The relationship between distal tubular proton secretion and dietary potassium depletion: evidence for up-regulation of H⁺-ATPase

Matthew Bailey¹, Giovambattista Capasso², Samuel Agulian³, Gerhard Giebisch³ and Robert Unwin¹

¹Centre for Nephrology, Department of Medicine, The Rayne Institute, University College London, London, UK.
²Chair of Nephrology, Second University of Naples, Naples, Italy and ³Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA

Abstract

Background. Dietary potassium depletion is associated with elevated plasma bicarbonate concentration and enhanced bicarbonate reabsorption in the distal tubule. The relationship between distal proton secretion and potassium status was investigated by in vivo micro-perfusion of the superficial distal tubule.

Methods. Experiments were performed on anaesthetized rats that had been maintained on either a low-potassium or control diet for 3–5 weeks prior to experimentation. The distal tubules were perfused at 10 nl/min with either a standard or a barium chloride-containing solution, and the late distal tubular transepithelial potential difference (Vte) and pH of the luminal fluid were recorded using a double-barrelled voltage and ion-sensitive microelectrode.

Results. In control rats, the Vte was −40.7 ± 2.4 mV and the tubular fluid pH was 6.44 ± 0.07; in potassium-depleted animals, the Vte was −15.0 ± 1.4 mV and the pH was 6.76 ± 0.03. The pH values in both groups of animals were significantly lower than would be predicted from the Vte and systemic pH for passive H⁺ distribution, indicating active proton secretion. Moreover, in hypokalaemic rats, this difference from predicted pH was significantly greater than in control animals (control = 0.27 ± 0.06 vs low-potassium = 0.46 ± 0.03; P < 0.01), suggesting enhanced active proton secretion. During perfusion with a solution containing BaCl₂, the late distal tubule Vte became lumen positive in potassium-depleted rats, contrasting with an increased lumen negativity in potassium-replete controls. The barium-induced lumen-positive potential difference observed in the hypokalaemic rats was abolished by intravenous administration of acetazolamide.

Conclusion. These data are consistent with enhanced electrogenic proton secretion (H⁺-ATPase) during dietary potassium deprivation.

Key words: late distal tubule; potassium depletion; proton secretion

Introduction

The late distal tubule, consisting of the short connecting tubule and the initial portion of the cortical collecting duct (CCD), typically displays a large, lumen-negative transepithelial potential difference (Vte), of the order of 30–50 mV. This lumen negativity arises primarily from the diffusion of Na⁺ into the principal cell down a favourable electrochemical gradient (created by basolateral Na⁺,K⁺-ATPase activity), which depolarizes the apical membrane with respect to the basolateral membrane. Early studies found that when either Na⁺ or K⁺ transport was reduced (during luminal perfusion with amiloride, basolateral superfusion with ouabain or in Na⁺- and K⁺-free solutions), the isolated rabbit CCD developed a stable, lumen-positive Vte [1,2]. The origin of the lumen-positive Vte could not, therefore, be readily explained on the basis of Na⁺ or K⁺ flux. However, if either acetazolamide or 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS) was added to the bath during simultaneous luminal perfusion with amiloride, the lumen positivity was markedly attenuated [1,2]. Moreover, in the absence of amiloride, these agents increased the lumen-negative potential difference. These experiments suggested that acidification of the tubular fluid was, to a degree, electrogenic, providing a positive component to the Vte that is normally obscured by the larger, negative potential resulting from the balance of Na⁺ and K⁺ fluxes (Figure 1).

In the present series of experiments, we have investigated the contribution of electrogenic proton secretion to the development of late distal tubular Vte in vivo. In addition, we have investigated the relationship between dietary potassium depletion, a condition associated with enhanced bicarbonate reabsorption in the distal tubule [3,4], and electrogenic proton secretion. The results obtained provide indirect evidence for
increased electrogenic proton secretion in the late distal tubule of potassium-depleted rats.

