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Acrylonitrile Produces Transient Cochlear Function Loss and Potentiates Permanent Noise-Induced Hearing Loss

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There is growing evidence that agents that produce oxidative stress in the cochlea have significant ototoxic potential by themselves and can potentiate noise-induced hearing loss as well. Acrylonitrile (ACN) metabolism entails conjugation with glutathione, resulting in rapid and pronounced depletion of this important antioxidant in many organs including brain, liver, and kidney. ACN metabolism also results in cyanide (CN) formation through a secondary oxidative pathway. The results of two physiological experiments are reported here. First, the acute effects of ACN (50 mg/kg sc) on auditory sensitivity are assessed using a within subject study. In the second study, persistent effects of ACN alone (50 mg/kg, sc and 2 × 50 mg/kg, sc) and ACN in combination with noise exposure (8 h, 108 dB octave-band noise) are evaluated using threshold sensitivity as the dependent measure. Auditory threshold shift and absolute thresholds were determined using the compound action potential (CAP) amplitude. Acute ACN administration produces a loss in auditory threshold sensitivity that reached a maximum 10–20 min following sc injection. Auditory thresholds returned to control levels 75–100 min following exposure. In the study of permanent auditory threshold shifts, ACN plus noise increased auditory threshold impairment relative to rats receiving noise only when thresholds were assessed 3 weeks following exposure. ACN by itself did not produce permanent threshold impairment 3 weeks following administration. Assays were undertaken in separate groups of rats to track the elevation in blood CN and the depletion of total glutathione in cochlea, brain, and liver following ACN treatment. Systemic blood CN levels were not significantly elevated until 60–120 min following injection, and cochlear glutathione levels showed significant depletion as little as 15 min after injection and remained depressed for about 4 h. The results confirm the prediction that ACN is acutely ototoxic and can enhance noise-induced hearing loss.

Key Words: **ototoxicity; acrylonitrile; oxidative stress; potentiation; noise; cyanide; glutathione depletion.**

Recent investigations from several laboratories have established that oxidative stress can be a prominent mechanism responsible for cochlear impairment following administration of a very broad range of agents. Oxidative stress has been implicated in cochlear injury following severe noise exposure (Henderson *et al*., 1999; Lautermann *et al*., 1997; Ohlemiller et al., 1999b; Seidman *et al*., 1993; Yamane *et al*., 1995; Yamasoba *et al*., 1999), and administration of aminoglycoside antibiotics (Garetz *et al*., 1994; Hester *et al*., 1998) and the antineoplastic agent, cisplatin (Kopke *et al*., 1997; Rybak *et al*., 1995). Oxidative stress is also thought to play a role in potentiation of noise-induced hearing loss associated with exposure to chemical asphyxiants (Fechter *et al*., 1997, 2002; Rao and Fechter, 2000). Most of the above studies demonstrate that the degree of cochlear impairment by noise and ototoxic agents can be enhanced using drugs that disrupt intrinsic reactive oxygen species (ROS) buffering systems and that the ototoxic potential of these agents can be reduced by augmenting the sequestration of free radicals.

Another line of research that also suggests a role of oxidative stress in cochlear susceptibility to noise has employed genetic strains with impaired mechanisms for reducing oxidative stress. Thus, Ohlemiller *et al*. (1999a, 2000) have shown that animals with a genetic deficiency in Ca/Zn SOD and mice with a targeted mutation to the gene for cellular glutathione peroxidase are more sensitive to noise-induced hearing loss than wild-type subjects. Sensitivity of specific cochlear cells has also been correlated with adequacy of intracellular ROS-reducing elements. Recently, Sha *et al*. (2001) suggested that the vulnerability of outer hair cells in the base of the cochlea to ototoxicants relative to the apex might reflect intrinsic glutathione (GSH) levels. They showed that GSH levels were higher in apical than in basal outer hair cells.

ROS are produced naturally during oxidative metabolism, and various intrinsic mechanisms exist for buffering these radicals. Among these are the tripeptide GSH, the enzymes superoxide dismutase (SOD) and catalase, and a variety of antioxidants such as ascorbate, tocopherols (vitamin E), and, possibly, melanin. All of these ROS scavenging or elimination systems appear to be present in the cochlea (e.g., Barbary *et al.*,

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1993; Pierson and Gray, 1982; Rarey and Yao, 1996; Takumi *et al*., 2001). When ROS levels exceed the capacity of intrinsic mechanisms, the resulting oxidative stress can produce cell damage by binding to macromolecules and by triggering lipid peroxidation.

