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Disruption of cochlear potentials by chemical asphyxiants Cyanide and carbon monoxide

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Abstract

While ischemia, hypoxic hypoxia, and carbon monoxide (CO) have received extensive study designed to characterize mechanisms by which they disrupt cochlear function, little data are available concerning cyanide's potential to disrupt auditory function. In this study, disruption of the compound action potential (CAP) and endocochlear potential (EP) by cyanide and CO was compared in rats treated with potassium cyanide (KCN) (7 mg/kg ip), saline, CO (35 ml/kg ip), and air. Acute KCN administration significantly suppressed CAP and EP transiently. The effect was seen initially on EP with CAP impairment occurring a few minutes later. Acute CO injection also suppressed the CAP significantly, but the effect was far smaller, occurred later in time, and lasted longer than the effect of KCN. The effect of CO on EP was equivocal. There was a good correspondence between blood cyanide levels and impairment of cochlear function; carboxyhemoglobin (HbCO) levels were elevated during the period when cochlear function was impaired, but recovery of cochlear function preceded the return of normal oxyhemoglobin. Both KCN and CO had somewhat preferential effects on high-frequency tones. Repeated cyanide administration caused a persistent CAP threshold elevation despite the rapid recovery of EP and CAP observed following acute KCN administration. The data suggest that acute KCN administration has a prominent disruptive effect at the stria vascularis presumably by disrupting the electron transport chain in this metabolically active structure. The principal target for acute CO ototoxicity in the cochlea is probably not the stria vascularis.

Keywords: Cyanide; Carbon monoxide; Ototoxicity; Auditory threshold; Hypoxia; Cochlea; Rat

1. Introduction

The mammalian cochlea represents a highly metabolically active structure that is vulnerable to the effects of hypoxia and chemical asphyxiants. Indeed, disruption of blood supply (ischemia) and reduction in available oxygen levels (hypoxia) have been suggested to be fundamental mechanisms that are responsible for many forms of sudden hearing loss and drug ototoxicity [17,23,45]. In addition, it is known that acute exposure to the chemical asphyxiant, carbon monoxide (CO), can yield profound hearing loss in humans [15,27,29,39] and laboratory animals [10,13,21,26]. And hypoxic hypoxia also disrupts cochlear function in laboratory animals [4,32,37].

Unlike the case for hypoxic hypoxia, CO, and ischemia, the effect of cyanide on the cochlea has not been well defined. Heijst et al. [18] studied 20 patients in Tanzania with sudden onset polyneuropathies that included hearing loss in nine cases. Blood cyanide and plasma thiocyanate levels were significantly elevated. The source of exposure was believed to be increased dietary intake of cassava due to food shortages. Direct experimental evidence that cyanide can produce hearing loss is limited. Konishi and Kelsey [22] showed a significant reduction in the endocochlear potential (EP), an indication of impaired stria vascularis function, when 50 mM NaCN–Ringer solution was perfused through the guinea pig cochlea. The stria vascularis contains very high concentrations of Na–K ATPase and plays a critical role in pumping potassium into the region of the cochlea surrounding the organ of Corti. The loss in EP was accompanied by a loss in the cochlear microphonic (CM) which is generated mainly by outer hair cells in response to sound and is dependent upon the EP [44]. In addition, the com-

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pound action potential (CAP) amplitude, a measure of auditory sensitivity to sound, was also depressed. By contrast, Evans and Klinke [11], working at a much lower concentration range (0.1–1 mM), perfused potassium cyanide (KCN) directly into the scala tympani and monitored both sharpness of tuning curves from individual neuronal units as well as the CAP and CM responses recorded from the round window in the cat. They reported a rapid, but reversible loss in N_1 sensitivity restricted to the characteristic frequency of the unit with no loss observed either in sensitivity of the broad low-frequency tail region of the tuning curve or in spontaneous firing rate. They rejected the view that cyanide impairs stria vascularis function at the low KCN concentrations employed by them largely because they observed an intact CM response and unit responses.

The basis for impairment of auditory function by CO is far better understood than is that for cyanide. The generation of the CAP is particularly vulnerable to CO with smaller shifts observed in the CM amplitude [13]. The loss in CAP sensitivity can be fully or partially blocked by the NMDA receptor blocker, MK-801 [26], and by putative inhibitors of free oxygen radicals such as PBN and allopurinol [12]. These findings have been interpreted to suggest that CO has its predominant effect at the inner hair cell or the synaptic contact between the inner hair cell and the spiral ganglion cell. However, EP has not been assessed following acute CO administration so that it is uncertain whether or not CO might impair stria vascularis function. The site of impairment in cochlear function by acute chemical asphyxiation has public health importance because it facilitates prediction of permanent threshold shifts, the prospect for potentiation of noise-induced hearing loss (NIHL) by the asphyxiants (e.g., [6,7,14]), and the potential to develop therapeutic strategies for preventing permanent auditory impairment.

