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Intermittent noise-induced hearing loss and the influence of carbon monoxide

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Abstract

Intermittent noise causes less hearing loss than continuous noise of equal intensity. The reduction in damage observed with intermittent noise may be explained by the fact that the auditory system has time to recover between the noise phases. Simultaneous carbon monoxide (CO) exposure produces greater noise-induced hearing loss than does noise alone (Chen and Fechter, 1999). In the present study, intermittent noise (octave-band with a center frequency of 13.6 kHz, 100 dB) of a 2 h total duration but with a different duty cycle (% of noise during exposure) was used. The intermittent exposure that had a shorter noise duty cycle induced a less permanent threshold shift (PTS) than those that had a longer noise duty cycle (or less rest periods). This relation between the loss in compound action potential (CAP) sensitivity and the noise duty cycle (or rest period) was abolished by the presence of CO. The cochlear microphonic (CM) amplitude revealed similar results to those seen using the CAP. While intermittent noise that had a short noise duty cycle did not cause hair cell loss by itself, the combined exposure to noise and CO (1200 ppm) caused remarkable OHC loss in the basal turn.

Key words: Intermittent noise; Noise-induced hearing loss; Carbon monoxide; Compound action potential; Surface preparation; Rat

1. Introduction

Acoustic overstimulation can damage the cochlea mechanically, especially the hair cell stereocilia, disrupting the micromechanics of the transduction process (see review by Lim, 1986). Overstimulation can also exhaust neurotransmitter storage in the hair cells and cause disruptions to the synapses, at least temporarily (Mulroy et al., 1990). This process may induce ischemia in the cochlea, the generation of free oxygen radical and further damage to the hair cell function (Hu et al., 1997; Liu, 1992; Quirk et al., 1994; Yamane et al., 1995a,b; Yamasoba et al., 1999). Increases of the intracellular calcium concentration have been directly observed in hair cells during intense noise exposure (Fridberger et al., 1998). Damage to hair cell membranes after acoustic trauma and changes in size and shape of hair cells have also been seen (Dew et al., 1993; Mulroy et al., 1998). Traumatic acoustic stimulation can alter the concentration of many proteins or enzymes in a complex function. These proteins and enzymes may play a variety of roles such as the cellular structure of auditory hair cells (F-actin), respiratory processes (succinate dehydrogenase (SDH)), cellular stress (heat shock protein, HSP 72) and free radical scavenging (Lim et al., 1993; Hu and Henderson, 1997; Jacono et al., 1998; Wang et al., 1990). Once hair cell loss occurs, nerve fiber degeneration begins and hearing loss becomes irreversible (see review by Canlon et al., 1998). Recovery of temporary threshold shifts (TTSs) within the first few days may reflect, in part, synaptic repair (Puel et al., 1998). Usually, the damage to the outer hair cells (OHCs) is greater than to the inner hair cells (IHCs). The IHC loss does not appear until the permanent threshold shift (PTS) exceeds approximately 30 dB (Hamernik et al., 1989). TTS may also be mediated via inactivation of mechano-electrical transduction channels at the apex of the OHCs (Patuzzi, 1998).

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Intermittent noise exposures produce less TTS and PTS and less cochlear damage than continuous exposure of equal energy (Campo and Lataye, 1992; Clark et al., 1987; Clark and Bohne, 1992; Fredelius and Wersall, 1992; Patuzzi, 1998). The reduction of the damage can be explained by the fact that the auditory system has time to recover between the noise phases (Campo and Lataye, 1992; Clark and Bohne, 1992).

Carbon monoxide (CO) can reduce oxygen supply to tissues and acute exposure to a high level CO can cause temporary hearing loss at high frequencies (Baker and Lilly, 1977; Fechter et al., 1987; Kowalska, 1980, 1981). CO toxicity is believed to be secondary to tissue hypoxia and may involve free radical generation (Fechter et al., 1997; Makishima et al., 1977). CO exposure, which alone does not induce PTS, can potentiate permanent noise-induced hearing loss (Chen and Fechter, 1999; Fechter et al., 1988; Young et al., 1987) when presented simultaneously with noise. Interestingly, measurements taken shortly after exposure reveal that subjects exposed to noise and CO or noise alone had similar threshold shifts. However, after sufficient recovery time, subjects exposed to noise alone exhibit recovery of threshold while those receiving combined exposure fail to recover the normal auditory threshold (Chen and Fechter, 1999).

If auditory impairment to the combined exposure of noise and CO does not show recovery, the ear will not benefit from the rest periods of the intermittent exposure. Since noise is often presented intermittently, it is important to know the influence of CO exposure on intermittent noise-induced hearing loss. This report addressed this issue and revealed that the rest period-related reduction of the noise-induced hearing loss was abolished by the simultaneous CO exposure.

2. Materials and methods

Forty-eight experimental animals (Long Evans pigmented rats, approximately 2 months of age) were acquired from Harlan Sprague Dawley and housed in the University of Oklahoma Health Sciences Center animal facility. All animal facilities at OU are registered at 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 IACUC serving the University of Oklahoma Health Sciences Center. The background noise level in the colony room is below 50 dB and the room temperature is controlled at 23°C. The light is turned on at 06:30 and turned off at 18:30. Food and water are available at all times.