Based upon the relationship that has been established between oxidative stress and cochlear dysfunction, we hypothesized that acrylonitrile (ACN), a chemical with important commercial uses, might be ototoxic itself or might potentiate noise-induced hearing loss based upon its ability to produce oxidative stress. The ultimate hypothesis to be tested is that by depleting glutathione and generating cyanide (CN), an inhibitor of SOD, ACN would enhance the impairment in auditory function produced by noise. Beyond an interest in understanding more about fundamental mechanisms of ototoxicity and developing a means of predicting which chemicals may be ototoxic, the practical importance of this research is based upon the very widespread use of this nitrile in commercial processes.

ACN is among the highest production volume chemicals, with more than 3 billion pounds of ACN produced in the United States annually (Kirschner, 1995). ACN is used in the production of manmade fibers (e.g., nylon), elastomers, and plastics, and as a chemical intermediate in the synthesis of a variety of products including dyes and pharmaceuticals (IARC, 1999).

A strong relationship has been demonstrated between administration of ACN and reduction in GSH levels in systems other than the cochlea. Benz *et al*. (1997) have shown that, at a dose of 20 mg/kg of ACN, liver GSH was depleted by 50% whereas a dose of 50 mg/kg depleted approximately 85% of liver GSH. Liver GSH depletion was followed by rapid recovery. Brain GSH levels were reduced by 50% in 30 min following ACN administration (75 mg/kg, sc) (Cote *et al*., 1984). While the metabolic pathways for ACN have been carefully mapped in a variety of organs including brain, liver, and kidney, there are no data on the possible effects this compound might have on cochlear glutathione and very few data showing that specific nitriles can disrupt cochlear cell function.

MATERIALS AND METHODS

Subjects. A total of 46 Long-Evans male pigmented rats, 2–3 months of age, obtained from Harlan (Indianapolis, IN) were employed in two physiological experiments. Additional rats of the same age and strain and from the same vendor were used; 90 for blood CN determination and 72 rats for glutathione analysis on liver, brain, and cochlea. The subjects were housed with free access to food and water in their home cages. Background sound levels in the colony room were below 50 dB using an A-weighting scale that largely eliminates low-frequency building vibration that is far lower than the rat's auditory range. A spectral analysis of this background noise level showed that sound levels in the frequency range used for threshold assessments (2–40 kHz) were below 40 dB using an unweighted or linear measurement scale. Temperature was maintained at 21 ± 1°C. Lights were on from 0630 h to 1830 h. All exposures and testing were performed during the daytime.

Procedures. Two auditory experiments were conducted. In the initial study, 10 rats were anaesthetized with ketamine (87 mg/kg, im) and xylazine (13 mg/kg, im) to produce a surgical level of anaestheia. The rats were then surgically prepared for assessment of the compound action potential (CAP). This potential represents the synchronous neural activity elicited from the primary auditory neurons (spiral ganglion cells) and provides a direct assessment of auditory threshold sensitivity. The CAP was assessed prior to administration of ACN or vehicle and, then repeatedly at 5-min intervals over the next 100 min. CAP was measured differentially between a silver wire electrode, insulated except at the tip and placed on the round window, and a silver chloride reference electrode that was placed in neck musculature. The methods have been described previously in greater detail (Fechter *et al*., 1986). The subjects were anaesthetized with xylazine (13 mg/kg, im) and ketamine (87 mg/kg, im), and normal body temperature was maintained using a DC heating unit built into the surgical table. The temperature of the cochlea was also maintained using a low-voltage high-intensity lamp. The auditory bulla was opened via a ventro-lateral approach to allow the placement of a silver wire electrode onto the round window. A silver chloride reference electrode was inserted into the neck muscle. The CAP signals evoked by pure tones were amplified 1000× between 0.1–1.0 kHz with a Grass AC preamplifier (Model P15). The sound level necessary to generate a visually detectable CAP response on a digital oscilloscope (approximate response amplitude of 1 mV) was identified. The CAP response was not averaged.

