Single channel recordings of calcium-activated potassium channels in the apical membrane of rabbit cortical collecting tubules

(barium blockade/selectivity)

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ABSTRACT Recordings of single potassium channels from the apical membrane of rabbit cortical collecting tubule have been achieved using the patch-clamp technique. The conductive properties of the channel have been studied in inside-out patches. The slope conductance of the open channel is ≈ 90 pS. The channel is selective to potassium over sodium, with a selectivity ratio of 9:1. Decreasing the calcium concentration of the solution bathing the cytoplasmic face of the patch results in a decrease of the open-channel probability. Decreasing the calcium concentration to 10 nM or less completely inhibited channel activity. The channel is also inhibited by barium in a dose-dependent fashion.

The initial and cortical collecting tubules are the major sites of potassium secretion by the kidney, and it appears that a passive transport mechanism, located in the apical membrane, can account for this translocation process (1). Evidence from perfused tubule studies suggests that there is a barium-sensitive potassium conductance in the apical membrane of the rabbit cortical collecting tubule (2-4).

The objective of the present study was to examine this potassium conductance in the apical membrane by single-channel analysis.

Using the well established techniques of manual dissection and isolation of individual tubules from rabbit kidneys (5), we were able to expose the apical membranes of the cells by tearing the tubule wall (6). We have been able to demonstrate the existence in these cells of barium-sensitive potassium channels that are calcium-activated.

MATERIALS AND METHODS

Cortical collecting tubule segments (≈ 1 mm long) were isolated from the kidneys of female New Zealand White rabbits by manual dissection (5). The apical membrane was exposed by ripping the tubule lengthwise with a sharp needle. The tubule was then secured by means of two strips of aluminum foil, which were wrapped around a small glass coverslip ($\approx 12 \times 4$ mm). The tubule, together with the coverslip, was transferred to a chamber, containing a Hepes-buffered Ringer (pH, 7.3), mounted on a microscope stage. The composition of this bath solution was 135 mM sodium/5 mM potassium/1.8 mM calcium/1 mM magnesium/145.6 mM chloride/10 mM Hepes/2 mM glucose. The tubule was then examined at a magnification of $\times 400$ using Hoffman optics, and the apical surface of the cells was identified. The apical membrane of a cell was approached with a patch pipette under micromanipulator control and high-resistance seals (>10 G Ω) were generally achieved spontaneously (without suction).

The patch pipettes always contained a filtered solution of the following composition: 75 mM sodium/75 mM potassi-

um/1.8 mM calcium/1 mM magnesium/10 mM Hepes/155.6 mM chloride (adjusted to pH 7.3 with NaOH). When potassium channels were present in the patch, they were seen to open and close spontaneously. The pipette was then withdrawn from the cell, bringing a small patch of membrane with it. If the patch showed signs of vesicle formation [loss of channel activity or distortion of channel currents (7)], it was briefly pulled through the liquid/air interface to rupture the external membrane of the vesicle. We have not attempted to record channel currents while cell attached. The bath solution was changed to contain 15 mM potassium, with sodium being the osmotic replacement, so the current was directed outward from the pipette to the bath.

In a second series of experiments, we have used solutions with differing calcium concentrations. The solutions contained free calcium concentrations of 1, 0.1, and 0.01 mM, calculated from the affinity constants of EGTA (5 mM), magnesium (1 mM), and hydrogen ions (pH, 7.3), calcium being added to give the desired free calcium ion concentration.

In a final series of experiments, barium was added to the 15 mM potassium bath solution from a stock solution of 100 mM barium chloride, to give a final concentration of 1, 10, 100, and 1000 μ M barium.

FIG. 1. Upper trace shows a typical recording of a potassium channel when the potassium current is directed from the pipette to the bath (75 mM potassium inside the pipette, 15 mM potassium in the bath). The holding potential is 0 mV. The arrow indicates the current when the channel is closed. The I/V curve for this channel is shown in the lower panel. The extrapolated reversal potential is +26 mV.

FIG. 2. Calcium dependence of two potassium channels. Holding potential is 0 mV. The arrows indicate the current when both channels are closed. The upper trace shows channel activity at $1 \mu\text{M}$ calcium. The middle trace shows partial inhibition of channel activity at $0.1 \mu\text{M}$ calcium. The lower trace shows the complete inhibition of channel activity at $0.01 \mu\text{M}$ calcium.

