

## Experimental estrogen-induced hyperprolactinemia results in bone-related hearing loss in the guinea pig

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**Horner KC, Cazals Y, Guieu R, Lenoir M, Sauze N.** Experimental estrogen-induced hyperprolactinemia results in bone-related hearing loss in the guinea pig. *Am J Physiol Endocrinol Metab* 293: E1224–E1232, 2007. First published August 21, 2007; doi:10.1152/ajpendo.00279.2007.—Our group (Horner KC, Guieu R, Magnan J, Chays A, Cazals Y. *Neuropsychopharmacology* 26: 135–138, 2002) has earlier described hyperprolactinemia in some patients presenting inner ear dysfunction. However, in that study, it was not possible to determine whether hyperprolactinemia was a cause or an effect of the symptoms. To investigate the effect of hyperprolactinemia on inner ear function, we first developed a model of hyperprolactinemia in estrogen-primed Fischer 344 rats and then performed functional studies on pigmented guinea pigs. Hyperprolactinemia induced, after 2 mo, a hearing loss of ~30–40 dB across all frequencies, as indicated by the compound action potential audiogram. During the 3rd mo, the hearing loss continued to deteriorate. The threshold shifts were more substantial in males than in females. Observations under a dissection microscope revealed bone dysmorphology of the bulla and the cochlea. Light microscopy observations of cryostat sections confirmed bone-related pathology of the bony cochlear bulla and the cochlear wall and revealed morphopathology of the stria vascularis and spiral ligament. Scanning electron microscopy revealed loss of hair cells and stereocilia damage, in particular in the upper three cochlear turns and the two outermost hair cell rows. The data provide the first evidence of otic capsule and hair cell pathology associated with estrogen-induced prolonged hyperprolactinemia and suggest that conditions such as pregnancy, anti-psychotic drug treatment, aging, and/or stress might lead to similar ear dysfunctions.

hormones; deafness; prolactin; estrogen; osteoprotegerin

PROLACTIN IS A POLYPEPTIDE hormone, originally believed to be uniquely secreted by the lactotroph cells of the anterior pituitary and was shown to have a major role in inducing lactation. Now, it is recognized to be synthesized in a wide range of tissues, including the brain (33). Receptor binding sites have been identified in a wide range of tissues, including the brain and peripheral organs, which include the otic capsule (78). Indeed, the biological action of prolactin is so widespread that it is now known to have at least 300 different functions (10, 33). Furthermore, prolactin is now recognized, together with other hormones such as cortisol, growth hormone, and endorphins, as a major stress-related hormone and is known to participate in the psychoneuroendocrinology of depression (67).

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Some ear pathologies, such as tinnitus, are known to be affected by situations of stress (45). Stress is widely believed to trigger episodes of Ménière disease, characterized by tinnitus, fluctuant hearing loss, and episodic vertigo. Recently, our group (49) investigated stress hormones in patients with Ménière disease and, unexpectedly, revealed several cases of Ménière disease with marked hyperprolactinemia and subsequent MRI-confirmed pituitary adenoma. A further study in Sweden confirmed the association of hyperprolactinemia in several patients with Ménière disease, and one had a pituitary adenoma (30). The data are particularly intriguing because hyperprolactinemia has never before been described in this pathology; furthermore, clinical diagnostic schema for hyperprolactinemia do not include any otological pathological features (6). On the other hand, giant pituitary masses can be associated with hearing loss (9, 16, 21, 65), tinnitus (27), and vertigo (82).

To shed some light on the effect of hyperprolactinemia on inner ear function, in the present study, we first reproduced the well-established rat model of estrogen-induced hyperprolactinemia (19, 66). With this model, we could demonstrate that estrogen implants did create hyperprolactinemia. We then went on to provoke hyperprolactinemia in the pigmented guinea pig for which our group has long experience with audiometry. We hypothesized that experimentally induced hyperprolactinemia would modify cochlear function.

### MATERIALS AND METHODS

Experimental procedures were reviewed and approved by the committee of the French Ministry of Agriculture and carried out under license (to K. C. Horner). The experimental protocols followed the guidelines of the Centre National de la Recherche Scientifique and the French and European laws on laboratory animal care and use.

Fischer 344 (F344) male rats (total of 30 rats) weighing ~200 g at the start of the study were supplied by Charles River (Lyon, France). Pigmented guinea pigs (total of 30 males and 30 females) weighing ~250 g at the start of the study were supplied by Centre de Production Animale (Olivet, France).

**Protocol chronology.** This study was carried out over a period of 3 yr. Most of the first year was devoted to investigations on F344 rats, which is a long-standing model used to induce hyperprolactinemia but which proved not to be a good model for the present study of auditory function (see RESULTS and DISCUSSION). The investigation was continued on pigmented guinea pigs. Guinea pigs were first chronically implanted with electrodes on the cochlear round window. Some animals were implanted with bilateral electrodes, and other animals

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received only one electrode. The choice of unilateral or bilateral electrodes depended on time and material availability. The eighth nerve audiograms were tested several times over a period of 2 wk to ensure the stability of the recordings from each animal. The animals then underwent operations to introduce implants subcutaneously. Thereafter, the audiograms were monitored weekly to detect short-term and long-term effects of the hormonal changes. At the end of the observation period, the animals were anesthetized and perfused for histological investigations.

**Implantation of the round window electrodes in guinea pigs.** Guinea pigs were anesthetized with a mixture of 10 mg/kg xylazine (Rompun from Bayer) and 50 mg/kg ketamine (Imalgène from Merial). A Teflon-coated platinum electrode was surgically implanted on the round window of the cochlea. The electrode was led, under the skin, to a connector that was fixed by four stainless steel screws to the skull. The connector was secured with dental cement. All materials were purchased from Phymep (Paris, France).

