Cisplatin-Induced Hyperactivity in the Dorsal Cochlear Nucleus and Its Relation to Outer Hair Cell Loss: Relevance to Tinnitus

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Cisplatin-induced hyperactivity in the dorsal cochlear nucleus and its relation to outer hair cell loss: relevance to tinnitus. J Neurophysiol 88: 699–714, 2002; 10.1152/jn00893.2001. Cisplatin causes both acute and chronic forms of tinnitus as well as increases in spontaneous neural activity (hyperactivity) in the dorsal cochlear nucleus (DCN) of hamsters. It has been hypothesized that the induction of hyperactivity in the DCN may be a consequence of cisplatin’s effects on cochlear outer hair cells (OHCs); however, systematic studies testing this hypothesis have yet to appear in the literature. In the present investigation, the relationship between hyperactivity and OHC loss, induced by cisplatin, was examined in detail. Hamsters received five treatments of cisplatin at doses ranging from 1.5 to 3 mg · kg⁻¹ · day⁻¹, every other day. Beginning 1 mo after initiation of treatment, electrophysiological recordings were carried out on the surface of the DCN to measure spontaneous multiunit activity along a set of coordinates spanning the medial-lateral (tonotopic) axis of the DCN. After recordings, cochleas were removed and studied histologically using a scanning electron microscope. The results revealed that cisplatin-treated animals with little or no loss of OHCs displayed levels of activity similar to those seen in saline-treated controls. In contrast, the majority (75%) of cisplatin-treated animals with severe OHC loss displayed well-developed hyperactivity in the DCN. The induced hyperactivity was seen mainly in the medial (high-frequency) half of the DCN of treated animals. This pattern was consistent with the observation that OHC loss was distributed mainly in the basal half of the cochlea. In several of the animals with severe OHC loss and hyperactivity, there was no significant damage to IHC stereocilia nor any observable irregularities of the reticular lamina that might have interfered with normal IHC function. Hyperactivity was also observed in the DCN of animals showing severe losses of OHCs accompanied by damage to IHCs, although the degree of hyperactivity in these animals was less than in animals with severe OHC loss but intact IHCs. These results support the view that the loss of OHC function may be a trigger of tinnitus-related hyperactivity in the DCN and suggest that this hyperactivity may be somewhat offset by damage to IHCs.

INTRODUCTION

There is growing evidence that changes in spontaneous activity are an important neural correlate of tinnitus, an auditory disorder characterized by the perception of sound in the absence of a corresponding acoustic stimulus. Increases in spontaneous activity (hyperactivity) occur at various levels of the auditory system following treatment with salicylate (Chen and Jastreboff 1995; Eggermont and Kenmochi 1998; Evans and Borerwe 1982; Manabe et al. 1997; Wallhauser-Franke 1997), an agent that causes tinnitus both in humans (Day et al. 1989; Karlsson and Flock 1990; McCabe and Dey 1965; McFadden et al. 1984; Mongan et al. 1973) and animals (Bauer et al. 1999; Jastreboff et al. 1988a,b, 1991). Hyperactivity induced in the inferior colliculus following salicylate treatment displays a similar time course and pitch as the tinnitus that is induced in humans using the same agent (Jastreboff et al. 1988a,b; Manabe et al. 1997). Hyperactivity can also be induced in the dorsal cochlear nucleus (DCN) following intense sound exposure (Kaltenbach and McCaslin 1996; Kaltenbach et al. 1998–2000; Zhang and Kaltenbach 1998). Intense sound exposure is one of the most common causes of tinnitus in humans (Axelsson and Barrenas 1992; Coles 1995; Meikle and Taylor-Walsh 1984; Penner and Bilger 1995), and behavioral evidence indicates that animals test positive for tinnitus after being exposed to the same sound conditions that cause hyperactivity in the DCN (Heffner and Harrington 2002; Kaltenbach et al. 1999).

It has been hypothesized that the abnormal spontaneous activity that underlies some forms of tinnitus results from shifts in the balance of inputs from the two cochlear hair cell systems (Jastreboff 1990, 1995; Tonndorf 1987). These include the inner hair cells (IHCs) with their associated type I primary afferents and outer hair cells (OHCs) with their associated type II afferents (Kiang et al. 1982; Spoendlin 1973, 1981). Imbalances between these two systems would seem most likely to occur when there is greater loss of OHC function than of IHC function. Jastreboff (1995) hypothesized that tinnitus may be triggered by this type of imbalance, citing several observations in the literature. He noted that intense sound exposure, which can cause chronic tinnitus, tends to cause greater damage to OHCs than IHCs (Saunders et al. 1985, 1991). He also noted that salicylates, which are well known inducers of transient or acute tinnitus, reversibly block the function of OHCs, altering their membrane properties and causing reversible reductions of spontaneous otoacoustic emissions and attenuation of active processes in the cochlea (Ashmore 1989; Brownell et al. 1990; Puel et al. 1988; Shehata et al. 1991; Styulpkowski 1990). Despite these suggestions, direct evidence linking hyperactiv-
ity to an imbalance of inputs from the two hair cell systems and, specifically, to the loss of OHC function, has yet to appear in the literature. Indeed, it remains unclear whether tinnitus or its underlying hyperactivity is caused exclusively by the loss of OHC function or might be more related to some subtle effects on IHCs.

Attempts to address these possibilities have thus far been inconclusive. A recent study from our laboratory (Melamed et al. 2000) showed that hyperactivity in the DCN as well as OHC loss could be induced by treating animals with cisplatin, an anti-tumor agent that is known to cause both hearing loss and tinnitus (Bokemeyer et al. 1998; Kawakita et al. 1999; Lerner et al. 1995; Nakai et al. 1982; Reddel et al. 1982). However, close inspection of the cochlear receptor epithelium after cisplatin treatment revealed that the induced hyperactivity in the DCN was usually associated with loss of OHCs as well as significant damage to IHCs. The possibility that hyperactivity might have been triggered by the IHC damage rather than OHC loss could therefore not be ruled out.

