5-29-1987

The Effects of Hypoxia on Sensory Cells of the Cochlea in Chinchilla

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THE EFFECTS OF HYPOXIA ON SENSORY CELLS OF THE COCHLEA IN CHINCHILLA

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Abstract

The effects of hypoxia on the sensory epithelium of the cochlea were investigated in the chinchilla. Systemic hypoxia was produced by increasing the dead space of the respiratory tidal volume.

A disarrangement of hair-cell stereocilia, and cytoplasmic protrusions from sensory cells are the main findings in cochleas from hypoxic animals; these changes take place firstly at the inner hair-cells then, with the increase in degree of hypoxia, at the outer hair-cells.

These degenerative changes of sensory cells correlate well with both respiratory suppression and with the elevation of auditory threshold to click stimulation as monitored using the compound action potential recording from the cochlear nerve. The latter measure appears to be a useful indicator of cochlear hypoxia.

Our morphological findings are similar to other studies including those which have reported on post-mortem cochlear hair-cell degeneration.

Our studies indicate the deleterious effects of long term hypoxia on cochlear mechanisms and point to the need for careful monitoring of cardiovascular and respiratory functions in animals under anaesthesia for physiological studies of the auditory system.

Introduction

The effects of hypoxia on the cochlea have been previously studied, for example, by Kimura and Perlman (4) who obstructed the artery to the inner ear and observed degenerative changes to the cochlear sensory epithelium. Changes of a similar nature were also reported in investigations of post-mortem degenerative processes in the cochlea (2,3,4), and can be attributed to tissue hypoxia caused by interruption of blood flow or, more profoundly, by animal death. On the basis of these observations we have carried out a more systematic study of the effects of cochlear hypoxia on both the morphological and functional integrity of the cochlea.

We have attempted to clarify the relationship between the degree/duration of hypoxia, the extent of cochlear damage and the functional consequences. There are a number of research groups investigating the physiology of the auditory system using experimental animals maintained for long periods under anaesthesia, and our results emphasize the importance of monitoring cardiovascular and respiratory function; depression of these can cause serious changes in cochlear condition.

Materials and Methods

Induction of Hypoxia and Monitoring of Cochlear Function and Physical Signs

Eight chinchillas weighing from 300g to 600g and free from middle ear disease were used. Animals were anaesthetized with sodium pentobarbitone (35mg/kg,IP) and diazepam (1mg/kg,IM) together with atropine (0.1mg/kg,IP) to prevent cardiovascular depression and airway obstruction by secretions in the respiratory tract. Maintenance doses of barbiturate (7mg/kg,IP) were given every 2 hours. Following this anaesthesia, tracheostomy was performed and a small tube was tightly inserted into the trachea. This tube was then connected to a syringe with a small hole at the distal end, adding dead space to tidal volume. Usually an increment of less than 20ml of dead space could be compensated for by an increase in tidal volume producing no change in cochlear function.

However, when dead space was increased beyond around 20ml, the cochlear thresholds of response started to deteriorate. By this adjustment, the smallest volume of dead space required to change the cochlear thresholds was determined and kept constant for the period of 120 to 200 minutes.

To monitor cochlear function, the mastoid bulla was opened to allow placement of a gross platinum electrode on the round window. Stimuli were delivered via an ear-phone sealed into the external auditory meatus and consisted of 3msec (1msec rise-fall time) tone bursts (repetition rate 11.5/sec) at frequencies between 0.5kHz and 24kHz. The cochlear action potential (CAP) was determined by averaging 200 sweeps (amplification 10^4, 150Hz to 3kHz bandpass filter). CAP threshold was estimated from N1 amplitude versus intensity curves by extra-polation to zero amplitude. Several frequencies were used so as to determine a CAP audiogram for each ear before and during periods of hypoxia. In addition, CAP thresholds to click stimuli were measured continuously.

Duration of hypoxia was 120 minutes for 4 animals and 200 minutes for 2 animals. Two control animals underwent exactly the same procedure but without the dead space addition to tidal volume. Respiratory and cardiac rates were monitored in all animals throughout the experiment.

Assessment of Cochlear Cells by SEM

Immediately after the end of exposure to hypoxia, animals were sacrificed for SEM observations of the cochleas.

The chest wall was opened for perfusion of fixative (phosphate buffered 1% glutaraldehyde/ 4% formaldehyde) via the aorta. Then the cochleas were quickly removed, oval and round windows were opened and fixative was gently perfused through scala tympani and scala vestibuli with a small pipette. Perfusion of the cochlea was completed within ten minutes of animal death. Cochleas were post fixed with 1% OsO4 and dissected in 70% alcohol. After dissection, specimens were prepared using OTO procedures and critical point dried. All our SEM observations were of the upper surface of the sensory epithelium.

Results

Control Animals

Figures 1 and 2 show the time course of physical signs and CAP audiograms during experiments from two control animals. In spite of some small fluctuations in the heart and respiratory rates, the click thresholds (lower diagram) maintained quite a constant level throughout the experiments. CAP audiograms measured at two different points in the course of experiments (A and B in the lower diagram) showed very good agreement indicating no deterioration in cochlear function across frequency.

Typical SEM results from these animals are shown in Figures 3A and 3B from apical and basal turns of the cochlea, respectively. Even in these

control animals, very slight cytoplasmic protrusion at the site of the basal body of the inner hair-cells was sometimes detected.

Otherwise, sensory hair-cells and other structures in the organ of Corti appear normal.

Animals with Hypoxia

In contrast to the normal animals, significant functional and histological deterioration was observed in the cochleas of animals exposed to hypoxia of various duration.