2.1. Noise and CO exposure

Broadband noise was generated by a function generator (Stanford Research System, Model DS335) and an octave-band noise was obtained by passing the broadband signal through a filter network (Frequency Devices, 9002) that provides a band-pass noise. The center frequency of the octave-band noise was 13.6 kHz (amplitude drops 48 dB/octave at the cutoff frequency). The octave-band noise was amplified with a power amplifier (SAE 2200) and delivered to two speakers (Vifa D25AG-05-06, 709) in the exposure chamber. The animal cages were located under the speakers with a vertical distance (to cage floor) of about 18 cm. The noise intensity was measured with a Sound Level Meter (Quest Electronics) using 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. The intensity of the noise used was 100 dB (Ln). The background noise level in the chambers with no added noise was about 40 dB. The air exchange rate in the exposure chamber was 8.5 l/min (providing for approximately 12 air changes per hour) and the airflow was monitored by a Top Trak 821-1-PS flowmeter. CO gas was metered into the chamber using a microflow valve and the CO level in the chamber was monitored by an Industrial Scientific Sensor and Controller with the nominal CO level used at 1200 ppm. The CO level in the exposure chamber reached a stable level within 1 h, with about 95% of the nominal value achieved within 40 min of exposure onset. 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). The noise was presented intermittently. The total noise period used for all noise-exposed subjects was 2 h. The silence periods varied in different experiments to provide intermittent noise with a 67% noise duty cycle (1 h noise, 1 h silence, 1 h noise), 50% noise duty cycle (40 min noise, 1 h silence, 40 min noise, 1 h silence, 40 min noise) and 40% noise duty cycle (30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise). The CO or air exposure time therefore varied from 4.5 to 6.5 h according to a different noise duty cycle. The rats were divided into groups randomly and exposed to each noise pattern, CO, air and their combinations. The animals were not anesthetized and could move freely in separated cages. During exposure (daytime, inactive phase for rats) food and water were not available.

