Comparative Study of Effects of Impact Tone and Steady State Tone Exposure: EP and Concentration of K^+ ion and Na^+ ion

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INADA, N., ITOH, T. and YAMAMURA, K. *Comparative Study of Effects of Impact Tone and Steady State Tone Exposure: EP and Concentration of K^+ ion and Na^+ ion.* Tohoku J. Exp. Med., 1999, 188 (3), 227-238 ——— To test the adequacy of equal energy principle (EEP), guinea pigs were exposed to impact tone. The changes in electrophysiological data, namely endocochlear potential (EP) and the change in K^+ ion and Na^+ ion concentrations in the endolymph were investigated. The frequency of impact tone was 1 pulse/second or 1 pulse/3 seconds. The steady state tone had Leq_{24h} = 100, 95, 90 or 85 dB, and impact tone had Leq_{24h} = 95, 90 or 85 dB. The results are the following. Both steady state and impact tone exposure cause changes of electrophysiological data. The effects on the absolute value of negative EP induced by impact tone exposures were smaller than that of steady state tone of the same Leq . The rate of pulses was also an important factor for impact tone exposure. Impact tone exposure of 1 pulse/second caused smaller absolute value of negative EP than that of 1 pulse/3 seconds. The K^+ ion concentration time course in the endolymph remained similar to the control (Exp. 1) only in Exp. 8 (85 dB; the lowest steady state noise exposure in our experiments), but no decrease in the K^+ ion concentration was detected in the other experiments, suggesting an alteration in the K^+ ion flow. The Na^+ ion concentration time course was also influenced showing no increase in Na^+ ion concentration compared to the control (Exp. 1c) and the lowest steady-state exposure experiment (Exp. 8c). Our experimental results suggest that both the K^+ ion and Na^+ ion movement are altered by tone exposure. We found also that the different types of noise exposure with the same Leq value does not exhibit the same changes. Leq_{24h} is not an accurate damage risk criteria. ——— impact tone exposure; equal energy principle EP; EP; K^+ ion; Na^+ ion © 1999 Tohoku University Medical Press

Temporary threshold shifts (TTS) and decrease of maximum cochlear microphonics (CM) occur following intense sound exposure (Glorig et al. 1961; Nixon et al. 1977; Sugisawa et al. 1994a, b; Hotta et al. 1996). Recently, the equal energy

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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 of steady-state tone. 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 (110 dB [SPL]) with same power spectrum (frequency; 30 Hz-10 kHz, spectrum of peak value: 3 kHz). Both noise exposures were having the same noise spectra. TTS induced by impact noise exposure was larger than that of pink noise, 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 the same Leq .

However we wanted to know if relative high level of impact tone (impact tone with fast rise time having trapezoidal form) has the same effect on the inner ear or not. The purpose of this study is to examine the applicability of the Leq_{24h} as DRC using several intensity of impact tone (trapezoidal intermittent tone with fast rise time) (8 kHz). Using EP obtained at the basal turn of the cochlea, the effects of impact tone (Leq_{24h} = 95, 90 and 85 dB) on the inner ear were compared to that of the 100, 95 and 85 dB steady state tone by measuring the electrophysiological parameters (EP and, K^+ and Na^+ ions in the scala media of the basal turn).

MATERIALS AND METHODS

Animals

One hundred forty two 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 19 groups (in Table 1).

The care and use of the animals reported in this study were approved by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan.

Tone exposure conditions

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.

The rise-decay time of above tone was within 200-300 μ seconds (Fig. 1). Noise exposure was carried out in a $4 \times 2.5 \times 3$ m soundproof room (sound insula-