Figures 4A (CH121) and 5A (CH128) show the results from two animals exposed to hypoxia for 200 minutes. Time zero on the abscissa indicates the start of dead space imposition on the tidal volume. CAP audiograms were measured before and during hypoxia at times labelled A and B. In animal 121, the decrease in respiratory and heart rates are considerable after the dead space imposition. In line with these changes, cochlear function deteriorated dramatically as a function of time. CAP response to click stimuli have elevated thresholds, by about 20dB, and the CAP audiogram measured at about 3 hours after respiratory loading showed 10 to 25dB deterioration particularly for frequencies above 4kHz. These changes are less evident in animal 128 with the threshold elevation being less than 10dB to click stimulation. From these data, we note a clear relationship between click threshold and respiratory rate; click threshold elevation corresponds to the suppression of respiratory function which presumably therefore relates to cochlear hypoxia. These correlations are evident, for example, at the time of 90 minutes in Figure 4A.

SEM observation of cochleas from 200 minute hypoxic animals are shown in Figures 4 and 5. In animal 121, cytoplasmic protrusions and disarrangement of sensory hairs at the level of inner hair-cells are prominent along the length of the cochlea (Figures 4B and 4C from the apical and basal turns of cochlea respectively). Pathological changes of a similar type, but less severe are observed in the cochlea of animal 128 (Figures 5B and 5C).

Four animals were made hypoxic for 120 minutes. Of these, one animal (CH170, Figure 6) had severe cardio-respiratory suppression after introduction of respiratory loading; thi caused an increase in click threshold o: :bout 30dB (Figure 6A, lower diagram). Animals 125 and 126 (Figures 7 and 8) had less severe changes in their cardio-respiratory and cochlear functions; thresholds for click stimulations were elevated by about 10dB (Figures 7A and 8A, lower diagram). Only in animal 186 were we unable to detect a deterioration in cochlear function despite the decrease in both respiratory and cardiac rates (Figure 9).

Of the 120 minute exposure animals most cochlear damage was observed in animal 170 (Figures 7B, 7C, 8B and 8C). The smallest changes were observed in the cochlea of animal 186 (Figures 9B and 9C).

These pathological changes, i.e., cytoplasmic protrusions and disarrangement of hairs mainly at inner hair-cell level, were common in all animals exposed to hypoxic conditions. The degree of

The effects of hypoxia on cochlear sensory cells.
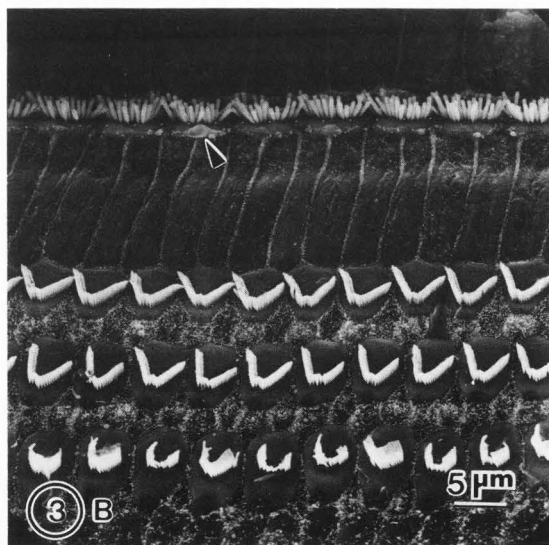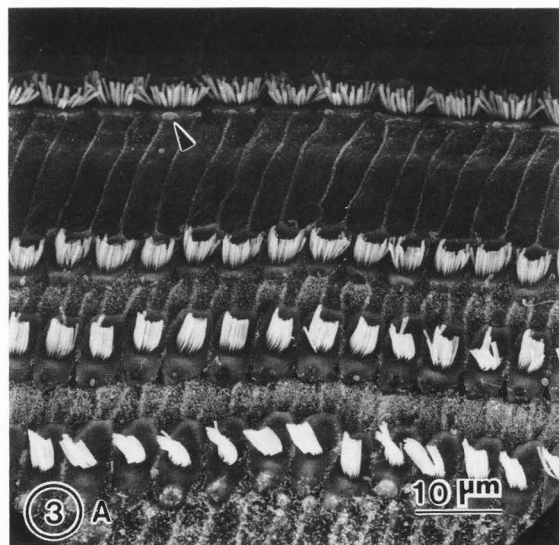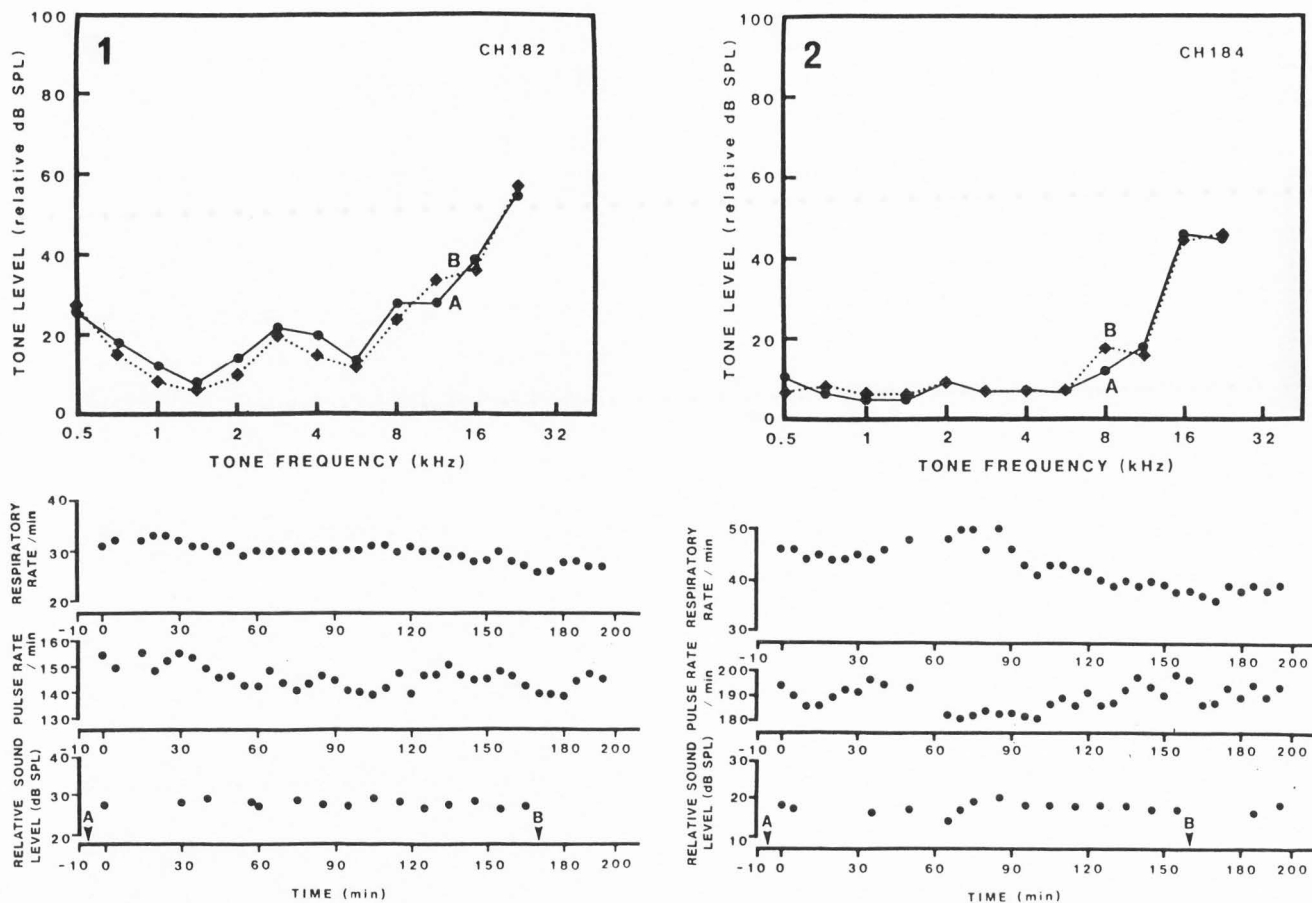
Figures 1 and 2: The lower graphs indicate respiratory rate, cardiac rate, and cochlear nerve compound action potential threshold of response to a click stimulus, monitored from a normal chinchilla during 200 minutes of general anaesthesia. The upper curves are audiograms derived from the compound action potential of the cochlear nerve, measured at the beginning and towards the end of the experimental period (at times A and B indicated on the lower abscissa).

