals, similar to the response observed in the first experiment, mechanical stimulation induced no significant change in bone formation in TPTX rats (Fig. 2).

TPTX animals given a single injection of PTH 45 min before mechanical stimulation showed a dose-dependent increase in the dynamic parameters of bone formation, compared with TPTX rats injected with vehicle alone (Fig. 3; BFR/BS: \( r = 0.47, P = 0.01 \); dLS/BS: \( r = 0.47, P = 0.01 \)). An increase in the extent of mineralizing surface (dLS/BS) represented the predominant contribution, but MAR was also increased significantly by loading in animals administered 60 and 600 µg/kg of PTH.

The increase in osteoblast surface in mechanically stimulated vertebrae compared with nonstimulated vertebrae in animals administered PTH was not statistically significant. There was no significant difference in ES/BS of the groups of animals (data not shown).

Fig. 3. BFR, dLS/BS, MAR, and osteoblast surface of nonloaded (C 6) and loaded (C 8) vertebrae of TPTX animals given a single dose of vehicle or 6, 60, or 600 µg/kg hPTH-(1—34) before loading. Results are expressed as means ± SE; n = 8 animals/group. Obs/BS (%), osteoblast surface. \( * P < 0.05 \) vs. nonloaded C 6 in TPTX animals given vehicle; \( ** P < 0.05 \) vs. loaded C 6 in TPTX animals given vehicle; \( \dagger P < 0.05 \) vs. nonloaded C 6 in TPTX animals given 6 µg/kg hPTH; \( \ddagger P < 0.05 \) vs. loaded C 6 in TPTX animals given 6 µg/kg hPTH; \( \S P < 0.05 \) vs. nonloaded C 6 in TPTX animals given 60 µg·kg \(^{-1}\)·day \(^{-1}\) hPTH; \( \mathsection P < 0.05 \) vs. nonloaded C 6 in TPTX animals given 600 µg/kg hPTH (ANOVA).
A similar pattern was observed in rats given three daily administrations of PTH starting 45 min before mechanical stimulation (Fig. 4). BFR was no greater in the loaded vertebrae of animals given three vs. one dose of PTH, although there was a nonsignificant increase in bone formation in nonloaded sixth vertebrae after three injections. Mechanically stimulated vertebrae from animals administered 600 µg/kg PTH showed an increase in osteoblast surface compared with nonloaded vertebrae (Fig. 4).

In animals administered PTH daily 3–5 days after mechanical stimulation, there was no change in the dynamic indexes of bone formation indicated by dLS/BS (Fig. 5). Nevertheless, both loaded and nonloaded vertebrae from the animals given 600 µg/kg showed a significant increase in osteoblast surface, reflecting the late induction of bone formation by PTH. Unlike in the groups administered PTH immediately before mechanical stimulation, there was no suggestion of an increase in bone formation in mechanically stimulated vs. nonstimulated vertebrae (Fig. 5). The absence of mechanical responsiveness, whether measured by fluorochrome labeling or as osteoblast surface, in the groups administered PTH 3 days after the mechanical stimulus supports the conclusion made from the results of the formal TPTX control group, that in TPTX rats there is no detectable response to mechanical stimulation.

Because TPTX is associated with a reduction in bone formation, it is not surprising that administration of one or three doses of PTH over a 7-day period was insufficient to support a normal BFR. To assess the extent to which mechanical stimulation proportionately increased bone formation above the baseline, we calculated BFR in loaded vertebrae as a proportion of that in nonloaded sixth caudal vertebrae. We found (Fig. 6) that a single dose of PTH (60–600 µg/kg) was sufficient to induce a relative increase in BFR in TPTX rats similar to that seen in sham-operated animals in contradistinction to the rats given no PTH or those with delayed administration of PTH.

No hybridization for c-fos mRNA was observed in cortical osteocytes of the eighth caudal vertebrae 1 h after 30 cycles of loading. Similarly, no hybridization was observed in the eighth caudal vertebrae of pinned nonloaded animals given vehicle or PTH. However, in vertebrae from rats both administered PTH and mechanically stimulated, a significant proportion of osteocytes showed hybridization for c-fos mRNA (Table 1, Fig. 7). This was significantly different compared with all other groups of animals. No hybridization of c-fos mRNA was detected in the control sections, comprising sense probe, or in the sixth caudal vertebrae of loaded animals treated with vehicle.

