Final Report
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"Models for Assessing Risk of Occupational Hearing Loss"

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Abstract

The research undertaken under this grant was designed to determine the ability of the chemical asphyxiants, carbon monoxide and cyanide, to potentiate noise induced hearing loss. Such mixed exposure to noise and asphyxiant can be predicted to occur in a number or workplace settings such as those that firefighters and truck drivers experience. The results of experiments conducted in rats demonstrate that these asphyxiants do potentiate noise induced hearing loss in a concentration dependent fashion and with an estimated lower bound benchmark concentration of approximately 200-300ppm. Asphyxiants may also increase vulnerability under conditions of intermittent noise exposure. A probable mechanism responsible for potentiation of noise induced hearing loss by asphyxiants is enhancement of free radical species with subsequent tissue damage.

Significant findings:

1. Carbon monoxide potentiates noise induced hearing loss (NIHL)
2. The lower bound benchmark dose for such potentiation lies between 200-300ppm CO
3. Carbon monoxide disrupts the relationship between increasing noise duty cycle for intermittent noise and increasing auditory impairment
4. The potentiating effect of CO on NIHL can be reduced or blocked by administration of the free radical scavenger PBN but not by the glutamate receptor blocker, MK-801
5. Direct measurement of free radicals in cochlear tissue shows a significant increase in free radicals with combined exposure to CO and noise, but no measurable change above baseline for CO and for noise given individually
6. Hydrogen cyanide produces a dose dependent increase in NIHL

Usefulness of findings

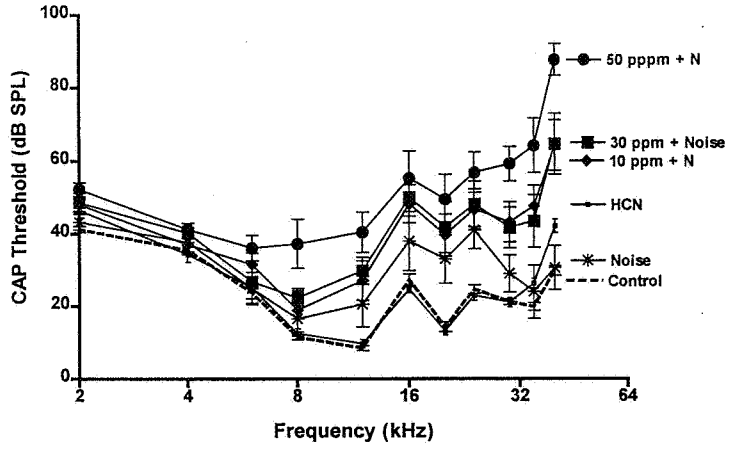
These findings should provide the Institute with important new data for conducting risk assessment analysis for workers exposed to noise and chemicals in the workplace

These findings identify mechanisms responsible for potentiation of NIHL and suggest chemical agents that are also likely to promote NIHL.

The specific aims were to determine

1. whether the potentiation of NIHL by chemical asphyxiants is more likely to occur under specific exposure conditions (with respect to dose and duration) and whether the equal energy principle and time weighted averaging offer adequate protection.
2. whether potentiation of NIHL by CO and HCN is influenced by the frequency characteristics of the noise.
3. whether pharmacological agents that block NMDA receptors and/or impair free oxygen radical formation protect against the potentiation of NIHL by chemical asphyxiants.

Figure 1



SPECIFIC AIM 1: How do asphyxiant dose and noise intensity/patterning affect potentiation?

We have focused most of our research on exposures to 2 hr 100 dB (lin) OBN with center frequency of 13.6 kHz and 1200 ppm CO based upon outcomes of some key initial experiments. We adopted the 13.6kHz OBN in large part because it produced auditory impairment restricted to relatively high frequencies providing us with confidence in the validity of our measurement of high frequency impairment when low-mid frequency auditory function was normal in exposed subjects. Based upon a 5dB trade-off for doubling noise duration, the 100dB 2 hr exposure would be equivalent to 90dB for 8 hr—matching OSHA exposure limits.