Materials and methods

Experiments were performed on male Sprague–Dawley rats (Teklad 88239; Madison, WI; <750 μmol K⁺ per kg dry weight) and companion control diet (Teklad 88238; 250 mmol K⁺ per kg dry weight) for 3–4 weeks. Rats were anaesthetized with an injection of 5-sec-butyl-5-ethyl-2thio barbituric acid (Inacitin; Byk-Gulden, Constance, Germany; 100 mg/kg body weight, i.p.) and prepared surgically for microperfusion of the superficial distal tubule, as previously described [5,6].

Briefly, the left jugular vein and right carotid artery were cannulated for infusions and for withdrawal of blood, respectively. A tracheotomy was performed and the bladder catheterized. The left kidney was exposed by a flank incision, cleared of perirenal fat and placed in a Perspex dish which was clamped to the operating table. Following immobilization of the kidney in a 5% agar solution, the exposed surface was bathed in heavy mineral oil for the duration of the experiment. The ureter was cannulated close to the hilum.

All animals received an i.v. infusion of NaCl (125 mmol/l) and NaHCO₃ (25 mmol/l) throughout the course of the experiment at 4 ml/h; during the final hour of surgery, an additional volume of infusate was administered to compensate for surgical losses. Two hours after the completion of surgery, superficial distal tubules were identified (following random insertion of the microperfusion pipette into proximal tubular segments) and perfused using a thermally shielded microperfusion pump (Hampel, Neu-Isenburg, Germany) inserted in the early-distal loop.

Following the insertion of a proximal oil block, the distal tubule was perfused sequentially in an orthograde manner with a perfusate designed to mimic native early distal tubular fluid, containing (in mmol/l): 50 NaCl, 2 KCl, 2 NH₄Cl, 1 CaCl₂, 100 urea and 5 HEPES. The pH of the perfusate was adjusted to 7.5. In the second set of experiments, BaCl₂ (5 mmol/l) was added to the perfusate, in an attempt to unmask electrogenic proton secretion. Subsequently, in potassium-depleted animals only, the effect of an i.v. injection of acetazolamide (20 mg/kg) on the Vₑₑₑₑ of the late distal tubule during perfusion with the solution containing Ba²⁺ was assessed.

Voltage and pH measurements were made using double-barrelled micropipette electrodes, fabricated from two separate borosilicate glass capillaries with an internal glass filament (1.2 and 1.0 mm o.d., respectively; Clark Electromedical Instruments, Pangbourne, UK) and bevelled to give a total tip diameter of 3 μm. The larger barrel was silanized for 1 h by cold-vapour silanization (hexamethyldisilazone; Fluka; Buchs, Switzerland) as previously described [5]. The tip of this barrel was filled with a proton-sensitive ion exchange resin (Hydrogen ionophore 1-cocktail A; Fluka) and the electrode was then backfilled with a 2-[N-morpholino]ethanesulphonic acid solution (MES; 20 mmol/l in 100 mmol/l NaCl; pH 7.0). The second, reference barrel, which was used to measure Vₑₑₑₑ, was filled with a solution containing sodium acetate (250 mmol/l), sodium chloride (250 mmol/l) and potassium chloride (2 mmol/l).

Both halves of the electrode were connected differentially to a high-impedance electrometer (World Precision Instruments, New Haven, CT). On the reference side of the circuit, a silver–silver chloride electrode, immersed in a potassium chloride solution (500 mmol/l) was connected to a bath of sodium chloride (150 mmol/l) by an agar bridge. In order to complete the connection, the end of the rat’s tail was skinned and placed in the bath of isotonic saline.