**DISCUSSION**

We have found that a single administration of 6 or 60 µg/kg of PTH immediately before mechanical stimula-
tion significantly augments the ensuing osteogenic response. We also found that TPTX rats show no detectable response to mechanical stimulation but that mechanical responsiveness can be restored in a dose-responsive manner by a single administration of PTH immediately before mechanical stimulation. This suggests that PTH is required, for mechanical responsiveness, either during or immediately after mechanical stimulation. This conclusion is consistent with the augmentation of osteocytic c-fos expression by PTH that we observed. We also found that the response to mechanical stimulation was not further increased by

Fig. 5. BFR, dLS/BS, MAR, and osteoblast surface of nonloaded (C6) and loaded (C8) vertebrae of TPTX animals given vehicle or 3 daily doses of 6, 60, or 600 µg·kg⁻¹·day⁻¹ hPTH-(1—34) commencing 3 days after loading. Results are expressed as means ± SE; n=8 animals/group. *P<0.05 vs. nonloaded C6 in TPTX animals given vehicle; bP<0.05 vs. nonloaded C8 in TPTX animals given 6 µg/kg hPTH; cP<0.05 vs. loaded C8 in TPTX animals given vehicle; dP<0.05 vs. loaded C8 in TPTX animals given 6 µg/kg hPTH; eP<0.05 vs. loaded C8 in TPTX animals given 60 µg·kg⁻¹·day⁻¹ PTH; fP<0.05 vs. loaded C8 in TPTX animals given 600 µg/kg hPTH.

Fig. 6. Percentage increase in BFR of loaded (C8) vertebrae of TPTX and sham-operated animals compared with their own nonloaded (C6) vertebra in animals given vehicle or 6, 60, or 600 µg/kg hPTH-(1—34) before loading (A); vehicle or 3 daily doses of 6, 60, or 600 µg/kg hPTH-(1—34) commencing 45 min before loading (B); and vehicle or 3 daily doses of 6, 60, or 600 µg/kg hPTH-(1—34) commencing 3 days after loading (C). Results are expressed as means ± SE; n=8 animals/group. *P<0.05 vs. TPTX animals given vehicle (ANOVA). In B and C, none of differences between groups was statistically significant.
three daily administrations of PTH and was absent in rats in which PTH was started 3 days after loading.

The abrogation of mechanical responsiveness by TPTX suggests that physiological levels of hormones from the thyroid and/or parathyroid are necessary for normal mechanical responsiveness in bone. Although the possible contribution of thyroxine and calcitonin cannot be excluded, the presumption that PTH is the hormone responsible is supported by the ability of PTH administration to restore mechanical responsiveness to normal levels. The possible role of thyroxine in mechanically induced bone formation requires further investigation.

Administration of a single subcutaneous dose of PTH to rats results in detectable plasma levels of immunoreactive PTH 15 min later, peaking at 30–60 min, before becoming undetectable 4 h after the injection (19, 25). In our experiments, the mechanical stimulus was timed to coincide with peak PTH levels. The very rapid fall in plasma PTH concentrations after a single dose suggests that PTH supports mechanically induced osteogenesis by sensitizing either the strain-sensing mechanism itself or early responses of bone to strain-generated signals. It is unlikely that the augmentation of bone formation response was due to raised serum calcium levels, since bone formation in the nonloaded sixth caudal vertebra was unaffected by a single PTH administration. Moreover, PTH administration causes a transient hypocalcemia in rats (30), and the dose that effectively restores and enhances mechanical responsiveness (60 µg/kg) has been shown not to cause hypercalcemia in rats (13, 15, 19).

We did not attempt to recapitulate physiological PTH replacement in these experiments; restoration of mechanical responsiveness in TPTX rats by a single dose of PTH and augmentation of the osteogenic response in intact animals by pharmacological levels of PTH at the time of loading identify a role for PTH in the mechanical responsiveness of rat bone.

In previous experiments, we have found that a maximal osteogenic response (300 loading cycles) induced detectable c-fos expression in a substantial minority of osteocytes (24). Although the role of c-fos expression in osteocytes is unknown, its induction within 30 min of mechanical stimulation is consistent with a role for osteocytes in the transduction of mechanical stimuli and suggests that c-fos represents a marker for mechanical responsiveness in these cells. Because the present experiments were designed to detect synergistic interactions between PTH and mechanical stimuli, we used a suboptimal loading regime (30 cycles), which was insufficient alone to induce detectable osteocytic c-fos expression. Although PTH is known to induce c-fos expression in a variety of bone cells, it has not been shown to induce osteocytic c-fos expression (25). The augmentation of osteocytic c-fos expression to detectable levels by PTH supports the notion that PTH acts by sensitization of the strain-sensing mechanism to mechanical stimulation.