Potentiation of NIHL by asphyxiants increases with increasing concentrations of CO and HCN

Parallel experiments have demonstrated more potentiation of NIHL with higher concentrations of HCN and CO. HCN exposures of 2 hr duration, at exposure levels as low as 10ppm potentiate NIHL significantly with even greater effect observed with 50ppm HCN for 2 hr. (fig 1). Ten ppm HCN is the 8 hr PEL established by OSHA. CO exposures of 500ppm and greater for 8 hr significantly potentiate NIHL (fig 2). Benchmark dose software (version 1.1b) published by the USEPA, permit us to extrapolate downward to a CO concentration having a small but appreciable potentiation of NIHL. Fitting a linear function to the data with this software, allows us to determine the intersection of the lower bound of the confidence interval about this regression line representing a 5 dB potentiation of NIHL. The data show (fig 3A) that the lower bound for this benchmark lies at approximately 320 ppm. Selecting as a benchmark a 10% elevation in threshold above the effect of noise alone produces a lower bound benchmark dose of 195 ppm CO (fig 3B). This 10% criterion has been applied by others in undertaking risk assessment. Risk assessment models that have been used in both neurotoxicology and general toxicology commonly select a safety factor of 10 as a default in extrapolating between species. This approach suggests that a "safe" CO level with respect to potentiation of NIHL would be 20-32 ppm—somewhat *lower* than the current PEL under OSHA and ACGIH guidelines (50ppm). The primary uncertainty factors here are contributed by the small number of subjects that can necessarily be tested (thus yielding variability in measurement) and the variability that is an inherent feature of noise exposure.