2.2. Compound action potential (CAP) and cochlear microphonic (CM) recording and analysis

Four weeks after exposure, the animals were anesthe-

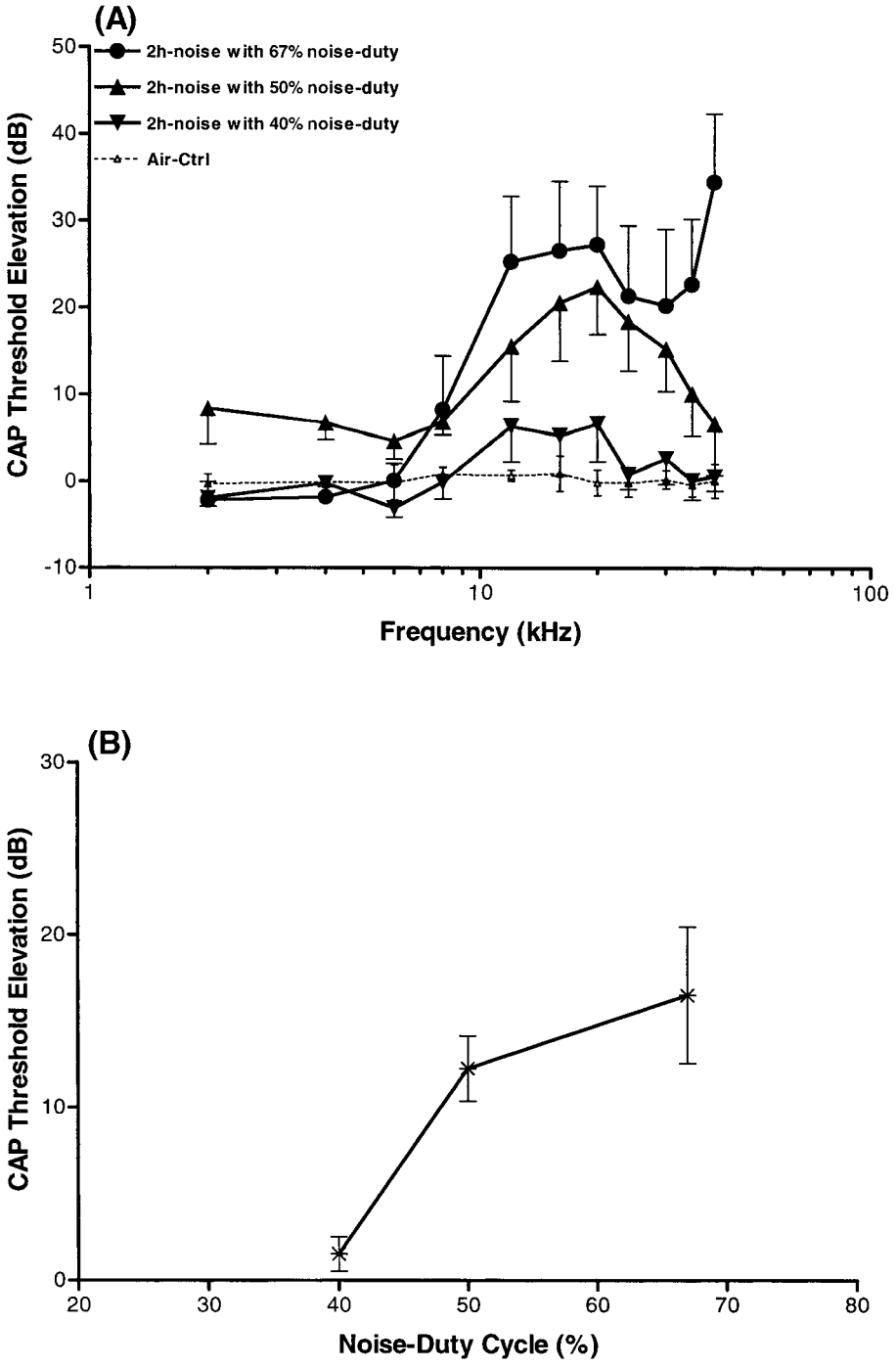

Fig. 1. CAP threshold elevations caused by intermittent noise with a different noise duty cycle. Octave-band noise centered at 13.6 kHz, 100 dB (Ln), 2 h noise presented in a 67% noise duty cycle (●, 1 h noise, 1 h silence, 1 h noise), 50% noise duty cycle (▲, 40 min noise, 1 h silence, 40 min noise, 1 h silence, 40 min noise) and 40% noise duty cycle (▼, 30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise). The rate of air flow was 8.5 l/min. Open triangles and a dotted line represent control levels. Vertical bars are S.E.M. (A) CAP threshold elevations as functions of the test frequency. $n = 6$ animals for each group. (B) Mean noise-induced CAP threshold elevations to each noise duty cycle averaged across six subjects and 11 frequencies.

tized with xylazine (13 mg/kg, intramuscular i.m.) and ketamine (87 mg/kg, i.m.). The round window 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 and CM. A silver chloride electrode was placed in the neck muscle as the reference. The CAP and CM signals were amplified with a Grass A.C. pre-

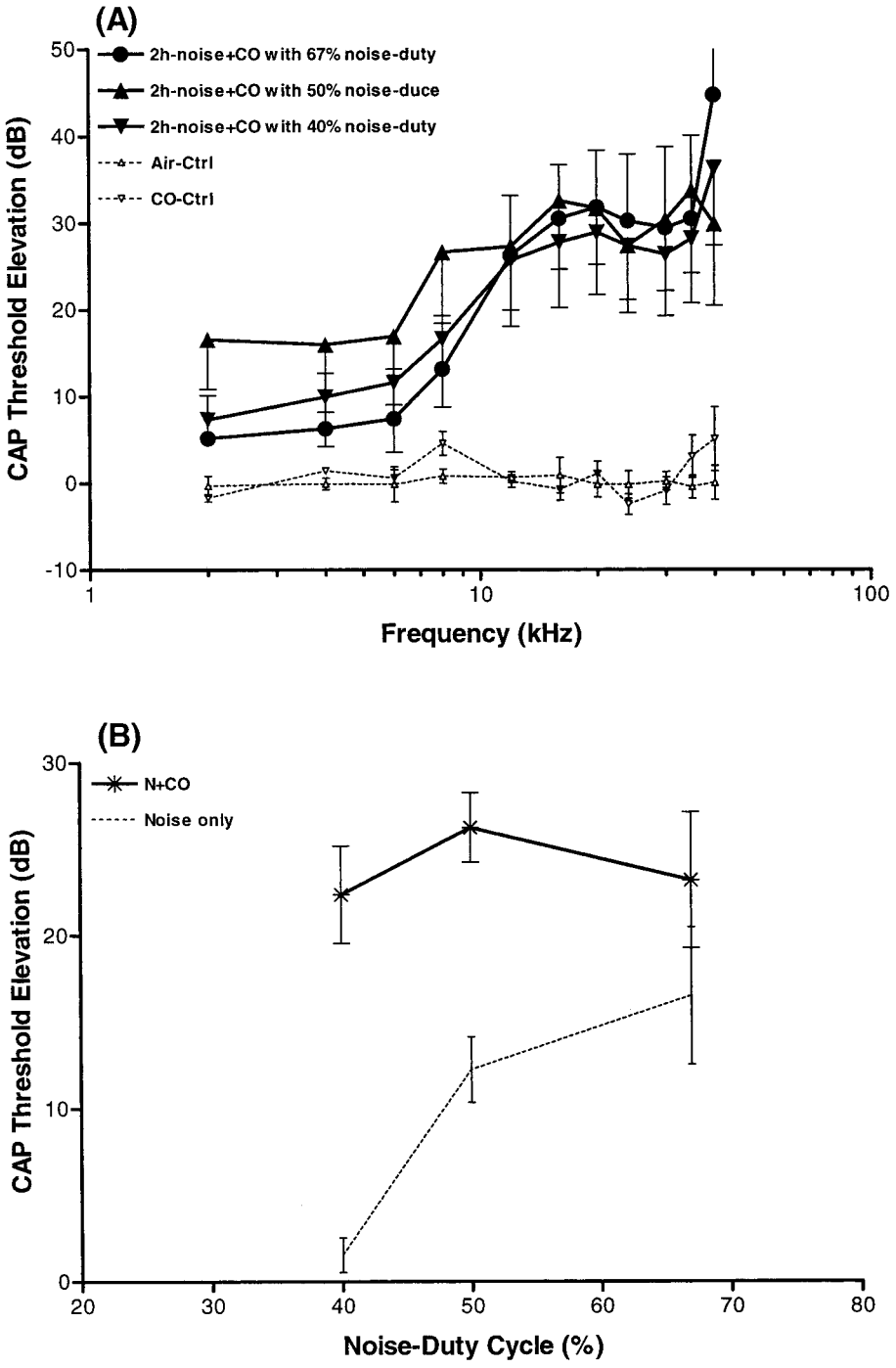

Fig. 2. CAP threshold elevations caused by combined exposure to intermittent noise and CO. The intermittent noises are the same as in Fig. 1. The CO level in the exposure chamber was 1200 ppm. CO was turned on 1.5 h prior to the onset of the noise and kept constant throughout the experiment (noise phase and silence phase). Lab air is 8.5 l/min. Open triangles and inverse triangles represent control levels and CAP threshold elevations to CO exposure alone. Vertical bars are S.E.M. (A) CAP threshold elevations as functions of the test frequency. $n=6$ animals for each group. (B) Mean noise-induced CAP threshold elevations to each noise duty cycle averaged across six subjects and 11 frequencies. Dotted line from Fig. 1B.

