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THE GENETIC-INDUCED HEARING IOSS CAN BLOCK THE EFFECT OF NOISE TRAUMA IN WAITZING GUINEA PIG

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Abstract

The waltzing guinea pig may be a good model to investigate if genetic factor can change the sensitivity in noise-induced hearing loss. A total of 34 waltzig guinea pigs were studied and we found that there is no any significant increased sensitivity to noise trauma if the age-induced hearing loss was considered in waltzing guinea pig.

Key words: Sensitivity, auditory evoked potential response, hair cell, morphology, hearing loss, waltzing guinea pig, postnatal.

Introduction

The waltzing guinea pig is a strain of guinea pig that has a geneitcally induced progressive hearing loss. In 1929, Ibsen and Risty were the first to describe the waltzing guinea pig to have tendency to whirl or waltz, similar to Japanese waltzing mice. Later, other groups continued to investigate this waltzing guinea pig. It was Ernstson who studied the waltzing guinea pig more extensively and observed the degeneration of the sensory cells of the hearing organ and the vestibular apparatus[1-3]. Other studies on the waltzing guinea pig followed[4-6], but these studies concentrated on the function of vestibular system of the waltzing guinea pigs. Wit[7] investigated the relationship between the gross cochlear potentials and hair cell pathology in the waltzing guinea pig, the conclusion was the similar to Ernston that the waltzer guinea pig was deaf at 4 weeks postnatal. The previous studies concluded that frequency specific auditory brainstem response (ABR) threshold shift increased with age and ABR thresholds could not be measured beyond 110 dB using our equipment in postnatal day 30, and the percent hair cell loss increased as age increased in waltzing guinea pig[8]. It is known that noise could induce hearing loss not only in normal species but also in genetic ones. Borg [9] investigated noise trauma effects to normotensive and spontaneously hypertensive rats, the conclusions were that old spontaneously hypertensive rats were more susceptible to noise than young ones and normotensitive rats. Li and Borg[10] demonstrated that sensitivity to noise trauma was significant difference only at one month age at middle frequency in CBA/CA mice and that susceptibility to noise trauma was significant different at earlier age and at middle frequency in C57b/6J, whereas there were no different at later age and at lower and higher frequency. However, in the waltzing guinea pig, is it the same or much different sensitivity to noise trauma as that of the normal guinea pig? Therefore, the aim of present study is to investigate the different sensitivities to noise between the normal and the waltzing guinea pigs by noise trauma, and morphological change is also studied.

Materials and Methods

Animals and acoustic overstimulation

The waltzing guinea pigs are maintained in our own colony, a normal adult female guinea pig is mated with a waltzing adult male guinea pig, and the litter consists of

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both normal and waltzing guinea pigs in a ratio of 1:1. It is easy to distinguish the normal and the waltzing guineapigs because the waltzing guinea pigs have a circling gait and lack of a nystagmus when rotated. There are no differences between the controls and waltzing guinea pigs in body weight and age.

A total of 34 guinea pigs which were used are from above strain in this study. Six groups were divided. Group I (n=5) are waltzing guinea pigs, for 105 dB 72 hours pure tone noise exposure at 1 kHz; group II (n=6) are control guinea pigs, the condition of noise exposure is the same as group I. Group III (n=5) waltzing guinea pigs, for 100 dB 24 hours pure tone noise exposure at 6.3 kHz; Group IV (n=6) control guinea pigs, the same noise exposure as Group III. Group V (N=7) are waltzer guinea pigs and act as age contrast without noise exposure. Group VI (N=5) are normal guinea pigs without noise exposure. All animals exposed to noise were at postnatal day 8. The unanesthetized animals were sound exposed in an open field acoustic chamber (225x125x100cm) with the loud speakers placed above the animal cage. Sound calibration was performed within the wire mesh animal cage with a 12.5 mm condenser microphone (Bruel and Kjaer. Model 2213).

