Molecular Motor and Electrokinetic Contributions to Outer Hair Cell Electromotility

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Jerry, Rocco A. and Ashim Dutta. Molecular motor and electrokinetic contributions to outer hair cell electromotility. J. Neurophysiol. 79: 471–473, 1998. The outer hair cell of the inner ear is believed to be responsible for the high sensitivity and selectivity of mammalian hearing. Molecular motors are generally believed to cause the electrically-driven length change (electromotility) of the outer hair cell. It has been suggested that electrokinetic effects might also play a significant role in electromotility, along with the molecular motors. This paper describes a new technique that can be used to experimentally determine the percentage of the electromotile response that is caused by electrokinetic effects. The technique is based on the novel idea that molecular motor activity cannot in itself generate a net force on the cell, but that electrokinetic effects can. Our method is the first that can separate molecular motor behavior from electrokinetic behavior, during experiments on the outer hair cell.

INTRODUCTION

The cylindrically shaped outer hair cell can change its length when it is electrically stimulated. This phenomenon is known as electromotility, and was first observed by Brownell et al. (1985). Electromotility is believed to be responsible for the high sensitivity and the sharp tuning of mammalian hearing.

Molecular motors have been suggested to cause electromotility. These motors are assumed to lie in the cylindrical wall of the cell. When an external electric field is changed, the motors change their conformation and this conformational change causes the cell to change its length. Dallos et al. (1993) and Jerry and Dutta (1998a,b), among others, have included molecular motors in their models for the outer hair cell.

It is currently not known whether electromotility is caused by molecular motors alone, or whether other mechanisms (such as electrokinetic effects) may also contribute to the total electromotile response. Electrokinetic forces act on the external wall of the outer hair cell and these forces can cause the cell to elongate or shorten. As discussed in Jerry et al. (1995b), electrokinetic forces on the cell wall can be classified into two categories: electrophoretic forces and electro-osmotic drag forces. Electrophoretic forces are simply forces that act on stationary charge sites, because of the presence of an electric field. The wall of the outer hair cell is expected to possess many of these charge sites, and thus electrophoretic forces may be present (Jerry et al. 1995b). Electro-osmotic drag forces also can be experienced by the cell wall. An electric field can cause the fluid near the cell wall to move (electro-osmosis) and this fluid motion can cause drag forces on the cell wall. Thus it is possible that part of the cell’s length change could be produced by both these electrokinetic effects, in addition to the action of the molecular motors.

Jerry et al. (1995b) have shown that the electrokinetic effect could be significant in electromotility of the outer hair cell and should not be ignored. In contrast, certain experiments suggest that electrokinetic effects could be relatively small. For example, see the experiments of Kakehata and Santos-Sacchi (1996) that treat the outer hair cell with salicylate and lanthanides and also the experiments of Kakehata and Santos-Sacchi (1995) that show the influence of intracellular pressure on electromotility. However, in the experiments that were performed thus far, there is no obvious way of quantitatively evaluating how much of the electromotile response is produced by electrokinetic effects and how much is produced by molecular motors. Below, we describe a method that can be used to determine the percentage of the electromotile response, which is produced by electrokinetic effects.

Two different types of electrical stimulation are commonly used with the outer hair cell. In the voltage-clamp experiments of Ashmore (1987), Kakehata and Santos-Sacchi (1995, 1996) and others, the voltage difference across the cell wall is controlled; it is assumed that the electric field is directed normal to the cell wall. The second type of electrical stimulation may be called transcellular stimulation and was used in the original electromotility experiments of Brownell et al. (1985). Transcellular stimulation simply means that the cell is situated between two electrodes. The electrodes are positioned along the axis of the cell, but far from the cell. One end of the cell is attached to a stationary support. Thus applying a voltage across the electrodes causes the cell to change its length.

Transcellular stimulation was investigated by Jerry et al. (1995b), who found that there can be a significant electrokinetic force on the nonstationary end of the cell; they also found that the net force on the cylindrical wall of the cell was negligible, because of a balancing of the electrophoretic and electro-osmotic drag forces. Thus the electrokinetic force simply acts like an axially-directed load on the nonstationary end of the cell. [Transcellular stimulation can produce electro-osmosis inside the cell, but the forces produced by intracellular electro-osmosis were shown to be negligibly small in Jerry et al. (1995a, 1996).]