amplifier (model P15). The gain was 1000. The bandpass frequency for CAP was 0.1–1.0 kHz and 0.1–50 kHz for CM. The CAP signals were observed using a digital oscilloscope (Nicolet Instrument, 2090-IIIA). The CM signals were sent to a SR530 Lock-in amplifier (Stanford Research Systems) and then to a PC computer for automatic determination of the 1 μV RMS amplitude. 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 fre-

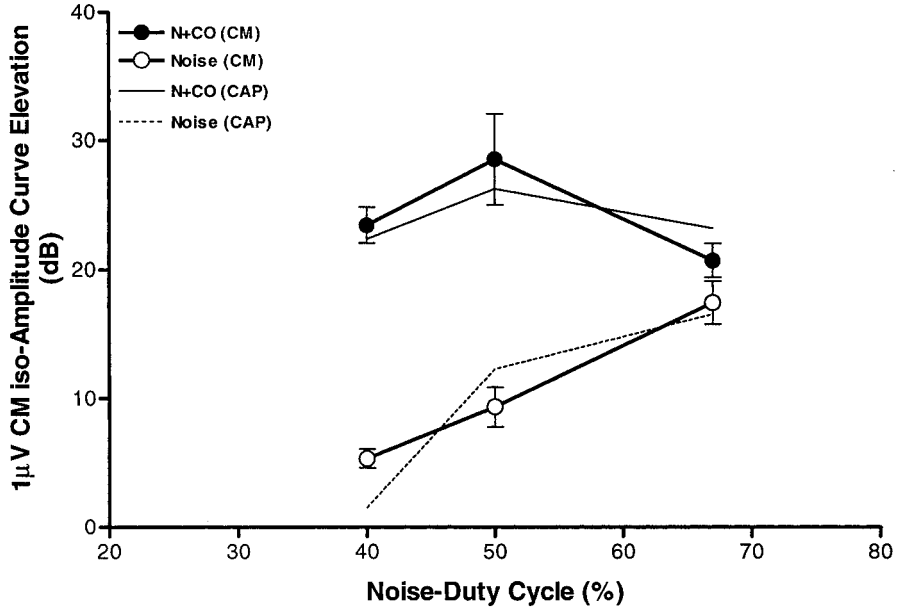

Fig. 3. Intermittent noise-induced elevations of 1 μV RMS CM iso-amplitude curve. The data are averaged across six animals and 11 test frequencies. Open circles represent the data from subjects exposed to noise alone. The elevation value varies with the noise duty cycle. The dotted line represents CAP data for subjects exposed to the noise alone (see Fig. 1B). Filled circles represent the data from subjects receiving the combined exposure to noise and CO. The solid line represents CAP data to the combined exposure (see Fig. 2B).

quency. The sound levels that evoked 1 μV RMS CM at each test frequency were determined by the computer program and the 1 μV RMS CM iso-amplitude curve was evaluated. CAP threshold elevation was calculated as the difference between each CAP threshold for each treated subject and the mean control value (from subjects exposed to air alone) at the same frequency. CM iso-amplitude curve elevation was obtained in a similar way.

Pure tones for eliciting CAP and CM were generated with the SR530 Lock-in amplifier (Stanford Research Systems). 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 fits into the exposed external auditory meatus. The frequencies of the tones were 2, 4, 6, 8, 12, 16, 20, 24, 30, 35 and 40 kHz. Continuous tones were used for eliciting CM. Tone bursts were used for CAP-eliciting. 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.

Fisher's least square difference (Super ANOVA software) was used to evaluate the overall significance between different noise exposures and test frequencies and their interactions. The frequency was evaluated as a within subject variable and the treatment group was a between subject variable.

2.3. Histology

The animals deeply anesthetized were decapitated after CAP and CM recording. Cochleae were removed immediately. Both the round and oval windows were opened and the apex of the cochlea was drilled open to facilitate perfusion. The cochleae were perfused with SDH incubation 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 were obtained with the Optimetic Image system. Hair cell counting and the measurement of the basilar membrane were achieved using Scion Image software. All hair cells, more or less stained, have been counted. Hair cell missing is easy to be identified with a scarred basilar membrane.

