Transepithelial potential difference in proximal tubules of anaesthetized potassium-depleted rats

Transepithelial electrochemical gradients in the proximal convoluted tubule during potassium depletion in the rat

D. G. Shirley, S. J. Walter, E. J. Folkerd, R. J. Unwin * and M. A. Bailey

*Division of Biomedical Sciences, Imperial College School of Medicine, Charing Cross Hospital, London W6 8RF and *Centre for Nephrology, University College London Medical School, London WC1E 6JJ, UK*

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1. In order to examine the electrochemical gradient for potassium reabsorption across the S_2 segment of the proximal convoluted tubule, transepithelial potential differences and transepithelial potassium concentrations were measured in anaesthetized potassium-replete and potassium-depleted rats.

2. Potassium-depleted rats were markedly hypokalaemic (plasma potassium, $1{\cdot}4 \pm 0{\cdot}1$ *vs.* $4{\cdot}1 \pm 0{\cdot}1$ mmol l^{-1} in potassium-replete rats) and had a significantly reduced muscle potassium content. In confirmation of previous reports, glomerular filtration rate was slightly reduced, while fractional reabsorption in the proximal convoluted tubule was enhanced.

3. In potassium-replete animals, the transepithelial potential difference (PD) at the late proximal convoluted tubule was $+2{\cdot}1 \pm 0{\cdot}3$ mV (lumen positive) and the tubular fluid to plasma ultrafiltrate concentration ratio for potassium (TF_K/UF_K) at the same site was $1{\cdot}03 \pm 0{\cdot}01$. In potassium-depleted rats, there was a striking reversal of the transepithelial PD (to $-4{\cdot}0 \pm 0{\cdot}4$ mV), while the TF_K/UF_K was increased to $1{\cdot}19 \pm 0{\cdot}03$.

4. The data from both potassium-replete and potassium-depleted animals are consistent with accumulating evidence that potassium reabsorption in the proximal convoluted tubule is passive in nature and depends partly on diffusion down an electrochemical gradient.

Recent evidence suggests that most, if not all, potassium reabsorption in the proximal convoluted tubule is passive (Giebisch & Wang, 1996). It is believed that a substantial amount of this reabsorption takes place by solvent drag through the paracellular route (Kibble *et al.* 1995; Wilson *et al.* 1997), but a possible second component is diffusion down an electrochemical gradient. Although the transepithelial potential difference (PD) is lumen negative in the very early (S_1) segment, in most of the proximal convoluted tubule (S_2 segment) the transepithelial PD is lumen positive, thus favouring potassium reabsorption. However, the transepithelial concentration gradient for potassium is uncertain. Although most reported values for the proximal tubular fluid to plasma concentration ratio for potassium (TF_K/P_K) are close to unity (e.g. Stanton *et al.* 1987; Walter *et al.* 1988), some early studies found a value below unity (e.g. Marsh *et al.* 1963; Malnic *et al.* 1964). It should be noted, however, that determinations of TF_K/P_K may underestimate the true concentration gradient, since there is evidence that not all plasma potassium is ultrafilterable (Shalmi *et al.* 1994). If this were taken into account, a significant electrochemical gradient favouring potassium reabsorption in the S_2 segment would be a real possibility.

The effect of potassium depletion on the proximal tubular electrochemical gradient for potassium is unknown. In this context, a recent investigation of renal lithium handling during potassium depletion may be relevant since, like potassium, filtered lithium ions are believed to be reabsorbed in the proximal tubule by passive processes (Greger, 1990). We found that in potassium-depleted rats the proximal TF_{Li}/P_{Li} was increased from its usual value of ~1·18 to 1·50 (Shirley & Walter, 1997). If proximal lithium reabsorption is indeed passive, we reasoned that an increase in the TF_{Li}/P_{Li} might indicate that the transepithelial PD is disturbed during potassium depletion. Furthermore, any such disturbance might be associated with a parallel increase in the transepithelial concentration gradient for potassium.