Baseline or zero voltage was measured initially with the electrode tip in the fluid bathing the kidney surface. The electrode was then advanced into the lumen of the last accessible portion of the late distal tubule, causing the measured voltage to deflect from the baseline. Once the recording had stabilized, the positioning of the electrode within the tubule lumen was verified by observation of the change in voltage in response to a change in perfusion rate [6]. Subsequently, voltage measurements were made during perfusion at 10 ml/min before the electrode was removed and a second baseline, or zero measurement was made. The tubule was then filled with silicone rubber (Microfil; Flowtech Inc., Carver, MA) so that the impalement site could be confirmed later by microdissection.

At the end of the experiment, a 75 μl sample of arterial blood was taken for measurement of systemic pH; following this, a large (2 ml) blood sample was taken for measurement of plasma Na⁺ and K⁺ concentration.

Analyses

The ion-sensitive electrodes were calibrated at the start and end of each recording by immersion both in standard solutions (with pH adjusted to 6.3 and 7.3 with either 0.1 M NaOH or HCl) and in the perfusate (pH 7.5). The mean slopes per decade change in proton concentration (control = 57.6 ± 1.2 mV; low-potassium = 58.2 ± 1.8 mV; NS) were not significantly different from the calculated Nernstian linear regression slope for this ionophore, and no significant interference from other cations has been found for these electrodes [5]. H⁺ electrodes typically had an input tip resistance of 70–100 GΩ, whereas the reference barrel had a resistance between 50 and 100 MΩ.

Na⁺ and K⁺ concentrations in plasma were measured by...
flame photometry (model 543, Instrumentation Laboratory, USA). The pH and the bicarbonate concentration in arterial blood were measured by blood-gas analysis (Corning model 170, Medfield, MA).

Calculations

The distal tubular V_{te} was taken as the mean voltage deflection from the mean of the pre- and post-impalement baselines, as recorded by the reference barrel. This change in voltage is the sum of two components: the first is the true V_{te} and the second results from altered tip potentials that may arise from differences between the ionic composition of the interstitial fluid (in which baseline voltage was measured) and that of the perfusate. In order to estimate the magnitude of the second component, the electrode was moved from the surface fluid to a pool of the perfusate, and the change in voltage was measured. In both groups of rats, the change was always <0.2 mV. Late distal tubular pH was calculated from the voltage difference recorded between the reference barrel and the H^+ -sensitive barrel of the double-barrelled microelectrodes. All values are expressed in pH units, on the assumption that the proton activity coefficient was the same in the standards used for calibration as it was in the tubular fluid.

Statistics

All values are presented as means ± SE. Comparisons between groups were made using Student’s t-test for unpaired or paired samples, as appropriate. In all cases, a difference was taken as statistically significant if P < 0.05.

Results

Whole-kidney and blood/plasma data

Blood and plasma data for the two groups of animals are shown in Table 1. As expected, the animals maintained on the potassium-deficient diet were markedly hypokalaemic compared with the potassium-replete rats. In addition, potassium-depleted rats were alkalotic and had an elevated plasma bicarbonate concentration.

Transepithelial potential difference and pH

The V_{te} of the late distal tubule was measured in groups of potassium-replete and potassium-depleted rats during distal tubular perfusion with either a control solution or a solution containing BaCl_2; sample traces are shown in Figure 2. The measured late distal tubular pH and the V_{te} are shown in Table 2. In both groups of animals, tubular fluid pH was significantly lower than could be accounted for solely on the basis of Nernstian, passive distribution of protons across the epithelium, thus confirming an active component.

Table 1. Plasma data

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>P</th>
<th>Low-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_K (mmol/l)</td>
<td>4.4 ± 0.1</td>
<td>&lt;0.01</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>P_Na (mmol/l)</td>
<td>143.8 ± 1.1</td>
<td>NS</td>
<td>142.3 ± 1.6</td>
</tr>
<tr>
<td>P_HCO_3 (mmol/l)</td>
<td>29.4 ± 0.6</td>
<td>&lt;0.01</td>
<td>38.9 ± 0.9</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.38 ± 0.01</td>
<td>&lt;0.01</td>
<td>7.47 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. P_K, plasma potassium concentration; P_Na, plasma sodium concentration; P_HCO_3, plasma bicarbonate concentration; and blood pH for rats that had been maintained on either a control diet (n = 10) or a potassium-deficient diet (Low-K; n = 10). Column 'P' denotes statistical comparisons between groups; NS = not significant.

