Tohoku J. Exp. Med., 1999, **188**, 161-175

The Effects of Tone Exposure on the Inner Ear Functions in the Guinea Pig: Impact Tone vs. Steady State Tone

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INADA, N., HOTTA, S., ITOH, T. and YAMAMURA, K. *The Effects of Tone Exposure on the Inner Ear Functions in the Guinea Pig-Impact Tone vs. Steady State State.* Tohoku J. Exp. Med., 1999, **188** (2), 161-175 —— The damage-risk criterion (DRC) for hearing supposes that sound exposure with equal energy implies equal risk for noise-induced hearing loss (NIHL). We measured cochlear microphonics (CM), compound action potential (CAP), endocochlear potential (EP) and K^+ ion concentration in the scala media, to see if the same level of Leq_{24h} (impact tone and steady state tone) induced the same physiological changes in the inner ear function or not. Regarding the equal energy principle (EEP), we also examined if the EEP is appopriate or not at exposure of moderate level tone. We also checked how the time interval between impact tones affects or not the inner ear functions at the same Leq_{24h} tone exposure. Therefore we used exposure at 1 pulse/second or 1 pulse/3 seconds and steady state tone exposure at $Leq_{24h} = 90$, 85 and 80 dB. The results are the following. Both steady state and impact tone exposure causes change of the electrophysiological data. First, CM maximum output voltage after exposure to impact tone of 115 dB ($Leq_{24h} = 90$ dB) was lower than after exposure to a 8 kHz steady state tone of 90 dB. CAP threshold (below 10 μV) obtained after the 115 and 110 dB exposure of impact tone were 5-10 dB higher than that of steady state tone of 90 dB. The negative EP induced by impact tone exposures showed the same tendency as the CM experiments. Having more frequent pulses (1 pulse/second vs. to 1 pulse/3 seconds) showed more inhibition. The K^+ concentration time course remained similar to the control when the Leq_{24h} was low (80 dB). Impact tone exposure induced stronger effects to the inner ear at exposure of moderate level tone than that of steady state tone of Leq_{24h}. ———— impact tone exposure; equal energy principle; CM; CAP; EP; K^+ ion

It is well known that temporary threshold shifts (TTS) and decreases of maximum cochlear microphonics (CM) occur following intense sound exposure (Fujii 1970; Sugisawa et al. 1994a; Hotta et al. 1998). Noise induced TTS was

Received March 16, 1999; revision accepted for publication June 23, 1999.

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considered to be integral part of the noise induced permanent threshold shift (NIPTS) process (Glorig et al. 1961). Moreover, it is an essential problem to prevent noise-induced hearing loss (NIHL). Recently, the equal energy principle (EEP) has been proposed as a useful device to measure the hazard of noise exposures in different conditions of tone exposure (Hamernik et al. 1987; Ward 1991).

However, Danielson et al. (1991) reported that high sound level impulse presented at "1 pulse/second" produces more hearing loss and hair cell damage than that of equal amount of energy in chronic experiment. They concluded that some combinations of temporal spacing and amplitude level produced totally different pattern of hearing loss. Dunn et al. (1991) measured auditory evoked response (AER) of chinchillas divided into two groups. One group was exposed to impact noise (peak value; 120 dB [SPL]) and the other group to the continuous pink noise of $L\mathrm{eq}_{24\mathrm{h}}$ (110 dB [SPL]) with the same power spectrum (frequency; Ca 30 Hz-10 kHz, spectrum of peak value: 3 kHz). Hearing threshold shift induced by the impact noise exposure was larger than that by the pink noise exposure, although the Leq was identical. In agreement with those results, Hamernik et al. (1987) reported that impulse noise (peak value; 131–147 dB) with same spectrum and energy can generate 20 dB higher threshold than that of continuous noise of $L\mathrm{eq}_{24\mathrm{h}}$.

The purpose of this study is to examine the applicability of the $L\mathrm{eq}_{24\mathrm{h}}$ as damage lisk criterion (DRC) using several intensity of impact tone (trapezoidal intermittent tone with fast rise time)(8 kHz). The effects of impact tone ($L\mathrm{eq}_{24\mathrm{h}}=90$, 85 and 80 dB) on the hearing were compared to that of the 90 dB steady state tone by measuring the electrophysiological parameters (CM, compound action potential (CAP), endocochlear potential (EP) and K^+ ion concentration in the endolymph).

Materials and Methods

Animals

One hundred forty five healthy albino (Hartley) guinea pigs with normal Preyer's reflexes, more than 5 weeks old, (weight; 250–300 g) were examined. Animals were fed the diet ad libitum and assigned to one of the 18 groups (Table 1).

The care and use of the animals reported in this study were approved by the animal comittee of our college.

Tone exposure conditions

(1) The Control a (CM, CAP); (2) Control b (EP); (3) Control c (K^+) chemical potential measurement with double-barrel K^+-sensitive liquid membrane electrodes in the following 3 experiments; (4) Exp. 1a (CM, CAP), (guinea pig had been exposed to 90 dB tone of steady-state); (5) Exp. 1b (EP); (6) Exp. 1c

