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"Neural" Responses to Acoustic Stimulation after Destruction of Cochlear Hair Cells*

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Summary. Electrophysiological and histological observations in guinea pig's cochleas after amikacin treatment (14 × 450 mg/kg) confirm the results obtained in a former experiment: clear, short-latency, click-evoked responses were recorded in cochleas with only very few hair cells remaining at the extreme apex. Detailed analysis of these responses strongly indicates a neural origin and confirms their low-frequency sensitivity. Careful histological observations confirm the extensive hair cell loss and the preservation of nerve fibers in the remnants of the organ of Corti and of the vestibular sense organs. These results suggest that the acoustical vibrations either stimulate the vestibular receptors or act directly or through some kind of mechano-electrical transduction on the remaining cochlear nerve fibers.

Key words: Click-evoked responses – Total cochlear hair cells loss – Cochlea – Vestibule – Ototoxicity

In a former experiment, routinely conducted for the study of the ototoxicity of the aminoglycoside antibiotic amikacin, clear neural responses to acoustic clicks could be recorded from the round window of guinea pig cochleas in which, during later histological observation using light and electron microscopy, we have not been able to detect any outer or inner hair cells on the basilar membrane except for a very few outer hair cells at the apex (Aran et al., 1979).

Unless a considerable number of inner hair cells have remained undetected in the lower basal turn of these cochleas, it was hypothesized that the responses were originating either in the remaining unmyelinated nerve fibers in the cochlea which would be acoustically stimulated without the mediation of sensory hair cells, or in the vestibular nerve fibers triggered by acoustic stimulation of the vestibular sense organ whose structure and function appeared to be preserved.

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In order to precise (1) the nature of the observed responses, and (2) the effective destruction of the hair cells along the basilar membrane, a second experiment, using an identical protocol, but more sophisticated electrophysiological and histological techniques, has been performed. The results, which confirm our initial impression, are presented here.

Material and Methods

Treatment

Exactly as in the former experiment (Aran et al., 1979), 12 healthy colored guinea pigs of about 400 g weight, presenting with a normal Preyer reflex, received 14 i.m. injections of amikacin 450 mg/kg/day, the drug being administered daily except during weekends. One guinea pig died during the treatment, two others died 10 days after the end of the treatment.

Click-evoked responses were recorded from the round window of the left ears in five of the nine remaining guinea pigs between 34 to 48 days after the end of the treatment. They were killed for histological preparation of the cochleas from 0 to 7 days after the recordings. One other guinea pig presented with otitis media and recordings were impossible to perform. Three remaining guinea pigs were kept for the long-term evaluation of the responses and of the cochleas. One has been equipped with an electrode permanently implanted on the round window of the left ear (Aran and Darrouzet, 1975; Aran and Erre, 1979), 34 days post Rx and by now, six months later, responses are still monitored. Thus the results presented here refer only to the five guinea pigs for which we actually dispose of recordings and histology.

Electrophysiology

For the recordings, a nickel-chrome 50 μm wire loop electrode is placed on the round window membrane of the left cochlea under Ketamine anesthesia, through a retro-auricular approach. After sealing of the hole in the bulla, a 10-cm Silastic tube is glued (using histoacryl) inside the external auditory meatus and coupled to either a Brüel-and-Kjaer condenser microphone cartridge or a TDH 39 Telephonics receiver. The signal is amplified 1 to 16 thousand times with a frequency band between 0.5 and 30 kHz. Responses are sampled and stored in a Histomat S data acquisition system with a sampling rate of 5 μs/point in a window of 5.12 ms (1024 points).

The stimuli, which in this case, due to the elevated thresholds, were presented in the TDH 39, consisted of broad frequency spectrum click produced by an 0.1 ms electrical impulse, and third-octave filtered clicks from 0.5 to 10 kHz. Both rarefaction and condensation stimuli were used alternately but the averaged responses to each polarity were stored separately.

Threshold and input-output functions for the click were systematically determined while other different specific studies, such as frequency threshold curves (CAP audiograms), high-pass masking, effect of anoxia, adaptation, etc. were occasionally performed on the various guinea pigs.

Histology

The five guinea pigs were killed by decapitation and the two bullas removed within a few minutes.