In order to test these hypotheses and thereby shed further light on the mechanism of proximal tubular potassium reabsorption, the present micropuncture study was performed using potassium-replete and potassium-depleted rats. The transepithelial PD was measured in late proximal tubules (S_2 segment) while, in order to assess the transepithelial concentration gradient for potassium, the tubular fluid potassium concentration was compared with

that of an ultrafiltrate of plasma obtained using an artificial dialysing membrane.

METHODS

Experimental protocol

Male Sprague–Dawley rats were maintained for 5–7 days on a potassium-deficient diet (K^+ content $< 0{\cdot}8$ mmol (kg dry wt)$^{-1}$) or a matched diet with a normal potassium content (250 mmol (kg dry wt)$^{-1}$) (Harlan Teklad, Bicester, Oxon, UK). The sodium content of both diets was 130 mmol (kg dry wt)$^{-1}$. The rats were then anaesthetized with Intraval (110 mg kg^{-1}, I.P., May & Baker, Dagenham, Essex, UK) and prepared for micropuncture using a flank incision to expose the left kidney as described previously (Walter *et al.* 1979; Shirley *et al.* 1990). The depth of anaesthesia was monitored by testing the limb withdrawal and pupillary reflexes at regular intervals; when necessary, supplementary doses of Intraval were administered I.V. All animals were infused I.V. with 0·9 % NaCl solution at 30 μl min^{-1}. Animals were used for one of two experiments: measurement of transepithelial potential difference (PD) in late proximal convoluted tubules or free-flow micropuncture assessment of proximal tubular function.

Measurement of transepithelial PD. Measurements were begun 2 h after the completion of surgery. First, a narrow-tipped micropipette (tip diameter, 2–3 μm), containing Sudan Black-coloured mineral oil, was inserted at random into a superficial proximal tubule segment. A small droplet of oil was released from this pipette, allowing the segments distal to the injection site to be visualized. Once the final superficial loop had been identified, a microelectrode (tip diameter, 1–2 μm) was inserted into the lumen in order to measure the transepithelial PD as described below. After the recording had been made, a third pipette, containing choline chloride solution (150 mmol l^{-1}), was inserted proximal to the microelectrode. This was done in order to confirm that the electrode tip was in the lumen and that there were no electrical leaks. Under these circumstances, perfusion of the proximal tubule with (sodium-free) isotonic choline chloride will result in a marked (> 25 mV) lumen-positive PD (Frömter & Gessner, 1974). Following this procedure, the tubule was filled with silicone rubber solution in order that the impalement site could later be confirmed by microdissection (Cortell, 1969).

The microelectrodes were pulled (Narishige PN3 pipette puller; Tokyo, Japan) from borosilicate glass capillaries with an internal glass filament (GC120-F10, 1·2 mm o.d. × 0·69 mm i.d.; Clark Electromedical Instruments) and filled with a solution containing sodium acetate (250 mmol l^{-1}), sodium chloride (250 mmol l^{-1}) and potassium chloride (2 mmol l^{-1}). The microelectrode was then mounted in a half-cell filled with the same solution and connected to a high-impedance electrometer (Duo 773 electrometer, World Precision Instruments), which allowed simultaneous injection of current and measurement of voltage. Thus, by passing current of known magnitude through the electrode, the resistance of the tip was determined; only electrodes with a tip resistance of less than 25 MΩ were used. By measuring tip resistance before and after impalement of the nephron, the continuity of tip integrity was assessed. On the reference side of the circuit, a silver–silver chloride electrode was immersed in potassium chloride solution (500 mmol l^{-1}) connected to a bath of sodium chloride solution (150 mmol l^{-1}) 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. The offset potential of this circuit remained constant during the experiment, indicating that individual junction potentials did not change.

Baseline or zero voltage was measured by lowering the microelectrode tip into the surface fluid. The microelectrode was then advanced into the tubule lumen, causing the measured voltage to deflect sharply from this baseline. Once the recording had stabilized, late proximal transepithelial PD was measured and the electrode was then removed from the tubule and a second baseline measurement made. Measurements were recorded using a Macintosh LC2 computer and Maclab Chart software (AD Instruments, Hastings, East Sussex, UK).