3. Results

3.1. Hearing loss induced by intermittent noise exposure

In this experiment, subjects received a total of 2 h noise exposure (100 dB SPL octave-band noise centered at 13.6 kHz) and were permitted a 4 week recovery

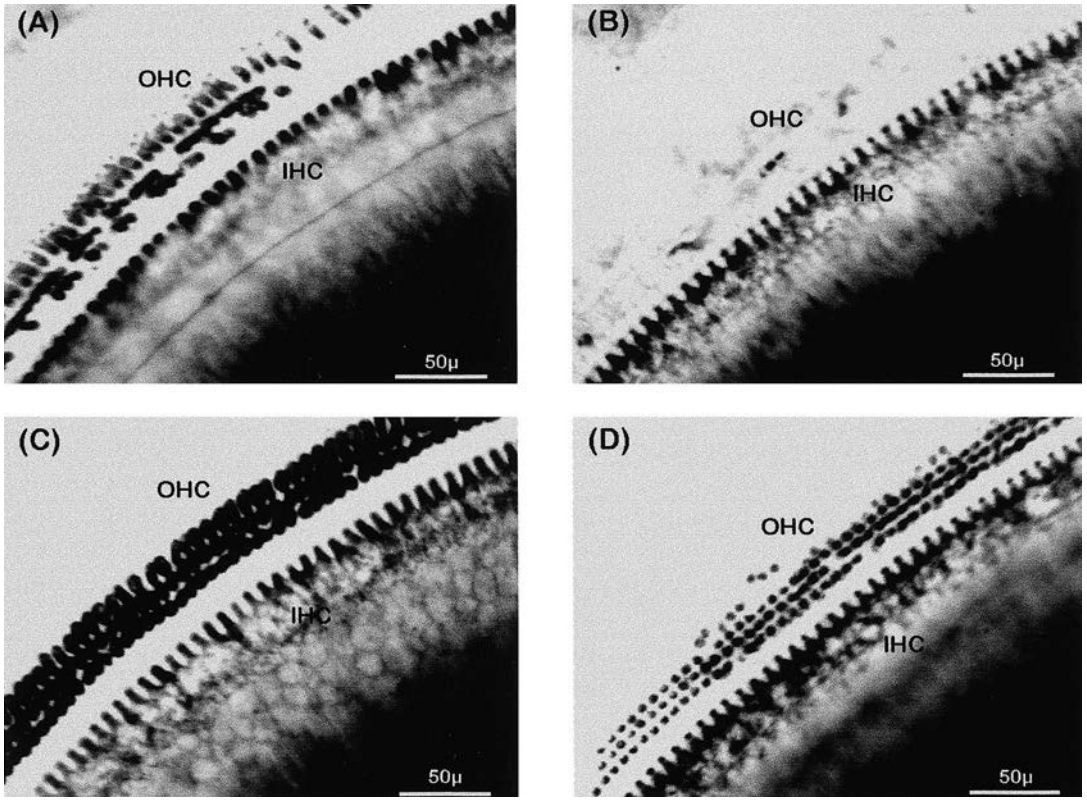

Fig. 4. Sample surface preparations by SDH staining showing OHC loss to the combined exposure and normal hair cells to the noise alone. Octave-band noise centered at 13.6 kHz, 100 dB (Ln), 2 h noise duration presented in 40% noise duty cycle (30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise). Horizontal bars represent 50 μm length. (A) Section located 58% to the apex (about 18 kHz) to the combined exposure of noise and CO (1200 ppm). (B) Section located 85% to the apex (about 35 kHz) to the combined exposure of noise and CO (1200 ppm). (C) Section located 55% to the apex to the noise alone. (D) Section located 87% to the apex to the noise alone.

period. CAP thresholds were compared between groups. Fig. 1 portrays the loss of CAP sensitivity that results from different noise duty cycles compared to animals receiving no noise exposure. Subjects that received 2 h noise exposure with a 67% noise duty cycle (1 h noise, 1 h silence, 1 h noise) had about a 25 dB threshold elevation across the frequency region of 10–40 kHz (Fig. 1A, filled circles). The animals that received noise exposure with a 50% noise duty cycle (40 min noise, 1 h silence, 40 min noise, 1 h silence, 40 min noise) showed CAP threshold elevations that averaged 15 dB above 10 kHz (Fig. 1A, filled triangles). The subjects that received noise exposure with only a 40% noise duty cycle (30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise) had only an average of 3 dB threshold elevation across the high frequency region (Fig. 1A, filled inverse triangles). Thus, the permanent hearing loss resulting from octave-band noise was less severe as the proportion of silence periods increased. Repeated measures ANOVA showed a significant difference in CAP threshold elevations between the four groups receiving different intermittent noise exposure or no noise (between treatment, $F_{[3,20]} = 5.48$, $P = 0.0065$; between frequency, $F_{[10,200]} = 12.63$, $P = 0.0001$; frequency-treatment interaction, $F_{[30,200]} = 4.62$, $P = 0.0001$). The CAP threshold elevations of 67 and 50% noise duty cycle groups (filled circles and triangles) were significantly different from the control group ($P = 0.003$ and $P = 0.02$, respectively) and from the 40% noise duty cycle group ($P = 0.006$ and $P = 0.039$, respectively), but the threshold elevations of the 40% noise duty cycle group (filled inverse triangles) were not different from the control group.

Fig. 1B presents the averaged CAP threshold elevations across animals and all frequencies in the three noise-exposed groups to show the relation between threshold elevation and the noise duty cycle. The mean CAP threshold elevation (±S.E.M.) is plotted as a function of the noise duty cycle. Clearly, the threshold elevation is smaller in the exposure with a shorter noise duty cycle than those of the exposure with a longer noise duty cycle. The data replicate the finding that introduction of silent periods during noise exposure can reduce or prevent auditory impairment from noise exposure.