TABLE 1. *Experimental conditions*

	No. of Experiment	Animals	Tone exposure conditions
1	Control a (CM, CAP)	9	
2	Control b (EP)	8	
3	Control c (K^+)	8	
4	Experiment 1a (CM, CAP)	8	
5	Experiment 1b (EP)	8	8 kHz, steady-state 90 dB
6	Experiment 1c (K^+)	8	
7	Experiment 2a (CM, CAP)	8	8 kHz, impact tone: peak
8	Experiment 2b (EP)	8	value 115 dB, 1 pulse/seconds,
9	Experiment 2c (K^+)	8	duration 3 milliseconds $Leq_{24h}=90$ dB
10	Experiment 3a (CM, CAP)	8	8 kHz, impact tone: peak
11	Experiment 3b (EP)	8	value 110 dB, 1 pulse/seconds,
12	Experiment 3c (K^+)	8	duration 3 milliseconds $Leq_{24h}=85$ dB
13	Experiment 4a (CM, CAP)	8	8 kHz, impact tone: peak
14	Experiment 4b (EP)	8	value 105 dB, 1 pulse/seconds,
15	Experiment 4c (K^+)	8	duration 3 milliseconds $Leq_{24h}=80$ dB
16	Experiment 5a (CM, CAP)	8	8 kHz, impact tone: peak
17	Experiment 5b (EP)	8	value 115 dB, 1 pulse/3 seconds,
18	Experiment 5c (K^+)	8	duration 9 milliseconds $Leq_{24h}=90$ dB

K^+; chemical potential measured with double-barrel K^+-sensitive liquid-membrane electrodes.

(K^+); (7) Exp. 2a (CM, CAP) 8 kHz impact tone, peak value 115 dB, 1 pulse/second, duration 3 milliseconds, $Leq_{24h}=90$ dB); (8) Exp. 2b (EP), (9) Exp. 2c (K^+); (10) Exp. 3a (CM, CAP) 8 kHz impact tone, peak value 110 dB, 1 pulse/second, duration 3 milliseconds, $Leq_{24h}=85$ dB); (11) Exp. 3b (EP); (12) Exp. 3c (K^+); (13) Exp. 4a (CM, CAP; 8 kHz intermittent tone, peak value 105 dB, 1 pulse/second, duration 3 milliseconds, $Leq_{24h}=80$ dB), (Impact tone of 8 kHz of 115 dB was shown in Figs. 1 and 2); (14) Exp. 4b (EP); (15) Exp. 4c (K^+); (16) Exp. 5a (CM, CAP; impact tone, peak value 115 dB, 1 pulse/seconds, duration 9 milliseconds, $Leq_{24h}=90$ dB); (17) Exp. 5b (EP); (18) Exp. 5c (K^+).

The exposure duration was 24 hours (from 10 : 00 a.m. to 10 : 00 a.m. the following day) in every experiments. During noise exposure, animals had access to food and water ad libitum.

Above mentioned experiments, frequency of impact tone exposure were examined from following reason. The acoustic reflex induced by tone exposure maintained during 0.5 second in the middle ear muscle. Therefore, low frequency of impact tone (1 pulse/second) was considered strong effect to the inner ear (Coles and Rice 1970). In the truth, frequency of press hammer was low.

The rise-decay time of above tone was instantaneous (Figs. 1 and 2). Noise

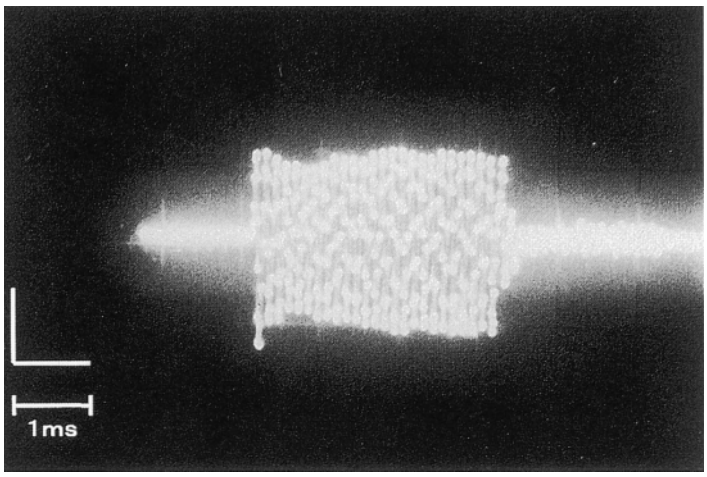

Fig. 1. Time course of exposed tone (8 kHz)-peak value 115 dB (all pass).

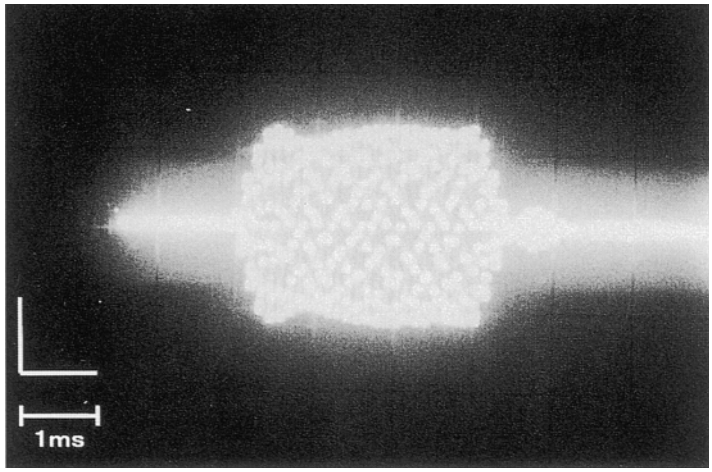

Fig. 2. Exposed tone (8 kHz)-peak value 115 dB (1/3 octave analysis, center frequency: 8 kHz).

exposure was carried out in a $4 \times 2.5 \times 3$ m soundproof room (sound insulation >50 dB at 1 kHz). The apparatus used for sound exposures included speakers (JBL 2426H, Northridge, CA, USA), an audio signal generator (LAG-126, Leader, Tokyo), pre and main amplifier (Model P 300 and Model CII, Accuphase, Yokohama) and electronic switch (TG 048, SB10A, Rion Co., Tokyo). In order to limit the movement, each guinea pig was put in wire cage of narrow width. The right ear of each animal faced the speakers at a distance of 20 cm. In every experiment, two guinea pigs in separate cages, guinea pig were exposed to tone. The sound pressure level was monitored at the side of the animals, using a precision sound meter with a 1/2 inch microphone (NA-40, Rion Co.) and a 1/3 octave analyzer (SA-59A, Rion Co.).