Auditory thresholds at each frequency were subsequently recorded at 5-min intervals over a period extending to 100 min postinjection. Thus, thresholds were obtained for each rat at each of the 11 test frequencies on 20 different occasions. The time period between electrode placement, assessment of baseline auditory function, and injection of ACN or water was approximately 10 min. Auditory thresholds were determined at 11 frequencies between 2–40 kHz. These were: 2, 4, 6, 8, 12, 16, 20, 24, 30, 35, and 40 kHz.

Stabilized ACN (Sigma, St. Louis, MO) was used in all studies to minimize the accumulation of peroxides and explosion hazard. For the study of acute effects of ACN, baseline auditory thresholds were determined as described above. Immediately following this baseline threshold determination, five rats were injected with 50 mg/kg ACN sc and five rats received distilled water sc as a control. The injection volume was 1 ml/kg body weight. In the second experiment, the effects of ACN administration on permanent noise-induced hearing loss were assessed using a between subjects design. For the study of permanent threshold shifts, the rats were assigned randomly to one of six groups ($n = 6$ per group). These groups received:

- 50 mg/kg ACN alone;
- 2 injections of ACN (50 mg/kg each) on two successive days with a 16-h interval between injections;
- Noise alone (108 dB octave-band noise for 8 h);
- Combined treatment with 50 mg/kg ACN followed immediately by noise;
- Combined treatment with 2 × 50 mg/kg ACN with the noise given following the second ACN administration; and
- No experimental treatment.

Auditory thresholds were assessed under ketamine/xylazine anaestheia 3 weeks following the appropriate treatment.

Noise exposure. Subjects were exposed to octave-band noise (OBN) at 108 dB using an unweighted or linear measurement scale for 8 h in an exposure chamber. Subjects not scheduled to receive noise were placed in a similar chamber, but with no supplemental noise provided. The OBN was defined as being centered at 13.6 kHz and extending from 9.6 to 19.2 kHz. Noise was generated using a TDT RP-2 Real Time Processor/Filter (Tucker Davis Technologies, Gainesville, FL) in conjunction with software developed by TDT (RP vds Version 1.1). This noise was amplified by a Parasound HCA-1000A High Current Amplifier (Parasound Products Inc., San Francisco, CA) and fed to speakers (Vifa D25AG-05, Videbaek, Denmark). The animal cages were located under the speakers with a vertical distance of approximately 5 cm from the top of the cages. Sound intensity was measured at the level of the rats' pinnae by a Quest Type 1 sound pressure meter with 1/3 octave filter set (models 1700 and OB300, Oconomowoc, WI). Acoustic measurements in the exposure chamber showed that levels were maximal and essentially flat be-

tween 10 and 16 kHz. Sound levels were approximately 10 dB lower at 20 kHz. The acoustic intensity was decreased about 40 dB below maximum between 5 and 10 kHz. Noise levels varied less than 2 dB within the space available to each animal. Background noise intensity with no added sound was approximately 40 dB using an unweighted or linear measurement scale. Air exchange rate within the chamber was 8.5 lpm (providing approximately 12 changes per h) with airflow being monitored by a Top Trak 821–1-PS flow gauge.

Blood cyanide determinations. The elevation and elimination of CN from blood was determined spectrophotometrically after 20 min, 1, 2, 3, and 4 h following ACN administration (20, 50 and 80 mg/kg, sc) using a between subjects design. At different time intervals following injection of ACN or saline sc, rats were rapidly anaesthetized using isoflurane. Three ml of blood was taken by cardiac puncture. One ml of whole blood was placed in a Conway cell and was acidified with sulfuric acid (0.25 M). The Conway cells were gently shaken for 3 h so that liberated CN could be trapped in 0.1 M NaOH solution contained in a separate compartment within the cell. Standards and blanks were utilized by adding known concentrations of KCN to blood samples of untreated control subjects. Blood CN levels were determined using methods of Feldstein and Klendshoj (1954) as described in Baselt (1987). NaOH solution containing unknown amounts of CN liberated from the blood sample was reacted with chloramine T and pyridine-barbituric acid solution, yielding a color reaction measured spectrophotometrically at 580 nm.

Glutathione measurement. Total glutathione levels were assessed in whole brain, whole liver, and paired cochleae from rats receiving a single injection of 50 mg/kg at time intervals ranging from 15 min to 8 h following ACN administration. Because of loss of tissue, different subjects were used to obtain the three different organs tested. Four tissue samples were obtained at each time period from separate rats. An additional group of control rats were treated with saline and four sets of tissue harvested 8 h later. None of these subjects was actually exposed to noise or used for audiometric testing.