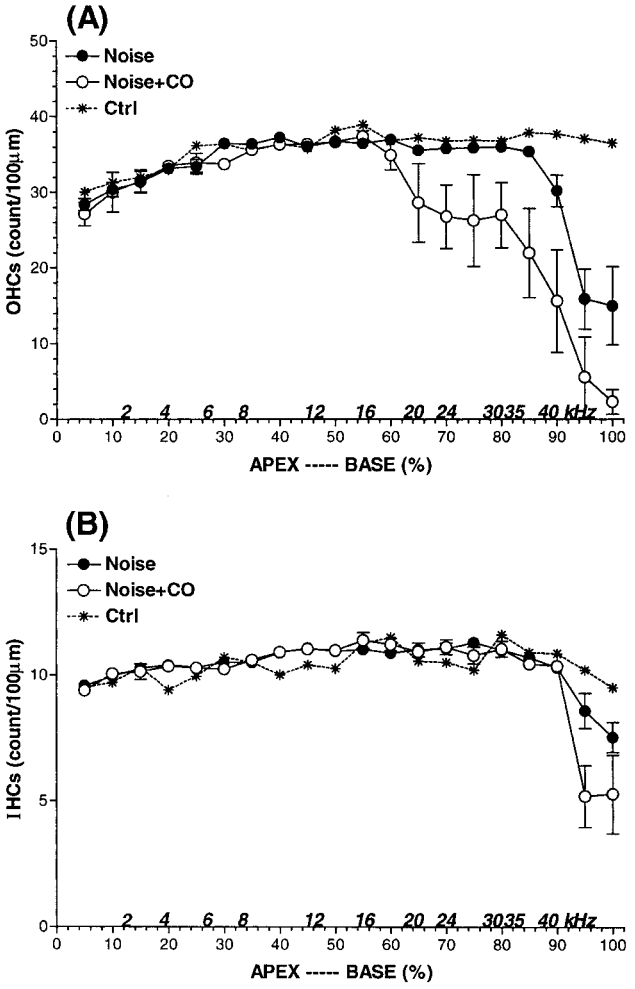

Fig. 5. Hair cell number per 100 μm length as functions of the basilar membrane length (in %). Filled symbols represent averaged data from animals exposed to the intermittent noise alone (30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise, $n=4$ animals). Open symbols represent averaged data from animals to the combined exposure of the noise and CO (1200 ppm, $n=4$ animals). Asterisks are control data. Vertical bars are S.E.M. The approximate frequency encoded at each portion of the cochlea (based on Muller, 1991) is indicated along the X axis. (A) OHCs, (B) IHCs.

3.2. Intermittent noise-induced threshold elevations in the presence of CO

Fig. 2 represents the effect of the same intermittent noises as used above in animals that were simultaneously exposed to noise and to CO. Subjects in all groups exposed to the combination of noise and CO had obvious threshold elevations (Fig. 2A, filled symbols), but the CO alone did not cause threshold elevation (open inverse triangles). Repeated measures ANOVA showed a significant difference in CAP threshold elevations between five groups receiving exposures to noise plus CO, CO alone and air alone (between treatment, $F_{[4,25]}=4.76$, $P=0.0054$; between frequency, $F_{[10,250]}=1.48$, $P=0.15$; frequency-treatment interaction, $F_{[40,250]}=1.26$, $P=0.15$). However, differing from threshold elevations caused by intermittent noise alone, the threshold elevations to the combined exposure of noise and CO did not significantly change with intermittent noise. All three groups of subjects receiving combined exposure to noise and CO had equivalent high threshold elevations, though the silence periods between noise phases varied in the exposures.

Fig. 2B presents the averaged CAP threshold elevations across animals and frequencies in the three groups of the combined exposures (asterisk) to show the relation between the threshold elevation and the noise duty cycle. Unlike the data from the animals to the intermittent noise only (dotted line, copied from Fig. 1B), the threshold elevations with combined noise and CO were much greater and independent of noise duty cycle. The greater threshold elevation with combined treatment indicates the potentiation of noise-induced hearing loss by CO. Importantly, the data show that an intermittent noise exposure that causes very small hearing loss in an air environment can produce a severe hearing loss in the presence of CO. The independence of the threshold elevation from the noise duty cycle indicates that the rest period between noise phases cannot protect the ear from noise-induced damage in the presence of CO.

3.3. Intermittent noise-induced elevation of the CM iso-amplitude curve

CM mainly reflects the electrical activity of OHCs. In normal animals, the 1 μV CM iso-amplitude curve is similar in shape to the CAP thresholds across frequencies. Similar to the CAP thresholds, a noise duty cycle dependent elevation was produced for the CM iso-amplitude curve. With a decrease of the noise duty cycle of the intermittent noise exposure, the elevation became less (Fig. 3, open circles). While the noise duty cycle decreased from 67 to 40%, the mean CM curve elevation (across animals and frequencies) decreased about 12 dB. This is very similar to the decrease of the CAP threshold elevation (about 15 dB, dotted line). Similar to the CAP threshold elevations (solid line), the noise duty cycle dependence on elevation of the CM iso-amplitude curve was abolished by the presence of CO (Fig. 3, filled circles). The similarity between the CM and CAP data suggests that the OHC might be the site that is impaired by combined exposure to noise and CO.

