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Evoked-potential thresholds and cubic distortion product otoacoustic emissions in the chinchilla following carboplatin treatment and noise exposure

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Abstract

Twenty-two chinchillas were given either a single intraperitoneal (i.p.) or intravenous (i.v.) injection (50 or 75 mg/kg) of Paraplatin™, an asymptotic threshold shift-producing noise or a combination of the drug and noise in series. Auditory evoked potential (pure-tone) audiograms and cubic distortion product otoacoustic emissions were obtained on each animal before and after treatment, and the sensory epithelium of the cochlea was evaluated using the surface preparation method. Anatomical analysis indicated that the carboplatin alone caused relatively severe but scattered losses of inner hair cells throughout most of the cochlea which were dependent on dose and administration route. The outer sensory cell population remained essentially intact. In animals that had up to 40% scattered losses of only inner hair cells, evoked potential thresholds were near normal and the emission functions either were normal or showed an enhanced output. The severe losses of inner hair cells produced by the drug had no effect on the threshold shift dynamics produced by a five-day uninterrupted noise exposure. In general, there was not a consistent relation between the emission data and both the permanent threshold shift and outer hair cell losses.

Keywords: Hearing loss; Otoacoustic emission; Carboplatin

1. Introduction

Otoacoustic emissions, a product of normal cochlear function tied to the integrity of the outer hair cell system (Brownell et al., 1985), are rapidly developing into a tool for evaluating peripheral pathology (Lonsbury-Martin and Martin, 1990). However, the ultimate diagnostic value of emission measurements is proportional to the correlations that can be developed with specific pathologies of the cochlea. Ototoxic drugs and noise are not only common hazards for the peripheral auditory system but are also tools for experimentally creating pathologies for investigating the viability of various diagnostic procedures. This paper focuses on the relations among cubic distortion product otoacoustic emissions, pure-tone thresholds as reflected in auditory evoked potentials from the inferior colliculus, and the cochlear sensory cell population following the creation of a series of unusual sensory cell losses as a result of the administration of the drug carboplatin and an asymptotic threshold shift-producing noise exposure.

In the mammalian cochlea most ototoxic drugs as well as noise have their greatest and earliest effect on the outer hair cells (OHC) leading to a partial loss of this group of sensory cells while the inner hair cells (IHC) are normally much less affected. Consequently, there is considerable information on the physiology and psychoacoustics in various animal species in which the predominant pathology is an OHC lesion. Preparations in which the OHC population is normal in the presence of substantial IHC losses are not common (Schrott et al., 1991) and the properties of such cochleas not well examined.

Recently Wake et al. (1993, 1994) and Takeno et al. (1994a,b) have shown that the anti-neoplastic drug carboplatin administered to the chinchilla could cause a dose-related loss of IHCs throughout the cochlea but leave the OHC population essentially intact. In the guinea pig, how-

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ever, carboplatin produced the typical ototoxic configuration of sensory cell loss (Takeno et al., 1994b).

In their studies of carboplatin in the chinchilla Takeno et al. (1994a,b measured the compound action potential (CAP), the cochlear microphonic (CM), and the auditory brainstem response (ABR). The sensory cell lesions, described in terms of OHC and IHC damage in the 1, 2, 4 and 8 kHz regions of the cochlea consisted primarily of IHC damage. The overall status of the entire sensory epithelium was not indicated. Their results showed that the CM was near normal when 50–70% IHCs were damaged and the OHCs were more than 80% intact in the appropriate region of the cochlea. However, with this sensory cell configuration, the CAP and ABR were elevated 40–60 dB.

In the present study we examined the changes in the auditory evoked potential (AEP), pure-tone thresholds recorded from the inferior colliculus and the distortion product otoacoustic emissions in animals treated with various regimes of carboplatin. Recognizing the unusual pattern of sensory cell losses reported in the literature, we were also interested in how an animal whose IHC population had been severely reduced would respond to noise trauma which typically affects the OHC population earliest and most severely. The ultimate objective of this work, however, was to evaluate the relation among the two diagnostic indices of peripheral pathology (i.e., a threshold metric and otoacoustic emissions) and the sensory cell population.

2. Methods

Thirteen chinchillas were treated with carboplatin (Bristol Laboratories, Paraplatin-AQ™). Two dosages of Paraplatin™ were used: 50 mg/kg and 75 mg/kg. Three animals were given a 50 mg/kg intravenous (i.v.) injection using a vein in the pinna; three animals were injected intraperitoneally (i.p.) at 50 mg/kg; four animals were given a 75 mg/kg i.p. injection and three animals were given a 75 mg/kg i.v. injection and allowed to recover for 30 days and then exposed to an asymptotic threshold shift (ATS) producing impact noise-exposure paradigm. The impact noise exposure consisted of a narrow band of noise centered at 1.0 kHz and presented one impact per second for 5 days, 24 h/day. The impact was presented at 121 dB peak SPL. Nine additional chinchillas were exposed to only the impact noise in order to define the pattern of threshold shifts, both ATS and PTS, produced by the noise in non-drug-treated animals.

2.1. Surgical preparation

All animals were made monaural by the surgical destruction of the left cochlea and an AEP-recording electrode was implanted into the left inferior colliculus. Details of the AEP procedures and surgery can be found in Ahroon et al. (1993). Briefly, each animal was anesthetized (i.m. injection of ketamine (35 mg/kg b.wt.) and xylazine (1 mg/kg b.wt.)) and made monaural by the surgical destruction of the left cochlea. A bipolar, platinum EEG electrode with electrode lengths of 7.5 mm (probe) and 2.5 mm (ground) was implanted into the region of the inferior colliculus (IC) under stereotaxic control for single-ended recordings of the AEP (Henderson et al., 1973; Salvi et al., 1982). A xylazine reversing agent (yohimbine (2 mg/kg b.wt. i.m.) was administered after the surgical procedure. The animals were allowed to recover for at least 2 weeks before AEP testing began.

2.2. Threshold testing

The animals were awake during testing and restrained in a yoke-like apparatus to maintain the animal's head in a constant position within the calibrated sound field (Blakeslee et al., 1978). AEPs were collected to 20 ms pure-tone bursts with 5 ms rise/fall times, presented at a rate of 10/s. A general purpose computer was used to acquire the evoked potential data and control the frequency, intensity, and timing of the stimulus. The electrical signal from the implanted electrode was amplified (50,000 ×), filtered (30–3000 Hz), and sampled using an analog-to-digital converter at 20,000 samples/s (50 μs period) over 500 points to obtain a 25 ms sampling window. Each digitized waveform was analyzed for large amplitude artifacts and, if present, the sample was rejected from the average and another sample taken. Averaged AEPs were obtained from 250 presentations of the 20 ms signal. Each waveform was stored to be used in threshold determination following the completion of the test stimulus intensity series.