However, 1/3 octave band level of 6.3 kHz and 10 kHz (center frequency) were lower by 10–15 dB than that of 8 kHz. An electronic switch (Digital Pulse Generator, Rion Co.) and transient memory (RAT-1100, Nihon Kohden, Tokyo), was used to generate the impact tone. Control experiments were carried out

without noise exposure.

Electrophysiological data

The guinea pig was anesthetized with 0.5 ml/kg (50 mg/ml) sodium pentobarbital. After tracheotomy, 1 ml/kg (20 mg/ml) succinylcholine chloride was injected into the leg muscle, and the animal was connected to a respirator (SN-480-7, Shinano K.K., Tokyo).

Room temperature was maintained above 20°C and hand warmer (Tokai Kagaku Co., Nagoya) was laid under a guinea pig during the experiment.

The apparatus used to measure the CM, and CAP included a generator (Iwatsu FG-350, Tokyo), an amplifier (Iwasaki Electronic, Sapporo), an electronic switch (Digital Pulse Generator, Rion Co.), transient memory (RAT-1100, Nihon Kohden, Tokyo), an oscilloscope (622J, Nihon Kohden) and a biophysical amplifier (MEG-1100, Nihon Kohden). Test sound stimuli produced by the speaker were delivered by a vinyl tube to the outer ear canal. The sound pressure level of the test tone was set by the dial of the above amplifier. Under a stereomicroscope (SZ, Olympus, Tokyo) the bulla was opened to expose the cochlea by a ventral approach. A dental reamer was used to make small (50 μm) holes in the scala vestibuli and tympani in the basal turn of the cochlea (about 3–4 mm from the oval window).

About 30 minutes after the noise exposure, the CM was measured by the method of vestibulo-tympanal differential recording (Tasaki and Spyropoulos 1959). Silver wires (30 μm in diameter) were inserted into the holes with a micromanipulator. The CM responses at 6 and 8 kHz were recorded from a pair of these electrodes and introduced into a high-impedance amplifier connected to transient memory and an oscilloscope.

The CAP was recorded by a 30 μm silver wire electrode inserted into the small hole in the scala vestibuli and an indifferent electrode using a small silver plate inserted into the guinea pig's neck muscle. Using an electronic switch (TG 048, SB-10A, Rion Co.), the stimulus sound of the tone burst (1 pulse/second) was set as follows. The rise time was within 1 millisecond and the decay time was within 2.5 milliseconds following 7 kHz pure tone. The pseudothreshold of the control (i.e., the threshold obtained under 10 μV N_1 potential of CAP) at 7 kHz was 15 dB in above amplifier (Iwasaki Electronic) at peak equivalent sound pressure level at control experiment. Using biophysical amplifier (MEG 1100 Nihon Kohden) CM (Low cut: 1.5 Hz-10 kHz: High cut) and CAP (Low cut: 1.5 Hz-1 kHz: High cut) were obtained. We usually carried out the experiment from clock 10 μseconds and duration 10 milliseconds (Transient Memory RAT-1100, Nihon Kohden). The amplifier used in this experiment had limit of maximum frequency 10 kHz. After the CM examination was completed, CAP was measured on the same guinea pigs.

A small hole in the scala media was perforated using a model SMZ-U

microscope (Nikon, Tokyo) at a magnification of ×60–65.

The electrode for EP measurements was made from a glass micropipette with a tip diameter of 1–3 μm filled with 3 mM KCl solution, in which a silver chloride wire (diameter, 200 μm) was immersed. The glass microelectrode was inserted into the small hole in the basal turn of the scala media using an electronic microdriver (model MW-81, Narishige, Tokyo).

As EP parameter, the resting potential (80–90 mV in the control animals) the minimum potential during asphyxia (−25 to −35 mV in the control animals), and the potential after asphyxia (overshoot). However, 3 minutes asphyxia was examined at measurement of the minimum potential of EP. The typical EP change induced by 3 minutes asphyxia is shown already (Sugisawa et al. 1994a).

Chemical potential of K^+ ion using a double-barrel electrode

The method of fabrication of the double-barrel K^+ electrode was according to Konishi and Salt (1983) and Abe et al. (1981) as follows. Two segments of capillary tubes (diameter, 1 mm) with internal filaments were glued together and pulled into a double-barreled glass microelectrode using a puller (PD-5, Narishige). The tip diameter of each barrel was less than 1–2 μm. The inside surface of the ion barrel was silanized by exposing it to dichlordimethyl silane (for 45 minutes at 50°C). The capillaries were then cured for 60 minutes at 200°C. The tip of the ion barrel was filled with the K^+ exchanger (IE190, WPI Inc., Sarasota, FL, USA) by introducing a small droplet from the back of the capillary. Thereafter, the shafts were filled with reference solution (using 150 mM KCl solution). The differential barrel was filled with 150 mM NaCl solution.