Table 2. Late distal tubular transepithelial potential difference and fluid pH

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>P</th>
<th>Low-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control perfusate</td>
<td>(n = 28)</td>
<td></td>
<td>(n = 57)</td>
</tr>
<tr>
<td>V_{te} (mV)</td>
<td>−40.7 ± 2.4</td>
<td>&lt;0.001</td>
<td>−15.0 ± 1.4</td>
</tr>
<tr>
<td>Measured pH</td>
<td>6.44 ± 0.04</td>
<td>&lt;0.001</td>
<td>6.76 ± 0.04</td>
</tr>
<tr>
<td>BaCl_2 perfusate</td>
<td>(n = 9)</td>
<td></td>
<td>(n = 12)</td>
</tr>
<tr>
<td>V_{te} (mV)</td>
<td>−49.6 ± 3.5</td>
<td>&lt;0.001</td>
<td>+9.9 ± 1.5</td>
</tr>
<tr>
<td>Measured pH</td>
<td>6.25 ± 0.06</td>
<td>&lt;0.001</td>
<td>7.25 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE; n in parentheses. Transepithelial potential difference (V_{te}) and late distal tubular fluid pH in rats maintained on either a control or potassium-deficient (Low-K) diet. Column 'P' denotes statistical comparisons between groups.
of proton secretion in the late distal tubule. Despite the fact that the \( V_{te} \) was significantly less lumen negative in the potassium-depleted rats (Table 2), the difference between measured and 'predicted' pH was markedly enhanced (Figure 3).

In control rats, addition of \( \text{BaCl}_2 \) to the perfusate caused a significant hyperpolarization of the lumen-negative \( V_{te} \) (\( P < 0.05 \)). In contrast, the late distal tubular \( V_{te} \) became lumen positive during perfusion with the solution containing \( \text{Ba}^{2+} \) in potassium-depleted animals (Figure 2 and Table 2; \( P < 0.01 \)). It should be noted that acidification of the tubular fluid was also affected by inclusion of \( \text{Ba}^{2+} \): although tubular fluid was still significantly more acidic than could be predicted on the basis of systemic pH and \( V_{te} \) alone, it was lower in potassium-replete and higher in potassium-depleted rats than values obtained during control perfusions (Table 2). The difference between measured and predicted pH, however, was still greater in the hypokalaemic rats (control = 0.31 ± 0.05 vs low-potassium = 0.41 ± 0.03; \( P < 0.05 \)).

In the final series of experiments, the nature of the lumen-positive potential difference observed in potassium-depleted rats during perfusion with barium chloride was investigated. \( V_{te} \) was recorded during perfusion of the late distal tubule with the barium-containing solution. Once a stable recording had been obtained, the carbonic anhydrase inhibitor acetazolamide was administered i.v.: in all cases, the lumen-positive \( V_{te} \) was abolished by acetazolamide (Figure 4). In a few experiments, we confirmed that these results were unaffected by the addition of amiloride (0.1 mmol/l) to the barium-containing perfusate (data not shown).

**Discussion**

In the present study, we have investigated the link between systemic potassium status and hydrogen ion secretion in the superficial distal tubule of the rat. The results show that during dietary potassium depletion, the late distal tubular \( V_{te} \) becomes less lumen negative; enhanced electrogenic proton secretion may contribute to this change.