Figure 3: Surface view of the cochlear sensory epithelium as seen by SEM. Inner hair-cells are shown towards the top of each micrograph. 3A is from an apical region of a control animal (CH182) and 3B from a basal area (note difference in length of stereocilia). Note the small cytoplasmic protrusions at inner hair-cells even in this control animal (see arrows).
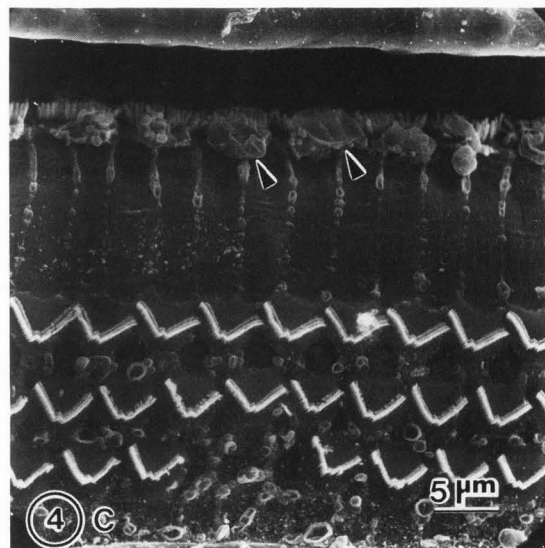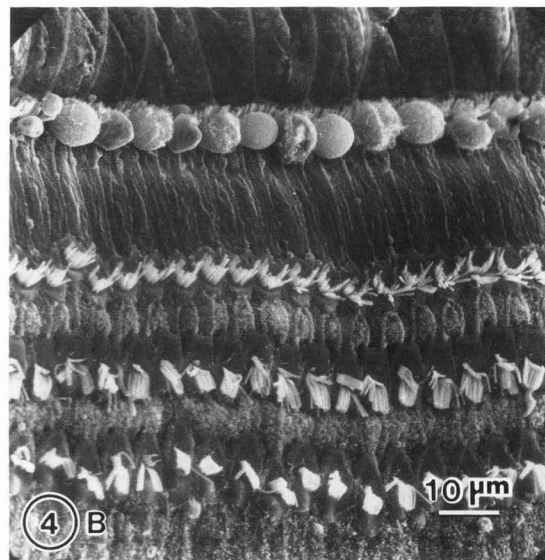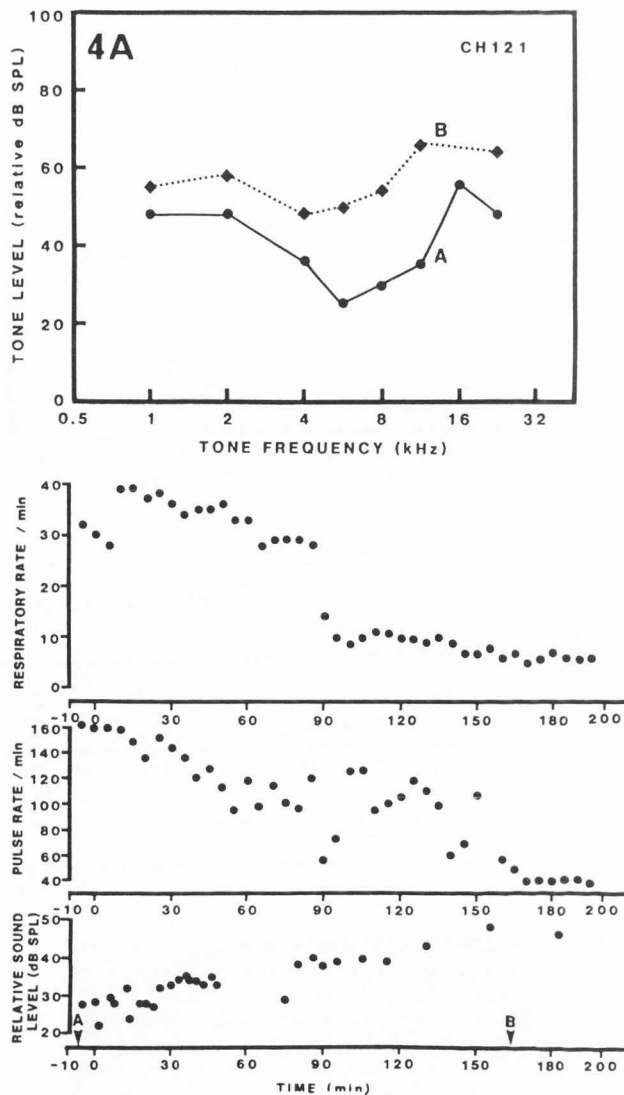
Figure 4: A. Changes in respiratory and heart rates and threshold elevations of cochlear responses (monitored via the CAP) resulting from a 200 minute period of hypoxia. The CAP audiograms before and during this period are shown in the upper curves of 4A. At the time of 90 minutes, acute respiratory suppression corresponded well with the rapid increase in the click threshold. B. and C.: Representative examples of the cochlear sensory epithelium from apical and basal regions of this animal. At the level of inner hair-cells, huge cytoplasmic protrusions, which are collapsed at the basal turn (arrows), can be seen. Sensory hairs are also disarranged at the apical regions.