TABLE 1. *Conditions of Exp. 1-8*

No. of Experiment	Animals	Tone exposure conditions
1 Experiment 1a (EP)	7	Control
2 Experiment 1b (K ⁺)	8	
3 Experiment 1c (Na ⁺)	7	
4 Experiment 2a (EP)	8	8 kHz, impact tone: peak value 120 dB,
5 Experiment 2b (K ⁺)	8	1 pulse/second, duration 3 milliseconds, $Leq_{24h} = 95$ dB
6 Experiment 3a (EP)	8	8 kHz, impact tone: peak value 115 dB,
7 Experiment 3c (Na ⁺)	8	1 pulse/second, duration 3 milliseconds, $Leq_{24h} = 90$ dB
8 Experiment 4a (EP)	7	8 kHz, impact tone: peak value 110 dB,
9 Experiment 4b (K ⁺)	8	1 pulse/second, duration 3 milliseconds, $Leq_{24h} = 85$ dB
10 Experiment 5a (EP)	8	8 kHz, impact tone: peak value 120 dB,
11 Experiment 5b (K ⁺)	6	1 pulse/3 seconds, duration 9 milliseconds,
12 Experiment 5c (Na ⁺)	8	$Leq_{24h} = 95$ dB
13 Experiment 6a (EP)	8	8 kHz, steady-state tone, 100 dB
14 Experiment 6b (K ⁺)	8	
15 Experiment 7a (EP)	7	8 kHz, steady-state tone, 95dB
16 Experiment 7b (K ⁺)	8	
17 Experiment 8a (EP)	8	8 kHz, steady-state tone, 85 dB
18 Experiment 8b (K ⁺)	8	
19 Experiment 8c (Na ⁺)	6	

K⁺, chemical potential measured with double-barrel K⁺-sensitive liquid-membrane electrodes, Na⁺, chemical potential measured with double-barrel Na⁺-sensitive liquid-membrane electrodes.

tion > 50 dB at 1 kHz). The apparatus used for sound exposures included an audio signal generator (Leader LAG-126, Tokyo) and main amplifier (Accuphase Model P 300 and Model CII, Yokohama), speakers (JBL 2450J, Northridge, CA, USA). In order to limit the movement, each guinea pig was put in a wire cage of narrow width. The right ear of each animal faced the speakers at a distance of 20 cm usually. In every experiment, two guinea pigs were exposed to tone, but in separate cages. The sound pressure level was monitored at the side of the

Fig. 1 Impact tone (8 kHz, Experiment 2).

animals, using a precision sound meter with a 1/2 inch microphone (NA-40, Rion Co., Tokyo) and a 1/3 octave analyzer (SA-59A, Rion Co.).

However, 1/3 octave band level of 6.3 and 10 kHz (center frequency) were lower by 10–15 dB than that of 8 kHz. Using electronic switch (Digital Pulse Generator, Rion Co.) and transient memory (RAT-1100, Nihon Kohden, Tokyo), the guinea pigs were exposed to the impact tone. Each group of animals was exposed to sound as schematized in Table 1 for 24 hours (10:00 a.m.–10:00 a.m.). 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). Using a hand warmer (Tokai Kagaku Co.), the animal holder was warmed. Room temperature was more than 20°C. Above condition was same in our all examination.

The bulla was opened to expose the cochlea by a ventral approach. A small hole in the scala media of the basal turn of the cochlea was perforated using a microscope (model SMZ-U, Nikon, Tokyo) at a magnification of $\times 60$ –70.

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 scala media using an electronic microdriver (model MW-81, Narishige, Tokyo).

EP parameters were the resting potential (80–90 mV in the control animals); the minimum potential during asphyxia (–25 to –35 mV in control animals), during which the respirator was stopped for 3 minutes to induce asphyxia state and the potential after asphyxia (overshoot). The typical EP change induced by

3 minutes asphyxia is shown already in our previous paper (Sugisawa et al. 1994b).

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

The method of fabrication of the double-barrel K^+ ion electrode was according to Konishi and Salt (1983) and Abe et al. (1981). 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, Tokyo). The tip diameter of each barrel was less than $1\text{--}2\text{ }\mu\text{m}$. The inside surface of the ion barrel was silanized by exposing it to dichlorodimethyl silane (for 45 minutes at 50°C). The capillaries were then heated for 60 minutes at 200°C . The tip of the ion barrel was filled with the K^+ ion 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^+ ion (the additional cations being Na^+ ion).

Respirator was stopped for 40 minutes. From the beginning of the asphyxia, K^+ ion concentration at 3, 5, 10, 20, 30 and 40 minutes were measured.

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

According to the method of Abe (1981), 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. The inside surface of the ion barrel was silanized by exposing it to dichlorodimethyl silane during 40 minutes. The capillaries were then cured for 60 minutes at 200°C . The tip of the ion barrel was filled with the Na^+ ion exchanger (IE110, WPI Inc.) by introducing a small droplet from the back of the capillary. Thereafter, the shafts were filled with reference solution (using 150 mM NaCl 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 Na^+ ion (the additional cations being K^+ ion). EP, K^+ and Na^+ ion measurements were carried out in another space nearby noise exposure room.

RESULTS

Statistical method

Statistical methods in this experiment were following. Non paired t -test and analysis of variance in one way classification were used. After analysis of variance was observed, the multiple comparison were calculated.