**Round-window compound action potential audiograms.** Guinea pigs were restrained in a holding tube in which head movements were prevented by the use of neck and nose rings. Recordings were carried out in a sound-proof booth (Amplifon, Milan, Italy). After several sessions, the animals became familiarized with the protocol and remained calm during testing.

The compound action potential (CAP) audiogram was constructed from the visual detection of averaged evoked CAP responses. The CAP was filtered (30 Hz to 10 kHz) and averaged (~200 responses) via a Cambridge Electronic Design computer/averager. Visual threshold detection was determined in response to tone pips (2-ms rise/fall, no plateau) at different frequencies (500 Hz to 32 kHz) presented at a rhythm of 30 per second. The step size for CAP thresholds was 10 dB down toward thresholds, and thereafter it was 3 dB. The reproducibility of threshold determination was close to 3 dB across different recording sessions and across experienced raters. The tone pips were presented in free field via an earphone (Sennheiser HD 424) at a distance of 1 cm from the ear pinna. The acoustic levels at the entrance of the acoustic meatus were determined by placing a 0.5-in. condenser microphone (Bruel and Kjaer 4191) at the position of the animal's pinna. Acoustic levels were calibrated and expressed in dB sound pressure level (SPL).

Only ears presenting normal CAP audiograms, at the end of the 2-wk observation period, were included in the study. According to our criteria for normality of audiogram, threshold at 8 kHz was <10 dB SPL; threshold at 16 kHz, 4 kHz, and 2 kHz was below 15 dB SPL; and threshold at 32 kHz and 1 kHz was below 30 dB SPL. These thresholds were similar to the best estimates from behavioral or other electrophysiological studies (31). We therefore eliminated animals that manifested signs of pathology such as otitis media. In addition, we did not include data from animals that were not followed regularly every week and those that had lost their electronic connector or electrode before the end of the observation period.

**Estrogen implants.** Silastic medical tubing (1.98-mm inner diameter, 3.18-mm outer diameter; Dow-Corning) was cut into 3-cm lengths. One end was sealed with silicon adhesive. The tubes were incubated overnight at 38°C. Then, 25 mg of estradiol (Sigma-Aldrich, Lyon, France) were introduced into the test tubes, and some control tubes were left empty. The open end was sealed with silicon adhesive, and the tubes were again incubated overnight at 38°C. Under Rompun/Ketalar anesthesia, an incision was made in the scapula region of the skin of the animal, and one implant, filled or empty for controls, per 250 g body wt was introduced. The incision was sutured.

**Blood sampling.** At the end of the observation period, animal groups (treated: 10 males and 10 females; and controls: 2 males and 2 females) were decapitated under anesthesia. Whole trunk blood was collected in dry tubes, allowed to clot at 4°C, and then centrifuged at 2,000 rpm. The serum was then stored at -20°C until assay.

**Serum prolactin determination.** The serum prolactin level was determined with the use of a colorimetric enzyme immunoassay kit (Biotrak, RPN 2563) supplied by Amersham Life Sciences. The protocol supplied with the kit was followed, and serum samples were diluted 1:5 in assay buffer (supplied). Briefly, samples, as well as the blanks and standards, were run simultaneously on the same assay plate. All incubations were carried out at room temperature. All samples were added to the coated wells and incubated with antiserum for 3 h. Prolactin conjugate was added and incubated for 30 min. The plate was decanted and washed four times with wash buffer. Amplex amplification reagent (horseradish peroxidase coupled) was added and again incubated for 30 min. After samples were washed four times with wash buffer, substrate was added and incubated for a further 30 min. The coloration was arrested by adding 1 M sulfuric acid to the wells. The plate was read on a spectrophotometer plate reader at 450 nm. A calibration curve was constructed from working standards supplied (0.82–200 ng/ml), and the experimental values were read directly from the curve. The assay kit had an analytic sensitivity of 0.44 ng/ml.

**Serum estrogen determination.** Serum estrogen was determined by the double antibody estradiol <sup>125</sup>I radioimmunoassay kit supplied by DPC France. The protocol supplied with the kit was followed, and serum samples were diluted 1:10 in the zero calibrator (supplied). Incubations were carried out at room temperature. Briefly, the serum samples were pipetted, antiserum was added, and samples were vortexed, followed by incubation for 2 h. <sup>125</sup>I-labeled estradiol was added, vortexed, and incubated for 1 h. Cold precipitating solution was added, vortexed, and incubated for 10 min. After centrifugation for 15 min at 3,000 g, the tubes were decanted and inverted on absorbent paper for 10 min. The tubes were then passed in a gamma counter, and counting was carried out over 1 min. A calibration curve was constructed from supplied calibrators (5–500 pg/ml), and the experimental values were read off directly from the curve. The assay kit had an analytic sensitivity of 1.4 pg/ml.

**Light microscopy.** The animals were anesthetized and then underwent intracardiac perfusion with ice-cold saline followed by paraformaldehyde (4% in phosphate buffer 0.1 M). The cochleae were removed and immersed in fixative for at least 2 h or up to overnight. They were then rinsed in phosphate buffer (0.1 M). The cochleae were then examined under the dissection microscope for detection of indexes of pathology. They were then decalcified in EDTA for up to 3 wk. The cochleae were then rinsed in phosphate buffer and immersed in sucrose (30%) overnight. The specimens were then bathed in cryoblock and rapidly frozen to -20°C in methyl butane. The cochleae were sectioned (15 µm) on a cryostat and colored with hematoxylin-eosin. Observations were carried out on a Nikon Eclipse E600 light microscope, and photography was carried out via a camera (Nikon) linked to ACT-1 software.

