A new approach to endocochlear potential and potassium ion concentration measures in mini pig models

Lili Ren [a], Ling Zhang [b], Weiwei Guo [a], Wei Sun [c], Shiming Yang [a,*]

[a] Department of Otorhinolaryngology, Chinese PLA General Hospital, Beijing, 100853, China
[b] Department of Otolaryngology, PLA Air Force Command College Hospital, Beijing, 100097, China
[c] Department of Communicative Disorders & Sciences, Center for Hearing and Deafness, State University of New York at Buffalo, Buffalo, New York, NY 14214, United States

Abstract

Mini pig models are large mammals and their ears are more similar with human beings in structure and development than other animals. However, the study on porcine ears is still in the initial stage and there is no description of an ideal operation approach to endocochlear potential and potassium ion concentration measurements. In this article, we describe a pre-auricular surgical approach to access the middle and inner ear for endocochlear potential and potassium ion concentration measures in mini pig models. Ten one-week old normal mini pigs were used in the study. The bulla of the temporal bone was accessed via a pre-auricular approach for endocochlear potential and potassium ion concentration measurements. The condition of the animals during the first post–experiment 24 h was observed. One animal died during surgery. The pre-auricular approach improved protection and preservation of relevant nervous and vascular elements including the facial nerve and carotid artery. So, the pre-auricular approach can be used for endocochlear potential and potassium ion concentration measurements with improved nerve and artery preservation mini pigs.

1. Introduction

Human biology is often based on the study of animal species models. For understanding human diseases, the development of adequate animal models is of immediate importance. Although some animal models like fruit fly (Drosophila), zebrafish and rodents are highly informative about human diseases, these models do not always closely reflect human biology. For example, rodent models of Parkinson's disease (PD) do not recapitulate human pathophysiology (Fernagut and Chesselet, 2004; Perez and Hastings, 2004; Yamada et al., 2004). So, it is necessary to find large mammals as models of human diseases. The pig is a non-primate mammal that closely resembles human being in anatomy, physiology and genetics. Recent research has facilitated the biological experimentation with pigs, and helped develop the pig into a novel model organism for biomedical research. Genomic comparisons between the pig and human show more structural resemblance than, for example, between mouse and human (Hart et al., 2007; Rettenberger et al., 1995; Thomas et al., 2003; Tumbleson, 1986; Swindle, 1992). Pig models of human diseases have already been used in biomedical research, such as in diabetes, dermatology, eye diseases, degenerative diseases and skeletal growth (Aigner et al., 2010; Bendixen et al., 2010). Valuable data have been

obtained from these pig biomedical models during the past 20 years (Dodds, 1982). As a special type of swine, mini pig models have already proven particularly valuable in biomedical research because of their small size, strong disease resistance, convenient experiment operation and other advantages (Soucek et al., 2001; MOY et al., 1996; VODIČKA et al., 2005; Gottlow et al., 2012).

Like human being, sensory transduction in the cochlea and the vestibular labyrinth in mini pig models depends on the cycling of K^+ (Hibino and Kurachi, 2006; Hibino et al., 2010; Nin et al., 2008; Wangemann, 2002; Zdebik et al., 2009). These potassium channels exist in the stria vascularis in the cochlea, and provide the major source of endocochlear potential (EP) (Wangemann, 2006). So endocochlear potential and potassium ion concentration are essential for hearing. At present, though rodent animals are often used in research in endocochlear potential and potassium ion concentration measure, their application in inner ear research is limited, because their inner ear anatomy and hearing development are not similar with human. Mini pig models are large mammals and their ear are more similar with human beings in structure and development, so these models will have better prospects and potentials in otology field. But the study on porcine ears is still in the initial stage and there is no exact operation approach to endocochlear potential and potassium ion concentration measures. In this article, we describe a pre-auricular surgical approach to access the middle and inner ear for endocochlear potential and potassium ion concentration measurements in mini pig models to lay a foundation for future studies on surgical treatment of inner ear diseases.

2. Materials and methods

2.1. Animals

Ten one-week old normal mini pigs were used in the study. Care and use of the animals in this study were approved by the China Agricultural University.

2.2. ABR measurements

Details of ABR measurements were provided elsewhere (Zheng et al., 1999). For ABR measurements, pigs were anaesthetized with chloral hydrate (0.4 g/kg, intraperitoneal). Needle electrodes were inserted at the vertex and pinna. ABRs were evoked with clicks and/or 5-ms tone pips (0.5-ms rise/fall, at 30/sec) at 4, 8, 16 and 20 kHz. The response was amplified, filtered, and averaged using an Intelligent Hearing System. Sound level was raised in 20- and/or 5-dB steps. At each level, 1024 responses were averaged. Threshold was determined by visual inspection.

2.3. The pre-auricular approach

Mini pigs were anesthetized with intraperitoneal chloral hydrate (0.4 g/kg) and the body temperature was maintained at 37 °C. The operation ear was placed upward. The skin was prepared with iodophor. A skin incision was made near the zygomatic and the mandible bone (Fig. 1). Muscle and connective tissue were carefully dissected to expose the anterior wall of external auditory canal, which was then open together with the tympanic membrane to expose the cochlea.

2.4. Exposure of the cochlea

The head of the mini pig was fixed in a stereotaxic frame or a fixing pole using dental cerement. The bulla of the temporal bone was exposed by a pre-auricular approach. The bony cochlear wall between the cochlear window and vestibular window was thinned using a custom blade (Fig. 2). A small hole was made using a custom tool, without causing the leakage of endolymph, for EP and potassium ion concentration measures.

2.5. EP and potassium ion concentration measures

2.5.1. Double barreled potassium electrode preparation

The glass pipettes (1.5 mm in diameter) were cleaned first by immersion in nitric acid for 24 h and then washing with deionized water for 2–3 h. The pipettes were then transferred into a clean glass oven and baked at 100 °C for 2–3 h and cooled down at room temperate. The double barreled glass pipettes were hand-made by tightening up two single glass pipettes together with copper wires. The double-barred pipette was pulled on an up-right glass micropipette puller (PE-2, Narishige). The tips of the electrode were broken to an outer diameter of 1–2 μm. One pipette for the ion-selective barrel was silanized with dichlorodimethylsilane vapor and then baked at 145 °C for 2 h. After the electrode cooled down at room temperature, the silanized barrel was first filled with 150 mMKCl. Under a microscope, using a custom-made device, the K ligand (IE-190, World Precision Instruments) was sucked into the tip of the silanized barrel to a depth of about 200 μm (air bubbles were carefully avoided during this procedure). The reference barrel (for EP recording) was filled with 0.5 M NaCl.

The electrode was then mounted on a motorized micromanipulator (PC–N5, Narishige) for calibration. The solution for the K electrode was connected with an Ag–AgCl wire and its output was connected to a high impedance microelectrode amplifier (JL-H2003, Shanghai, China). The electrodes were calibrated at 37 °C in series solutions of KCl and NaCl (total 150 mM) that contained $[K^+]$ at 1, 5, 25, 50, 100 and 150 mM. The readouts were recorded and printed using the GraphPad Prism software. The slope of a linear regression curve best fitting the readouts was calculated as the potassium responding factor based on the following formula (Formula 1):

$$V = V_0 + S \times \text{Log}_{10} \left[K^+ \right]$$

where V = voltage readout, S = slope of the potassium responding factor (mV/decade), and $[K^+]$ = concentration