Thresholds were estimated from each tone-burst intensity series using 5 dB steps at octave intervals from 0.5 to 16 kHz and at 11.2 kHz. Threshold was determined to be one half step size (2.5 dB) below the lowest intensity that showed a 'response' consistent with the responses seen at higher intensities. The average of at least three separate threshold determinations at each frequency obtained on different days was used to define the pre-exposure audiogram. Thirty days following drug injections, thresholds were tested again using the pre-treatment protocol and the average of three threshold measurements at each frequency was used to construct the post-drug-injection audiogram. Permanent threshold shift (PTS) was defined as the difference between the post- and pre-drug-injection thresholds at each test frequency.

For those animals that received the drug and noise, the noise exposure (see below) began immediately following the 30-day post-drug-injection test protocol. During the 5-day noise exposures, one complete AEP audiogram was obtained daily on each animal to establish the level of asymptotic threshold shift (ATS), that is, the difference between the threshold during noise exposure and the pre-

injection threshold. The mean ATS was computed as the difference between the thresholds during exposure (averaged over 5 days) and the pre-injection threshold at each test frequency. At least 30 days after the noise exposure, final AEP audiograms were constructed using the average of three separate threshold determinations at each of the seven pre-exposure frequencies using the pre-exposure AEP protocol. PTS was defined as the difference between the post-exposure and pre-injection thresholds at each individual test frequency.

2.3. Cubic distortion product otoacoustic emissions (3DPE)

Otoacoustic emissions were measured in the ear canal of the awake but restrained (Hargett et al., 1986) animal with the Etymotic ER-10C instrument using CUB^{e}DIS v2.40 software. The $2f_1-f_2$ distortion product was measured at 32 points per octave. The set of 81 distortion product emissions collected at 81 different frequencies for a fixed set of primary levels is referred to as the DPEgram. The following parameters were used in collecting the 3DPEs: 1.0 kHz $\leq f_2 \leq$ 10 kHz, where f_2 is the higher frequency primary tone; $f_2/f_1 = 1.22$; the levels L_1 and L_2 of f_1 and f_2, respectively, were equal and the averaging time was constant at 2 s. The primary levels were varied between 20 and 70 dB in 10 dB steps to produce six DPEgrams. All 3DPE data were plotted as a function of the geometric mean frequency of the primaries. The same number of DPEgrams was collected and at the same times during the experimental sequence as the AEP audiograms. The average of the three pre- and three post-treatment 3DPE measurements was used to establish permanent treatment effects. During the ATS producing noise exposure a single set of DPEgrams (i.e., one DPEgram for each primary level) was acquired daily and the average DPEgram at each primary level over the 5 days of exposure was used to establish the effect of the ATS state of the animal on the emissions.

2.4. Noise exposure

The noise impacts were synthesized using a Macintosh 840AV computer system and the LabVIEW™ graphical programming language. A digital pulse was synthesized and passed through a 400-Hz-wide digital Butterworth filter centered at 1.0 kHz. The impulse was played at one impulse per second through an AB International amplifier and an Altec/Lansing Model 299-8A high-frequency driver with Model 84666 transition piece and MR 94B horn. The pressure-time waveform and spectrum of the 121 dB peak SPL impact are shown in Fig. 1.

2.5. Histology

All animals were killed under sodium pentobarbital anesthesia 32 days after either carboplatin injection or noise exposure. Cochleas were immediately removed and perfused with 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide, both with veronal acetate buffer at 7.3 pH. Following complete removal of the basilar membrane with the attached sensory epithelium, the tissue was mounted in glycerin on glass slides. The sensory cell population was assessed using differential interference contrast microscopy. Cochleograms were constructed by computing the percent IHC and OHC loss across adjacent 0.24 mm segments of the sensory epithelium over the entire length of the organ of Corti. Cell loss as a function of basilar membrane position was plotted as a function of frequency using the chinchilla frequency-place map constructed by Eldredge et al. (1981).

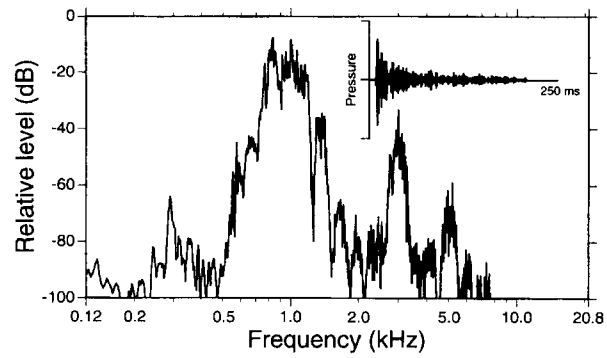


Fig. 1. The pressure-time history and the spectrum of the 121 dB impact noise used to produce an asymptotic threshold shift.

2.6. Animal care

The care and use of the animals reported on in this study were approved by the SUNY-Plattsburgh Institutional Animal Care and Use Committee. In conducting the research described in this report, the investigators adhered to the *Guide for Care and Use of Laboratory Animals*, as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources Commission on Life Sciences, National Academy of Sciences-National Research Council, revised 1985.

3. Results

3.1. Effects of carboplatin on AEP thresholds and sensory cell populations

A total of 10 animals received a carboplatin injection and were killed 32 days after the injection. Our intent was not to study the dose-response relation but rather to investigate the IC evoked potential threshold and the otoacoustic emission changes produced by the resulting pathological cochlea. Thus, the results from the animals that received only the drug will be described as accurately as possible in general terms, as well as with examples of data

Table 1
Percent IHC and OHC losses averaged over lengths of the organ of Corti corresponding to the indicated octave band frequencies and permanent threshold shifts (PTS; dB) at the indicated frequency in individual animals treated with the indicated doses of Paraplatin™. "Total" refers to the average percent loss in the entire cochlea

	50 mg/kg i.p.			50 mg/kg i.v.			75 mg/kg i.p.		
Octave band(kHz)	IHC	OHC	PTS	IHC	OHC	PTS	IHC	OHC	PTS
	Chinchilla 2039			Chinchilla 2082			Chinchilla 2056		
0.125	6.6	1.5		22.4	4.5		25.5	2.3	
0.25	10.6	1.6		27.2	1.8		32.3	1.7	
0.5	12.2	1.2	0.0	31.7	0.6	3.3	37.5	1.0	−1.7
1	10.1	0.7	−1.7	25.7	0.6	−10.0	34.5	0.8	1.7
2	22.4	0.4	1.7	43.7	0.4	−10.0	31.9	1.4	1.7
4	18.5	0.9	−1.7	60.6	0.6	− 5.0	52.0	0.3	1.7
8	4.7	0.2	−6.7	51.4	0.0	1.7	26.6	0.9	−3.3
16	0.0	0.0	−1.7	21.8	0.2	− 1.7	6.0	0.0	6.7
Total	11.5	0.8		39.7	1.0		33.5	1.0	
	Chinchilla 2040			Chinchilla 2084			Chinchilla 2057		
0.125	6.7	15.7		6.8	35.4		31.8	2.5	
0.25	9.4	2.4		11.2	7.0		41.0	1.5	
0.5	11.9	2.1	6.7	17.1	2.4	0.0	35.8	1.6	3.3
1	12.9	1.9	5.0	10.0	2.4	− 8.3	35.5	1.4	8.3
2	17.8	0.4	1.7	10.0	1.3	− 3.3	30.6	0.4	1.7
4	20.1	0.8	23.3	8.9	3.8	− 5.0	48.2	0.5	−1.7
8	6.4	0.4	11.7	19.8	1.1	6.7	37.6	0.4	−3.3
16	0.4	0.0	8.3	5.0	0.4	− 1.7	6.4	0.1	0.0
Total	12.5	2.7		11.8	5.4		36.3	1.0	
	Chinchilla 2055			Chinchilla 2088			Chinchilla 2061		
0.125	4.0	1.8		3.4	14.1		20.3	6.2	
0.25	2.7	1.5		5.8	16.2		29.0	1.4	
0.5	1.1	0.5	−6.7	6.2	10.6	− 1.7	34.9	0.8	−6.7
1	1.6	0.6	−6.7	3.6	10.8	− 1.7	28.1	0.5	6.7
2	1.6	1.0	3.3	7.2	4.8	3.3	40.3	0.4	1.7
4	11.4	1.0	−3.3	22.2	3.2	− 1.7	80.3	1.0	0.0
8	4.8	0.1	−1.7	18.1	3.3	− 1.7	68.0	0.6	6.7
16	0.4	0.1	−3.3	5.0	2.7	− 3.3	13.4	0.2	−1.7
Total	3.6	0.8		9.7	8.3		39.3	1.1	

