THE EFFECT OF PERILYMPHATIC TETRODOTOXIN ON PERIPHERAL AUDITORY SYSTEM FUNCTION

H. Alexander Arts, M.D.,* Susan J. Norton, Ph.D.,† and Edwin W Rubel, Ph.D.‡

Abstract Several studies have indicated that efferent activity influences otoacoustic emissions. The present studies have been designed to evaluate the influence of auditory nerve activity on otoacoustic emissions. The distortion product otoacoustic emission \(2f_1 - f_2\) (DPOAE) was studied in Mongolian gerbils before and after placement of tetrodotoxin (TTX) within the round window niche. The release compound used was ethylene vinyl-acetate copolymer resin (Elvax®). Elvax alone had no effect on the DPOAE, auditory brainstem response (ABR), or cochlear microphonic (CM). Elvax with TTX resulted in preservation of the DPOAE and CM, but elimination of the ABR. This study demonstrates that the generation of action potentials in efferent and afferent axons of the auditory nerve is not required for the maintenance of DPOAEs. Further, in the anesthetized gerbil, complete blockade of action potentials in the auditory nerve has no systemic influence on DPOAE magnitude.

Keywords: Otoacoustic emissions, tetrodotoxin, hair cell

Current models of cochlear function assume that active, nonlinear biomechanical elements are responsible for the ear's high sensitivity and sharp tuning. The active elements are presumed to be the outer hair cells (OHCs) for the following reasons: (1) outer hair cells have been shown to have motile responses to electrical and/or chemical stimulation in vitro (Ashmore, 1987; Brownell et al., 1985; Crawford & Fettiplace, 1985; Kachar et al., 1986; Zenner et al., 1985; Zenner, 1986), (2) the majority of efferent auditory nerve fibers are directed toward OHCs rather than inner hair cells (IHCs). Similarly, the majority of fibers synapsing on OHCs are auditory nerve fibers (Brown, 1987; Spoendlin, 1972; Spoendlin, 1978). On the other hand, IHCs are the primary innervation for auditory efferent fibers, suggesting that they function...

*Department of Otolaryngology—Head & Neck Surgery, University of Michigan School of Medicine, Ann Arbor, Michigan. †Dept. of Otolaryngology—Head & Neck Surgery, University of Washington School of Medicine, Seattle, Washington

Reprint requests: Dr. Arts, Department of Otolaryngology—Head & Neck Surgery, University of Michigan Medical Center, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0312.
primarily as receptors, and that OHCs function as a sort of mechanical modulator; (3) the stereocilia of OHCs are embedded in and apparently attached to the tectorial membrane, whereas the stereocilia of the IHCs are not (Engström & Engström, 1978). This would appear to be necessary if OHC motility is to have a mechanical effect on basilar membrane (BM) motion; (4) the cochlea has been shown to be capable of generating acoustic energy in the form of otoacoustic emissions. The presence of these energy-requiring emissions would appear to imply the existence of a motile element in the cochlea, such as the OHCs, and emissions are lost when OHCs are damaged or missing (Kemp, 1978; Horner et al., 1985; Schrott et al., 1991; LeCalvez et al., 1998).

It is likely that the processes or elements responsible for the cochlea’s sharp tuning are also responsible for its nonlinear behavior in general. One manifestation of the cochlea’s nonlinear behavior is the presence of intermodulation distortion products. Distortion product otoacoustic emissions (DPOAEs) are generated when the cochlea is presented simultaneously with two or more tones of different frequency \((f_1, f_2, \ldots)\). In general, the most robust and most well studied distortion product is the cubic difference tone, \(2f_1 - f_2\). Distortion products can be perceived psychoacoustically and have been detected in auditory nerve recordings (Smoorenberg, 1970, 1976). Several studies show a relationship between the DPOAE intensity for any given primary frequencies and the psychoacoustic threshold at those frequencies (Lonsbury-Martin & Martin, 1990; Martin et al., 1990). Like spontaneous and transient-evoked otoacoustic emissions, DPOAEs are extremely sensitive to the physiological state of the cochlea (Rubel & Norton, 1991). Thus, it seems reasonable to postulate that the OHCs are at least a component of the nonlinearity responsible for generating DPOAEs.

