Parathyroid hormone-parathyroid hormone-related peptide receptor expression and function in otosclerosis

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Otosclerosis is a bone dystrophy localized to the otic capsule, an embryonic structure from which develop the inner ear and the stapes footplate (9, 13). This disease is a frequent cause of deafness in adults, affecting over 10% of deaf adult patients seen in outpatient activity by otolaryngologists (9). Its prevalence is estimated as 0.2–0.3% of the population in Western Europe and North America (9). About 10% of Caucasian adult temporal bones present histological foci of otosclerotic lesions (9, 14). In the early forms, otosclerotic foci are found only in the stapes and disturb the sound transmission, whereas advanced lesions can involve the cochlea, producing sensorineural hearing loss, or the vestibule, causing vertigo (9, 12). The otosclerotic process in the otic capsule is initiated by an increase in bone resorption with the presence of numerous resorption foci rich in blood vessels, also designated as otospongiotic foci (6, 13, 14, 23). In response to this increase in bone resorption, a reconstruction phase is conducted by numerous osteoblasts present in otosclerotic tissue leads to fibrous bone foci (13, 23). These lesions showing a high bone turnover are similar to those observed in Paget’s disease (23). Although the clinical signs and the histological aspects of otosclerosis are widely described (9, 13, 14, 17), the pathogenesis of this disease remains unclear and many hypotheses including autoimmune and viral origins have been advanced (19, 22, 26).

The precise cellular mechanisms leading to the increased bone turnover in otosclerosis are not elucidated (9). Considering the major role of parathyroid hormone (PTH) in the physiology of bone turnover mediated by osteoblasts (1) and the histological aspect of otosclerotic foci, the possibility of an abnormality related to PTH action can be raised. The aim of this study was to investigate such a possibility. In order to do so, PTH-related peptide (PTHrP) receptor expression and function were compared in pathological stapes and external auditory canal (EAC) cortical bone samples (used as control bone) in patients undergoing functional surgery for otosclerosis.

Patients, Materials, and Methods

Patients and bone samples. Eleven patients undergoing surgery for hearing loss due to stapedovestibular ankylosis were included in this study. Ten patients presented otosclerosis (patients A–F and H–K), and one had osteogenesis imperfecta involving both stapes and EAC (patient G). Ethics committee approval and patients’ consent were previously obtained for the bone samplings. Clinical data were obtained from medical files. The diagnosis of otosclerosis was based on clinical, audiometric, and computed tomography scan findings and confirmed by the peroperative aspect of the stapes. Pathological stapes and bone chips from the EAC were...
obtained during surgery. Bone samples were immediately immersed in either culture medium for subsequent cell cultures (patients A, B, C, and H-K) or liquid nitrogen for RNA extraction (patients D, E, F, and G). Stapes and EAC from the same patient were always studied in parallel. The volume of the bone samples obtained from the stapes and EAC was similar and did not exceed a few tenths of a cubic millimeter.

Primary cell cultures and passages. Stapes and EAC bone fragments were placed in 10-cm² culture wells in culture medium composed of 4.5% DMEM glucose (GIBCO, Gaithersburg, MD) containing 25 mg/l vancomycin (Lilly France SA, St. Cloud, France) and 30% FCS in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Cells migrated from bone explants and maximal cellular growth from the explants were obtained in ~21 days, at which time cells were trypsinized, plated homogeneously on the culture surface, and allowed to grow to confluence during 14–21 additional days in contact with the explants. At confluence, cells were trypsinized, counted, distributed in 48-well plates (0.8 x 10⁶ to 1.6 x 10⁶ cells/cm²), and cultured for 7 days at which time they reached confluence and were used for measurement of osteocalcin secretion, cAMP production, and PTH-PTHrP receptor mRNA expression. Cell cultures were photographed under a phase-contrast microscope (Fig. 1A). SaOS-2, a human osteoblast-like cell line, was cultured in parallel in 12-well plates with DMEM with 100 U/ml penicillin G, 100 µg/ml streptomycin, and 10% FCS. For patients I, K, and H, cellular morphological changes were studied after PTH stimulation on primary cell cultures. For these experiments, nonconfluent first passage cells were incubated with 80 nM bovine PTH-(1–34) (bPTH) during 60 min at 37°C. Morphological changes were easily evidenced in three samples: stapes of patient H and EAC of patients I and K. In these cultures, a cellular contraction appeared in elongated cells, whereas an elongation could be observed in already contracted cells. Thus the morphological changes induced by PTH appeared to depend on the initial shape of the cells as previously observed (2). Morphological studies in the rest of the samples (EAC of the patient H and stapes of patients I and K) before and after PTH were not conclusive.

