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Research article

Establishment of an experimental system to study the influence of electrical field on cochlear structures

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HIGHLIGHTS

- New experimental model of the effects of electrical field on cochlear tissues is presented.
- Electrical field can induce the loss of auditory hair cells.
- Electrical field can induce neuronal degeneration.

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ABSTRACT

Treatment of partial hearing loss with the combined electrical and acoustical stimulation (EAS) aims at restoring the hearing while preserving the residual hearing. The aim of present study was to establish an in vitro system to study the effects of an electrical field on the auditory hair cells and spiral ganglion cells. Cochlear tissues containing the organ of Corti, spiral limbus and spiral ganglion neurons were dissected from post-natal Wistar rats (p3–p5) and cultured in the micro-channels. Electric current was homogenously applied on the apical, medial and basal parts of explants. Biphasic rectangular pulses were applied continuously over a period of 30 h or 42 h and the explants were fixed and stained to visualize the hair cells and neurites. Application of electrical field for 30 h has not induced significant changes in the number of inner or outer hair cells when compared to the control. However, after 42 h of electric stimulation, the number of hair cells decreased significantly by about 30%. The medial and basal fragments were particularly affected. The number of neurites has not been influenced but significant neuritic beading, consistent with neurodegeneration, was observed after 42 h of electric stimulation. Although performed with immature auditory tissues, our findings hint at the possibility of particular electric current inducing damage or loss of auditory hair cells, which should be considered when designing EAS electrodes.

1. Introduction

Sensorineural hearing loss is characterized by the loss of outer and inner hair cells (OHC and IHC) and by degeneration of the spiral ganglion neurons (SGN). To restore the ability to hear and increase the quality of life [1], cochlear implantation is used as a methods of choice [2,3]. Cochlear implant (CI) converts sound waves to an electrical current and stimulates SGN via an electrode array inserted into the cochlea. The clinical indication for implantation includes patients with residual hearing. In such patients, great efforts are made to preserve their remaining hearing despite the implantation [4]. Combined electrical and acoustic stimulation CI devices (EAS) were recently introduced [5]. When compared to the conventional CIs, the EAS electrodes are shorter, have smaller diameter, and greater flexibility [5,6]. However, a review of 25 clinical studies has demonstrated that the post-implantation hearing loss has increased in 24% of EAS users and a total hearing loss has affected 13% of them [7]. Although the post-implantation hearing loss could be related to the insertion trauma and inflammation, the

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possibility of tissue damage due to the electrical stimulation should not be excluded [8]. Contemporary CI-related research focuses on the biological effects of implants on the remaining functional auditory tissue. The impact of the electric current on survival and integrity of different cell types in the organ of Corti (OC) is still unclear. Non-specific chronic stimulation via a ball electrode on the round window membrane has not affected the residual hearing [5]. Also, the long-term electrical stimulation has not caused the hair cell loss in cats [9]. By contrast, an adverse effect on the OC was observed after chronic in vivo intra-cochlear electrical stimulation as compared to the unstimulated ear [10,11]. One explanation of the contradictory results may be the use of various cochlear regions, in which the outcome was measured. Of two studies that have concentrated on the tissues that are adjacent to the active electrodes [12,13], only in one the loss of IHC and OHC was observed [12]. The number of SGN as well as the neurites in the peripheral process also decreased in the electrode-proximal region. Moreover, decreased numbers of neurites following electrical stimulation were also reported [9].

The aim of our present study was to introduce an in vitro system for electro-toxicity screening of explanted tissues or organs. Using this system, we wanted to determine changes in auditory hair cells and cochlear neurites exposed to the electrical stimulation.

2. Material and methods

2.1. Preparation of the OC

The membranous cochleae were prepared from 3 to 5 day old Wistar rats. All experiments were approved by the Ethics Committee (T0232/12). Details of the preparation of OC cultures have been described elsewhere [14,15]. Briefly, after decapitation, the head was bisected and the temporal bones were removed and placed in Dulbecco's Phosphate Buffered Saline (cat# 14,190-094 Gibco Life Technologies, Darmstadt, Germany). Next, the cochlear epithelium consisting of the OC, spiral limbus, and spiral ganglion neurons was transferred to DMEM (cat# 14,190-094, Gibco/Life Technologies), dissected, separated from modiolus, and split into the apical, middle, and basal parts.