Transepithelial PD was taken as the mean deflection from the mean of the pre- and post-impalement baselines. This change in voltage is the sum of two factors: the first is the true transepithelial PD and the second results from altered tip potentials that may arise from differences between the ionic composition of the interstitial fluid (in which zero voltage was measured) and that of the perfusate. The magnitude of the latter was estimated empirically by moving the electrode from a solution designed to mimic late proximal tubular fluid into one approximating plasma, and noting the voltage change. In addition, the change in tip potential was estimated theoretically, using the Goldman constant field equation. In both situations (control and low K^+) the change in tip potential was found to be less than 0·5 mV.

A recording was accepted if: (i) the deflection from zero was stable for at least 1 min; (ii) the correct position of the electrode tip was confirmed by choline perfusion; (iii) the first and second baselines differed by less than 1 mV; (iv) the post-impalement tip resistance was identical to the pre-impalement value; and (v) the impalement site was in the final surface loop of the proximal tubule.

Free-flow micropuncture. One hour after the completion of surgery, [^{3}H]inulin (Amersham International, Aylesbury, Bucks, UK) was included in the saline infusion (60 μCi primer, 60 μCi h^{-1}). After a 1 h equilibration period, renal clearance measurements were made for 3 h, while free-flow micropuncture collections were taken from late proximal convoluted tubules (4–6 collections per rat) using procedures described previously (Walter *et al.* 1979). Puncture sites were confirmed following injection of silicone rubber solution (Cortell, 1969). Micropuncture collections were deposited under oil, their volumes measured using calibrated constriction pipettes, and triplicate samples taken for measurement of [^{3}H]inulin, sodium and potassium.

Femoral arterial pressure was monitored throughout using a Druck (Groby, Leics, UK) transducer linked to a Lectromed MX 216 pen recorder. Arterial blood samples were taken regularly for measurement of [^{3}H]inulin; an extra sample was taken at the beginning and end of the micropuncture period for assessment of acid–base status and measurement of plasma sodium and potassium concentrations. An ultrafiltrate of a terminal blood sample (~2 ml) was obtained using a Centrifree Micropartition System (Amicon, Stonehouse, Glos, UK); 1 ml of plasma yielded ~200 μl of ultrafiltrate.

At the end of each experiment, a sample of thigh muscle was taken and desiccated to constant dry weight, then digested in concentrated nitric acid for analysis of skeletal muscle potassium content. Animals were killed with an I.V. injection of sodium pentobarbital.

Analyses

[^{3}H]Inulin activities in urine, plasma and tubular fluid samples were measured by β-emission spectroscopy (model 2000 CA, Canberra Packard, Pangbourne, Berks, UK) after dispersal in Aquasol 2 scintillation cocktail (Canberra Packard). Tubular fluid sodium and potassium concentrations were measured, after appropriate dilution, by electrothermal atomic absorption (Perkin

Table 1. Furnace programmes used for measurement of sodium and potassium on the atomic absorption spectrophotometer

Element	Furnace temperature (°C)	Ramp time (s)	Hold time (s)	Internal gas flow (ml min^{-1})
Sodium	90	1	10	300
	120	20	10	300
	900	1	20	300
	20	1	15	300
	1750	0	5	0 (Read step)
	2600	1	5	300
Potassium	90	1	10	300
	120	20	10	300
	950	1	5	300
	20	1	15	300
	1550	0	8	0 (Read step)
	2400	1	5	300
Tantalum coating	90	20	10	300
	120	99	30	300
	600	5	1	300
	2400	1	3	300