For guinea pigs N° 188, 192, and 193, both cochleas were fixed by perfusion of osmic acid (1%) in veronal buffer. The left cochleas were placed in 70% alcohol and studied by the surface preparation method. Two of them were entirely dissected and observed in Ann Arbor at the Kresge Hearing Research Institute (guinea pigs N° 188 and 192) and cochlear cytograms of the entire length of the basilar membrane were obtained. Only portions of each turn of the left cochlea of guinea pig N° 193

were examined in our laboratory, the total length of the basilar membrane thus observed being about 60% of its entire length. The right cochleas were prepared for transmission electron microscopy.

For the two last guinea pigs (N° 190 and 194), a different technique (Aran, 1977), allowing a precise reconstruction along the entire length of the basilar membrane without the hazards of the manual dissection, was used for the left and right ears. The cochleas were fixed by perfusion with glutaraldehyde (5%) in cacodylate buffer. Then decalcification was obtained by immersion in EDTA (10%) during 14 days. After dehydration, these decalcified cochleas were imbedded by perfusion of a low viscosity resin (Spurr). Then they were cut in serial sections on an LKB pyramitome with a glass knife which is large enough to cut the entire cochlea in a direction parallel to the modiolus according to the Guild's method. Sections of 10 μm were made every 50 μm, placed on a glass plate in liquid Spurr which then polymerized in an oven. Such slides are quickly obtained and the organs of Corti and spiral ganglions of the various turns can be observed in light microscopy (phase or interference Nomarski contrasts) with magnification up to 100 × 10, thus allowing the detailed spiral reconstruction of the entire cochlea as in the Guild's method. Moreover, after each 10 μm section, a thicker section (20 μm) was made which could be dissected and mounted for thin sections and transmission electron microscopy.

Results

It must be emphasized that the results are strikingly similar to those obtained in the former experiment (Aran et al., 1979) and that they are, here also, quite identical from one guinea pig to another.

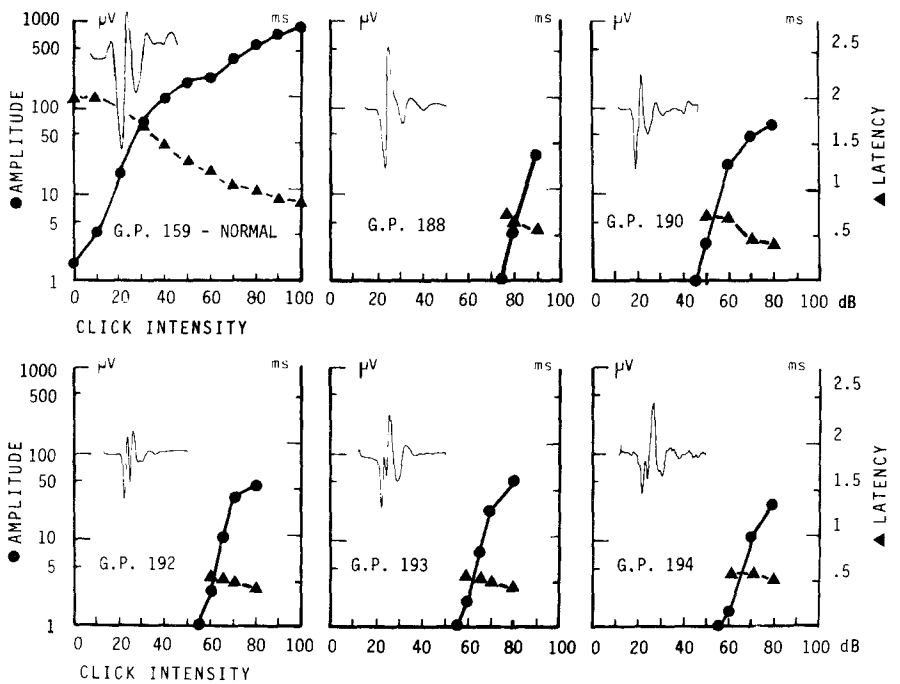

Fig. 1. Amplitudes and latencies of the averaged click-evoked responses (condensation + rarefaction) in a normal guinea pig (*GP*) (N° 159) and in five amikacin-treated GPs (14 × 450 mg/kg – 34 to 48 days post Rx). For each GP a typical pattern of the response to a high intensity click is represented (trace duration: 5 ms)