Figure 2

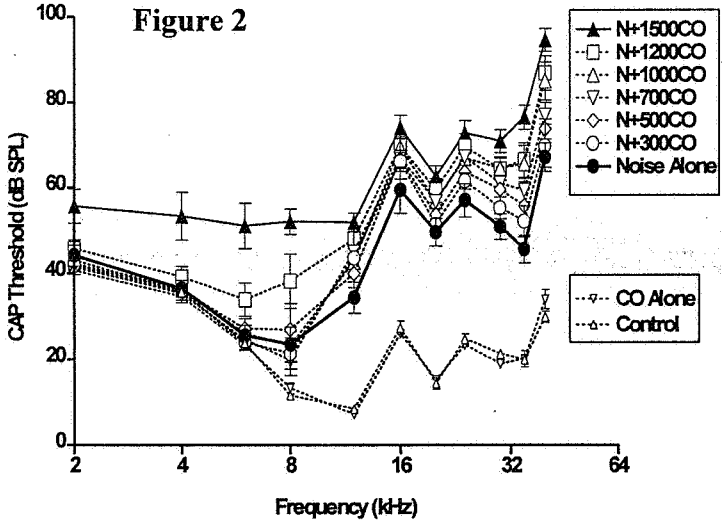


Figure 3A

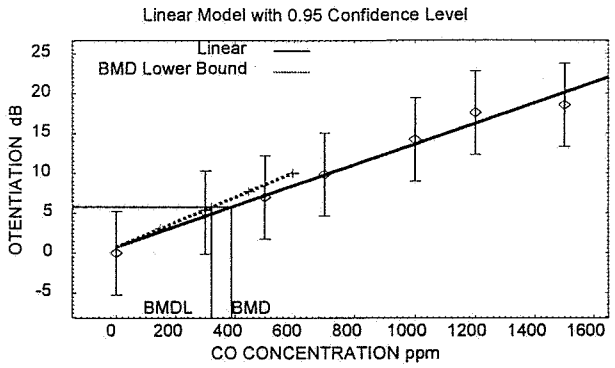


Figure 3B

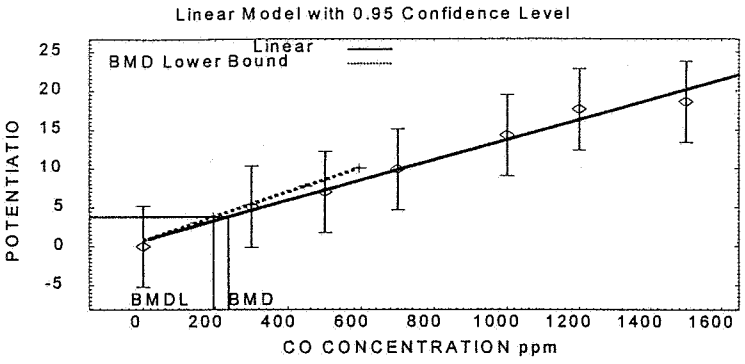


Figure 4A

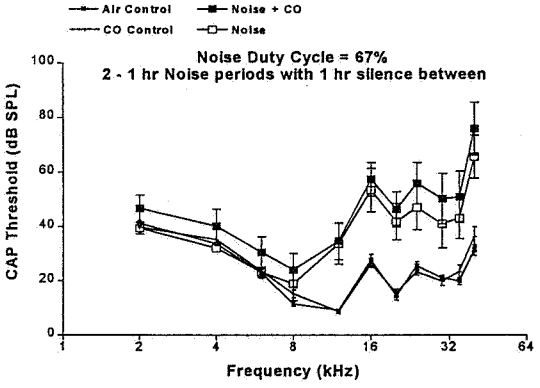


Figure 4B

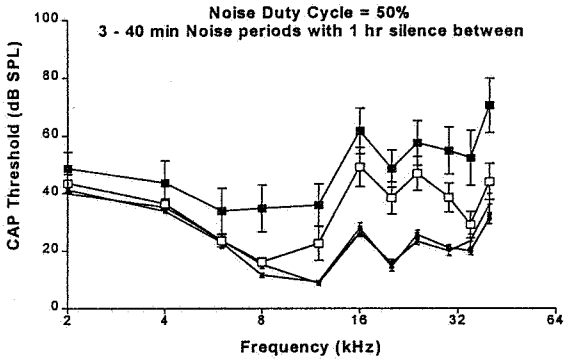
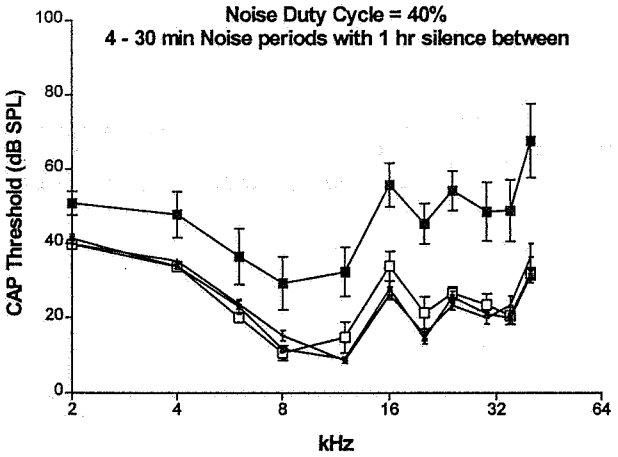


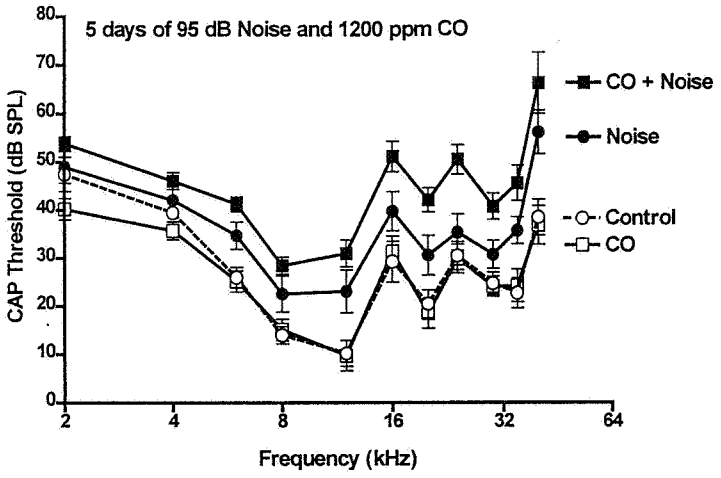
Figure 4C



CO blocks the protective effects of quiet rest periods in intermittent noise exposure:

