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Succinate dehydrogenase (SDH) activity in hair cells: a correlate for permanent threshold elevations

Guang-Di Chen *, Misty L. McWilliams, Laurence D. Fechter

University of Oklahoma, Health Sciences Center, College of Pharmacy, P.O. Box 26901, 1110 N. Stonewall, Oklahoma City, OK 73190, USA

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Abstract

Hair cell loss is often used as a histological correlate of hearing loss. However, the histological and the physiological data are not always well correlated. This paper investigates the use of succinate dehydrogenase (SDH) activity in the hair cells as a marker of cellular dysfunction and so the loss of auditory sensitivity. In our previous studies, potentiation of noise-induced auditory threshold elevation by carbon monoxide (CO) was observed [Chen and Fechter, 1999; Chen et al., 1999]. However, its histological basis is still unclear. In this study, rats were exposed to 100-dB octave-band noise (center frequency = 13.6 kHz, 2 h) or to the combination of the noise and CO (1200 ppm). Threshold elevation of compound action potential (CAP) and cochlear histological changes were assessed 4 weeks after exposure. The noise alone caused CAP threshold elevations with little if any or without hair cell loss. However, the SDH activity in the hair cells decreased after the exposure. The SDH reduction, especially in the inner hair cells, was well related to the loss of auditory sensitivity. The combined exposure to noise and CO caused more severe CAP threshold elevation and SDH activity reduction than did the noise alone and it also caused significant outer hair cell loss. However, across all the test frequencies, neither the hair cell loss nor the SDH reduction alone had good correlation to the reduction of the auditory sensitivity. Under this situation, CAP threshold elevation seemed to follow OHC loss at high frequencies and to follow SDH reductions in the IHCs at low frequencies, where no hair cell loss occurred.

Key words: Cochlea; Succinate dehydrogenase activity; Surface preparation; Noise-induced hearing loss; Carbon monoxide ototoxicity; Rat

1. Introduction

Noise-induced hearing loss stems in most cases from cochlear disruptions and especially impairments of the hair cells. However, the relationship between the damage in the hair cells and the loss of auditory sensitivity is unclear leading to inconsistent reports. Hamernik et al. (1989) found a good relationship between permanent threshold shift (PTS) and the hair cell loss in pooled data, obtained over many years induced by different noise exposure in chinchilla. Outer hair cell (OHC) loss was observed with less than 5 dB PTS and inner hair cell (IHC) loss began when the PTS exceeded 30 dB. Thus they concluded that the first 30 dB of PTS was related to losses of OHCs. Other reports based on the study of guinea pig, cat, monkey and chinchilla obtained similar conclusions; that the first approximately 40 dB PTS was due to OHC losses (Lurie, 1937; Schuknecht, 1953; Ryan and Dallos, 1975; Stebbins et al., 1979). Total loss of OHCs without IHC losses was found to cause 30–50-dB hearing loss (Ryan and Dallos, 1975). OHCs are believed to contribute to the cochlear active process and loss of all OHCs may lead to complete loss of this process. However, in some reports, hearing loss and hair cell loss were not correlated or were poorly correlated. Hair cell loss was observed without hearing loss in some cases (Hunter-Duvar and Elliot, 1973; Henderson et al., 1974; McFadden et al., 1998) and permanent hearing loss (up to about 15–40 dB) was observed without hair cell loss in others (Engstrom, 1983; Engstrom and Borg, 1983; Hunter-Duvar and Elliot, 1972; Lataye et

* Corresponding author. Tel.: +1 (405) 271-6593, ext. 47235; Fax: +1 (405) 271-7477; E-mail: guangdi-chen@ouhsc.edu

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al., 2000). Even in cases where both hearing loss and hair cell loss are observed, the comparison between the audiogram and the cytocochleogram does not always show good correlation at all frequencies. Hearing losses at some frequencies have been seen without corresponding hair cell loss and hair cell loss at some locations without corresponding threshold shift (Campo et al., 1997, 1998; Fechter et al., 1988; Hamernik et al., 1994).

There have been attempts to investigate the relationship between non-lethal impairment of hair cells, such as damage to stereocilia, and functional impairment of the cochlea. Engstrom and Borg (1983) observed a 30–40 dB PTS in rabbits after noise exposure without hair cell loss. However, the damage to stereocilia of IHCs correlated well with the threshold shifts (Engstrom and Borg, 1983; Borg and Engstrom, 1989). Their conclusion was that the stereocilia of the IHCs are the structure most susceptible to damage by noise (Engstrom, 1983; Engstrom and Borg, 1983). In rabbit, the OHCs are frequently left unaltered even when the stereocilia of most IHCs exhibited pronounced alterations.

Auditory hair cells in rats may also survive following noise exposure generating a moderate-level hearing loss. Lataye et al. (2000) recently reported that octave-band noise at 97 dB SPL induced a PTS of about 15–20 dB without hair cell loss. The noise-induced hearing loss in rats under this condition was mainly related to the injury of the stereocilia (of both the IHCs and the OHCs), though the hearing loss induced by chemical ototoxic agents such as toluene or styrene in the rat was mainly related to OHC losses (Lataye and Campo, 1997; Lataye et al., 2000).