The primary target organ for both CO and cyanide is the nervous system because of its high metabolic demand [46]. Symptoms of acute cyanide toxicity include convulsions, uncoordinated movements, seizures, and tremors [2,5,9,40] in addition to respiratory arrest and cardiac failure [3,25,46]. The most significant effect of cyanide appears to be on cytochrome *c*, and consequently, on oxygen utilization in the tissues [36]. Cyanide blocks the enzyme, cytochrome oxidase, thus leading to a breakdown of energy generation through aerobic metabolism. This enzyme is abundantly present in cochlear hair cells, in the stria vascularis, and in the afferent nerve terminals [19,41].

Acute CO toxicity produces marked cardiovascular and neurobiological effects thought to result predominantly from its affinity for heme proteins [16], the subsequent reduction in available oxygen binding sites in blood [10], and conformational changes resulting from formation of carboxyhemoglobin (HbCO) which inhibit the dissociation of oxygen from oxyhemoglobin [36]. Consequently, direct impairment of oxygen delivery occurs in tissue. In addition to actual tissue hypoxia resulting from this interaction with hemoglobin, CO is known to bind to many other proteins including myoglobin, cytochrome *c* oxidase, and other cytochrome *P*450s (cf., [8]).

The principal objective of this study was to compare the effects of high doses of CO and KCN on specific cochlear potentials that are known to be sensitive to disruption in oxygen delivery. The purpose was to determine whether both of these agents disrupt the same cochlear cells as indexed by alterations in cochlear potentials. Because initial data suggested that the effects of KCN administration were short-lived, a second study was designed in which subjects received repeated daily exposure to KCN under exposure conditions that produce central nervous system impairment. The objective was to determine whether repeated KCN injection would yield persistent ototoxic effects.

2. Materials and methods

2.1. Subjects

Forty-eight Long–Evans pigmented rats about 2 months old were acquired from Harlan Sprague–Dawley and housed in University of Oklahoma Health Sciences Center animal facility. Background noise level in the colony room is below 50 dB (A) and spectral analysis showed that sound levels centered at octave bands of 2 kHz and above were 40 dB or lower. Room temperature is controlled at 21°C with the light turned on at 0630 h and turned off at 1830 h. Food and water are available at all times. All procedures regarding the use and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) serving the University of Oklahoma Health Sciences Center.

2.2. CAP and CM recording

The methods used for assessing CAP and CM have been described previously [12,26]. Briefly, the animals were anesthetized with xylazine (13 mg/kg im) and ketamine (87 mg/kg im). The round window was exposed surgically using a ventro-lateral approach and an insulated silver wire electrode was carefully placed on the round window for CAP and CM recording. A silver chloride electrode was inserted into the neck muscle as the reference. The CAP and CM signals were amplified (gain = 1000) with a Grass AC pre-amplifier (Model P15). The band-pass frequency for CAP was 0.1–1.0 kHz and for CM 0.1–50 kHz. The CM signals were sent to a SR530 Lock-In Amplifier (Stanford Research Systems, CA) and then to a PC computer. The sound level that evoked a 1-μV RMS CM at each test frequency was determined by the computer program and the CM iso-amplitude curve was evaluated as previously described [12,26].

The CAP signals were displayed using a digital oscilloscope (Nicolet Instrument, 2090-IIIA,WI). The sound level

of the test frequencies that evoked a just detectable CAP (approximately 1 μV) was used as an estimate of the threshold at each frequency.

2.3. Stimulus generation for CAP and CM assessment

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 sound source (made from an ACO 1/2 in. microphone, 7013) placed within a speculum that fit into the exposed external auditory meatus. CAP thresholds were assessed sequentially for frequencies of 2, 12, and 40 kHz for the acute exposure studies. These frequencies were selected to assess auditory function in the region of the rats' best hearing (12 kHz) and a high and low frequency that lie outside this region of best hearing. Eleven frequencies between 2 and 40 kHz were employed in studying persistent effects of repeated KCN administration. 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. CM was recorded only in the study of persistent effects of KCN using continuous tone stimuli. Sound levels at all testing frequencies were calibrated with a probe microphone located near the eardrum.

2.4. Assessment of acute KCN effects on CAP threshold

Ten rats were used to study the effect of cyanide on CAP threshold; five received KCN injection (7 mg/kg) and five received saline injection as control. CAP was measured prior to and then continuously following the injection.

