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NMDA receptor blockage protects against permanent noise-induced hearing loss but not its potentiation by carbon monoxide

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Abstract

While a clear role has been proposed for glutamate as a putative neurotransmitter at the inner hair cell type I spiral ganglion cell synapse, the possible role of excessive glutamate release in cochlear impairment and of NMDA receptors in such a process is uncertain. The present study compares the protective effects of (+)-MK-801, an NMDA receptor antagonist, and the relatively inactive isomer (−)-MK-801 against permanent noise-induced hearing loss (NIHL). The study also asks whether (+)-MK-801 can protect against the NIHL potentiation by carbon monoxide (CO). Rats ($n = 6$) were exposed to 100-dB, 13.6-kHz octave-band noise for 2 h after receiving injection of (+)-MK-801 hydrogen maleate (1 mg/kg), (−)-MK-801 hydrogen maleate (1 mg/kg), or saline. Other groups of animals were exposed to the combination of noise and CO (1200 ppm) after receiving (+)-MK-801 or saline. Additional subjects received (+)-MK-801, saline or CO exposure alone. Compound action potential (CAP) threshold sensitivities were compared 4 weeks after the exposures. The results show significant protection by (+)-MK-801 against the permanent CAP threshold elevation induced by noise alone, but no protective effect of (−)-MK-801. (+)-MK-801 produced limited protection against threshold shifts induced by the combination of noise and CO. Outer hair cell (OHC) loss was not protected by (+)-MK-801 administration. The data suggest that NMDA receptor stimulation may play a role in NIHL resulting from fairly mild noise exposure. The data do not support a role for NMDA receptor stimulation in the potentiation of NIHL that results from simultaneous exposure to CO and noise.

Key words: NMDA receptor antagonist; MK-801-stereo isomers; Noise-induced hearing loss; Carbon monoxide ototoxicity; Rat

1. Introduction

There is strong experimental evidence that glutamate serves as an afferent neurotransmitter in the cochlea, responsible for the fast synaptic transmission from the inner hair cells (IHCs) to afferent nerve endings (e.g. Altschuler et al., 1989; Bobbin, 1979; Bobbin and Ceasar, 1987; Bobbin and Thompson, 1978; Bobbin et al., 1990; Jenison and Bobbin, 1985; Jenison et al., 1985; Puel, 1995; Ryan and Schwartz, 1984). Most subtypes of glutamate receptors, including NMDA receptors, are believed to exist in the cochlea based on physiological (Kleinlogel et al., 1999; Puel et al., 1991) and immunocytochemistry and molecular techniques (Knipper et al., 1997; Niedzielski and Wenthold, 1995; Niedzielski et al., 1997; Safieddine and Wenthold, 1997). However, pharmacological studies have not demonstrated a clear functional role for NMDA receptors in the cochlea (Bobbin, 1979; Bobbin and Thompson, 1978; Jenison et al., 1986; Puel et al., 1991).

Excessive glutamate release has been identified as a basic mechanism of neurotoxicity through the process of excitotoxicity (Choi and Rothman, 1990), but its relevance to ototoxicity and noise-induced hearing loss are less certain. Acute auditory impairments induced by different ototoxic agents (intense noise and carbon monoxide) are partially blocked by administration of

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Abbreviations: ABR, auditory brainstem response; ANOVA, analysis of variance; CAP, compound action potential; CF, center frequency; EDTA, ethylenediamine tetraacetic acid; NIHL, noise-induced hearing loss; IHC, inner hair cell; NMDA, *N*-methyl-D-aspartate; OHC, outer hair cell; SDH, succinate dehydrogenase; SGC, spiral ganglion cell

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glutamate receptor antagonists (Duan et al., 2000; Liu and Fechter, 1995; Puel et al., 1998). It is not certain whether these antagonists can also protect against noise exposure that yields a permanent threshold shift, though protective effects of NMDA antagonists on permanent hearing loss induced by aminoglycoside antibiotics have been reported (Basile et al., 1996; Duan et al., 2000).

NIHL potentiation by CO has been observed in several previous reports (Fechter et al., 1988; Chen and Fechter, 1999; Chen et al., 1999). The mechanism underlying the NIHL potentiation by CO is unclear, but the relationship established between hypoxia and ischemia and excitotoxicity (Choi, 1988; Meldrum, 1985; Raichle, 1983; Rothman and Olney, 1986) suggests this as one possible mechanism of such potentiation. Moreover, there is evidence that the acute auditory threshold elevation that results from high-dose CO exposure is reduced by (+)-MK-801 (Liu and Fechter, 1995).