Elmer atomic absorption spectrophotometer, model 3110, with HGA 600 furnace and AS 60 autosampler; Perkin Elmer, Beaconsfield, Bucks, UK), essentially as described by Shalmi *et al.* (1994) but with modifications as indicated below. The graphite tubes and platforms were coated *in situ* with saturated ammonium heptafluorotantalate (100 g l^{-1}; Aldrich; 2 × 75 μl injections into the tube, followed by 2 × 20 μl injections onto the platform) using a method based on that described by Sampson (1991). Argon was used as the purge gas. Background correction was not required. Working standards for sodium and potassium were prepared by dilution of Perkin Elmer atomic spectrometry standards using fresh 18 MΩ water (Elga UHQ water purification system, Elga Ltd, High Wycombe, Bucks, UK) in polypropylene volumetric flasks which had been washed repeatedly. Pipette tips and atomic absorption cups (Clinicon, Petworth, West Sussex, UK) were washed repeatedly with fresh 18 MΩ water and air dried before use. The furnace programmes and instrument settings are shown in Tables 1 and 2, respectively. For sodium, the within-assay and between-assay coefficients of variation at 13 μmol l^{-1} were 6·5% ($n = 10$) and 4·8% ($n = 13$), respectively. Corresponding values for potassium at 130 nmol l^{-1} were 6·6% ($n = 16$) and 5·5% ($n = 18$).

Sodium and potassium concentrations in urine, plasma and plasma ultrafiltrates, and potassium concentration of digested muscle extracts, were determined by flame photometry (model 543, Instrumentation Laboratory, Warrington, UK). Arterial pH and plasma bicarbonate concentration were measured with an ABL 500 Blood Gas System (Radiometer, Copenhagen, Denmark).

Calculations

The renal clearance of [^{3}H]inulin (used as a measure of glomerular filtration rate, GFR) was calculated by the standard formula. For measurement of tubular fluid/plasma concentration ratios of [^{3}H]inulin (TF_{In}/P_{In}) and sodium (TF_{Na}/P_{Na}), plasma values were interpolated from those measured. For measurement of tubular fluid/plasma ultrafiltrate concentration ratios of potassium (TF_K/UF_K), the ultrafiltrate value was that measured in the plasma ultrafiltrate of the terminal blood sample. (Plasma potassium did not change measurably during the experiment.) Values for plasma sodium, potassium and bicarbonate, and for arterial pH, were calculated as the mean of the two samples taken in each experiment. Similarly, for each variable derived from tubular fluid collections, a single mean value was calculated per rat. In each case, these mean values were used for subsequent statistical comparisons.

Table 2. Instrument settings used for measurement of sodium and potassium on the atomic absorption spectrophotometer

	Wavelength (nm)	Slit width (nm)	Integration (s)	Sample volume (μl)
Sodium	330·2	0·7	5	20
Potassium	769·9	0·7	8	20

Statistics

Values are expressed as means ± S.E.M. Comparisons between the two groups of rats were made using Student's unpaired t test. In the case of ratios, this was preceded by logarithmic transformation of the data. A value of $P < 0·05$ was considered to be statistically significant.

RESULTS

Tables 3 and 4 show data from the groups of rats subjected to free-flow micropuncture collections; values from the animals in which PD measurements were made were qualitatively similar (data not shown).

Table 3 confirms that the degree of potassium depletion sustained by rats on the low-K^+ diet was substantial: a reduced muscle potassium content and marked hypokalaemia were both present. However, no evidence of disturbed acid–base status was found.

As found previously when using this particular low-K^+ diet (Shirley & Walter, 1997), weight gain was well maintained on the days leading up to micropuncture. Consequently, body weights in control and potassium-depleted rats were similar. However, as seen in previous studies (Peterson, 1984; Walter *et al.* 1988), kidney weight was increased in the potassium-depleted animals, while GFR was slightly reduced (Table 4).

Values for late proximal transepithelial PD measurements from control rats ($n = 6$) and potassium-depleted animals ($n = 7$) are shown in Fig. 1. In the control group, every individual value except one was lumen positive, and the mean PD (+2·1 ± 0·3 mV, $n = 33$) was very similar to values reported in the literature (e.g. Frömter & Gessner, 1974). In marked contrast, in only three out of thirty-eight determinations in the potassium-depleted rats was the transepithelial PD lumen positive; the mean value was −4·0 ± 0·4 mV.