2.5. Assessment of persistent effects of repeated KCN on CAP threshold

For the study of persistent effects on CAP threshold by repeated cyanide administration, additional 10 rats were used; five were injected with KCN (7 mg/kg) daily for 3 days and five were injected with saline as control. CAP was recorded once 24 h after the last injection.

2.6. Assessment of acute effects of CO on CAP threshold

Ten rats were used to study the effect of CO on CAP threshold; five rats received CO injection (35 mg/kg) and five received air injection as control. CAP was measured once prior to and then continuously following the injection.

2.7. KCN and CO exposure

A single dose of CO and KCN was selected to determine whether CO and KCN could alter CAP threshold. The doses selected were below the LD_{50} and were found in pilot studies to produce a moderate acute impairment of CAP threshold. Injection of KCN solution (7 mg/kg ip) was used in this experiment since it is below the LD_{50} and previous research has shown that such a dosage produces significant neurological effects such as activation of voltage-sensitive calcium channels and stimulation of glutamate release in the brain [20,31,33]. KCN has been reported to have a LD_{50} of 10.2 mg/kg in mice [42] and 10 mg/kg in rats [43]. Pilot studies performed in this laboratory showed that a dosage of 9 mg/kg KCN was lethal in three of four rats. KCN was purchased from Fisher Scientific (Springfield, NJ). In the study of repeated cyanide exposure, experimental animals received 7 mg/kg ip KCN injection daily for 3 days.

For the CO study, a dosage of 35 ml pure CO gas per kilogram was selected based on earlier studies (e.g., Ref. [13]) showing this dose to yield a loss in threshold sensitivity without producing lethality. The approximate LD_{50} for CO gas administered in this manner to rats is 40 ml/kg. CO gas was trapped into a bladder bag. The CO was withdrawn from the bag and injected (intraperitoneal) into the rats using a 25-ml gas-tight no. 1025 syringe from Hamilton (Reno, NV).

2.8. Measurement of blood CN and CO levels

Blood cyanide and CO levels were assessed in six additional subjects evaluated in parallel with the electrophysiological studies. The subjects were anaesthetized in the same manner as for subjects receiving auditory testing. Blood was sampled from the heart before and at multiple time intervals following injection of KCN and CO. For measurement of blood cyanide, four animals were used. In two of the animals, the blood was sampled prior to and at 3, 10, and 17 min following KCN injection. In another two rats, blood was sampled prior to and then 6, 13, and 20 min following the injection. Blood samples (2 ml) were placed in 83 mm OD Conway diffusion cells (VWR, Chicago, IL) and mixed with 2 ml of 10 M H_2SO_4. Two milliliters of NaOH (10 M) was placed in the center chamber of the Conway diffusion cell. The concentration of CN^- was determined after 2 h using an ion-specific electrode (Accumet Research AR25 pH/Ion meter and AccuTupH Rugged Bulb pH Combination Electrode; Fisher, Springfield, NJ).

For measurement of HbCO, two additional animals were used. Blood samples were collected before the CO injection and at 30-min intervals following CO injection over a 150-min interval. The blood was stored in capped glass syringes on ice until they could be analyzed at the conclusion of each experiment. The HbCO was measured using an IL282 CO-Oximeter calibrated for rat blood.

2.9. EP recording

Twelve additional rats were anesthetized as described above. The right cochleae were surgically exposed using a ventro-lateral approach and a small hole was carefully made

in the lateral wall of the basal turn. This permitted a glass micropipette electrode (with tip about 1 μm, filled with 2 M KCl solution) to be carefully lowered into the scala media using a micromanipulator with hydraulic drive. In those subjects for which the electrode was positioned successfully ($n=10$), stability of the EP was assessed over a 10-min period. Then subjects received injection of KCN (7 mg/kg ip) ($n=3$), saline ($n=2$), CO gas (35 ml/kg ip) ($n=3$), or air ($n=2$) and EP was monitored continuously. At the conclusion of the study, the subjects were euthanized by lethal injection of sodium pentobarbital as a control to assure that the negative EP of about −30 mV that is normally observed immediately following death was present in these animals. The electrode was then withdrawn from the cochlea and the potential assessed with the electrode just touching the outside of the cochlea to insure that there had been no drift from a 0-mV setting established at the beginning of the experiment. CAP thresholds were not evaluated in these subjects.

2.10. Statistics

In studies of the acute effects of CO and KCN, statistical analysis was performed using a repeated measures ANOVA (NCSS software) with Treatment serving as a between-subjects variable and Frequency and Time following toxicant as within-subjects variables. The analyses are based upon all of the CAP threshold determinations made. These are indicated in Figs. 1 and 5. Post-hoc analyses used the Scheffe's multiple comparison tests. Blood CN^- and HbCO data were not analyzed statistically. Rather, the blood gas data from individual subjects are presented.