Any damage to the hair cells caused by ototoxic agents, even mechanical impairments, may induce alterations of cellular energy metabolism directly or indirectly. The energy metabolic level may be a good indicator of the cellular functional level. Succinate dehydrogenase (SDH) is one of the Kreb's cycle enzymes, the activity of which may reflect the cellular energy metabolic level. A decrease in SDH activity in auditory hair cells after noise exposure has been observed (Wang et al., 1990; Zhai et al., 1998). The noise-induced reduction of SDH activity can recover with auditory sensitivity (Zhai et al., 1998). However, the noise-induced SDH activity reduction was assessed either simply by visual comparison (Wang et al., 1990) or by 'five grade' evaluation of SDH staining (Zhai et al., 1998). The relationship between the SDH activity along with the cochlea and audiograms especially the permanent hearing loss is still unclear. In the present study, the SDH staining density in auditory hair cells is measured by a designed computer system. The successive measurements make it possible to compare the histochemical alterations to the physiological changes.

Not only the noise, the decrease of SDH activity can also be induced by many other ototoxic agents, such as ethacrynic acid (Horn et al., 1978), carboplatin (Saito et al., 1989) and gentamicin (Yang and Han, 1991). Some of the drugs only influenced SDH in the OHCs, but not in the IHCs (Saito et al., 1989) or influenced SDH in high frequency cells, but not in low frequency cells (Yang and Han, 1991). Anoxia (Li et al., 1994) and iron deficiency (Sun et al., 1990) can also cause a decrease of SDH staining. It seems that the SDH activity reduction is commonly related to the damage to the hair cells, no matter by which ototoxic agent it is caused.

This experiment focused on the study of the correlation between noise-induced permanent hearing loss and SDH reduction in the hair cells, since the noise-induced hearing loss in rats may stand without hair cell loss under some conditions (Chen et al., 1999; Lataye et al., 2000). Based on the cochlear electrophysiological responses in our previous reports, it was suggested that the potentiation of noise-induced hearing loss by carbon monoxide (CO) (more hearing loss to the combined exposure to noise and CO than to the noise alone) might occur at the OHC level instead of IHCs (Chen and Fechter, 1999; Chen et al., 1999). This study also determined the histological basis for the potentiation of the noise-induced hearing loss by CO.

2. Materials and methods

2.1. Subjects

Experimental animals (16 Long Evans pigmented rats approximately 2 months of age) were acquired from Harlan Sprague Dawley and housed in University of Oklahoma Health Sciences Center animal facility. All animal facilities at OU are registered with the US Department of Agriculture and are inspected semi-annually by the members of the Institutional Animal Care and Use Committee (IACUC). All procedures regarding the use and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) serving the University of Oklahoma Health Sciences Center. Background noise level in the colony room is below 50 dB(A) and 75 dB_{Lin}. The sound energy in any octave band above 2 kHz is less than 40 dB SPL. The room temperature is controlled at 21°C. The light is turned on at 06:30 and turned off at 18:30. Food and water are available at all times.

2.2. Procedure

Four subjects were exposed to octave-band noise (100 dB_{Lin}), two to 1200 ppm CO and four to the combination of the noise and CO. The animals were raised in

the colony room after the exposure for 4 weeks before physiological and histological examining. Six animals were exposed to lab air only for physiological control and three for histochemical control. All the animals were exposed in an exposure chamber for 3.5 h. CO was turned on immediately and noise was turned on 1.5 h after the animal was placed in the chamber. Thus the noise duration was 2 h and the CO exposure was 3.5 h.

Exposures were conducted in a reverberant 40-l glass cylinder equipped with stereo speakers for delivering sound, a Quest 1″ microphone and sound level meter for monitoring sound level, and a CO monitor (Industrial Scientific) for measuring the chamber gas concentration. Air exchange rate in the exposure chamber was 8.5 l/min (providing for approximately 12 air changes per hour), and airflow was monitored by a Top Trak 821-1-PS flowmeter. Background noise level in the chamber with no added noise was about 40 dB(A) and 85 dB_{Lin} with the highest energy at a frequency of 31.5 Hz and below. The energy in any octave band higher than 2 kHz is less than 34 dB SPL. The subjects were placed within small wire-cloth enclosures ($15\times13\times11$ cm) within the chamber, and were conscious and free to move within the enclosures.

2.3. Noise and CO exposure

Broadband noise generated by a function generator (Stanford Research System, Model DS335) was band-pass-filtered through a filter network (Frequency Devices, 9002) to produce an octave-band noise with a center frequency (CF) of 13.6 kHz and 48 dB/octave roll-off at the cutoff frequency. The octave-band noise was amplified and delivered to the two speakers in the exposure chamber. Noise intensity used in this experiment was 100 dB measured with a linear weighting at the approximate level of the animals' ears. The noise level varied less than 2 dB within the space available to the animal.

CO gas was metered into the chamber using a microflow valve. The nominal CO level was 1200 ppm. The CO level in the exposure chamber reached a stable level within 1 h (Chen and Fechter, 1999). When the noise was turned on (at 1.5 h after the onset of CO), the CO in the exposure chamber and the HbCO concentration in the blood had already reached its plateau (Chen and Fechter, 1999).