NIHL can be reduced if rest periods of silence are interspersed within the noise. This fact underlies the time-weighted average for specifying noise exposure. Thus, less impairment is predicted if 2 hours of noise exposure are separated by one hour of quiet (67% noise duty cycle) as compared to 2 hours of continuous noise (100% noise duty cycle). We conducted a study to determine whether *reduced* impairment by noise with *reduced noise duty cycle* would result under conditions of CO exposure. Subjects received continuous exposure to 1200ppm CO for 2.5 to 6.5 hr. Noise exposure (OBN at 13.6kHz) was presented for a total of 2 hr duration, but was scheduled to occur either in 2- 1 hour blocks, 3- 40 min blocks, or 4-30 min blocks with 1 hr periods of silence interspersed between blocks of noise. Thus the noise duty cycles were 40, 50, and 67%. Combined exposure to noise and CO blocked the ameliorating effects of rest periods within noise exposure (fig. 4) even though CO exposure by itself for the maximal time period of 6.5 hours did not produce impairment of auditory function. Thus, CO can potentiate hearing loss induced by intermittent noise more than that induced by continuous noise. Perhaps as an extension of this finding, we have shown that repeated exposure to CO (1200ppm) + noise (95dB OBN) for 2 hr daily over 5 days yields potentiation of NIHL (see below).

Figure 5



Development of cumulative potentiation of NIHL by CO with repeated exposures

The purpose of this initial experiment was to determine whether repeated exposure to CO + noise at levels of noise that do not produce marked auditory impairment resulted in potentiation of NIHL. The subjects were exposed for 5 consecutive days to 95dB(lin) OBN noise +1200ppm CO, CO alone, or no treatment. Four weeks later CAP and CM were measured. Noise exposure alone elevated CAP thresholds by 10-15dB, but did not alter the 1uV RMS CM isopotential curve. Exposure to Noise + CO, however, potentiated NIHL significantly above the impairment produced by noise alone (fig 5) and also elevated the CM isopotential curve by approximately 10dB. This experiment may be relevant for workers exposed repeatedly to asphyxiants and noise. Additional experiments are planned in the renewal application to determine whether similar findings will be seen with repeated HCN and whether the effects can be observed when noise exposure is reduced to a level that does not yield a PTS.

Potentiation of NIHL by CO does not increase linearly with noise severity

Potentiation of NIHL by CO does *not* increase linearly with noise severity. Combined exposure to CO + noise produces the greatest potentiation at moderate noise levels that produce 10-20dB NIHL. At more severe sound levels, NIHL continues to grow in subjects receiving noise alone, but subjects receiving combined exposure to CO + noise do not show increasing auditory impairment beyond that shown by the noise-only group. However, while the peak impairment produced by CO + Noise appears to asymptote with increasing noise, there is a broadening of the frequencies at which auditory impairment is seen with the combined exposure. A second experiment performed using a 5 dB trade-off for doubling of the noise duration found that once sufficient noise was used to produce a slight auditory impairment by itself, the potentiation by CO that occurred was equivalent irrespective of increasing noise intensity or duration. These data underscore the fact that the extent of threshold elevation does seem to have an upper limit which may be a result of the cellular targets for toxicity (e.g. damage to OHCs) and mechanism of toxicity. Perhaps the extent of "initiation" of ROSs by noise is less important than the "promotion" of ROSs via inhibition of ROS buffering mechanism. This hypothesis will be addressed pharmacologically in a new proposal.

SPECIFIC AIM 2: Does noise frequency spectrum influence extent or pattern of potentiation of NIHL by CO?

