Basolateral Na^+/H^+ exchange maintains potassium secretion during diminished sodium transport in the rabbit cortical collecting duct

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Stimulation of the basolateral Na^+/K^+-ATPase in the isolated perfused rabbit cortical collecting duct by raising either bath potassium or lumen sodium increases potassium secretion, sodium absorption and their apical conductances. Here we determined the effect of stimulating Na^+/K^+-ATPase on potassium secretion without luminal sodium transport. Acutely raising bath potassium concentrations from 2.5 to 8.5 mM, without luminal sodium, depolarized the basolateral membrane and transepithelial voltages while increasing the transepithelial, basolateral and apical membrane conductances of principal cells. Fractional apical membrane resistance and cell pH were elevated. Net potassium secretion was maintained albeit diminished and was still enhanced by raising bath potassium, but was reduced by basolateral ethylisopropylamiloride, an inhibitor of Na^+/H^+ exchange. Luminal iberitoxin, a specific inhibitor of the calcium-activated big-conductance potassium (BK) channel, impaired potassium secretion both in the presence and absence of luminal sodium. In contrast, iberitoxin did not affect luminal sodium transport. We conclude that basolateral Na^+/H^+ exchange in the cortical collecting duct plays an important role in maintaining potassium secretion during compromised sodium supplies and that BK channels contribute to potassium secretion.

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K^+ secretion in the cortical collecting duct (CCD) is tightly coupled to Na^+ reabsorption. Increased Na^+ entry across the apical membrane stimulates the Na^+-K^+-ATPase, which enhances Na^+ extrusion and K^+ uptake across the basolateral membrane.[1] This is followed by passive diffusion of K^+ across the apical membrane along a favorable electrochemical gradient. We have previously demonstrated that in the isolated perfused rabbit CCD raising bath K^+ from 2.5 to 8.5 mM in the presence of luminal Na^+ increases K^+ secretion and Na^+ reabsorption as well as apical Na^+ and K^+ conductances.[2–4]

However, K^+ excretion may not completely depend on apical Na^+ entry. For example, rats maintained on a low-Na^+ diet can increase renal K^+ secretion in response to acute K^+ loading.[5] Furthermore, a significant kaliuretic response after acute K^+ loading has been observed in dogs receiving amiloride, which inhibits apical Na^+ reabsorption in the distal nephron.[6] Thus, it is conceivable that the kidney maintains K^+ secretion by a mechanism, which does not require apical Na^+ entry in principal cells. The aim of the present study is to test the hypothesis that basolateral Na^+ entry via Na^+/H^+ exchange (NHE) could sustain Na^+-K^+-ATPase activity and K^+ secretion in the CCD during compromised luminal Na^+ absorption.

RESULTS

First we examined the electrical properties of principal cell in response to raising basolateral K^+ in the absence of luminal Na^+. As shown previously,[2,4] raising basolateral K^+ from 2.5 to 8.5 mM in the presence of luminal Na^+ induced hyperpolarization of both transepithelial voltage (V_T) and basolateral membrane voltage (V_B) followed by depolarization. The initial transient hyperpolarization was the result of stimulating Na^+-K^+-ATPase whereas the subsequent depolarization was due to alterations of apical Na^+ and K^+ conductances as well as basolateral K^+ conductance. Figure 1 is a typical recording showing the effect of raising basolateral K^+ concentration on V_T and V_B. In contrast to the condition

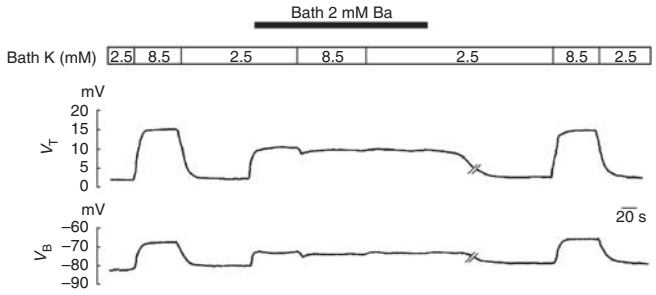

Figure 1 | Typical tracings showing effects of raising bath K^+ from 2.5 to 8.5 mM in absence and presence of bath Ba^{2+} (2 mM) on V_T and V_B in the CCD. The luminal Na^+ concentration was 0 mM.

Table 1 | Effects of raising bath K^+ from 2.5 to 8.5 mM in the absence of luminal Na^+ on barrier voltages and conductances in the CCDs

Bath K (mM)	2.5	8.5	2.5 (recover)
V_T, mV (n=53)	1.0 ± 0.4	8.3 ± 0.5*	0.8 ± 0.4
V_B, mV (n=53)	−82.7 ± 1.2	−75.1 ± 1.1*	−82.9 ± 1.3
V_A, mV (n=53)	83.8 ± 1.2	83.4 ± 1.2	83.7 ± 1.3
G_T, mS/cm^2 (n=53)	6.5 ± 0.1	7.4 ± 0.1*	6.3 ± 0.1
fR_A (n=53)	0.56 ± 0.01	0.60 ± 0.01*	0.56 ± 0.02