2.4. Compound action potential (CAP) recording

Four weeks after the exposure, the animals were anesthetized with xylazine (13 mg/kg, intramuscular (i.m.)) and ketamine (87 mg/kg, i.m.). The round window of the right ear was surgically exposed using a ventro-lateral approach and a silver wire electrode was carefully placed on the round window under a surgery microscope for recording CAP. A silver chloride electrode was placed in the neck muscle as the reference. The CAP signals were amplified with a Grass A.C. preamplifier (Model P15). The gain was 1000. The band-pass frequency for CAP was 0.1–1.0 kHz. The CAP signals were displayed using a digital oscilloscope (Nicolet Instrument Co., 2090-IIIA). The sound level of test frequencies that evoked a just detectable CAP was determined and this value was used to estimate the threshold at the frequency. CAP threshold elevation was obtained by comparing each examined animal to control animals.

Pure tones for eliciting CAP were generated with the SR530 Lock-in amplifier (Stanford Research Systems, Inc.). The signals were attenuated by a programmable attenuator and then amplified by a high voltage amplifier and delivered to a high frequency earphone (made from an ACO 1/2″ microphone, 7013) placed within a speculum that fit into the exposed external auditory meatus. Frequencies of the tone bursts were 2, 4, 6, 8, 12, 16, 20, 24, 30, 35 and 40 kHz. The duration of the tone bursts was 10 ms with a rise/fall time of 1.0 ms and a repetition rate of 9.7/s. Sound levels at all testing frequencies were calibrated with a probe microphone located near the eardrum.

2.5. Histology

The deeply anesthetized animals were decapitated after CAP recording. Cochleae (usually right ones) were removed immediately. Both the round and the oval windows were opened and the apex of the cochlea was drilled open to facilitate perfusion. The cochleae were perfused with SDH incubative solution (0.05 M sodium succinate, 0.05 M phosphate buffer and 0.05% tetranitro blue tetrazolium) and immersed in the solution for 1 h (37°C). Then the cochleae were fixed with 10% formalin for at least 2 days. After fixation, the cochleae were decalcified in 10% ethylenediamine tetraacetic acid solution for 3 days or longer as needed. Cochlea microdissection was accomplished under a light microscope. Successive image pictures (covering 200–300 μm of the basilar membrane) were obtained with Optimetic Image system with microscope objective magnification of $10\times$. Hair cell counting and measurement of SDH staining density in the hair cells were achieved using Scion Image software. Hair cell loss was obtained by comparing experimental and control animals. All hair cells with detectable SDH staining were counted. All IHCs in each image picture (a length of 20–30 cells) were selected and the mean SDH staining density was measured. Effort was also made to exclude afferent nerve endings, but the whole hair cell was