Evoked Response Audiometry

Auditory brainstem response thresholds were obtained from 17 waltzing guinea pigs and 17 age-matched controls (100-180g) after being anaesthetized with an intramuscular injection of 50mg ketamine and 10mg rompun per kilogram body weight. Supplementary doses of ketamine were given when needed. Only animals without evidence of middle ear infection, as determined otoscopically, were used. Measurements were made prior to exposure at postnatal day 7 and again after the exposure at postnatal day 15. In group V and VI, the guinea pigs were measured only at postnatal day 15 without noise trauma. Responses were differentially recorded between subcutaneous stainless steel electrodes at the vertex and mastoid, while the lower back served as ground. Frequency specific auditory responses were elicited by single full cycle sine waves filtered through a one-third octave band pass filter (Bruel and Kjaer, type 1612) with the centre frequencies at 0.5, 1.0, 2.0, 4.0, 8.0 and 12.5 kHz. Stimuli were presented through a 10 cm tube connected to an earphone (Telephonics TDH39) and sealed to the external auditory canal. The response was amplified 10,000 times and averaged (2,048 epochs) by a signal average (Medelec MS92). Stimulus intensity was varied in 5 dB steps in the beginning and then adjusted in 1.5 dB steps when near threshold until a visual detection threshold was obtained. All threshold measurements used in this study represent the stimulus intensity needed to produce a visual detection threshold of the brainstem response. The visual detection threshold was determined from a number of repetitive measurements made for those stimuli at each frequency. Stimulus intensity was calibrated in a 0.5 cc coupler with a one-quarter inch condenser microphone (Bruel and Kjaer, model 2607). All sound pressure levels were expressed in sound pressure level (SPL) re: 20μPa. Testing was performed in a sound attenuated box.

Morphological Analysis of the Cochleae

Following the postnatal day 15 measurement, the cochleae were removed from the temporal bone and placed in 4% paraformaldehyed in phosphate buffered saline (pH 7.4) for 1 hour. The apical portion of the bony cochlea was opened and the oval and round windows perforated in order to allow the fixative to perfuse through the tissues. The tissue was then exposed to 0.3% Triton X-100 for 10 minutes, rinsed in phosphate buffered saline and incubated in fluorescent labeled Phalloidin (TRITC) (1:100) (Molecular probes, USA) for 1 hours followed by several washes in phosphate buffered saline. The organ of Corti was dissected into approximately 2 mm coils and placed on a microscope slide in Citi-flour, and covered with a cover slip and sealed with nonfluorescent nail polish. Phalloidin was used to label structures containing filamentous actin. Since the apical parts of the hair cells contain actin bearing structures, they react specifically with Phalloidin. The use of phalloidin is eased the burden of counting the hair cells as well as scar formations. All hair cells throughout the cochleae were examined using a 40x objective. The length of the guinea pig cochlea is 18 mm. The field of analysis for each portion of the cochlea was 0.24 mm.

Statistical Analysis

The statistical analysis used in this study was a student´-test at a significance level of 0.05.

Results

Auditory brainstem response thresholds and the different of age

The auditory brainstem response thresholds for the waltzing and normal guinea pigs showed the thresholds of waltzer guinea pigs were elevated 10-18 dB when comparing the age match control guinea pigs at postnatal day 7, especially at higher frequency. The thresholds of the waltzer guinea pigs had 16-38 dB rising than those of control age match guinea pigs. Age-induced ABR

threshold shift in control and waltzer guinea pigs , for the control, there were no threshold shifts between the postnatal day 7 and postnatal day 15, whereas for the waltzer guinea pigs, there were 5-17 dB thresholds shifts, especially at 8 and 12.5 kHz. The difference all above was significant at 0.05 levels.

The different of noise trauma

The auditory brainstem response thresholds were measured at postnatal day 15 without noise trauma and postnatal day 15 after different noise trauma, Group I was waltzer guinea pigs which was exposed to 1 kHz 105 dB 72 hours pure tone noise trauma at postnatal day 8, and had 5 days interval and measured ABR threshold again. There were no threshold shifts at 8 and 12.5 kHz, while there were 11 dB and 16 dB shifts at 0.5 and 1 kHz, 25 dB shifts at 2 and 4 kHz.

