Endotoxin induced damage to the cochlea in guinea pigs

M J Tarlow, S D Comis, M P Osborne

Abstract
An apparently unique form of cochlear damage was produced in guinea pigs by perfusing the cochlea or injecting the cerebrospinal fluid with bacterial endotoxin. This developed rapidly (within two hours) and was characterised by swelling of the tectorial membrane and damage to both inner and outer hair cells, with parallel functional damage demonstrable electrophysiologically. All these changes could be attenuated by pretreatment with dexamethasone. Such endotoxin mediated lesions may be the mechanism by which hearing loss occurs in bacterial meningitis.

Deafness is a well recognised complication of bacterial meningitis. Ten per cent or more of children who contract bacterial meningitis develop significant sensorineural deafness, and it is the most important cause of acquired permanent deafness in children. The mechanism by which deafness is produced has not been clearly defined. Recent studies have shown that most of the other clinical characteristics of meningitis can be produced by bacterial cell wall material alone—endotoxin in the case of Gram negative organisms and lipoteichoic acid in the case of the Gram positive Streptococcus pneumoniae. Endotoxin itself is relatively harmless to most mammalian cells in vitro and is thought to act in vivo by provoking the release of cytokines such as tumour necrosis factor and interleukin-1, which themselves initiate the inflammatory response. This release of cytokines can be suppressed by dexamethasone. Because so many of the other features of bacterial meningitis result from the inflammatory response to endotoxin, we wondered whether the hearing loss that so commonly occurs could be accounted for similarly.

Methods
After urethane anaesthesia and tracheal cannulation in the guinea pig the left temporal bulla was exposed and a round hole approximately 50 μm in diameter was drilled into the basal turn of the scala tympani, about 2 mm from the round window. A slightly larger hole was made at the tip of the cochlea to allow the perfusate to flow out. A glass micropipette filled with artificial perilymph and linked to a 'Trionic' microinjection pump (IP-3, Vickers Medical) was inserted into the basal hole in the scala tympani and fitted tightly into it. Artificial perilymph containing endotoxin (Escherichia coli 026:B6 lipopolysaccharide (Sigma Chemicals), 100 ng·l μg) was introduced into the cochlea in a total volume of 17 μl over a period of one minute. Some leakage occurred from the apical hole, and this was mopped up with a tissue wick.

Recordings of cochlear microphonics and compound action potentials were made at 30 minute intervals for at least two hours using an electrode sealed within the glass micropipette.

At the end of the experiment the cochlea was immediately fixed by intravital perfusion of glutaraldehyde and prepared for scanning electron microscopy as previously described. The opposite cochlea was similarly fixed immediately after death. These studies were repeated after pretreatment of the guinea pigs with dexamethasone (1 mg/kg intraperitoneally) one hour before the infusion of endotoxin. Similar experiments were performed in which 100 ng·l μg of E coli endotoxin were injected intracerebrospinal into the cerebrospinal fluid. These studies too were repeated after pretreatment with dexamethasone. Control studies were performed perfusing the cochlea with artificial perilymph alone, without endotoxin. Each experiment was repeated several times.

Results
Administration of endotoxin directly into the scala tympani has shown that sound evoked...
electrical responses such as compound action potential and the cochlear microphonics are affected. In our experiments the compound action potential was elicited by stimulation with a 1 msec 10 kHz tone pip.

The amplitude of the first negative wave (N1) of the complex represents the synchronous activation of primary auditory nerve fibres by the stimulus and is thus a measure of cochlear output. Administration of endotoxin produced a consistent drop in the size of the compound action potential; the extra intensity of sound necessary to return the amplitude to its original value was measured, and expressed in decibels (dB). Readings were taken at half hourly intervals up to a maximum of 2-5 hours.

In most cases no change was seen in the first hour after the endotoxin administration but thereafter there was a marked drop in the height of the response (fig 1). By contrast, the endotoxin caused a fall in the height of the cochlear microphonic (an electrical response of the hair cells alone) starting in most cases in the first half
hour and continuing for the remainder of the experiment (fig 2). Perfusion of the cochlea with artificial perilymph alone showed no significant change in compound action potential or cochlear microphonics, and no detectable ultrastructural alteration to hair cell morphology.

When guinea pigs were pretreated with dexamethasone the electrophysiological impairment produced by endotoxin was considerably reduced (figs 1 and 2). Two main morphological changes were noted in the cochlea after endotoxin treatment alone: the first was a generalised swelling of the tectorial membrane that frequently touched the stereocilia of inner hair cells—this does not occur in the normal cochlea. The second effect was damage to hair bundles of both inner and outer hair cells. Mild damage led to splaying of stereocilia; more serious damage was associated with kinking or bending of stereocilia, which were sometimes completely flattened so that they lay on the surface of the hair cells. Occasionally stereocilia were torn from their attachment on the hair cells and adhered to the tectorial membrane (fig 3). Pretreatment with dexamethasone reduced the severity of this damage (fig 4).