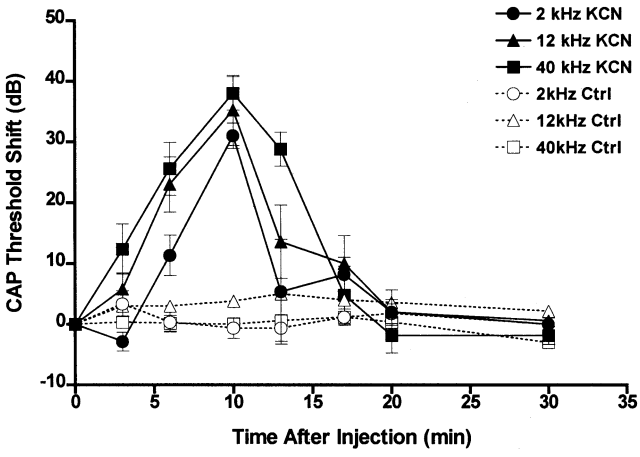

Fig. 1. CAP threshold shift as a function of time following the injection of KCN (7 mg/kg ip). Filled symbols are CAP threshold shifts obtained in animals treated with cyanide ($n=5$ rats). Filled circles: 2 kHz; filled triangles: 12 kHz; filled squares: 40 kHz. Open symbols are CAP threshold shifts obtained in saline control animals ($n=5$). Open circles: 2 kHz; open triangles: 12 kHz; open squares: 40 kHz. Vertical bars indicate the standard error (S.E.).

3. Results

Fig. 1 presents CAP threshold shifts as a function of time following KCN and saline injection at three test frequencies. The injection of KCN causes obvious CAP threshold elevation which is apparent at the initial CAP assessment 1–3 min following injection and becomes more obvious between 3 and 6 min after the exposure. The injection of saline did not cause threshold loss. Interestingly, the influence of KCN injection on CAP threshold seems more severe for high-frequency tones. The cyanide influence on auditory sensitivity at high frequency occurred earlier and recovered slower than at low frequency. A threshold shift of 12 dB was observed at 40 kHz 1–3 min after the injection, while a similar threshold shift was not observed at 2 kHz until 3–6 min after the injection. In KCN-treated subjects, the CAP threshold at 2 kHz had almost recovered at 13 min, while a remarkable threshold shift was observed at the other two frequencies at this time period. However, the peak threshold shift (at 10 min) following KCN was not markedly different across frequencies being 31 dB at 2 kHz, 35 dB at 12 kHz, and 38 dB at 40 kHz. In general, the influence of cyanide exposure on auditory function lasted for only a short period; CAP threshold recovered completely within about 20 min of KCN administration.

Repeated measures ANOVA showed a significant difference in CAP threshold shifts between animals treated with KCN and saline ($F_{(1,8)}=78.32$, $P<.0001$) and significant Treatment × Time ($F_{(7,56)}=35.43$, $P<.0001$) and Treatment × Time × Frequency ($F_{(14,112)}=2.63$, $P<.0025$) interactions. Subsequent post-hoc pair-wise comparisons showed that significant threshold elevation occurred in KCN-treated subjects at 3, 6, 10, and 13 min following treatment ($P<.05$, .001, .001, .01, respectively). At 12 kHz, KCN-treated subjects showed threshold elevation only at 6 and 10 min ($P<.001$). Finally, for 2-kHz tones, threshold was elevated only at 10 min following KCN ($P<.001$). Significant differences were also observed between frequencies in terms of threshold elevations among the KCN-treated rats. Threshold elevation at 40 kHz was significantly greater than that observed for 2-kHz tones 3, 6, and 13 min following KCN ($P<.05$, .01, and .01, respectively). Significant differences between the effect seen at 40 and 12 kHz were observed at 13 min only ($P<.05$). Finally, threshold elevations for the KCN-treated subjects were significant between 2 and 12 kHz only at 6 min ($P<.05$).

Fig. 2 shows the effect of KCN injection on EP in three subjects (A) compared to two saline control animals (B). The EP began to drop 2–4 min following KCN injection, a time period that is slightly shorter than either the initial loss of CAP (3–6 min) or the peak of the CAP threshold shift (at 10 min). The cyanide influence varied quite widely among individuals with two of the subjects showing a very marked reduction in the potential and the third subject showing a distinct, but small decrease in EP. The mean decrease of EP in these subjects was 68 ± 29%. The corresponding CAP