3.4. Intermittent noise-induced hair cell loss and the CO influence

Hair cell loss in the animals exposed to the intermit-

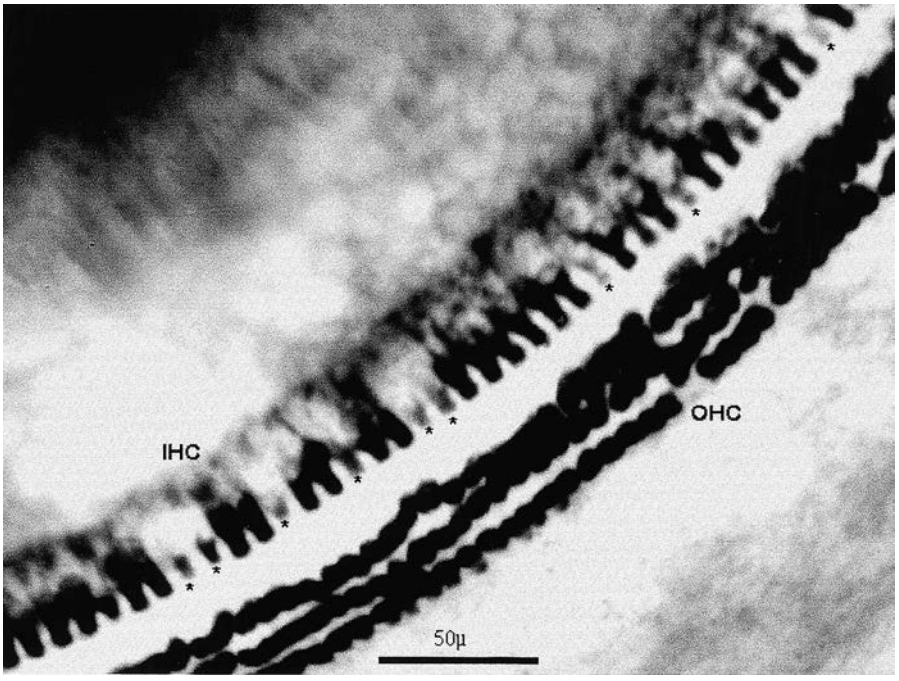

Fig. 6. Example of hair cells with frequencies (about 12 kHz) within the noise band (9.6–19.2 kHz). Lighter stained IHCs can be seen and are marked by stars.

tent noise of the shortest noise duty cycle (30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise, 1 h silence, 30 min noise) with or without CO exposure was compared, since these two groups showed the greatest difference in auditory function (see Figs. 1 and 2, filled inverse triangles). The animals exposed to the noise alone did not show evident hair cell loss in any region of the test frequencies. However, the animals exposed to the combination of noise and CO showed OHC loss in the high frequency region. Hair cell loss was not found in any of the animals close to the cochlea apex (low frequencies).

Fig. 4 presents four examples to show this difference. Fig. 4A,B is from an animal that received simultaneous noise and CO exposure. Fig. 4A shows OHC loss, especially in the first and second rows of a section from the basilar membrane located about 58% to the apex (about 18 kHz according to Muller, 1991). Fig. 4B is from a location much closer to the round window (85% from apex corresponding to a frequency of about 35 kHz). At this location, all OHCs were gone, while the IHCs were still present. Fig. 4C,D is from the similar basilar membrane locations as A and B, respectively, in an animal exposed to the noise alone (55 and 87% to the apex, respectively). Hair cell loss did not occur in C and only a few cells were lost in the high frequency region (D). Usually, the first row of OHCs was more susceptible to the noise damage.

Fig. 5 presents cytocochleograms from the subjects exposed to the intermittent noise alone and those receiving the combined exposure of noise and CO. Fig. 5A shows the averaged number of OHCs. Each point represents the number of cells present in the three rows at the corresponding location. Clearly, the animals receiving the combined exposure to noise and CO (open circles) show pronounced OHC loss in the high frequency region in comparison with control animals (asterisks). The animals to noise alone (filled circles) did not show obvious OHC loss in the region of the test frequencies. In the region very close to the round window, hair cell loss was seen. No animals in the experiment show IHC loss, except in the region above the test frequency (Fig. 5B).

Though CAP threshold elevation was seen within the noise band, hair cell loss was not. The hair cell staining was dependent on the activity of the SDH in the cells or in other words, on the respiratory level of the cells. Interestingly, hair cells showing lighter staining were observed in the frequency region beginning from the region showing hearing loss in many cases. In the frequency region corresponding to the noise band, the lighter-stained cells were often seen among the heavy-stained cells. Fig. 6 shows an example in an animal to the combined exposure of noise and CO. The section was taken from the basilar membrane 45% to the cochlea apex that corresponded to a frequency (12 kHz) within the noise band. The lighter-stained cells are marked with stars. The lighter staining may indicate a lower energy metabolism level of the cells, even though the cells still survive. This phenomenon was not ob-

served in the low frequency region and was more often seen in animals exposed to combined exposure of noise and CO than in animals exposed to noise alone. The longer the duration of continuous exposure of noise, the staining difference of the hair cells is more prominent. This will be reported separately.

4. Discussion

Intermittent noise exposure induces less threshold shifts and less hair cell loss than continuous noise exposure of equal intensity (Campo and Lataye, 1992; Clark et al., 1987; Clark and Bohne, 1992; Fredelius and Wersall, 1992; Patuzzi, 1998). The decreased damage could be due to the recovery periods between noise phases (Campo and Lataye, 1992; Clark and Bohne, 1992). It is well known that multiple processes are disrupted during noise exposure and that recovery processes may be dependent upon an energy supply, since synthesis of many substances may be involved. Providing the ear with extra oxygen can protect the ear from noise-induced hearing loss (Hatch et al., 1991; Hu et al., 1991). Combined exposure of noise and CO induces a much greater hearing loss than noise alone (Chen and Fechter, 1999; Fechter et al., 1988). CO can reduce the oxygen supply to the cells and possible interrupt with oxidative metabolism. If the CO exposure reduces or abolishes the recovery process during the rest periods between noise phases, these rest periods will no longer be of any benefit to the ear and hearing loss will persist. On the other hand, if the combined exposure induces some cellular damage that cannot be recovered, the rest periods during the noise phases will also not be of any benefit. We examined intermitted noise-induced auditory impairment and the effects of CO exposure on the noise-induced impairment via electrophysiological and histological methods.