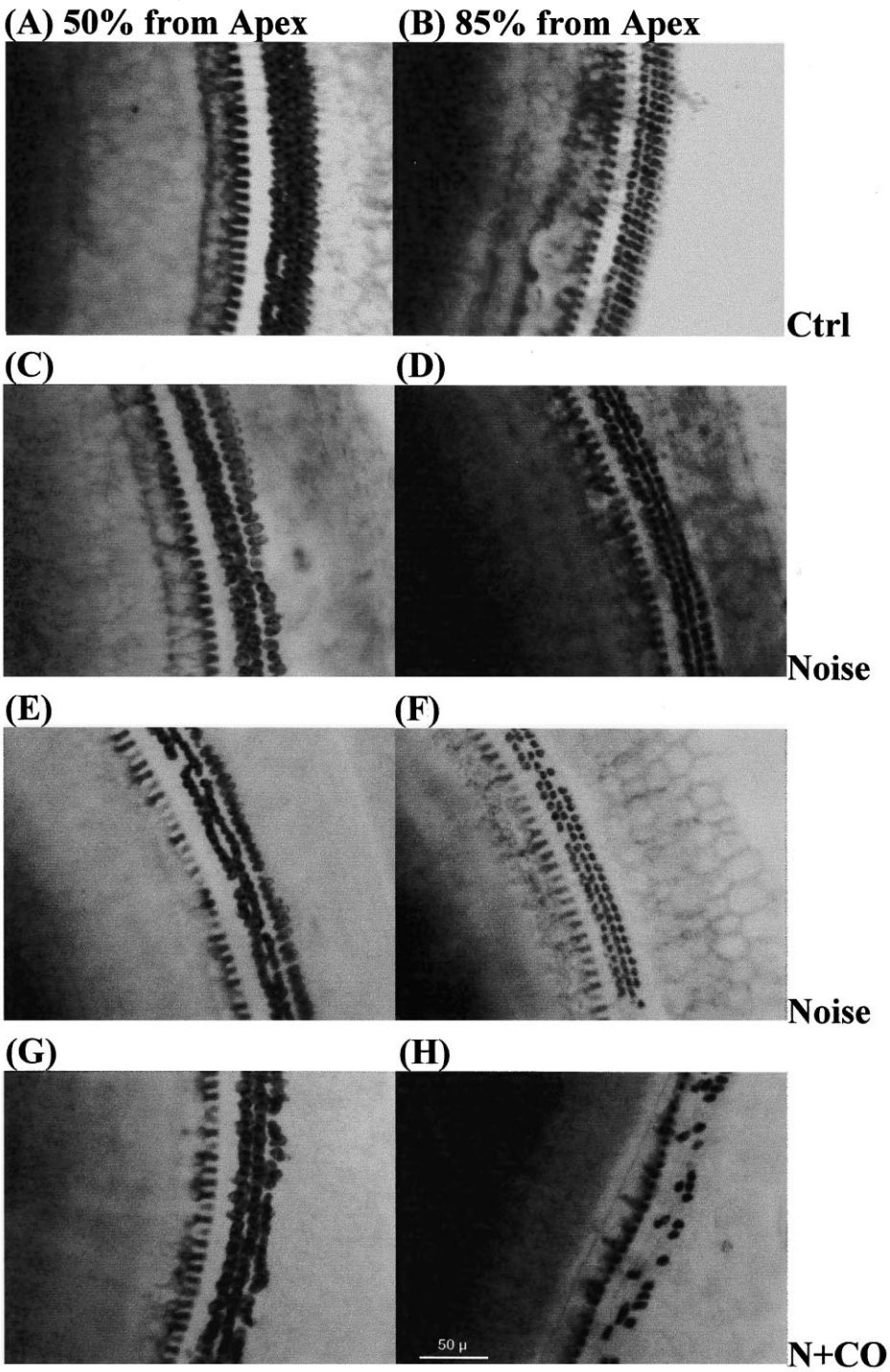

Fig. 1. Examples showing SDH-stained auditory hair cells exposed to noise or noise+CO or air only. Octave-band noise: CF = 13.6 kHz, 100 dB (Ln), 2 h; CO: 1200 ppm, 3.5 h. Left panels: 50% from the apex; right panels: 85% from the apex. Horizontal bar: 50 μm. (A) and (B): Showing SDH-stained auditory hair cells in a control rat. (C) and (D): Showing SDH-stained auditory hair cells in a rat exposed to noise alone but without CAP threshold elevation caused. (E) and (F): Showing abnormal SDH staining in auditory hair cells in a rat exposed to noise alone and with CAP threshold elevation induced. (G) and (H): Showing SDH-stained auditory hair cells in a rat exposed to the combination of noise and CO.

included. The density of SDH staining in the hair cells was normalized to that in the hair cells located at the apical turn since the high frequency noise does not impair threshold sensitivity for low frequencies (Chen et al., 1999).

2.6. Statistical analysis

Statistical analysis was performed using a repeated measures ANOVA (NCSS software).

3. Results

Cochlear hair cells have much higher SDH activity than supporting cells. Therefore the hair cells are easily recognized in the surface preparation stained for SDH activity. Fig. 1A,B are examples to show SDH-stained hair cells in a control subject from the middle (50% from the apex) and basal turns (85% from the apex), respectively. Based on a rat cochlea map developed by Muller (1991), the middle turn at 50% corresponds to the frequency about the center of the octave-band noise (13.6 kHz) and the location at 85% from the apex is about 40 kHz, which is the highest test frequency used in the present study. Fig. 1C and D are examples from similar cochlea locations as Fig. 1A and B in a subject exposed to octave-band noise (CF = 13.6 kHz, 100 dB, 2 h). The noise exposure did not cause either abnormal SDH staining or permanent CAP threshold elevation in this animal. Fig. 1E and F are examples from an animal exposed to the same noise as used in Fig. 1C and D. This animal had threshold elevation up to about 35 dB. Only limited OHC loss was found in the basal turn, though severe decreases of auditory sensitivity began from 12 kHz through the high frequency region (see Fig. 3A, filled circles). However, SDH staining density in this animal varies noticeably among hair cells. The SDH activity even varied between adjacent IHCs (see Fig. 1E). While some IHCs were well stained (heavy staining), others were stained poorly (light). The reduction of SDH activity (reflected by the staining density) may reflect cellular function decrease and may correlate to the impairment of auditory sensitivity. This will be described in detail in the following sections. Fig. 1G and H are examples from a subject exposed to the combination of noise and CO. Abnormal SDH staining in IHCs from the middle turn (Fig. 1G) is also seen as that in Fig. 1E. Losses of OHCs were observed in the basal turn (Fig. 1H). The animal had threshold elevations of about 35–50 dB from 8 kHz to 40 kHz.