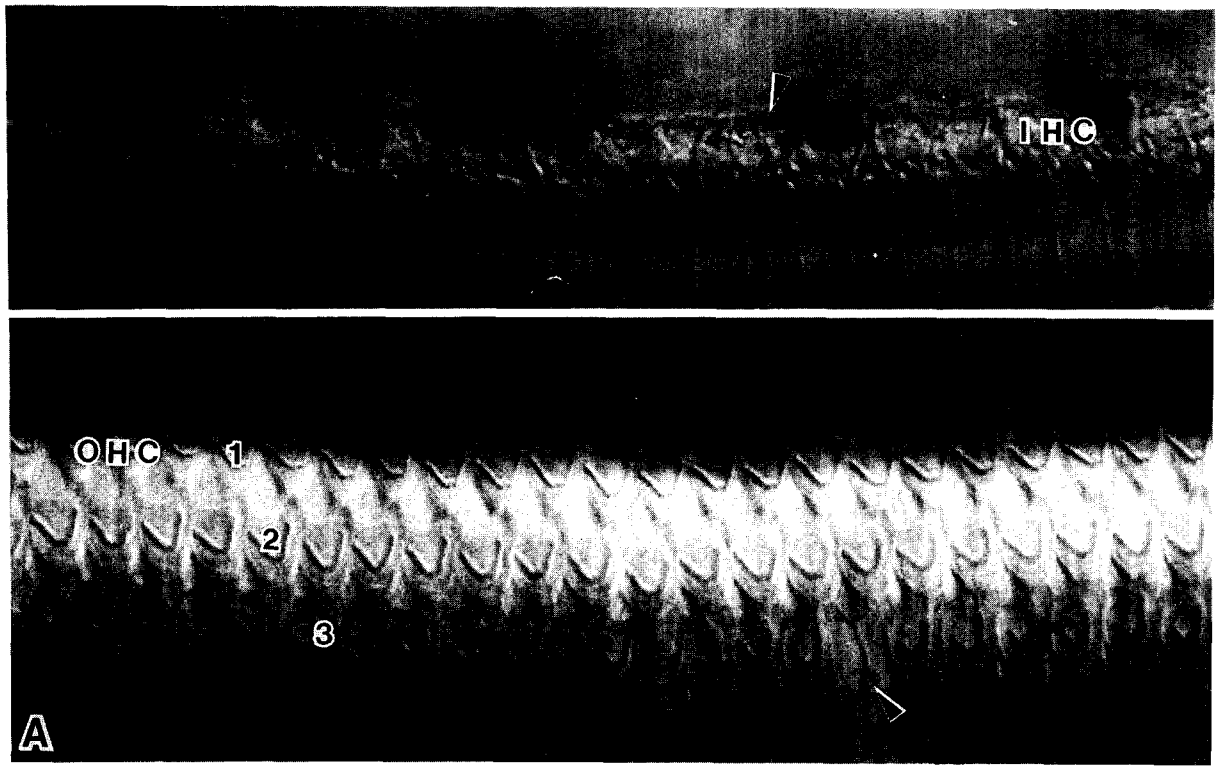
from four individual animals, each chosen to illustrate various features of the drug-treated group.

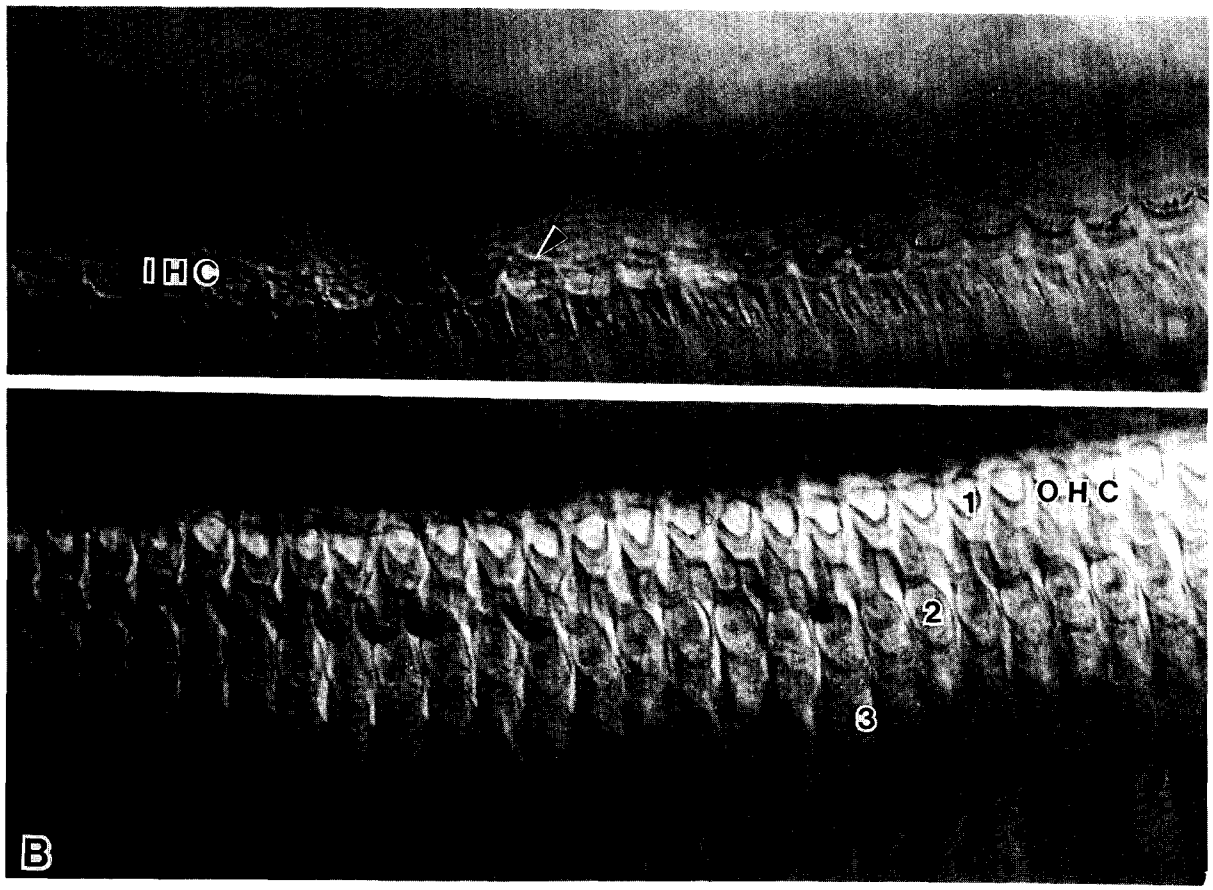
Both doses of the drug and administration routes caused substantial scattered losses of IHCs throughout most of the cochlea with usually very little OHC loss. A summary of the percent sensory cell loss within adjacent octave band lengths of the cochlea and the total sensory cell loss and PTS measured in nine of these animals is shown in Table 1. Of the nine animals presented in this table, eight showed normal AEP thresholds despite severe IHC losses. Fig. 2 illustrates the appearance of the surface of the sensory epithelium in two animals from the 75 mg/kg i.p. group. In order to get both the IHCs and the OHCs in focus two photographs were taken. However, each pair of micrographs was taken from the same location on the organ of Corti and only the focal plane was changed. The OHCs generally appear normal at this level of analysis with only an occasional missing cell while the scattered pattern of IHC loss (identified by a typical 'x' configured scar at the level of the reticular lamina) seen in these micrographs is typical of that seen throughout the cochlea. OHC cilia, when clearly visible, appeared normal. No estimate of the distribution of cilia disturbances or other more subtle pathologies which may have resulted from the drug administration (or noise exposure) was undertaken in these animals. Thus, using the surface preparation only the lower bound to the sensory cell pathology existing in these preparations can be estimated with confidence.