At the appropriate time point, the rats were rapidly anaesthetized under isoflurane anesthesia, and tissues were quickly harvested and frozen immediately in liquid nitrogen. The tissues were stored at −80°C until assayed. When assayed, tissues representing each survival time point following ACN administration were included in a given run. Total glutathione was assayed using a kinetic spectrophotometric method described by Akerboom and Sies (1981), in which GSH or glutathione disulfide (GSSG) in the presence of glutathione reductase brings about the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to 5-thio-2-nitrobenzoate (TNB). Formation of TNB is tracked spectrophotometrically over time at 412 nm.

The frozen tissue was transferred to tubes containing ice-cold perchloric acid. For the cochlear samples, the tissue, including the bony shell, was pulverized under liquid nitrogen. The samples were centrifuged to precipitate the tissue, and the supernatant was neutralized with potassium carbonate. Following precipitation of potassium perchlorate, an aliquot of sample was incubated with nicotinamide adenine dinucleotide phosphate (NADPH), DTNB, and glutathione reductase. Readings were taken every 30 s for a period of 3 min and compared to a standard curve run with each set of samples.

Statistical analysis. The data were analyzed using parametric tests. For the study of acute ACN effects, a one-between, two-within factorial ANOVA was conducted with treatment as the between subject's factor and time following treatment and frequency as the within-subject factors. The analysis used each rat's preexposure baseline threshold, the most deviant thresholds following exposure, and the final threshold readings taken 75–100 min following ACN, when thresholds had essentially returned to normal for treated subjects. For the rats treated with water sc, threshold shifts were evaluated at comparable time periods. Subsequently, an additional repeated measures ANOVA was undertaken comparing the maximal shift in auditory threshold for treated and control subjects *regardless* of the time following injection. Thus, the comparison for treated subjects compared preexposure thresholds with those assessed 10–20 min following the ACN. For the control subjects, the comparison was between pretreatment with vehicle and a time close to the termination of the experiment 100 min following treatment, when thresholds showed maximal changes from baseline.

The statistical model used to study the persistent effects of ACN on hearing employed a one-between subjects (treatment) and one-within subjects (frequency) ANOVA. Glutathione and blood CN data were evaluated using one-way ANOVA.

RESULTS

ACN (50 mg/kg, sc) elevated auditory thresholds temporarily in all rats tested acutely, although the specific time of maximal threshold elevation and threshold recovery varied somewhat among experimental subjects receiving this agent. Consequently, Figure 1a presents the averages of the maximal acute effects of ACN administration on auditory thresholds and the point of maximal threshold recovery. These timepoints vary slightly for different subjects. Similarly, Figure 1b provides auditory thresholds for control subjects at time intervals that match those used for ACN subjects. ACN produces a preferential high frequency impairment that can be observed