The objectives of this study are to determine, using pharmacological methods, whether NMDA receptor stimulation plays a role in permanent threshold loss resulting from noise exposure and whether potentiation of NIHL by CO also results from this mechanism.

2. Materials and methods

2.1. Subjects

Sixty experimental animals (Long Evans male 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 OUHSC are registered with the US Department of Agriculture and are inspected semi-annually by the members of the Institutional Animal Care and Use Committee (IACUC). All procedures regarding the use and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) serving the University of Oklahoma Health Sciences Center. The background noise level in the colony room is below 50 dB(A) with energy in any octave band higher than 2 kHz lower than 40 dB SPL. The room temperature is controlled at 21°C. The light is turned on at 06.30 and turned off at 18.30. Food and water are available at all times.

2.2. Exposure procedures

Exposures were conducted in a reverberant 40-l glass cylinder equipped with stereo speakers for delivering sound, a Quest 1″ microphone and sound level meter for monitoring sound level, and a CO monitor (Industrial Scientific) for measuring the chamber gas concentration. Air exchange rate in the exposure chamber was 8.5 l/min (providing for approximately 12 air changes per hour), and airflow was monitored by a Top Trak 821-1-PS flow meter. The background noise level in the exposure chamber was 40 dB(A) with energy in any octave band higher than 2 kHz lower than 34 dB SPL. The subjects were placed within small wire-cloth enclosures ($15 \times 13 \times 11$ cm) within the chamber, and were conscious and free to move within the enclosures.

2.3. Noise exposure

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

2.4. CO exposure

Carbon monoxide (CO) gas was metered into the chamber using a microflow valve. The nominal CO level was 1200 ppm with actual level achieved of 1201 ± 18 ppm (S.D.). The CO level in the exposure chamber reached its nominal level and the blood HbCO concentration reached its plateau about 1 h following the CO exposure (Chen and Fechter, 1999; Chen et al., 1999).

2.5. MK-801 treatment

(+)-MK-801 hydrogen maleate (*(5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine hydrogen maleate*; RBI, cat# M-107) or (−)-MK-801 hydrogen maleate (RBI, cat# M-108) was dissolved in saline (1 mg/ml). (+)-MK-801 is a non-competitive NMDA receptor antagonist that acts at the NMDA receptor-operated ion channel as an open channel blocker, and (−)-MK-801 is a much less active isomer of MK-801, that has a low affinity for the NMDA receptor (Griesbach and Amsel, 1998). Injection (i.p.) dosage was 1 mg/kg. Saline injection was in an equivalent volume (1 ml/kg). The half-life of (+)-MK-801 in the blood is 1.4 h (maximal level appeared at about 46 min), and the drug is detectable 48–72 h after

dosing (Hucker et al., 1983). Though the maximal drug level in the blood may delay after injection, the drug was injected in the present study just before the noise onset, since in previous studies the drug was injected this way and showed protection against hearing loss (Duan et al., 2000; Liu and Fechter, 1995). The dosage of 1 mg/kg was chosen based upon a previous study (Liu and Fechter, 1995) showing that injection of 1 mg/kg (+)-MK-801 was protective against auditory threshold loss 30 min after CO injection. Protection by MK-801 against ischemia-induced neurodegeneration has also been reported with dosages that exceeded 0.3 mg/kg (Gill et al., 1987).

Ten groups of rats with six in each group were used. To determine the effect of NMDA antagonism on NIHL, animals were exposed to noise with pretreatment of (+)-MK-801 hydrogen maleate, (−)-MK-801 hydrogen maleate, or saline. The effect of (+)-MK-801 alone was also determined. The effects of the two stereo isomers of MK-801 on NIHL were studied sequentially and separate control subjects were tested in parallel with each form of the drug. To determine whether NMDA antagonism could protect against NIHL potentiation by CO, rats were exposed to noise+CO with saline or (+)-MK-801 pretreatment. The effect of CO exposure alone on auditory function was also assessed in a separate group. All subjects included in this study were placed in an exposure chamber for 3.5 h. CO (1200 ppm) or clean compressed air was turned on immediately to permit equilibration of CO in blood and noise was turned on 1.5 h after the animal was placed in the chamber. Thus the noise duration was 2 h and the CO and air exposures were 3.5 h. The animals were taken out of the chamber just before noise onset for a short period (<5 min) for the injection (i.p.) of the drugs or saline.