The Responses

The responses to the click are presented in Fig. 1 and compared with that classically obtained, in the same conditions, in a normal guinea pig. The thresholds range between 45 and 75 dB (above the mean threshold in normal guinea pigs: dB HL). The maximum amplitude is about 50 μV, some 20 times smaller than in the normal, but the responses are quite clear, with a very fast, diphasic pattern. The latencies are always very short (around 0.5 ms at 80 dB), quite shorter than in the normal at the same intensity (1 ms). There is very little difference between the responses to the rarefaction and condensation clicks (Fig. 2A). We also observed, using trains of clicks with inter-clicks intervals of 10 ms and inter-trains intervals of 100 ms, that no adaptation was present at any intensity level. Finally, the response appears quite dependent upon the oxygen supply as evidenced by its rapid disappearance when such a supply is suppressed (Fig. 2B).

The frequency sensitivity of the responses seems to be restricted to the low frequencies. Responses to third-octave filtered clicks are obtained only for frequen-

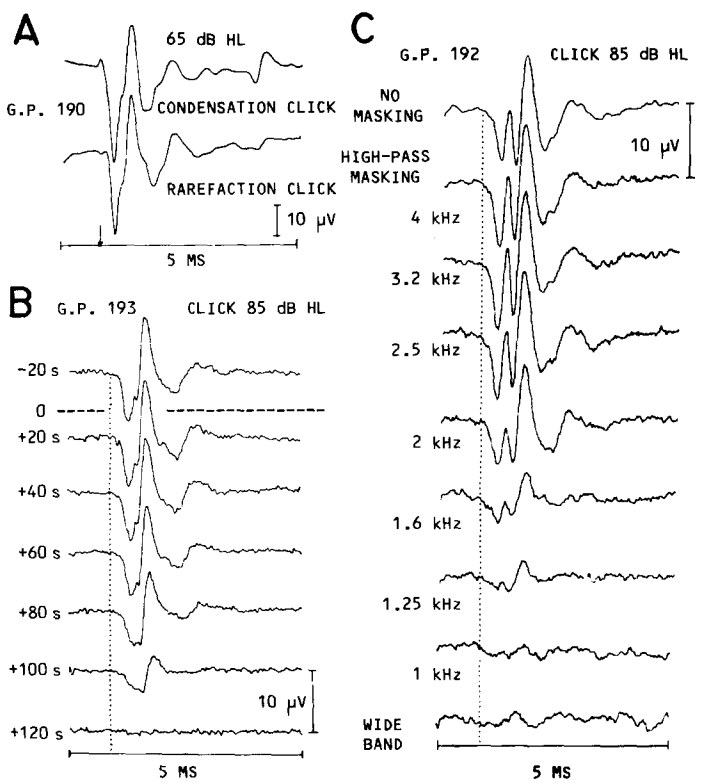

Fig. 2. A Comparison of the responses to the rarefaction and condensation clicks in GP N° 190. **B** Responses to the 85 dB click recorded in GP N° 193 every 20 s before and after respiration block followed by cardiac arrest (time 0). The response disappears totally within 2 min. **C** Effects of a masking high-pass filtered white noise on the click-evoked response in GP N° 192 for various cut-off frequencies, as compared to the total masking produced by the unfiltered wide-band noise (*bottom*). Masking is effective between 2.5 and 1 kHz only

cies below 4 kHz and best thresholds are found between 0.5 and 2 kHz (Fig. 3). The efficient frequency band in the spectrum of the wide-band click in eliciting the response is also limited to the low frequencies between 1 and 2.5 kHz as shown by the effect of high-pass masking noise in Fig. 2C, which begins to mask below 2.5 kHz.