The present study was undertaken to re-examine the relationship between hyperactivity and the type of hair cell loss. Unlike previous studies, we studied animals in three different cisplatin dose groups with the aim of producing animals in which the lesions spanned a wide range of hair cell lesion severities and patterns. Indeed, many of the animals we treated displayed lesions that were restricted to OHCs, whereas others involved significant damage to both IHCs and OHCs. This range of lesion severities enabled us to test for a correlation between the level of hyperactivity induced in the DCN and the degree of OHC loss and to determine how the level of hyperactivity was affected when the lesions became more severe and involved injury to the IHCs.

METHODS

Animal subjects and treatment groups

Adult Syrian golden hamsters were obtained from Charles River in the age range of 45–55 days. The animals were maintained at 21–22°C. Food and water were available ad libitum. All procedures were approved by the Animal Investigation Committee (AIC) at Wayne State University, and all subjects were housed by AIC-approved facilities.

The hamsters were divided into three groups, each receiving five intraperitoneal injections (1 injection/day, every other day) of cisplatin at one of the following doses: 1.5, 2.25, or 3 mg/kg. Each group had a corresponding subgroup of control animals that received injections of isotonic (0.9%) saline. During and after the injection period, the animals were maintained in the animal care facilities for a recovery period. The vast majority of animals were studied after recovery periods of 1–2 mo. Four additional cisplatin-treated animals and two controls were allowed recovery periods of 5–6 mo.

Recordings of spontaneous multiunit activity

Following the postinjection recovery period, animals were prepared surgically to study the effects of cisplatin electrophysiologically. The animals were anesthetized with intramuscular injections of ketamine/xylazine (113/17 mg/kg). Each animal was placed in a sound attenuation booth, a parieto-occipital craniotomy was performed, and the left DCN was exposed by partial aspiration of the cerebellum. Recording electrodes were micropipettes filled with 0.3 M NaCl. The tip impedance of each electrode was 0.4 MΩ. Electrode position was controlled remotely using a Narashige XYZ micromanipulator. A camera mounted onto a microscope inside the booth, together with a video monitor outside the booth, allowed remote visualization of the DCN surface and placement of the electrode. The electrode was lowered until contact was established between the electrode tip and the DCN surface as signaled by a sudden appearance of neurogenic activity. Neural signals were filtered (300–10,000 Hz), amplified 1,000 times, and conveyed to an oscilloscope and level discriminator. The signals consisted of compound waveforms that resulted from temporal overlap of multiple single unit action potentials (i.e., multiunit activity, MUA). The level discriminator was adjusted to trigger on neural voltages more negative than −100 mV (after amplification). The output of the discriminator was fed to a universal counter that was used to count spontaneous voltage events. Neither the level discriminator nor the counter displayed any measurable dead time that affected potential fluctuations below 500 Hz (Kaltenbach and Afman 2000; Kaltenbach and McCaslin 1996; unpublished observations). Recordings were carried out in two to three parallel rows, each row consisting of 10–15 recording sites spaced about 100 μm apart and spanning the medial-lateral axis of the DCN. Previously, we have shown that this axis corresponds to the tonotopic axis of the DCN (Kaltenbach and Afman 2000; Kaltenbach and Lazor 1991; Kaltenbach and McCaslin 1996). At each site, counts of spontaneous events were performed over an interval of 90 s. To avoid possible experimental bias, all recordings were performed blind, without knowledge of which animals had been treated with cisplatin and which had been treated with saline.

The map of sites was converted to a map of coordinates that were localized relative to the 5-kHz isofrequency contour line. This line was identified following recordings of spontaneous MUA by determining the locus of neurons tuned to 5 kHz in each of the three rows of recording sites. The methods used for testing multiunit tuning properties were the same as those described previously (Kaltenbach and Lazor 1991; Rachel et al. 2002).

Data analysis and evaluation of neural activity

Counts of spontaneous events from each recording were converted to spontaneous multiunit rates, expressed as the number of voltage events per second. For each animal, the level of spontaneous MUA was plotted as a function of distance relative to the 5-kHz isofrequency contour line on the surface of the DCN. This contour typically lay within the lateral third of the DCN as viewed dorsally. The plots for all animals were then averaged to obtain a curve representing the mean level of spontaneous MUA versus distance for each of the treatment groups as well as for controls. The effects of cisplatin were evaluated by comparing plots for each cisplatin treatment group with those of controls. Tests of statistical significance of differences between points at corresponding DCN loci were performed using a two-tailed t-test. The criterion for statistical significance was P ≤ 0.05.

Cochlear histology

At the end of each recording session, the animal was killed. The left temporal bone was removed, and the bony ceiling of the apical turn of the cochlea was punctured with the tip of fine jewelers forceps. The cochlea was then perfused through the round and oval windows with 3% glutaraldehyde and placed overnight in the same solution. The next day, the fixed tissue was transferred to a 1% solution of OsO4 for darkening. Microdissection of the cochlea was accomplished by separating the apical turn from the basal turn using extra sharp forceps and microsurgical scissors. The tissue was then critical point dried and sputter coated with gold-palladium.