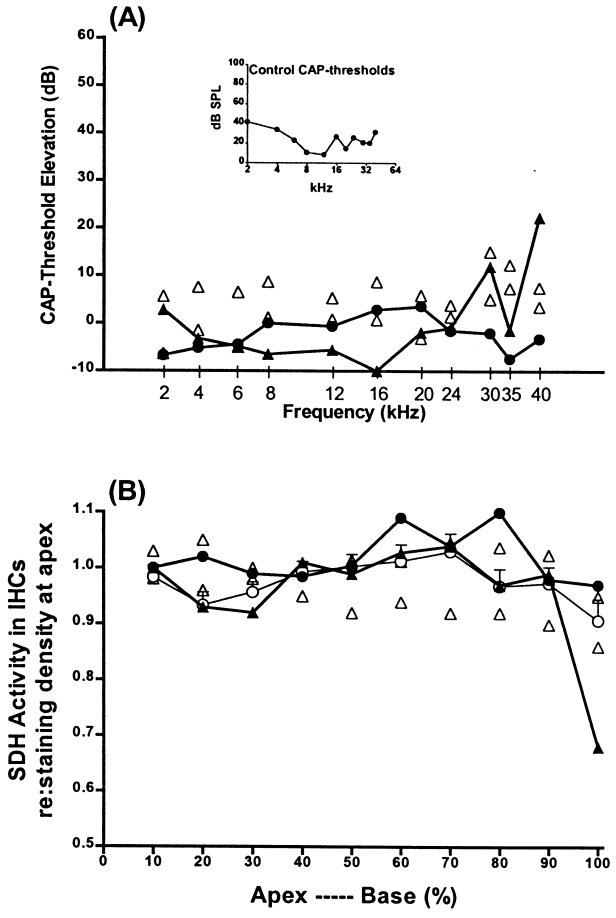

Fig. 2. CAP threshold elevations (A) and normalized SDH activity levels in the IHCs (B) as functions of frequency or basilar membrane length. Filled symbols: two rats exposed to the noise alone, but without noise-induced CAP threshold elevation; open triangles: two rats exposed to CO alone; open circles: control animals ($n = 3$). Insert: CAP thresholds of six control rats. Vertical bars: S.E.M. Noise: octave-banded (CF = 13.6 kHz) at 100 dB_{Lin} for 2 h. CO: 1200 ppm for 3.5 h.

3.1. Correlation between CAP threshold elevations and cochlear histological changes in animals exposed to the noise alone

Subjects exposed to the noise alone showed a highly variable response with some subjects showing no impairment in auditory function while other subjects showed threshold elevations of about 30 dB at frequencies of 12–40 kHz. Minimal or no hair cell loss was seen in these animals examined 4 weeks after the exposure. However, a relationship was seen between SDH activity reduction in hair cells and impaired auditory thresholds in the present report.

Fig. 2 compares CAP threshold elevations (Fig. 2A) to normalized SDH activities in the IHCs (Fig. 2B) in animals with little or no hearing loss (< 10 dB at most frequencies). The CAP thresholds of two rats exposed to the noise alone (filled symbols) and two rats to CO alone (open triangles) are at the control level with threshold elevations distributed around the zero level

(Fig. 2A). The CAP thresholds of six control rats are shown in the insert in Fig. 2A. Corresponding to the small or no CAP threshold elevations, the SDH activity in the IHCs varied less along with the basilar membrane in three control rats (open circles), two rats exposed to CO alone (open triangles) and two rats exposed to the noise alone (filled symbols, Fig. 2B). There is no significant difference between animals exposed to noise, CO, and the controls.

Fig. 3 compares the CAP threshold elevations in two rats exposed to the noise alone and having remarkable noise-induced impairments (Fig. 3A) to the normalized SDH activities in the IHCs (Fig. 3B). Corresponding to the noise band (9.6–19.2 kHz), there was a peak of threshold elevation at 16 kHz. High frequency-tuned hair cells had increased susceptibility to the noise, though the noise did not contain high frequency components above 20 kHz. Differing from the data presented in Fig. 2, the SDH activity in the IHCs in these animals with noise-induced hearing loss varied remarkably along the basilar membrane (Fig. 3B). The SDH activity level in the IHCs decreased towards the basal turn (high frequency). Interestingly, the reduction of the SDH activity in the IHCs seems well correlated with the CAP threshold elevation (comparing Fig. 3B with Fig. 3A). At a location 50–60% from the apex, SDH activity in the IHCs reached the lowest level comparing to other locations, which seems to correspond to the threshold elevation peak at 16 kHz. At locations around 80%, the relative high SDH activity level may correspond to the relatively smaller CAP threshold elevation at 30 kHz. Open circles are the controls. Statistic analysis between SDH measurements of the two noise-exposed animals and that of the control animals shows a significant difference ($P = 0.0018$). The measurement of OHC SDH activity in these animals did not reveal a significant difference from the control level.

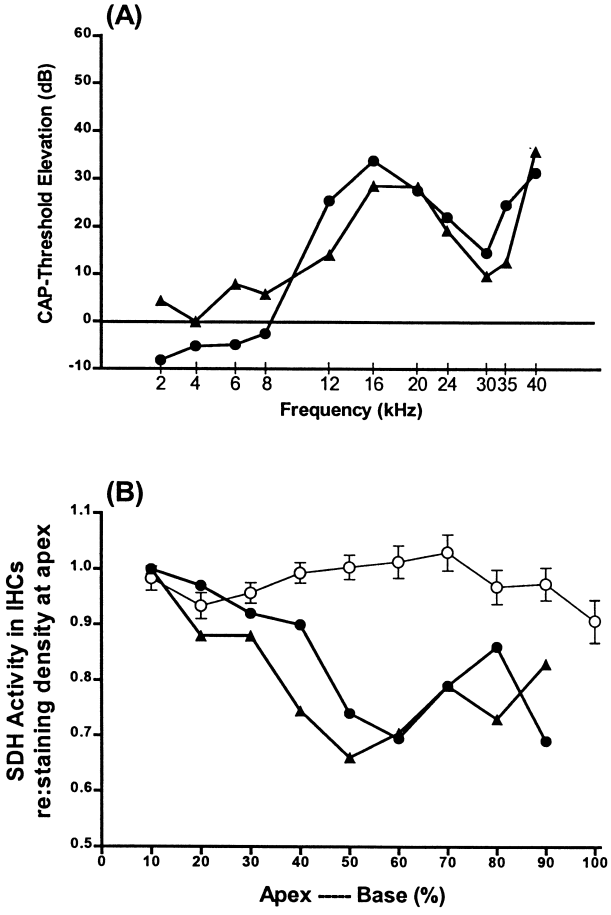

Fig. 3. CAP threshold elevations (A) and normalized SDH activity levels in the IHCs (B) as functions of frequency or basilar membrane. Filled symbols: two rats exposed to the noise alone (13.6-kHz octave-band noise at 100 dB_{Lin} for 2 h); open circles: control animals ($n = 3$). Vertical bars: S.E.M.