histological damage correlated with the degree of cochlear threshold elevation caused by the hypoxic period.

Discussion

There have been a number of studies which investigated the effects of hypoxic conditions on cochlear function and histology. Kimura and Perlman [4] described a series of pathological changes to hair-cells as a function of the duration of blood interruption; cytoplasm edema took place at the inner hair-cells within 30 minutes and hair-cells degenerated totally within 6 hours. In other studies, it is

reported that post-mortem changes of the sensory epithelium initially (within one hour) occur at the level of inner hair-cells progressing to the outer hair-cells [2,3,6].

In our experiments the first degenerative changes were also noted to be at the inner hair-cell and to progress toward outer hair-cells. The deterioration correlates with the degree of cochlear function as indicated by the CAP, which is clearly an accurate indicator of the cochlear hypoxia [1,5].

On the basis of these observations it is assumed that the inner hair-cells are more vulnerable than the outer hair-cells to hypoxia independent of cause, e.g. from respiratory
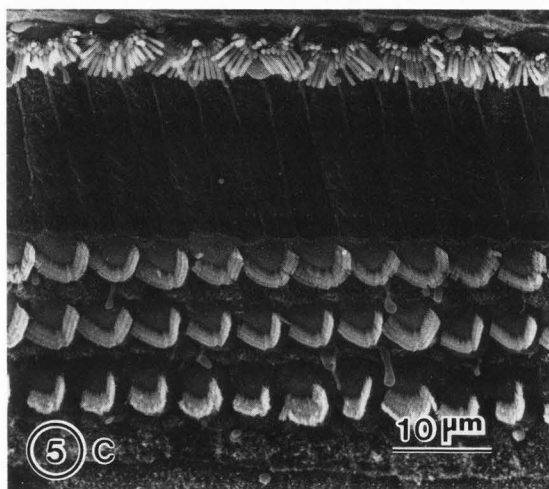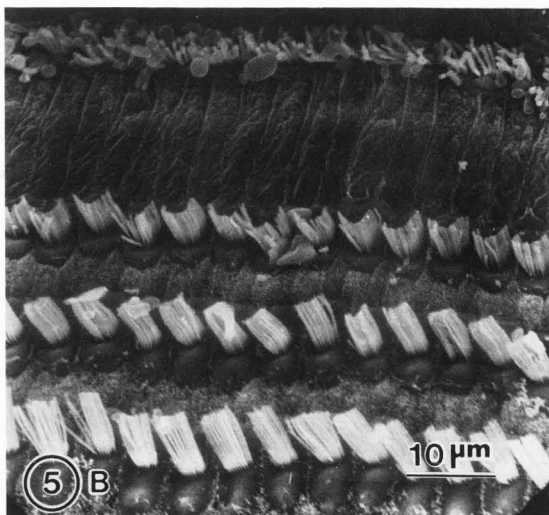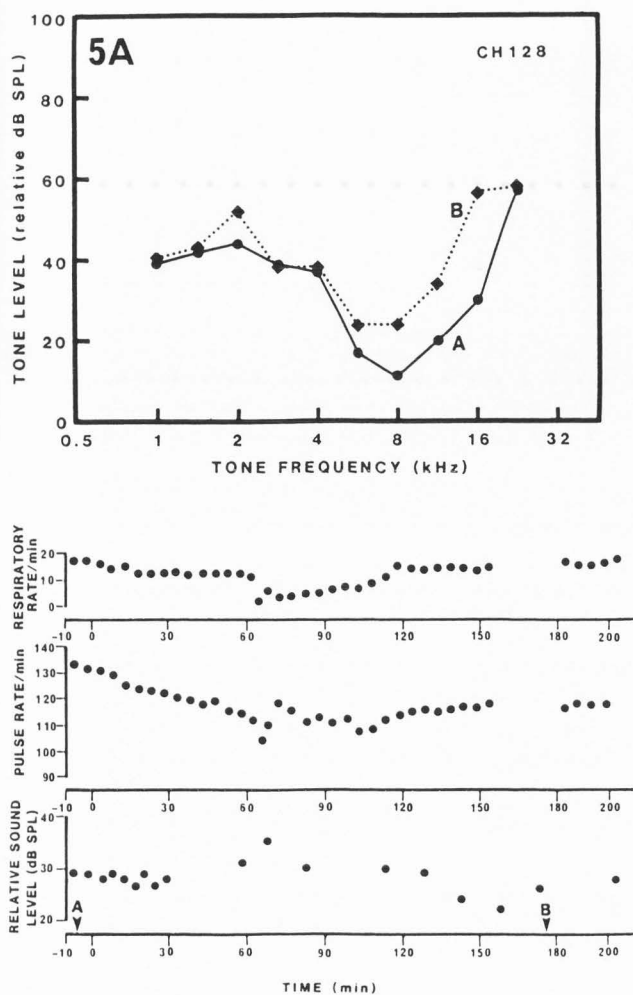
Figure 5: A. Systemic and cochlear changes, both functional and morphological resulting from a period of 200 minutes hypoxia. B. and C.: The cochlear sensory epithelium from apical and basal regions of this animal. Disarrangements and swelling of stereocilia and cytoplasmic protrusions are seen at inner hair-cell level.