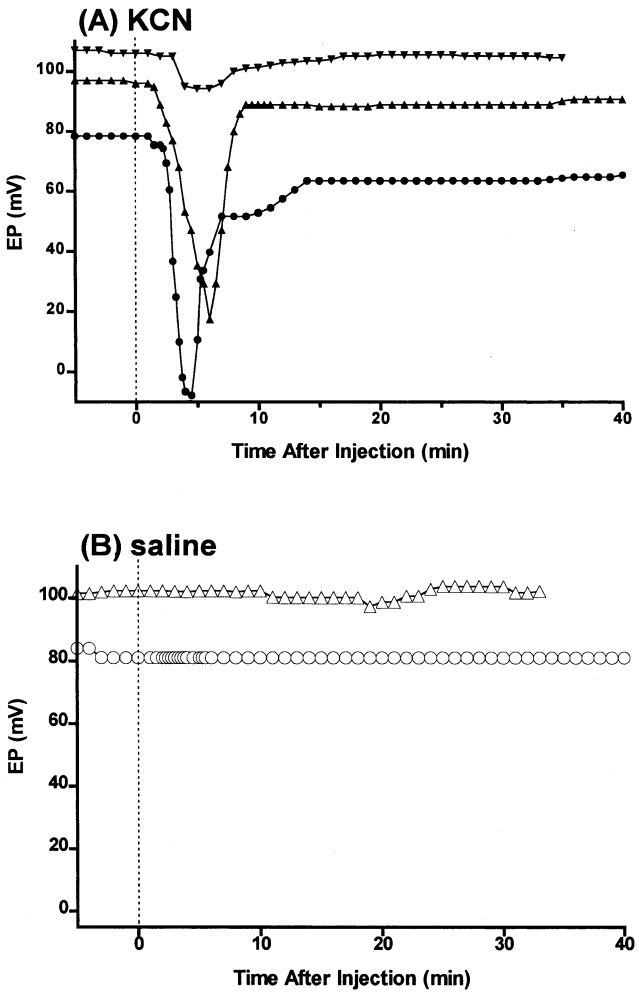

Fig. 2. EP as a function of time following KCN injection 7 mg/kg ip (A) or saline (B) for individual rats.

shift in these specific subjects is not available. The EP recovered substantially within 10–15 min after the injection reaching 93 ± 4% of the initial values. Fig. 2B demonstrates the stability of the EP among saline-treated control subjects.

Fig. 3 presents individual data points showing blood CN level (ppm) as a function of time on a log scale following KCN administration. The highest CN concentration was obtained with the first blood sample taken 3 min after the injection. The concentration was about 6 ppm, which is close to the whole body average KCN level of 7 ppm (7 mg/kg). The KCN was eliminated quickly showing a linear relation to the log of time post-injection ($r = -.96$). Based on this relationship, blood KCN would be completely eliminated within 30 min.

While a single exposure to KCN causes only a transient CAP threshold shift, Fig. 4 presents CAP thresholds and the 1 μV CM isopotential curves for subjects receiving three daily KCN injections and saline controls as a function of frequency. Repeated KCN injection caused elevation in auditory threshold (see Fig. 4A) that was especially noticeable at the extreme portions of the auditory range sampled.

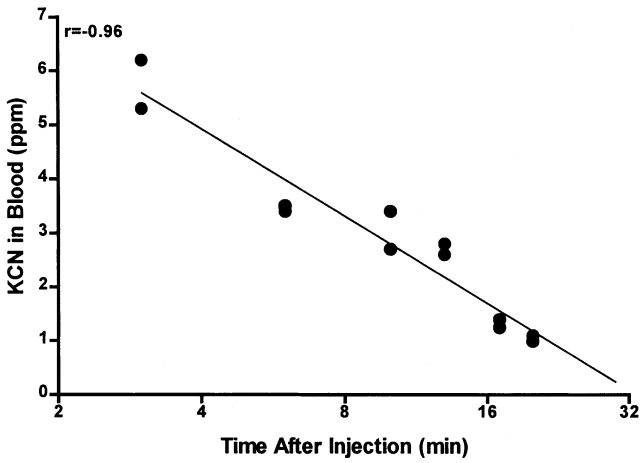

Fig. 3. Elimination of KCN in blood from individual rats ($n = 4$) following cyanide administration. KCN dosage: 7 mg/kg ip. Expressed on a log scale. The linear regression is indicated as a solid line with correlation coefficient of −.96.

