

Intratympanic Injection of Dexamethasone: Time Course of Inner Ear Distribution and Conversion to Its Active Form

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Hypothesis: Intratympanically injected dexamethasone 21-phosphate is converted to its active form dexamethasone in the inner ear and follows the distribution of the glucocorticoid receptor.

Background: Although dexamethasone is routinely delivered intratympanically for hearing loss, we know little of its inner ear pharmacokinetics. Dexamethasone 21-phosphate is the pharmaceutical compound available for injection, but it must be converted to its biologically active form (dexamethasone) to bind to the glucocorticoid receptor. Therefore, the current study was conducted to determine the time course of dexamethasone 21-phosphate movement from the middle ear into the inner ear, its conversion to dexamethasone, and the distribution of both forms relative to the glucocorticoid receptor.

Methods: BALB/c mice were injected intratympanically with the prodrug dexamethasone 21-phosphate and inner ears collected at postinjection times ranging from 5 minutes to 7 days.

Ears were immunohistochemically stained for dexamethasone 21-phosphate, dexamethasone, and the glucocorticoid receptor.

Results: Both forms of dexamethasone were seen in the inner ear within 15 minutes, reaching their highest staining intensity at 1 hour. Neither drug was seen after 24 hours. The strongest staining occurred in the spiral ligament, organ of Corti, spiral ganglion, and vestibular sensory epithelia. Distribution of the drug paralleled locations of the glucocorticoid receptor except in the stria vascularis marginal cells, which stained heavily for the receptor but not the drug.

Conclusion: Dexamethasone rapidly travels from the middle ear into the inner ear and converts to its active form. The drug distribution follows that of the glucocorticoid receptor. However, it probably has little impact on ear tissues after 24 hours.

Key Words: Dexamethasone—Glucocorticoid receptor—Inner ear—Mouse—Transtympanic steroid delivery.
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Glucocorticoids (prednisone, dexamethasone, methylprednisolone) traditionally have been used to treat a variety of hearing disorders, including sudden hearing loss and autoimmune inner ear disease (1). However, despite a half century of administering steroids for hearing loss (2), we still have little insight into the abnormal cellular mechanisms within the cochlea that underlie steroid-responsive ear disease. Knowledge of these mechanisms is critical for our understanding of normal cochlear function and for the design of appropriate clinical therapies. Furthermore, in spite of the effectiveness of glucocorticoids, their

severe side effects prevent long-term management of inner ear dysfunction (1,3,4). These side effects include increased susceptibility to infection, sodium and fluid retention, hypertension, muscle weakness, osteoporosis, increased ocular pressure, cushingoid state, fat deposition (face), nervousness, and insomnia. Minimizing or preventing such severe side effects while effectively controlling hearing loss is a major challenge for otologists.

Our understanding of the role of steroids on inner ear function is becoming even more critical with the current practice of injecting steroids directly into the middle ear to reverse cochlear disease (5–11). The advantages of middle ear injection are that it avoids the systemic effects of steroid treatment and increases the amount of steroid entering the inner ear compared with systemic injections (5,6). Despite this, several questions remain regarding steroid-responsive processes in the ear and whether treatments are targeting these appropriately. Little is known regarding the efficacy of intratympanic steroid administration, such

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as the route of steroid travel through the inner ear fluid compartments, its eventual anatomic distribution, the amount of time the steroid remains in the inner ear, and the functional impact on gene expression by cochlear tissues. Furthermore, it is unknown whether there are differences in distribution, concentration, and efficacy between the various glucocorticoids, such as those with and without an affinity for the mineralocorticoid receptor.

Dexamethasone is one such glucocorticoid used for intratympanic delivery. However, little has been studied regarding its pharmacokinetics in the ear. It is supplied for clinical use as the prodrug dexamethasone sodium phosphate (Dex-21P). When used as a systemic medication, dexamethasone sodium phosphate is converted to active dexamethasone (Dex) in the plasma and liver by native phosphatases (12). Previous inner ear studies have evaluated the inner ear concentration of dexamethasone after intratympanic injection, but they have not addressed the prodrug conversion to its active form. It is unknown whether similar enzymatic processes can occur in the ear for there to be any clinical utility of such a treatment.