2.6. CAP recording

Four weeks after the exposure, the animals were anesthetized with xylazine (13 mg/kg, 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 the compound action potential (CAP). A silver chloride electrode was placed in the neck muscle as the reference. The CAP signals were amplified with a Grass A.C. preamplifier (Model P15). The gain was 1000×. The band-pass frequency for the potential was 0.1–1.0 kHz. The CAP signals were displayed using a digital oscilloscope (Nicolet Instrument Co., 2090-IIIA). The sound level that evoked a just detectable CAP was determined at 11 test frequencies and this value was used to estimate the threshold at each frequency. The CAP was used in this study instead of the auditory brainstem response (ABR) to facilitate comparison with previous research published for this laboratory demonstrating that NIHL can be influenced by other agents (Chen and Fechter, 1999; Liu and Fechter, 1995; Fechter et al., 1997; Rao and Fechter, 2000).

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

2.7. Histology

Histological study was undertaken on a random sample of the subjects. The deeply anesthetized animals were decapitated after CAP recording. Cochleae were removed immediately. Round and oval windows and the apex of the cochlea were opened to facilitate perfusion. The cochleae were perfused with SDH incubative solution (0.05 M sodium succinate, 0.05 M phosphate buffer and 0.05% tetranitro blue tetrazolium) and immersed in the solution for 1 h (37°C). Tetranitro blue tetrazolium is an electron acceptor that, on reduction, precipitates as insoluble and highly colored formazan. SDH catalyzes sodium succinate and provides electrons for the reduction of the electron acceptor. Thus the staining depends on SDH activity (Chen et al., 2000). After the staining, the cochleae were fixed with 10% formalin for at least 2 days. After fixation, the cochleae were decalcified in 10% EDTA solution (ethylenediamine tetraacetic acid) for 3 days or longer as needed. Cochlea microdissection was accomplished under a light microscope. Successive image pictures (covering 200–300 μm basilar membrane) were obtained with Optimetic Image system. Counting of hair cell loss was achieved using Scion Image software.

2.8. Statistical analysis

Statistical analysis was performed using a repeated measures ANOVA and between groups variables (NCSS software) in which treatment was a between subjects variable and frequency was evaluated as a within subjects variable. Orthogonal comparisons were used for comparing individual groups with different treatments.