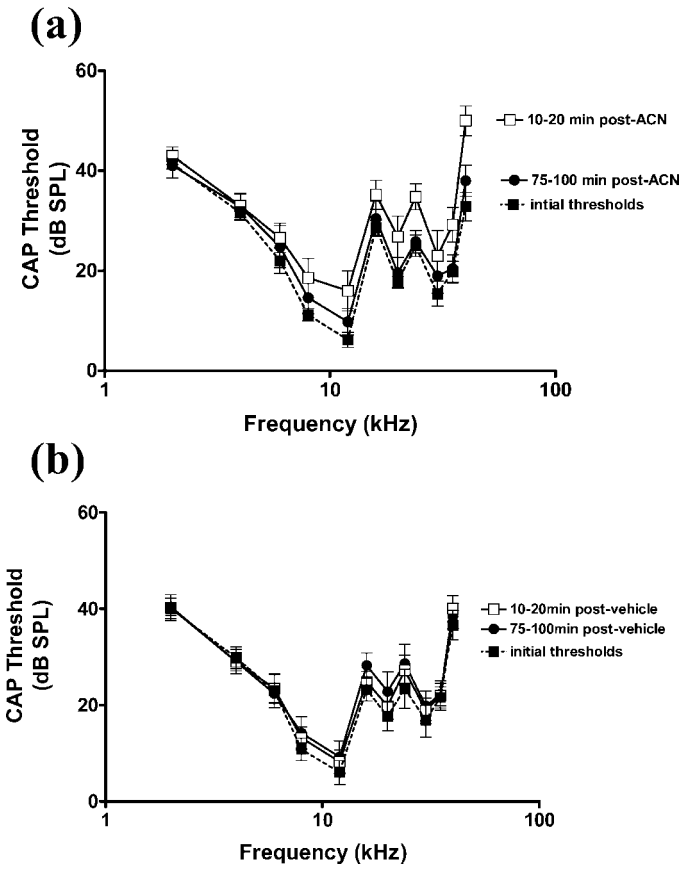

FIG. 1. Acute effects of ACN injection (a) and water injection (b) on compound action potential threshold (mean ± SEM) for 5 rats per treatment condition. Open boxes represent the thresholds at the time following injection when auditory thresholds were most impaired by ACN and matching threshold measurements at the same time points for control rats. Closed circles represent the point of maximal recovery of function after injection.

between 10 and 20 min following sc administration. This loss in sensitivity is approximately 10–20 dB over the stimulus range of 8–40 kHz. However, the loss appears to be transient (a temporary threshold shift) and substantially all of the impairment recovers between 75 and 100 min following injection of the toxicant.

The ANOVA showed a significant interaction between treatment and time postinjection ($F_{2/158} = 8.16$, $p < 0.005$) confirming that the two treatments resulted in differential effects with time. The main effects of frequency ($F_{10/158} = 135$, $p < 0.0001$) and time ($F_{2/158} = 13.67$, $p < 0.0005$) were also significant. A separate repeated measures analysis focused upon the *maximal* change in auditory threshold sensitivity relative to each rat's baseline response rather than the shift at specific time periods was also performed. The treatment main effect ($F_{1/8} = 10.70$, $p < 0.02$) as well as the effect of frequency ($F_{10/80} = 5.54$, $p < 0.0001$) were significant. The interaction of treatment and frequency was also significant ($F_{10/80} = 2.15$, $p < 0.05$). While the mean loss of auditory threshold sensitivity for control rats was 2 ± 1 dB measured across all frequencies, the corresponding loss for ACN-treated rats was 8 ± 1 dB. The loss in threshold sensitivity tended to be flat for control rats, while it showed a clear high frequency bias for ACN-treated rats (Fig. 1a).

Figure 2 summarizes the effect of ACN administration (50 mg/kg × 2 injections) on the extent of permanent noise-induced hearing loss that was measured three weeks following the ACN and ACN + noise treatment. The auditory thresholds for rats receiving only a single ACN treatment + noise were intermediate between those receiving noise only (data not shown) and those receiving the second ACN administration prior to noise. Their data were, however, included in the statistical analysis, and the results are described below. Consistent with the effects of acute exposure, ACN alone did not produce a persistent loss in auditory threshold sensitivity (Fig. 2). Auditory thresholds for subjects receiving ACN were within 5 dB of those recorded for control subjects and tended to be, if anything, slightly *more* sensitive than for control subjects. Rats receiving both ACN (2 × 50 mg/kg) and noise showed auditory impairments as large as 40 dB relative to untreated controls. The thresholds for rats receiving combined treatment were especially poor at high frequencies. Between 12 and 40 kHz, the region of maximal auditory loss, the average impairment by ACN (2 × 50 mg/kg) + noise rats was 27 dB. By comparison, rats receiving a single ACN treatment + noise showed an average threshold shift of 11 dB between 12 and 40 kHz. Noise treatment by itself also elevated auditory thresholds substantially at all frequencies tested, but the extent of threshold elevation was always less than 20 dB. A one-between (treatment) one-within (frequency) ANOVA demonstrated a significant effect of treatment ($F_{4/25} = 20.73$, $p < 0.0001$), a significant effect of the within subject factor, test frequency, ($F_{10/250} = 90.76$, $p < 0.0001$), and a significant treatment by frequency interaction ($F_{40/250} = 6.83$, $p < 0.0001$). Post hoc comparisons using Sheffe's multiple comparisons test showed that subjects treated with both ACN (2 × 50 mg/kg) + noise had significantly more auditory function loss overall at test frequencies between 12 and 40 kHz relative to rats receiving noise alone, ACN alone, and no experimental treatment. Noise-exposed rats also differed significantly from controls over this frequency range, but no difference was observed between control subjects and those treated only with ACN.