TABLE 2. *Change of EP induced by several tone exposure (impact tone and steady state tone)*

Group	Number (<i>n</i>)	Pre <i>p</i> (mv)	Negative <i>p</i> (mv)	Post <i>p</i> (mv)
Exp. 1a (Control)	7	86.4 ± 3.8	−29.1 ± 4.2	91.8 ± 3.9
Exp. 2a	8	89.9 ± 1.1	0.5 ± 3.1	94.1 ± 3.0
Exp. 3a	8	89.0 ± 4.0	−3.1 ± 2.6	94.4 ± 4.7
Exp. 4a	8	90.7 ± 2.1	−12.9 ± 4.0	93.7 ± 3.2
Exp. 5a	8	88.0 ± 2.7	−8.6 ± 4.9	92.0 ± 3.9
Exp. 6a	8	87.2 ± 2.7	0.8 ± 3.0	89.1 ± 2.6
Exp. 7a	7	87.7 ± 2.7	−2.7 ± 2.0	93.0 ± 6.3
Exp. 8a	8	89.3 ± 1.6	−26.4 ± 5.4	93.1 ± 2.6

^a*p* < 0.01. ^b*p* < 0.05 (Student's *t*-test). See Table 1 for tone exposure conditions.

EP measurement

The results of the EP measurements of control and in Exp. 1a, 2a, 3a, 4a and 5a, 6a, 7a and 8a are summarized in Table 2. We compared the static level, the negative potential, and the after potential.

A reduction in the absolute value of negative potential (Exp. 1a vs. Exp. 2a, 3a, 4a, 5a, 6a and 7a) were observed. There was significant difference either between negative value of EP obtained Exp. 7a (95 dB steady state tone) and that of Exp. 2a (impact tone exposure, $Leq_{24h} = 95$ dB) ($p < 0.05$). But, there were not significant differences between that of Exp. 2a vs. that Exp. 6a (steady-state tone of 100 dB) and that of impact tone of 115 dB (Exp. 3a) vs. steady-state tone of 95 dB (Exp. 7a). Also, there were significant differences in negative potential between that of Exp. 4 (peak value 110 dB) and that of Exp. 8 (steady-state tone, 85 dB). Impact tone exposure showed a dose dependency (Exp. 2a, 3a, 4a [steady-state] and, 6a, 7a, 8a [impact tone exposure]). Impact (1/second) tone of 120 dB ($Leq_{24h} = 95$ dB) has the biggest effect in these experiments. However when the pulse rate was decreased from 1/second to 1/3 seconds, the inhibition decreased significantly in the same Leq level (comparable to the steady state 95 dB condition: $-2.7 \text{ mv} \pm 2.0$ in Exp. 7a, $0.5 \text{ mv} \pm 3.1$ in Exp. 2a and $-8.6 \text{ mv} \pm 4.9$ in Exp. 5a).

Fig. 2 K^+ ion concentration (mM) in endolymph during 40 minute asphyxia (mean $\pm$ S.D.)

Mean $\pm$ S.D. * $p < 0.05$ (Multiple Range Test: modified LSD test).

Fig. 3 Na⁺ ion concentration (mM) in endolymph during 40 minute asphyxia (mean $\pm$ S.D.)

Mean $\pm$ S.D. * $p < 0.05$ (Multiple Range Test: modified LSD test).

K⁺ ion measurement

The K⁺ ion concentration for 40 minute asphyxia is shown in Fig. 2. A decrease with time of the K⁺ ion concentration can be seen in Exp. 1 and Exp. 8. However, no significant decrease in the K⁺ ion concentrations were found in

animals of Exp. 2b (impact tone 120 dB), 4b (impact tone 110 dB, 1 pulse/second), 5b (impact tone 120 dB, 1 pulse/3 seconds) and Exp. 7a (steady-state 95 dB), indicating an alteration in the K^+ ion transport throughout the hair cells and other tissue (Modified LSD Test, $p < 0.05$).

Na⁺ ion measurements

The Na^+ ion concentration during 40 minute asphyxia is shown in Fig. 3. In the control animals (Exp. 1) and those from Exp. 8, significant increases in Na^+ ion concentration were observed (Modified LSD Test, $p < 0.05$). However, there were no significant differences in the Na^+ ion concentrations in the animals of Exp. 3 and 5.

DISCUSSION

Using relative high level of impact tone and steady state tone, the effect of tone exposure on inner ear function was examined by measuring EP, K^+ ion and Na^+ ion concentration in the endolymph which is thought to be a function of the stria vascularis in the scala media of the cochlea in guinea pigs.