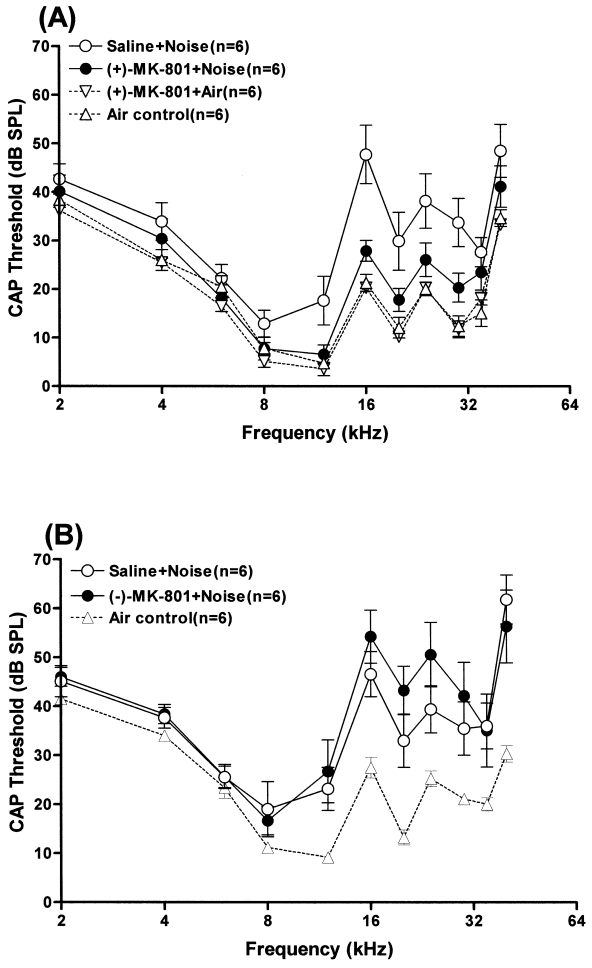

Fig. 1. CAP thresholds of rats receiving different treatments. (A) Showing protection of (+)-MK-801 against noise-induced threshold elevation. (B) Showing that the noise-induced CAP threshold losses are not reduced by (−)-MK-801. Vertical bars indicate standard error of the mean (S.E.M.). Octave-band noise with center frequency at 13.6 kHz, 100 dB_{Lin} for 2 h; (+)-MK-801 (1 mg/kg), (−)-MK-801 (1 mg/kg) or saline were injected (i.p.) at noise onset; CAP thresholds were measured 4 weeks after the exposure.

3. Results

3.1. Effects of (+)-MK-801 and (−)-MK-801 on noise-induced threshold elevations

Fig. 1A presents CAP thresholds of rats measured 4 weeks after different experimental treatments. The group exposed to 100-dB octave-band noise for 2 h with saline injection had moderate CAP threshold elevations (17.6 ± 1.9 dB elevation averaged across frequencies of 12–40 kHz) comparing to the control group. However, (+)-MK-801 injection prior to the same noise exposure markedly reduced the threshold elevation relative to the animals receiving saline prior to noise. The average threshold elevation among subjects receiving (+)-MK-801 and noise was 6.1 ± 0.8 dB averaged across frequencies of 12–40 kHz. (+)-MK-801 alone did not alter auditory thresholds measured 4 weeks later.

The repeated measures ANOVA showed a significant difference in CAP thresholds between the four groups presented in Fig. 1A (between treatment, $F_{3/20} = 8.59$, $P = 0.0007$; treatment–frequency interaction, $F_{30/240} = 2.95$, $P < 0.0001$). Post hoc analysis shows that the saline+noise group is significantly different from the control group of saline+air ($P = 0.0004$) and also significantly different from the group of (+)-MK-801+noise ($P = 0.0106$).

Fig. 1B presents CAP thresholds measured in the animals exposed to 100-dB octave-band noise and pretreated with (−)-MK-801, which has little activity at the NMDA receptor, or with saline. The experiment was ran separately from that shown in Fig. 1A. Unlike (+)-MK-801, (−)-MK-801 administration did not protect against the noise-induced CAP threshold elevation. Instead, the animals with (−)-MK-801 and those with saline pretreatment showed equivalent noise-induced threshold elevation compared to the control animals. The average threshold elevation among subjects receiving (−)-MK-801 and noise was 23.1 ± 2.0 dB averaged across frequencies of 12–40 kHz and the group receiving saline and noise was 18.4 ± 2.3 dB.

The repeated measures ANOVA showed a significant difference in CAP thresholds between the three groups presented in Fig. 1B (between treatment, $F_{2/15} = 8.40$, $P = 0.0036$; treatment–frequency interaction, $F_{20/150} = 3.98$, $P < 0.0001$). While noise exposure elevated auditory thresholds significantly above control ($P = 0.0066$), post hoc analysis shows that the saline+noise group is

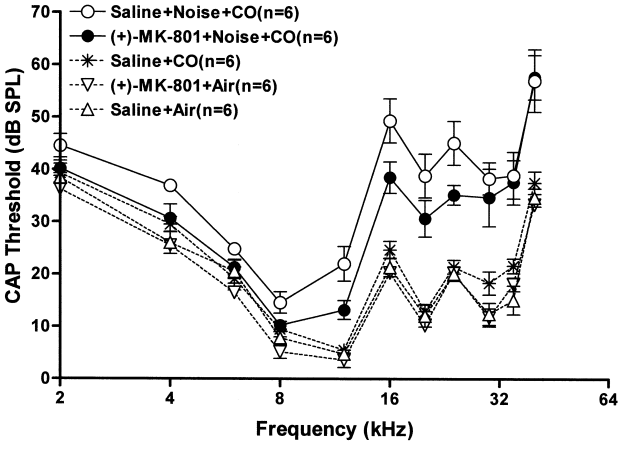

Fig. 2. CAP thresholds versus test frequencies showing protection of (+)-MK-801 against CAP threshold elevations caused by combined exposure to noise and CO. Vertical bars indicate standard error of the mean (S.E.M.). Noise exposure, treatment of (+)-MK-801 and saline and CAP threshold measurement are the same as that shown in Fig. 1; CO exposure: 1200 ppm, 3.5 h with 1.5 h prior to the onset of noise.

not different from the group that received (−)-MK-801+noise ($P = 0.4968$).