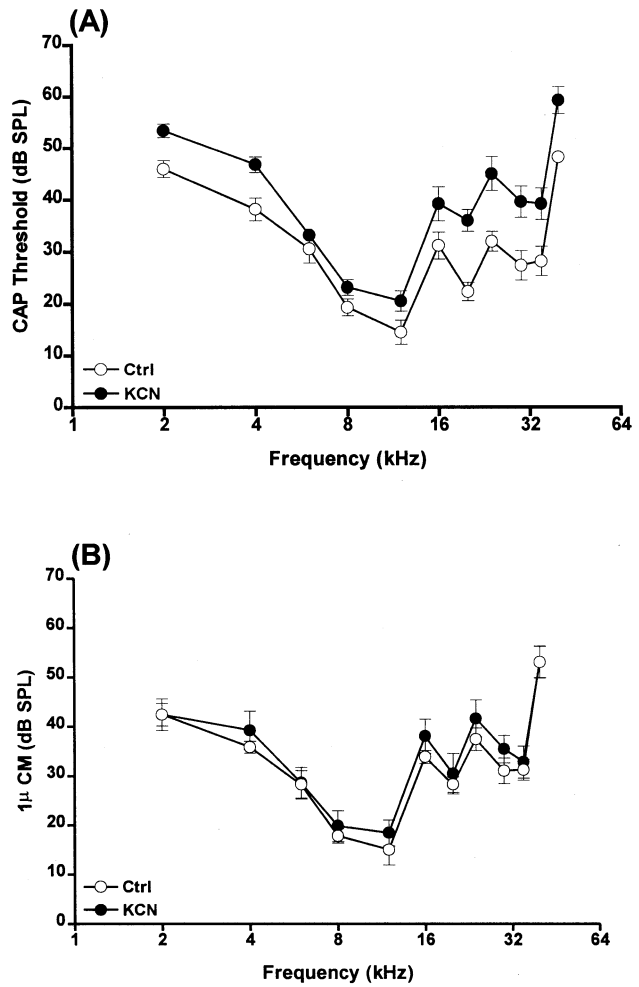

Fig. 4. CAP thresholds (A) and loss of the 1-μV RMS CM (B) after 3-day repeated cyanide administration. Filled circles: treated with KCN (7 mg/kg ip) once a day for 3 days, $n = 5$. Open circles: treated with saline ($n = 5$). CAP threshold and CM were measured 24 h after the last administration. Vertical bars: standard error (S.E.).

The losses are especially large at the high frequencies. There was no evidence (see Fig. 4B) that CM amplitudes were altered by the KCN treatment; the difference between the isopotential curves as a function of frequency for the two groups was less than 5 dB. Repeated measures ANOVA showed a significant difference in CAP threshold elevations between the KCN-treated group and the control group ($F_{(1,8)}=14.12$, $P<.006$), and a significant Treatment–Frequency interaction ($F_{(10,80)}=2.33$, $P<.02$). No significant differences were seen between treatment groups with respect to CM ($F_{(10,80)}=0.78$).

Fig. 5 presents CAP threshold at three different frequencies (2, 12, 40 kHz) in rats injected with CO gas (35 ml/kg ip) and air controls as a function of post-injection time. CO elevated CAP thresholds by as much as 15 dB, while CAP thresholds of control animals varied by less than 5 dB from pre-injection levels. The effect of CO on threshold appeared to be slightly smaller for 2-kHz tones than for 12- and 40-kHz tones. Compared to KCN-injected subjects, the CO injection induced a longer-lasting threshold loss, though the threshold shift was smaller than that obtained in the cyanide-treated animals. The maximal threshold shift was only about 15 dB, comparing with about a 40-dB maximal shift in the cyanide-treated animals (see Fig. 1). The CO-induced CAP threshold shift reached its peak at about 1.5 h, and the threshold recovered about 2.5 h after the injection. Repeated measures ANOVA showed a significant difference in CAP threshold shifts between the animals that received CO injection and air injection ($F_{(1,8)}=26.37$, $P<.0009$) and a significant Treatment × Time interaction ($F_{(15,120)}=3.73$, $P<.0001$). Neither frequency nor interactions that included frequency as a term were significant.

CO injection can also cause EP reduction although the effect is less apparent than that seen following KCN. Fig. 6 presents CO-induced EP reduction in three rats (A). Reduction of EP occurred in two of the three subjects studied with full or partial recovery observed. Generally, the CO-induced EP reduction was less than the KCN-induced EP reduction

