Cochlear degeneration in minipigs after repeated hyperbaric exposures


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Wilkes MK, Palmer AC, Pearce PC, Luff NP, Halsey MJ, Calder IM. Cochlear degeneration in minipigs after repeated hyperbaric exposures. Undersea Biomed Res 1989; 16(2):139–152.—Auditory function and cochlear pathology were investigated in 4 minipigs subjected to compression to 4 ATA, held for 1 h, and decompressed using a standard schedule (Blackpool Tables) on 21 occasions. Three minipigs were used as controls. Brainstem auditory evoked response testing was carried out after the last hyperbaric exposure and showed no response bilaterally in 3 and unilaterally in 1 of the test animals. Light microscopy demonstrated a loss of hair cells throughout the cochlea in all the compressed animals; in one the tectorial membrane was detached and adherent to Reissner’s membrane. Vestibular changes were confined to the saccule. Hemorrhage was not a prominent feature. On scanning electron microscopy the pathologic changes included missing hair cells and fused and giant stereocilia. Possible causes of the pathology include barotrauma or direct effects of repeated compression and decompression on the inner ear, or both. The compression-decompression profile used was one that is thought to be safe for compressed air workers who are repeatedly exposed to hyperbaric conditions.

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In this paper we describe hearing defects and pathologic changes in the cochleas of minipigs which were subjected to compression-decompression in air on 21 separate occasions. These findings were incidental to the original purpose of the project which was to determine whether such exposures would lead to neuropathologic changes in the spinal cord. Experiments previously carried out on minipigs using a similar hyperbaric protocol had indicated that such spinal degeneration would occur, but this work had not been adequately controlled (J. Stothard, I. H. Thomas, and A. C. Palmer, personal observations). In the event, no lesions were found in the spinal cord in the present study. However, as other investigations were at the time being carried out on naturally occurring hearing defects in animals, using brainstem auditory evoked
responses (BAERs), the opportunity was taken to apply the test to the experimental minipigs at the end of the series of compressions-decompressions, in view of the known association between diving and deafness (1).

The results showed an abnormal BAER bilaterally in 3 of the 4 exposed animals and unilaterally in the 4th. In view of these findings, the histologic protocol was immediately changed to include examination of the inner ears by light and scanning electron microscopy. This report is therefore a preliminary one. Retrospectively, omissions in the experimental procedure are apparent, but it is hoped that these can be corrected in subsequent investigations.

MATERIALS AND METHODS

Animals

Eight Gottingen miniature pigs (minipigs), aged 5–7 wk, were used initially, but 1 died early in the experiment from an unrelated cause, leaving 3 control pigs (pigs 1, 3, and 6) and 4 experimental pigs (pigs 2, 4, 5, and 7). Their weights at the start were approximately 6 kg, rising to 20–25 kg after 5 mo.

Pressure chamber

The pressure chamber used in this experiment was a vertically mounted carbon steel cylinder with hemispherical ends, the topmost of which acted as a lid. The volume of the chamber was 1200 liters and the internal dimensions were 1.83 m in length and 0.91 m in diameter.

The internal environment of the chamber was controlled by an external purification system which removed carbon dioxide, water vapor, and volatile contaminants. Temperature was kept constant and the partial pressure of oxygen was raised from 0.21 ATA before pressurization to 0.4 ATA during compression.

The sound level in the chamber during inflow of compressed air was 58 dB(A), measured on a Precision sound level meter type 2206B (Bruehl and Kjaer, Copenhagen).

Protocol

Pressure group

The 4 pigs were placed in two plastic circular open-top containers inside the pressure chamber. The lid of the chamber was placed in position and bolted down. The chamber was then compressed with air to 4 ATA at 0.2 ATA per minute and held at this pressure for 1 h. Decompression took place over 2 h at a rate of 0.2 ATA per minute according to the following schedule (Blackpool Tables):

4.0 ATA–1.8 ATA hold 5 min
1.8 ATA–1.6 ATA hold 20 min
1.6 ATA–1.4 ATA hold 35 min
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1.4 ATA–1.2 ATA hold 45 min
1.2 ATA–1.0 ATA

A total of 21 simulated dives took place over a 5-mo. period.

Control group

The control group was also placed in the open-top containers in the chamber on 21 occasions and for the same total length of time as the experimental group. Intervals between these occasions were no more than 1 d more or less than the intervals between the exposures of the experimental group. The animals were subjected to the same environmental noise levels as the experimental group but without actual pressurization. The control pigs were kept together in the same environment and moved the same distances as the experimental pigs.