Our objective was to determine whether auditory impairment always occurred at the high frequency portion of the cochlea or whether the impairment could be "modulated" by adjusting the noise spectrum employed. The results show that CO is able to potentiate NIHL produced by *any* octave band tested and that the specific frequencies affected reflect the nature of the noise exposure. Thus, low frequency noise, that stimulates a broad section of the basilar membrane, produces a relatively flat auditory impairment when CO is added. High frequency OBN (13.6kHz CF) produces more selective high frequency impairment with noise. We also showed that potentiation of NIHL by CO was observed only when the noise intensity at each OBN was sufficiently loud that the noise by itself was able to produce at least a slight impairment of auditory function.

SPECIFIC AIM 3: The role of free radical damage and excitotoxicity in potentiation of NIHL: Mechanisms of chemical asphyxiant potentiation of NIHL

We completed experiments to determine the cellular targets of acute CO and CN injection. The purpose here was to search for mechanisms by which chemical asphyxiants given by inhalation might potentiate permanent NIHL. This is not a foolproof search strategy, however, since differences in the route and dose

of exposure to chemical asphyxiant between acute single toxicant injection studies and chronic effects from mixed exposure (noise + asphyxiant) studies may identify outcomes from the acute studies that are not relevant to the chronic effect mixed exposure studies. The protocol for assessing acute asphyxiant ototoxicity relies on within subject designs in which we record from the cochlea of anaesthetized rats prior to and then immediately following very large CO gas or KCN injections ip. The protocol for assessing chronic impairment of auditory function by combined exposure to noise + HCN or CO involves a between subjects approach in which subjects are exposed to toxicant by inhalation in the presence of noise, and, for comparison, subjects receiving exposure to CO alone, HCN alone, noise alone or control exposure.

Figure 6A

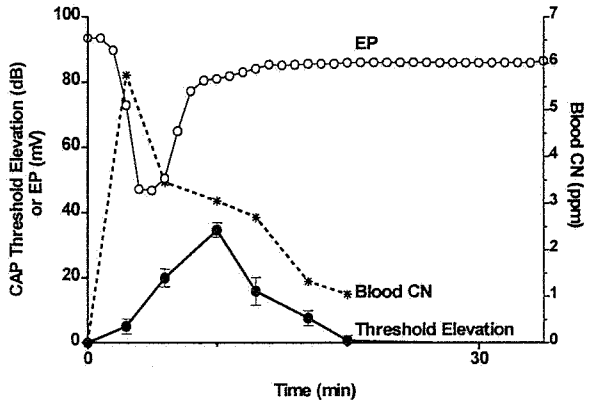
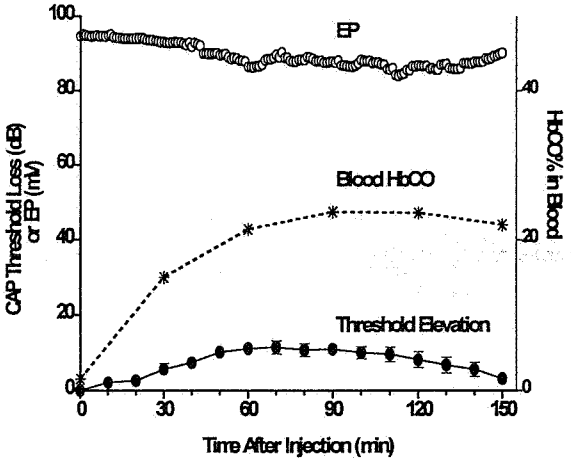


Figure 6B



Cochlear Targets for Acute Injection of CO and KCN

Pharmacological evidence shows that acute CO injection promotes excessive glutamate release in the cochlea and also increases ROS "load". The data summarized below suggest that while excess ROS load may be responsible for potentiation of NIHL by CO, that excitotoxicity cannot explain the potentiation. The CAP and CM were recorded before and after CO and KCN injection at the round window. In additional subjects, the EP—a measure of stria vascularis function—was assessed along with blood cyanide and COHb. The results are reported in Tawackoli, et al. They show transient impairment of CAP sensitivity following injection of either chemical asphyxiant that is correlated closely to the blood levels of these agents (fig 6A). In addition, KCN injection produced a rapid and profound decrement in the net EP recorded from the second turn, indicating disruption of stria vascularis function. CO produced a small and inconsistent effect on this measure, indicating that impairment of the stria is not a prominent target for acute CO toxicity (fig 6B). That KCN disrupts the stria profoundly is consistent with its known inhibition of the electron transport chain and consequently with ATP generation and Na-K ATPase.

