·Basic Research·

ET-1 expression and ultrastructural changes in the retina after exposure to infrasound in rats

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Abstract

• AIM: To evaluate the expression of ET-1 and ultrastructural changes in the retina of Sprague-Dawley rats after exposure to infrasound.

• METHODS: Twenty-five male Sprague-Dawley rats were randomly divided into 5 groups, 5 in each group, and 4 groups were exposed to infrasound of 16Hz, 130dB for 2 hours each day, and 1 group served as control. The exposure time for the 4 groups was respectively 1, 7, 14 and 21 days. Then, the rats were sacrificed after intravenous injection with lanthanum nitrate (La), and the retina was examined with electron microscopy and immunochemistry with antibodies against ET-1.

• RESULTS: After infrasonic exposure for 1 day, ET-1 was expressed mainly in the endothelia of retinal and choroidal blood vessels, so was it in the control group. After infrasonic exposure for 7 days, it was expressed mainly in the retinal pigment epithelial cells, outer nuclear layer and outer plexiformlayer. After exposure for 14 days, its expression was enhanced in the outer nuclear layer, inner plexiform layer and ganglion cell layer. After exposure for 21 days, its expression was weakened in all layers. With exposure going on, the injury in retina gradually extended from the outer to the inner. Under the electronic microscope, La was observed to have infiltrated into several layers of the retina after 1 day of exposure and it became denser and denser from the 1st to the 21st day except at the 7th day. Meanwhile, the retinal layers showed cellular edema, swollen organelles such as mitochondria and endoplasmic reticula, karyopyknosis and edging, cell membrane and karyotheca dilation, rupture and myelin changes.

• CONCLUSION: The expression of ET-1 in retina is related to the location of infrasonic injury, and ET-1 may have a protective effect on retina against the infrasonic injury.

• KEYWORDS: endothelin; infrasound; lanthanum; retina; immunohistochemistry; electronic microscopy; rats

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INTRODUCTION

Infrasound, with its frequency from 0.0001Hz to 20Hz, exists widely in our environment. It can produce many biological effects and to some extent, affect the central nervous system, retina and the optic nerve [1,2]. Endothelin (ET), as a repairing cytokine in the nervous system, increases its expression in case of optic nerve injury[3]. However, its effect on retina against the infrasonic injury has not been reported. This study aimed to explore the effect of ET-1 on retina exposed to infrasound using immunohistochemistry and electron microscopy.

MATERIALS AND METHODS

Materials Twenty-five male Sprague-Dawley rats, weighing from 200-250g, were randomly divided into 5 groups, 5 rats in each group, and 4 groups were exposed to infrasound of 16Hz, 130dB, for 2 hours each day. The other group served as control. The infrasonic pressure cabin and infrasonic measuring system were made by Xijing Hospital of the Fourth Military Medical University, Institution of Acoustics of CAS (Chinese Academy of Sciences) and the 41st Institution of Aerospace Industrial Company. After irrigated with lanthanum nitrate (La) intravenously, the rats in the 4 experiment groups were sacrificed after infrasonic exposure for 1, 7, 14 and 21 days respectively.The globes were removed and the samples were prepared for electron microscopy. The tissues for light microscopy were soaked in polymerisatum 40g/L at 4℃ for 30 minutes and then made into wax pieces. Finally, they were made into slices of 5μm

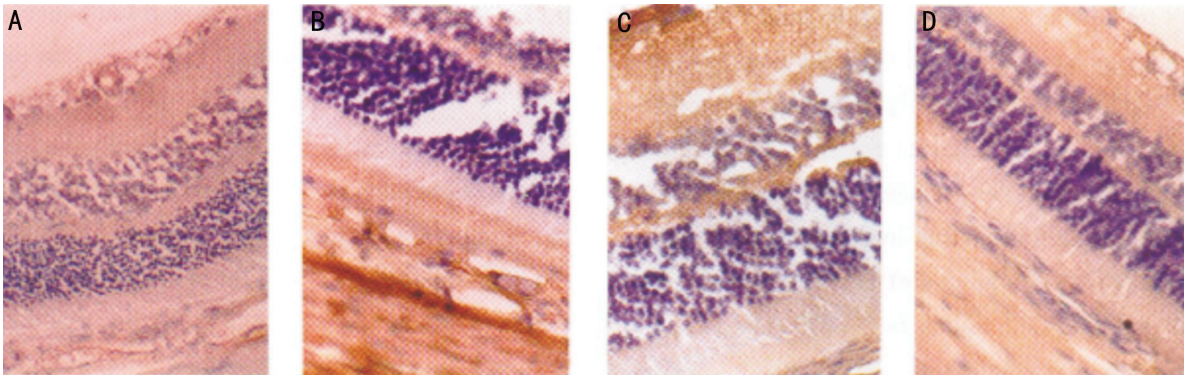

Figure 1 The retina of SD rats±exposure to infrasound (SACB×400) A:Without infrasound×200;B:To infrasound for 7 days;C:To infrasound for 14 days;D:To infrasound for 21 days

in thickness and stuck with polylysine. The mouse anti-rats monoclonal antibody againat ET-1, SABC immunohistochemistry assay kit and DAB assay kit were from Wuhan Boster Biological Technology Company.