**Scanning electron microscopy.** Two alternative fixation procedures were employed. Some animals underwent intracardiac perfusion with ice-cold saline followed by paraformaldehyde (4% in phosphate buffer, 0.1 M). The cochleae were removed and perfused with phosphate buffer followed by glutaraldehyde (2.5% in phosphate buffer, 0.1 M). After 2 h, the cochleae were perfused and rinsed three times (each 15 min) in phosphate buffer (0.1 M).

For animals that had been decapitated for trunk blood sampling, some cochleae were rapidly removed and perfused via the round and oval windows with glutaraldehyde (2.5% in phosphate buffer). After 2 h, the cochleae were perfused and rinsed three times (each 15 min) in phosphate buffer (0.1 M).

In all cases, the cochleae were then perfused with osmic acid (1% in phosphate buffer) and further fixed for 1 h. This was followed by perfusion and rinsing three times (each 15 min) in phosphate buffer. Dehydration was carried out to up to 70% alcohol (each 15 min). Dissection was performed to remove the bony wall of the cochlea and to expose the organ of Corti. The specimens were then prepared for

scanning electron microscopy (SEM) after critical point drying and coating with gold-palladium.

**Statistical analysis.** Statistical analysis was carried out with the SigmaStat 3.1 program (Systat Erkrath). Data at 1, 2, and 3 mo were considered for statistics. For analysis of acoustic sensitivity data, a three-way (sex or laterality, time, frequency) ANOVA was performed followed by examination of all pair-wise comparisons using the Holm-Sidak method.

## RESULTS

Attempts to follow auditory function in F344 rats, by brain stem recordings, were abandoned for two reasons. We did find some hearing sensitivity loss ( $\sim 20$  dB after 1 mo) in the F344 rats. However, several recent publications, which appeared after the start of our study, showed that hearing function in untreated F344 rats deteriorates with aging faster than in other strains of rats (74, 75). In addition, we encountered technical complications of instability of long-term recordings associated with rejection of the screws from the skull after  $\sim 1$  mo. Because this did not happen in control rats and because we used the same type of screws and cement as for guinea pigs, we suggest that one plausible explanation would be a modified immune response related to hyperprolactinemia in these rats (33).

**Pigmented guinea pigs.** The outward sign of the changing hormonal status in the guinea pigs was a break in the body weight growth curve, which could be detected 1 mo after insertion of the estrogen implants (data not shown). At the end of 3-mo treatment, some animals started to lose their hair.

**Hormone assays.** Standard control prolactin values are between 10 and 50 ng/ml in normal young rat. In the absence of published data, to our knowledge, the standard control values for guinea pigs were assumed to be similar. Control values for one male and one female were below the lower end of the calibration curve (ends at 10 ng/ml) calculated to be  $\sim 10$  ng/ml. In treated animals, prolactin had minimum-median-maximum values of 78–143–438 ng/ml for males ( $n = 10$ ) and 23–83–625 ng/ml for females ( $n = 10$ ). There was no significant difference between males and females according to a Mann-Whitney rank sum test ( $P = 0.121$ ; Fig. 1, left).

The experimental estrogen levels increased above standard control values of 5–20 pg/ml (reference in normal young rat). Control values for one male and one female were below the lower end of the calibration curve (ends at 50 pg/ml) but calculated to be  $\sim 10$  and  $\sim 15$  pg/ml, respectively. For estrogen, the minimum-median-maximum values were 138–861–1,688 pg/ml for males ( $n = 10$ ) and 175–751–8,900 pg/ml for females ( $n = 10$ ). There was no significant difference between males and females according to a Mann-Whitney rank sum test ( $P = 0.91$ ; Fig. 1, right).

At the time of sampling, after 3 mo of treatment, we found no significant relationship, according to Pearson product-moment correlation, between prolactin and estrogen for males ( $P = 0.19$ ), females ( $P = 0.33$ ), or males and females together ( $P = 0.15$ ).

**Audiogram changes.** Twelve test males (4 bilateral and 8 unilateral; total ears:  $n = 16$ ) and 13 test females (7 bilateral and 6 unilateral; total ears:  $n = 20$ ) presented good audiograms at the onset of the study and could be followed over 3 mo. Control animals with empty tubes (5 males and 5 females) showed no change in the audiogram over the observation

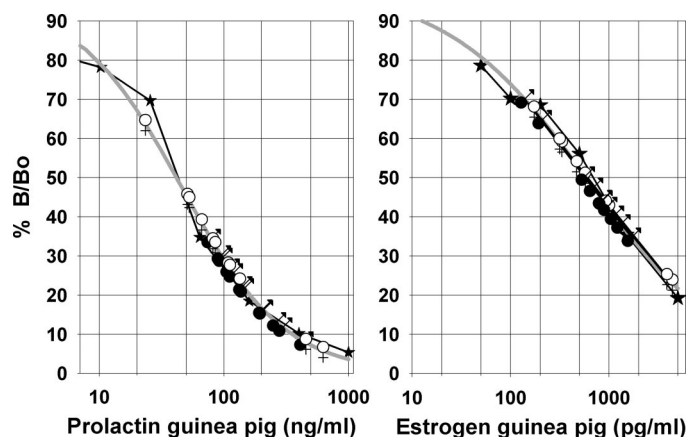


Fig. 1. Immunoassay for determination of guinea pig prolactin and estrogen. Blood serum from estrogen-primed guinea pigs was employed for determination of prolactin (ng/ml) by enzyme immunoassay (left) and for estrogen (pg/ml) by radioimmunoassay (right). ●, Males; ○, females. In each case, the calibration curve was supplied and adjusted here to take into account the respective dilutions (black plot with fixed reference values represented as black stars). On the y-axes, %B/Bo represents the percentage of immunobinding for the calibration values from which the experimental values were calculated according to the kit instructions and plotted (gray plot).