Electrodes were calibrated in isotonic mixtures of 150 mM NaCl-KCl solutions. The responses of the K^+-selective barrels were determined in 4.8, 15, 100 and 150 mM K^+ (the additional cations being Na^+). When respirator was stopped during 40 minutes, the concentration of K^+ ion in the scala media was observed. From the beginning of the sphixia, K^+ ion concentration at 3, 5, 10, 20, 30 and 40 minutes were observed temporary. EP and K^+ ion measurement were observed in another space nearby noise exposure room.

Results

Cochlear microphonics

In Fig. 3, the CM intensity function to 8 kHz test frequency were shown (the control and Exp. 1a-3a). There are significant differences in CM maximum output voltage to sound stimulus at 75 dB SPL among that of control and those of Exp. 1a, 2a and 3a. There are significant differences in CM maximum output voltage between that of Exp. 2a and that of Exp. 3a. Asymptotic threshold shift (ATS; threshold shift induced more than 12–16 hours tone exposure) of Exp. 1a-3a elevated more 10 dB than that of control. However, ATS of Exp. 2a was 5 dB greater than those of Exp. 1a and 3a. In Fig. 4, there is significant difference

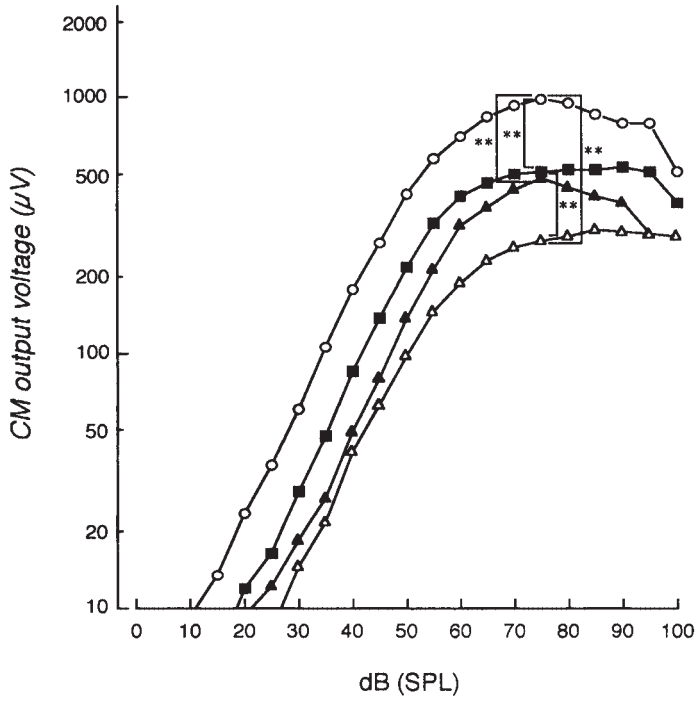

Fig. 3. Mean intensity function of CM (test frequency; 8 kHz). ○, Control; ▲, Experiment 1a (90 dB steady state tone exposure of 8 kHz); △, Experiment 2a (impact tone of 8 kHz exposure, peak value 115 dB); ■, Experiment 3a (impact tone exposure, peak value 110 dB).
$^{**}p < 0.01$ (t-test).

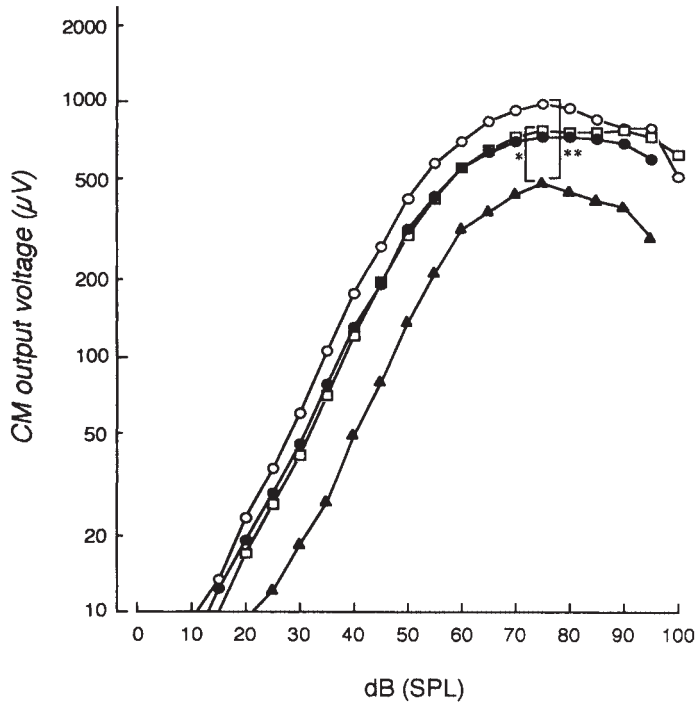

Fig. 4. Mean intensity function of CM (test frequency; 8 kHz). ○, Control; ▲, Experiment 1a (90 dB steady state tone exposure of 8 kHz); □, Experiment 4a (impact tone exposure, peak value 105 dB); ●, Experiment 5a (impact tone exposure [1 pulse/3 seconds], peak value 115 dB).
$^{*}p < 0.05$, $^{**}p < 0.01$ (t-test).

in CM maximum output voltage to test sound stimulus at 75 dB (SPL) among that of Exp. 1a and those of control, Exp. 4a and 5a, and also threshold of Exp. 1a was elevated 5 dB greater than control, Exp. 4a and 5a. The Exp. 2a conditions (namely, 115 dB, 1 pulse/second) has the most pronounced effect. The above experimental data were close to each other, so we show these data on separate figures.