Methods

Immunohistochemistry staining The expression of ET-1 in different layers of retina after exposure to infrasound was detected with mouse anti-rats microclonal antibody against ET-1, according to the illustration of immunohistochemistry kit. The trial was controlled with the retina of SD rats free from infrasonic exposure and the controls with PBS or normal goat serum in substitution of antibody of ET-1 were also set up.

Electron microscopy The permeation of lanthanum through various retinal layers from choroid and the corresponding ultrastructural changes at different time points were detected after infrasonic exposure.

RESULTS

Immunostaining and Microscopy In retina free from infrasonic exposure, there was weak ET-1 expression in the retinal blood vessels and choroidal endothelial cells; there was no ET-1 expression in the retinal pigment epithelial (RPE) cells; the retina tissues in all layers were tight and compact (Figure 1A). The retina of the group exposed for 1 day did not change in the staining results compared with the controls. However, the RPE cells of the group exposed for 7 days were stained weakly. A stained zone appeared between RPE cells and the outer nuclear layer, about the position of the outer segment of photoreceptors (Figure 1B). After exposure for 14 days, the RPE cells were still stained and the nucleus was fusiform; the staining in the outer segment of photoreceptors vanished and was replaced by vacuolation; outer nuclear layer, outer plexiform layer and inner nuclear layer were stained strongly; inner plexiform layer was also stained and some vacuolation was found; the ganglion cell layer contained many stained cells (Figure 1C). After exposure to infrasound for 21 days, the staining in retina weakened and was replaced by vacuolation; RPE cell nucleus is shaped obviously different but still stained positive; the number of ganglion cells reduced dramatically and the cells appeared vacuolated; the structure of retina became loose compared with the control (Figure 1D).

Electron Microscopy In the controls, La permeated into intercellular space of the RPE cells and discontinued at the tight junction of the RPE cells (Figure 2). In the outer segment of photoreceptor, La permeated and cell membranes were integrated but the organelles including mitochondria and endoplasmic reticula swelled in the group with exposure for 1 day (Figure 3); in the group of 7-day exposure, La permeation decreased; in the group of 21-day exposure, La permeated, cell membranes were not integrated, myelin figure and organelles were obscure, and there were myelin figure and cell fragment between cells (Figure 4). In the group of 7-day exposure, outer nucleus layer, outer plexiform layer and inner nucleus layer showed less La, integrated cell membrane and swollen organelles (Figure 5). In the group of 14-day exposure, outer nuclear layer exhibited increased La, integrated cell membrane, less cytoplasm and edge-gathered and condensed chromatin (Figure 6). In the group of 1-day exposure, the nerve fiber layer and inner limiting membrane showed La granules, integrated cell membrane and swollen organelles (Figure 7). In the group of 21-day exposure, cell membrane became less integrated, organelles were obscure and fragments could be seen between cells (Figure 8).

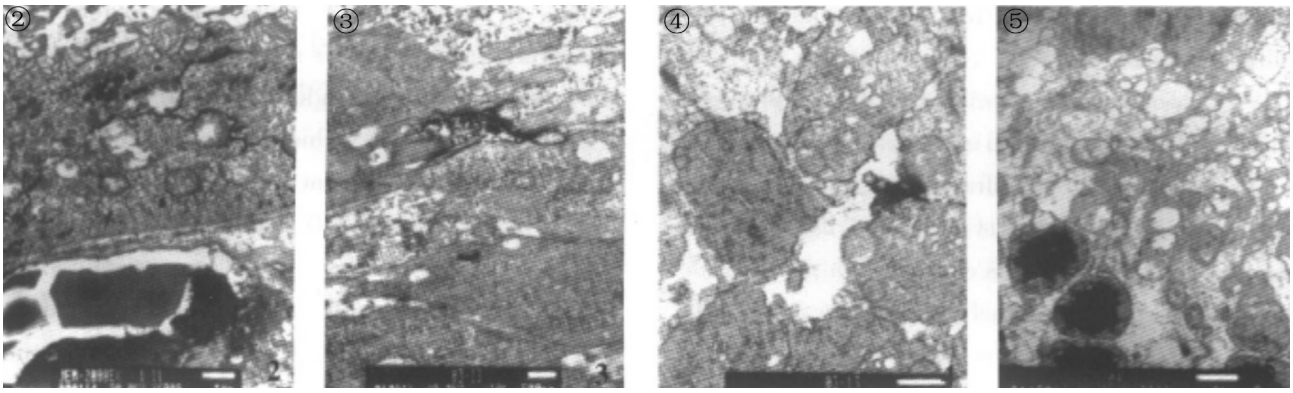

Figure 2 The retina of SD rats without exposure to infrasound (×6 000)