period. In test animals with estrogen implants, some threshold fluctuations could be observed over several weeks (not shown here). These fluctuations were considered particularly interesting but not detailed for the purpose of the present long-term study. No consistent deterioration of the audiogram could be detected before several weeks. After 2 mo, definite threshold shifts could be detected, and, after 3 mo, the threshold shifts progressed, in general, in males and in females. At this stage, some threshold shifts from males ( $n = 7$ ) were more than 60 dB and in one case above 80 dB at the limit of the calibration curve of the acoustic setup (Fig. 2).

For all animals (males or females) with bilateral data, ANOVA (3 way: laterality, months, frequency) showed no statistical difference between left and right ears at any month or frequency. Thereafter, all results from all ears were considered in further statistical analysis. A three-way ANOVA (sex, months, frequency) indicated significant differences between months ( $P < 0.001$ ), between sexes ( $P < 0.001$ ), and between frequencies ( $P < 0.001$ ), with significant interactions between months and sex ( $P < 0.019$ ) and frequency and sex ( $P < 0.01$ ). All pair-wise multiple comparisons were then made with the Holm-Sidak method, and a significance level of 0.05 was chosen. All results cannot be listed here; however, it appeared, as can also be seen in Fig. 2, that threshold shifts increased and differed significantly at all 3 mo for males and for females. Males differed significantly from females at all 3 mo.

**Light microscopy observations.** All cochleae were visualized under the dissection microscope during the perfusion for fixation. At dissection, the bulla wall was perceived to be thicker and harder than in control specimens. When the bulla was opened, the middle ear was clearly visible, and the absence of otitis media was confirmed in all cases (Fig. 3). At this stage, the presence of some type of bone remodeling could be clearly identified within the cochlear bulla (arrow 1) and the cochlea (arrow 3) compared with the normal specimens. In addition, the dark band corresponding to the position of stria vascularis was less easily identified than normal (arrow 2). Within the