Clinical tests

At the end of the series of compressions-decompressions each animal was exercised in a long corridor to see whether there were signs of incoordination, vestibular derangement, visual defect, or obvious neurologic abnormality.

BAER testing

Twenty-four hours after the last pressurization, BAER testing was carried out on both ears of each pig. For this procedure the pigs were sedated with 1 mg/kg xylazine and 10–20 mg/kg ketamine given by i.m. injection. Subcutaneous stainless steel reference and active needle electrodes were placed at the vertex of the skull and immediately ventral to the external meatus of the ear to be tested. A silver earclip ground electrode was attached to the opposite ear pinna. An alternating polarity square wave generated click stimulus of 0.1 msec duration was played into the pig’s ear at a rate of 20 clicks a second through a TDH 49P earphone (Telephonics Corporation). Evoked responses were recorded over 10 msec using a Medelec MS6 signal-averaging recording system (Medelec Ltd.). A bandpass filter of 32 Hz–3.2 KHz was used and the responses were averaged over 1024 epochs. Both ears of each pig were tested. The reference level used for click intensity was the average hearing threshold for clicks for normal young adult humans (OdBHL).

Fixation, tissue processing, and examination

Immediately after BAER testing the pigs were killed with an i.v. overdose of sodium pentobarbital. The temporal bones were removed immediately and the tympanic bullae opened to expose the cochleas, which were then fixed by perilymphatic perfusion (2). This was achieved by perforating the round window membrane, removing the footplate of the stapes from the oval window, and gently syringing fixative through the cochlea. A small hole was made in the wall at the apex of the cochlea to aid perfusion. Because the aim was to carry out fixation as soon as possible after the animal had been killed, it was not possible to examine the round window membrane in detail for evidence of rupture before this procedure. One temporal bone from each
pig was prepared for light microscopy, and the other cochleas from control pig 3 and experimental pigs 4 and 5 were processed for scanning electron microscopy. The second cochlea from control pigs 1 and 6 and experimental pigs 2 and 7 were processed for transmission electron microscopy. Examination of this tissue has not yet been completed.

The temporal bones for light microscopy were perfused with 10% formol saline. They were immersed in the fixative for a minimum of 24 h and then decalcified in 10% formic acid for 16 days. After washing in tap water and dehydration the temporal bones were embedded in Taab epoxy resin (Taab Laboratories Equipment Ltd). Sections 2–5-μm thick were cut in the midmodiolar plane and stained with toluidine blue.

The cochleas for scanning electron microscopy were perfused using 4% glutaraldehyde in phosphate buffer (pH 7.2) with 8 ml/liter of 1% calcium chloride. They were left immersed in the fixative overnight and then washed in buffer and postfixed in 1% phosphate buffered osmium tetroxide. Microdissection of the cochleas was carried out under 70% alcohol to expose the organ of Corti. The tissue was dehydrated then critical-point dried, coated with gold, and viewed in a Joel JSM 35CF scanning electron microscope.

Other organ samples taken at postmortem examination included the brain, spinal cord, liver, heart, kidney, adrenal, pancreas, and sciatic nerve. All these tissues were fixed in 10% formol saline. The eyes were fixed in Davidson’s solution. Transverse blocks of the fixed brain were cut from the cerebral hemispheres at the level of the hypothalamus, the diencephalon, midbrain, cerebellum, and medulla. Transverse sections of the spinal cord were taken at the levels of C3, C8, T8, and L3. After dehydration, clearing and embedding in paraffin wax, sections were cut at 10 μm and stained with hematoxylin and eosin.

RESULTS

Clinical tests

None of the pigs showed any abnormal behavior either during or after compression or decompression. The pig is notoriously difficult to assess neurologically, but when examined before euthanasia no evidence was found of incoordination, visual defect, or signs of vestibular deficit, such as spontaneous nystagmus, head tilt, or circling. One experimental pig (pig 5) was occasionally seen to drag its left hindfoot.

BAER results

Responses were obtained from all the control pigs at thresholds varying from 70 to 95 dBHL (Table 1). In the experimental pigs no responses were obtained at the highest intensity stimulus used (95 dBHL) except in 1 pig (pig 7) where there was no response in the left ear but the threshold in the right ear was 55 dBHL. The left cochlea of this animal was used for light microscopy.
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TABLE 1
RESULTS OF BRAINSTEM AUDITORY EVOKED RESPONSE TESTS

<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Experimental (E), Control (C)</th>
<th>Response Threshold in dBHL</th>
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<tr>
<td></td>
<td></td>
<td>Right Ear</td>
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<tr>
<td>1</td>
<td>C</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>55</td>
</tr>
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Pathology

Brain, spinal cord, and other organs

No significant changes were found in the brains or spinal cords apart from a slight loss of Purkinje cells in the cerebellum of dived pig 5. In this pig there were also small collections of inflammatory cells in the kidney indicative of a mild pyelonephritis. No abnormalities were found in the auditory nerves or brainstem auditory pathways of any of the animals.