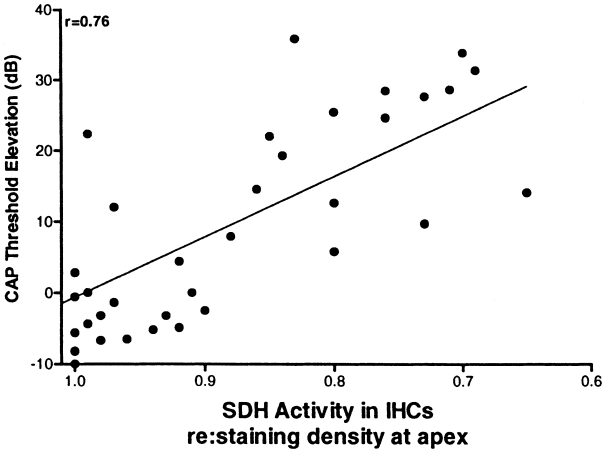

Fig. 4. CAP threshold elevations as a function of normalized SDH activity in IHCs in four rats exposed to the noise alone. Octave-band noise (CF = 13.6 kHz) at 100 dB_{Lin} for 2 h.

In Fig. 4, CAP threshold elevations at different frequencies obtained in the four animals exposed to the noise alone are plotted as a function of normalized SDH activity levels in the IHCs in the corresponding basilar membrane locations. Obviously, the CAP threshold elevation increased with the decrease of SDH activity in the IHCs, indicating a relationship between noise-induced SDH activity reduction in the hair cells and loss of auditory sensitivity. The linear regression line (coefficient = 0.76) predicts that each 10% SDH activity reduction is associated with about an 8.5-dB threshold elevation.

3.2. *Correlation between CAP threshold elevations and cochlear histological changes in animals exposed to the combination of noise and CO*

All four animals exposed to the combination of noise

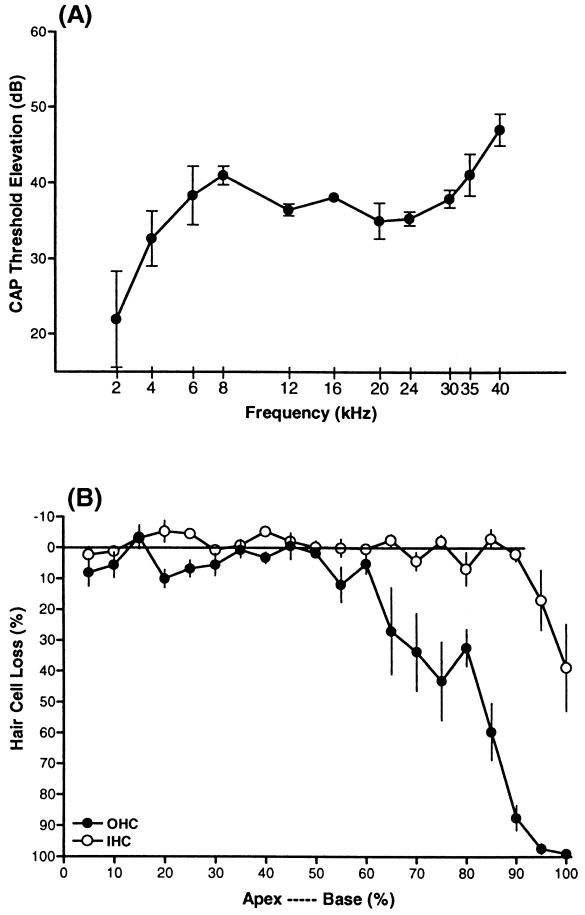

Fig. 5. Losses of CAP threshold and auditory hair cells in rats exposed to the combination of noise and CO. Noise: octave band at 13.6 kHz, 100 dB for 2 h; CO: 1200 ppm for 3.5 h (1.5 h prior to noise onset). Vertical bars: S.E.M. (A) CAP threshold elevations ($n=4$). (B) Losses of hair cells. Open circles: IHCs; filled circles: OHCs. $n=4$.

and CO had a larger CAP threshold elevation than that obtained in the animals exposed to the noise alone (potentiation) as well as a broader loss of CAP threshold sensitivity spreading towards the low frequency region (Fig. 5A). No IHC loss was observed in the animals except at the very basal turn ($>95\%$ of the distance from apex to base, Fig. 5B, open circles). However, OHC losses were observed within the high frequency region beginning at the middle turn (corresponding to about 12–16 kHz). Increased OHC loss was seen towards the basal turn (see Fig. 5B, filled circles). Reduction of SDH activity and abnormal SDH staining in the IHCs were also observed (see Fig. 1G as an example).

Comparing Fig. 5A and B, CAP threshold elevations and the hair cell losses are not well correlated. While both OHC losses and CAP threshold elevations in the high frequency region (>20 kHz) were observed, in the low frequency region, the CAP threshold elevations did not have a corresponding hair cell loss.