Osteocalcin secretion assessment. Osteocalcin concentration was measured by a radioimmunological method (ELSA Osteo, IS Bio, France) in cell culture media from stapes and EAC cell cultures obtained from patients A, B, and C.

Van Kossa staining of the primary cell cultures. Primary passage cells from stapes and EAC samples of patients H, I, and K were grown to confluence in 12-well plates. Cells were fixed by 4% neutral Formalin during 10 min at room temperature, washed three times with distilled water, covered by 1% silver nitrate solution, and exposed to ultraviolet light during 60 min. Wells were subsequently washed, incubated with 2.5% sodium thiosulfate solution during 5 min, washed again with distilled water, and dried. Mineralization foci were photographed under a phase-contrast microscope (Fig. 1B).

RNA extraction from bone cell cultures and bone samples. Cells grown in 48-well plates were lysed by repetitive aspiration in 80 µl/well of a lysis buffer containing 10 mM Tris, 1% NP-40, 0.3 U RNAsine, and 10 mM 1,4-dithiothreitol. Cellular debris was eliminated by centrifugation at 12 x 10³ rpm during 5 min, and the cell supernatant was used for RT. To extract total RNA from bone fragments, the bone fragments were crushed in 1 ml of lysis buffer (4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% N-laurylsarcosine, and 0.1 M β-mercaptoethanol) with a polytron. RNA was then extracted with phenol water and isoamyl alcohol–chloroform (1:24, vol/vol) and precipitated by isopropanol (7). The pellet was resuspended in 50 µl of water and RNA suspension used for RT.

RT of RNA extracted from cultured cells and bone fragments. Complementary DNA (cDNA) was synthesized by RT of RNA in solution (27 µl of cell supernatant or 5 µl RNA solution extracted from bone fragments) with 400 U Moloney murine leukemia virus RT (200 U/µl, GIBCO) in a buffer containing 250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl₂, and 1 mM d-nucleotide triphosphate (final volume: 40 µl).

PCR. cDNA amplification of the PTH-PTHrP receptor was obtained by a nested PCR with two oligonucleotide pairs designated C/B and E/D (Table 1). The pair C/B amplified a 430-bp cDNA fragment, and the internal pair E/D amplified a final product of 181 bp. A 784-bp fragment of glyceraldehyde-

Fig. 1. Photomicrograph of a representative stapes bone cell culture at confluence (A) and Van Kossa staining of mineralization foci surrounded by cells in a stapes bone cell culture (B).
3-phosphate dehydrogenase (GAPDH) was amplified with a pair of oligonucleotides designated 10/11 (Table 1). The cDNA segments were amplified from 10 μl of the RT product in a PCR buffer (10 mM Tris·HCl, 0.1% Triton X-100, and 0.2 g/l BSA) and in the presence of 0.2 mM of dNTP and 1 U of Taq polymerase at 5 U/μl (Appligen, Gaithersburg, MD). Each PCR cycle comprised denaturation at 94°C, annealing at 60°C, and elongation at 72°C, each step during 1 min. Thirty-three cycles were used for each stage of PTHrP receptor and GAPDH cDNA amplification. The reaction was stopped by a temperature decrease to 10°C. Preliminary experiments, measuring PCR product signal as a function of PCR cycle number on comparable quantities of RNA in SaOs-2 and with the same primers, had shown that at 33 cycles amplification was not saturated for GAPDH or PTH receptor cDNA in these conditions. Stapes and EAC samples of each patient were assessed in parallel. A 10-μl sample of the PCR product was run on a 2% agarose gel with ethidium bromide. A 100-bp molecular weight ladder permitted verification of the length of the amplified fragment.