Axial loading experiments were performed on the outer hair cell by Hallworth (1995). In his experiments, a flexible
glass fiber was attached to one end of the cell, while the other end of the cell was attached to a stationary support. The glass fiber allows the experimenter to apply an axially directed force to one end of the cell. Because the fiber is flexible, Hallworth was able to estimate the magnitude of the applied force, by measuring how much the fiber bends. (The applied force is linearly related to the amount of bending and the fiber is calibrated in advance.) Starting with a cell having an unloaded length \( L_1 \), Hallworth was able to apply different loads to the cell and measure both the applied force \( f \) as well as the new length of the cell \( L_2 \). From this information, he was able to estimate the axial stiffness of the cell.

Elements of both the Hallworth (1995) and the Brownell et al. (1985) experiments can be utilized to develop a novel method for estimating the importance of electrokinetic effects. We describe the way to combine these experiments below.

Suppose that transcellular stimulation is applied to the outer hair cell. However instead of attaching the cell to a stationary support, the cell should rather be attached to a flexible glass fiber. When a voltage is applied to the electrodes, not only will the cell change its length, but the fiber will also bend. The amount of bending may be measured and this value can be used to estimate the net force that the cell experiences in the electric field. This additional measurement, namely the net force on the cell, was not made in the experiments of Brownell (1985) and it is this measurement that is the key to estimating the importance of electrokinetic effects in electromotility. If molecular motors were entirely responsible for the length change of the cell, then the net force should be zero. The reason is that molecular motor activity should not produce any net force on the cell. If the net force is measured to be nonzero, then some fraction of the cell’s length change must be caused by electrokinetic effects. This fraction can be estimated as follows.

A two-step method is necessary in order to evaluate the importance of electrokinetic effects. First, a voltage is applied to the electrodes and two measurements are taken: the net force on the cell \( f \) and the new length of the cell \( L_2 \). In the second step, the Hallworth experiment should be performed without electrical stimulation: an external force should be applied by using the glass fiber to achieve a cell length equal to \( L_2 \) and this external force should be measured. Let \( f_2 \) be the value of this force. Note that \( f_2 \) can be described in words: \( f_2 \) is the value of the externally applied load that is required to produce the same cell length as that achieved through electrical stimulation. If electromotility were caused entirely by electrokinetic effects, then \( f \) would be equal to \( f_2 \). If electromotility were entirely caused by molecular motors, then \( f \) would equal zero. Thus the ratio \( f/f_2 \) is a measure of the relative importance of electrokinetic effects in electromotility of the outer hair cell.

Alternatively, the measured value of \( f \) can be used to estimate the portion of the cell’s length change that is caused by electrokinetic effects alone. This may be done simply by using the force vs. length data from the Hallworth experiment, to estimate the length change that would be caused by a load having a value of \( f \). Then this estimated length change is due entirely to electrokinetic effects and can be compared to the total length change of the cell. The ratio of the electrokinetically generated length change to the total length change is a measure of the fraction of the electromotile response that is caused by electrokinetic effects.

There are some practical considerations that we mention here. One question that immediately arises is whether or not the Hallworth (1995) glass fiber is sufficiently sensitive to measure the net electrically generated force on the cell. In the process of measuring the cell’s axial stiffness, Hallworth had accurately measured the force required to shorten the cell by a few microns. In general, electrical stimulation usually produces a cell length change that is also approximately a few microns (Brownell et al., 1985). If electrokinetics were entirely responsible for this length change, then one would expect to measure a net force that is approximately the same magnitude as that measured by Hallworth. Thus his glass fiber is suitable for our purposes.

In the original experiment of Hallworth (1995) , the glass fiber can easily be detached from the cell, because of very weak bonding between the cell and the glass fiber. In our proposed experiment, however, it is recommended that the glass fiber be firmly attached to the cell. Thus it may be necessary to pretreat the surface of the glass fiber with a material that can adhere to the cell wall. Alternatively, one end of the cell could be pretreated with an adhesive agent. The experimenter would be wise to select a surface treatment that possesses little surface charge of its own. There is an additional consideration that we point out. Our analysis ignores the surface charges on the end of the cell that is attached to the glass fiber. These particular surface charges could contribute to the net electrokinetic force on the cell. If this end of the cell were completely covered by the glass rod, then the surface charge of this cell end may be ignored. Even if the glass rod does not completely cover the cell end, then the surface charge may still be ignored if the cell end is pretreated with an adhesive having a low surface charge.

Current models of electromotility include the molecular motor behavior but ignore electrokinetic effects. Our method should allow experimentalists to calculate the electrokinetic contribution to electromotility and then subtract this quantity from the total electromotile response. This allows molecular motor behavior to be investigated independently and the molecular motor properties to be estimated more accurately.

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