Table 1. c-fos mRNA in situ hybridization

<table>
<thead>
<tr>
<th>Group</th>
<th>%Positive Osteocytes</th>
<th>%Positive Bone Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pin/vehicle</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pin/PTH</td>
<td>0</td>
<td>0.94 ± 0.62</td>
</tr>
<tr>
<td>Load 30/vehicle</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Load 30/PTH</td>
<td>4.18 ± 2.14*</td>
<td>0.34 ± 0.23</td>
</tr>
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Values are means ± SE. *P < 0.05 vs. all other groups.

A reduction in bone formation in TPTX animals has been noted before (17, 18, 27). Conversely, there is abundant evidence that PTH stimulates bone formation in rats (14, 20, 29, 32, 36). However, the anabolic action of PTH has been shown to be transient and to diminish considerably by 48 h after administration (9). Thus, although a single dose at the time of mechanical stimulation was sufficient to restore mechanical responsiveness, we would not necessarily expect to restore the basal level of bone formation to normal with the regimes of PTH administration used in these experiments. However, we did detect an increase in osteoblast surface even 2 days after three injections of PTH, consistent with the known ability of PTH to stimulate osteoblastic activity.

An alternative explanation for suppression of bone formation in TPTX animals is hypocalcemia (33, 34). Although mild hypocalcemia is a possible contributory factor to the low bone formation rate observed in the TPTX animals in the current experiment, it is unlikely to account for lack of mechanical responsiveness: this was restored in TPTX rats by a single injection of PTH before loading, was not significantly increased by two further doses, and was not evident if PTH administration was delayed 3 days after loading. Moreover, the serum calcium is unlikely to be markedly decreased in the TPTX rats given a relatively high calcium diet. It has been shown that bone formation is inhibited in hypocalcemic but not in normocalcemic TPTX rats (34), and other workers (35) have also demonstrated PTH response in bones of TPTX rats despite hypocalcemia.

Our results suggest that PTH in some way sensitizes bone cells to mechanical stimulation. The mechanism by which this occurs is uncertain. One way in which this might occur is through the ability of PTH to stimulate bone resorption. However, previous work has suggested that the anabolic action of PTH in rats is not dependent on bone resorption and occurs despite suppression of bone resorption (13, 15, 26, 32, 36). Similarly, we have found that suppression of bone resorption is without influence on the mechanical response, possibly because mechanical responsiveness is the mechanism by which bone resorption leads to bone formation (16). Moreover, we observed sensitization of osteocytes to mechanical responsiveness by PTH within a very short time compared with the length of time occupied by the resorption phase of the coupling mechanism.

Alternatively, PTH might activate bone cells in such a way that their responsiveness to mechanical signals is increased through the secretion of PTH-induced cytokines by osteoblastic cells or by cytoskeletal modifications that might be associated with altered mechani-
cal responsiveness (2, 12, 21, 28). We have recently found that both prostaglandin and nitric oxide generation is required for mechanical responsiveness in bone (5, 11), and it is tempting to speculate that a requirement for dual signaling molecules, suggested by that data, might underlie the synergy between PTH and mechanical stimulation in the osteogenic response. These interactions will be clarified by further investigations. Whatever the explanation, our results provide an insight into the interactions between the major hormonal and mechanical influences on bone physiology and an opportunity to analyze the cellular and molecular basis for this synergy as a means to the identification of novel approaches to the induction of osteogenesis.

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Fig. 7. A (low magnification) and B (higher magnification of part of A): In situ hybridization (ISH) of cortex of C8 for c-fos 1 h after mechanical stimulation of PTH-treated animals. Some osteocytes show strong hybridization (arrows); others are unlabeled (representatives indicated by arrowheads). C and D: ISH of cortex of C8 for c-fos 1 h after stimulation of C8. No osteocytes show labeling. Representative unlabeled osteocytes are indicated by arrowheads. Magnifications: ×140 (A and C); ×350 (B and D).
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