2.2. Tissue culture

DMEM/F12 (1:1) (cat# 21,331-020, Gibco) supplemented with 10% v/v heat-inactivated fetal bovine serum (FBS, Biochrom AG, Berlin, Germany), 0.6% glucose, insulin-transferrin-Na-selenit-mix 2 μL/mL (Roche Diagnostics GmbH, Mannheim, Germany), and penicillin 100 U/mL (Grünenthal GmbH, Aachen, Germany) was used. The cultures were carried at 37 °C and 5% CO_2 in a humidified tissue culture incubator.

2.3. Electrical stimulation

Explants were placed in a micro channel (μ-Slide I, ibiTreat, ibidi, Germany). Cochlea isolated from contralateral ear has served as a control without an electrical stimulation. The rectangular channels (50 mm long, 2 mm width, and 400 μm height) contained 100 μL medium, the whole μ-slide was filled with 1 mL medium. The explants were placed at the bottom of channel and the μ-slides were moved into stimulation chamber. Following 18 h or 6 h incubation without electrical field application, electrical stimulation was applied for 30 h or 42 h, respectively (Fig. 1).

For the electrical stimulation, the explants were placed in a customized, sterile stimulation chamber, allowing a parallel and independent stimulation of up to three channels. The electrical pulses were generated by a multi-channel pulse generator (master 8, A.M.P.I, Israel) and applied via customized amplifier in the stimulation chamber. Platinum electrodes (1 cm^2) covered with platinum black were used to improve electrically active electrode surface by reducing the current density on the surface. The electrodes were placed in PBS buffer (PBS, cat # 18,912-014, Gibco/Life Technologies, Darmstadt, Germany) and connected with the micro slides via glass bridges filled with 1.5% agarose gel (Tiny III, InnoTrain Diagnostics, Germany). The rectangular cross-section of the channel enables homogeneous application of the electrical field in the channel.

The explants were exposed to biphasic rectangular pulses (phase duration: 40 μs each, cathodic phase 1st, interphase interval: ~225 μs, frequency: 1 kHz, electrical current: 0.35 mA per pulse). These pulses were symmetric and charge balanced, therefore no electrical DC-field component was applied. The applied electrical current of 0.35 mA corresponds with a current level of 176CL generated by a cochlear implant, which is a moderate stimulation level. The current density in the micro channel was 18 mA per cm^2 with a charge of 15 nC per phase. The applied electric field strength was around 100 V/m per phase calculated with a conductivity of the cell culture media measuring 1.7 S/m [16].

2.4. Immunofluorescence and microscopy and hair cell score

The fixing, staining and hair cell scoring procedures were performed as previously described [15]. Briefly, after fixation with 4% paraformaldehyde, the filamentous actin of the inner and outer hair cells was visualized with phalloidin and the intact cells, defined as located within the classical hair cell arrays, with clearly defined cuticular plate and distinct, non-fused stereocillia, were quantified under epifluorescent microscope. Three different fields of 100 μm length were scored in each explant.

2.5. Spiral ganglion neurites and neuritic beading score

Digital images were taken under fluorescent microscope using AxioImager (AxioImager, Zeiss, Germany). Within each image, at least three different 100 μm-sections were selected randomly for the quantification of the neurite number. To avoid scoring damage induced during preparation, edges of explants and fragments with partially or totally disrupted neuronal cell bodies were excluded.

Following criteria were set for scoring of neuritic beading:

0: intact neurites distinguishable from the spiral ganglion cell body up to the hair cell region without visible neuritic beading

1: intact distinguishable neurites with obvious neuritic beading

2: neurites with predominant neuritic beading

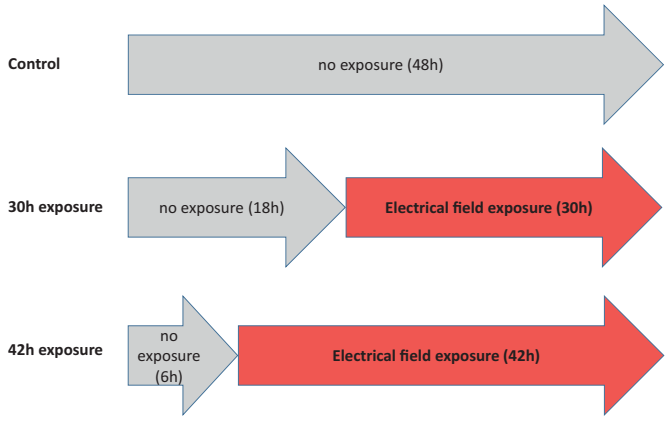

Fig. 1. Experimental flow depicting incubation condition for the control and the electrical field-exposed cochlear tissues. The explants in all experimental groups were cultured for a total of 48 h.