CAP threshold elevations in subjects receiving the combined exposure did not follow SDH activity in the IHCs either. The correlation between elevations of CAP thresholds and SDH activity levels in the IHCs was only 0.10, though marked SDH reduction was observed. However, in the low frequency region (2–8 kHz), the CAP threshold elevation (see Fig. 5A) seemed to follow the reduction in SDH activity in the IHCs (see Fig. 6A). When the CAP threshold elevations in this region are plotted as a function of the SDH reduction in the IHCs in the corresponding basilar membrane locations, correlation of 0.57 is seen.

Fig. 6 presents the averaged CAP threshold elevations for the combined exposure to noise and CO as a function of mean reduction of SDH activity in the IHCs (Fig. 6A) and as a function of OHC loss at corresponding locations (Fig. 6B). Mean threshold eleva-

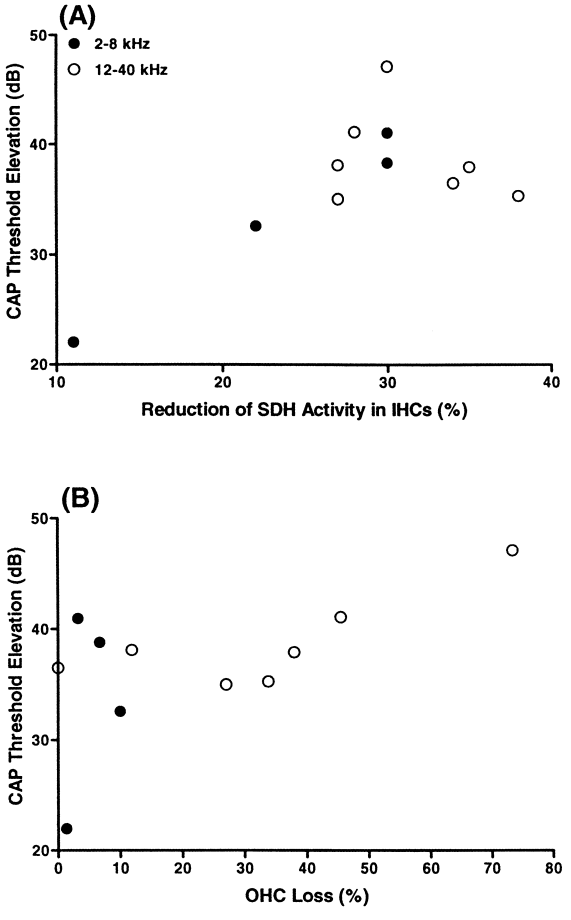

Fig. 6. Mean CAP threshold elevation at each frequency as a function of SDH activity reduction in corresponding IHCs (A) and as a function of OHC loss at corresponding locations (B) in the rats to the combined exposure to noise and CO. Noise and CO exposures are the same as that in Fig. 5.

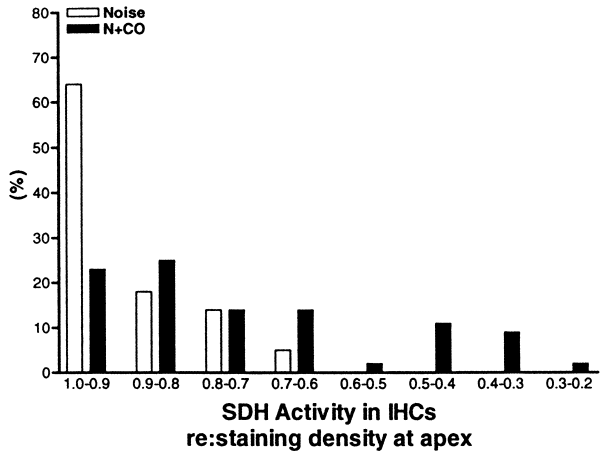

Fig. 7. Distribution of normalized SDH activity in IHCs. Data are obtained at basilar membrane locations corresponding to each test frequency.

tion at low frequencies (2–8 kHz, filled circles) follows the drop in SDH activity level (Fig. 6A) better than the OHC loss (Fig. 6B). Linear regression analysis of the CAP threshold elevations in the low frequency region along with the reduction of SDH activity in the IHCs (Fig. 6A, filled circles, $r = 0.99$) indicates a 9.3-dB increase of the CAP threshold for each 10% reduction of SDH activity in the IHCs. This is similar to that (8.5 dB) seen in the animals exposed to noise alone (see Fig. 4). However, the mean threshold elevations at high frequencies (12–40 kHz, open circles) follow OHC loss (Fig. 6B) better than the SDH activity in the IHCs (Fig. 6A). In Fig. 6B, clearly the CAP threshold elevation (open circle) increases with the loss of OHCs.

3.3. *Histological basis for potentiation of noise-induced threshold elevation by CO*

The combined exposure to noise and CO caused more hearing loss than noise exposure alone (see Figs. 2A, 3A and 5A), and caused significant losses of OHCs, which noise alone and CO alone did not (Chen and Fechter, 1999; Chen et al., 1999, see Fig. 5B). Clearly, the OHC loss may contribute to the extra CAP threshold elevation (potentiation) by the combined exposure to CO and noise at this level.