Figure 2 shows the data from free-flow micropuncture collections from late proximal convoluted tubules. Fractional

Table 3. Arterial pressure, muscle K^+ content, plasma electrolyte concentrations and arterial pH

	Control ($n = 9$)	K^+ depleted ($n = 9$)	P
MAP (mmHg)	102 ± 2	96 ± 3	n.s.
Muscle K^+ (mmol (kg dry wt)$^{-1}$)	407 ± 11	317 ± 12	< 0·001
Plasma Na^+ (mmol l^{-1})	140 ± 1	138 ± 1	n.s.
Plasma K^+ (mmol l^{-1})	4·1 ± 0·1	1·4 ± 0·1	< 0·001
Plasma bicarbonate (mmol l^{-1})	26·8 ± 0·5	27·7 ± 0·8	n.s.
Arterial pH	7·40 ± 0·01	7·41 ± 0·01	n.s.

Values are means ± S.E.M. n, number of rats; MAP, mean arterial pressure; n.s., not significant.

Table 4. Body weight, kidney weight and renal functional data

	Control ($n = 9$)	K^+ depleted ($n = 9$)	P
Body weight (g)	288 ± 5	284 ± 7	n.s.
Kidney weight (g)	1·00 ± 0·03	1·23 ± 0·06	< 0·01
GFR (ml min^{-1})	1·36 ± 0·08	1·16 ± 0·07	< 0·05
Na^+ excretion (nmol min^{-1})	866 ± 196	1019 ± 217	n.s.
K^+ excretion (nmol min^{-1})	1229 ± 85	12 ± 2	< 0·001

Values are means ± S.E.M. Renal data apply to the left kidney only. n, number of rats; GFR, glomerular filtration rate.

water reabsorption was increased significantly in the potassium-depleted animals, as evidenced by the rise in TF_{In}/P_{In}. As expected, in control rats the late proximal TF_{Na}/P_{Na} was close to unity. Although the mean value in potassium-depleted animals was also close to unity (1·02 ± 0·01), it was significantly higher than the corresponding control value. In both groups of rats, sodium concentrations in plasma and plasma ultrafiltrate were virtually identical. However, the concentration of potassium in plasma ultrafiltrate was, on average, lower than that in plasma ($UF_K/P_K = 0·97 \pm 0·01$ in the control group, 0·95 ± 0·02 in the potassium-depleted group). Therefore, for potassium determinations, tubular fluid concentrations were compared with those in plasma ultrafiltrate. The late proximal TF_K/UF_K in control rats (1·03 ± 0·01) was significantly greater than unity ($P < 0·05$, 95% confidence limits); in the potassium-depleted group, TF_K/UF_K was raised considerably (to 1·19 ± 0·03). Thus, fractional reabsorption of potassium in the proximal convoluted tubule of potassium-depleted rats was only modestly elevated, values in control and potassium-depleted groups being 0·53 ± 0·02 and 0·59 ± 0·02 ($P < 0·05$), respectively.

DISCUSSION

The potassium content of the potassium-deficient food used in the present study was low enough to ensure that after only 5–7 days on the diet the rats were severely potassium depleted, as shown by the marked hypokalaemia and the reduced muscle potassium content. Nevertheless, no disturbance of acid–base status was apparent. Although it is generally assumed that hypokalaemia induces a metabolic alkalosis (Capasso *et al.* 1986; Walter *et al.* 1988), the present results concur with those in our previous study of short-