suppression, interference of blood supply to cochlea or animal death.

Although two differing time periods of dead space imposition were employed in these experiments, there was no obvious correlation between the duration and the degree of hair-cell damage. Indeed, there were large variations in the degree of sensory cell degeneration between subjects. These inter-subject variations can be attributed to the respiratory tolerance of each animal which is influenced by many factors including the level of general anaesthesia. In general, animals which showed severe respiratory rate suppression had marked deterioration in both cochlear function and histology. On the other hand, animals which showed good tolerance to respiratory loading had little deterioration

to the cochlea even when the dead space imposition lasted more than 3 hours.

In addition to the effects of cochlear hypoxia on sensory cell degeneration, very mild but similar changes sometimes occurred in the cochleas of control animals fixed at death. The fixative was perfused through the aorta while the animal is deeply anaesthetized. However, there is a time lapse of a few minutes between the opening of the chest wall and the perfusion of fixative during which severe hypoxia may rapidly develop. The cytoplasmic protrusions observed in the control animals are attributed to this hypoxia. It is suggested that if the hypoxia is severe enough, then such a condition for only a few minutes duration will cause degenerative changes to cochlear epithelium.
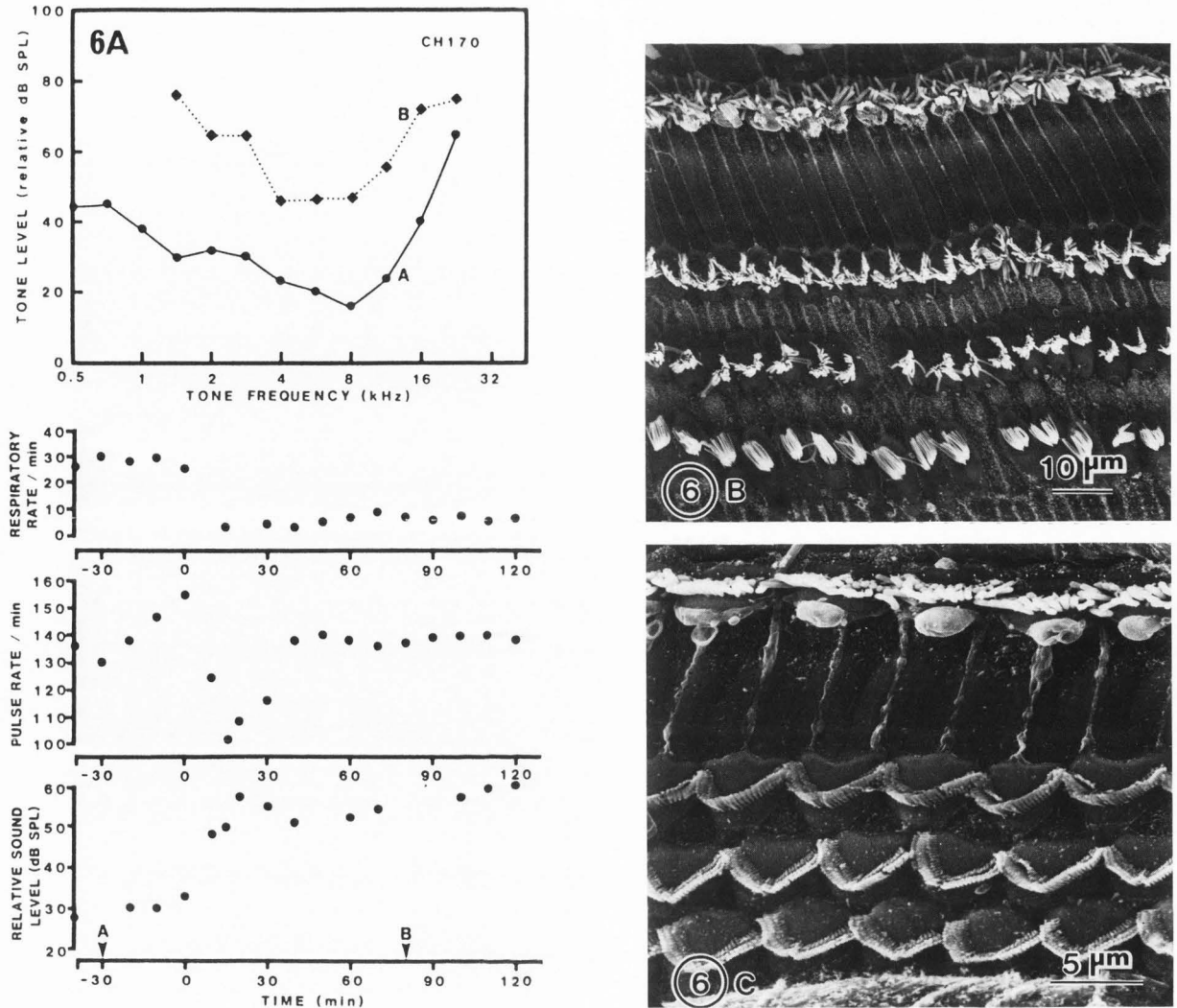
Figure 6: Effects of a period of 120 minutes of systemic hypoxia on cochlear thresholds and cochlear morphology. In line with the severe deterioration in cochlear function, vesticular or collapsed cytoplasmic protrusions are marked. Stereociliar disarrangement is prominent at apical regions (B).