Fig. 7 compares distribution of normalized SDH activities in the IHCs at locations corresponding to the test frequencies in the subjects exposed to the noise alone (open bars) with those to the combined exposure to noise and CO (filled bars). Obviously, animals exposed to the combination of noise and CO had greater reduction of SDH activity in the IHCs. It seems that the simultaneous CO exposure not only causes more damage to the OHCs (more cell loss), but also to the IHCs (more SDH activity reduction).

4. Discussion

In the present study, rats were observed with hearing loss up to about 35 dB after exposure to a 100-dB octave-band noise alone (13.6 kHz, 2 h), but with few or no hair cell loss. In the animals exposed to the combination of the noise and CO (1200 ppm), though OHC losses were observed in the high frequency region with CAP threshold elevation, no hair cell loss was observed in the low frequency region where CAP threshold elevation was also observed (see Fig. 5A). It is not surprising to see hearing loss without hair cell loss, since hair cell death is only the extreme case of cellular dysfunction and may take many weeks to stabilize. Hair cells may also survive with reduced function. Dysfunction of auditory hair cells may cause reduction of auditory sensitivity. However, it is still unclear whether the reduction in cellular function (SDH activity reduction) will recover to the normal level or whether the dysfunctional cells will die after a longer post-exposure period.

Many reports suggest that OHCs are the primary target for noise trauma. Loss of IHCs occurred only in the cases with great hearing loss (Lurie, 1937; Schuknecht, 1953; Ryan and Dallos, 1975; Stebbins et al., 1979; Hamernik et al., 1989). The data obtained in the present study in the rats exposed to intense noise or to the combination of noise and CO are consistent with the previous finding at this point. CAP threshold elevation up to about 50 dB was observed without IHC loss (see Fig. 5). In one case, even a 75-dB permanent CAP threshold elevation at 40 kHz was observed after combined exposure to noise and 1500 ppm CO for 8 h. All IHCs in the subject still existed on the cochlear surface preparation, though the SDH staining in the IHCs was abnormal (unpublished data).

No hair cell loss does not always mean no impairment occurred in the cells. A 30–40-dB noise-induced hearing loss was observed in rabbit with correlated impairment to the stereocilia of the IHCs, but without hair cell loss (Engstrom and Borg, 1983; Engstrom, 1983; Borg and Engstrom, 1989). The OHCs were frequently left unaltered even when the stereocilia of most IHCs exhibited pronounced alteration (Engstrom, 1983). It seems that in rabbit, the IHC stereocilia are the structures most susceptible to damage by noise (Engstrom, 1983; Engstrom and Borg, 1983). However, in chinchilla, while severe noise-induced damages on OHC stereocilia were seen, the stereocilia on most IHCs looked normal (Boettcher et al., 1992). In rats, the noise-induced moderate hearing loss measured 4 weeks after the exposure seems mainly related to the injury of the stereocilia of the hair cells instead of cell loss (Lataye and Campo, 1997; Lataye et al., 2000).

In the present study, the measurement of SDH activ-

ity in auditory hair cells was used as a marker of cellular dysfunction. SDH is one of the Kreb's cycle enzymes, which is tightly bound to the mitochondria. Any damage to the cell may cause a reduction of cellular energy metabolic level. SDH activity can easily be assessed by a histochemical method.

It has been reported that SDH activity in auditory hair cells decreased after noise exposure (Wang et al., 1990; Zhai et al., 1998). Limited data indicated that the reduction of the SDH activity recovered with the recovery of auditory sensitivity. However, detailed comparison between SDH activity in auditory hair cells and hearing loss (temporary or permanent) has not been documented yet.

The present study provides a detailed comparison between SDH activities in the hair cells and permanent CAP threshold elevations induced by exposure to intense noise. In the animals exposed to 100-dB octave-band noise alone (13.6 kHz, 2 h), permanent CAP threshold elevations less than about 35 dB were observed within the noise band and in the higher frequency region, but with few or without hair cell loss. The loss of CAP threshold was closely correlated to the reduction of the SDH activity in the IHCs (see Fig. 4). Measurement of SDH activity in OHCs in the present study did not reveal a significant difference from the control. It seems that the SDH activity level in the IHCs is a good marker of the cellular functioning and auditory sensitivity.

Auditory sensitivity is dependent upon the functional state of IHCs, OHCs, supporting cells and probably other related structures. If only one kind of cell (such as IHCs) was impaired (or prevalently impaired) by a toxicant, the functional reduction in these cells would correlate to the loss of auditory sensitivity. In the present study, the noise alone may only or dominantly influence the IHCs and influence the OHCs less, such that the CAP threshold elevations are correlated with the SDH activity reduction in the IHCs. When both IHCs and OHCs are impaired, the reduction of auditory sensitivity would be determined by the interaction of them. In a different frequency region, the hearing loss could be caused by different histological impairment in the cochlea. OHCs may survive the noise exposure alone given the level used in the present study (100 dB for 2 h), but many OHCs may be destroyed by the noise exposure when the CO is presented simultaneously. In the high frequency region, the extra damage to the OHCs, that causes OHC loss, by the combined exposure to noise and CO may be the dominant histological basis for the potentiation of the noise-induced hearing loss. In the low frequency region, the combined exposure to noise and CO did not cause loss of OHCs. However, the combined exposure to noise and CO caused more reduction of SDH activity in the IHCs. The more severe reduction of cellular function may account for the potentiation.

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