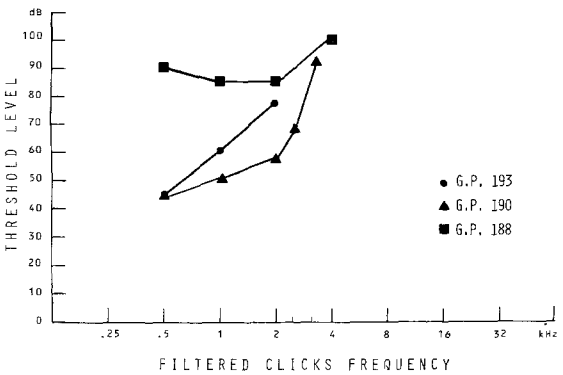

Fig. 3. Thresholds of responses to filtered clicks of various frequencies (CAP audiograms) in three amikacin-treated GPs. Responses above 4 kHz could not be obtained

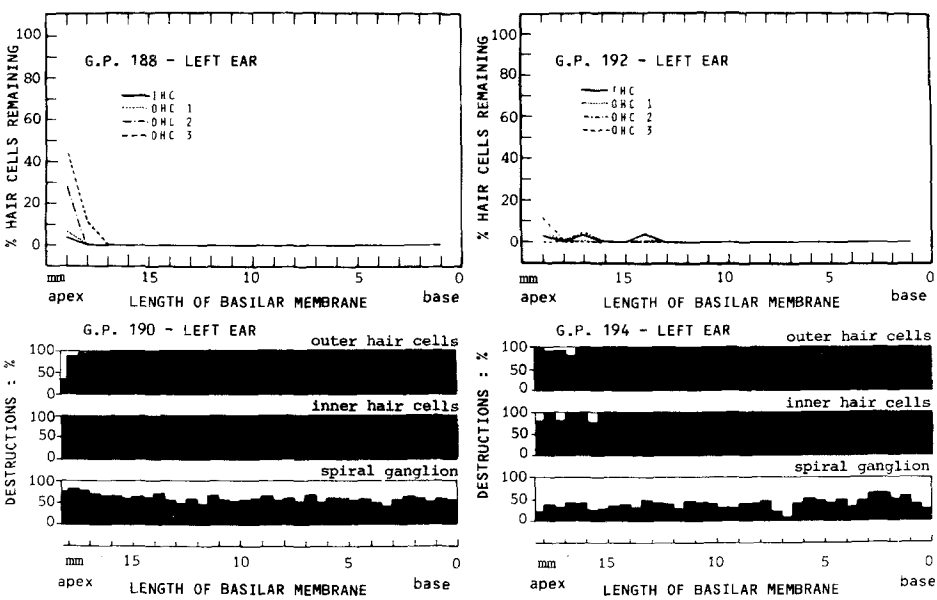

Fig. 4. *Top:* Cochlear cytograms obtained from surface preparations of the left cochleas of two of the amikacin-treated GPs (N° 188 and 192), expressed in % of the remaining inner hair cells (*IHC*) and outer hair cells (*OHC*) of the three rows as a function of distance from the base on the basilar membrane. *Bottom:* Histo-cochleograms obtained with the modified Guild's method for the left cochlea of 2 other amikacin treated GPs (N° 190 and 194) representing % of loss of hair cells (inner – outer) and of spiral ganglion cells as a function of distance from the base on the basilar membrane. See details of light microscopy, reconstruction and electron microscopy of the cochlea of GP N° 190 in Figs. 5, 6 and 7