The purpose of this study was to conduct a preliminary investigation into the inner ear pharmacokinetics of intratympanic dexamethasone delivery. Of particular interest were its diffusion parameters (time course, distribution) into the ear and its conversion to the functionally active form. It also was of interest to determine whether the anatomic distribution of drug followed that of the glucocorticoid receptor or whether some other pattern was observed.

MATERIALS AND METHODS

Mice

Adult BALB/c mice were used to determine the transport of the dexamethasone prodrug (Dex-21P) from the middle ear to the inner ear and the conversion there to its active form (Dex). These mice also were used to identify the location of the glucocorticoid receptor within the inner ear. All mice were 3 to 5 months old.

Dexamethasone Injections

BALB/c mice were anesthetized with a ketamine-xylazine cocktail and their eardrums observed under a surgical stereomicroscope. Dexamethasone sodium phosphate (Dex-21P; American Regent Laboratories, Shirley, NY, U.S.A.) was injected (5 μ L) into the middle ear at a concentration of 4 mg/ml, the standard hospital pharmacy formulation for patients. The distribution of drug in the inner ear was evaluated at postinjection survival times of 5, 10, 15, and 30 minutes; 1, 2, 4, 8, and 12 hours; and 1, 3, 5, and 7 days. A total of four mice were evaluated at each survival time. The opposite ear control was either uninjected or injected with phosphate-buffered saline.

Immunohistochemical Staining

Mice were anesthetized and intracardially perfused with fixative (4% paraformaldehyde in 0.1 mol/L phosphate buffer) at the various postinjection survival times. The inner ears were dissected free of the middle ear bulla and skull and postfixed overnight. After 5 days of microwave decalcification in ethyle-

nediamine tetra-acetic acid, the ears were cryostat sectioned and placed on slides for immunohistochemistry.

Dexamethasone

An assessment was made of the distribution of the two dexamethasone forms. Rabbit antibodies (Biogenesis, Kingston, NH, U.S.A.) to both the prodrug (anti-Dex-21P) and the active drug (anti-Dex) were applied to adjacent sections on the same slide to minimize differences in staining caused by processing. The two primary antibodies are specific for their antigenic target, with no affinity for the other chemical form of dexamethasone. The primary antibodies were identified with an anti-rabbit secondary antibody conjugated to Alexa Fluor 488 (Molecular Probes, Eugene, OR, U.S.A.). Ears receiving the two control treatments (phosphate-buffered saline or no injection) served as negative controls and underwent the same immunohistochemical evaluation as the drug-treated ears. All sections were viewed with a Nikon TE300 (Nikon, Tokyo, Japan) inverted fluorescence microscope fitted with the Bio-Rad MRC1024 laser confocal system (Bio-Rad Laboratories, Hercules, CA, U.S.A.) for a qualitative analysis of the anatomic distribution of the two steroid forms.

Glucocorticoid Receptor

Immunohistochemical staining for the glucocorticoid receptor was conducted to determine whether the anatomic distribution of the drug forms correlated with the locations of the glucocorticoid receptor. Cochlear sections were incubated with an anti-glucocorticoid receptor primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) followed by the same secondary antibody above. Stained sections were observed on the laser confocal microscope for the presence of fluorescent label staining to determine the location of the dexamethasone receptor. All animal procedures were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee.

RESULTS

Dexamethasone

The fluorescent signal was first detected in the inner ears harvested 15 minutes after middle ear injection. None of the sections harvested after 24 hours displayed any of the fluorescent label, which was interpreted as resulting from the steroid having cleared the cochlea by this time. The highest intensity staining appeared to occur at the 30-minute and 1-hour postinjection times. Sections treated with antibodies to the prodrug and the active drug both demonstrated increased fluorescence in the perilymphatic spaces during this time period (Fig. 1). None of the cochlear tissues from control mice stained with either of the dexamethasone antibodies.