Essentially similar electrophysiological and ultrastructural changes occurred whether endotoxin was introduced directly into the cochlea, or injected intracranially into the cerebrospinal fluid, although the changes after intracisternal injection were more variable. In some guinea pigs treated with intracochlear endotoxin, hyperaemia of the brain surface was noted after two hours. No similar effect was observed in the animals treated with dexamethasone.

Discussion

A pronounced reduction in cochlear output, reflected both in compound action potential and in cochlear microphonics, occurred in guinea pigs within two hours of the microperfusion of endotoxin into the scala tympani. This was accompanied by ultrastructural changes to the stereocilia of both inner and outer cochlear hair cells, which seem to be characteristic of endotoxin induced damage and some of which (adherence of stereocilia to tectorial membrane) have not to our knowledge been reported in association with any other type of cochlear insult.

This effect can confidently be attributed to the endotoxin rather than the mechanical interference, because neither the surgical procedure nor the perfusion of artificial perilymph alone produced any ultrastructural or electrophysiological changes in the cochlea. Previous studies similarly confirmed that the procedure itself does not affect cochlear function.11,12 In addition, intracisternal injection of endotoxin without any mechanical interference to the cochlea produced similar ultrastructural damage.

The morphological changes that we saw were sufficient in themselves to account for the electrophysiological losses that we detected, as hair cell stereocilia are essential for the transduction process that converts sound waves to electrical impulses.

A direct connection (the cochlear aqueduct) exists between the cerebrospinal fluid and the perilymph; this is patent both in guinea pigs13 and in humans.14 Bacterial endotoxin might well reach the inner ear in meningitis by this route. Consistent with this concept is the appearance of white cells in the perilymph rather than the endolymph, both in association with human meningitis15,16 and with experimental meningitis in rodents.16

The auditory lesion leading to deafness in meningitis has up till now not been clearly defined. Most workers consider that it is likely to be cochlear,17 but auditory nerve damage has not been excluded as a contributory factor. No specific site of cochlear injury has been found. Our work would suggest that the cochlear hair cells may be the primary site of this hearing damage, and may be particularly vulnerable to the effects of endotoxin; other sites of damage within the cochlea cannot, however, be excluded. Cochlear damage is also consistent with the well recorded association between deafness in meningitis and associated vertigo or labyrinthitis,18 and with the electrophysiological studies that have been performed on young children left deaf after meningitis.19,20

Our findings also suggest a potential mechanism by which hearing loss can be limited in bacterial meningitis. Lebel et al. and Girgis et al., in a series of well controlled clinical studies, found that steroids (used as adjuncts to antibiotics) improved the prognosis in children with meningitis.21-23 The most pronounced effect that they found was the reduction of deafness in the steroid treated patients. Pretreatment of patients with meningitis with steroids is not a practical proposition, but it is of interest, to note that until antibiotic treatment is started endotoxin concentrations in the cerebrospinal fluid both of experimental animals and of patients with meningitis remain relatively low, only rising rapidly in association with bacterial cell wall lysis.24,25 Thus treatment with steroids starting at the same time as the antibiotic treatment may be sufficient to block much of the cochlear damage.

This concept is supported by the recent findings of Mustafa et al. who showed that steroids reduced inflammation if given with (or before) antibiotics in experimental meningitis, but had little effect if their administration was delayed for one hour after the antibiotics had been given.26

The concentration of endotoxin present in the perilymph was difficult to assess accurately because much of it leaks rapidly out of the cochlea immediately after infusion, but as only 100 ng were sufficient to produce a clear effect despite this leakage, it seems likely that the effect we have described mimics that in natural infection and in experimental meningitis in rabbits, in which doses of 20 ng have produced a considerable inflammatory response.27 The maximal loss of cochlear response was noted within two hours of the onset of perfusion with endotoxin. This time course fits in well both with the inflammatory effect noted in experimental meningitis in the rabbit when endotoxin was injected into the cerebrospinal fluid, and with the associated release of tumour necrosis factor.5
If this inflammatory process is mediated by cytokines their site of origin is unclear. It is possible that they are released from known cytokine producing cells in the central nervous system, such as astrocytes or microglia. If this is the case, endotoxin would have been expected to gain access to the central nervous system through the cochlear aqueduct after cochlear microperfusion, and the cytokines to have diffused back in a similar manner. It is not impossible that the cytokines were released locally within the cochlea, especially as early evidence of hearing damage (within 60 minutes) was detected after local microperfusion.

Although we have shown a modulating effect of dexamethasone on cochlear damage induced by endotoxin, other anti-inflammatory drugs may have a similar effect and may have a role in the control of the inflammatory response in the ear.

We conclude that this endotoxin mediated effect may be the primary mechanism by which deafness is produced in meningitis, and that it may be possible to ameliorate this with drugs.

We thank Dr George McCracken for his advice and encouragement throughout this study, and Mr T Hayward for expert technical assistance.