Summary

The functional and morphological effects of cochlear hypoxia have been described. Extended periods of mild hypoxia can produce significant deterioration in cochlear function. Because of this, considerable attention should be paid to the history of cardiovascular and respiratory function when assessing the cochlear pathology of animals which have been under general anaesthesia for long periods.

Acknowledgements

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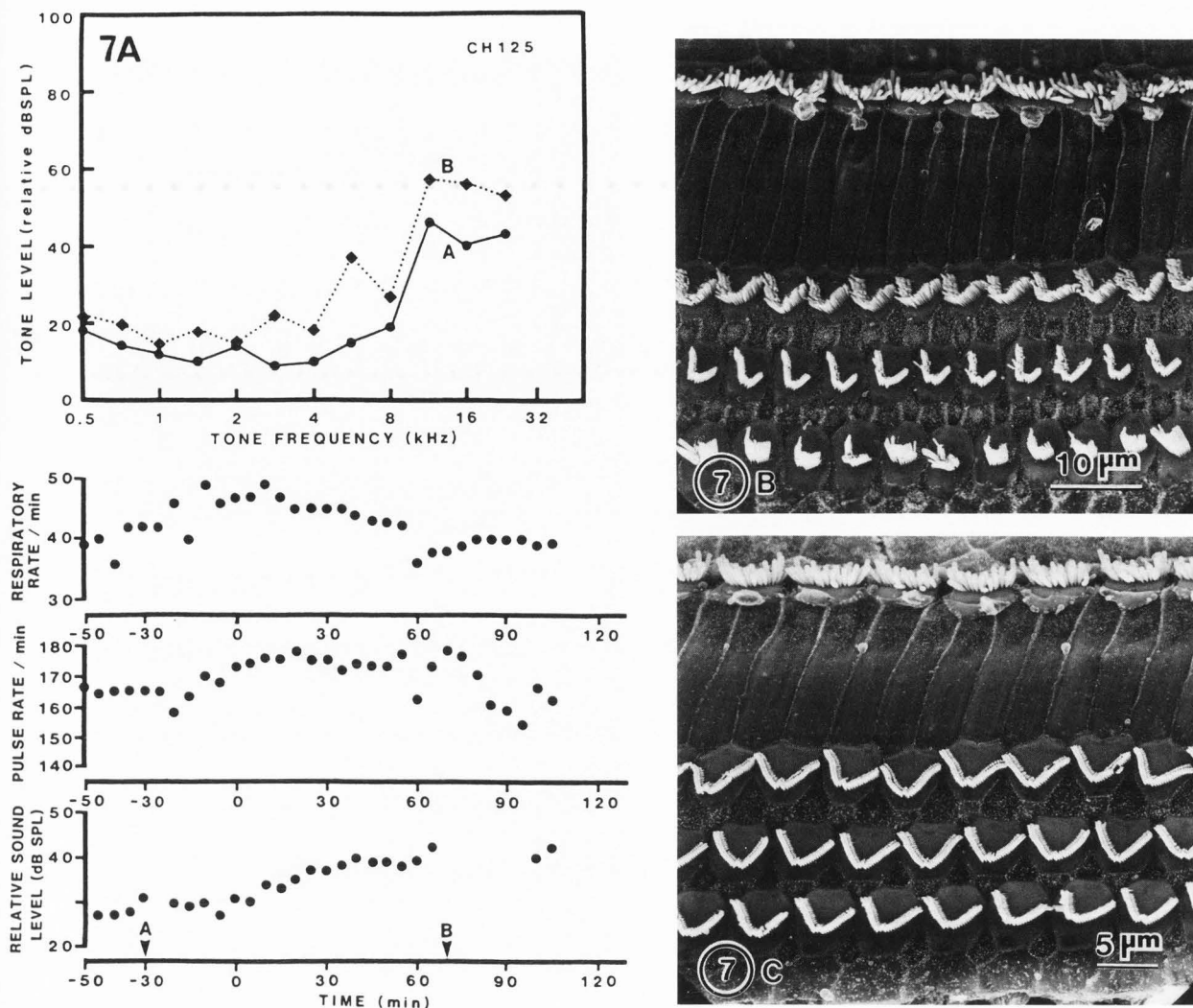The effects of hypoxia on cochlear sensory cells.

Figure 7: Effects of a period of 120 minutes of systemic hypoxia on cochlear thresholds and cochlear morphology. Histological changes are less severe in both apical (B) and basal (C) regions as well as the cochlear function.

