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Research paper

Does BAPTA leave outer hair cell transduction channels closed?

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Abstract

The calcium chelator BAPTA was iontophoresed into the scala media of the second turn of the guinea pig cochlea. This produced a reduction in low frequency cochlear microphonic (CM) measured in scala media and an elevation of the cochlear action potential (CAP) threshold that lasted for the duration of the experiment. Using two pipettes, one filled with KCl and the other KCl and BAPTA (50, 20 and 5 mM) it was possible to observe the effect of passing current through one electrode while measuring the endolymphatic potential (EP) with the other. The results demonstrated that current passed via the BAPTA pipette caused a sustained increase in EP of 8.2, 12.9 and 7.8 mV in the three animals used. This increase coincided with the decrease in low frequency CM that indicated a causal connection between the two. In a second series of experiments, pipettes with larger tips were inserted into scala media in the first cochlear turn and BAPTA was allowed to diffuse from the pipette. The results confirmed the relationship between EP increase and the fall of scala media CM. One interpretation of these results is that lowering the Ca^{2+} concentration of endolymph with BAPTA inhibits mechano-electrical transduction in outer hair cells (OHCs) and leaves the hair cell transduction channels in a closed state, thus increasing the resistance across OHCs and increasing the EP. These findings are consistent with a model of hair cell transduction in which tension on stereo cilia opens the transduction channels.

Keywords: Cochlea; Endolymphatic potential; Cochlear microphonic; Outer hair cells; BAPTA; Tip links; Stereocilia; Mechanotransduction

1. Introduction

Mechanoelectrical transduction (MET) in auditory and vestibular hair cells relies on ionic conductance changes brought about by mechanical deflection of stereocilia. Individual stereocilia are joined by filamentous strands (tip links) that connect the tips of shorter stereocilia with the shafts of their taller neighbours (Assad et al., 1991; Furness et al., 2002; Gillespie and Walker, 2001; Goodyear and Richardson, 2003; Pickles and Corey, 1992; Pickles et al., 1984; Tsuprun and Santi, 2000; Zhao et al., 1996). According to the "tip link hypothesis", shearing deflection of the stereocilia bundle modifies the tension on the tip links, which are mechanically coupled to the MET channels. The geometry is such that deflection in the direction of the tallest stereocilia should increase the tip link tension. Because this is the direction in which the MET conductance is greatest, it has been proposed that the tip links directly gate the MET channels, causing them to open when the tension is increased. Evidence suggests that the resting tension of the tip filaments is regulated by an "adaptation motor" (intrinsic to the MET channels and sensitive to the extracellular calcium levels) and that tension is increased in low $[Ca^{2+}]$ and reduced in high $[Ca^{2+}]$ (Assad and Corey, 1992). When extracellular $[Ca^{2+}]$ is reduced in the presence of the calcium chelator BAPTA, the tip links are disrupted, presumably because tension increases to breaking point (Meyer et al., 1998). In vitro, the normal mechanical sensitivity of hair cells is abolished when the tip link connections are broken, and it has been assumed that low tip link tension would leave the MET channels closed. However, Meyer (2005) and Meyer et al.

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(1998) have presented evidence that MET channels are inactive but open after exposure to the low Ca^{2+} concentrations produced by the presence of BAPTA. These authors concluded that while the tip links were necessary for transduction, they alone did not directly gate the channels.

The vulnerability of tip link connections to low [Ca^{2+}] induced by BAPTA and the resulting loss of mechanical sensitivity have been determined from studies of isolated hair cells. It seems an obvious step to investigate the effects of BAPTA in vivo. In particular, the OHCs of the cochlea are of interest by virtue of their role in contributing to the "cochlear amplifier" (Davis, 1983; Robles and Ruggero, 2001). Our aim in this paper was to introduce BAPTA into scala media by iontophoresis and by passive diffusion and to measure changes in EP and the CM. If BAPTA leaves the MET channels closed, we would expect an increase in access resistance of scala media and hence an increase in EP that would be correlated with a fall of CM (CM being the extracellular manifestation of OHC receptor current). On the other hand, if lowering the [Ca^{2+}] disrupts the tip links and leaves the MET channels open, as suggested by Meyer et al. (1998) and Meyer (2005), we would expect a reduction in EP caused by a decreased resistance across the apex of the OHCs that would correlate with a decrease in CM.

