Bending the Primary Cilium Opens Ca\textsuperscript{2+}-sensitive Intermediate-Conductance K\textsuperscript{+} Channels in MDCK Cells

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Abstract. Increasing tubular fluid flow rate has previously been shown to induce K\textsuperscript{+} secretion in mammalian cortical collecting duct. The mechanism responsible was examined in the present study using MDCK cells as a model. The change in membrane potential difference (\(E_M\)) of MDCK cells was measured with a fluorescent voltage-sensitive dye, DiBAC\textsubscript{4}(3), when the cell’s primary cilium was continuously bent with a micropipette or by the flow of perfusate. Bending the cilium produced a hyperpolarization of the membrane that lagged behind the increase in intracellular Ca\textsuperscript{2+} concentration by an average of 36 seconds. Gd\textsuperscript{3+}, an inhibitor of the flow-induced Ca\textsuperscript{2+} increase, prevented the hyperpolarization. Blocking K\textsuperscript{+} channels with Ba\textsuperscript{2+} reduced the flow-induced hyperpolarization, implying that it resulted from activation of Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels. Further studies demonstrated that the hyperpolarization was diminished by the blocker of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels, charybdotoxin, whereas iberiotoxin or apamin had no effect, results consistent with the activation of intermediate-conductance Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels. RT-PCR analysis and sequencing confirmed the presence of intermediate-conductance K\textsuperscript{+} channels in MDCK cells. We conclude that the increase in intracellular Ca\textsuperscript{2+} associated with bending of the primary cilium is the cause of the hyperpolarization and increased K\textsuperscript{+} conductance in MDCK cells.

Key words: DiBAC\textsubscript{4}(3) — Ca\textsuperscript{2+} — Fluo-4 — Flow — Ba\textsuperscript{2+} — Charybdotoxin

Introduction

The primary cilium, a solitary non-motile structure arising from the centriole and extending into the apical bathing solution, enables Madin Darby Canine Kidney (MDCK) cells to sense fluid flow (Praetorius & Spring, 2001). The intracellular Ca\textsuperscript{2+} concentration begins to increase a few seconds after the primary cilium is bent by manipulation with a micropipette or by the initiation of fluid perfusion. The maximal increase in intracellular Ca\textsuperscript{2+} concentration is achieved about 40 seconds after bending is initiated, and nearly five minutes are required for the Ca\textsuperscript{2+} concentration to return to baseline level. The increase in intracellular Ca\textsuperscript{2+} concentration caused by flow is accompanied by a large hyperpolarization of the membrane toward the calculated equilibrium membrane potential for K\textsuperscript{+} (\(E_K\)) (Praetorius & Spring, 2001). Since MDCK cells are known to have Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels (Breuer, Mack & Rothstein, 1988), this hyperpolarization might be due to activation of these channels.

In our previous study (Praetorius & Spring, 2001), MDCK cell \(E_M\) was measured with conventional glass microelectrodes. Several technical difficulties arose that severely limited the number of successful experiments. We showed that merely contacting the apical membrane of MDCK cells with a micropipette or microelectrode caused a transient increase in intracellular Ca\textsuperscript{2+} that lasted for about a minute. Thus, microelectrode punctures were required to remain stable for approximately two minutes before the experimental increase in perfusion rate could be performed. Such a response to mechanical contact is not unique to MDCK cells since nasal epithelial cells were reported to respond to mechanical stress with a transient increase in intra-
cellular Ca$^{2+}$ of similar duration and amplitude and as a result of the release of ATP and UTP across both the apical and basolateral cell membranes (Homolya, Steinberg & Boucher, 2000). Frequently, increases in fluid flow rate caused dislodging or small displacements of the microelectrode that resulted in leaks or changes in the membrane potential ($E_m$). Finally, we could not manipulate a cell’s primary cilium with a micropipette and simultaneously monitor its $E_m$ with a microelectrode. Therefore, we employed an approach for $E_m$ measurement in MDCK cells using a voltage-sensitive fluorescent dye, bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC$_4$(3)).