If OHC motility is fundamental to the function of the “cochlear amplifier,” then OHC motion would have to be tightly controlled by a feedback network, with the feedback perhaps coming from the IHC’s or basilar membrane motion itself. If this is the case, it brings into question the role of the efferent fibers to the OHCs in this process. Several studies have shown that efferent stimulation has an effect on otoacoustic emissions. Mountain (1980) demonstrated that stimulation of the crossed olivocochlear bundle altered electrically-evoked DPOAE in guinea pigs. Similar results were noted in chinchilla by Siegel and Kim (1982), and they demonstrated that these effects were blocked by perfusion of the cochlea with curare. Guinan showed that crossed olivocochlear bundle stimulation altered stimulus frequency emissions in cats (Guinan, 1986); Mott et al., (1989) demonstrated that contralateral acoustic stimulation altered spontaneous emissions in humans.

While these studies show that efferent activity has an effect on emissions, it is of interest to know whether auditory nerve activity, either efferent or afferent, is required for emission generation, and whether “spontaneous activity” of auditory nerve fibers influences emission properties. To test this hypothesis, we blocked auditory nerve action potentials using tetrodotoxin (TTX) in a gerbil. The gerbil was chosen as the experimental animal, as it has robust DPOAEs and an easily accessible round window niche which facilitates placement of a slow-release vehicle for noninvasive delivery of TTX to the perilymph. This technique has been previously described by Pasic, Born, and Rubel and has been shown to reversibly block auditory nerve activity in birds and mammals (Born and Rubel, 1988; Pasic and Rubel, 1989).

**MATERIALS AND METHODS**

**TTX Preparation**

TTX was formulated into sustained release pellets containing ethylene vinyl-acetate copolymer resin (Elvax®, DuPont), by using previously described techniques (Langer et al., 1985; Pasic & Rubel, 1989). Pellets were manufactured, weighing 0.5 mg
each and containing 250–750 ng of TTX. Elvax pellets without TTX were also manufactured as controls.

**Subjects**

Mongolian gerbils were obtained from Tumblebrook Farms (West Brookfield, MA) and given free access to food and water. All gerbils were between 3 and 9 weeks of age. Animals were anesthetized with ketamine (75 mg/kg IM) and xylazine (5 mg/kg), and anesthesia was maintained with supplemental injections as needed to prevent nociceptive reflexes. Body core temperature was maintained at 38°C by a thermostatically controlled heating pad during the experiment.

**Measurements**

After the animal was suitably anesthetized, it was placed in a head holder and onto a thermostatically controlled heating pad. A postauricular skin incision was made and the posterior ear canal skin identified. The ear canal skin was then transected posteriorly and the pinna reflected anteriorly. A low-noise microphone (Etymotic ER-10B) was used to monitor the ear canal distortion products. The microphone tip was connected to a short segment of silicone rubber tubing, creating a volume of approximately 0.5 cc. With the aid of a micromanipulator, the end of the tubing was sealed snugly to the transected external auditory canal. Within the microphone assembly was a self-contained, battery-powered heater to prevent moisture buildup within the sealed cavity. The output of the microphone was routed to its own preamplifier/power supply. The equalized output of the preamplifier was flat (+5 dB) from 700 Hz to 10 kHz. The preamplified microphone signal was then analyzed with a digital signal analyzer (Hewlett-Packard HP3561A). The preamplifier output was sampled by the signal analyzer over eight contiguous 2-second time windows for a period of 16 seconds. A 1024 point fast fourier transform (FFT) was computed for each sample, with the center frequency equal to the cubic difference tone, \(2f_1 - f_2\) (2153.75 Hz). The data were transferred over an IEEE-488 bus to a DEC PDP-11/73 minicomputer for storage and analysis.

The stimulus tones were generated by a two-channel signal generator (Hewlett-Packard HP8904A) driving two transducers (Etymotic ER-3A). The transducers were attached to the ear canal via silicone rubber tubing passed through small holes made for this purpose in the ER-10B microphone assembly. The ear canal stimulus intensity was verified prior to each measurement by using the ER-10B microphone and the previously calibrated signal analyzer. Input-output functions for DPOAE intensity versus stimulus intensity were obtained for each animal and each experimental condition for stimulus intensities from 20–80 dB SPL. Stimulus frequencies were \(f_1 = 3077\) Hz and \(f_2 = 4000\) Hz, resulting in a \(f_1/f_2\) ratio of 1:1.3. The stimulus levels \((L_1, L_2)\) were equal in all cases.