Group II was control guinea pigs which had the same noise trauma condition as the group I. There were only 10 dB threshold shifts at 8 and 12.5 kHz, 25 dB shifts at 2 and 4 kHz

Group III was waltzer guinea pigs which were exposed to 6.3 kHz 100 dB 24 hours pure tone noise trauma at postnatal day 8. After one week interval recovery, The ABR threshold were measured again. It had only 1 and 5 dB threshold shifts at 0.5 and 1 kHz, 12 dB threshold shifts at 2 kHz, however, there were 28, 27, and 25 dB threshold shifts at 4, 8, and 12.5 kHz.

Group IV was control guinea pigs which had the same noise trauma as the group III, it showed that there were 10, 6, 3, 11 dB threshold shifts at 0.5, 1, 2, and 4 kHz, whereas there were 28 and 40 dB threshold shifts at 8 and 12.5 kHz.

In order to better understand the different sensitivity to noise trauma between the control and waltzer guinea pigs, we compared noise-induced ABR threshold shifts between the control and waltzer guinea pigs at the same noise trauma condition. Results showed that there were 10 dB different at 0.5, 8, and 12.5 kHz, and no different at 1, 2, 4 kHz when exposed to 1 kHz 105 dB 72 hours. There were 15 dB different at 4, and 12.5 kHz, no different at the other frequencies when exposed to 6.3 kHz 100 dB 24 hours. However, there were no significant different between the control and waltzer guinea pigs at 0.05 level.

Quantification of Hair Cell Loss

The phalloidin labeled surface preparations of the organ of Corti were analyzed to quantify the degree of hair cell loss caused by noise trauma. A missing hair cell is readily identified by a scar formation when the preparation is viewed in fluorescent illumination. The percent of hair cell loss for the different rows of hair cell along the length of the cochlea was analyzed. The analysis began at 4 mm distance from the round window and extended up to the apex of the cochlea at 18mm from the round window.

Group I had 33 dB threshold shifts at 2 kHz and 4 kHz, demonstrating more hair cell loss, the highest in the third row of outer hair cell, in turn, second and first rows outer hair cell. The inner hair cell was gently affected. There were 82% third row outer hair cell loss between 8 and 12 mm distance from the round window and 78% third row outer hair cell loss between 12 and 16 mm distance from the round window, also 51% the second row outer hair cell loss between 8 and 12 mm distance from the round window, 51% second row outer hair cell loss in 12-16 mm from the round window, 25% first row outer hair cell loss between 10 and 12 mm distance from the round window, and 4-8% inner hair cell loss located in 4-8 mm distance from the round window.

In group II, threshold shifts were 20-25 dB by noise-induced at 0.5, 1, 2, and 4 kHz that showed 30-50% second row outer hair cell loss between 10 and 14 mm distance from the round window. There was 40-50% second row outer hair cell loss and 20-30% first row outer hair cell loss in above distance. However, no inner hair cell loss was found in this Group.

Group III had 25-28 dB noise-induced threshold shifts demonstrated that there was 15-35% the third row outer hair cell loss between 8 and 12 mm distance from the round window, the second row outer hair cell loss was only below 10% which occurred in 8-12 mm distance from the round window, first row outer hair cell loss was below 5% along the axis of the cochlea. The inner hair cells loss was scattered in the cochlea.

Group IV had only 5% third row outer hair cell loss in 10-12mm distance from the round window. Other rows of outer hair cells and inner hair cell were not affected. However, the noise-induced threshold shifts were 28-40 dB at and 12.5 kHz.