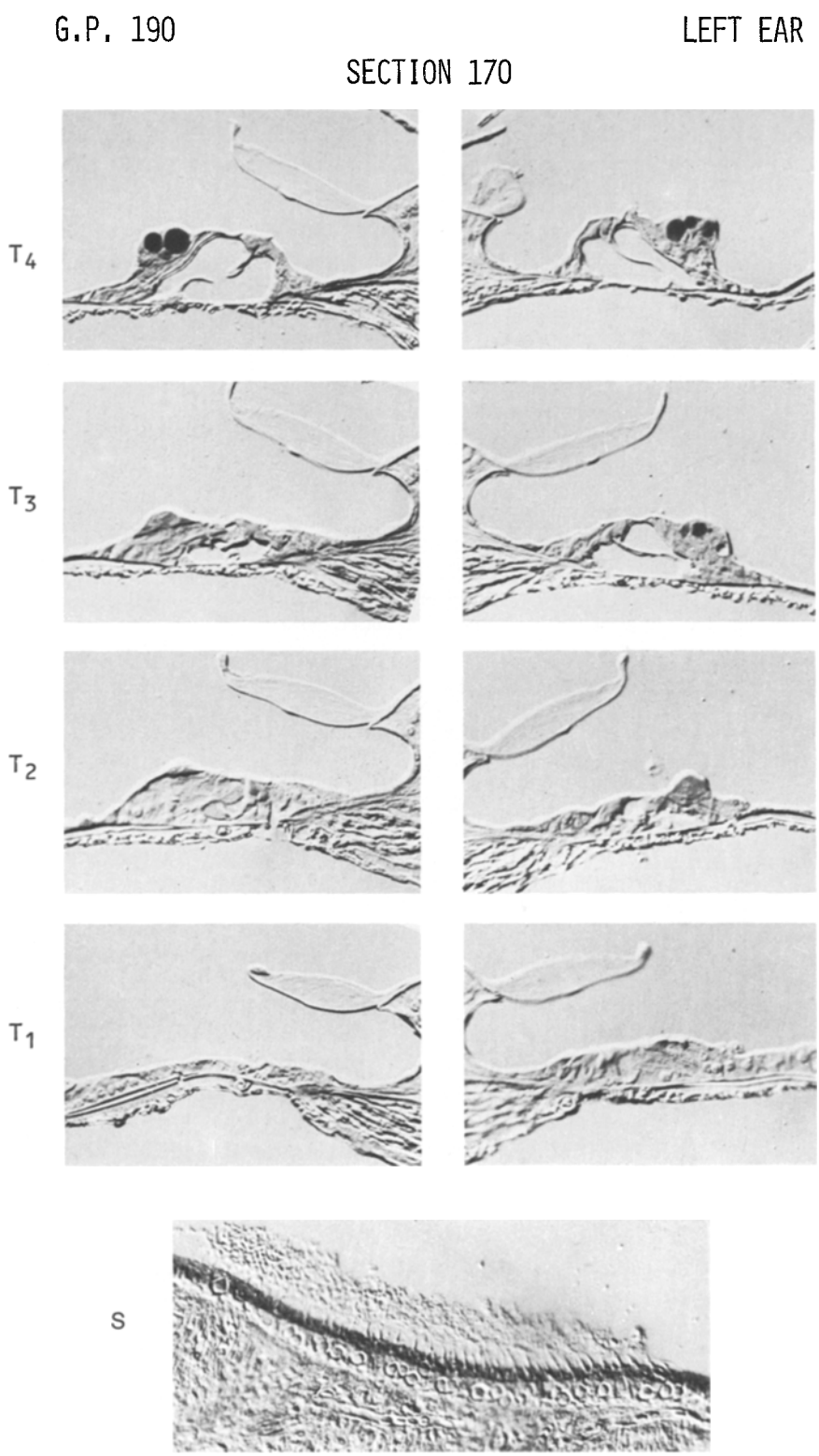

Fig. 5. (Legend see page 67)

Condition of the Inner Ears

Whichever histological method was used, entire surface preparations – serial sections – electron microscopy, it has been impossible to detect any remaining outer or inner hair cell in the cochleas from which the responses described above were obtained, except for very few, mainly outer hair cells at the extreme apex (Figs. 4 to 7). No hair cell could be observed also in the left cochlea of guinea pig N° 193. However, in all these cochleas there still remains a more or less undifferentiated epithelium along most of the basilar membrane (Fig. 5), where numerous nerve fibers can be observed, mainly in the internal spiral bundle, and a few in the external spiral bundle and spiral tunnel fibers. In the osseous spiral lamina and spiral ganglion there is a loss of about 50% (Fig. 4, guinea pigs 190 and 194). However, the nerve fiber density in the remnants of the organ of Corti appears markedly reduced as compared to a normal cochlea.