Compound action potential

In this experiment, compound action potential (CAP) value below 10 μV was considered as threshold. In Figs. 5 and 6, CAP intensity function is shown. In this experiment, CAP was observed directively. Compared CM output voltage, an extent of that of CAP was small. In this experiment, CM data and CAP data were shown to close by many experimental series. The points are above to each other so we expresssed these data in separate figures. The threshold value of whole-nerve CAP was different for each experiment. Under the Exp. 2a, CAP maximum output voltage at 115 dB (SPL) was much lower than that of the control, Exp. 1a and 3a. ($p<0.01$) and elevation of threshold between Exp. 2a and control was also observed (Ca, 20 dB). In Fig. 6, there are significant differences of maximum output voltage among that of control and those of Exp. 1a, 4a and

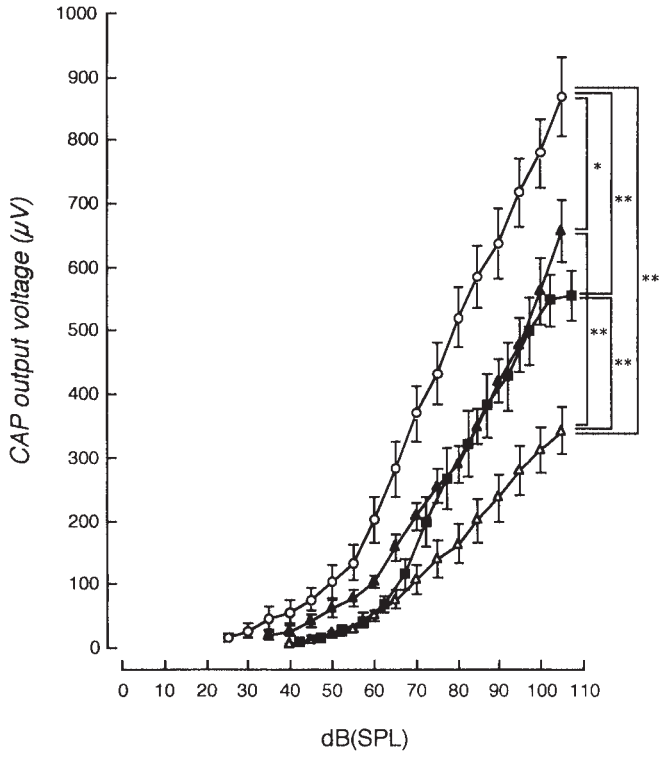

Fig. 5. Mean intensity function of CAP (test frequency; 7 kHz). ○, Control; ▲, Experiment 1a (90 dB steady state tone exposure of 8 kHz); △, Experiment 2a (impact tone of 8 kHz exposure, peak value 115 dB); ■, Experiment 3a (impact tone exposure, peak value 110 dB).
$^{*}p<0.05$, $^{**}p<0.01$ (t-test).

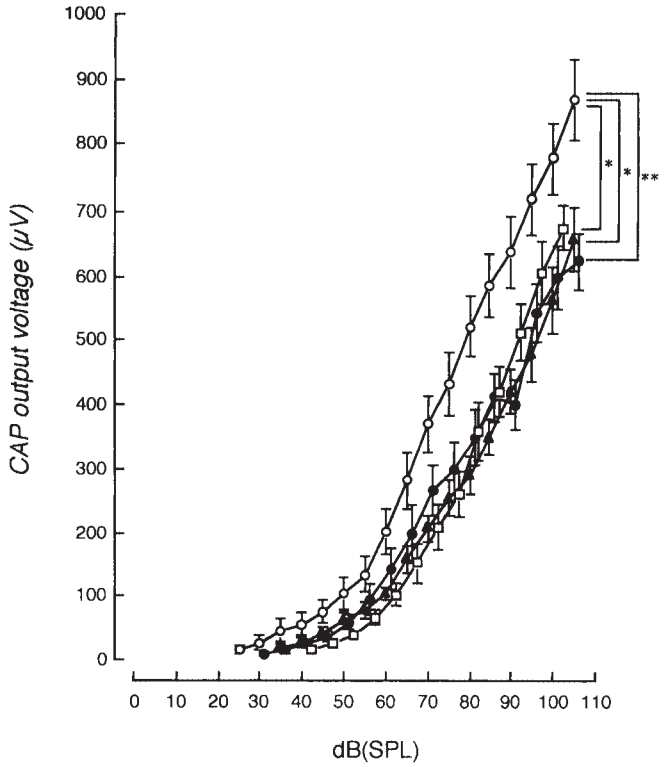

Fig. 6. Mean intensity function of CAP (test frequency; 7 kHz). ○, Control; ▲, Experiment 1a (90 dB steady state tone exposure of 8 kHz); □, Experiment 4a (impact tone exposure, peak value 105 dB); ●, Experiment 5a (impact tone exposure [1 pulse/3 seconds], peak value 115 dB).
$^{*}p<0.05$, $^{**}p<0.01$ (t-test).

TABLE 2. *Threshold (below 10 μV) of CAP*

Exp. No.		
Exp. 2 (impact 115 dB = Leq_{24h} = 90 dB)	48.1±5.9	a
Exp. 3 (impact 110 dB = Leq_{24h} = 85 dB)	42.1±8.6	a
Exp. 4 (impact 105 dB = Leq_{24h} = 80 dB)	33.8±8.3	a
Exp. 1 (st-st = 90 dB)	32.1±9.5	a
Exp. 5 (impact 115 dB = 1 pulse/3 sec = Leq_{24h} = 90 dB)	28.4±6.5	a
Control	20.0±5.3	

[a]Multiple comparison test (Bonferroni), $p<0.05$.

5a.