In the present study, the relationship between the threshold elevation and the noise duty cycle has been demonstrated. The noise-induced threshold elevations varied with the noise duty cycle of a 2 h noise exposure with a lower noise duty cycle (or longer silence periods between noise phases) corresponding to less threshold elevation. It is still unclear whether it is related to the number of intermittent silence periods or to the total rest time during the exposure. More data are needed to answer this question.

This study also evaluated the effect of CO exposure on the noise-induced impairment. Our previous report showed that animals exposed to continuous noise and CO had little recovery of function compared to continuous noise alone and therefore maintained an elevated threshold (Chen and Fechter, 1999). However, under these conditions, if noise alone did not generate a significant threshold elevation, the combined exposure of noise and CO did not induce a significant threshold elevation (Chen and Fechter, 1999). When the noise is presented in an intermittent condition, an exposure of noise alone that again shows no significant effect, now in the presence of CO, can generate threshold elevations equal to those observed under much more severe conditions. Therefore, simultaneous CO exposure abolished the relationship between threshold elevation and the noise duty cycle. The threshold elevations to the combined exposure of noise and CO were higher than that to the noise alone in any group and independent of the noise duty cycle. The elevation of CM iso-amplitude curves was similar to the corresponding CAP threshold elevations, indicating that changes in cochlear function might occur at the OHC level or at some level that may influence both the CM and CAP. Elevation of the CM iso-amplitude curve was dependent on the noise duty cycle. Exposure to combined exposure to noise and CO caused more elevation than to noise alone.

The histological study found predominant OHC loss in the high frequency region but little to no loss of IHCs. The location of maximal damage on the basilar membrane was reported to be consistent with the noise frequency in some reports (Eddins et al., 1999; Fredelius and Wersall, 1992), but discrepancies were also observed, especially at a high noise level, in other cases (Borg et al., 1992; Campo and Lataye, 1992). In this report, threshold elevations to low noise duty cycle exposure may be limited in the frequency region of the noise band. When the noise duty cycle increases or to the combined exposure of noise and CO, the damaged area expanded to high frequencies. Though the threshold elevations were observed in the frequency region of the noise band, OHC loss rarely happened in the region corresponding to the noise band. This indicates that normal histology does not always represent normal functionality of the hair cells. It was often observed that staining of the cells (SDH activity dependent) became lighter towards the frequency region of the noise band and higher frequencies. Some IHCs within the noise band appeared to have light staining, though no cell loss was observed.

The CO level used in these experiments, 1200 ppm, is high when compared to permissible exposure levels to this pollutant either in the ambient or work environment. In the USA, the Environmental Protection Agency permits ambient exposure levels of 9 ppm averaged over 24 h and 35 ppm averaged over 1 h. For work environments, the standards proposed by ACGIH and by OSHA are 50 ppm averaged over an 8 h work day, with a peak level of 200 ppm. Fechter et al. (in press) have shown a linear relationship between the CO concentration and the extent of potentiation of noise-induced hearing loss with statistically significant differ-

ences in threshold elevation observed with 500 ppm CO exposures and higher when continuous 8 h noise and CO exposures are used. The theoretical 'no adverse effect' CO level predicted by linear regression analysis for such a continuous exposure lies between 200–400 ppm CO in rats for an 8 h exposure (Fechter et al., in press). In developing permissible exposure standards for humans, it is common to determine in a laboratory animal model an estimate of the lowest exposure dose that might produce adverse effects (e.g. Ohanian et al., 1997; Slikker et al., 1996). This 'benchmark' level is then adjusted by a safety factor designed to provide sufficient protection knowing the uncertainties involved in extrapolation of data across species, extrapolation from sub-chronic to chronic exposures and extrapolation to particularly sensitive groups within the population such as the elderly or very young. It is common, for example, to adopt a safety factor of 10 in setting permissible human exposures based upon laboratory animal data (cf. Slikker et al., 1996). In the current experiments, we have seen robust potentiation of hearing loss induced by intermittent noise exposure when simultaneous CO is present. The magnitude of the threshold elevation seen in subjects exposed to intermittent noise and CO is similar to that observed when continuous 8 h noise exposure and CO is employed (Fechter, et al., in press). Assuming that an equivalent potentiation of noise-induced hearing loss by CO occurs with continuous and intermittent noise, we would predict statistically significant potentiation with intermittent noise and CO concentrations of 500 ppm. A theoretical no effect level would lie in the range of 220–400 ppm CO. When modified to account for a possible greater susceptibility of people compared with rats, we obtained a reference dose of 22–50 ppm (depending upon whether one extrapolates a no effect level or accepts a statistically significant lowest adverse effect level). Thus, using currently accepted standards for assessing risk, human CO exposures are approximately at or somewhat above the reference dose required to maintain safety from the potential of NIHL by CO based on the current findings.

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