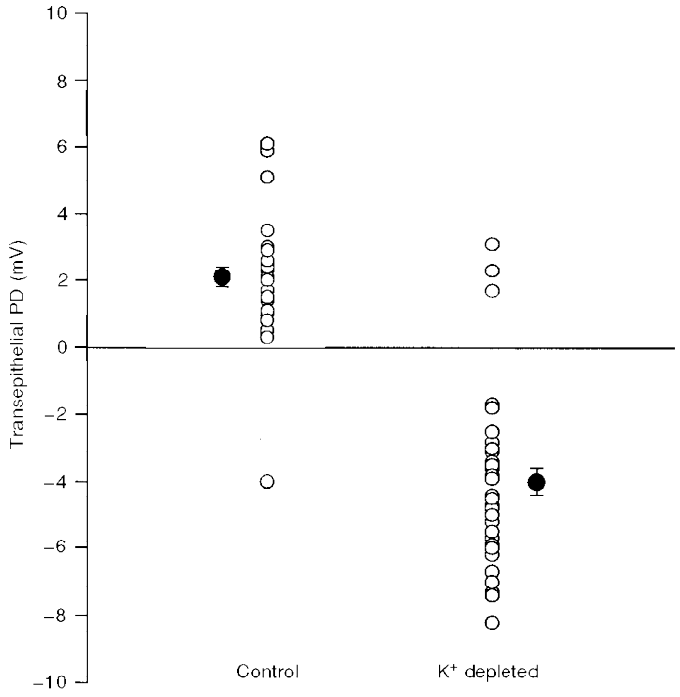

Figure 1. Transepithelial PD at the late proximal convoluted tubule

Individual values (○) and means ± S.E.M. (●) are shown. $P < 0·001$.

term potassium depletion (Shirley & Walter, 1997). It seems that, in the rat at least, a more prolonged hypokalaemia is necessary for the alkalosis to develop.

The well-documented renal effects of potassium depletion were evident in the present study: renal enlargement (Peterson, 1984; Walter *et al.* 1988), reduced GFR (Luke *et al.* 1978; Walter *et al.* 1988; Shirley *et al.* 1990) and enhanced fractional fluid reabsorption in the proximal tubule (Capasso *et al.* 1986; Walter *et al.* 1988; Shirley & Walter, 1997). Previous studies, however, have not examined the effect of potassium depletion on the transepithelial PD in the proximal tubule. Cemerikic *et al.* (1982) reported that the basolateral membrane of proximal tubular cells was hyperpolarized in potassium-depleted rats, but no information was available concerning the PD across the luminal membrane. The present finding of a reversal of the normal PD in the late proximal convoluted tubule (S_2 segment) is striking; before accepting the data, possible artifactual influences on our electrophysiological measurements should be considered.

The first point to make is that the values for late proximal transepithelial PD in control animals (~ +2 mV) were entirely consistent with previously published values (e.g. Frömter & Gessner, 1974; Seely & Chirito, 1975). Furthermore, when the Goldman constant field equation was used to provide an estimate of the liquid junction potential between electrode tip and tubular fluid, a value of no more than 0·5 mV was obtained in either setting; clearly this error factor is insufficient to account for the ~6 mV difference between the two groups of rats. Finally, it could be argued that incorrect positioning of the tip of the microelectrode might account for a negative PD in the potassium-depleted rats (i.e. if the tip were not always positioned wholly within the lumen). However, there is no obvious reason why such an artifact would apply to only one group of animals and, more importantly, perfusion of tubules with choline chloride was found to cause a marked positive PD in every case; this possibility can therefore be dismissed.

Having discounted identifiable artifacts, a pathophysiological explanation for the reversed transepithelial PD in potassium-depleted rats must be sought. The normal lumen-positive transepithelial PD in the S_2 segment is believed to be a diffusion potential arising as a consequence of Cl^- exit from the tubule down its concentration gradient, the latter having been established in the S_1 segment as a result of preferential reabsorption of bicarbonate (Seely & Chirito, 1975; Aronson