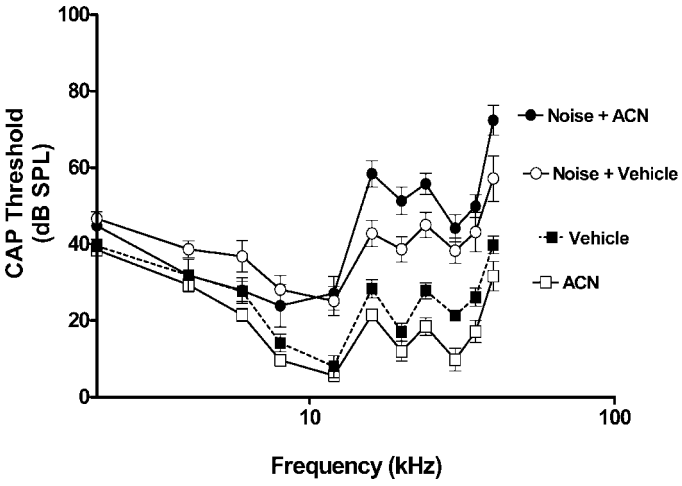

FIG. 2. Effects of ACN (2 × 50 mg/kg), noise alone, and combined exposure to ACN and noise on the compound action potential threshold (mean ± SEM) for six rats per treatment condition compared to untreated air controls. ACN significantly impaired auditory thresholds relative to both untreated controls and noise-exposed rats. ACN by itself did not impair auditory function.

Measurements of blood CN and of cochlear, brain, and liver glutathione were made in order to assess the status of these markers of ACN metabolism at various time points utilized in the study of acute effects of ACN on auditory function. Blood CN levels were measured in animals that were exposed to three different doses of ACN (20 mg/kg, 50 mg/kg and 80 mg/kg). All three doses yielded significant CN production followed by recovery to near-normal levels (Fig. 3). The time of CN elimination is dose-dependent and was longest after 80 mg/kg ACN injection. In addition, the maximal CN level also tended to show a dose dependency, although group differences emerged only after the 1-h time point. It is noteworthy that equivalent blood CN levels were observed prior to and at the 1-h time point across ACN doses. An analysis of variance with both ACN dose and time following ACN as between subject factors showed a significant effect of dose ($F_{2/86} = 21.74$, $p < 0.00001$) and time ($F_{5/86} = 23$, $p < 0.00001$). Finally, a significant interaction of dose and time was seen ($F_{10/86} = 7.18$, $p < 0.00001$).

Figure 4 portrays the decrease in liver, brain, and cochlear glutathione levels that occurs following injection of a single dose of 50 mg/kg ACN, expressed as a percentage of control values. These control values (± SEM) were 8.575 ± 0.81 μmol/g tissue in liver, 3.05 ± 0.40 μmol /g tissue in brain, and

