Reciprocal effects of Ca$^{2+}$ and Mg-ATP on the ‘run-down’ of the K$^+$ channels in opossum kidney cells

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Abstract. Using the patch clamp technique, we identified an inwardly rectifying K$^+$ channel in the membrane of opossum kidney cells. The single channel conductance was about 90 pS for inward currents and 30 pS for outward currents under a symmetrical high-K$^+$ condition. The activity of the channel was found to decrease with time during recording from inside-out patches. In the solution with submicromolar Ca$^{2+}$, the activity disappeared within 4-20 min. Intracellular Ca$^{2+}$ promoted the run-down of the channel activity at 0.1-1 mM, whereas millimolar Mg-ATP restored the activity after run-down. The run-down channels could never be reactivated by ATP in the absence of Mg$^{2+}$, or by a nonhydrolyzable ATP analog, AMP-PNP, even in the presence of Mg$^{2+}$.

Key words: Patch clamp - Kidney cell - K$^+$ channel - Run-down - Ca$^{2+}$ - ATP

Introduction

Intracellular recordings with conventional micro-electrodes have demonstrated the presence of K$^+$ conductive pathways in basolateral membranes of proximal tubular epithelial cells (see Giebisch and Aronson 1986, for a review). Changes in the K$^+$ conductance were associated with alterations in Na$^+$ transport. Although the precise mechanism still remains unknown, the functional link between Na$^+$ transport and K$^+$ channel activity was speculated to be somehow mediated by actions of Ca$^{2+}$ and metabolic activity within the cell.

Recently, the K$^+$ channels have directly been demonstrated with patch clamp technique (Kawahara et al. 1987, Parent et al. 1988). This approach enables us to investigate the regulatory factors of the K$^+$ channels. In the present study, applying this technique to opossum kidney (OK) cells, which have been used as a model for the proximal tubular epithelia (Ubl et al. 1988), we found that the K$^+$ channel activity was reciprocally controlled by intracellular Ca$^{2+}$ and Mg-ATP.

Methods

OK cells, which were kindly supplied by Dr. L. R. Forte, were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were harvested from confluent monolayers with trypsin and incubated in the culture medium for 4-8 hours before use. All experiments were performed at room temperature.

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Cell-attached and inside-out patch experiments followed the method described by Hamill et al. (1981), using an EPC-5 amplifier (List). Patch pipettes were filled with the solution containing (in mM) 145 KCl, 1 MgCl$_2$, 10 HEPES-NaOH (pH 7.3). The electrode resistance ranged between 4-8 MΩ. The
control solution (free Ca\(^{2+}\) 50 nM) was composed of 115 KCl, 1 MgCl\(_2\), 3 CaCl\(_2\), 10 EGTA, 30 KOH and 5 HEPES (pH 7.3). When necessary, some modified solutions were employed, such as a 30 mM K\(^+\) solution made by replacing KCl in the control solution with NaCl, a Mg-free solution prepared by omitting MgCl\(_2\), and substituting EGTA for HEPES in the control solution, and high Ca\(^{2+}\) solutions containing 145 KCl, 1 MgCl\(_2\), 0.01-1 CaCl\(_2\), and 10 HEPES-NaOH (pH 7.3). One millimolar Mg-ATP or Mg-AMPPNP (adenylylimidodiphosphate) was applied by adding 1 mM Na\(_2\)ATP (or Li\(_4\)AMPPNP) and 1 mM MgCl\(_2\) to the control solution after readjusting pH to 7.3.

Values for the potential represent the patch membrane potential (V_p), and in the case of cell-attached patches, they were estimated on the assumption that the cell membrane potential was 0 mV at a K\(^+\) concentration of 145 mM. Currents flowing from the cytoplasmic side to the external side were designated as positive and shown as upward deflections in the figures.

Results

The activity of ion channels as shown in Fig. 1A was observed in about 80% of the cell-attached patch membranes on the OK cells. The current-voltage (I-V) curve exhibits inward rectification (Fig. 1B, open circles). The single channel conductance determined from 13 patches was 89.3 \(\pm\) 2.3 pS (mean \(\pm\) SEM) for inward currents and 34.4 \(\pm\) 1.9 pS for outward currents. The I-V relationship of the channel was not altered after excision of the patch membrane (Fig. 1B, closed circles). The reversal potential was around 0 mV under a symmetrical K\(^+\) condition across the patch membrane. It was shifted to 41.5 \(\pm\) 1.9 mV (n=4), when the internal K\(^+\) concentration was reduced from 145 to 30 mM (Fig. 1C). Since this value is close to the equilibrium potential of K\(^+\) (40.5 mV at 25°C), it appears that the channel is selective for K\(^+\) over Na\(^+\) and Cl\(^-\).

During the long-lasting recordings from inside-out patches, the K\(^+\) channel activity was found to decrease with time ('run-down'). The time course of the run-down varied from patch to patch. In most cases, the activity disappeared within 4-20 min in the control solution with submicromolar Ca\(^{2+}\) (Fig. 2A, C). The run-down of the channel was promoted by 0.1-1 mM Ca\(^{2+}\) applied to the internal surface of the membrane. The channel activity was usually lost after 30 sec-application of 1 mM Ca\(^{2+}\) (Fig. 2B). In the figure, the trace of the recording during Ca\(^{2+}\)-application is not shown because of a dominant activity of Ca\(^{2+}\)-activated channels coexisting in the patch membrane. Such a promoting effect of Ca\(^{2+}\) on the run-down was also observed at 0.1 mM in four experiments, although the data are not shown. In lower concentrations, it was difficult to clearly demonstrate the effects of Ca\(^{2+}\) because of a large variability of the time course of the non-maximal run-down.

Since reduction of the intracellular Ca\(^{2+}\) ions down to submicromolar concentrations failed to prevent the run-down, another cytosolic factor should be involved in maintenance of the K\(^+\) channel activity. Thus, we tested effects of ATP, one of the candidates. As shown in Fig. 2A-C, the channel activity partially recovered a few minutes after application of 1 mM ATP in the presence of Mg\(^{2+}\). Since the channels activated by Mg-ATP showed essentially the same characteristics as the previous channels with respect to the I-V relationship, K\(^+\)-selectivity and Ca\(^{2+}\)-induced inactivation (Fig. 2C),