3.2. 3DPEs following carboplatin treatment

In a preliminary study of over 40 monaural but untreated animals from which five DPEgrams (i.e., one DPEgram for each of the five primary levels) were collected on each animal on five different days, the standard deviations of the 3DPEs, at all frequencies on every animal, for a

Fig. 2. Surface preparation micrographs taken from two different cochleas (A,B) of animals treated with 75 mg/kg i.p. of Paraplatin™, showing the scattered loss of IHC (arrow head) and the nearly normal OHC population. A single OHC (arrow head) is missing in the bottom panel of A. (Number refers to rows of OHCs.)


IHC
OHC
1
2
3
A


IHC
1
OHC
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B

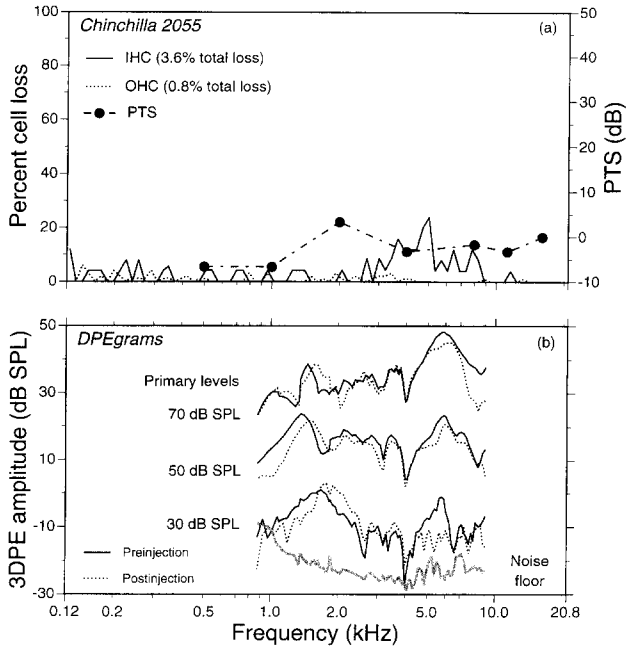

Fig. 3. A summary of data for chinchilla 2055 injected with 50 mg/kg i.p. Paraplatin™. a: 30-day post-injection cochleogram and PTS audiogram; b: pre- and post-injection DPEgrams obtained using primary levels of 30, 50, and 70 dB SPL.

given level of the primary tones, were on the order of 3–4 dB. Thus for the purpose of discussing the individual animal 3DPEs, post-treatment changes in the 3DPE at a given primary level and frequency that exceed the pre-treatment mean for that animal by more than 10 dB (better than a 95% confidence interval) were considered meaningful. Cochleograms, PTS audiograms, and DPEgrams from four individual animals given only the drug are shown in Figs. 3–6. These particular animals were chosen because they reflect four distinctively different outcomes of the drug treatment.

The animal least affected by the drug is shown in Fig. 3. Fig. 3a shows this animal's cochleogram and PTS audiogram. With virtually no OHC loss and a relatively small IHC loss in the 4 kHz region, there is no PTS in this animal. DPEgrams measured with 30, 50, and 70 dB SPL primaries shown in Fig. 3b indicate essentially no post-treatment changes at most frequencies. However, systematic decrements exceeding 10 dB of pre-treatment values are seen in the 4.5–6.5 kHz region of the DPEgram at the lower (and presumably more diagnostic) primary levels (30 dB SPL). This is also the region in the cochlea having the largest IHC loss but a near normal OHC population.

Fig. 4 shows a parallel data set for chinchilla 2061. This animal had a 39% overall loss of IHCs; particularly large IHC losses in the 3–10 kHz region of the cochlea; a normal OHC population; and an essentially normal (within 10 dB) audiogram. The 3DPE functions (Fig. 4b) show a consistent increase in output across a number of frequencies at the higher (60 dB SPL) primary levels. This is the only change observed in an animal having a severe IHC loss. At the lower primary levels (e.g., 40 dB SPL), other than a very narrow loss of 3DPEs just above 2 kHz, there were no changes in the 3DPE output.

The PTS audiogram, cochleogram and 3DPE functions for chinchilla 2040 are shown in Fig. 5. This is the only animal in the group of nine animals summarized in Table 1, receiving only the drug, that showed a substantial PTS of 23 dB at 4 kHz. For low level primaries (30–50 dB) the emissions, shown in Fig. 5b, were consistently reduced below about 3 kHz. In this region of the cochlea, AEP thresholds and the OHC population were very near normal. Although the emissions were consistently enhanced above 4 kHz at higher primary levels (50–70 dB), the enhancement was within the 95% confidence interval established for meaningful changes in 3DPE measurements.

Emissions and threshold data on the remaining animals presented in Table 1 were unremarkable, that is, there were no systematic pre- versus post-treatment changes in the emissions that exceeded the 95% confidence interval established for meaningful change. That is, in animals 2082, 2056, and 2057 showing 30–40% IHC loss with essentially normal OHC populations and normal AEP thresholds, the emissions were also normal.