Well known character of impact tone was to have fast rise time (within 1 millisecond), a moment peak time and decay time (1–200 milliseconds) (Coles and Rice 1967; Ward 1968). However in our experiments, relative high sound level (120 dB) tone with fast rise time (within 300 μ seconds) but trapezoidal form (duration 3 milliseconds and 9 milliseconds) were used because good reproducibility of tone exposure and relatively simple structure of tone spectrum.

Impact tone exposure has a dose dependent effect. Higher Leq_{24h} causes larger changes in the parameters examined. Impact tone with the same Leq_{24h} but changing the rate of pulse shows different effects. Nine milliseconds long pulses/3 seconds exposure seems to have milder effect on the inner ear function than that of 3 milliseconds/second. Long interval of time between impact to impact was considered to be an important factor.

Using CM and CAP, we reported the effects of impact tone below 115 dB on the cochlea (Inada 1999). In this experiments, we report the effect of impact tone with higher sound pressure level on the cochlea of guinea pigs using EP, K^+ ion and Na^+ ion concentration in the endolymph.

High level of impact tone exposure has larger physiological effect on the guinea pig inner ear than that of steady-state tone of equal energy reported by Borg and Engstrom (1989). However, Spondlin (1976) proposed that damage risks were difficult to predict for impulse noise, and damage risks could be considerably higher when compared with same total energy in steady-state noise.

Based on the above observation, it is speculated that sound level, rate of presentation, rise time of intermittent tone, etc. are important parameters.

The absolute value of the negative EP in Exp. 2a was significantly different from those of impact tone (peak value of 115 dB; $Leq_{24h} = 90$ dB and peak value

of 110 dB; $Leq_{24h}=85$ dB) and steady-state tone of 95 dB, but significant difference was not observed between that of Exp. 2a and that of Exp. 6a. Impact tone of 120 dB exposure (1 pulse/second) were shown to have stronger effect on EP of the guinea pig than that of Exp. 5a (peak value 120 dB, 1 pulse/3 seconds, duration 9 milliseconds). The mechanical injury of the cochlea induced by every impact tone exposure was compared the cochlear damage by the vasoconstriction induced by steady-state tone exposure (Davis et al. 1953).

A positive EP is considered to be an oxygen-sensitive, electrogenic K^+ ion potential (Oftner 1987), while a negative potential represents a diffusion potential across the endolymph perilymph barrier during asphyxia. In order to prevent death of animal, time limit of asphyxia was planned to 3 minutes. K^+ ion permeability of the hair cell is important for the generation of the CM (Gibson et al. 1983).

Pickles et al. (1984), emphasized that K^+ ion 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, and floppiness of the cilia considered to be dysfunction of channels. Dysfunction of the pump of marginal cell in the stria vascularis was induced by asphyxia and flow of K^+ ion into the endolymph was stopped. Ikeda and Morizono (1990) reported that K^+ influx occurs through transduction channels located at the hair bundle. K^+ ion movement through channel of the stereo cilia in the hair cell from endolymph to perilymph also stopped by strong tone exposure. Therefore, high K^+ ion concentration in the endolymph (Ma et al. 1996) maintained static level (Abe et al. 1981).

K^+ ion diffusion also disturbed by strong tone exposure. In this experiment, K^+ ion concentration, Exp. 4b (peak value 110 dB, 1 pulse/second, $Leq=85$ dB) did not increase along time course but, that of Exp. 8 (85 dB, steady state) decreased. As mentioned above impact tone exposure induce stronger effect of the inner ear.

Following Hodgkin and Katz equation, K^+ ion and Na^+ ion permeability ratio of cochlear partition in conjunction with the ionic concentrations in the endolymph and perilymph, have important role to the diffusion potential (Mori and Konishi 1985). Also Na^+ ion concentration did not show any increase at moderate level (Exp. 8c peak value 115 dB, 1 pulse/second, $Leq=90$ dB) of impact tone exposure. But, increase of Na^+ ion concentration was observed in the Exp. 8c (85 dB steady state tone). Impact tone exposure disturbs the Na^+ movement through the marginal cell (Oftner et al. 1987).

We suppose that impact tone exposure of relative high sound level induced distortion of K^+ ion movement from the endolymph to the perilymph by channel of the hair cell (Sugisawa et al. 1994b; Hotta et al. 1996) and other channels, and disturbed Na^+ ion movement from the perilymph to the endolymph.

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