Analysis of histological results

Scanning electron microscope (SEM) images of the cochlear hair cell fields were obtained at ×150 and were assembled into a montage.
that showed the surface of the entire receptor epithelium in both turns of the cochlear spiral. The length of the organ of Corti in this magnified montage was then divided into numbered bins, each measuring 1 cm along the axis of the cochlear spiral. The average number of bins for each cochlea was 83. Each bin was ranked on a scale of 0–10 according to the percent survival of IHCs and OHCs. A grade of 10 was given to animals in which the number of surviving hair cells was within 1.25 SD of the average number of hair cells/bin in corresponding portions of the normal cochlea (n = 6). Scores of 9 were given to bins in which the number of surviving hair cells was 90% of the mean normal hair cell number, 8 for scores of 80%, and so on. Percent IHC and OHC survival was then plotted as a function of bin number, yielding a pair of cytocochleograms for each animal. Animals were categorized according to the degree of OHC loss caused by the cisplatin treatment. To compute average cochleograms for each treatment group, the scores from corresponding bins were summed and divided by the number of animals in the group. Mean scores were calculated for all bins in this way and used to generate mean cochleograms.

In those animals having high degrees of OHC loss but in which IHCs were present throughout the preserved portions of the cochlea, we also took into consideration more subtle factors that might have compromised IHC function without necessarily resulting in their degeneration. Two features were examined in this regard. The first was the condition of the IHC stereocilia tuft. This factor was considered important because a previous study found a relationship between the survival of the tallest IHC stereocilia and the level of spontaneous discharge rates of auditory nerve fibers (Liberman and Dodds 1984). The condition of the stereocilia tuft was scored for each cell. We ranked each IHC on a scale of 1–4 based on the proportion of the tallest stereocilia that remained intact and fused. A score of 4 was given to IHCs with more than 90% of the stereocilia intact, 3 to cells with 50–89% intact, 2 to cells with 10–49%, and 1 to cells with 0–9% stereocilia intact. These scores were averaged across IHCs for each bin and plotted as stereociliograms that displayed the stereocilia tuft scores/bin as a function of distance from the basal to apical extremity. The second factor was the condition of the inner pillar cell head plate immediately adjacent to the IHCs. This factor was considered important because bulges of the head plates, which are common products of cochlear trauma, can impinge on the IHC stereocilia, thus possibly affecting transduction and neurotransmitter release that could alter spontaneous activity. To quantify this factor, we noted the number of bins showing either upwellings (i.e., blebs) or ripples of the head plates that impinged on the IHC stereocilia.

RESULTS

Successful recordings were completed in a total of 46 animals including 24 treated with cisplatin and 22 saline-treated controls. Usable histological measurements of the cochlea were obtained from 22 cisplatin-treated animals. Four cochleas, taken from control animals, were also evaluated histologically to verify that hair cells were not affected by saline treatment.

Histological results

HAIR CELL CONDITION IN SALINE-TREATED ANIMALS. Cochleas of four saline-treated controls were analyzed histologically. Data from one of these animals are presented in Fig. 1. The appearance of the hair cell fields in the middle of the basal turn is shown in Fig. 1A and the cochleogram is shown in Fig. 1B. Note the presence of three rows of OHCs and one row of IHCs. Note also the good condition of both types of hair cells. As illustrated by the flatness of the cochleogram, the hair cell populations were complete throughout all preserved parts of the cochlea. Gaps of single missing hair cells were seen at random locations along the cochlea of two control animals (not shown). These deletions were manifested as shallow notches in the cochleograms for these animals. While the animal represented in Fig. 1 displayed no missing hair cells, the occasional absence of OHCs in the other animals are reflected in the mean cochleogram shown in Fig. 5A which is based on an average of the histograms for all four animals in this group.

There was no indication of significant damage to IHC stereocilia in the animal represented in Fig. 1. This is indicated by the flatness of the stereociliogram in Fig. 1C. The other three control animals also contained nearly complete stereocilia tufts, although a few gaps of missing stereocilia were seen in one of these control animals. Tilting and splaying of stereocilia in control animals, such as that shown in Fig. 1D, were probably artifacts of tissue preparation. The reticular lamina appeared smooth and unwrinkled in control animals, and there were no signs of blebbing of inner pillar cell head plates.

HAIR CELL CONDITION IN CISPLATIN-TREATED ANIMALS. Cisplatin-treated animals displayed normal OHCs and IHCs in much of the apical turn of the cochlea, but varying degrees of OHC loss in the basal turn. With few exceptions, IHC loss was highly restricted, involving loss of no more than 1% of the IHCs in the basal turn of the cochlea. The severity of OHC loss varied enough for each cisplatin dosage group that it was not possible to predict the magnitude of the lesion based on the dose of cisplatin administered. Because the quantity of OHC loss was critical to the central focus of this study, animals were categorized according to the percentage of missing OHCs in the basal half of the cochlea where damage was greatest. The categories are described as follows.

Animals with mild OHC lesions. Four animals that were treated with cisplatin fell into this category, which was characterized by loss of less than 15% of the OHCs in the basal half of the cochlea. The apical turns contained normal populations of OHCs. IHCs were present and in excellent condition throughout the basal turn. Data obtained from a representative animal in this category are presented in Fig. 2. The photomicrograph in Fig. 2A shows the relatively modest loss of OHCs from the middle of the basal turn. The effect of cisplatin was evidenced by a slightly higher incidence of OHC deletions than seen in controls. These deletions occurred randomly in each of the three OHC rows and mainly in the basal-most 20% of the cochlea. Gaps of single missing hair cells were seen at random locations along the cochlea of two control animals (not shown). These deletions were manifest as shallow notches in the cochleograms for these animals. While the animal represented in Fig. 1 displayed no missing hair cells, the occasional absence of OHCs in the other animals are reflected in the mean cochleogram shown in Fig. 5A which is based on an average of the histograms for all four animals in this group.