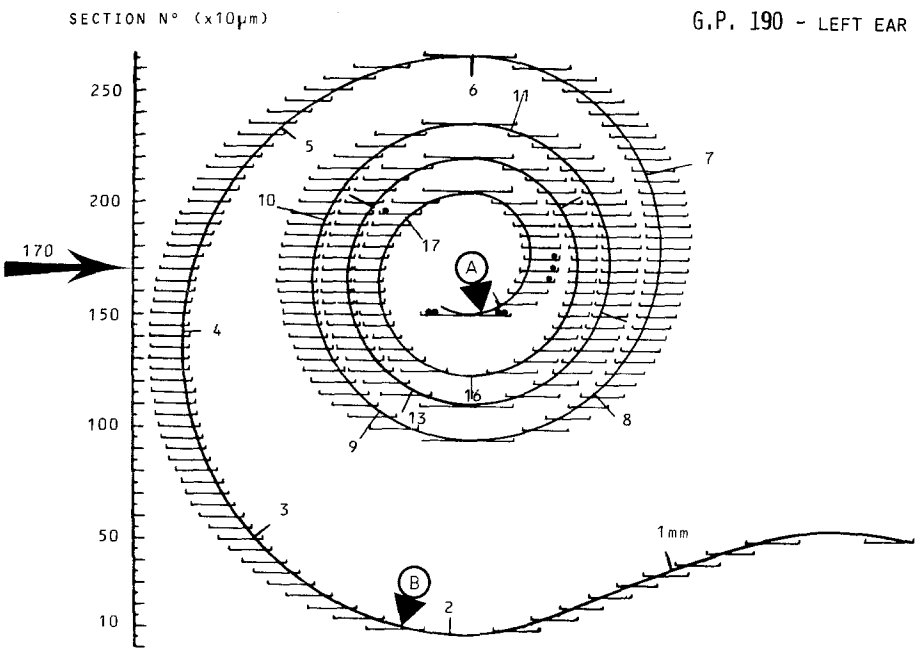

Fig. 6. Typical Guilds's spiral reconstruction of the basilar membrane from optic examinations of the serial sections of the left cochlea of GP N° 190 (Fig. 5). Each frame (——) represents the basilar membrane for each section of the organ of Corti which could be observed on the serial sections. Only hair cells which could be detected have been represented (●). They are only very few at the apex. All the empty frames correspond to sections where no hair cell could be observed. The triangles in A and B indicate the places where the electron micrographs of Fig. 7 have been taken. The arrow indicates the place of the section N° 170 shown in Fig. 5

Fig. 5. Views of the organs of Corti displayed on section N° 170 of the left cochlea of GP N° 190 (see total reconstruction in Figs. 4 and 6) for the various turns (*T*) from the base (T1, left) to the apex (T4, right) where there only an outer hair cell can be observed. At the bottom (*S*) the saccule from the same section showing a near to normal population of hair cells

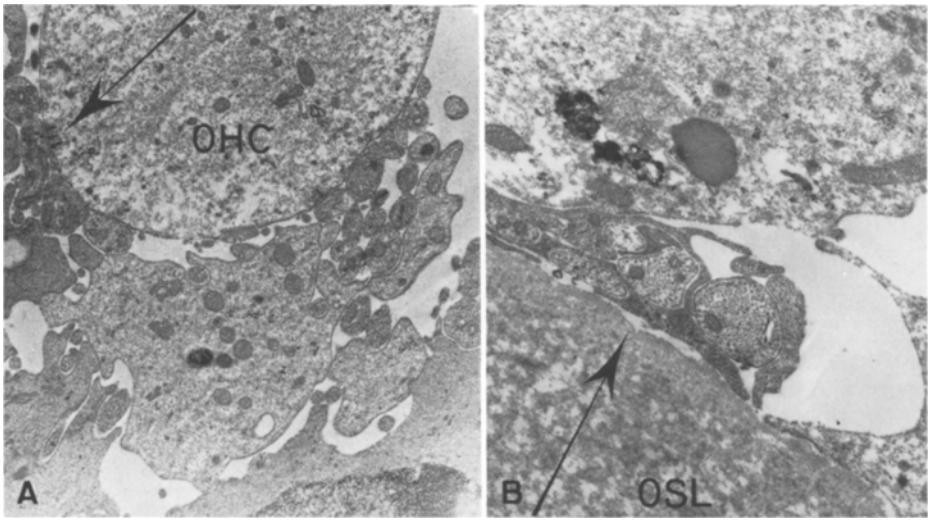

Fig. 7. Example of electron-microscopic details from the left cochlea of GP N° 190 in the places (**A** and **B**) corresponding to that indicated in Fig. 6 at about 18 and 2 mm from the base, respectively. **A** Base of one of the very few outer hair cells (*OHC*) of the apex with afferent nerve endings and synaptic bars (*arrow*). **B** Nerve fibers (arrow) of the internal spiral bundle between the osseous spiral lamina (*OSL*) and a supporting cell at the base of the cochlea where hair cells are totally missing