The ABR was used to monitor integrity of the auditory nerve before and after pharmacologic manipulation. The stimulus used was a 0.1 ms click, with alternating polarity generated by a single channel click generator at a rate of 10 Hz. This signal was then attenuated with a IEEE-488 bus-controlled attenuator. The attenuated signal was used to drive one of the previously mentioned transducers attached to the ear canal. The stimuli were calibrated in peak-equivalent SPL (peSPL) with reference to an 80 dB SPL, 1 kHz tone using the peak-to-peak method (Burkhard, 1984). The cochlear microphonic potential (CM) was also measured in many cases. The stimulus for this was a 1 kHz tone windowed to give two complete cycles and presented in an identical manner as the ABR stimuli. To eliminate the ABR in the response, averages were taken with both positive and negative polarity, and the results subtracted and divided by 2. Both ABR and CM were measured via subdermal needle electrodes placed behind each ear, with the positive electrode behind the test ear. A ground electrode was placed subdermally
over the animal’s lumbar spine. The signal was bandpass-filtered at the preamplifier from 30-3000 Hz, and then further bandpass-filtered from 150–4000 Hz with an external filter (Krohn-Hite model 3550). The signal was amplified x1000 with an isolation preamplifier (Grass P15), then amplified further as necessary, and monitored with a storage oscilloscope. The output of the oscilloscope was then averaged with respect to the stimulus at a typical sampling rate of 28.5 kHz on the DEC PDP 11/73 computer.

After control measurements were taken (DPOAE I/O function, ABR, and CM), the sustained-release pellet with TTX was placed in the gerbil’s round window niche through a mastoidotomy approach. The mastoid bulla was readily accessible through the postauricular incision and easily opened with sharp forceps. The measurements were then repeated at various intervals. In the early experiments, both ears were used as separate experiments. This was found to be impractical, due to the length of anesthesia and the technical difficulties involved. Thus, in later experiments, only one ear was tested. In control animals, either no manipulation was administered except for opening the bulla, or a sustained-release pellet without TTX was placed in the round window niche with the same measurements taken. In each animal, and for each experimental condition, DPOAE intensity versus stimulus intensity was measured and the input-output function plotted. Difference functions were calculated by subtracting the experimental DPOAE value from the control DPOAE value for each stimulus intensity. The auditory brainstem response as a function of stimulus intensity, as well as the cochlear microphonic response, were also recorded.

RESULTS

ELVAX—CONTROL

When Elvax pellets without TTX were placed in the round window niche, no sig-

Figure 1. Typical ABR and CM waveforms before and after placement of Elvax pellets without TTX.
significant changes were noted, compared to the control state. No significant changes were noted between control and Elvax conditions in ABR morphology, threshold, or input-output curves. Similarly, no systematic changes were seen in cochlear micromphonic responses. Figure 1 is a typical example of ABR and CM waveforms seen before and after placement of Elvax pellets. Figure 2 is a typical example of the DPOAE amplitude as a function of stimulus intensity before (control) and after placement of the Elvax pellet. No significant changes were seen in the ABR levels after placement of Elvax pellets in the round window niche. With the Elvax pellet in place, the click-evoked ABR threshold was similar to the control value (approximately 40–45 dB peSPL) and the CM in response to a 1000 Hz sine wave was recordable at low to moderate levels.

**ELVAX—TETRODOTOXIN**

When Elvax pellets containing TTX were placed in the round window niche, prompt disappearance of the ABR and preservation of the CM was observed in each case, as predicted (Pasic and Rubel, 1989). Figure 3 shows the ABR and CM before and after placement of an Elvax/TTX pellet in each of two animals. Vestibular nerve function was, apparently, blocked as well, as the animals in this group consistently exhibited marked vestibular dysfunction upon awakening, i.e., walking in circles, frequently stumbling, and spinning longitudinally when held in the air by their tails. This effect disappeared within 24–48 hrs. After verifying that auditory nerve function was blocked using ABR, DPOAE levels were measured as a function of stimulus levels. In each case, DPOAE levels were preserved to within one standard deviation of the mean of the control values averaged over all animals tested (n = 34 ears, 54 measurements). Figure 4 shows the DPOAE I/O functions from these two animals. The Elvax/TTX measurements were taken at 6 and 47 minutes after placement of the implant in the animal shown in Figure 4a, and at approximately 10 minutes of TTX exposure in the data shown in Figure 4b. Although no consistent pattern is seen, there appears to be a slight tendency for DPOAE levels to decrease slightly after placement of Elvax/TTX.
Figure 3. ABR and CM waveforms for two animals (A & B) using Elvax with TTX pellets. The control waveforms were obtained prior to placement of pellets in the round window niche, and the "TTX" waveforms obtained approximately 10 minutes after TTX/Elvax pellet placement. ADP amplitude versus stimulus level for the control condition and the Elvax with TTX condition in two animals. Error bars indicate ±1 standard deviation about the mean of all control values across all animals.
Figure 4. DPOAE amplitude vs. stimulus level for the control condition and the Elvax with TTX condition in two animals (A & B). Error bars indicate ±1 standard deviation about the mean of all control values across all animals.
DISCUSSION