Inner ears

On light microscopy the most consistent abnormality seen in the experimental pigs was the loss of cochlear hair cells. On mid-modiolar sections there was almost complete absence of inner and outer hair cells in all the experimental pigs, although these were clearly visible in all regions of the cochlea in the control pigs (Figs. 1 and 2). The pillar cells and tunnel of Corti were also absent in the basal part of the cochlea in 2 of the exposed pigs. In 1 experimental pig Reissner’s membrane was collapsed onto the organ of Corti throughout most of the cochlea and the tectorial membrane was curled into the sulcus (Fig. 3), whereas in the other 3 experimental pigs Reissner’s membrane was slightly ballooned into the scala vestibuli in the apical turns but was otherwise normal. The stria vascularis seemed normal in all except the most severely affected pig (pig 5), in which it contained large vacuoles. In some of these a homogeneous acellular material was seen (Fig. 4). In this animal the tectorial membrane was detached from its normal position at the limbus throughout the cochlea, and could be seen either free in the endolymphatic space or adherent to Reissner’s membrane and covered with a layer of flat cells (Fig. 5). There was also a floccular precipitate containing degenerated cells in the scala media. Precipitate was seen in the endolympathic and perilymphatic spaces of two other experimental and two control cochleas, but this was acellular and present to a much lesser degree. A small amount of hemorrhage was present in 3 of the experimental pigs, in the scala media of the basal turn of pig 7 and in the scalae tympani of pigs 2 and 4, but this was not a
Fig. 1. Organ of Corti from control minipig 3; IHC = inner hair cells; OHC = outer hair cells; T = tectorial membrane; R = Reissner’s membrane. ×220.

Fig. 2. Organ of Corti from experimental minipig 4 showing absence of outer hair cells (arrow). ×220.
Fig. 3. Inner ear from experimental minipig 2. There is loss of hair cells, Reissner’s membrane (R) is collapsed and the tectorial membrane (T) is curled into the sulcus. Hemorrhage and cellular debris (CD) are present in the scala tympani (ST). × 220.

Fig. 4. Stria vascularis from experimental minipig 5. The stria (S) is vacuolated and contains homogeneous acellular material (arrow). × 480.
Fig. 5. Inner ear from experimental minipig 5. There is degeneration of the organ of Corti and detachment of the tectorial membrane (T) which is covered with a layer of flat cells and is adherent to Reissner's membrane (R). × 190.

major feature. The spiral ganglion seemed normal in the control and dived pigs, but measurements to assess changes in spiral ganglion cell population were not carried out.

In the vestibular labyrinth a small amount of hemorrhage occurred in the endolymphatic and perilymphatic spaces of control pig 1 and experimental pig 2. Some red blood cells, flocular precipitate, and cellular debris were also found in the endolymphatic space of the saccula and posterior ampulla of experimental pig 5. In this animal and in experimental pig 4 the otoconia had become displaced from the saccula macula although the macula itself appeared normal. In experimental pig 2 the saccula membrane had collapsed onto the macula and the sensory and supporting cells seemed severely atrophied. Some swelling of sensory cells occurred in the anterior and lateral cristae ampullares from both control and experimental pigs, but the utricular maculae and cristae were otherwise normal in all animals.

The part of the round window membrane that had not been perforated during fixation was also examined by light microscopy. No consistent difference in membrane thickness or cellularity was found between the experimental and control groups.

Scanning electron microscopy was carried out on the organ of Corti from 1 control and 2 experimental pigs. In both experimental pigs there was loss of hair cells. In pig 4 there was scattered outer hair cell loss in the basal turns of the cochlea and some stereocilia were fused and degenerate, but the inner hair cell row was less affected (Fig. 6). Toward the apex there was less outer hair cell loss but progressively more missing inner hair cells. The stereocilia appeared disorganized, and giant stereocilia were present, particularly in the inner hair cell row (Fig. 7). This was not seen in the
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Fig. 6. Scanning electron micrograph of 2nd cochlear turn from experimental minipig 4. Some outer hair cells (OHC) are missing and there is fusion of stereocilia (arrows). × 1800.

control pig (Fig. 8) where complete rows of outer and inner hair cells were present throughout the cochlea and giant or fused stereocilia were not observed. In the other experimental pig (pig 5) no stereocilia were visible at all and the hair cells were replaced by supporting cells in all turns of the cochlea (Fig. 9). In the basal turn the pillar cells were also absent. Some regions of the reticular lamina were obscured by cellular debris.