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Animals with intermediate OHC lesions. In four cisplatin-treated animals, OHC loss was more widespread than the group showing mild OHC loss. In this group, between 27 and 45% of OHCs were missing in the basal turn, while IHCs remained intact throughout. Data from a typical example are presented in Fig. 3. As can be seen in the photomicrograph of Fig. 3A, taken as part of the histological data from a saline-treated control animal (JR120898-C), the scanning electron micrographs of the organ of Corti showing the appearance of the outer (OHC) and inner (IHC) hair cells. The large arrow points to an OHC and the small arrow to an IHC. B: cochlear histograms for the 2 types of hair cells. The height of the bar for each bin is proportioned to the percent survival of hair cells. Distance along the cochlear length is relative to the apical extremity. The lines under the histograms underscore bins where hair cells were either destroyed accidentally during microdissection or were blocked from view by overhanging tissue. C: IHC stereociliogram plotting the percent survival of stereocilia in each bin as a function of location. D: high-magnification view of the IHCs showing the condition of the stereocilia tuft. The tilting and splaying of stereocilia were artifacts of tissue preparation. E: spontaneous multiunit activity (MUA) plotted as a function of distance across the dorsal cochlear nucleus (DCN) surface. Note the level of activity in this animal did not exceed about 19 events/s. All photomicrographs in this figure and Figs. 2–4 were obtained from positions along the cochlear spiral between 25 and 40% of the distance from the base to the apex.
from the middle of the basal turn, missing hair cells occurred in all three OHC rows. The cochleograms of Fig. 3 indicate that OHCs were normal in the apical turn, and there was no evidence of significant IHC loss in any region of the cochlea. This was also true of the IHCs of the other three animals in this category. The histograms for this animal are thus qualitatively similar to the mean cochlear histograms averaged across the four animals (Fig. 5C). Preservation of IHC integrity was also seen at the level of stereocilia. In three animals, no more than 1% of the stereocilia were missing in the basal turn. In the
other animal, only 3% of the stereocilia were missing from this region. Some splaying of IHC stereocilia was observed but did not appear to differ from that seen in controls (compare Fig. 3D with Fig. 1D). Two animals (not including the one represented in Fig. 3) showed only a few IHCs that were flanked by blebs or bulges of the adjacent inner pillar cell head plates. The reticular laminas of the other two animals were smooth and did not show any such bulges. This group as a whole thus showed
moderate loss of OHCs but little or no indication that the normal function of IHCs might have been compromised significantly.

**Animals with severe OHC lesions.** Fourteen cisplatin-treated animals displayed severe lesions characterized by loss of the majority (i.e., more than 50%) of OHCs in the basal turn. These OHC losses were spread more broadly than in the intermediate group, invading more than half of the apical turn of the cochlea in some animals. Generally, IHCs were almost completely present throughout the basal turn, although as will be described in following text, there was evidence that IHCs may not have been normal in some animals. Data obtained from one animal in this category are presented in Fig. 4. The photomicrograph of Fig. 4A, taken from the middle of the basal turn, shows how the majority of OHCs in this region were missing, while IHCs remained intact. The vast majority of bins in the basal half of the cochlea were missing between 50 and 95% of the OHC population (Fig. 4B, top). IHCs were intact, except for minor deletions at positions corresponding to 70 and 90% of the distance to the basal extremity (Fig. 4B, bottom). The IHC stereociliogram for the animal with severe OHC loss is shown in Fig. 4C. IHC stereocilia loss was very minimal (i.e., 2% of bins in the basal turn) in this animal. The greatest loss was seen at a position of 70% of the distance from the apex to the basal extremity.

All except 1 of the 14 animals in this group showed excellent survival (i.e., 97–100%) of IHCs in the basal turn, similar to the example shown in Fig. 4. The mean IHC histogram thus appeared nearly flat (Fig. 5D, bottom). In contrast, the mean loss of OHCs ranged between 75 and 90% across the basal turn (Fig. 5D, top). Half (n = 7) of the cochleas from this group, when examined at high magnification, also showed nearly intact IHC stereocilia, with no more than 2% of the original stereocilia population missing from the basal half of the cochlea. In six other animals, however, stereocilia loss was slightly more marked, reaching between 3 and 5% missing stereocilia in this region. One other cochlea showed loss of 21% of its basal turn stereocilia. The most prominent indication that IHCs may not have been normal in some animals with severe OHC lesions is suggested by the presence of irregularities of the reticular lamina. These irregularities included blebs or ripples of the reticular lamina immediately adjacent to the IHC cuticular plates. They appeared to represent a bulging or protrusion of the reticular lamina. These bulges often appeared to be pushing against the IHC stereocilia tuft causing the latter to lean toward the cochlear modiolus (arrow in Fig. 4D). They were confined to the most basal turn, being most frequent in the hook region, grading into a smooth, regular surface in the apical direction. Eight of the 14 animals in this treatment group showed blebs in more than 10 of the bins of the basal turn. The other six displayed such irregularities only in the hook region of the cochlea.

**Electrophysiological results**

**SPONTANEOUS MUA IN SALINE-TREATED CONTROLS.** Figure 6A presents measures of spontaneous MUA in the 22 saline-treated controls. Activity in this group of animals was mostly below 20 events/s with a slight trend toward higher levels of activity in the middle portion of the DCN (i.e., between 0.2 and 0.8 mm from the 5-kHz isofrequency contour) where levels in three animals reached between 25 and 30 events/s. The most typical example is the animal represented in Fig. 1E, which displayed a maximal rate of 19 events/s. Mean activity for this group is shown by the curve marked (●) in Fig. 7.

**SPONTANEOUS MUA IN CISPLATIN-TREATED ANIMALS WITH MILD OHC LESIONS.** The levels of spontaneous MUA recorded in this group of animals (n = 4) were generally similar to those observed in saline treated controls. As in the example of Fig. 2E, rates were under 30 events/s across the entire DCN surface (Fig. 6B). Also similar to the saline-treated controls, there was a slight tendency for activity to increase toward the middle of the DCN. The mean level of activity, represented by the curve marked (○) in Fig. 7, was only slightly higher than the mean level of activity in controls. Thus mild loss of OHCs was associated with only a very modest increase in spontaneous MUA in the DCN.