A large body of evidence has accumulated over the past 25 years suggesting that the motion of the basilar membrane is affected by active, energy-requiring processes. This was first suggested by Gold and Hearing (1948) who calculated that the mechanical damping of the cochlear partition would be too great to account for the psychophysical threshold and frequency discrimination observed. Rhode (1973) later demonstrated that the basilar membrane response became less nonlinear, less sensitive, and less frequency-selective, following death of the animal or interruption of cochlear blood supply. The same manipulations were found to result in a progressive loss of tuning (Robles et al., 1976). These changes, and others like them, would seem to be best explained by a change in the dampening coefficient of the cochlear partition. Gold’s hypothesis was supported by the work of Kim et al (1980), who showed that the postmortem changes were best explained by a loss of an energy source and a change from negative to positive damping. The discovery of otoacoustic emissions, i.e., acoustic energy generated within the cochlea, provided further support to Gold’s original hypothesis (Kemp, 1978; Kemp, 1979; Kemp & Chum, 1980).

Like Rhode’s nonlinearities, DPOAEs are highly vulnerable to the physiologic state of the cochlea. After death, the intensity and number of the distortion products decreases progressively. Some distortion products (to high-level signals) remain hours or more after death, and these are thought to result from passive nonlinearities. This physiologic vulnerability of DPOAEs and other data, suggest that the nonlinear behavior of the cochlear partition is due to bidirectional coupling between the outer hair cells and the basilar membrane. The discovery of the motile properties of the outer hair cells further supported this hypothesis. (Brownell et al., 1985; Zenner, 1986)

Thus, it is now widely accepted that the OHC generates mechanical forces that are powered by local membrane potentials and which act to counteract the viscous damping forces within the cochlea. In other words, they supply the active “negative damping” necessary for the high degree of tuning seen. If this were the case, spontaneous and evoked otoacoustic emissions could be generated when the OHC feedback force reaches a critical phase, or is larger than the viscous damping force by an amount that cannot be dissipated locally. The nonlinear behavior of such a system would be expected to result in distortion products. It is likely, therefore, that the elements responsible for spontaneous and evoked otoacoustic emissions are also responsible for DPOAEs.

The results of this study indicate that DPOAEs are not dependent on auditory nerve activity. DPOAEs may be modulated by auditory nerve efferent activity through alteration of membrane potentials of the OHCs, but in the absence of efferent stimuli, they are still present. Our experiment verifies this hypothesis. Even though auditory nerve activity was completely blocked by TTX, distortion products were only slightly altered. The DPOAE amplitude did have a tendency to be slightly lower after placement of TTX in the round window, however this effect was not statistically reliable and was not systematically evaluated in this study. If this effect is real, it is likely due to elimination of the efferent effect on the OHCs.

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REFERENCES


**ARTICLE ONE**

**SELF-ASSESSMENT QUESTIONS**

1. Distortion product otoacoustic emissions (DPOAE) are generated when the cochlea is:
   (a) Presented with a pure tone
   (b) Presented simultaneously with two or more tones of different frequencies
   (c) Presented with a transient signal
   (d) Not stimulated

2. Tetrodotoxin (TTX) has been shown to:
   (a) Enhance outer hair cell activity
   (b) Reversibly block auditory nerve activity
   (c) Alter the frequency of the cochlear microphonic
   (d) None of the above

3. Elax pellets with TTX have been shown to:
   (a) Abolish the ABR
   (b) Abolish DPOAEs
   (c) Abolish the cochlear microphonic
   (d) All of the above

4. The results of this study indicate that:
   (a) DPOAEs are not dependent upon auditory nerve activity
   (b) DPOAEs depend substantially upon auditory nerve activity
   (c) DPOAEs are closely related to the ABR
   (d) DPOAE amplitude increases proportionate to the amount of the TTX administered

5. Efferent auditory activity:
   (a) is responsible for the non-linear behavior of the cochlea
   (b) results in distortion product otoacoustic emissions
   (c) persists despite TTX blockade
   (d) may modulate distortion product otoacoustic emission amplitude