3.2. Effect of (+)-MK-801 against potentiation of noise-induced CAP threshold loss by CO exposure

Fig. 2 presents CAP thresholds of animals exposed to 100-dB OBN and 1200 ppm CO. The combined exposure caused 24.1 ± 1.3 dB CAP threshold elevation averaged across the frequencies of 12–40 kHz (open circles). Pretreatment with (+)-MK-801 injection reduced the threshold elevation relative to that seen in subjects exposed to CO+noise, but the extent of this reduction was limited. The average threshold elevation among subjects receiving (+)-MK-801+noise+CO was 18.1 ± 2.0 dB averaged across frequencies of 12–40 kHz. CO exposure alone did not cause a CAP threshold loss relative to the air controls.

The repeated measures ANOVA showed a significant difference in CAP thresholds between the five groups in Fig. 2 (between treatment, $F_{4/25} = 34.41$, $P < 0.0001$; treatment–frequency interaction, $F_{40/250} = 5.22$, $P < 0.0001$). The saline+noise+CO group has significantly higher thresholds than the control group that received saline+air ($P < 0.0001$). Additionally, the subjects receiving saline+noise+CO differ significantly from the group that received (+)-MK-801+noise+CO ($P = 0.0117$). Thresholds of (+)-MK-801+noise+CO are also significantly different from the control ($P < 0.0001$). There is no significant difference between the three groups of saline+CO and (+)-MK-801+air and saline+air.

Comparison between Fig. 1A and Fig. 2 suggests that while (+)-MK-801 can reduce NIHL, it does not block the potentiation of NIHL by CO exposure (extra threshold loss by combined exposure). The saline+noise group had a 17.6-dB threshold loss averaged across frequencies of 12–40 kHz (see Fig. 1A, open circles) while subjects receiving saline+noise+CO had a 24.1-dB average threshold loss (Fig. 2, open circles), giving a 6.5-dB threshold loss potentiation by CO. With (+)-MK-801 treatment, while threshold loss by noise alone reduced to an averaged 6.1-dB threshold loss (Fig. 1A, filled circles), subjects receiving (+)-MK-801+noise+CO only reduced to an averaged 18.1-dB threshold loss (Fig. 2, filled circles), giving an extra 12.0-dB loss by the combined exposure.

Though CAP threshold loss caused by noise+CO was partially protected against by (+)-MK-801 (see Fig. 2), hair cell losses were not reduced by the (+)-MK-801 administration. Fig. 3 plots OHC losses caused by the combined exposure to noise and CO with and without (+)-MK-801 as a function of distance from the cochlea apex. Hair cell loss is not presented for other groups since OHC loss was rarely observed in animals exposed to the noise alone. Hearing loss with few or without hair cell loss is possible (Hunter-Duvar, 1975). IHC loss was rarely observed in any groups of animals. The OHC losses were not significantly different between the two groups pretreated with saline or (+)-MK-801 and then exposed to the noise ($P = 0.8000$).

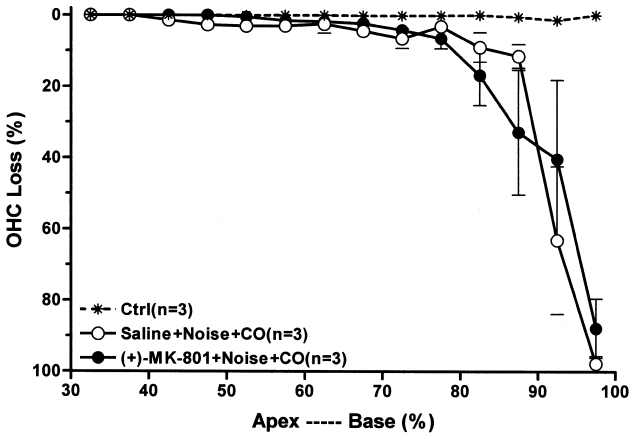

Fig. 3. Outer hair cell loss as function of cochlear distance from apex showing no evident protection of (+)-MK-801 against cell loss. Vertical bars indicate standard error of the mean (S.E.M.). *, Control animals; ○, exposed to noise+CO with saline injection; ●, exposed to noise+CO with (+)-MK-801 injection (1 mg/kg).