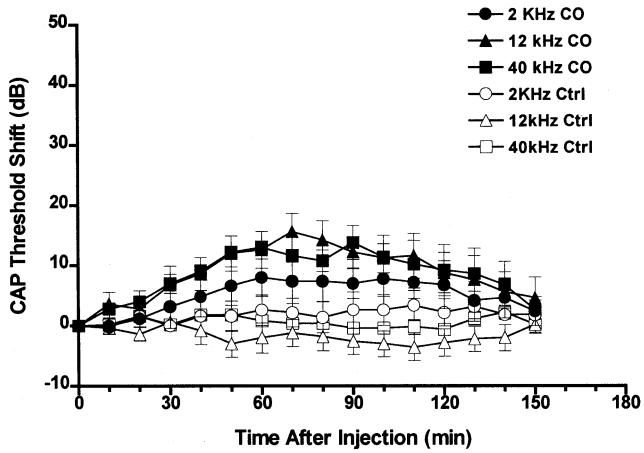

Fig. 5. CAP threshold shift as a function of time following the injection of CO (35 ml/kg ip). Filled symbols are CAP threshold shifts obtained in animals treated with CO ($n=5$ rats). Filled circles: 2 kHz; filled triangles: 12 kHz; filled squares: 40 kHz. Open symbols are CAP threshold shifts obtained in air control animals ($n=5$). Open circles: 2 kHz; open triangles: 12 kHz; open squares: 40 kHz. Vertical bars are standard error (S.E.).

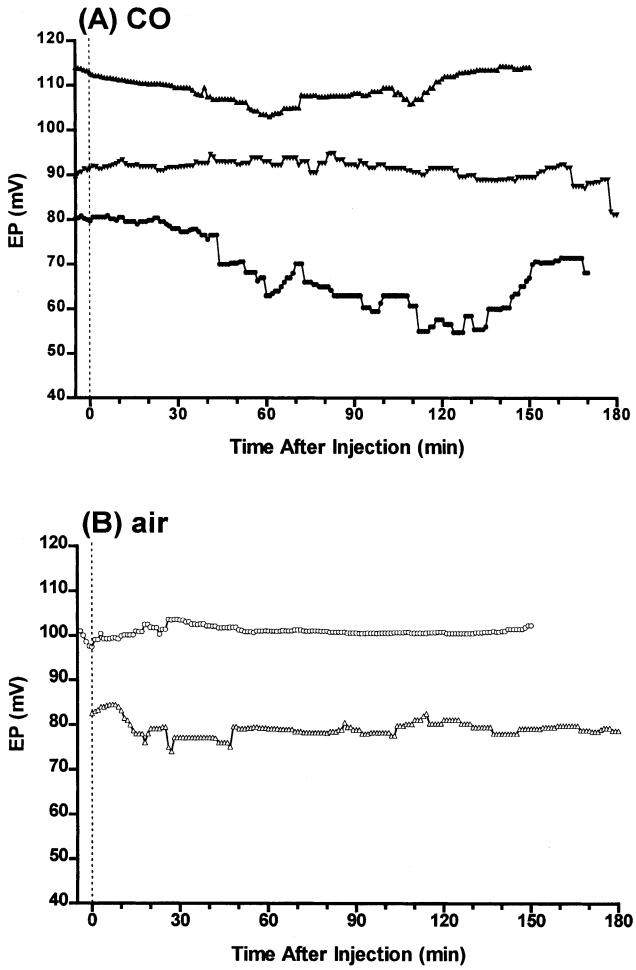

Fig. 6. EP as a function of time following (A) CO administration 35 ml/kg ip or (B) air.

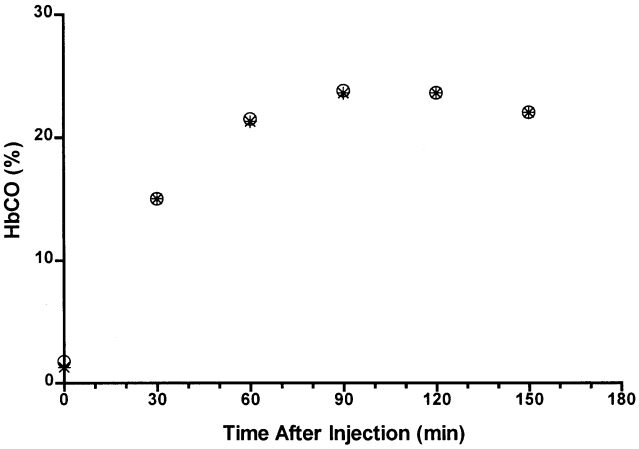

Fig. 7. Elimination of HbCO in blood from individual rats ($n=2$) following injection of CO gas (35 ml/kg ip).

(see Fig. 2). The maximum mean EP suppression by the CO injection was about 13 ± 9%. EP levels returned to about 96 ± 4% of baseline levels by the conclusion of the study. The largest EP reduction occurred during 1–2 h following CO administration and recovered between 2 and 2.5 h following the injection. Fig. 6B presents control level obtained in two rats injected with air.

Fig. 7 presents data from two individual rats showing blood HbCO concentration after CO injection (35 mg/kg ip). HbCO level in the blood increased gradually and reached its peak at about 90 min after the injection. At 150 min, the HbCO level was still close to the peak level (about a 7% drop), though at this time cochlear potentials (CAP and EP) had already recovered substantially.