In opposite, examination of the utricles and saccules revealed an almost normal population of healthy sensory hair cells (Fig. 5, bottom) corresponding to a normal vestibular function as evaluated by nystagmographic observations. Indeed, for the right ears, the histological findings were similar.

Discussion

The aims of this study were to reproduce the responses and the cochlear pathology already obtained with this amikacine treatment and then to analyze the nature of these peculiar responses, trying not to miss any possible surviving hair cell anywhere along the basilar membrane in the histological observations.

The set of electrophysiological data suggests very strongly the neural nature of the responses: there is an immediate effect of anoxia, a precise threshold, and the pattern of the responses is quite comparable to that of a classical compound action potential and it does not follow the phase of the acoustic stimulus. Moreover, it is possible to mask this response with a wide-band noise and there is a frequency specificity. These latter points rule out the possibility for the response to be some kind of microphonic potential. The fact that adaptation, which in normal cochleas occurs with such an inter-click interval of 10 ms is not present, does not oppose to the neural nature of the response. This inter-click interval is much larger than the relative refractory period of the 3—4 μm diameter cochlear-nerve fibers, not considering any transducing mechanism.

If we then assume that these responses are neural, the question rises of their

origin, since it is very likely that, as indicated by the two histological methods, all the hair cells, outer and inner, are missing except for very few at the extreme apex.

The first possibility is that these responses are triggered by the few remaining mainly outer hair cells of the apex. However, they are very scarce although apparently functional (Fig. 7A) and it is difficult to think that they are sufficiently many to be responsible for such clear responses. Similar clicks thresholds as well as comparable response amplitudes have been obtained in cochleas which, after treatment with other antibiotics, revealed many more inner and outer hair cells in the upper turns (Aran and Darrouzet, 1975; Aran et al., 1979): moreover, in such cases the responses waveforms were much broader and the latencies much longer (above 2 ms). Although the frequency sensitivity of the responses in this study would be in favor of their apical origin, it is in complete opposition with their very short latency which cannot include the travelling wave delay, and which are even much shorter than the shortest latency ever observed in the normal guinea pig for compound action potentials (Aran and Cazals, 1978) as well as for unit recordings (Evans, 1972).

The fact that the vestibular sense organs are relatively normal with a majority of preserved sensory cells would suggest that the responses are triggered by acoustic stimulation of these structures. Such a possibility has been often pointed out in lower vertebrates (Lowenstein and Roberts, 1948, 1951) up to man where an acoustically evoked myogenic response (inion response) could be attributed to saccular excitation (Townsend and Cody, 1971). Then, the low-frequency sensitivity of the responses in our experiments fits well with such saccular origin. However, there still remains the problem of latency which is still too short (0.2 ms between click stimulus and the beginning of the response trough) with respect to the classical delay of the hair cell-nerve ending synapse.

On the contrary the last possibility which could agree with such a short latency would be the direct acoustical stimulation of the remaining nerve fibers in the cochlea, whether this excitation would act directly on the fiber membrane or through some kind of mechano-electrical transduction process in the surrounding remnants of the organ of Corti. However, then it is impossible to observe, in our records, any of the classical characteristics of the travelling wave.

Thus, it is obvious that, in any of these hypothesis, we cannot find a satisfying agreement between the various characteristics of these responses, particularly between latency and frequency specificity.

Finally, in the interpretation of these results, it appears essential to be able to determine if such low-amplitude, short-latency responses are already present in the records from normal guinea pigs. In such a case they would show up as soon as the responses normally triggered by the hair cells progressively disappear with hair cells degeneration. Otherwise they would be produced through a new process which would develop during or after this extreme biological modification of the cochlea.

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