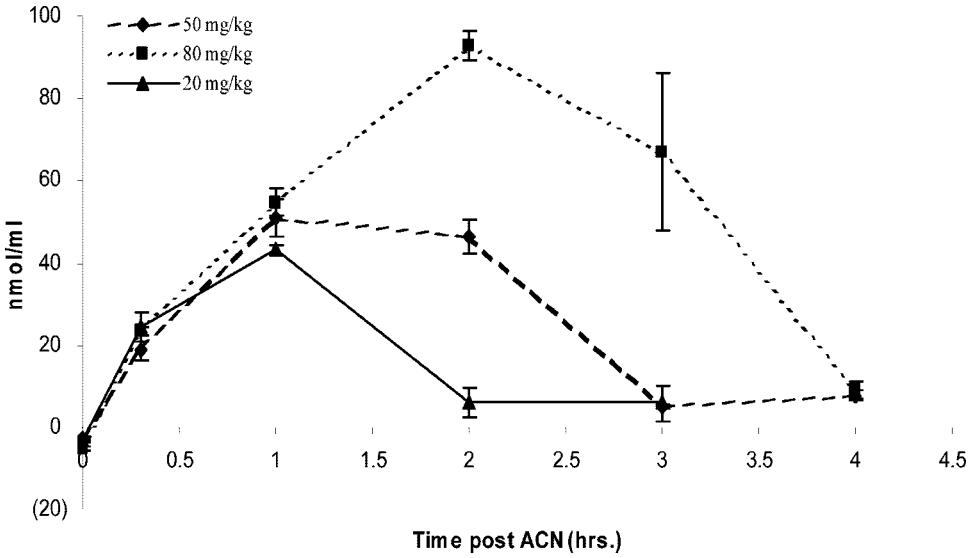

FIG. 3. Accumulation of cyanide in systemic blood following injection with ACN (20, 50, and 80 mg/kg, sc). Values at each time point represent the mean ± SEM of 5 different subjects at each time point. Cyanide levels were significantly elevated from the initial reading at 20 min. Significant dose related effects are apparent between 2 and 3 h postadministration.

18.68 ± 7.2 nmol/pair of cochleae. While all three tissues show the same general pattern of glutathione loss and recovery following treatment, there appear to be some differences between tissues in the extent and duration of glutathione depletion. Specifically, liver shows the most profound and rapid decrease in glutathione levels, reaching 33% of baseline levels 15 min following treatment. Brain and cochlear samples appear to show a slower and less severe loss in glutathione, although the data suggest that ultimately the cochlea is more severely affected both in terms of extent of depletion and duration of glutathione depletion than is the brain. This conclusion is offered cautiously, however, because the cochlear data are based upon very small tissue samples, with associated difficulties in undertaking glutathione assessment. A two factor between subjects analysis of variance showed a significant difference in extent of glutathione depletion among tissues ($F_{2/50} = 3.70$, $p = 0.03$) with liver showing a greater depletion than brain or cochlea at different time intervals following ACN ($F_{4/50} = 4.16$, $p = 0.005$).

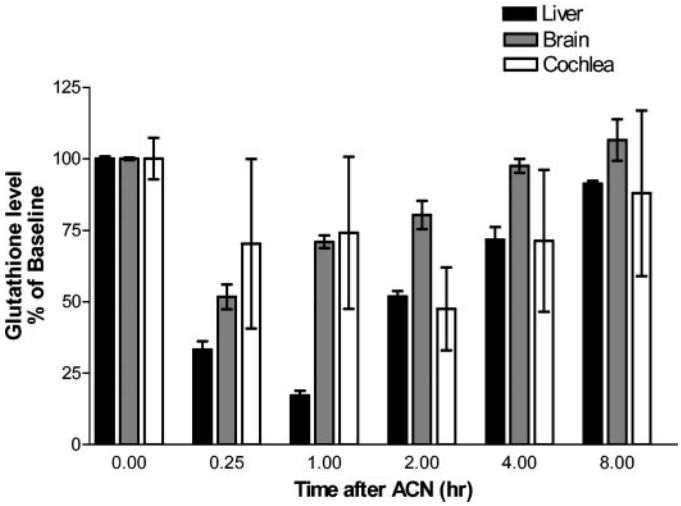

FIG. 4. Total cochlear, liver, and brain glutathione content in control rats and rats treated with ACN (50 mg/kg, sc) between 15 min and 8 h prior to harvesting tissue.

DISCUSSION

The current investigation demonstrates that ACN can produce a transient elevation in pure-tone high-frequency auditory thresholds. The threshold loss occurs rapidly and also recovers rapidly. There are no persistent impairments of hearing that can be identified three weeks following ACN administration, given by itself, even at the more severe dose regimen of 50 mg/kg ACN × 2 days. While we did not produce a permanent impairment in auditory function with ACN, there are several studies that have shown ototoxicity following chronic exposures to other nitriles including 3-butenenitrile (Balbuena and Llorens, 2001; Gagnaire *et al*., 2001), cis-crotonitrile (Balbuena and Llorens, 2003; Gagnaire *et al*., 2001), and cis-2-pentenenitrile (Gagnaire *et al*., 2001). However, in these other studies, either prolonged treatment (e.g., 12 weeks of treatment, 5 days/week, Gagnaire *et al*., 2001) or higher dose and slightly longer treatments have been used (Balbuena and Llorens, 2003). Thus there is a possibility that higher dose or chronic ACN exposure might also carry potential for producing permanent impairment of auditory function.