Table 1. Sequences of oligonucleotides used to assess PTH-PTHrP receptor and GAPDH mRNA expression

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>5′-3′ Sequence</th>
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<tbody>
<tr>
<td>B</td>
<td>TICCAATCACAAAGGGCATTGCC</td>
</tr>
<tr>
<td>C</td>
<td>CCGCGGGATCAGCCACCCC</td>
</tr>
<tr>
<td>D</td>
<td>TGAGAGACAGAAAGAGGACCACC</td>
</tr>
<tr>
<td>E</td>
<td>GTCACTGCTTTTNGCCAGT</td>
</tr>
<tr>
<td>10</td>
<td>AAGGCTGGGCGCATTG</td>
</tr>
<tr>
<td>11</td>
<td>GTCTGCTGGGCGACCTGA</td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone; PTHrP, PTH-related peptide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Reverse primers are in bold.

Sequen of oligonucleotides used to assess PTH-PTHrP receptor and GAPDH mRNA expression.

Table 2. Bone cell number obtained from stapes and EAC bone samples at confluence around explant from each patient

<table>
<thead>
<tr>
<th>Cell No. x 10^5</th>
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<tbody>
<tr>
<td>A</td>
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<tr>
<td>B</td>
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<tr>
<td>C</td>
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<tr>
<td>H</td>
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<tr>
<td>I</td>
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<tr>
<td>J</td>
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<td>K</td>
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</table>

Cells were grown from bone explants as described in PATIENTS, MATERIALS, AND METHODS until maximal growth from explant (21 days). At this stage, cells were fluorescently stained and plated homogeneously on culture surface and allowed to grow to confluence during 14–21 additional days. At confluence, cells were trypsinized again, counted, and replated in 48-well plates. EAC, external auditory canal. A–C and H–K are patients.
Normalized PTH-PTHrP receptor mRNA expression obtained in stapes was compared with that in EAC by paired t-test. P < 0.05 was considered significant.

RESULTS

Clinical, audiometric, and radiological findings. The otosclerosis population (patients A-F and H-K) was composed of five males and five females. The mean age was 37 yr, ranging between 24 and 55 yr. A bilateral involvement was noted in seven cases (64%). A hereditary factor was noted in three cases (27%). No other past medical history of systemic, otologic, or bone disease was observed. Patient G was a 25-yr-old female who presented an osteogenesis imperfecta involving the EAC in addition to typical lesions of the stapes.

The mean hearing loss assessed on tonal audiometric test at 500, 1,000, and 2,000 Hz was 55 dB, ranging from 35 to 80 dB. A pure conductive hearing loss was observed in six cases (55%), and the conductive loss was associated with a sensorineural component in five cases (45%). Middle ear computed tomography scan revealed a demineralization focus at the oval fenestra in six cases of otosclerosis.

Cell culture phenotype. Cells grew in a centrifugal manner from the explants. Due to the small size of the bone samples, the cell number obtained in stapes and EAC bone cultures at confluence showed variability. From the explants, primary cultures were obtained from EAC and stapes on the one hand or between first passage and primary cell cultures on the other hand (data not shown). Osteocalcin secretion, when assessed, was detected in the cell culture media from both stapes and EAC cell cultures (Table 3).

The mean hearing loss assessed on tonal audiometric test at 500, 1,000, and 2,000 Hz was 55 dB, ranging from 35 to 80 dB. A pure conductive hearing loss was observed in six cases (55%), and the conductive loss was associated with a sensorineural component in five cases (45%). Middle ear computed tomography scan revealed a demineralization focus at the oval fenestra in six cases of otosclerosis.

Parathyroid hormone (PTH)-PTHR receptor expression was measured in media from bone cells cultured to confluence in 1.25 cm²/well as described in PATIENTS, MATERIALS, AND METHODS by a direct antibody method.