Acute CO injection may generate excitotoxicity:

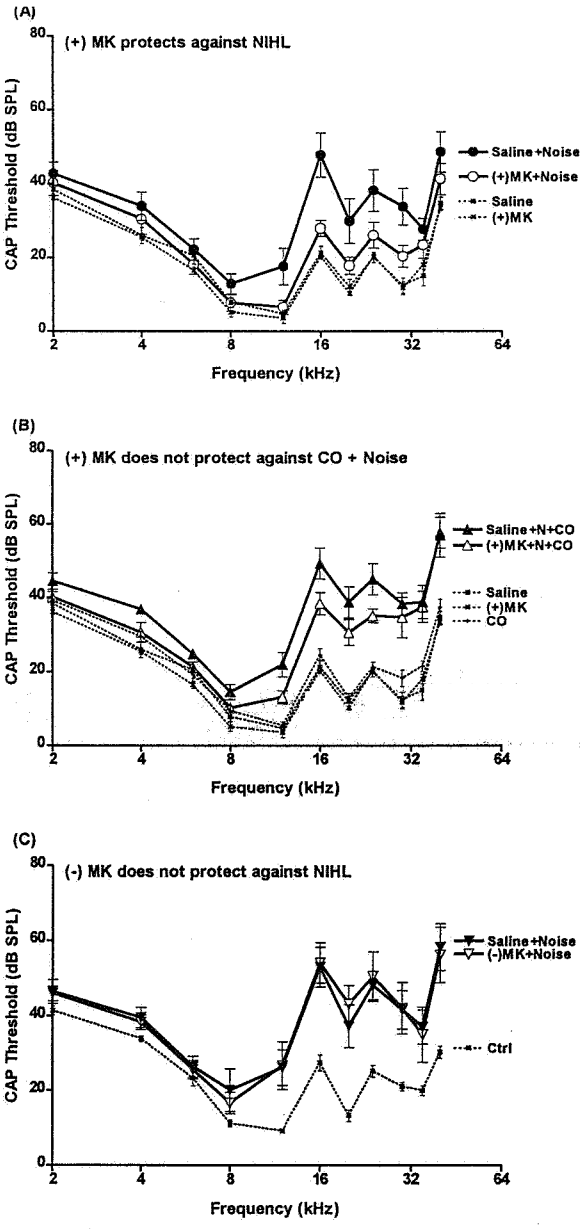
Because hypoxia has been shown to promote release of glutamate in the brain, we sought to determine whether acute CO exposure enhances glutamate release from the IHC yielding excitotoxicity. We asked whether the NMDA receptor blocker, (+) MK 801, could protect against the acute ototoxicity of this agent. We found that (+) MK801 can block the acute loss in auditory function resulting from CO injection suggesting that excessive release of glutamate is occurring at the IHC-SGC synapse with resulting impairment of the CAP.

Can excitotoxicity account for potentiation of NIHL by CO inhalation exposure?

We found that (+) MK-801 did not protect against potentiation of NIHL by inhalation exposure to CO indicating that potentiation of permanent NIHL cannot be explained as an excitotoxic event (fig 7B). While it is essential to undertake additional confirmatory studies to rule out an excitotoxic mechanism for potentiation of NIHL by CO, these data have led us to look to other explanations (e.g. ROS promotion) to understand the basis for potentiation of NIHL by CO.

We did determine, however, that (+) MK-801 could reduce permanent hearing loss induced by 2-hr noise only- (a finding that is consistent with an excitotoxic mechanism in the case of noise exposure alone). Interestingly, the stereo isomer (-) MK-801 with very limited NMDA blocking activity was not protective against noise- reinforcing the view that NMDA receptor blockade is important to achieving protection against NIHL at exposure levels used here (fig 7A, 7C). This is a notable finding because molecular approaches and pharmacological studies aimed at elucidating the type(s) of glutamate receptors in the cochlea have not demonstrated clear evidence for NMDA receptors or a role for such receptors in normal auditory function. We hypothesize that such receptors do exist in the cochlea and that, while they do not play a major role in normal cochlear function, they can be active under conditions of cochlear over stimulation and contribute to an excitotoxic impairment. Again, confirmatory studies using drugs with selectivity for the NMDA receptors are on-going.