Figure 3 The outer segment of photoreceptor of the retina of SD rats with exposure to infrasound for 1 day (×10 000)

Figure 4 The outer segment of photoreceptor of the retina of SD rats with exposure to infrasound for 21 days (×15 000)

Figure 5 The outer nuclear layer, out er plexiform layer and inner nuclear layer of the retina of SD rats with exposure to infrasound for 7 days (×3 000)

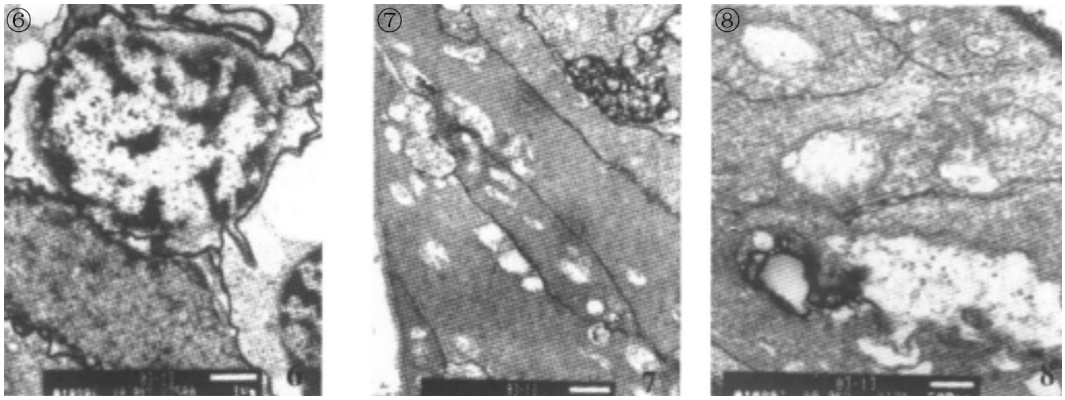

Figure 6 The cell of the outer nuclear layer of the retina of SD rats with exposure to infrasound for 14 days (×7 500)

Figure 7 The nerve fiber layer and inner limiting membrane of the retina of SD rats with exposure to infrasound for 1 day (×7 500)

Figure 8 The nerver fiber layer and inner limiting membrane of the retina of SD rats with exposure to infrasound for 21 days (×12 000)

DISCUSSION

Infrasound leads to the injury of organisms by its special biological resonance effect, which results in functional and structural damage of the central nervous system [1,4]. In eyes, infrasound can cause the changes of visual field, blood flow and blood vessels[5-7], and affect the metabolism of the lens[8]. The amplitude of ERG-a, b and ΣOPs waves decreased in the retina of SD rats after exposure to infrasound, which indicates that it has some biological effects on both visual function and retinal blood flow [2]. ET, a known vasocontrictor, is composed of 21 amino acid residues, the sequence of which is similar to that of neurotoxin of some snake [9,10]. Therefore, it has close relationship with the nerve system. Stimulated by hypoxia, the RPE cells *in vitro* excrete ET-1 [11]. Miyata *et al* [12] reported that ET-1 inhibited adrenalin-induced stellation of pituicytes. Rogers *et al* [3] found that in the normal CNS, astrocytes expressed low but detectable level of ET (B) receptors, and after CNS injury, both astrocytes and microglia expressed high levels of ET(B) receptors. ET (B) receptors provide a therapeutic target for regulating glial proliferation and the release of neurotrophic factors from glia. These prove that ET-1 has a positive effect on stabilization of nerve tissues.

The results of our experiment showed that, in the controls ET-1 was only expressed in the vascular tissue of the retina. After exposure to infrasound, ET-1 began to be present in several layers of the retina besides blood vessels, and its expression changed with the exposure time and the injury of retinal tissues under optical microscope. The result of electron microscopy indicated that La had permeated through retina after exposure for 1 day, and increased and accumulated with increase of the exposure time; with exposure for 1-7 days, organelles including mitochondrion and endoplasmic reticulum swelled; with exposure for 14

days, membranous disc of photoreceptors was ruined, nuclear membrane expanded, chromatin condensed, cells became edematous and cytoplasma was vacuolated; with exposure for 21 days, there were less integrated cell membrane, myelin figure, obscure organelles and cell fragments appeared in and between the cells.