Data from one animal (chinchilla 2192) that received the 75 mg/kg i.p. dose and was not included in Table 1, is shown in Fig. 6. The animal was very unusual in that it had, by far, the most severe IHC losses throughout most of the cochlea (total IHC loss = 84%) and only a scattered 5% overall loss of OHCs (Fig. 6a). AEP thresholds at and

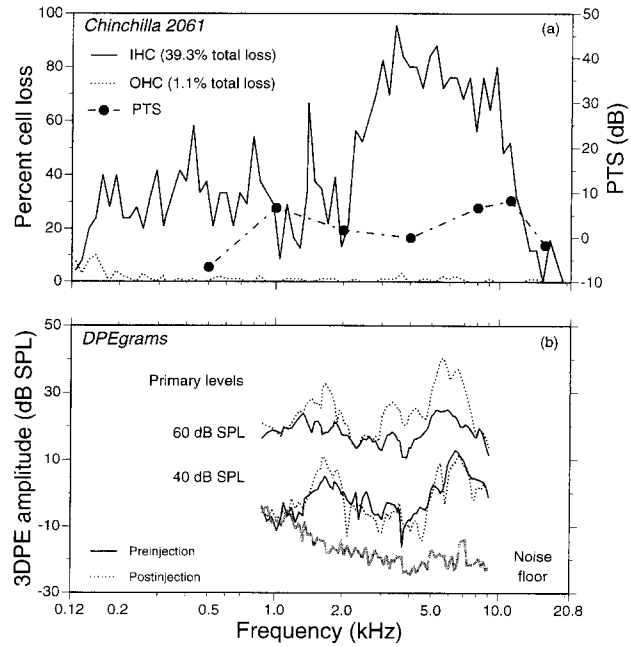

Fig. 4. Summary of data for chinchilla 2061 injected with 75 mg/kg i.p. Paraplatin™. a: 30-day post-injection cochleogram and PTS audiogram; b: pre- and post-injection DPEgrams obtained using primary levels of 40 and 60 dB SPL.

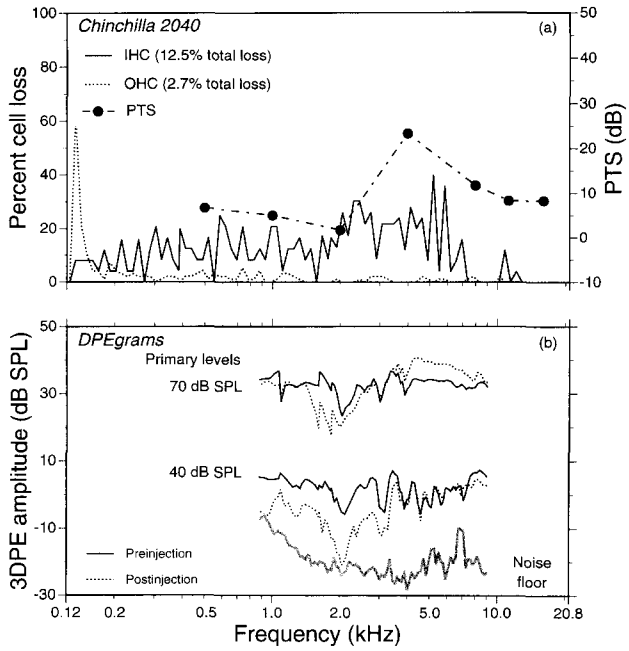

Fig. 5. A summary of data for chinchilla 2040 injected with 50 mg/kg i.p. Paraplatin™. a: 30-day post-injection cochleogram and PTS audiogram; b: pre- and post-injection DPEgrams obtained using primary levels of 40 and 70 dB SPL.

below 2 kHz and at 16 kHz were near normal, while they were elevated about 40 dB at 4 and 8 kHz. When the AEP waveforms for this animal were reviewed the AEP was robust and did not appear any different from what is

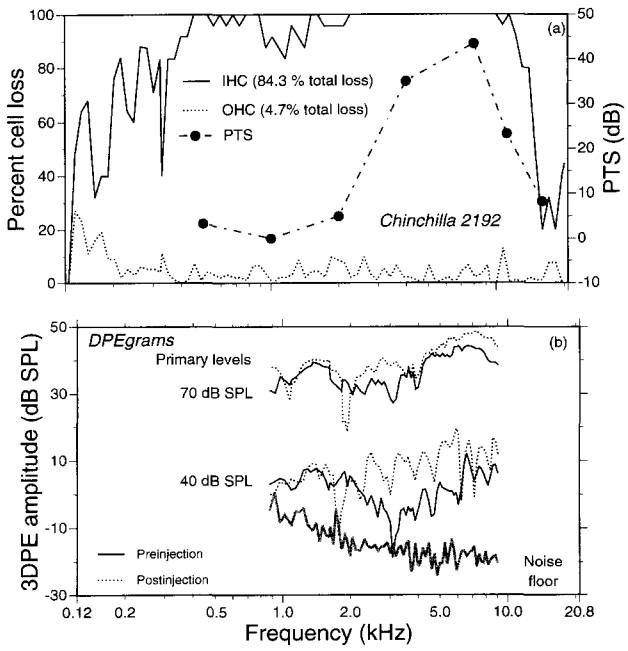

Fig. 6. A summary of data for chinchilla 2192 injected with 75 mg/kg i.p. Paraplatin™. a: 30-day post-injection cochleogram and PTS audiogram; b: pre- and post-injection DPEgrams obtained using primary levels of 40 and 70 dB SPL.

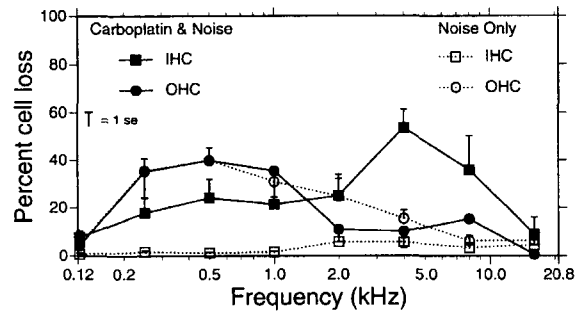

Fig. 7. The group mean cochleograms, showing the mean percent sensory cell loss within adjacent octave band lengths of the cochlea, for the noise-alone ($n = 9$) and noise-plus-drug ($n = 3$) groups (SE = standard error of the mean.)

typically seen following a noise exposure that produces a similar PTS but not the IHC loss. In this subject (2192), the 3DPE output in the 2–6 kHz region shown in Fig. 6b was enhanced especially at the lower primary levels. Except for the narrow band around the 2 kHz region, there were no systematic decrements in the 3DPE functions despite the large PTS.