**Late distal tubular potential difference and pH**

The data obtained in control rats are in accord with previously published values, i.e. the late distal tubule displays a large, lumen-negative potential difference. This potential difference, \( V_{te} \), is the sum of the potential differences across individual cells of a heterogeneous epithelium, but is largely determined by the balance of \( \text{Na}^+ \) and \( \text{K}^+ \) transport across the principal cell. Electrogenic proton secretion in the \( \alpha \)-intercalated cell may provide a small positive component to the overall \( V_{te} \) (Figure 1). The measured pH of the late distal tubular fluid was significantly lower than could be accounted for by passive (Nernstian) distribution, alone, and is consistent with active proton secretion.

In potassium-depleted animals, the late distal tubular \( V_{te} \) was markedly less negative than that measured in control rats (Figure 5 summarizes the data obtained from these experiments). The \( V_{te} \) is determined by the relative polarization of the apical membrane with respect to the basolateral; and thus, in the distal tubule, variable events at the apical membrane play an important role in determining the final magnitude of this difference. Therefore, the reduction in the lumen-negative \( V_{te} \) observed in potassium-depleted rats could reflect both a decrease in apical membrane permeability to \( \text{Na}^+ \) and maintenance of a high electrochemical gradient for \( \text{K}^+ \) across this membrane of the principal cell (i.e. the electrochemical gradient is not reduced by \( \text{K}^+ \) secretion as normally occurs along the distal tubule); however, enhanced electrogenic proton secretion by the \( \alpha \)-intercalated cell could also contribute. Indeed, the difference between measured and predicted pH was greater in potassium-depleted rats than in controls, suggesting that active proton secretion in the
late distal tubule is stimulated by dietary potassium depletion. This is consistent with previous studies in which dietary potassium depletion was associated with enhanced bicarbonate reabsorption in the distal tubule [3,4,7].

It is, however, difficult to reconcile enhanced proton secretion with the observation that the pH of the tubular fluid was significantly higher in hypokalaemic animals than in controls. There are two possible explanations for this paradox. The depolarization of $V_{te}$ by $H^+$ secretion reduces the electrochemical driving force for further proton secretion and is, therefore, self-limiting. Nevertheless, it should be noted that despite the reduced driving force for proton secretion, the tubular fluid was markedly more acidic than would be predicted, suggesting perhaps more effective tubular buffering of secreted $H^+$ in potassium-depleted animals. Ammoniagenesis is enhanced by dietary potassium depletion [8], and an increased ammonium concentration is present in late distal tubular fluid of hypokalaemic rats [9]. Although, the perfusate buffer concentration in the present study is identical in the two groups of animals, augmented, ‘passive’, entry of ammonia into the distal tubule from the cortical interstitium could well occur. The renal cortex consists mainly of proximal tubules in diffusion equilibrium with ammonia; the cortical interstitial free ammonium concentration, similar to that in the late proximal tubular fluid [10], would be increased in potassium depletion. Thus it is possible that potassium depletion leads to enhanced entry of ammonia and increased buffering of protons in the distal tubule. In addition, the distal convoluted tubule is a significant site of ammonia production [11], although its response to potassium depletion is unknown.

**Effect of barium on $V_{te}$ and tubular fluid pH**

In potassium-replete animals, blockade of apical $K^+$ channels with BaCl$_2$ resulted in a small but significant hyperpolarization of the late distal tubular $V_{te}$, as expected in an epithelium displaying a marked $K^+$ conductance in the apical membrane (Figures 2 and 5). The associated reduction in the pH of the tubular fluid (and an increase in the difference between predicted and measured pH) may reflect the increased electrical gradient for proton secretion.

In contrast, the late distal tubular $V_{te}$ became lumen positive during perfusion with the solution containing Ba$^{2+}$ in potassium-depleted rats (Figures 2 and 5), thus reducing the driving force for electrogenic proton secretion and resulting in a higher steady-state tubular fluid pH (Table 2). Yet, the difference between predicted and recorded pH, used as an index of active proton secretion in the present study, was still significantly greater in these animals than in controls.