The result of CAP threshold elevation was shown in Table 2. There were significant differences among CAP threshold of Exp. 2 (impact 115 dB) and those of Exp. 4 (impact 105 dB: Leq_{24h} = 80 dB), Exp. 1 (steady-state tone of 90 dB)

TABLE 3. *Change of EP induced by tone exposure*

	Pre P (mV)	Negative EP (mV)	After P (mV)	N
Control	86.0±2.4	−28.4±2.0	90.4±2.9	8
Exp. 1b (st-st. 90 dB)	87.6±1.9	−12.0±6.0	92.1±1.8	8
Exp. 2b (impact 115, Leq_{24h}=90 dB)	88.0±2.7	− 2.7±6.0	93.1±4.1	8
Exp. 3b (impact 110, Leq_{24h}=85 dB)	85.3±3.0	− 5.6±2.3	88.2±1.9	8
Exp. 4b (impact 105, Leq_{24h}=80 dB)	87.0±2.2	−13.2±5.6	91.8±3.7	8
Exp. 5b (impact 115, Leq_{24h}=90 dB, 1 pulse/3 seconds)	87.3±1.9	−10.8±4.2	91.8±1.9	8

[a]Multiple comparison test, Duncan test ($p<0.05$).

and Exp. 5 (impact 115 dB, 1 pulse/3 seconds). There were significant differences among CAP threshold Exp. 3 (impact 110 dB) and those of Exp. 4, Exp. 1, Exp. 5 and Control. Increased value of Leq_{24h} caused inhibition of the CAP output value (Leq_{24h}=90 dB has much stronger effect than Leq_{24h}=80 dB). However as Figs. 3 and 4 show changing the pulse from 1 pulse/1 second to 1 pulse/3 seconds results in much less inhibition.

EP measurement

The results of the EP measurements of control and in Exp. 1b, 2b, 3b, 4b and 5b (n=8) are summarized in Table 3. We compared the static level, the negative potential, and the after potential. In Fig. 7, one example of EP time course was shown (Exp. 2b). A reduction in the absolute value of negative potential (control vs., Exp. 1b, 2b, 3b, 4b and 5b [multiple comparisons test: Duncan $p<0.05$]) was observed. There was no significant difference either between the negative value of EP obtained in Exp. 1b (90 dB steady state tone) and that of Exp. 4b (impact tone exposure, Leq_{24h}=80 dB). Impact tone exposure showed a dose dependency (Exp. 2b, 3b, 4b). Impact (1 pulse/second) tone of 115 dB (Leq_{24h}= 90 dB) has the biggest effect in these experiments. However where the pulse rate was decreased from 1 pulse/second to 1 pulse/3 seconds, the inhibition got significantly less in same Leq level (comparable to the steady state 90 dB condition: −12.0±6.0 in Exp. 1b, −2.7±6.0 in Exp. 2b and −10.8±4.2 in Exp. 5b).

K^+ ion measurement

Using the double-barrel electrode, the chemical potential of endolymph (K^+)

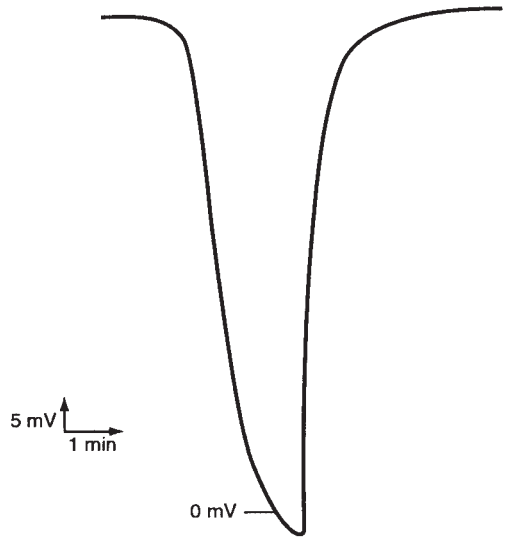

Fig. 7. Time course of EP. One example (Exp. 2b).

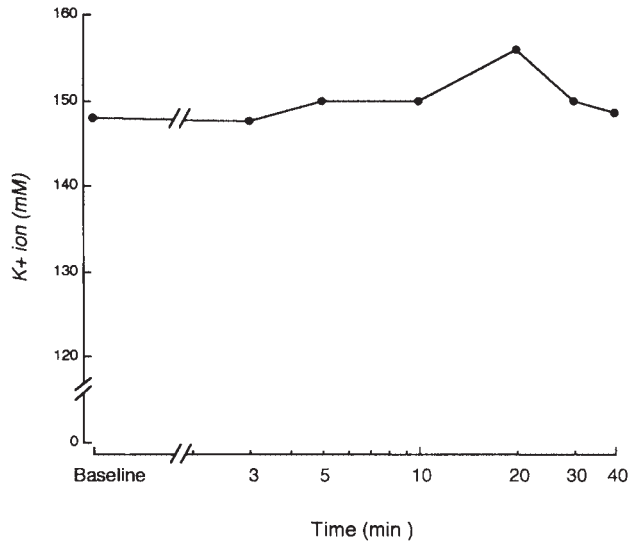

Fig. 8. Time course of K^+ ion concentration in the scala media. One example (Exp. 2c).

was measured. The regression equation (potential vs. KCl concentration) was calculated for each electrode, allowing the determination of K^+ concentrations. The K^+ ion concentration for 40 minutes asphyxia is shown in Table 4. In Fig. 8, one example of K^+ ion concentration (time course: 40 minutes) was shown (Exp. 2c). A systematic decrease of the K^+ concentration can be seen over the time in control animals, Exp. 4c and 5c. However, no significant decrease in the K^+ ion concentrations were found in animals of Exp. 1c (steady state 90 dB), 2c ($Leq_{24h} = 90$ dB) and 3c ($Leq_{24h} = 85$ dB), indicating an alteration in the K^+ transport throughout the hair cells (Multiple range test, $p < 0.05$). In the case of K^+ transport also the pulse rate is an important factor. Applying the same Leq_{24h} but using different pulse pattern results in different K^+ transport. When the pulse is less frequent (1 pulse/3 seconds), the K^+ transport is comparable to the control, while 1 pulse/second stimuli at the same Leq_{24h} cause no alteration.