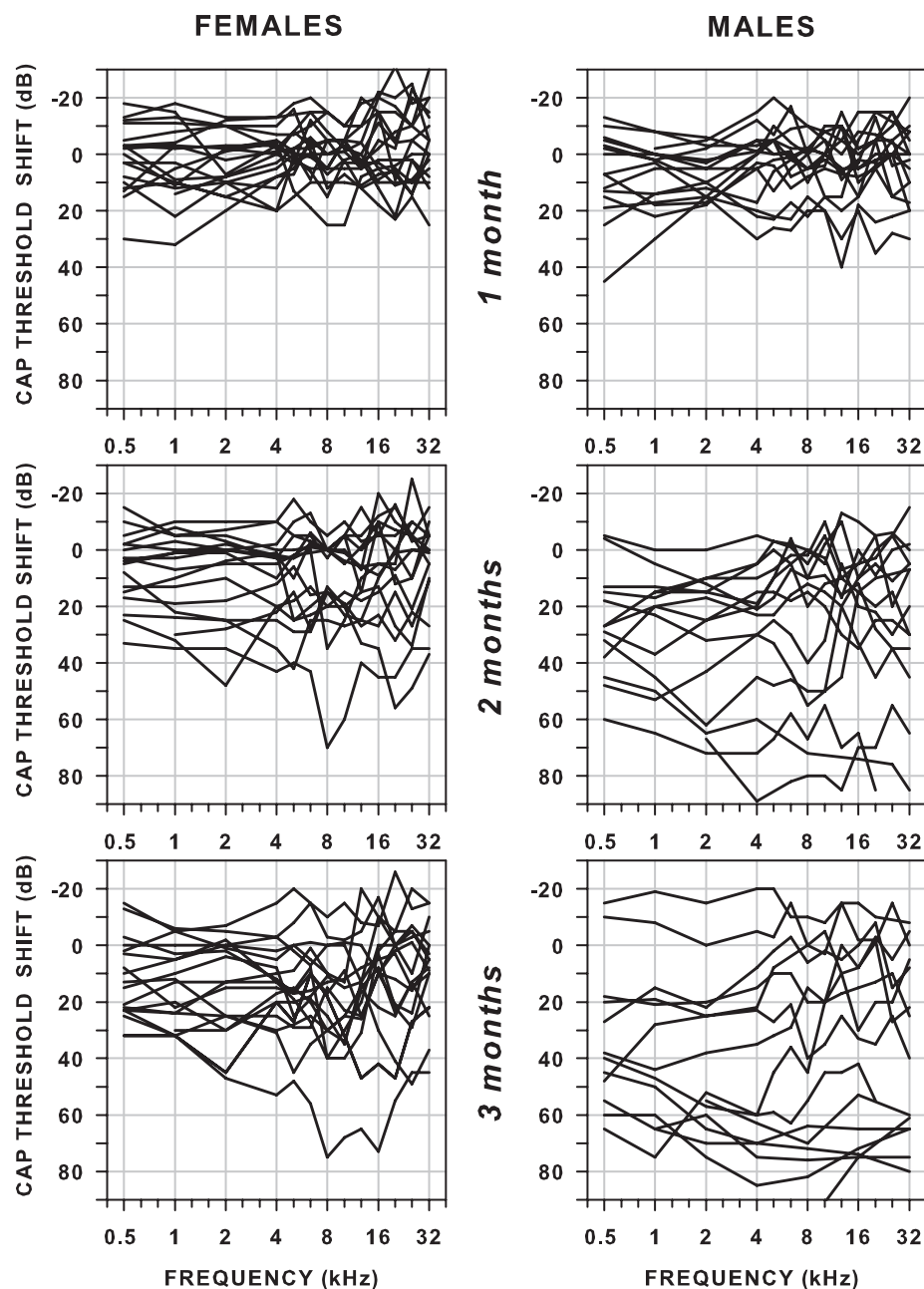


Fig. 2. Audiogram changes in estrogen-primed guinea pigs over 3 mo. Auditory nerve compound action potential (CAP) threshold shift (dB) is plotted as a function of frequency (kHz). There was no significant difference between right and left ears for males or females; therefore, data from both ears are presented without distinction. *Left:* females ( $n = 20$ ). *Right:* males ( $n = 16$ ). Progressive threshold shifts observed throughout 3 mo demonstrated more loss for males than for females.

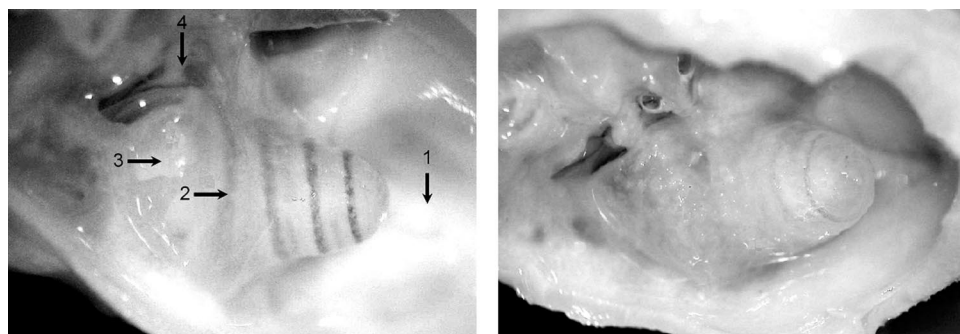
limits of the macro-observations under the dissection microscope, the middle ear ossicles appeared normal in the majority of cases, but clear bone dysmorphology of the middle ear ossicles could clearly be identified in two specimens (*arrow 4*).

Light microscopy was carried out on cochleae from treated females (4 unilateral and 3 bilateral; total ears:  $n = 10$ ) and males (4 unilateral and 2 bilateral; total ears:  $n = 8$ ) and control cochleae (3 unilateral female and 3 unilateral male;  $n = 6$ ). The cochlea images presented in Fig. 4, *D–F*, were from a male animal and are representative of the cochlear pathology observed. There was no evidence for otitis media. The middle ear ossicles could be clearly identified at the base of the cochlea (Fig. 4*D*). The most obvious pathological feature was the bone dysmorphology throughout the cochlear bone, which could represent otospongiotic-like foci (Fig. 4*D*). This was particu-

larly evident immediately behind the stria vascularis/spiral ligament (Fig. 4*E*). In addition, the spiral ligament showed signs of deterioration and the collagen fibers were less easily identified than in control cochleae (Fig. 4*F*). The stria vascularis also appeared degenerated (Fig. 4*F*). No control cochlea presented such anomalies.

One specimen from a female presented normal thresholds in both ears for frequencies between 16 and 32 kHz together with a sensitivity loss of more than 40 dB for all frequencies below. Because the two ears had similar audiograms, one cochlea was observed in light microscope and the other in SEM. Light microscopy did not reveal changes to either bone or the stria vascularis. The other cochlea was observed in SEM, where pathological changes could be detected at hair cell level, as detailed below.

Fig. 3. Macroscopic view of cochleae illustrating the bone dysmorphology. Dissection microscope view of a control cochlea (*left*) and a cochlea from a treated animal (*right*). Obvious pathological features could be observed, including thickening of the bulla wall (arrow 1) and cochlear wall dysmorphology (arrow 3). Stria vascularis was less visible through the bony wall of the cochlea (arrow 2); however, middle ear bones were exceptionally calcified (2 cases), as presented here (arrow 4).



**Scanning microscopy.** SEM observations were carried out on cochleae from male (2 unilateral,  $n = 2$ ) and female (1 bilateral and 1 unilateral,  $n = 3$ ) guinea pigs. The cochleae were observed throughout their length.

The two cochleae from the male guinea pigs presented at least 30-dB losses across the audiogram, and SEM observa-

tions confirmed the loss of hair cells and stereocilia pathology throughout the cochlea with a slightly better preservation in the upper basal turn.

