Intracellular potentials of the organ of corti in guinea pigs

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Introduction

Cochlear microphonic potentials (CM) can be recroded from the intact organ of Corti, but it is still an open question whether they are essential phenomena in the sound reception of hair cells. In order to determine what motivates the release of chemical transmitters at the synaptic end of hair cells, potentials in response to sound stimulations have to be intracellularly recorded. The intracellular potentials are identified by means of a marking technique using Alcian Blue dye.

Methods

Guinea pigs weighing 250 to 500 g were employed as experimental animals. The animals were anesthetized with pentobarbital sodium injected intraperitoneally in a dose of 30 mg per kg of body weight and were kept in artificial respiration after tracheotomy and muscle relaxation with gallamine triethiodide were performed. The right tympanic bulla was opened through a ventrolateral approach. For the purpose of intracochlear illumination a piece of glass rod was attached to the surface of the bony wall of the basal turn.

Recording and dye-application electrodes were inserted into the organ of Corti through the round window. Sound stimulation in bursts of 2500 to 3000 Hz was delivered through a coupler between a Mitsubishi Diatone TW-25 tweeter and the external ear canal. DC potentials were fed to a Nihonkohden MZ-4 preamplifier and recorded with a pen recorder. AC potentials were led from a Tektronix 565 cathod-ray oscilloscope to an X-Y recorder through an averaging data processor.

Immediately after the potential recording the dye marking of the recording sites of potentials was performed. The glass micropipett electrodes, less than 1 μm in tip diameter were filled with 3 % Alcian Blue - 1.5 M KCl solution. The ionized Alcian Blue was expelled from the electrode by applying 1×10^{-7} A current pulses of 500 msec duration, once a second for 1 to 2 min. The cochlea was rapidly removed from the skull after decapitation. The widely-fenestrated cochlea at the round window and apex was fixed for about 12 hr at 4°C in 2.5 % glutaraldehyde solution. The organ of Corti on the hook portion of the basilar membrane was carefully picked out and was observed as a surface preparation. Following that the material was dehydrated in ethanol and embedded in Epon. The Alcian Blue spot was precisely located in cross sectional preparations.

Results and discussions

During the electrode penetration into the organ of Corti from the scala tympani to the scala media, a few negative deflections can be obtained prior to the recording of the positive endocochlear dc potential (EP). These negative potentials in the organ of Corti were thought to originate intracellularly when first recorded,[1)] and it was later ascertained

by using marking techniques.[2~4] In electrode tip positions where the negative deflections were usually obtained, very unstable negative or sometimes positive potentials were recorded. The dye marking to determine the recording sites of these potentials was executed. The detection of marked spots on surface preparations was insufficient for cell identification because it is very difficult to localize the marked spot in the vertical plane of the organ of Corti even by the microscope's foucussing. Therefore, marked spots were located in cross sections. Fourteen marked spots were found in outer hair cells and twenty three were in supporting cells. The other spots were in inner and outer pillor cells, Boetcher's cells and Claudius' cells.

The intracellular resting potentials of Deiters' cells were between $-45 \sim -90.0$ mV and their mean value was -67.8 ± 4.2 mV. The magnitude of CM inside the Deiters' cells, expressed as the ratio to that measured in the scala tympani, was 1.32 and its amplitude was 3.1 ± 0.4 mV. The negative dc potentials of outer hair cells were -5.2 to -29.3 mV and they were obviously smaller than the resting potential in the supporting cells. The intracellular dc potentials in several outer hair cells were found to have a positive polarity. The mean CM was 1.86 as the relative values of the measurement in the scala tympani and the mean amplitude was 3.8 ± 0.7 mV. The CMs of both outer hair cells and supporting cells were the same in phase as the CM in the scala tympani.

The potentials of the outer hair cells identified are compared with those of supporting cells in Figure. The intracellular negative dc potentials in the outer hair cells are recorded smaller than in supporting cells and the decrease of the dc potentials tends to relate to diminution of the CM amplitude. In supporting cells there is no relationship between the dc potentials and the CM.

Until recently there has been a controversy as to whether the negative potentials in the organ of Corti are intercellular or intracellular. The present marking study using Alcian Blue confirmed that the origin of the negative potential is intracellular through the observation of the marked spots on cross sections.

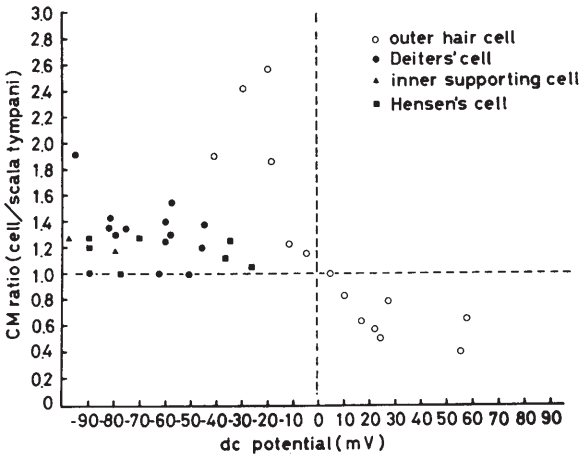

Fig. 1. Potentials in outer hair cells and supporting cells. CMs in those cells are expressed as the relative value of the measurement in the scala media.

References

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Scanning electron microscopic image of neuromuscular junctions

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Introduction

Although recent electron microscopical studies have added much information to the structure and function of neuromuscular junctions(NMJs), there is very little information in literature concerning three dimensional organization of NMJs at the fine structural level.

So far scanning electron microscopic studies of NMJs have not been achieved; the main obstacle resides in the presence of intramuscular connective tissue components which totally conceal the surface of NMJs when examined under the scanning microscope.

To overcome this problem, we have applied a modification of the HCl-Collagenase method which has been introduced by Evan *et al.*[1] in the survey of the basal aspect of tissues and cells.

Materials and method

The sternothyroid muscle of the chinese hamster were used for this study. The muscles were fixed *in situ* buffered glutaraldehyde followed by postosmification. In order to remove connective tissue components, the muscle strips were treated with 8N HCl for 20 to 40 min at 60°C after rinsing them in distilled water according to the method reported by Evan *et al.*[1] It was found that the treatment with HCl alone removes collagen and basal lamina almost completely, and that the successive digestion with collagenase recommended in the original method was found to be omissible. Another method for the same purpose which consists of enzymatic digestion prior to fixation[2] was also tested but resulted in serious distortion of the tissues caused by muscle contraction during the specimen preparation. They were dehydrated through a graded series of ethanol and were immersed in isoamyl acetate for 30 min. After drying by the critical point method and spatter coating with gold, the specimens were examined in Hitachi S-500A scanning electron microscope.

Results and discussion

In the specimens where intramuscular connective tissues are adequately removed, such structures as muscle fibres, intramuscular nerves and blood capillaries are immediately recognized under the scanning microscopy.