TABLE 4. *K^+ ion concentration (mM) in endolymph during 40 minutes asphyxia*

Exp. No.	Baseline	Time (minutes)					
		3	5	10	20	30	40
Control	145.6±5.3	147.3±9.2	144.6±11.1	142.4±10.7	141.9±11.3	140.8±10.8	126.3±5.4
Exp. 1c (st-st. 90 dB)	144.0±6.7	141.4±4.9	143.5±4.3	144.3±3.9	146.9±6.5	141.9±7.8	141.4±5.9
Exp. 2c (impact 115, Leq_{24h}=90 dB)	141.9±7.8	143.4±8.8	146.3±8.2	148.3±6.9	144.4±8.2	145.6±4.9	146.8±5.8
Exp. 3c (impact 110, Leq_{24h}=85 dB)	145.9±7.3	143.3±7.7	138.0±14.1	146.7±5.5	143.1±6.0	143.0±13.3	142.9±5.0
Exp. 4c (impact 105, Leq_{24h}=80 dB)	150.8±6.6	148.4±9.3	140.8±10.4	143.5±9.5	154.9±8.5	130.7±15.6	120.8±7.1
Exp. 5c (impact 115, 1 pulse/3 seconds, Leq_{24h}=90 dB)	148.8±8.6	148.1±9.0	146.6±12.9	143.8±9.8	142.3±9.5	131.6±14.7	121.3±8.7

[a]significance, $p<0.05$ (Multiple comparison test: modified LSD test).

Discussion

In the present experiment, the effects of impact tone exposure on inner ear function was examined by measuring CM, CAP, EP and K^+ ion concentration in the endolymph which is thought to be a function of the organ of Corti, cranial eighth nerve and the stria vascularis (Tasaki and Spyropoulos 1959) in guinea pigs. Relative high sound level (115 dB) tone with fast rise time ($Leq_{24h} = 90$ dB) but trapezoidal form (duration 3 and 9 milliseconds) were used because good reproducibility of tone exposure could be achived. The character of impact tone was usually to have fast rise time (within 1 millisecond), a moment peak time and decay time (1–200 milliseconds) (Coles and Rice 1967: Ward 1968).

The following points were examined. Whether effect of impact tone exposure to the inner ear of the guinea pigs was equal to that of same Leq_{24h} of steady state tone or not. Moreover, impact tone exposure with the same Leq_{24h} but different for the rate of pulse shows differences or not.

Nine milliseconds long pulses/3 seconds exposure seems to have milder effect on the ear function. Interval of time between impact to impact was considered to be an important factor. Low frequency pulse of impact tone exposure induce larger effect to hearing than that of high frequency (ex: 10 pulses/seconds). However, too long interval of impacat tone induce recovery of the influence to hearing by impact tone exposure at one by one (Coles and Rice 1970).

However, exposure of high level of intermittent tone with fast rise time has different physiological effect on the guinea pig inner ear than that of steady-state tone of equal energy reported by Borg and Engstrom (1989). Hamernik and Henderson (1974) reported that the result of number of missing hair cell induced by impulse tone exposure were different at several times of after exposure. Above results didn't show the liner dysfunction of the hair cell of the organ of the Corti. Moreover, Spoendlin (1976) proposed that damage risks were difficult to predict for impulse noise, but they could be considerably higher when compared with same total energy in steady noise. Based on the above observation, it is speculated that sound level, rate of presentation, rise time of intermittent tone, etc. are important parameters.

Decrease of the absolute value of negative EP in all tone exposure group were also observed. The absolute value of the negative EP in steady state tone exposure was not significantly different from low level impact tone (peak value of 105 dB: $Leq_{24h} = 80$ dB). Expose of 115 dB impact tone (1 pulse/second) were shown to have strong effect on the guinea pig EP.

A positive EP is considered to be an oxygen-sensitive, electrogenic K^+ ion potential (Oftner et al. 1987), while a negative potential represents a diffusion potential across the endolymph perilymph barrier.

K^+ ion permeability of the hair cell is important for the generation of the CM (Gibson et al. 1983). In order to obtain diffusion potential of K^+ ion, time limit

of asphyxia was planned to 3 minutes.

Pickles et al. (1984), emphasized that K^+ permeability occurred in the organ of Corti through channels in the tips of stereocilia. Lim (1980) found that noise exposure induced a loss in stereocilia rigidity, with floppiness of the cilia considered to be abnormal. Based on the above mentioned results, it was considered that tone exposure induced disturbance of function in the channel of stereocilia.

High concentration of potassium was observed in the scala media by Ma et al. (1996). Because we wanted to measure the change of K^+ ion concentration, we planned our experiments in a way to stop the function of stria vascularis (K^+ ion pump) by asphyxia. We stopped the respirator for 40 minutes. Our current experiments have not shown any decrease in the K^+ ion concentration at high level of impact tone exposure indicating an alteration in the K^+ ion movement across the membrane.

Impact tone exposure of relatively high sound level induced distortion of K^+ ion movement from the endolymph to the perilymph by channels of the hair cell (Sugisawa et al. 1994b; Hotta et al. 1996) and other channels.