One female guinea pig presented a substantial sensitivity loss across the audiogram ( $>75$  dB) for which SEM confirmed the degeneration of stereocilia bundles and loss of hair cells

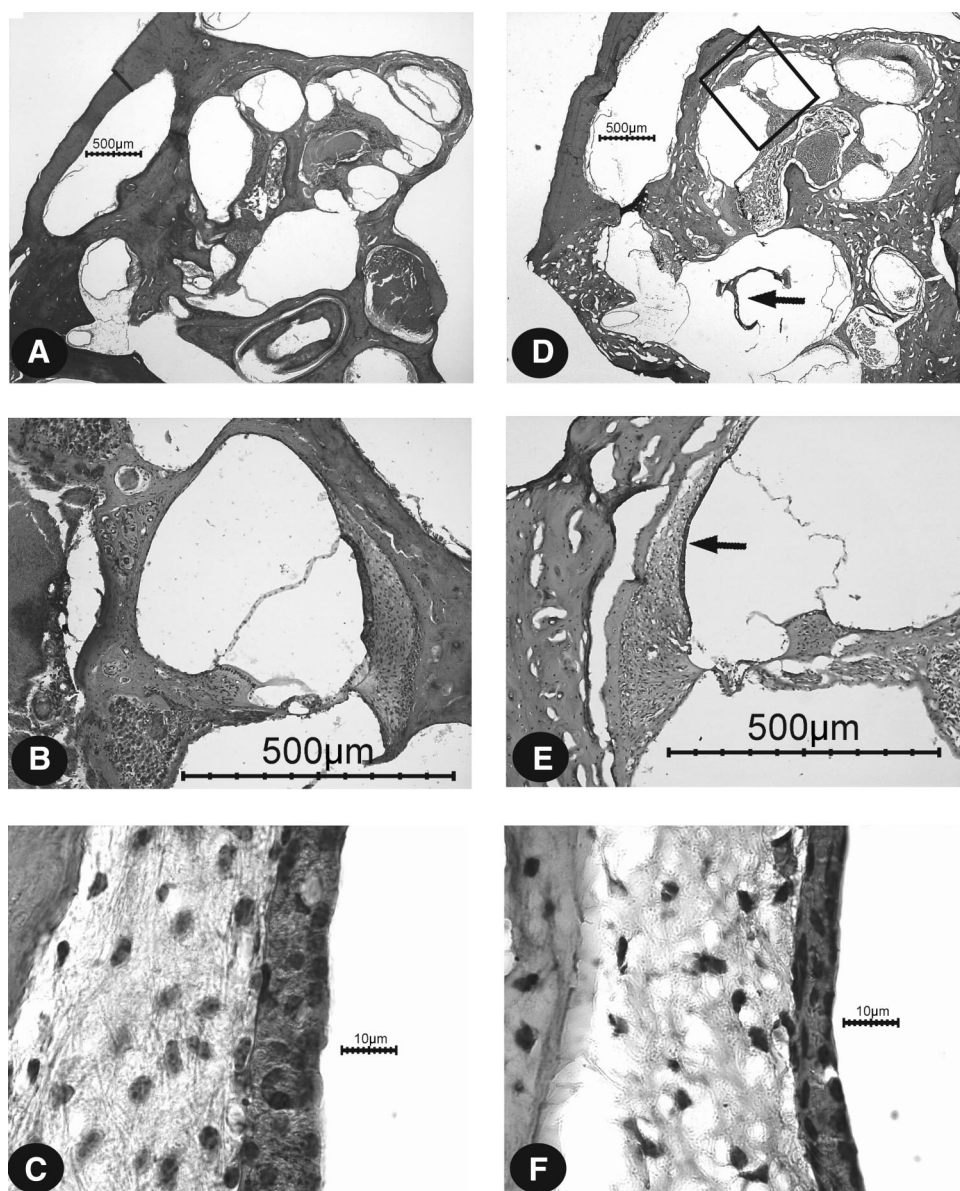


Fig. 4. Light microscopy showing pathology of the bony cochlea. Cryostat sections (15  $\mu$ m, hematoxylin-eosin stained) from a control cochlea (A–C) are compared with those from a treated animal (D–F). For the experimental cochleae, there was complete absence of otitis media, and the middle ear ossicles could be clearly identified (arrow in D). The most striking pathology was the apparent osteoporosis of the bony wall of the cochlea. At higher magnification (rectangle in D presented in E), the bone appeared particularly fragile close to the spiral ligament. In addition, the stria vascularis (arrow in E presented in F) showed thinning. The spiral ligament appeared fragile, and collagen fibers were less easily identified.



throughout the cochlea. The other ear, on the other hand, presented very little sensitivity loss, and indeed the SEM observations confirmed the excellent condition of the stereocilia and hair cells. Indeed, they were comparable to those seen in the organ of Corti of a control guinea pig (Fig. 5, A–C).

For the female guinea pig presenting very similar audiograms in the two ears, one cochlea was observed in light microscopy and the other in SEM. Light microscope observations did not reveal modifications. In contrast, SEM revealed stereocilia pathology and hair cell loss above the basal turn of the cochlea. The damage essentially involved loss of hair cells from the two more external rows. The stereociliary bundles of the remaining hair cells of rows 2 and 3 appeared limp or folded, in particular for row 3 (Fig. 5, D–F).

## DISCUSSION

Estrogen treatment in pigmented guinea pigs led to body-weight loss, in agreement with first studies on this animal model (19, 66) and in accordance with its anorectic property described more recently (34, 35). In the longer term, estrogen induced hyperprolactinemia, for the large part via inhibition of dopamine synthesis in tuberinfundibular dopaminergic neurons (3, 5). The treatment resulted in body hair loss in accordance with prolactin modulation of hair growth in humans (32) and in mice (24). Beyond these expected results, our study revealed two major new findings concerning the ear. Long-term estrogen treatment led to hearing loss, which was more substantial

in males than in females. There was a close link between threshold shifts and bone dysmorphology of the cochlear bulla and cochlea.