The ratio of PTH-PTHrP receptor over GAPDH mRNA signal was calculated for each sample (normalized PTH-PTHrP receptor mRNA expression) as described in PATIENTS, MATERIALS, AND METHODS.
and in one EAC sample over two tested (patient C). Bars: ratio of normalized PTH-PTHrP receptor mRNA expression determined as described in Fig. 2 and PATIENTS, MATERIALS, AND METHODS. For patients A–F, this ratio in stapes is lower than that in EAC (P < 0.05).

lower in bone cells cultured from stapes compared with that obtained in EAC cells (ranges: 0.8–4.5 and 1.5–7 in stapes and EAC cells, respectively, P < 0.05; Fig. 4, A and B). PTH at 0.8 nM concentration induced a significant cAMP stimulation in two stapes (patients A and B) and in two EAC samples (patients A and C) over seven patients tested. At 8 nM, PTH produced a cAMP stimulation in one stapes (patient B) over three tested and in one EAC sample over two tested (patient C). In SaOS-2, cAMP response was submaximal with 8 × 10⁻¹⁰ M PTH (see Fig. 6). Forskolin (5 × 10⁻⁵ M) induced a significant stimulation of cAMP production in all otosclerotic and control samples (ranges: 20.7–83.1 and 4.9–99.8 in stapes and EAC, respectively, P < 0.05; Fig. 5). The stimulation increase of cAMP production by 50 µM forskolin over basal was similar in bone cells cultured from stapes and EAC and in SaOS-2 (Figs. 5 and 6). In SaOS-2, 50 µM forskolin elicited a lower cAMP response than 30 nM PTH. Basal cAMP production was significantly higher in the stapes than that in the EAC for the patient I. There was no significant difference between basal values of cAMP production measured in bone cells cultured from stapes and EAC bone chips in the rest of the cases (Table 4).

### DISCUSSION

To investigate a possible role of PTH-PTHrP receptor in abnormal bone turnover observed in otosclerosis, the function and expression of the receptor were studied in the involved tissue (stapes) in comparison with that in normal bone. The expression and function of the PTH-PTHrP receptor were measured in stapes and EAC bone chips from patients with otosclerosis. The results showed that the expression and function of the PTH-PTHrP receptor were significantly lower in stapes than in EAC bone chips from patients with otosclerosis. The results suggest that the PTH-PTHrP receptor plays a role in the abnormal bone turnover observed in otosclerosis.

### Table 4. cAMP production in cell cultures

<table>
<thead>
<tr>
<th></th>
<th>Basal cAMP</th>
<th>cAMP + PTH</th>
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<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stapes</td>
<td>28.8 ± 6.27</td>
<td>75.0 ± 17.02*</td>
</tr>
<tr>
<td>EAC</td>
<td>28.5 ± 2.98</td>
<td>65.0 ± 10.38</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stapes</td>
<td>37.1 ± 2.51</td>
<td>93.1 ± 17.23†</td>
</tr>
<tr>
<td>EAC</td>
<td>39.9 ± 5.20</td>
<td>70.1 ± 9.61*</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stapes</td>
<td>54.5 ± 7.15</td>
<td>56.3 ± 5.75</td>
</tr>
<tr>
<td>EAC</td>
<td>33.2 ± 6.99</td>
<td>234.1 ± 24.94†</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stapes</td>
<td>20.6 ± 7.40</td>
<td>52.5 ± 3.86†</td>
</tr>
<tr>
<td>EAC</td>
<td>25.6 ± 7.31</td>
<td>137.4 ± 35.91*</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stapes</td>
<td>142.7 ± 27.12‡</td>
<td>89.0 ± 24.48</td>
</tr>
<tr>
<td>EAC</td>
<td>18.8 ± 1.94</td>
<td>36.7 ± 1.70†</td>
</tr>
<tr>
<td>J</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stapes</td>
<td>49.3 ± 5.37</td>
<td>46.0 ± 5.65</td>
</tr>
<tr>
<td>EAC</td>
<td>335.7 ± 104.84</td>
<td>1095.0 ± 20.92†</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stapes</td>
<td>120.8 ± 23.15</td>
<td>215.3 ± 81.77</td>
</tr>
<tr>
<td>EAC</td>
<td>97.1 ± 37.32</td>
<td>161.5 ± 52.73</td>
</tr>
</tbody>
</table>