**SPONTANEOUS MUA IN CISPLATIN-TREATED ANIMALS WITH INTERMEDIATE OHC LESIONS.** Spontaneous MUA recorded from the four animals in this group are presented in Fig. 6C. Two of the animals showed activity that was not elevated above control levels. For these two animals the activity did not exceed 10 events/s at any position along the medial-lateral axis of the DCN. In the other two animals, however, activity was clearly higher than control levels, ranging between 30 and 60 events/s over at least a third of the DCN. Among the four animals in this lesion category, the animal with the highest activity is the one represented in Fig. 3E, with a peak rate of 59 events/s. This animal was also the one with the greatest amount (45%) of basal turn OHC loss. Mean activity for this category as a whole is shown by the curve marked (■) in Fig. 7. The increases in mean rate above control levels were maximal in the medial half of the DCN that corresponds to the location of OHC loss in the basal turn of the cochlea. These results indicate that intermediate loss of OHCs was associated with a moderate increase in spontaneous MUA in the DCN.

**SPONTANEOUS MUA IN CISPLATIN-TREATED ANIMALS WITH SEVERE OHC LESIONS.** This category of 14 animals displayed much higher spontaneous activity overall than did either control animals or animals with mild or intermediate OHC loss. Nine of the 14 animals with severe OHC loss (75%) showed rates that were appreciably higher than the mean upper limit (12 events/s) of the control group (Fig. 7). Six animals showed rates in excess of 50 events/s (see example in Fig. 4E). Three others showed rates that were either 37 or 38 events/s. The remaining four showed rates that did not differ significantly from those in controls. Mean activity in this group shown by the curve marked (□) in Fig. 7, was significantly higher than control levels at nine positions along the medial-lateral axis of the DCN. Most of these (7/9) were found in the medial (high-frequency) half of the DCN (i.e., at positions equal or medial to the 0.4-mm locus relative to the 5-kHz contour line). Activity in the lateral (low-frequency) half differed significantly from control levels but by a smaller magnitude. This distribution pattern is consistent with the histological findings that OHC loss occurred mostly in the basal (high-frequency) half of the cochlea (Fig. 5D). Activity also decreased in the extreme medial portion of the DCN where it converged toward control levels.

The generally higher activity seen in animals with severe
OHC lesions was not related to the amount of IHC damage. Eight of the 14 animals in this category had high spontaneous MUA (≥30 events/s) but displayed no significant IHC loss. Indeed, several of these eight animals were among those with the highest spontaneous MUA (more than 50 events/s). In two of the three animals with the highest activity (≥75 events/s), IHC stereocilia loss and the number of bins with irregularities of the reticular lamina that would likely have impaired IHC function were negligible. The results indicate that severe OHC loss was associated with strong increases in spontaneous activity.

FIG. 4. Histological data obtained from a cisplatin-treated animal (JR121498) with severe loss of OHCs. A: the scanning electron micrographs of the organ of Corti show the widespread loss of OHCs typical of animals with severe OHC lesions. In this group the vast majority (>70%) of OHCs were missing from all 3 rows in the basal half of the cochlea. IHCs were relatively intact in most animals. B: cochlear histograms for the 2 type of hair cells showing that the loss of OHCs occurred mainly in the basal 50% of the cochlea, increasing in severity toward the basal extremity. C: IHC stereociliogram plotting the percent survival of stereocilia in each bin as a function of location. D: high-magnification view of the IHCs showing the condition of the stereocilia tuft. The spaying of stereocilia in this animal did not differ from that seen in controls (Fig. 1E) and was therefore likely to be an artifact of tissue preparation. E: spontaneous MUA plotted as a function of distance across the DCN surface. The level of activity in this animal reached a maximal value of 95 events/s. The arrow in D points to a bleb from the inner pillar cell head plate that impinged on the IHC stereocilia tuft.
MUA, but when the OHC loss was accompanied by subtle damage to IHCs or impingements on the IHC stereocilia, the increases in activity were smaller.

Relationship between OHC loss and changes in spontaneous MUA

The relation between OHC loss and changes in the level of spontaneous MUA was examined by plotting the mean percent OHC loss versus mean maximal spontaneous rate for each of the four lesion categories represented in Figs. 5–7. For each group, the mean maximal spontaneous rate was obtained by finding the peak of the corresponding curve in Fig. 7, and the mean OHC survival was the mean percentage of surviving OHCs calculated by averaging all bar heights in the basal half of each OHC histogram in Fig. 5. The data in Fig. 8 indicate a steady increase in the level of activity as a function of OHC loss, although the slope in the increase in spontaneous MUA was more gradual than for the increase in OHC loss.

The relationship between spontaneous MUA and OHC loss for individual animals was also examined. The results are presented in Fig. 8B, which plots peak activity versus the percent loss of OHCs in the basal turn for all 22 cisplatin-treated animals from which both histological and electrophysiological measures were obtained. This plot displayed two distinct phases. The first occurred between 0 and 80% OHC loss and consisted almost entirely of animals with OHC loss only and negligible damage to IHCs (●). The second phase occurred between 80 and 100% OHC loss and involved mostly animals with mixed lesions consisting of OHC loss with co-existing minor injury to IHCs (□). The cochleas of these animals showed 15 or more bins (roughly 20% of the basal turn) in which IHC stereocilia were either partially missing or were impinged on by blebs from the inner pillar cell head plates. Within the first phase, the level of activity increased linearly with the amount of OHC loss, yielding a correlation coefficient, r, of 0.89. When the amount of OHC loss exceeded 80% and was accompanied by more pronounced IHC damage, activity showed a sharp drop into a lower range, although the trend toward higher activity was still apparent with further increases in the amount of OHC loss. The r value for points in this second phase was 0.51. These data suggest that activity increases with OHC loss, but the increase is less dramatic and may be offset when a certain degree of IHC injury is reached.