Qiu *et al*[2] found that after exposure to infrasound there was a phase of time in which the waves of ERG-a, b went up to normal again and the waves didn't go down until the 14th day. This reflects the adaptation of retina to the effect of infrasound. The similar phenomenon was also identified in the research on the ultrastructure of cerebral cortex of SD rats after exposure to infrasound of 8Hz-130dB [13]. Our data indicated that La infiltration decreased and ultrastructural changes did not develop further in the group exposed to infrasound for 7 days in comparison with the group exposed for 1 day. La infiltration and ultrastructural changes did not develop until after 14-21 days of exposure. However, at the same time, ET-1 began to express in the RPE cells and its expression was gradually enhanced; it finally declined with the development of injury from RPE cells to inner limiting membrane. Therefore, the results of ultrastructural changes are agreeable with those of immunohistochemistry and optical microscopy, which hints that the cellular functions strengthen in compensation in early exposure to infrasound, and become exhausted with the deterioration of retinal injury in later exposure to infrasound. In this process ET-1 plays a positive role. The ultrastructure of the outer segment of photoreceptors showed an abundance of endoplasmic reticulum in which ET-1 was synthesized [10], which may explain the positive staining of the outer segment of the photoreceptors.

Comparative study of ET-1 expression in retina after exposure to infrasound for different durations, and the infrasonic injury of retina illustrates that ET-1 is induced and synthesized by infrasonic injury, and its expression in certain retinal tissue declines with the aggravation of infrasonic injury in the same tissue, which may be related to its effect of nerve protection.

REFERENCES

1 Fei Z, Zhang X, Wang XF, Liu XZ, Chen JZ, Jia KY. The changes and significance of blood-brain barrier permeability after infrasound damage in rats. *J Fourth Mil Med Univ* 1999;20(8): 678-680

2 Qiu P, Guo SY, Zhang ZM, Chen JZ, Jia KY, Wang B. Effect of infrasound on ocular electrophysiology of rats. *J Fourth Mil Med Univ* 1999;20(7):583-586

3 Rogers SD, Demaster E, Catton M, Ghilardi JR, Levin LA, Maggio JE, Mantyh PW. Expression of endothelin-B receptors by glia *in vivo* is increased after CNS injury in rats, rabbits, and humans. *Exp Neurol* 1997;145(1):180-195

4 Fu GY, Chen JZ, Jia KY. Changes of glutamate in brain of rats exposed to infrasound. *J Fourth Mil Med Univ* 1999;20(4):288-290

5 Parker DE, Tubbs RL, Littlefield VM. Visual-field displacements in human beings evoked by accoustical transients. *J Acoust Soc Am* 1978;63(6):1912-1918

6 Svidovyi VI, Kuklina OI. State of the hemolymph circulatory bed of the conjunctiva as affected by infrasound. *Gig Tr Prof Zabol* 1985;(6):51-52

7 Sidorenko EI, Obrubov SA, Dreval AA, Tumasian AP. Experimental study of morphologic changes in ocular tissue structures after exposure to infrasound pneumomassage. *Vestn Oftalmol* 1996;12(3):17-19

8 Yan H, Chen JZ, Jia KY. Effect of infrasound on molecular chaperone activity of alpha-crystallin. *J Fourth Mil Med Univ* 2002;23(12):1071-1073

9 Kloog Y, Sokolovsky M. Similarities in mode and sites of action of sarafotoxins and endothelins. *Trends Pharmacol Sci* 1989;10:212-214

10 Bhatt AD. Endothelins and anti-endothelins. *Curr Pharm Des* 2001;7 (6): 461-474

11 Udono T, Takahashi K, Nakayama M, Yoshinoya A, Totsune K, Murakami O, Durlu YK, Tamai M, Shibahara S. Induction of adrenomedullin by hypoxia in cultured retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 2001;42(5): 1080-1086

12 Miyata S, Furuya K, Nakai S, Bun H, Kiyohara T. Morphological plasticity and rearrangement of cytoskeletons in pituicytes cultured from adult rat neurohypophysis. *Neurosci Res* 1999;33(4):299-306

13 Ye L, Gong SM, Huang XF, Wang F, Liu J, Chen JZ. Changes of pallium ultrastructure after infrasound damage in rats. *J Fourth Mil Med Univ* 2002;23(9): 856-858