2. Methods

BAPTA was injected by iontophoresis and by passive diffusion into the first and second turns of scala media of adult pigmented guinea pigs. The animals were anaesthetised with Nembutal (30 mg kg^{-1} i.p. initially; 15 mg kg^{-1} every 2.5 h) and Hypnorm (0.15 ml i.m. repeated at 40 min intervals). Details of surgical procedures and maintenance of the animals have been described previously (Kirk, 2001; Sellick et al., 2005). Electrophysiological parameters were measured using LabView programs developed by Rob Patuzzi and Greg O'Beirne. The experimental procedures complied with guidelines of the National Health and Medical Research Council of Australia and were approved by the Animal Ethics Committee of The University of Western Australia.

2.1. Application of BAPTA with iontophoresis

In an initial series of three experiments, single micropipettes containing varying concentrations of KCl and BAPTA (tetrapotassium salt) (150 mM KCl plus 50 mM BAPTA, 200 mM KCl plus 20 mM BAPTA and 200 mM KCl plus 5 mM BAPTA) were placed in the scala media of the second cochlear turn and 5 μA of current (scala media negative) was passed for 5 min. The value of EP was noted upon penetration before current was applied and upon withdrawal of the pipette after the current was applied.

Because one micropipette was used for both EP measurement and iontophoresis, reliable measurement of the time course of EP change was not possible. Hence this method of measuring the effect of scala media BAPTA on EP was replaced with one in which two micropipettes ~2 μm outside diameter tips) were inserted into scala media in the second turn: one filled with 200 mM KCl and the other with 20 mM BAPTA in 180 mM KCl. The KCl pipette was used to measure changes in EP when current was applied to the BAPTA pipette and the BAPTA pipette was used to record EP when current was passed, as a control, through the KCl pipette. Both pipettes shared a common earth. Any change to the earth electrode caused by the passing of current would be common to both the BAPTA pipette and the KCl pipette recordings. The EP change due to the iontophoresis of BAPTA was calculated by subtraction of EP change produced when current was passed through the KCl pipette. Because iontophoresis of BAPTA into scala media produced a permanent changed in EP and CM, it was not possible to interleave control and BAPTA injections.

Measurement of the CM in response to an 86 Hz tone was performed using a high-quality PC sound card (Card Deluxe; Digital Audio Labs) and custom software. EP was monitored with a data logger (CEC webDAQ/100). CAP thresholds were measured using visual detection from a round-window electrode. Iontophoresis current (−5 μA dc) was applied from a constant-current source (A–M systems stimulator model 2100).

2.2. Application of BAPTA by passive diffusion

Micropipettes with tip diameters between 6 and 8 μm, filled with 100 mM KCl and 100 mM BAPTA or 130 mM KCl and 20 mM BAPTA (the latter solution was adjusted to pH 7.1 and had an osmolarity of 345 mosmoles) were inserted into scala media of the first turn via the round window and basilar membrane. EP and scala media CM (evoked by a 207 Hz tone) was measured with this pipette. The frequency of the probe tone of 207 Hz was used

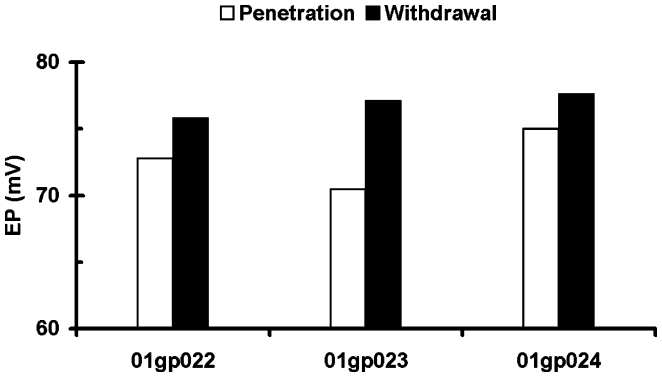

Fig. 1. In this series of three animals, a single pipette filled with 150 mM KCl and 50 mM BAPTA (01gp022), 200 mM KCl and 20 mM BAPTA (01gp23), 200 mM KCk and 10 mM BAPTA (01gp024) was placed in the second turn scala media and −5 μA of current was passed for 5 min. The histograms compares the EP measured during the initial penetration with that measured when the pipette was withdrawn after the current had been passed.