3.3. Effects of noise trauma in subjects pretreated with carboplatin

Fig. 7 shows the 30-day post-exposure group mean ($n = 9$) percent OHC and IHC losses over octave band lengths of the cochlea centered at the indicated frequencies for animals that received only the noise compared with those ($n = 3$) that received the drug (75 mg/kg i.v. dose of Paraplatin™) and after 30 days, the same ATS-producing noise. It is clear from this figure that the noise alone produces a primarily low frequency (< 2 kHz) based OHC loss and very little IHC loss, while the drug/noise group on average differs only in the amount of IHC loss that is scattered through the extent of the cochlea with the largest losses focused in the 4 kHz region. The cell loss produced by the drug does not appear to influence the cell loss resulting from the noise exposure (i.e., the cell loss is additive). Fig. 8 shows the mean pre- and 30-day post-drug-injection AEP thresholds for the three animals that were given a 75 mg/kg i.v. dose of Paraplatin™ and, following the 30-day post-injection testing protocol, were exposed to the ATS-producing noise. There was no statistically significant difference in the pre- and 30-day post-injection thresholds despite the presumed severe losses of IHCs. Thus, the three animals in this group had normal AEP thresholds but presumably a severely compromised IHC population when exposed to the noise.

The IHC loss in these animals as a result of the drug treatment alone can only be estimated by comparing the mean octave band sensory cell loss between the two sets of data shown in Fig. 7. Since the noise exposure alone did not produce any substantial IHC loss, the IHC loss seen in the drug/noise group should, on the basis of the results

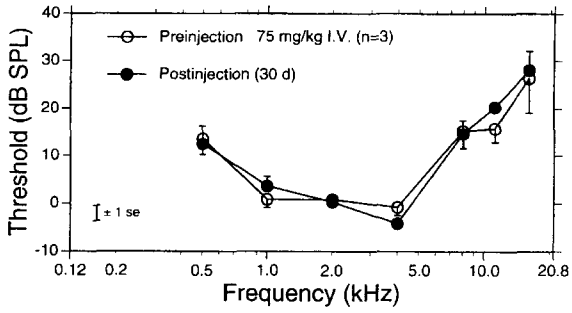

Fig. 8. The group mean pre- and 30-day, post-drug-injection audiograms for the three animals that received the 75 mg/kg i.v. injection of Paraplatin™ prior to exposure to noise (SE = standard error of the mean).

from the drug-only subjects, be attributable to the drug. One should similarly be able to estimate the effect of the drug on sensory cell populations in each of the three individual animals from the individual cochleograms shown in Fig. 9a–11a by assuming that the IHC loss is primarily due to the drug while the OHC loss is primarily the result of the noise.

The data shown in Figs. 9–11 from each of the three animals that comprised the drug/noise group had features in common as a group as well as some unique features. Panel (a) of these figures displays the individual cochleogram, 30-day post-drug-injection PTS, and 30-day post-noise-exposure PTS for each of the three animals. The mean ATS and PTS from the group of nine animals exposed to the noise but not having any drug treatment is compared with the PTS and ATS measured in each individual animal that received the drug and then the noise in Fig. 9b, 10b and 11b. Panel (c) in each of these figures shows the DPEgrams obtained using 50 dB primaries: prior to drug administration; 30 days post-injection; 30

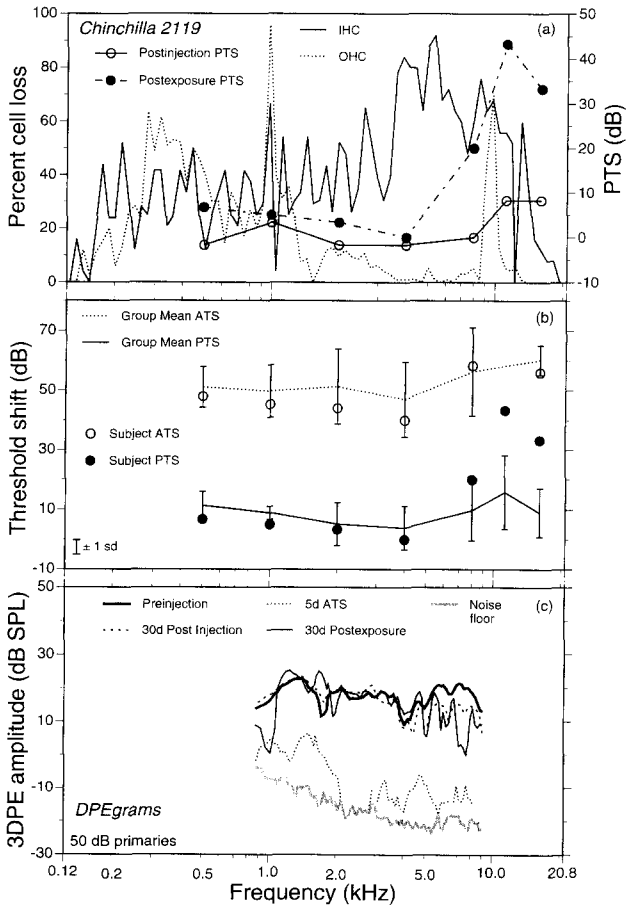

Fig. 9. A summary of data for chinchilla 2119. The animal was given a 75 mg/kg i.v. dose of Paraplatin™ and, after 30 days, exposed to the asymptotic threshold shift (ATS)-producing noise. a: Cochleogram; the 30-day post-injection, and 30-day post-exposure PTS audiograms. b: Mean ATS, and the mean PTS from the nine animals that were exposed to the 121 dB peak SPL impact noise for 5 days (solid and dotted lines, SD = group standard deviation), along with the comparable data (symbols) from chinchilla 2119. c: DPEgram obtained using 50 dB SPL primaries measured prior to drug injection, 30 days post-injection, during the ATS condition, and 30 days post-exposure.

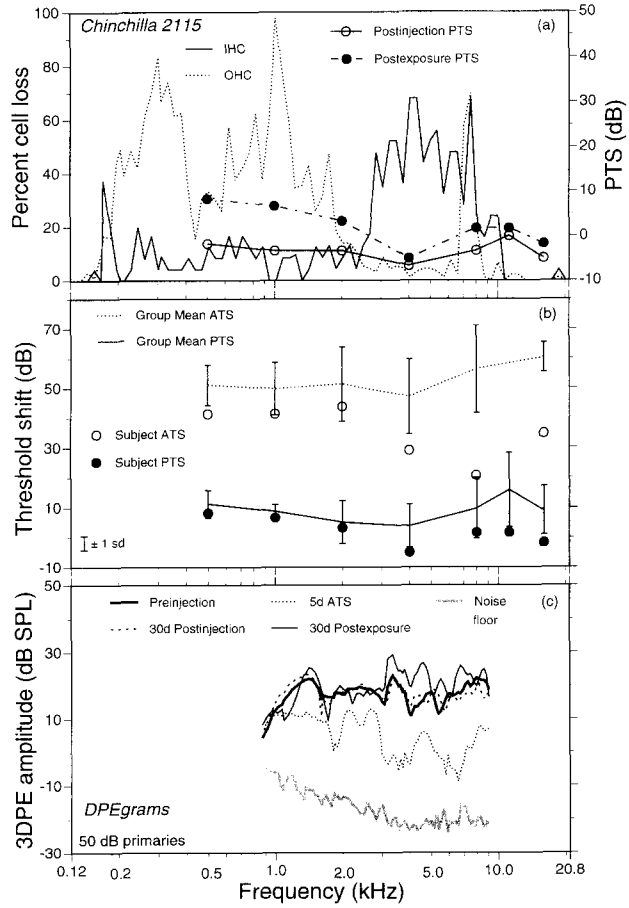

Fig. 10. A summary of data for chinchilla 2115. The animal was given a 75 mg/kg i.v. dose of Paraplatin™ and, after 30 days, exposed to the asymptotic threshold shift (ATS)-producing noise. a: Cochleogram; the 30-day post-injection, and 30-day post-exposure PTS audiograms. b: Mean ATS, and the mean PTS from the nine animals that were exposed to the 121 dB peak SPL impact noise for 5 days (solid and dotted lines, SD = group standard deviation), along with the comparable data (symbols) from chinchilla 2115. c: DPEgram obtained using 50 dB SPL primaries measured prior to drug injection, 30 days post-injection, during the ATS condition, and 30 days post-exposure.

days post-exposure; and the DPEgram averaged over the 5 days of noise exposure (i.e., the mean DPEgram during the ATS condition).