Relation of spontaneous MUA to body weight and cisplatin dose.

The possibility was considered that changes in spontaneous MUA might have been related to changes in body weight due to some toxic side effect of cisplatin on vital body functions. Because the degree of toxicity would be expected to increase with cisplatin dose, the influence of toxicity was examined by plotting the maximal level of spontaneous MUA for each animal versus the dose of cisplatin each animal received. The results, shown in Fig. 9A, indicate a poor rela-
tionship between level of activity and cisplatin dose. The correlation coefficient was only 0.08, indicating that dose was a negligible factor influencing spontaneous MUA. On the other hand, because toxicity may not necessarily be correlated with dose, a more meaningful way of testing the effect of a possible systemic toxicity on spontaneous activity is to plot the level of activity versus body weight. This relationship was tested in Fig. 9B. Again, the correlation coefficient was only 0.01. Thus assuming that body weight decreases with systemic toxicity, the data presented in this figure indicate that systemic toxicity was not an important factor determining the level of spontaneous MUA in the DCN of cisplatin-treated animals.

DISCUSSION

The evidence presented in the foregoing section lends further support to the hypothesis presented earlier (Melamed et al. 2000) that loss of OHCs is an important factor in the etiology of DCN hyperactivity caused by cisplatin administration. Several observations in the present study point to this conclusion: All cisplatin-treated animals showing multiunit spontaneous rates well above control levels displayed measurable losses of OHCs, and the mean level of activity in these animals increased and showed a significant correlation with the degree of OHC loss (Fig. 8, A and B). Moreover, significant increases in activity were not seen in individual control animals (Fig. 6A) and were generally absent or only slight in cisplatin-treated animals with mild OHC loss (Fig. 6B). The hyperactivity seen in cisplatin-treated animals with intermediate and severe OHC loss occurred mostly in the medial half of the DCN (Fig. 7). In previous studies, this half of the DCN has been shown to represent the higher frequency half of the hamster’s audiometric range (Kaltenbach and Lazor 1991; Kaltenbach and McCaslin 1996; Kaltenbach et al. 1998). This finding corresponds to the observation in the present study that OHC loss in cisplatin-treated animals was distributed mainly in the basal half of the
cochlea where frequencies in the higher part of the hamster’s audiometric range are analyzed.

Our data also suggest that the increases in activity in the DCN were not related to IHC injury. We found that many animals with very high activity showed loss of OHCs with no significant damage to IHCs. Among those animals with the severest OHC lesions, those showing damage to IHCs generally had lower activity than those with little or no apparent damage to IHCs (Fig. 8B). And finally, the level of peak spontaneous MUA in animals with mixed IHC and OHC damage was more weakly correlated with OHC loss than in animals with little or no IHC injury (Fig. 8B). This fact coupled with the observation that hyperactivity declined in the medialmost extremity of the DCN, where damage was more extreme and involved more impingements of pillar cell head plates on IHC stereocilia, suggests that damage to IHCs may offset the condition of hyperactivity triggered by OHC loss. This interpretation is consistent with previous studies showing that damage to IHCs or their stereocilia causes a decrease of spontaneous discharge rates of type I primary afferents (Liberman and Dodds 1984; Wang et al. 1997).

An alternative interpretation of our results is that the increases in DCN activity were not the result of OHC loss but rather to a direct toxic effect of cisplatin on the DCN or on some vital function that affected the DCN, independent of the effects on OHCs. Cisplatin is a potent nephrotoxin (Borch 1987; Gandara et al. 1990; Rybak 1986), and severe nephrotoxicity can lead secondarily to a number of complications that could, at least in theory, affect spontaneous neural activity. The degree of toxicity increases with dose and is typically manifest...
in its severe form as weight loss (Ammer et al. 1993; Masson and Rhodes 1992). However, we found a poor relationship between the degree of hyperactivity and the dose of cisplatin (Fig. 9A). Although many of the animals treated with cisplatin in the present study did show some weight loss, only a weak relationship was found between the amount of weight loss and the degree of hyperactivity (Fig. 9B). These findings do not conform to the pattern expected if hyperactivity were the result of a direct toxic effect of cisplatin on the DCN or on vital functions. Also, the possibility that cisplatin might have gained access to the DCN or brain stem directly is weakened by several previous studies showing that this hydrophilic compound generally does not cross the blood-brain (Gregg et al. 1992; Minami et al. 1996, 1998; Nakagawa et al. 1996) or blood-CSF barriers (Gormley et al. 1981; Nakagawa et al. 1996). A study examining the pharmacokinetics of cisplatin in humans and animals found that concentrations of cisplatin in CSF measured only 1–2% of serum levels and did not exceed 4% (Gormley et al. 1981). Also, this minimal cisplatin was cleared below the limits of detectability within 2.5 h after intravenous injection. It is probably because of this rapid clearance that central neurotoxicities following cisplatin chemotherapy are rare (e.g., Clamon et al. 1996; Verschraegen et al. 1995).

An aspect of our results that needs to be reconciled with the interpretation that hyperactivity in the DCN might be linked to OHC loss was the observation that a few animals showing OHC lesions with no apparent IHC damage did not display significant increases in spontaneous MUA (● in phase 2 component of Fig. 8B). The simplest explanation for this discrepancy is that high activity induced by OHC loss may have deteriorated into low activity as a result of damage to the DCN that may have been incurred during surgical exposure of the DCN. Surgical exposure requires aspiration of the cerebellum, a maneuver that can sometimes result in a leakage of blood onto the DCN surface or a tugging of the cerebellar peduncle, either of which can lead to spasms of DCN capillaries (W. S. Quirk and J. A. Kaltenbach, unpublished observations). Activity is most affected in the medial-most portion of the DCN, immediately caudal to the cerebellar peduncle, but can spread laterally to affect activity over the entire DCN surface. This explanation might also account for some of the decline in hyperactivity that was often seen in the medial extremity of the DCN of cisplatin-treated animals.