**Limitations of the present data.** Elevated experimentally induced levels of estrogen and prolactin were confirmed. We were not able to correlate hormone levels with threshold shifts because different experimental groups of animals were involved. On the other hand, when substantial threshold shifts were recorded, otic bone dysmorphology was systematically observed. At early stages of the pathology, hearing loss was fluctuant (not documented). In one case, bone dysmorphology was not detected by light microscopy, but SEM confirmed hair cell stereocilia disarray in the three upper cochlear turns, which followed a gradient from the third outer hair cell row to first row, similar to that described earlier for experimental endolymphatic hydrops in the guinea pig (50, 79, 80). Actin depolymerization of the stereocilia was hypothesized in that model to account for the fluctuant hearing losses (48). Interestingly, a causal link between F-actin disassembly and increased prolactin has been established for pituitary cells in culture (13). It would be interesting to pursue this hypothesis, to determine the relationship between stereocilia pathology and early threshold fluctuations.

We found that males were more sensitive than females, although at the end of 3-mo observation period the median levels of prolactin and estrogen in males and females were not significantly different. The sex difference might have been due

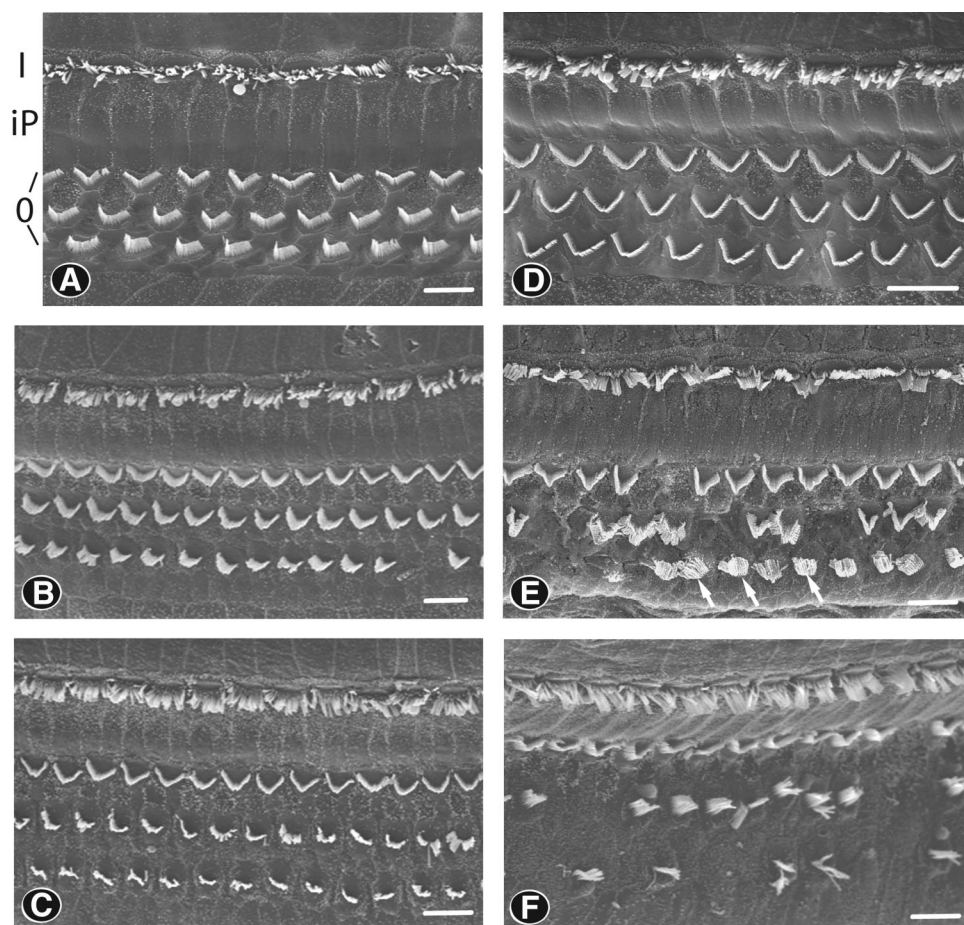


Fig. 5. Cochlear hair cells as seen in scanning electron microscopy. In cases where the hearing loss across the audiogram was moderate, the stereociliary bundles appeared, as shown for a control guinea pig (A–C). A more profound low hearing loss was associated with essentially normal basal turn of the cochlea (D) and substantial damage, including loss of hair cells, from the 2 more external rows in the middle turn (E) and apical turn (F). The stereociliary bundles of the remaining hair cells of rows 2 and 3 appeared limp and folded, particularly for row 3 (E and F). I, inner hair cells; O, outer hair cells; iP, pillar supporting cells.

to dynamic interactions between estrogen and prolactin or the interplay of other hormones such as cortisol, which can protect against acoustic trauma (12) and can be sex-difference sensitive in some patients with inner ear pathology (47).

*Estrogen and the cochlea.* Estrogen receptors  $\alpha$  and  $\beta$  have been identified within the inner ear of rats and mice in various cell types, including hair cells, spiral ganglion cells, stria vascularis, and spiral ligament (91). Observations on the isolated stria vascularis from gerbils have shown that estrogen rapidly inhibited the slow component of the potassium current channel in the apical membrane of the marginal cells, suggesting that estrogen could influence  $K^+$  secretion in vivo via a nongenomic mechanism (56). Data from human studies are equally scarce. Estrogen receptor  $\alpha$ -containing cells have been located selectively in the spiral ganglion and  $\beta$ -containing cells in the stria vascularis (90).