Results are in pmol·mg protein⁻¹·15 min⁻¹ and are means ± SE of triplicate determinations. A–C and H–K are patients. cAMP production was measured as described in PATIENTS, MATERIALS, AND METHODS. Basal cAMP: cAMP production in absence of agonist. cAMP + PTH: cAMP production measured after 80 nM bovine PTH (1–34) incubation at room temperature during 15 min. *P < 0.05 and †P < 0.01, comparison with basal value of same sample; ‡P < 0.05, comparison with basal value in EAC of same patient.
The observation that PTH evoked a cAMP response lower than that produced by forskolin in our series is interesting to point out, because PTH elicited a similar or even greater cAMP response than forskolin in SaOS-2 (this study) and in other primary bone cell cultures (24). The significance of this observation remains unclear. Importantly, a lower cAMP response to PTH than to forskolin was evidenced in both stapes and EAC cells. As regards the PTH-PTHrP receptor mRNA expression, this was found to be lower in the otosclerotic stapes, which is in accordance with the lower cAMP response to PTH in the otosclerotic stapes. The high sensitivity of RT-PCR, a technique used for the assessment of the mRNA expression, imposes a cautious interpretation of the results. In fact, the PTH-PTHrP receptor cDNA could not be amplified in a single PCR round. Despite this limitation, it is noteworthy that all patients with otosclerosis presented a lower PTH-

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**Fig. 6. Stimulation of cAMP production in SaOS-2 cell line by PTH and forskolin (FK).** Stimulation of cAMP production was performed as described in patients, materials, and methods. Values are means ± SD of triplicates from one representative experiment performed in parallel with primary bone cell cultures.

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that measured in EAC cells is in agreement with previous work by Fano et al. (11) who reported that a higher PTH concentration was required to stimulate adenyl cyclase (AC) activity in stapes to the same extent as in EAC cells. In contrast to our study, the basal AC activity reported by these authors was signifi-
cantly higher in otosclerotic cells compared with that in control cells. Difference in experimental conditions, and in particular the fact that we measured cAMP production in the presence of 3-isobutyl-1-methylxan-
thine in intact cells, whereas Fano et al. measured AC activity in cell membrane, may explain these results.

Different mechanisms could be responsible for a decreased PTH stimulation of cAMP production. Inadequate experimental conditions can be excluded because SaOS-2, used as positive control in our series, showed cAMP response to low PTH concentrations. Moreover, they would not explain the difference of cAMP response between stapes and EAC in our series. Observations by Della Torre et al. (8) and Maurizi et al. (21), based on an abnormal cAMP response to proprano-

ol and on a difference of AC activity between otoscle-

rotic and control cell homogenates in the presence of GTP, Ca^{2+}, Mn^{2+}, and calcitonin, suggested that a calcitonin receptor/G protein binding alteration may be responsible for the abnormal agonist-stimulated AC activity in otosclerotic cells. Other possibilities, which do not exclude a defect in receptor/G protein interac-
tion, comprise defects in AC activity and receptor expression or structure. The lower response in stapes samples reported here was not due to an AC defect because the stimulation of cAMP production by forsko-

lin was evidenced in all cases and was similar to that in EAC cells. In other types of osteoblast-like cell cultures, a wide range of cAMP response to PTH and forskolin has been reported (10, 25). In our series, the control bone samples were obtained from a near anatomic site the involvement of which by the otosclerotic lesions has never been observed in large autopsy series (14). However, excluding positively any difference of cAMP response between stapes and EAC not related to otoscle-

rosis would have necessitated the comparison of normal stapes and EAC, which was ethically impossible in this study.

The observation that PTH evoked a cAMP response lower than that produced by forskolin in our series is interesting to point out, because PTH elicited a similar or even greater cAMP response than forskolin in SaOS-2 (this study) and in other primary bone cell cultures (24). The significance of this observation remains unclear. Importantly, a lower cAMP response to PTH than to forskolin was evidenced in both stapes and EAC cells. As regards the PTH-PTHrP receptor mRNA expression, this was found to be lower in the otosclerotic stapes, which is in accordance with the lower cAMP response to PTH in the otosclerotic stapes. The high sensitivity of RT-PCR, a technique used for the assessment of the mRNA expression, imposes a cautious interpretation of the results. In fact, the PTH-PTHrP receptor cDNA could not be amplified in a single PCR round. Despite this limitation, it is noteworthy that all patients with otosclerosis presented a lower PTH-

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a normal bone sample obtained in a near anatomic site, the EAC, from the same patient.