In renal distal tubule, an increase in luminal flow rate is known to induce K$^+$ secretion and hyperpolarization of the transepithelial potential difference (Malnic, Berliner & Giebisch, 1989). K$^+$ secretion is flow-dependent for rates up to 6 nl/min in aldosterone-treated rabbit cortical collecting duct, above which it saturates (Engbretson & Stoner, 1987). In the rat distal tubule, flow-dependent K$^+$ secretion is reported to saturate between 20–30 nl/min (Malnic, Berliner & Giebisch, 1989). It has recently been suggested that this K$^+$ secretion in rabbit cortical collecting ducts is mediated through activation of Ca$^{2+}$-sensitive maxi K$^+$ channels (Tanigushi & Imai 1998; Woda et al., 2001; Woda et al., 2002). The exact nature of the K$^+$ channels and the signal transduction pathway for this response are, however, not well established.

It is our hypothesis that the intracellular Ca$^{2+}$ concentration increase consequent to flow-induced bending of the primary cilium opens Ca$^{2+}$-sensitive K$^+$ channels in the apical membrane of MDCK cells, leading to a large hyperpolarization of $E_m$. Because of the similarities in the time course of flow-induced Ca$^{2+}$ transients, transepithelial potential difference and K$^+$ permeability, we further hypothesize that a similar sequence of events occurs in renal tubules and is responsible for the flow dependence of K$^+$ secretion in the distal tubule and collecting duct.

Materials and Methods

Cell Culture

Wild-type MDCK cells (passages 62–76 from the American Type Culture Collection, Rockville, MD) were grown to confluence on 25-mm diameter cover slips in Dulbecco’s modified Eagle medium (DMEM) with 10% fetal bovine serum (Gibco, Grand Island, NY) and 2 mM glutamine, but without riboflavin, antibiotics or phenol red, as previously described (Xia et al., 1984).

Solutions

The perfusion solution had the following composition, in mm: [Na$^+$] 137, [K$^+$] 5.3, [Ca$^{2+}$] 1.8, [Mg$^{2+}$] 0.8, [Cl$^-$] 136.9, HEPES 14, glucose 5.6, probenecid 5. Low-chloride solution with Ba$^{2+}$ contained, in mm: [Na$^+$] 137, [K$^+$] 5.3, [Ca$^{2+}$] 8, [Mg$^{2+}$] 0.8, [Ba$^{2+}$] 5, [Cl$^-$] 116.6, gluconate 131.3, HEPES 14, glucose 5.6, probenecid 5. All solutions were adjusted to pH 7.4 at 37°C and an osmolality of 300 mOsmol.

Microscopy and Perfusion

Continuously perfused MDCK cell monolayers were viewed on the stage of an inverted microscope (Diaphot, Nikon, Melville, NY) equipped with differential interference contrast (DIC) combined with low light level fluorescence as described previously (Xia et al., 1998). Imaging was performed with a 100×1.3 N.A. lens (Nikon) and an intensified CCD camera (ICCD-1001, Video Scope, Sterling, VA).

Low-chloride solution with Ba$^{2+}$ contained, in mM: [Na$^+$] 131.3, HEPES 14, glucose 5.6, probenecid 5. All solutions were adjusted to pH 7.4 and an osmolality of 300 mOsmol.

Intracellular Calcium Measured by Fluo-4

The cells were incubated for 15 minutes with the Ca$^{2+}$-sensitive probe Fluo-4-AM (5 µM) at 37°C, washed to remove excess probe and allowed at least a 20-minute de-esterification period. Then they were placed in the perfusion chamber and Fluo-4 fluorescence was measured. The fluorescence measurements were initiated 50 seconds prior to the movement of the primary cilium. During these experiments, the cells were under constant slow perfusion of 2 µl sec$^{-1}$. In the flow experiments, the primary cilia of several cells were bent simultaneously by increasing the flow of perfusate to 8 µl sec$^{-1}$.

Membrane Potential Measurements with DiBAC$_4$(3)

Changes in the membrane potential were measured with the fluorescent probe DiBAC$_4$(3). The distribution of dye between the cell membrane and the cytosol is dependent on the membrane potential. Hyperpolarization results in more dye accumulation in the cell membranes, decreasing the signal arising from the cytosol and, therefore, the emitted fluorescence intensity of a region of interest within the cell (Briäüer, Häuser & Strasser, 1984). The relationship between the relative intensity of the fluorescence of a region of interest within the cell and the $E_m$ was determined experimentally.