Values are mean ± s.e. Data at 8.5 mM K^+ in the bath were taken at the peak of the depolarization. *P < 0.001 compared with preceding period.

in which luminal Na^+ is present, the transient hyperpolarization of V_T and V_B was absent following raising basolateral K^+ to 8.5 mM. Raising basolateral K^+ depolarized V_T from 1.0 ± 0.4 to 8.3 ± 0.5 mV and V_B from −82.7 ± 1.2 to −75.1 ± 1.1 mV without significant changes of apical membrane voltage (V_A; Table 1). Also, both transepithelial conductance (G_T) and the fractional apical membrane resistance (fR_A) significantly increased from 6.5 ± 0.1 to 7.4 ± 0.1 mS/cm^2 and 0.56 ± 0.01 to 0.60 ± 0.01, respectively. It is possible that the expected transient hyperpolarization of V_T and V_B was masked by a rapid depolarization induced by changing the K^+ equilibrium potential following raising basolateral K^+. Alternatively, removal of luminal Na^+ might weaken the stimulation of Na^+-K^+-ATPase induced by raising basolateral K^+ concentration. The first possibility was confirmed by experiments in which the above experiment was conducted in the presence of Ba^{2+} in the bath solution (Figure 1). It is apparent that raising bath K^+ concentration caused a transient hyperpolarization of V_T and V_B. Thus, raising K^+ concentration could still activate Na^+-K^+-ATPase in the absence of luminal Na^+. Moreover, in the absence of luminal Na^+, stimulation of Na^+-K^+-ATPase with 8.5 mM K^+ increased both basolateral membrane conductance (G_B) (from 11.7 ± 0.9 to 15.7 ± 1.4 mS/cm^2; n = 18, P < 0.001) and apical membrane conductance (G_A) (from 8.4 ± 0.5 to 9.2 ± 0.8 mS/cm^2; n = 18, P < 0.05; Figure 2). Because removal of luminal Na^+ is expected to abolish the apical Na^+ entry, the observed increase in the G_A is best explained by augmentation of the apical K^+

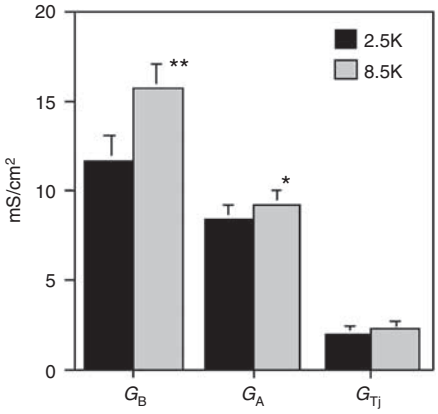

Figure 2 | Bar graph summarizing effects of raising bath K^+ from 2.5 to 8.5 mM on the basolateral membrane conductance (G_B), apical membrane conductance (G_A), and tight junction conductance (G_{TJ}). *P < 0.05 and **P < 0.001 compared with 2.5 mM bath K^+ (2.5K). The number of tubules examined is 18.

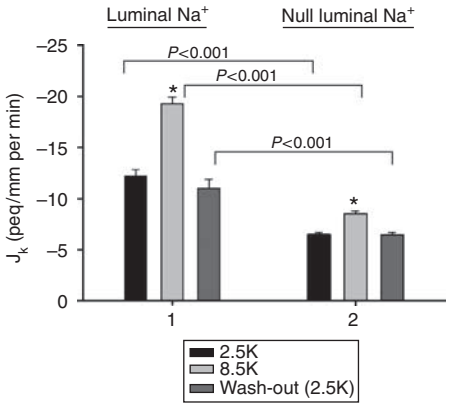

Figure 3 | Effects of raising bath K^+ from 2.5 to 8.5 mM on net K^+ secretion (J_K) in CCDs perfused *in vitro* in the presence of lumen Na^+ (146.8 mM) or absence of lumen Na^+. Asterisk indicates the significant difference with 8.5 mM K^+ in the bath (8.5K) in comparison to 2.5 mM K^+ in the bath (2.5K). The number of tubules examined in the presence and absence of lumen Na^+ is 6 and 4, respectively.

conductance. This view is also supported by the observation that adding amiloride to the lumen did not affect, whereas inhibition of apical K^+ conductance with luminal Ba^{2+} did abolish the high bath-K^+-induced changes in fR_A (data not shown).

To evaluate the role of Na^+-K^+-ATPase in stimulating K^+ secretion in the absence of luminal Na^+, we examined the effect of raising bath K^+ from 2.5 to 8.5 mM on net K^+ secretion (J_K). As shown in Figure 3, we confirmed that in the presence of luminal Na^+, raising bath K^+ from 2.5 to 8.5 mM stimulated J_K from −12.1 ± 0.8 to −19.3 ± 0.6 peq/mm/min (n = 6, P < 0.001). Removal of luminal Na^+ significantly attenuated net K^+ secretion at both basolateral 2.5 and 8.5 mM K^+ concentrations. However, raising bath K^+ from 2.5 to 8.5 mM still increased J_K from −6.5 ± 0.2 to −8.5 ± 0.1 peq/mm/min (n = 4, P < 0.001; Figure 3). Thus,