The results showed a lower stimulation of cAMP production by PTH in stapes cells in comparison with that measured in EAC cells. A wide range of values was measured for basal and agonist-stimulated cAMP pro-
duction for both stapes and EAC cell cultures. This may be, at least in part, explained by differences in cell composition of the individual cultures. This heterogeneity, which is inherent to our model, would partially mask the phenotype differences due to the pathological process depending on the proportion of the involved cells in our cultures. Although published results (20) and our observations suggest the presence of osteoblast-

like cells in these cultures, a difference of osteoblast-

like cell proportion between stapes and EAC cultures cannot be totally excluded. This difference could ex-

plain a higher cAMP response to the PTH stimulation in the EAC. However, the microscopic aspects of the bone cells cultured from stapes and EAC were similar, mineralization foci were observed in all cases, and osteocalcin was detected in the culture medium from stapes and EAC bone cells when it could be measured. These suggest that the stage of differentiation of cells grown from stapes and EAC was similar and thus that difference in cell differentiation does not explain the lower response obtained in stapes cells. Osteocalcin secretion in cell culture media was used as a specific indicator of osteoblast-like cell presence and osteoblastic high differentiation in the cultures. This marker could not be used to exclude differences of cell popula-
tion between the cultures, because this would have required the assumption that there is no difference in osteocalcin secretion activity in otosclerotic and control osteoblast-like cells. Moreover, no relation can be es-

tablished between osteocalcin production and the ability of these cultures to form mineralization foci, because these were similarly present in all cultures in spite of differences in osteocalcin concentrations in the media.

The observation that PTH induced a lower stimula-
tion of cAMP production in stapes cells compared with
PTH-related peptide receptor (PTHrP receptor) expression in the involved tissue (stapes) than that in the control sample (EAC). In addition, stapes-EAC normalized PTH-PTHrP receptor mRNA expression ratios were homogeneous and similar between cases studied by RNA extraction from bone and those studied through cell cultures, demonstrating that cell cultures conserved their original PTH-PTHrP receptor expression phenotype. Interestingly, the patient who did not have a lower PTH-PTHrP receptor mRNA expression in the stapes compared with that in EAC presented osteogenesis imperfecta involving both external and middle ear and not otosclerosis.

It is unlikely that the decreased PTH-PTHrP receptor expression and function are the triggering event leading to otosclerosis. More probable etiologic factors include a persistent measles infection (19, 22) and an abnormal regulation of bone matrix protein metabolism such as glycosaminoglycan (4). Abnormalities of PTH-PTHrP receptor expression and function can be easily integrated in the mechanisms leading to abnormal bone remodeling induced by these possible etiologies. PTH-PTHrP receptor function and expression abnormalities are among potential consequences of measles infection, because this virus induces multiple cytopathogenic effects such as upregulation of heat shock proteins and chromosomal fragmentation (3). Moreover, the PTH-PTHrP receptor may be directly involved in the regulation of glycosaminoglycan metabolism or may indirectly influence it through a modification of its sulfation (16).

The relation between the low PTH-PTHrP receptor expression and the high bone turnover in the otosclerotic stapes remains to be elucidated. Different hypotheses can be proposed concerning this relation. Observations in SaOS-2, a human osteoblastic cell line, show that the activation of the cAMP transduction pathway mimicked the PTH metabolic stimulatory effects (15) and decreased the PTH-PTHrP receptor mRNA expression (12). An anomaly of this pathway beyond cAMP production (e.g., an abnormal activation of the protein kinaseA) would explain a lower receptor mRNA expression associated with a high bone remodeling in otosclerotic tissue. It could also be speculated that PTH stimulated second messengers other than cAMP, such as intracellular Ca2+, could be incriminated in the increased bone turnover. In favor of that hypothesis, a higher basal intracellular Ca2+ concentration associated with a lower stimulation of this messenger’s concentration by PTH was observed in otosclerotic bone cultures compared with that obtained in EAC control samples (11).

In conclusion, our results are compatible with a lower PTH-PTHrP receptor mRNA expression in tissue samples and in early stage primary cell cultures associated with a lower cAMP response in the otosclerotic stapes compared with that in EAC. This difference supports the hypothesis that an abnormal cellular response to PTH contributes to the abnormal bone turnover in otosclerosis.

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REFERENCES