In distinction to the lack of permanent impairment produced by ACN given by itself, studies of noise potentiation by ACN do establish the ability of this nitrile to enhance the damaging effects of noise on hearing, particularly for high-frequency tones. The term potentiation is used specifically because, in our hands, ACN did not affect auditory function permanently, while it did promote or potentiate noise-induced auditory dys-

function. Because of the wide use of acrylonitrile in industrial settings where significant noise exposure is also present, the present study identifies an interaction that may be of considerable public health importance. Clearly, however, the route, dose, and frequency of ACN exposure used here are far different from that experienced by human workers. Therefore, further laboratory investigations and, most importantly, epidemiological studies will be essential in determining human risk from ACN and noise.

The current work, while establishing potentiation of noise-induced hearing loss by ACN, does not establish a mechanism by which this potentiation occurs. It was predicted that ACN might be ototoxic in its own right or capable of promoting noise-induced hearing loss based both on its potential to produce oxidative stress and its ability to produce CN *in vivo*. This study provides descriptive data on the depletion of cochlear glutathione by ACN and also on the time course of CN generation and elimination from systemic blood. These data do not permit a clear correlation with the acute loss in auditory function resulting from ACN. ACN produced maximal auditory threshold impairment within 20 min of administration. At this time point, systemic CN values are clearly elevated above control levels, but had certainly not reached peak levels. Indeed, systemic blood CN levels were higher both 1 and 2 h following ACN than they were 20 min following this agent; yet auditory thresholds had recovered completely at the 2-h time point. Similarly, cochlear glutathione levels were significantly depressed at the initial time interval when hearing loss was evident. However, auditory function recovered well in advance of glutathione recovery. Thus, the acute ototoxic effect of ACN cannot be readily associated with elevated CN levels, though admittedly direct cochlear assessment of this parameter was not possible. Nor can the acute reversible effect of ACN on hearing be associated with reduced cochlear glutathione. Rather, the acute effects of ACN might reflect other unidentified toxic actions of this chemical in the cochlea. A notable target would be the cells of the lateral wall of the cochlea. These cells have the highest energy requirements in the cochlea (e.g., Pujol *et al*., 1981), are responsible for maintaining a stable DC charge within the cochlea and a high potassium milieu in the region surrounding the hair cells. Both of these functions are essential to maintaining normal auditory sensitivity. Reversible impairment of cochlear function has been observed to occur on a similar time course to that observed in this study by a range of agents that disrupt the lateral wall. Noteably, these agents include hydrogen cyanide (Tawackoli *et al*., 2001) as well as loop diuretics (e.g., Rybak, 1985), and carbon monoxide (Tawackoli *et al*., 2001). Further studies will need to determine whether the acute loss in CAP sensitivity following ACN is due to loss of the DC charge generated at this lateral wall.

While the basis by which ACN promotes noise-induced hearing loss cannot be proven using these preliminary findings, this report does provide useful data for pursuing mechanistic studies and for characterizing the time course during which potentiation of noise-induced hearing loss by ACN is likely to occur. It is noteworthy that, at a dose of 50 mg/kg ACN, blood CN levels were elevated for less than 3 h, while the noise exposure continued for an additional 5 h. Glutathione also was depleted during this time interval, although some residual depletion was still observed at the 3-h time period. An important question will be whether the interaction of ACN and noise is restricted to the time period during which CN is elevated and GSH is depressed. If the promotion of permanent noise-induced hearing loss by ACN administration is related to oxidative stress in the cochlea resulting from production of ROS by noise, coupled with a reduced capacity to sequester these reactive moieties due to reduced levels of GSH, then manipulation of ROS sequestration should be an effective means of testing this proposal.

The current data are consistent with many other reports in demonstrating the accumulation of CN in blood following ACN administration and the depletion of glutathione. The current study shows a depletion of glutathione in liver of approximately 67% achieved at 15 min following exposure. This value corresponds closely to the 85% depletion of liver glutathione reported by Benz *et al*. (1997) 30 min following injection of the same ACN dose. While the production of CN in our study tends to be dose dependent, it is of interest that subjects in the three different exposure dose groups did not differ from each other in total blood CN level at 1 h postexposure. This finding may reflect saturation of CYP2E1 that is responsible for oxidative metabolism of ACN or of the limitation in reduced glutathione that ultimately conjugates cyanoethyleneoxide to form CN. That higher doses of ACN ultimately produce higher levels of blood CN probably reflects the accumulation of CN coupled with a delay in the metabolism of CN to form thiocyanate.

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