Acknowledgments

This investigation was supported by a grant from the Ministry of Education Science, Sports and Culture, Japan (09670351). We thank Dr. T. Sugano, Prof. U. Imai and Dr. M. Miyamoto for their kind comments concerning our study.

References

1) Abe, N., Ohyama, S. & Shida, T. (1981) Method of fabrication of double barrel K^+ ion electrode and its application to physiology of cochlea. *Audiology*, **24**, 397-398. (in Japanese)
2) Borg, E. & Engstrom, B. (1989) Noise level, inner hair cell damage, audiometric features and equal energy hypothesis. *J. Acoust. Soc. Am.*, **86**, 1776-1782.
3) Coles, R.R.A. & Rice, G.G. (1967) Hazard from impulse noise. *Ann. Occup. Hyg.*, **10**, 381-388.
4) Coles, R.R.A. & Rice, G.G. (1970) Toward criterion for impulse noise in industry. *Ann. Occup. Hyg.*, **13**, 43-50.
5) Danielson, R., Henderson, D., Gratton, L., Bianchi, L. & Salvi, R. (1991) The importance of "temporal pattern" in traumatic impulse noise exposures. *J. Acoust. Soc. Am.*, **90**, 209-218.
6) Dunn, D.E., Davis, C.J., Merry, C.J. & Franks, J.R. (1991) Hearing loss in the chinchilla from impact and continuous noise exposure. *J. Acoust. Soc. Am.*, **90**, 1979-1985.
7) Fujii, M. (1970) The experimental study of otoacoustic trauma to the inner ear. *Practica. Otologica.*, **63**, 557-608. (in Japanese)
8) Gibson, W.P.R., Prashor, D.K. & Kilkenny, G.P.C. (1983) Diagnostic significance of transtympanic electrocochleography in Meniere's Disease. *Ann. Otol. Rhinol. Laryngol.*, **92**, 155-199.
9) Glorig, A., Ward, W.D. & Nixon, J. (1961) Damage risk criteria and noise-induced hearing loss. *Archives Otolaryngology*, **74**, 413-423.
10) Hamernik, R.P. & Henderson, D. (1974) Impulse noise trauma. A study of histological susceptibility. *Archives Otolaryngology*, **99**, 118-121.

11) Hamernik, R.P., Patterson, J.H. & Salvi, R.J. (1987) The effect of impulse intensity and the number of impulses on hearing and cochlear pathology in the chinchilla. *J. Acoust. Soc. Am.*, **81**, 1118-1129.
12) Hotta, S., Sugisawa, T. Itoh, T. & Hasebe, M. (1996) A comparative study on the effect of pure-tone exposure of the guinea pig cochlea. *Eur. Arch. Otorhinolaryngol.*, **253**, 45-51.
13) Hotta, S., Takahashi, M., Itoh, T. & Yamamura, K. (1998) Experimental study of effects of gentamicin injection on the guinea pig—electrophysiological studies—. *Acta Otolaryngol. (Stockh)*, **118**, 362-368.
14) Konishi, T. & Salt, A.N. (1983) Electrochemical profiles for potassium ions across the cochlear hair cell membranes of guinea pigs. *Hear. Res.*, **11**, 219-233.
15) Lim, D.J. (1980) Cochlear anatomy related to cochlear microphonics: A review. *J. Acoust. Soc. Am.*, **67**, 1686-1695.
16) Ma, L.Y., Rarey, K.E., Gerhardt, K.J., Curtis, L.M. & Rybak, L.P. (1996) Electrochemical potentials and potassium concentration profiles recorded from perilymph, endolymph and associated inner ear tissues in adrenalectomized rats. *Hear. Res.*, **96**, 151-156.
17) Oftner, F.F., Dallow, P. & Cjeatham, M.A. (1987) Positive endocochlear potential: Mechanism of production by marginal cells of stria vascularis. *Hear. Res.*, **29**, 117-124.
18) Pickles, J.O., Comis, S.D. & Osborne, M.P. (1984) Cross-links between stereocilia in the guinea pig organ of Corti and their possible relation to sensory transduction. *Hear. Res.*, **15**, 103-112.
19) Spoendlin, H. (1976) Anatomical changes following various noise exposure. In: *Effects of noise on hearing*, edited by D. Henderson, R.P. Hamernik, D. Dosanjh & J.H. Mills, New York, Raven Press, pp. 69-89.
20) Sugisawa, T., Ishida, A., Hotta, S. & Yamamura, K. (1994a) The effect of 6 kHz tone exposure on inner ear function of the guinea pig: Relation to changes in cochlear microphonics, action potential, endocochlear and chemical potentials of K^+ ions and Na^+ ions, using a double barrel glass electrode. *Eur. Arch. Otorhinolaryngol.*, **251**, 154-159.
21) Sugisawa, T., Nemoto, R., Inada, N., Yamamura, K. & Ishida, A. (1994b) Effect of 4 kHz tone exposure on the guinea pig inner ear: Relation in the change of cochlear microphonics action potential, electrochemical potential and K^+ ion concentration induced by noise exposure. *ORL*, **56**, 263-268.
22) Tasaki, I. & Spyropoulos, C.S. (1959) Stria vascularis as source of endocochlear potential. *J. Neurophysiol.*, **22**, 149-155.
23) Ward, W.D. (1968) Proposed damage-risk criterion for impulse noise (gunfire) (u), Report Working group 57. Contract No. NONR 2300 (5) CHABA, Washington. D.C.
24) Ward, W.D. (1991) The role of intermittence in PTS. *J. Acoust. Soc. Am.*, **90**, 164-169.