Figure 7



Acute CO injection may generate ROSs:

To determine whether acute CO injection results in ROS generation in the cochlea, we asked whether the spin trap agent, PBN, and the xanthine oxidase inhibitor, allopurinol, can protect against the acute ototoxicity of this agent. Xanthine oxidase can be a source of superoxide and hydrogen peroxide while allopurinol can inhibit this process thus serving as an antioxidant. We found that both drugs reduced the acute loss in auditory function resulting from CO injection suggesting that ROS contributed to impairment of acute auditory function (Fechter et al., 1997). We hypothesize that acute CO injection alone does not produce permanent threshold shifts because the initiation of ROS generation (e.g. noise exposure) is not present and perhaps because the primary site for CO potentiation is via inhibition of ROS buffering capacity.

Can excess ROS account for potentiation of NIHL by CO inhalation exposure.

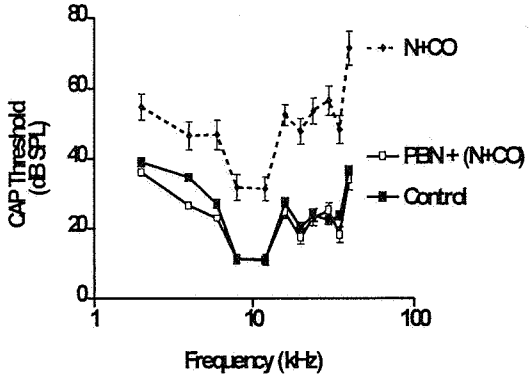
To test this hypothesis we asked whether the free-radical spin-trap, PBN, administered both pre- and post-combined exposure and also only post-exposure (as a potential therapeutic strategy) could protect against potentiation of NIHL by CO. While both administration strategies reduced the extent of NIHL by combined exposure groups, PBN given before and after exposure provided significantly better protection against potentiation of NIHL by CO when compared to post-exposure administration alone (fig 8). Repeated post-exposure administration within 4 hr of exposure revealed somewhat greater protection than a single administration of PBN. These data are consistent with the interpretation that ROS are generated very early during and following combined exposure to CO and noise.

Combined exposure to noise + CO yields ROS measured by electron paramagnetic resonance

We have worked with Drs. Kotake and Reinke to obtain direct EPR evidence that 1200ppm CO + 100dB OBN for 30 min exposure generates ROS in the cochlea (fig 9 & 10). The data indicate that combined exposure to CO and noise for a brief interval is sufficient to

generate a large free radical signal. Neither CO nor noise alone was able to produce a free radical signal in the cochlea of sufficient magnitude to be detected by EPR.

Figure 10



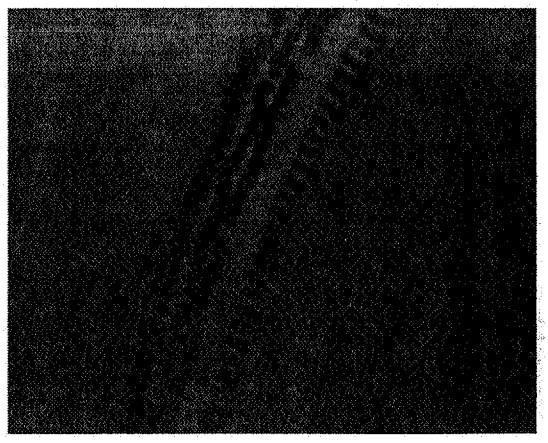
Noise + CO yields a persistent reduction of SDH activity in hair cells