Channel currents were measured using a high-gain amplifier (Yale Mark V patch clamp amplifier), recorded with an FM tape recorder (Tandberg), and hard copies of the events were obtained by playing the tape recordings back through a Clevite 220 Brush Recorder. The frequency response of this total system is limited by the chart recorder to ≈ 100 Hz.

RESULTS

The upper trace of Fig. 1 shows the normal channel activity through an inside-out patch, with potassium ions passing from the pipette to the bath, driven solely by the chemical gradient for potassium of 40 mV (clamp potential is zero). Under these conditions, with a high calcium concentration in the bath, the channel is seen to be mainly open, with a lot of short-lived closures and distinct long-lived closures [defined as slow closures by Moczydlowski and Latorre (8); see also upper trace of Fig. 3]. The lower panel of Fig. 1 shows the I/V curve for this particular channel, obtained by clamping the patch to various command potentials and measuring the current flow through the open channel. The slope conductance is 88 pS under these ionic conditions. We have not noted any marked voltage dependence of the channel over the narrow voltage range studied so far (± 20 mV). The extrapolated reversal potential for this channel was +26 mV, giving a sodium/potassium selectivity of 9.4:1.

In a series of experiments using inside-out patches (cytoplasmic face of the patch in the bath), decreasing the calcium

FIG. 3. Concentration-dependent inhibition of two potassium channels by barium. Arrows indicate the closed channel current. Holding potential is 0 mV. Barium concentrations are indicated above each trace.

concentration of the fluid in the bath caused a graded decrease in the probability of the channels being open (Fig. 2). In this experiment, the holding potential is zero, with an outwardly directed potassium current from the pipette to the bath (openings upward). There are two channels in the patch and they are seen to be mainly open at 1 μ M calcium (top trace). Decreasing the calcium concentration to 0.1 μ M effectively halves the open state probability (middle trace), whereas in five experiments at 0.01 μ M calcium (lower trace), we have observed no openings.

The effect of adding barium to the cytoplasmic face of an inside-out patch (clamp potential zero) is shown in Fig. 3. Over the range 1 μ M to 1 mM barium, there is a concentration-dependent decrease in channel-open probability.

DISCUSSION

The conductance, selectivity, and calcium dependence of the channels that we report here suggest that they belong to a group known as calcium-activated potassium channels, as reviewed by Latorre and Miller (9). These channels are characterized by being inactive at calcium concentrations $<0.1 \mu$ M, and by increasing open state probabilities as cytoplasmic calcium concentration is increased. A similar channel has recently been discovered in the basolateral membrane of collagenase-isolated rodent salivary gland acini (10). Similar to our findings, barium-blockade of the calcium-activated potassium channels from rat muscle, incorporated in bilayers, has also been reported by Vergara and Latorre (11).

Evidence from microelectrode studies suggests that there is a barium-sensitive potassium channel in the apical membrane of cortical collecting tubule (3, 4). We have not yet been able to ascertain whether barium inhibits the channel from the apical face, which is the face where barium was added in the microelectrode studies. However, barium is an effective blocker at both faces of the calcium-activated potassium channel isolated from rat muscle (11).

A calcium- and voltage-dependent potassium conductance has been demonstrated in the apical membrane of *Necturus* gall bladder (12). It was observed that an increase in intracellular calcium increased the potassium conductance over a certain voltage range. A similar channel has also been identified by noise analysis in this tissue when depolarizing the apical membrane (13).

In summary, we have isolated a barium-sensitive potassium channel from the apical membrane of cortical collecting

tubules of rabbits. We suggest that this is the pathway for potassium secretion by the cortical collecting tubule, and we also predict that procedures likely to alter the intracellular calcium activity will alter the apical membrane potassium conductance. For instance, if sodium/calcium exchange exists at the basolateral membrane, the following sequence of events could occur: a decreased sodium delivery and a decrease in luminal sodium concentration would cause a decline in cell sodium activity, a decline in cell calcium activity, and a decline in apical potassium conductance. The resulting decreased potassium secretion would serve as a mechanism to conserve potassium. Indeed, such a relationship between potassium and sodium handling is uniformly observed in the distal nephron (1).

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