As a group, all animals showed relatively large, presumably noise-induced OHC losses apicalward of the 2 kHz region of the cochlea and large scattered IHC losses (presumably drug-induced) throughout the cochlea with largest IHC losses focused in the 2–8 kHz region. Each animal had normal AEP thresholds and DPEgrams prior to noise exposure. Each of the three animals showed an ATS level at most frequencies that was similar to that produced in the noise-alone group. Thirty days following the noise exposure, PTS levels were similar to those measured in the group exposed to only the noise. Thus, pre-treatment with the drug with the attendant loss of IHCs did not seem to affect the levels of ATS or PTS. The 3DPEs in each animal were generally depressed during the ATS state and showed some depression 30 days after the noise exposure in some animals at some frequencies. The frequency correspondence of these changes in the emissions, however, did not always correspond with the frequency of PTS or location of OHC lesions.

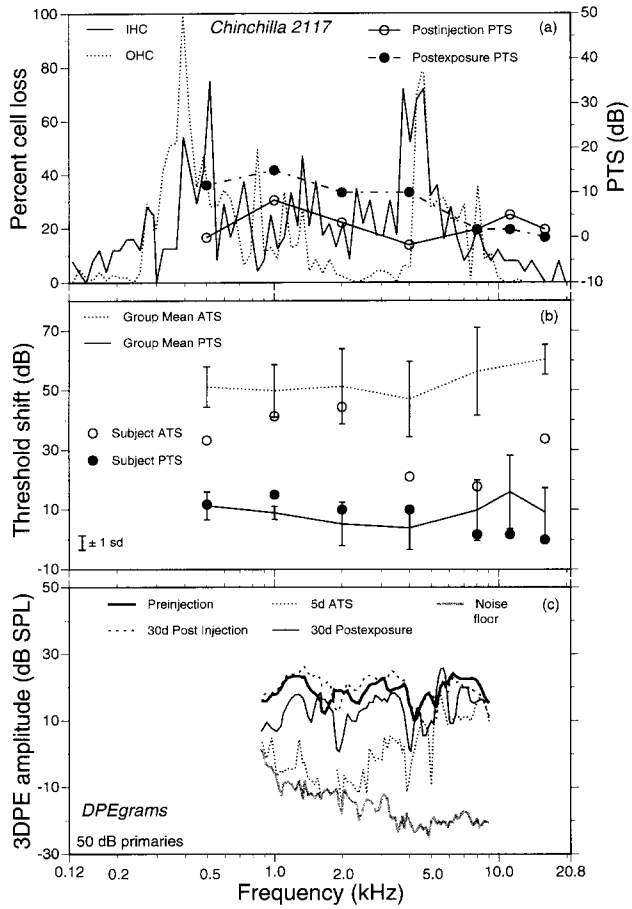

Fig. 11. A summary of data for chinchilla 2117. The animal was given a 75 mg/kg i.v. dose of Paraplatin™ and, after 30 days, exposed to the asymptotic threshold shift (ATS)-producing noise. a: Cochleogram; the 30-day post-injection, and 30-day post-exposure PTS audiograms. b: Mean ATS, and the mean PTS from the nine animals that were exposed to the 121 dB peak SPL impact noise for 5 days (solid and dotted lines, SD = group standard deviation), along with the comparable data (symbols) from chinchilla 2117. c: DPEgram obtained using 50 dB SPL primaries measured prior to drug injection, 30 days post-injection, during the ATS condition, and 30 days post-exposure.

3.4. Individual subject presentations

Chinchilla 2119 (Fig. 9) showed the most severe loss of both OHCs and IHCs of the three animals as well as the largest PTS which exceeded 40 dB at the 11.2 kHz test frequency. Despite large IHC and OHC losses in the 0.5–4 kHz region, AEP thresholds were either normal or elevated less than 10 dB. The ATS measured in this subject fell very close to the mean of the noise-only group as seen in Fig. 9b. DPEgrams before and after drug treatment exhibited no differences; at ATS, however, the emissions were severely depressed or eliminated (below the noise floor) across the entire frequency range in agreement with the ATS frequency profile. Thirty days after the noise exposure, emissions were reduced at the high frequencies where a large PTS also was measured and where there were large IHC and some OHC losses. In the vicinity of 1 kHz where thresholds were normal but there was a large loss of both IHCs and OHCs the 3DPEs also were reduced.

Chinchilla 2115 (Fig. 10) showed a bimodal sensory cell loss; below about 2 kHz primarily OHCs were missing, while above 2 kHz, the loss consisted primarily of missing IHCs. This subject showed less than a 10 dB loss of threshold at 0.5, 1, and 2 kHz compared to the post-drug-treatment thresholds which were normal, while thresholds were normal at and above 4 kHz. ATS was similar to the noise-only group at and below 2 kHz but more than a standard deviation below the noise-only group above 2 kHz. During the ATS state, the emissions (Fig. 10c) were depressed across most frequencies, but the largest depression was measured above 3 kHz, a region with relatively little OHC loss and less ATS than at the lower frequencies. Emissions pre- and post-drug administration were essentially the same while at 30-day post-noise exposure there is some evidence of an enhanced 3DPE output in the 4 kHz region. Thus, in this animal, the emissions responded to the ATS state, but not in as frequency-specific a fashion as might have been expected. Despite a low-frequency OHC lesion penetrating well into the 2 kHz region of the cochlea, the emissions were normal in this region 30-days post-exposure.

Chinchilla 2117 (Fig. 11) showed mixed OHC and IHC losses throughout the entire cochlea; less than a 10 dB increase in thresholds at and below 4 kHz relative to the thresholds recorded 30 days after drug administration. ATS levels at and below 2 kHz were similar to the noise-alone group mean while above 2 kHz the ATS was substantially less. ATS and PTS in this animal were very similar to Chinchilla 2115 (Fig. 10). The emissions shown in Fig. 11c were essentially the same before and 30 days after

drug administration. At ATS the emissions were depressed from the lowest frequency through about the 5 kHz region where there was a second sizable OHC lesion. Above 5 kHz, the emissions showed much less depression. The frequency profile of the 3DPE at ATS generally reflected the ATS profile. Thirty-day post-exposure emissions showed significant depression in spectrally narrow regions of the DPEgram that tended to reflect the pattern of OHC loss.