*Estrogen can positively affect hearing.* Estrogen does affect hearing, but the data are often contradictory (51). Aging or ovariectomy in rats resulted in increased latency of the acoustically evoked brain stem responses. Estrogen treatment was not found to change these observations in older rats (22) but did reverse the latency changes after ovariectomy in younger rats (20). Hearing in mice was best preserved in females relative to males until menopause, beyond which the advantage was lost (40). Indeed, when estrogen was suppressed in female mice, by administration of tamoxifen, reduced medial olivocochlear feedback was observed (93). Sex differences in age-related human hearing loss is well documented (37). Hearing sensitivity declines rapidly above the age of 20–30 yr in men and above the age of 50 yr in women (71). Better hearing has been reported to be correlated with higher serum estradiol levels in postmenopausal women (53).

*Estradiol can negatively affect hearing.* Several reports have indeed suggested that oral contraceptives may increase risk of hearing loss and in particular otosclerosis, although no clear picture has emerged (96). Otosclerosis is estimated to have a prevalence of 0.3% (36) according to clinical manifestations and up to 3% (26) or more according to histological examination of temporal bones postmortem. It is an age-related ear pathology that becomes manifest, in general, between ages 20 and 40 yr, affects twice as many women as men, and is usually bilateral (15, 25, 58). Hearing loss related to cochlear otosclerosis worsens during periods of intense hormonal activity (83). Otosclerosis often begins during a first pregnancy and is seriously aggravated during successive pregnancies (38).

Reversible (44) and irreversible hearing losses have also been described in women after the use of contraceptives (68), as well as with hormone replacement therapy (92). Elevated serum prolactin levels in women can occur with high-dose estrogen contraceptive pills (52). A recent report has revealed that hormone replacement therapy, using estrogen and progesterin together, negatively affects hearing in aged women, whereas estrogen alone does not (39). Hyperprolactinemia can be induced by an estrogen-progesterone synergy (72, 97). Interestingly, treatment with estrogen together with progesterone for 30 days in guinea pigs resulted in minor changes in latency and decreased amplitude of brain stem-evoked responses, as well as an inflammatory pattern within the cochlea (7).

*Hyperprolactinemia.* Hyperprolactinemia prevalence in an unselected normal population has been reported to be 0.4% but

can be as high as 17% in a population of women with reproductive problems (6). Hyperprolactinemia is associated with aging in rats (29, 89) and in women and men (4, 8, 77, 81, 95). Prolactin levels rise during pregnancy and in particular during the last trimester (73) as well as during lactation (94). In addition, schizophrenia is supposed to be associated with hyperactivity of dopamine, such that neuroleptic treatment has its therapeutic effect through inhibition of central dopamine functions (14). Hyperprolactinemia is a well recognized side effect of antipsychotic drug treatment (28) and in schizophrenia (41, 42).

*Hyperprolactinemia can lead to osteoporosis and possibly otosclerosis.* Prolactin influences calcium mobilization in rats (55, 69). One major side effect of hyperprolactinemia in humans is decreased bone mineral density (54, 64) and osteoporosis (76). Hyperprolactinemia is associated with decreased bone mass density, which occurs during pregnancy (2), lactation (70), and schizophrenia (1, 43, 62, 63).

Strangely, hyperprolactinemia has not previously been linked to otosclerosis. On the other hand, decreased bone mineral density has been found to be moderately associated with hearing loss in older men but not in women (46). In the presence of osteoporosis, the chances of having otosclerosis are increased by a factor of two (17) or four (18). Furthermore, abnormal *COL1A1* collagen gene expression might be implicated in the two pathologies (18, 61). At this point, it is particularly interesting to recall the earlier reports of middle ear bone remodeling in the gerbil after chemical sympathectomy by guanethidine sulfate (86) or hydroxydopamine (85). Although these neurotoxins do eliminate sympathetic activity, there are, in parallel, major central consequences. In particular, both treatments reduce hypothalamic dopamine, which leads to an increase in serum prolactin levels (57, 59). In light of the present data, the possibility of a prolactin involvement in chemical sympathectomy should certainly be considered.

Prolactin is known to have a direct inhibitory effect on osteoblast activity (23); in addition, prolactin regulates the expression of receptor activator of NF- $\kappa$ B ligand (RANKL), at least in mammary gland (88). RANKL, expressed in osteoblasts, is highly involved in bone remodeling and activates its receptor RANK on osteoclasts (60). Osteoprotegerin, which is an antagonist of RANKL, has been found to be produced within the cochlea in the spiral ligament (98) from where it is likely to be secreted into the perilymph and can hence diffuse into surrounding otic capsule bone (60). Estrogen-induced hyperprolactinemia, described in the present study, may lead to bone remodeling of the otic capsule by interfering with the fine balance between osteoprotegerin-RANKL-RANK within the otic capsule.

*Conclusions.* Although experimental chronic estrogen-induced hyperprolactinemia in the guinea pig may not represent all categories of hyperprolactinemia in humans, the data allow us to speculate that hyperprolactinemia observed by us earlier, in some patients with Ménière disease (30, 49), might have played some causative role in the symptoms. In fact, otosclerosis and Ménière disease can coexist, although the causal relationship remains controversial (84, 87). Because both can present low-frequency hearing loss, tinnitus, and vestibular dysfunction, otosclerosis can mimic the symptoms of Ménière disease (11), and the diagnosis of otosclerosis can even be missed at first (87).



The unexpected novel finding of prolactin-related otic capsule bone dysmorphology fits with recent concepts regarding the molecular biology of otosclerosis, and our data suggest that prolactin is acting via a osteoprotegerin-RANKL-RANK mechanism.

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