4. Discussion

The acute administration of both KCN and CO can disrupt auditory function transiently. However, the severity of the impairment, the pattern of effect on critical tissue beds within the cochlea, and the time scale of effect vary greatly for the two agents. KCN causes remarkable reduction in the EP which is indicative of dysfunction in the stria vascularis. Thus, the stria vascularis appears to be very sensitive to cyanide as it is for ischemia [44]. The probable basis of this sensitivity is the very high rate of oxidative metabolism in the stria vascularis required to maintain a stable EP coupled with low total energy reserves in the form of glycogen as compared to the organ of Corti [44]. In the case of KCN, the correlation between impaired EP and impaired CAP threshold was quite close. EP was suppressed shortly following the KCN injection and CAP reached maximal disruption 5 min later. Similarly, the recovery of EP anticipates by about 5 min recovery of CAP. Since CAP threshold sensitivity is influenced by the function of outer hair cells and these, in turn, are dependent upon a functional stria vascularis, it is likely that the loss in CAP threshold sensitivity reflects, in part if not in total, the impairment of stria vascularis function by KCN. The abrupt decrease in EP within 6 min after KCN injection (intraperitoneal) in the present study in rats is similar to that obtained by Konishi and Kelsey [22] in guinea pig by cochlea perfusion with NaCN.

Yet, the acute transient effects of KCN on auditory function cannot be related easily to the possible link suggested in humans between chronic cyanide exposure through dietary sources and permanent auditory impairment. Here, limited data from repeated KCN administration suggest the possible accumulation of ototoxicity as indicated by CAP threshold elevation even though electrophysiological measures suggest complete or near-complete recovery of function in the cochlea following a single KCN administration. It is important to note that the CM amplitude measured a day following the last KCN injection did not differ from that of control subjects. This finding is consistent with the very rapid recovery in EP amplitude that is observed following a single KCN injection, suggesting that the stria vascularis does recover from repeated KCN administration. In the central nervous system, cyanide inhibits brain antioxidant defenses, thus predisposing the brain to further oxidative injury [28]. In other studies, the repeated administration of cyanide causes catalepsy and akinesia in mice and a sustained elevation of oxidation products including malondialdehyde in brain [21]. We speculate that cyanide might produce similar oxidative stress in the cochlea resulting in the accumulation of impairment.

For the CO subjects, the loss of CAP sensitivity and of EP was relatively small, but long-lasting compared to the KCN influence. Unlike the case with KCN, the impairment in auditory threshold observed with CO did not appear to be closely correlated with impairment of the stria vascularis. The loss of EP tended to be of small magnitude, remote in time from CO administration, and gradual in onset and recovery. However, all of these factors contribute to the difficulty in relating CAP sensitivity loss to EP loss. While the role of the stria vascularis in CO ototoxicity cannot be ruled out, the increase in cochlear blood flow known to occur following CO administration [13] and the existence of alternative cochlear targets for CO established previously suggest that the stria vascularis is not central to CO ototoxicity. Previous studies (e.g., [21,26]) suggest that CO produces excessive glutamate release from the inner hair cell that may yield excitotoxic effects at the spiral ganglion cell. The presence of glutamate receptors at the spiral ganglion cells and of excitotoxicity has previously been established [30,34,35]. It is also impressive that the HbCO level in blood only dropped a little from its peak value when both the EP and CAP threshold had recovered 2.5 h following the CO administration. This may reflect an adaptive response to CO administration which is an increase in cochlear blood flow [13].

The present report revealed a more long-lasting cyanide influence on high-frequency thresholds than low-frequency thresholds. This finding is similar to the ototoxic effect of CO [12,13,26] that has been observed previously and which shows a trend in the current work indicating that the high-frequency cells are especially susceptible to chemical asphyxiants. However, the reason for this preferential effect might be quite different. Specifically, it is known in the guinea pig that resting EP amplitude shows a gradient with the largest potentials observed at the base, where high-frequency tones are encoded, and smaller potentials at the apex [1,38]. Since KCN depresses EP markedly, it may be that this results in a more severe or at least more rapid impairment of high-frequency cochlear function.

The investigation of persistent ototoxic consequences of repeated CO injection was not undertaken here. While such data might have provided an interesting comparison to data from the repeated KCN administration study, the repeated injection of large volumes of gas directly into the peritoneal cavity is not a procedure that we chose to undertake without

anesthetic. However, we have determined (unpublished observation) that repeated daily inhalation exposures to 1200 ppm CO for 3.5 h/day over 5 days does not have any measurable long-term consequences (measured 4 weeks following exposure) on CAP threshold or on CM amplitude, or on a non-invasive measure of outer hair cell function (distortion product otoacoustic emissions) either 1 or 3 days following exposure.

Acknowledgments

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