4. Discussion

While there is a consensus that otoacoustic emissions are primarily the result of the bi-directional transduction properties of the OHC (Brownell, 1990), a role for the IHCs in the generation or maintenance of emissions cannot be entirely ruled out. Since it is the OHCs that are first damaged by excessive noise and many drugs, emissions would seem to be ideal for the early detection of noise or drug-induced damage to the auditory system. The effects of noise combined with the ability of carboplatin to eliminate large numbers of IHCs in the chinchilla while leaving the OHCs intact allows for the creation of some very unusual patterns of sensory cell loss. These unusual lesions may be of use in better understanding the role of the two populations of sensory cells in hearing and in the development of more reliable diagnostic procedures.

The summary data shown in Table 1 are in essential agreement with the data of Takeno et al. (1994b), which showed that carboplatin has, in terms of sensory cell loss, its greatest effect on the IHC system of the chinchilla and that the group average IHC loss is increased with an increased drug dose and with an i.v. route of drug delivery. As shown in this table, there were very few OHCs lost as a result of the drug. One of the interesting features, however, of the summary data shown in Table 1 is that even for relatively large losses (30–40%) of IHCs (e.g., the 75 mg/kg i.p. group) AEP pure-tone thresholds recorded from the IC were not, in general, elevated. These IC AEP thresholds are not too different from those of the Group B ($n = 10$) animals in the Takeno et al. (1994b) study. In general, in our material, when the drug-treated animals showed normal IC AEP thresholds despite the large losses of IHCs their AEP waveform morphology was normal. A detailed comparison of the growth of the AEP waveform amplitudes with stimulus intensity or change in the latencies of the response in each of these animals was not undertaken. Qiu et al. (1996) recently presented data from carboplatin-treated chinchillas that also showed normal IC AEP thresholds in animals with a severely compromised IHC population but the AEP amplitudes were reduced at suprathreshold levels. They also showed normal thresholds and normal I/O functions in the auditory cortex of these animals. While all of these results are a little surprising considering the reduced afferent flow to the central nervous system, they do support the results of Schuknecht and Wollner (1953) who found normal behavioral thresholds following more than a 50% sectioning of the VIII nerve. Normal thresholds in certain strains of mice with intact OHCs but losses of IHCs have also been reported by Schrott et al. (1989).

In general, 30 days after injection with carboplatin, in regions of the cochlea where a large, nearly total IHC lesion exists; where there is a near normal OHC population, and where there is a severe PTS; the emissions were either normal or there was an increase in the 3DPE output at some primary levels (e.g., Figs. 4 and 6). As with the mouse data of Schrott et al. (1991), and the chinchilla data of Trautwein et al. (1996) the emissions are robust in cochleas having a normal OHC population but severe losses of IHCs. The only consistent change in the 3DPEs observed in our experimental population seems to be the increase in the output levels of the 3DPEs at some frequencies in some animals. An enhancement of 3DPEs has also been reported by Franklin et al. (1991) in some rabbits following a noise exposure. The possible mechanisms for such an enhancement effect are not obvious, but considering the severely reduced afferent flow to the central nervous system in some of these preparations, an efferent mechanism might be hypothesized. Unfortunately, the role of the efferent system in the modulation of the emissions is not clear and conflicting results can be found in the literature (e.g., Siegel and Kim, 1982; Littman et al., 1992; Lowe and Robertson, 1995; Liberman et al., 1996). In animals toughened by intermittent noise exposures Kujawa and Liberman (1996) also showed consistent increases in 3DPEs. Clearly there are situations in which enhanced emissions can be measured, but what seems to be more unusual as well as difficult to explain is the appearance of enhanced emissions over a broad range of frequencies that show a 30–40 dB PTS as measured with the IC AEP (Fig. 6).

Chinchilla 2040 shown in Fig. 5 was the only animal that consistently showed a reduction of 3DPEs despite a relatively low-level sensory cell loss and little PTS. In this animal at 4 kHz a 25 dB PTS did not elicit any 3DPE change. Based upon the data of the other eight animals summarized in Table 1, a number of which showed much larger IHC losses, the reason for the PTS and 3DPE changes in animal 2040 is not obvious and recourse to a damaged-cilia argument or other more subtle changes being responsible for the 3DPE reduction (Brown et al., 1989) is not satisfactory. Other animals (e.g., Fig. 4) with more substantial lesions and presumably more substantial subtle morphological changes in the OHCs did not show such 3DPE depression or PTS.

Another effect which can be seen in this and in other 30-day post-treatment animals is that the 3DPE output often displayed large excursions at relatively close frequencies. When present, these large peaks and valleys in the DPEgrams were consistent in magnitude and frequency

on the three, 30-day post-treatment test sessions which took place on three different days. While the fine structure of the DPEgram is different for each animal it is quite consistent within each animal over many days of testing. The extent to which some of the fine structure of the DPEgram might relate to sensory cell morphology must await more detailed anatomical analysis.

When animals, whose IHC population has been severely compromised by carboplatin, are exposed to an ATS-producing noise that results in some sensory cell loss and PTS, the animals respond as if they were completely normal prior to the exposure. On the basis of the DPEgrams and audiograms the animals were normal despite having 20–60% inferred IHC losses scattered throughout the cochlea. In some cochleas in which there is also substantial OHC loss as a result of a subsequent noise exposure, the changes (or lack of them) in the 3DPE output are not always clearly related either to the PTS or OHC loss. A lack of correspondence between cell loss, cilia damage and 3DPE changes was also reported by Subramaniam et al. (1994). Despite this lack of correspondence, the 3DPEs did respond to the transient changes induced by the ATS-producing noise although there was not always a congruence in the frequency dimension between the ATS and the emission changes. Franklin et al. (1991), however, reported close agreement in the frequency pattern of behavioral threshold shifts and emission changes, while Hamernik et al. (1996) showed that in the chinchilla exposed to high-level impulses, the 3DPE was a more sensitive, frequency-specific indicator of noise-induced cochlear effects than pure-tone thresholds.

5. Conclusions

Severe losses of IHCs accompanied by a normal complement of OHCs does not result in a parallel reduction of AEP thresholds measured at the level of the inferior colliculus. Near-normal thresholds can be consistently measured with as much as a 40% overall loss of IHCs and in the apical half of the cochlea, with nearly total IHC loss (Fig. 6). On the basis of the animals that received the drug/noise treatment, threshold shifts are clearly determined more by damage to the OHC system than by IHC loss.

The 3DPEs measured from cochleas with only IHC losses and little or no OHC loss or PTS are either normal or show an increased output.

The presence of large pre-noise-exposure IHC losses does not alter the ATS, PTS, or OHC loss that would be anticipated from the subsequent noise exposure.

The 3DPEs measured during ATS are shifted but not necessarily at the same frequencies that ATS occurs.

There is not always a clear relation in the frequency dimension between the changes in the 3DPEs and the OHC loss.

The peaks and valleys in the 3DPE functions (i.e., the DPEgram fine structure), that are consistent within an animal but differ across animals, increase in number and become exaggerated in many of the post-treatment animals.

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