DISCUSSION

Previous reports have shown that rapid decompression in the goat can lead to infarction of the white matter of the spinal cord, accompanied by vasogenic edema and hemorrhage; hemorrhage was also sometimes found in the brain (3, 4). A particular search was therefore made in the present experiments for evidence of these changes, but none was found. The absence of neuropathologic changes in the spinal cord of the minipigs indicated that our original observations on minipigs under similar conditions had not been adequately controlled.

The method of perilymphatic perfusion for fixation of the cochlea is a standard procedure (2) which was used because it gives very good preservation of the neuroepithelium of the inner ear. It is thus particularly valuable for fixation before scanning electron microscopy. However, it has the disadvantage that it involves damaging the middle ear and rupturing the round window membrane, which prevents full histologic examination of these structures.
The absence of BAERs in 3 of the 4 compressed-decompressed pigs was unexpected, especially as the diving schedule used was based on Blackpool Tables, a schedule regularly adopted by human compressed air workers. In all 4 experimental pigs there was loss of hair cells from the organ of Corti throughout the cochlea. In 1 severely affected pig, the tectorial membrane was detached and adherent to Reissner’s membrane and was covered by a layer of flat cells. In this animal the stria vascularis was vacuolated and contained unidentified homogeneous material, and a cellular precipitate was present in the scala media. Hemorrhage was not a major feature in any of the cochleas. In the vestibular labyrinth only the saccule was affected, with collapse of the saccular membrane and macular degeneration in 1 experimental animal and the presence of cellular debris and precipitate in another. The displacement of otoconia seen in 2 of the pigs may have been artifactually produced by the method of fixation; however, it was not found in any of the control animals.

Some audiologic studies (5, 6) have indicated that there is a greater risk of high frequency hearing loss developing in divers than in the nondiving population; however, the reason for this is still controversial. In addition there are numerous reports of sudden onset hearing losses occurring in divers, often associated with tinnitus and vestibular signs (7–13). These have in some cases been reversible changes. Possible causes of inner ear damage in divers include barotrauma of the inner ear, decompression sickness affecting the end organ, bubbles developing in the inner ear fluids, gas embolism, and excessive occupational noise exposure (1, 14, 15).
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Fig. 8. Scanning electron micrograph of the organ of Corti from the second cochlear turn of control minipig 3 showing normal inner and outer hair cell stereocilia (IHC and OHC) and heads of pillar cells (P). x 1800.

Inner ear barotrauma can occur when middle ear pressure is unequal to ambient pressure. This results from failure of equalization of pressure via the eustachian tube, sometimes exacerbated in man by forceful Valsalva’s maneuver (1). The round or oval window membranes may rupture leading to leakage of perilymph with consequent hearing loss or tinnitus and vertigo or both. Surgical repair of the fistula often leads to partial or complete recovery (1, 10, 12–15). The inner ear pathology associated with barotrauma in man has rarely been described. However, in 1 case reported by Gussen (16), where barotrauma occurred while flying, there was a rupture of Reissner’s membrane which healed to form a balloonlike structure. The stria vascularis was absent in the promontory and in the apex, and narrowed throughout the rest of the cochlea, but the organ of Corti was normal.

Studies of experimentally induced barotrauma have shown a range of pathologic changes in the inner ear when different compression and decompression regimens have been used. Lamkin et al. (17), applying pressure changes directly to the middle ear of guinea pigs, found a high incidence of hemorrhage in the basal turn of the scala tympani, but the round window membrane was intact and the organ of Corti normal in all cases as assessed by light and phase contrast microscopy. There was, however, a time- and pressure-related decrease in electrophysiologic function of the cochleas on application of negative pressure.

In contrast, studies by Takahashi (18), Hando et al. (19), and Nakashima et al. (20) in which guinea pigs were subjected to rapid compression or decompression did result in damage to the organ of Corti. This was seen both with and without round window membrane rupture, and in the work by Nakashima et al. (20) occurred largely inde-
Fig. 9. Scanning electron micrograph of the reticular lamina from the 2nd cochlear turn of experimental minipig 5. Heads of the pillar cells (P) are present but inner and outer hair cells have been replaced by supporting cells (arrows). ×700.