4. Discussion

The present study compares the efficacy of (+)-MK-801 hydrogen maleate and (−)-MK-801 hydrogen maleate to provide protection against permanent NIHL in rats. (+)-MK-801 is an NMDA receptor channel blocker, while (−)-MK-801 has a low affinity for the NMDA receptor (Griesbach and Amsel, 1998). The data show that (+)-MK-801 does protect against NIHL, while (−)-MK-801 does not protect against the NIHL. This difference in efficacy to protect against NIHL is consistent with the differential drug sensitivity at the NMDA receptor. The data are not consistent with a hypothesis that hydrogen maleate can reduce ototoxicity by acting as an antioxidant. The potentiation of the permanent NIHL by CO exposure was not reduced by the administration of the NMDA receptor blocker. This finding suggests that the potentiation of the permanent NIHL by CO does not stem from impairment resulting from NMDA-mediated stimulation, though these receptors do seem to be involved both in acute CO-induced hearing loss (Liu and Fechter, 1995) and acute NIHL (Duan et al., 2000; Puel et al., 1998).

Indirect evidence with the use of glutamate antagonists suggests that NMDA receptor stimulation is involved in hearing loss induced by exposure to various ototoxic agents, such as noise (Duan et al., 2000; Puel

et al., 1998), aminoglycoside antibiotics (Basile et al., 1996; Duan et al., 2000), and acute CO (Liu and Fechter, 1995). However, glutamate receptor antagonists, including NMDA receptor antagonists, in most cases, only partially protect against the auditory impairments (Basile et al., 1996; Duan et al., 2000; Janssen, 1992; Liu and Fechter, 1995; Puel et al., 1998). That glutamate receptor antagonists do not protect completely against noise-induced auditory impairments reflects the fact that other mechanisms are also involved in NIHL.

Noise-induced impairment is thought to reflect a number of mechanisms including mechanical damage (Borg and Engstrom, 1989; Clark and Pickles, 1996; Engstrom, 1983; Engstrom and Borg, 1983; Henderson et al., 1994; Lataye et al., 2000; Levine et al., 1998; Vertes et al., 1982; Ward et al., 1981) and metabolic alterations (Chen et al., 2000; Fridberger et al., 1998; Hu and Henderson, 1997; Jacono et al., 1998; Lim et al., 1993; Liu, 1992; Ohlemiller et al., 1999; Seidman et al., 1993; Wang et al., 1990; Yamane et al., 1995a,b; Yamasoba et al., 1999). The relative importance of such mechanisms is influenced, no doubt, by noise severity. Damage to the OHC is thought to be especially important for NIHL based largely upon histological studies using surface preparation (e.g. Hamernik et al., 1989; Lurie, 1937; Ryan and Dallos, 1975; Stebbins et al., 1979). Because glutamate receptors are believed to be restricted to the spiral ganglion cell (SGC) type I synapses (Littman et al., 1989; Matsubara et al., 1996; Niedzielski and Wenthold, 1995; Ruel et al., 1999; Ryan et al., 1991; Safieddine and Bybalin, 1992; Usami et al., 1995), though glutamate receptor expressions are transiently presented in the OHCs in the developing ear, the current results suggest a role for IHC–SGC in certain forms of NIHL (Knipper et al., 1997). Noise-induced swelling of the radial afferent dendrites under IHCs has also been observed (Duan et al., 2000).

Noise-induced permanent hearing loss can be potentiated by CO (Chen and Fechter, 1999; Chen et al., 1999; Fechter et al., 1988). The mechanism of the potentiation is still unclear, but previous data do show extra damage to the OHCs by the combined exposure to noise and CO (Chen et al., 1999). In the present study, though the combined exposure to noise and CO induced more CAP threshold elevation than the noise alone, the protection of (+)-MK-801 against the combined exposure-induced threshold elevation did not exceed that against the threshold elevation by the noise alone. The data indicate that the NIHL potentiation by CO may not stem from the excitotoxic process in the cochlea, though it is known that (+)-MK-801 can protect against both the acute effect of noise and CO (Liu and Fechter, 1995; Duan et al., 2000; Puel et al., 1998). It must be mentioned that this conclusion is limited due to the one time point and one dosage level administration of the NMDA receptor antagonist and also the one post-exposure time hearing loss measurement.

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