Prediction of auditory impairment based solely upon a cytocochleagram is difficult because there is only a rough correspondence between hair cell loss and cochlear dysfunction. We have found that the correlation between auditory impairment and SDH activity as long as 4 weeks following exposure may be useful in predicting dysfunction. Following exposure to 100-dB OBN for 2 hrs alone subjects showed highly variable auditory impairments ranging from 0-40 dB impairment. Combined exposure to noise + CO (1200 ppm) yielded threshold shifts of about 40 to 50 dB. The fact that noise alone produced highly variable threshold shifts among subjects is consistent with the notoriously variable effects of noise both in individual animals and people (and thus the notion of tough and sensitive ears). The lack of variability in the combined exposure group arises, we believe, from the fact that nearly all individuals are affected and that there is an upper limit to the extent of hearing loss our system can identify. When analyzing the cytocochleagrams, only the noise + CO subjects showed any hair cell loss and this loss was for OHCs within the basal-most 40% of the cochlea. In the animals with noise-induced PTS, lighter SDH staining was observed in IHCs. Usually the influence was intermittent with lighter stained cells found among heavily stained cells. It is of interest that noise-only subjects with hearing loss did not show abnormality in SDH density of OHC. Noise exposed subjects that did not show a NIHL had no such abnormal SDH stained IHCs. The abnormal SDH-stained IHCs appeared within the turn that corresponded to the frequency region of auditory impairment (fig. 2). This is an intriguing finding that might indicate that noise exposures at these levels have a persisting effect on the IHC-SGC units. This is possibly related to NMDA-mediated impairment with noise exposure (fig 11). The lack of effect of noise alone on SDH in OHCs may suggest that at least in the rat for moderate noise levels, these cells may not suffer prominent injury. Studies proposed to determine recovery of function following asphyxiants + noise or noise alone will elucidate the role of OHC function and SDH disruption during recovery following exposure.

Figure 11

DPOAE assessment

We have collected a substantial amount of DPOAE data both with rat and guinea pig that attest to the stability of such measurements within control subjects, the comparability between our control subjects and data published in other laboratories, and to the ability of DPOAE methods to detect the effects of noise and CO + noise. While such data have not yet seen submitted for publication, the results of one series of experiments designed to study solvent ototoxicity in guinea pig have been accepted for publication.



List of Publications Resulting from this Grant

Published and accepted manuscripts

Fechter LD. Mechanisms of ototoxicity by chemical contaminants: Prospects for intervention. *Noise & Health*, 1999, 2:10-27. Specific aims 1, 3

Chen GD and Fechter LD. Potentiation of octave-band noise induced auditory impairment by carbon monoxide, *Hearing Research*, 1999,132, 149-159. Specific aim 2

Chen GD, McWilliams M and Fechter LD. Intermittent noise induced hearing loss and the influence of carbon monoxide, *Hearing Research*, in press. Specific aim 1

Fechter LD, Chen GD, and Rao DB. Characterizing conditions that favor potentiation of noise induced hearing loss by chemical asphyxiants, *Noise and Health,* in press. Specific aim **1**

Chen GD, McWilliams M, and Fechter LD. Succinate dehydrogenase (SDH) activity in hair cells: a correlate for permanent threshold elevations. *Hearing Research,* 2000, 145:101-110.

Tawackoli W, Chen GD and Fechter LD. Acute disruption of cochlear potentials by chemical asphyxiants, *Neurotox and Teratol,* 2000, in press. Specific aim 1

Rao DB and Fechter LD. Increased noise severity limits potentiation of noise induced hearing loss by carbon monoxide, *Hearing Research*, 2000, 150:206-214.

Manuscripts under editorial review

Rao D and Fechter LD. Protective effects of phenyl-*N-tert*-butylnitrone (PBN) on the potentiation of noise induced hearing loss by carbon monoxide, *Toxicology and Applied Pharmacology*, 2000, 167:125-131. Specific aim 3

Fechter LD, Chen GD and Rao D. Predicting exposure conditions that facilitate the potentiation of noise-induced hearing loss by carbon monoxide. *Toxicological Sciences, in press.* Specific aim 1

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