Antibody binding to the active drug (Dex) was present as early as binding to the prodrug (Dex-21P), indicating that the chemical conversion occurred rapidly in the inner ear. In general, the fluorescent signal from the active dexamethasone appeared slightly brighter than the signal from the prodrug at the earliest time points. However, this slight qualitative difference was not judged significant.

The highest concentration of dexamethasone labeling was seen in the spiral ligament, basilar membrane, organ of Corti, and spiral ganglion (Fig. 2). Slight steroid staining was seen in the stria vascularis, although it showed

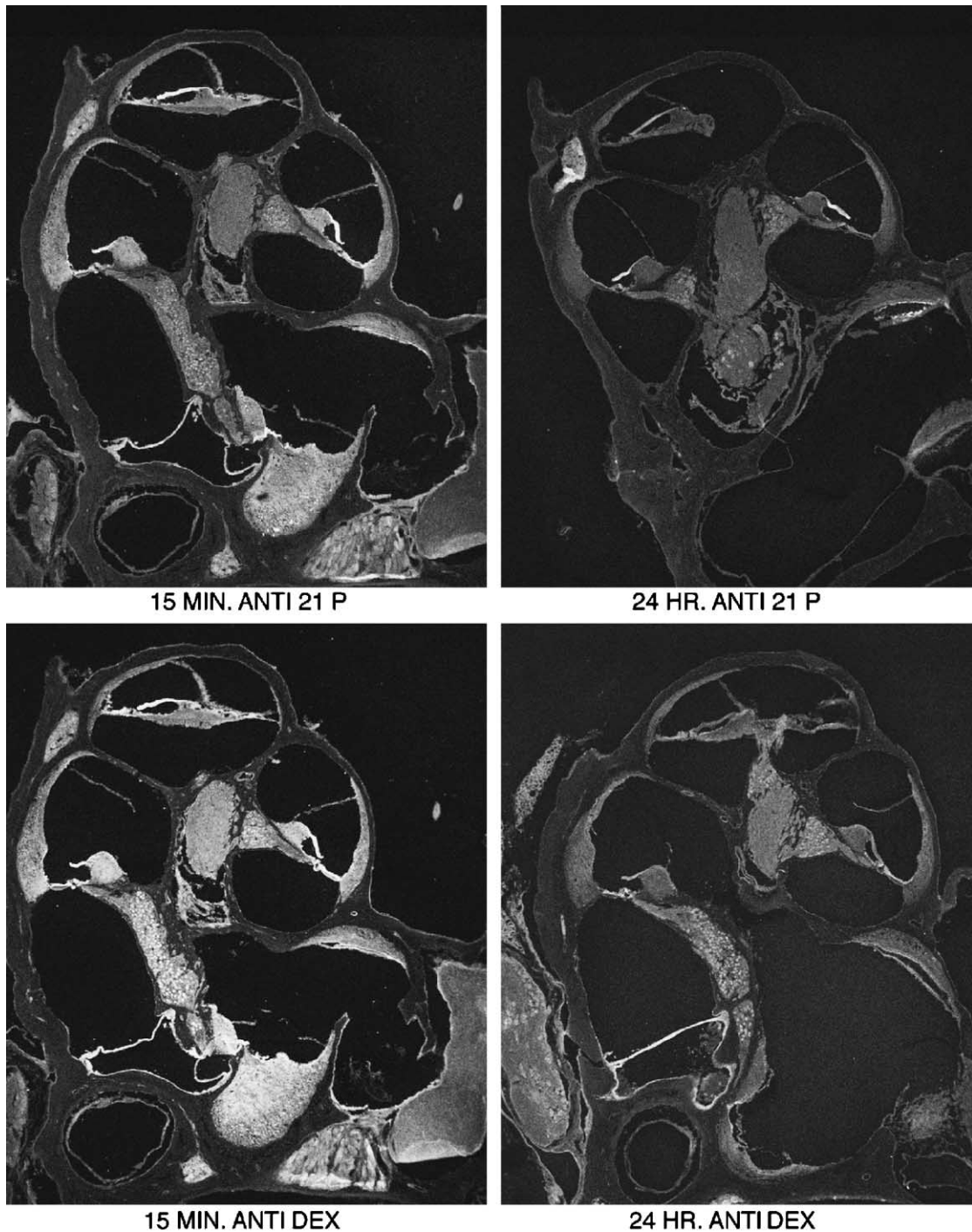


FIG. 1. Dexamethasone antibody staining demonstrating the distribution of the prodrug (21P) and active dexamethasone (Dex) within the inner ear at 15 minutes and 24 hours after injection into the middle ear. Most sensorineural structures are brightly labeled, indicating the widespread presence of both drug forms.

significantly less fluorescence than the spiral ligament. The strong staining within the spiral ligament and organ of Corti suggested the steroid distributed extensively into the perilymphatic spaces from the round window. It also appeared that the steroid reached the endolymphatic spaces because staining was seen in cells lining the

inner and outer sulcus. However, these cells also could be reached from their perilymphatic side, so it is not conclusive whether the drug reached the endolymph. The tectorial membrane showed strong labeling, but this also was seen in mice not receiving the steroid, suggesting this structure artifactually bound secondary