...pendently of the incidence of middle ear barotrauma lesions. Decompression caused more severe pathologic change than compression, and the effect was increased with more rapid pressure change or blockage of the eustachian tube (18). In the studies by both Takahashi (18) and Nakashima et al. (20) there was predominantly outer hair cell damage, and in most cases this was more severe in the basal than the apical turns of the cochlea. Outer hair cell loss was also demonstrated by Hando et al. (19) using scanning electron microscopy, and the pathologic abnormalities included swelling and fusion of sensory hairs, bleb formation, and derangement and breaking off or loss of stereocilia.

However, the detailed study in rats by Levendag et al. (21), using “safe” and “unsafe” decompression profiles from 20 ATA, failed to show any electrophysiologic changes in inner ear function or damage to the organ of Corti as assessed by scanning electron microscopy, even in the presence of barotrauma lesions in the middle ear.

The effects of rapid decompression on the inner ear have been investigated in experiments where barotrauma has been deliberately prevented from occurring. In studies by Landolt et al. (22, 23) and by Fraser et al. (24), using squirrel monkeys in which the tympanic membranes had been perforated before the experiment, rapid decompression from 28.2 ATA (274 msw) caused severe changes in the inner ears. These included hemorrhage and precipitate in the cochlear and vestibular fluids, fracture of the wall of the semicircular canals, and later the development of ectopic new bone in the labyrinth. Similar pathology has also been seen in a human case of decompression sickness (25). The postulated cause of these lesions was vascular...
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changes caused by the development of bubbles in the blood vessels or the direct pressure effects of bubbles developing in the inner ear fluids. In a study by McCormick et al. (26), cochlear hemorrhage was also a feature in 1 guinea pig which suffered a severe vestibular attack after decompression from 80 ATA, where barotrauma was prevented by prior opening of the middle ear. This animal also showed cochlear hair cell degeneration. In another experiment (26) the same author found reduced cochlear function in guinea pigs subjected to a different rapid decompression regimen from 10.1 ATA (300 ft). This was found to be largely prevented by prophylactic administration of heparin, supporting the theory of a thrombotic or embolic etiology.

Comparison of results between studies is difficult because of differences in species, compression-decompression protocols used, and whether barotrauma was prevented. However, the changes described in the inner ears of the minipigs seem to show features more in common with the barotrauma-associated lesions in the studies by Takahashi (18), Nakashima et al. (20), and Hando et al. (19) than the inner ear pathology associated with rapid decompression in the absence of barotrauma, in which intralabyrinthine hemorrhage was a prominent feature (22–24, 26). Similarly, the vestibular pathology observed in the minipigs was confined to the sacculus and did not resemble the severe changes described after decompression sickness in squirrel monkeys and man (22–25).

The present study differs from previous investigations in that inner ear changes occurred with a compression-decompression regimen that is generally thought to be safe for humans, and it also examined the effects on the inner ear after multiple exposures to these conditions. However, it is not known at what stage the inner ear damage occurred or whether it continued to progress during the course of the series of compressions and decompressions. The pathogenesis of the cochlear lesions in the minipigs is not known. It is unlikely that the damage was caused by noise trauma because of the relatively low level of noise in the pressure chamber. Barotrauma may have occurred because the tympanic membranes of the pigs were not perforated before the experiment. It was not possible to examine in detail at postmortem the round window membranes or middle ear for evidence of barotrauma. Species and age differences exist in the relative ability of the eustachian tube to open when ambient pressure is changed (18, 27), and it is not known how easily this occurs in the minipig. Clearly a major difference from man is that normal human divers make deliberate efforts to equalize middle ear pressure during dives whereas minipigs presumably do not. It is also possible that the pathology resulted from effects of repeated compression and decompression other than barotrauma. These could include either decompression sickness affecting the cochlea, although the pigs showed no generalized clinical signs of this, or possibly other changes such as the development of bubbles in the inner ear fluids. To distinguish between barotrauma and other pressure effects it would be necessary to repeat the procedure on minipigs in which barotrauma was prevented by prior perforation of the tympanic membranes. Future studies should also include assessment of auditory function and pathologic examinations of inner ears from animals at different stages throughout the course of the experiment.

It is disturbing to find that cochlear degeneration occurred in minipigs on a pressure schedule that is usually regarded as being safe for man. It may be that the minipig is particularly sensitive to this procedure. Whatever the reason, the minipig may prove to be a useful species in which to investigate this further.
REFERENCES