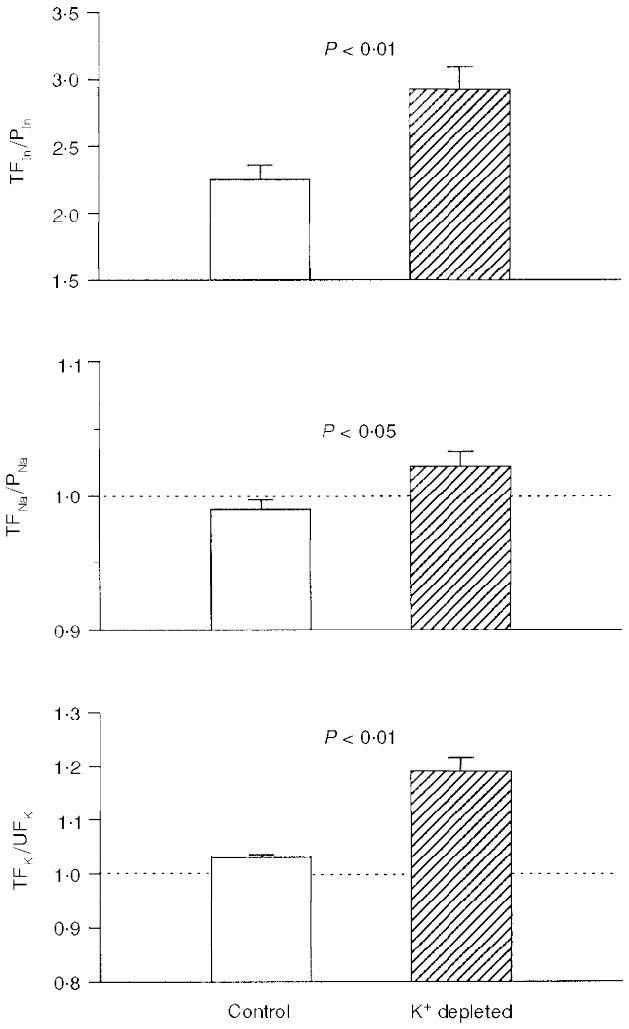

Figure 2. Tubular fluid (TF)/plasma (P) concentration ratios or tubular fluid/plasma ultrafiltrate (UF) concentration ratios at the late proximal convoluted tubule

Values (means and S.E.M.) are given for [^{3}H]inulin (TF_{In}/P_{In}), sodium (TF_{Na}/P_{Na}) and potassium (TF_K/UF_K) in control (□) and potassium-depleted rats (▨). There were nine rats in each group.

& Giebisch, 1997). It has been shown that during potassium depletion fractional chloride reabsorption in the proximal convoluted tubule is reduced (Kunau *et al.* 1968); indeed, there is evidence that net chloride *secretion* can occur (Luke *et al.* 1978). Whether such an alteration in chloride fluxes could have accounted for, or contributed to, the reversal of the transepithelial PD in the present study is unknown. An alternative possibility is that reduced insulin release as a consequence of the hypokalaemia (Weiner & Wingo, 1998) might result in hyperglycaemia and an increased intraluminal load of glucose at the S_2 segment, which in turn could generate luminal electronegativity (Giebisch & Wang, 1996). However, in separate experiments, performed under identical conditions to the present ones, we found that the plasma glucose concentration in the potassium-depleted rats ($8{\cdot}9 \pm 0{\cdot}3$ mmol l^{-1}, $n = 5$) was not significantly greater than that in the controls ($8{\cdot}6 \pm 0{\cdot}2$ mmol l^{-1}, $n = 5$) (D. G. Shirley & J. Skinner, unpublished observations). This possible explanation is therefore untenable.

Turning now to transepithelial potassium concentration gradients, the mean TF_K/UF_K in the S_2 segment of our control animals ($1{\cdot}03 \pm 0{\cdot}01$) was slightly greater than unity, which, together with the lumen-positive PD, demonstrates the existence of a finite electrochemical gradient favouring potassium reabsorption. The only other study of proximal TF_K/UF_K (Shalmi *et al.* 1994) reported a value of $1{\cdot}09 \pm 0{\cdot}05$. If the small discrepancy between the two studies is a real one, it is unlikely to have resulted from differences in tubular fluid measurements since we used essentially identical methods. Shalmi *et al.* (1994) recommended the use of TF_K/UF_K rather than TF_K/P_K because they found that a significant fraction of plasma potassium was not filterable. Although the present investigation confirmed this, we found that the extent of the apparent protein binding of potassium was variable: individual values for UF_K/P_K ranged between 0·90 and 1·00. The corresponding figures in the study of Shalmi *et al.* (1994) were not given, but it is possible that variations can be obtained as a consequence of the ultrafiltration procedure itself: it is crucial that a fixed-angle rotor, rather than a swinging bucket, is used in order to counteract build-up of retained protein at the filtering surface and thus minimize protein–protein interactions.