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Discussion with Reviewers

Y. Harada: You examined the correlation between the duration of hypoxia and morphological or physiological changes. Did you measure PaO_2 or $PaCO_2$ during the experiment?
Authors: No, we made no direct measures of arterial O_2 or CO_2 tensions.

Y. Harada: Changes of body temperature has a critical effect on nerve excitability. Did you measure the body temperature throughout the experiment?
Authors: Throughout the experiments the body temperature of the animals was kept constant using an homeothermic heating system.

Y. Harada: Were there any differences of degree of morphological change among each turn?
Authors: The apical turn tended to be more affected than the basal turn by the cochlear hypoxia.

E.R. Lewis: In terms of basic cellular structures and mechanisms, what do you think your observed pathological changes in morphology mean?
Authors: For the cause of cytoplasmic protrusions we can speculate that there may be: 1) membrane weakness due to mechanical or chemical effects on the cochlear epithelium; 2) membrane
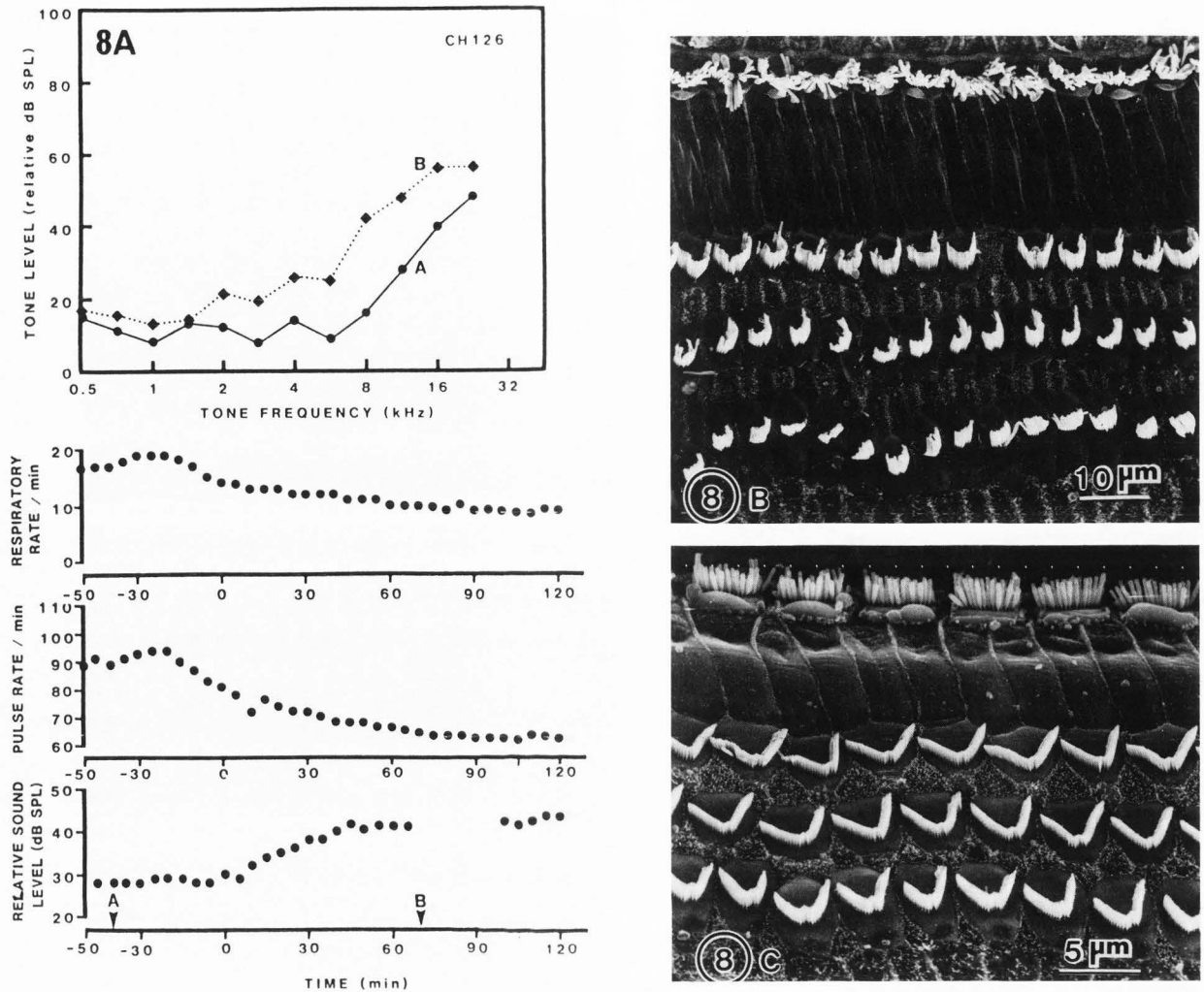
Figure 8: Effects of a period of 120 minutes of systemic hypoxia on cochlear thresholds and cochlear morphology. In accordance with the moderate functional deterioration of cochlea, inner hair-cells show moderate degree of change (apical (A) and basal (C) regions).

permeability changes leading to the osmotic swelling; 3) metabolic dysfunction causing membrane weakening; 4) lysosomal activity. Tissue hypoxia may cause these pathological states in the cochlear epithelium and eventually lead to the cytoplasmic protrusion of cells.

Y. Harada: Cytoplasmic protrusions are seen on the pillar cells and Deiter's cells in Figure 4C. Are these changes also due to hypoxia? Authors: This animal showed very severe degenerative changes of the cochlea along with much deterioration in cochlear function. It is considered that the degenerative change of the cochlea initially takes place at the level of the inner hair-cells then progress to the outer hair-cells. The cytoplasmic protrusions observed on the pillar cells and Deiter's cell are considered to indicate the next stage in the progression of degenerative changes due to severe hypoxia.