Possible mechanisms of cisplatin-induced hyperactivity

There are several mechanisms by which loss of OHCs could lead to hyperactivity in the DCN. One mechanism is that the hyperactivity is a consequence of altered cochlear mechanics that somehow causes an increase in the excitability of type I primary afferent auditory nerve fibers. An increase in type I afferent activity could then be relayed centrally causing an increase in the spontaneous activity of DCN neurons. Although this explanation cannot be entirely dismissed, it does nonetheless seem dubious in light of a previous report that loss of OHCs following aminoglycoside treatment did not significantly affect spontaneous discharge rates of type I primary afferents (Dallos and Harris 1978), despite dramatic effects on their response thresholds (Dallos and Harris 1978; Evans and Harrison 1975; Schmiedt 1982; Schmiedt et al. 1980).

A second mechanism by which hyperactivity might be induced in the DCN by cisplatin is through a change in the balance of inputs from the two primary afferent channels, in line with the hypothesis proposed by Jastreboff (1995). This model might involve the portion of DCN circuitry that is shown in Fig. 10, leading to a shift in the balance of excitation and inhibition of DCN neurons. Loss of OHCs could remove tonic activity of type II afferents, leading to a loss of excitatory input to central target neurons that might be inhibitory in function. Type II afferents terminate in the granular region of the cochlear nuclei (Berglund and Brown 1994; Brown and Ledwith 1990; Brown et al. 1988; Shore and Moore 1998), and there is evidence that granule cells may be among the recipients of type II input (Berglund and Brown 1994). Granule cells are excitatory neurons (Godfrey et al. 1977; Manis 1989; Molitor and Manis 1997; Oliver et al. 1983; Rubio and Juiz 1998; Waller et al. 1996; Wenthold et al. 1993), many of whose axons project as parallel fibers in the DCN molecular layer. Within this layer, the axons of granule cells spread across isofrequency sheets, synapsing directly on fusiform cells (Berrebi and Mugnaini 1991; Kane 1974; Mugnaini 1985).

![Diagram of pathways](http://jn.physiology.org/)

FIG. 10. Pathways that might underlie the effect of OHC loss on DCN spontaneous activity. In this model, there are 2 pathways reaching the DCN from the cochlea, including the type I pathway originating from IHCs and the type II pathway originating from OHCs. The type I axons synapse on the basal dendrites of the fusiform cells, 1 of the 2 classes of DCN principal neurons. Type II axons are shown synapsing on the granule cells. The granule cells project via parallel fibers onto 2 types of inhibitory interneurons, including the cartwheel cells and stellate cells. These inhibitory interneurons, in turn, provide input to fusiform cells. Loss of OHCs would be expected to lead to loss of spontaneous activity of type II axons, leading to a reduction of tonic drive of granule cells. The loss of granule cell drive would reduce the activity of cartwheel and stellate cells, leading to a disinhibition of fusiform cells and causing these principal neurons to increase their firing rate. Another possibility is that hyperactivity results from a compensatory change in sensitivity of granule cells to input from the medial olivococchlear bundle (MOCB). If the sensitivity increases (for example, by upregulation of cholinergic receptors or by sprouting of MOCB axons to take over vacant synaptic space left by the loss of type II input), granule cell activation of cartwheel or stellate cells would increase, leading to the observed hyperactivity. Alternatively, if the sensitivity to cholinergic input decreases (downregulation), granule cell activation of cartwheel cells or stellate cells would decrease, leading to a disinhibition of fusiform cells and accounting for the observed hyperactivity.
as well as on inhibitory interneurons (cartwheel cells and stellate cells), which in turn, synapse on fusiform cells (Berrebi and Mugnaini 1991; Golding and Oertel 1997; Mugnaini 1985; Mugnaini et al. 1980a,b). Although the type II fiber population composes only about 5–10% of the auditory nerve, the large number of granule cells in the cochlear nuclei coupled with the broad range of axonal spread of many granule cell axons, suggests that loss of type II input could cause major changes in the DCN. If it is assumed that at least some type II afferents are spontaneously active, as suggested by the recent work of Robertson et al. (1999) and that these type II afferents excite granule cells, the following effects on DCN neurons might be expected: excitatory input to fusiform cells via direct granule cell input would be reduced and excitatory input to cartwheel and stellate cells would be reduced, resulting in a disinhibiting of fusiform cells. Which of these two effects on fusiform cells would dominate would depend on the relative weight of these two sources of inputs to fusiform cells. Previous studies conducted in vitro suggest that the inhibitory input to fusiform cells, via cartwheel cells and stellate cells dominates over the excitatory input of granule cells onto fusiform cells (Davis et al. 1996; Waller et al. 1996). It might therefore be expected that the net effect of reduced drive to granule cells from type II primary afferents would be an increase in the level of fusiform cell spontaneous activity. This line of speculation is consistent with recent evidence suggesting that spontaneous activity of fusiform cells increases after exposure to noise, a manipulation that usually damages OHCs more than IHCs (Brozoski et al. 2002). However, it would need to be reconciled with recent findings reported by Chang et al. (2002) that intense sound exposure causes an increase in the number of spontaneously active bursting neurons in the DCN, which are thought to be cartwheel cells (Manis et al. 1994; Oertel and Wu 1989; Zhang and Oertel 1994), and a decrease in the number of regular discharging neurons in DCN, which are presumed to be fusiform cells.