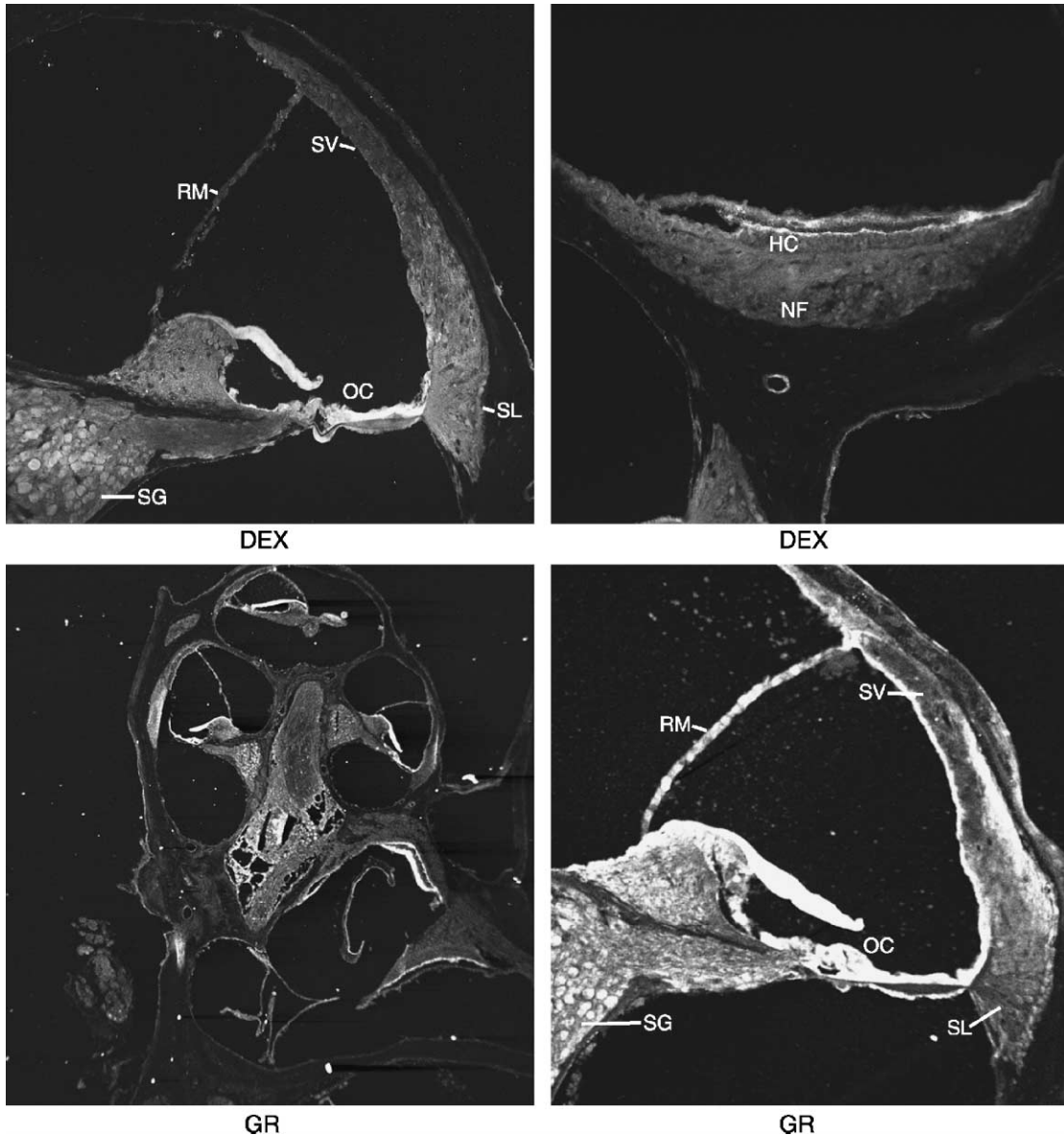


FIG. 2. Inner ear distribution of Dex and the glucocorticoid receptor. (*Above*) Staining for dexamethasone (Dex) 15 minutes after intratympanic injection shows label of the organ of Corti (OC), spiral ligament (SL), and spiral ganglion (SG), reflecting the rapid and broad diffusion of drug throughout the cochlear structures. Less staining is seen in the stria vascularis (SV) and Reissner's membrane (RM). Strong fluorescent staining was seen in the saccule hair cells (HC) and underlying neural fibers (NF). (*Below*) Staining for the glucocorticoid receptor (GR) labeled the spiral ligament (SL), organ of Corti (OC), spiral ganglion (SG), Reissner's membrane (RM), and marginal cells of the stria vascularis (SV).

antibody without the presence of antigen. All organ of Corti cells (e.g., hair cells, supporting cells) are surrounded by perilymph, so their staining does not conclusively demonstrate access of the drug to the endolymph.

Vestibular structures also stained heavily with the dexamethasone antibodies. Strong labeling was seen in the maculae of the utricle and saccule (Fig. 2) and the semicircular canal cristae. Because frozen sections were used, it was not possible to resolve exactly which cell types within these structures were predominantly labeled. However, it appeared that strong staining was seen in the epithelial structures (hair cells and supporting cells), oto-

conial membrane, and vestibular ganglia neurons and nerve fibers. The prominent staining of the tips of the hair cells suggests their surface structures were exposed to the drug within the endolymph.

Glucocorticoid Receptor

To determine whether the dexamethasone distribution matched that of its receptor, antibody staining for the glucocorticoid receptor in the inner ear was conducted. The distribution of this receptor matched virtually all areas in which dexamethasone was seen (Fig. 2). Strong receptor staining was seen in the lateral wall, although there appeared

to be less staining of the stria vascularis than of the spiral ligament. The marginal cells lining the stria often showed fluorescent labeling, whereas the other stria cell layers did not. The marginal cells did not appear to stain with the dexamethasone antibodies, suggesting a possible difference between the distribution of the drug and its receptor.

All structures of the organ of Corti stained heavily for the steroid receptor. Although exact cellular differentiation was difficult in the frozen sections, the antireceptor antibody appeared to stain hair cells, supporting cells, spiral limbus, tectorial membrane, basilar membrane, outer sulcus cells, inner sulcus cells, and Reissner's membrane. Strong fluorescent label also was seen in the spiral ganglion neurons and peripheral processes of the auditory nerve fibers between the ganglion and hair cells. Vestibular sensory epithelia also stained heavily for the glucocorticoid receptor. Antibody labeling was seen in the maculae of the utricle and saccule, otoconial membrane, the semicircular canal cristae, and vestibular ganglion neurons.