E.R. Lewis: Is there evidence that the pathological changes you observed are reversible? B.A. Bohne: What do you think the cytoplasmic protrusions mean as far as survival of the cell is concerned? Authors: Our experiment did not allow us to answer these questions. The reversibility of the effect of hypoxia will be investigated in future experiments.

B.A. Bohne: Have you examined other hypoxic cochleas with TEM or as whole mounts? If so, are the pathological changes in the bodies of the IHC's also more advanced than the OHC bodies? Authors: Cochleas were examined only with SEM in this preliminary study. The observation of degenerative change of intracellular structure due to hypoxia is in our schedule of future experiments.

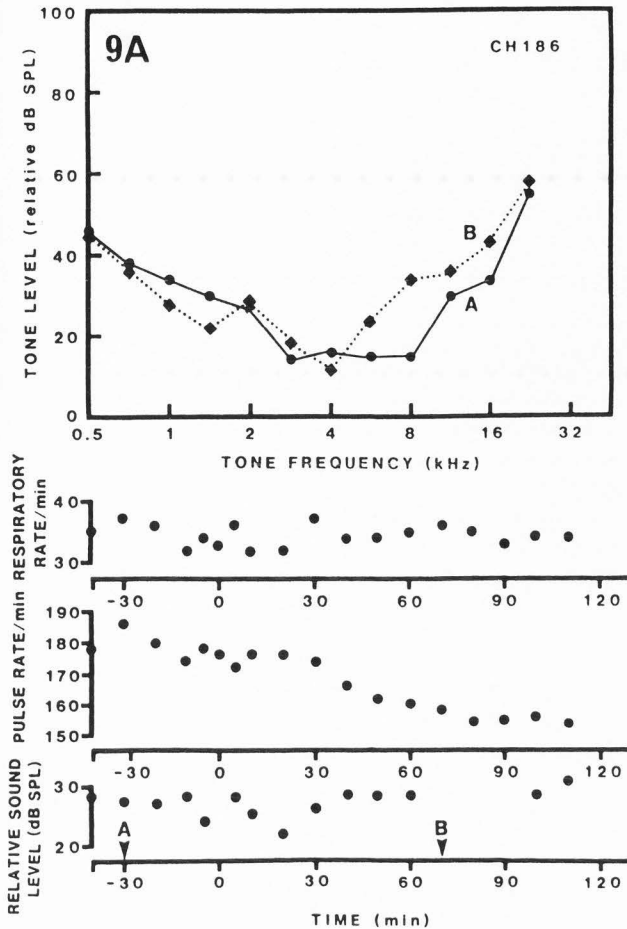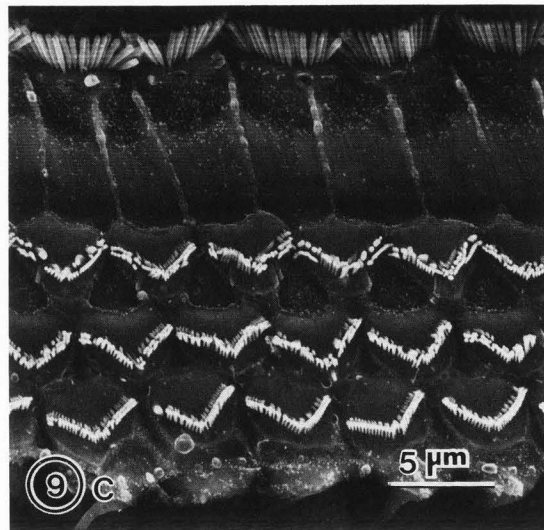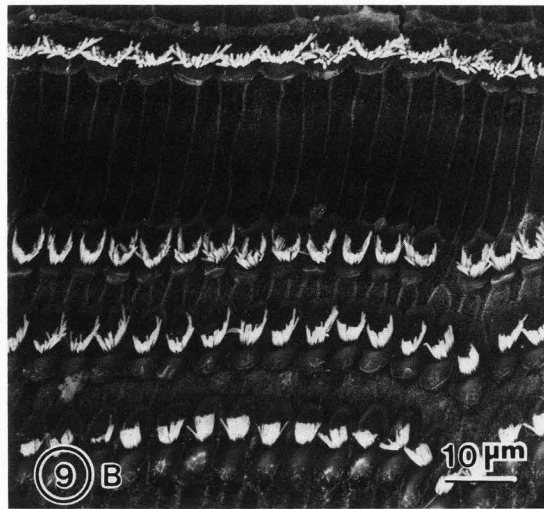The effects of hypoxia on cochlear sensory cells.

Figure 9: Effects of a period of 120 minutes of system hypoxia on cochlear thresholds and cochlear morphology. This animal showed the least change in the cochlear function (A). Cytoplasmic protrusion is not evident in both apical (B) and basal (C) regions.

B.A. Bohne: Your results tend to indicate that a period of hypoxia has a differential effect on the apex and base of the cochlea. There appears to be less disarrangement of stereocilia in the basal turn. Was this a consistent finding? If so, can you speculate on the cause of the apex and base difference?

Authors: In general, the trend is that the apical turn of the cochlea tends to be more affected than the basal turn by hypoxia. This is contrary to many other ototoxic influences for which the cochlear base is more vulnerable. There are some factors which may explain this difference. Firstly, taking the blood supply system within the cochlea into account, the basal turn of the cochlea first receives the oxygenated blood from the cochlear artery. As a result, the apical turn of the cochlea is supplied by blood which has passed through the basal part and is therefore with a reduced oxygen supply. Another speculation is that an autoregulatory system of blood flow, in the

cochlea, may result in uneven oxygenation of cochlear turns. Perhaps the base of the cochlea is given some priority which leads, in the long term, to more hypoxia apically.