In the present study, the reversal of the late proximal transepithelial PD in potassium-depleted rats was associated with a significant (~16%) rise in TF_K/UF_K. The effect of potassium depletion on proximal TF_K/UF_K has not previously been investigated directly. However, examination of the data from two studies in which tubular fluid potassium concentrations were measured in potassium-depleted rats reveals small (unremarked) increases in late proximal TF_K/P_K (Malnic *et al.* 1964; Walter *et al.* 1988), which accords with the present observation. In the light of the transepithelial PD data, our findings support current views on the passive nature of proximal tubular potassium transport in that the reversal of the transepithelial PD would be expected to oppose potassium reabsorption. The present findings are also consistent with the observation in normal rats that I.V. infusion of acetazolamide, which reduces the magnitude of the lumen-positive PD in the S_2 segment (Seely & Chirito, 1975), causes a small increase in TF_K/P_K in the late proximal convoluted tubule (Kibble & Shalmi, 1995). Dependence of proximal tubular fluid potassium concentration on the prevailing transepithelial PD is also suggested by the reported increase in intraluminal potassium concentration in the lumen-negative S_1 segment of Munich–Wistar rats (Le Grimellec, 1975).

In recent years, several types of K^+-ATPase activity have been located along the nephron, but the only one identified in the proximal tubules is the type II isoform which is sensitive to both the classical K^+-ATPase inhibitor SCH28080 and ouabain (Doucet, 1997). Its function is unknown. *In vivo* microperfusion experiments using SCH28080 failed to disclose a significant effect on net potassium fluxes, but interpretation of the data was hampered by the finding that the vehicle for SCH28080, dimethyl sulphoxide, itself affected potassium transport (Wilson *et al.* 1997). The latter authors speculated that the K^+-ATPase might provide a secretory component, in view of the observation that the activity of the pump is virtually abolished during potassium depletion (Younes-Ibrahim *et al.* 1995). Some support for this interpretation may be found in the results of the present study. The net driving force favouring passive potassium reabsorption can be calculated by comparing the measured PD with the potassium equilibrium potential (E_K) determined from the Nernst equation ($E_K = -61\log_{10}[TF_K/UF_K]$). This calculation yields a driving force of 3·0 mV in control rats but only 0·6 mV in potassium-depleted rats. It could be speculated that this difference results from a secretory influence of the K^+-ATPase which occurs only in control animals. It should be emphasized, however, that the small difference in calculated driving force is within the experimental error of the respective measurements.

Finally, the increase in late proximal TF_K/UF_K was accompanied by a much smaller, but nevertheless statistically significant, increase in TF_{Na}/P_{Na} (from 0·99 to 1·02). As far as we are aware, this is the only circumstance in which such an increase has been reported. Since a significant proportion of sodium reabsorption in the S_2 segment is believed to occur by electrodiffusion (Berry & Rector, 1991), an increase in TF_{Na}/P_{Na} when the PD is reversed is perhaps not surprising. In view of the fact that sodium reabsorption also has an active component, it is also unsurprising that the increase did not match that for potassium.

In conclusion, we have shown that potassium depletion results in a change in the transepithelial PD in the late proximal convoluted tubule, from +2 mV (lumen positive) to −4 mV (lumen negative). Although the cause of this striking observation is unknown, it is associated with a moderate increase in late proximal TF_K/UF_K and a much smaller increase in TF_{Na}/P_{Na}. The results are consistent with accumulating evidence that potassium reabsorption in the proximal convoluted tubule is passive in nature.

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Corresponding author

D. G. Shirley: Department of Cellular and Integrative Biology (Renal Section), Division of Biomedical Sciences, Imperial College School of Medicine, Charing Cross Hospital, London W6 8RF, UK.

Email: d.shirley@cxwms.ac.uk