A third possible mechanism is that loss of normal input from afferent auditory nerve fibers (either type I or type II) might cause a plastic or compensatory change in the influence of intrinsic and/or descending (i.e., efferent) inputs to DCN neurons (Fig. 10). Given the complexity of DCN circuitry and the multiple sources of intrinsic and descending inputs, one could speculate countless models. In the interest of simplicity, we will discuss only one “descending trigger mechanism” based on input from the most widely studied descending pathway to the cochlear nucleus, the medial olivocochlear bundle. Branches of medial olivocochlear neurons project to the granule cell domain, so a likely target of these neurons are granule cells (Godfrey et al. 1997). These inputs are cholinergic and have been shown to affect spontaneous activity of DCN neurons, especially cartwheel cells (Chen et al. 1994). As mentioned in the preceding paragraph, increases in the number of bursting neurons (presumed cartwheel cells) in the DCN were observed in DCN slices obtained from hamsters that had been exposed to intense sound (Chang et al. 2002). The increase in the number of active bursting neurons was associated with enhanced sensitivity of bursting neurons to carbachol, a cholinergic agonist (Chang et al. 2002). One model suggested by Chang et al. was that the increase in the number of spontaneously active bursting neurons after intense sound exposure might result from the enhanced sensitivity of granule cells to descending cholinergic input. This hypothesis is consistent with their earlier works showing that the spontaneous activity of presumed cartwheel cells can be increased by electrical stimulation of granule cell axons (i.e., parallel fibers) (Waller et al. 1996) and that the predominant synaptic effect of cholinergic agonists on cartwheel cells is excitatory (Chen et al. 1994). Based on these observations, an increase in granule cell sensitivity to cholinergic input would be expected to increase the spontaneous activity of granule cells, leading to an increased activation of cartwheel cells, raising the number of active neurons.

From the foregoing discussion, it is clear that hyperactivity could result from any of several mechanisms. A key to understanding which, if any, of the above-described mechanisms is correct is likely to be the identification of the neuronal cell class(es) from which hyperactivity originates. This is an issue on which our research is presently focused.

**Relationship to tinnitus**

Although no study has yet shown that cisplatin causes tinnitus in animals, there is an abundance of evidence that cisplatin causes tinnitus in humans (Bokemeyer et al. 1998; Kawakita et al. 1999; Lerner et al. 1995; Nakai et al. 1982; Reddel et al. 1982). Follow-up studies of patients treated with cisplatin for various types of cancers indicate that tinnitus is often a chronic problem that may endure for months or years following termination of treatment with cisplatin (Bokemeyer et al. 1998; Kawakita et al. 1999). The dose of cisplatin used in the present study was similar to the clinical dose known to cause tinnitus in human subjects.

Our findings with cisplatin extend the findings obtained from studies using sodium salicylate, which is known to be a potent inducer of acute tinnitus (Day et al. 1989; Karlsson and Flock 1990; McCabe and Dey 1965; McFadden et al. 1984; Mongan et al. 1973), and which is known to block OHC function (see review of Casals 2000) and cause changes in spontaneous activity throughout the auditory system (Chen and Jastreboff 1995; Eggermont and Kenmochi 1998; Evans and Borerwe 1982; Manabe et al. 1997; Wallhauser-Franke 1997). However, the results with cisplatin differ from those with salicylate in two ways. One concerns the reversibility of the tinnitus-inducing agent. Whereas the effects of salicylates cited in the preceding text are reversible, those with cisplatin appear to be more or less permanent, occurring many weeks after treatment. The extended and presumably irreversible effect of OHC loss induced by cisplatin offers to explain the chronic nature of tinnitus that often results from cisplatin chemotherapy (Boke- meyer et al. 1998; Lerner et al. 1995; Kawakita et al. 1999; Nakai et al. 1982; Reddel et al. 1982). In addition, whereas salicylate has generally been found to cause blockage of OHC function, cisplatin causes OHC loss. The fact that both types of manipulations cause hyperactivity and tinnitus suggests that any manipulation that compromises the functional status of the OHC system without damaging IHCs would be a major trigger of tinnitus.

Finally, it is important to emphasize that loss of OHCs is not the only trigger of tinnitus. Numerous mechanisms have been invoked to explain the various types of tinnitus that have been reported in the clinical literature. These have been reviewed previously (Kaltenbach 1999). Our results do nonetheless sug-
gest that greater loss of OHCs than IHCs may be responsible for the elevation of spontaneous activity leading to tinnitus. Previous studies have shown that intense sound exposure also leads to hyperactivity (Kaltenbach and Afman 2000; Kaltenbach and McCaslin 1996; Kaltenbach et al. 1998, 1999; Zhang et al. 1998) and tinnitus (Bauer and Brosowski 2001; Heffner and Harrington 2002). However, little relationship was found in those studies when the level of activity was compared with the degree of OHC loss (Kaltenbach and McCaslin 1996). It may be that the lack of a relationship in this previous study was due to the fact that the OHC loss was associated with damage to IHCs. As we showed in Fig. 5B, damage to IHCs appears to offset the increases in activity related to OHCs. Alternatively, it may be that the hyperactivity and tinnitus that are induced by intense sound exposure involve a different mechanism. Intense sound could, for example, cause an over-activation of primary afferent neurons, which could cause excess glutamate release in the cochlear nucleus. This excess could exert an excitotoxic effect on central target cells, causing a shift in the balance of excitatory and inhibitory inputs to DCN neurons without necessarily causing equivalent losses of OHCs. Recent work showing that intense noise causes greater fiber degeneration in the cochlear nuclei than in the auditory nerve is consistent with this possibility (Kim et al. 1997; Moster and Bohne 1983).

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REFERENCES


Heffner HE and Harrington IA. Tinnitus in hamsters following exposure to intense sound. Hear Res In press.