**Origin of lumen-positive potential difference in late distal tubule of potassium-depleted rats**

Studies *in vitro* have shown that, when Na$^+$ or K$^+$ transport is inhibited, the CCD develops a lumen-positive $V_{te}$. It has been suggested that this may reflect either electrogenic Cl$^-$ reabsorption [12] or electrogenic proton secretion [1,2]. The role of Cl$^-$ transport is controversial: two groups have reported that removal of Cl$^-$ from the bath or lumen was without effect on the $V_{te}$ of the rabbit collecting duct [1,2]. In contrast, Hanley and associates reported that similar manoeuvres caused the positive potential difference to fall to zero in the same species [12]. The reason for such a discrepancy is not known, but it may reflect the different anions used to substitute Cl$^-$: in the experiments performed by Hanley and associates, methylsulphate (an anion that has been reported to cause cell swelling [2]) as opposed to SO$_4^{2-}$ was used in place of Cl$^-$. The abolition of a lumen-positive potential difference may relate to cellular damage, rather than to cessation of Cl$^-$ reabsorption. Nevertheless, as H$^+$-ATPase activity has an absolute requirement for Cl$^-$ [13], the results of Hanley and colleagues may also reflect a reduction in electrogenic proton secretion. Whether Cl$^-$ dependence is due to biochemical interaction with the pump itself or is an indirect result of electrical shunting is unknown. A recent study has shown that bicarbonate reabsorption in the late distal tubule of the rat was reduced by half if Cl$^-$ transport was inhibited [14], although apical chloride channels have not been reported to be present in this segment. Moreover, proton pump-mediated pH recovery in MDCK cells depends on the presence of a Cl$^-$ conductance and is attenuated in the absence of Cl$^-$ [15]. It is much more likely then that the lumen-positive potential difference reflects electrogenic proton secretion rather than Cl$^-$ transport *per se*. In the isolated CCD, both acetazolamide and the HCO$_3$/Cl transporter inhibitor SITS, agents known to inhibit acidification of the tubular fluid *in vivo* [16,17], reduced the lumen-
positive $V_{te}$ to close to zero. Similarly, in the present study, i.v. administration of acetazolamide abolished the lumen-positive potential.

The processes underlying proton secretion in the late distal tubule have now been partially defined. Active secretion by the ‘proton pump’ ($H^+\text{-ATPase}$) predominates, although there may be some additional contribution from $Na^+/H^+$ exchange and $H^+/K^+\text{-ATPase}$ [8]. Of these mechanisms, the $H^+\text{-ATPase}$ is the only transporter known to be intrinsically electrogenic [18]. Therefore, the results from the present study suggest that during dietary potassium depletion, an increase in electrogenic $H^+\text{-ATPase}$ activity in the late distal tubule of the rat may contribute to the reduction in late distal tubular $V_{te}$ that was observed in the first series of experiments.

Supporting evidence for increased $H^+\text{-ATPase}$ activity during $K^+$ depletion also comes from morphological studies in which hypokalaemia was associated with hyperplasia of the $z$-intercalated cell apical membrane and an increase in the density of electron-dense studs in this membrane [19]. These studies are thought to be the structural manifestation of $H^+\text{-ATPase}$, suggesting that potassium depletion induces the fusion of pump-laden vesicles into the apical membrane in a manner analogous to that reported during acidosis [13]. Furthermore, preliminary findings in this laboratory have suggested that potassium depletion elicits a re-distribution of $H^+\text{-ATPase}$ in the CCD of the rat, with an increase in the percentage of cells displaying immunoreactivity in the apical membrane [20].

In conclusion, the present study provides electrophysiological evidence for enhanced active proton secretion in the late distal tubule of potassium-depleted rats. The lumen-positive potential difference observed in these animals during perfusion with barium, and its subsequent collapse during acetazolamide infusion, is compatible with electrogenic $H^+\text{-ATPase}$ activity.

References


Received for publication: 11.11.98
Accepted in revised form: 5.2.99