Discussion

The auditory sensitivity is affected by many factors, e. g. age, drugs, noise and hereditary etc. The waltzing guinea pig is a good model to study the relationship between the auditory sensitivity and morphology because the degeneration of this type of guinea pig is limited in the sensory cell of the hearing organ and of the vestibular system. The present results show that there is not significant difference sensitivity to noise trauma between normal and waltzer guinea pigs when studying the ABR threshold shifts. The morphologic analysis of the cochlea from groups I and II confirmed that the maximum hair

cell loss did seem to be quite different, but when we considered the age-induced hair cell loss in the waltzer guinea pigs, it was hard to say that there were difference between these two groups in morphologic change, the previous study found that there was near 20-40% third row outer hair cell loss in postnatal day 15[8]. The present results showed that there was 80% third row outer hair cell loss in noise-exposure waltzer guinea pigs (1 kHz 105 dB 72 hours), while there was also 30-50% third row outer hair cell loss in age-match normal guinea pig when exposure to the same noise trauma. Previous study also showed that there were 50% third row outer hair cell loss when adult normal guinea pigs were exposure to 1 kHz 105 dB 72 hours pure tone[11]. From these two groups, we did not yet find a significant difference in morphological change. In group III and group IV, we did not yet find a significant difference in morphological change. In group III and group IV, the hair cell loss was not significantly different when considering age-induced hair cell loss. The hair cell loss was not significantly different when considering age-induced hair cell loss. The noise trauma did not increase hair cell loss of the waltzer guinea pigs because the present results did not show that the third row outer hair cell loss was a significant increase when compared to the previous results[8]. An intriguing result was that there were more than 40 dB threshold shifts at the higher frequency after 7 days interval in 6.3 kHz 100 dB 24 hours noise trauma in group III, and also 28-40 dB threshold shift at higher frequency in group IV, but there were not an increase hair cell loss. In group III, there was 35% third row outer hair cell loss in 8 to12 mm from the round window, however, there was also 20-40% third outer hair cell loss at postnatal day 15 waltzing guinea pigs without noise trauma. In group IV, the third outer hair cell loss was below 5%. In actually, this noise trauma could only affect stereocilia while the hair cell body was intact. The finding that the inner hair cell stereocilia are more sensitive to noise trauma than the outer hair cell stereocilia has been reported [12]. Canlon et al[13] had shown that the inner hair cell stereocilia change could be correlated with the temporary component of the threshold shift. Unfortunately, in present study, the change of stereocilia was not investigated. Another reason could be partly due to the swelling of the afferent dendrites beneath the inner hair cells[14-15], one question is why there was no significant different auditory sensitivity to noise trauma between the control and waltzing guinea pigs? Danto[16] studied the auditory susceptibility of the infant and adult guinea pig to noise trauma, and demonstrated that the infant guinea pig had higher significant susceptibility to noise trauma than the adult. If it is case, in our present study, the noise exposure was at postnatal day 8, there could be also higher susceptibility to noise trauma than the adults. Because the waltzing guinea pig become deaf at postnatal day 30, so it is impossible to compare the susceptibility of guinea pig become deaf at postnatal day 30, so it is impossible to compare the susceptibility of these two types guinea pig to noise trauma at later stage. Li and Borg[10] investigated the auditory sensitivity to noise trauma in two genotypes of mice (CAB/Ca, C57BL/6J), and addressed that the younger CBA/Ca mice more susceptibility to noise trauma than the adult at middle frequency, whereas the C57BL/6J did not show significant different susceptibility to noise trauma between younger and adult. They also concluded that the noise effect could be blocked by age-induced hearing loss in genetic-induced mice. Our present results show that the noise trauma does not increase the threshold shifts of the waltzer than control guinea pig. On the other hand, the age-induced hearing loss was higher at high frequency in waltzing guinea pig, this is resistance with degeneration of the waltzing guinea pig beginning at more base of the cochlea, while, we can find that the susceptibility to noise trauma were more sensitive in the control than the waltzing guinea pig although there were no significant different, it could be surmised that the age-induced degeneration also block the noise effect in the waltzing guinea pig. It was demonstrated that the sensitivity of the guinea pig to noise trauma was specific to 4-6 kHz noise overstimulation[17-19], the reason was not clear. If this is case, it is reasonable to explain why there are larger threshold shifts in high frequency noise trauma. However, why there are not significant hair cell losses, so further study is necessary

In summary, the noise trauma sensitivity of the waltzer guinea pigs is similar to that of normal guinea pigs, it is possible that the age-induced hearing loss can block the noise effect in the waltzing guinea pig. The ABR threshold shifts are quite dependent on the original noise trauma.

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