DISCUSSION

This study localized dexamethasone in the inner ear after intratympanic injection. Antibodies specific for either the prodrug or its active form showed dexamethasone was detected within the inner ear at 15 minutes but was undetectable after 24 hours. Furthermore, the active form was present as early as the prodrug, indicating that the conversion occurred rapidly. This confirms that intratympanic administration of dexamethasone delivers the active drug to inner ear structures, but the medication likely has a limited functional window of 24 hours after administration. Higher concentrations of injected drug may last longer.

Previous studies of direct cochlear sampling of dexamethasone after transtympanic injection in guinea pigs (5–7) showed that the scala tympani and scala vestibuli steroid levels were essentially identical, endolymph levels were substantially higher than the perilymph levels, and perilymph levels were significantly higher than when the drug was delivered systemically. However, fluid sampling in these studies did not permit assessment of the distribution and duration of steroid within the inner ear after intratympanic administration. Therefore, the current study provides insight into these additional clinically relevant issues to add to our understanding of dexamethasone pharmacokinetics in the ear. Comparable rapid entry of steroid into the cochlea to a maximal concentration at 1 hour, followed by rapid elimination within a few hours, also is seen with intravenous injections (13).

Several studies have shown that the cochlea has glucocorticoid receptors (14–18), and the inner ear distribution of the receptor in the current study paralleled its reported location in these other studies. However, studies of receptor mRNA do not show it within the organ of Corti, in contrast to immunohistochemical staining (17,18), so some issues of this receptor distribution and control remain to be clarified. Nevertheless, the receptor is widespread throughout the

inner ear, which suggests that many molecular functions could come under this steroid's control. Although determining the means of transport of steroids through the inner ear after intratympanic administration is critical, the broader goal is to better understand the control of hearing loss by steroids. Currently, we have very little understanding of where steroids act within the cochlea, their receptor-binding functions, or the cellular and molecular processes in the ear that are regulated by receptor activation.

The glucocorticoids have numerous physiologic actions that may impact cochlear tissues (19). The common use of glucocorticoids in otology relates to their presumed immune suppression and anti-inflammatory functions in the cochlea (3,20,21). For example, the transcription factor nuclear factor- κ B regulates the synthesis of many proinflammatory cytokines involved in the general immune response (22–24), and this is suppressed by glucocorticoids (23,25,26). The mRNA for this transcription factor is expressed in the mouse cochlea after systemic lipopolysaccharide inoculation (27), and this presumably would be suppressed by glucocorticoid treatment. It is thus possible that this nuclear factor- κ B downregulation within specific inner ear compartments by glucocorticoids is responsible for reversing hearing loss.

Although considerable emphasis is placed on the immune suppressive functions of glucocorticoids, it must be remembered that this class of corticosteroids has considerable impact on carbohydrate and protein metabolism, as well as significant control over electrolyte and water balance. For example, glucocorticoids have a restorative effect on ion transport in dysfunctional autoimmune mouse stria vascularis (28). Also, glucocorticoids have rapid nongenomic effects on stria marginal cell K^+ ion transport (29), paralleling the stronger staining of marginal cells with receptor antibody in the current study. The strong staining of the glucocorticoid receptor in the spiral ligament further emphasizes its potential role in K^+ ion homeostasis. These ion homeostatic functions could explain glucocorticoid improvement in auditory brainstem response latencies in people (30) and increased aquaporin expression in rats (31). Glucocorticoids also have been shown to reduce cochlear damage after insults from ototoxic drugs (32), ischemia (33), mechanical injury (34), and noise (35,36).

Steroid treatments, although convincingly beneficial to some clinicians, have been shown by others to lack efficacy, and some have concluded that recovery is not enhanced (37,38). This controversy regarding steroid therapies must be clarified for effective treatment of sensorineural hearing disorders. This lack of consistent treatment outcome reflects our lack of understanding of steroid-responsive mechanisms in the ear. This may be related to timing of treatment after hearing loss onset, using the wrong steroid to counter the specific cochlear abnormality, or not using the steroid appropriately in light of other systemic disease processes. Once the intracochlear diffusion characteristics of steroids have been elucidated, investigations into the molecular and biochemical processes they control within various inner ear structures will be possible.

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