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Intracellular Potassium Activity in Mammalian Proximal Tubule: Effect of Perturbations in Transepithelial Sodium Transport

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Summary. Intracellular potassium activity (a_{K_i}) was measured in control conditions in mid-cortical rabbit proximal convoluted tubule using two methods: (i) by determination of the K^+ equilibrium potential (E_K) using Ba^{2+}-induced variations in the basolateral membrane potential (V_{BL}) during transepithelial current injections and (ii) with double-barrel K-selective microelectrodes. Using the first method, the mean V_{BL} was -48.5 ± 3.2 mV (n = 16) and the mean E_K was -78.4 ± 4.1 mV corresponding to a a_{K_i} of 68.7 mM. With K-selective microelectrodes, V_{BL} was -36.6 ± 1.1 mV (n = 19), E_K was -64.0 ± 1.1 mV and a_{K_i} averaged 40.6 ± 1.7 mM. While these last E_K and V_{BL} values are significantly lower than the corresponding values obtained with the first method ($P < 0.001$ and $P < 0.01$, respectively), the electrochemical driving force for K transport across the basolateral membrane ($\mu_K = V_{BL} - E_K$) is not significantly different for both techniques (30.1 ± 3.3 mV for the first technique and 27.6 ± 1.8 mV for ion-selective electrodes). This suggests an adequate functioning of the selective barrel but an underestimation of V_{BL} by the reference barrel of the double-barrel microelectrode. Such double-barrel microelectrodes were used to measure temporal changes in a_{K_i} and μ_K in different experimental conditions where Na reabsorption rate (J_{Na}) was reduced. a_{K_i} was shown to increase by 12.2 ± 2.7 (n = 5) and 14.1 ± 4.4 mM (n = 5), respectively, when J_{Na} was reduced by omitting in the luminal perfusate: (i) 5.5 mM glucose and 6 mM alanine and (ii) glucose, alanine, other Na-cotransported solutes and 110 mM Na. In terms of the electrochemical driving force for K exit across the basolateral membrane, μ_K, a decrease of 5.4 ± 2.0 mV ($P < 0.05$, n = 5) was measured when glucose and alanine were omitted in the luminal perfusate while μ_K remained unchanged when J_{Na} was more severely reduced (mean change = -1.7 ± 2.1 mV, NS, n = 5). In the latter case, this means that the electrochemical driving force for K efflux across the basolateral membrane has not changed while both the active influx through the Na-K pump and the passive efflux in steady state are certainly reduced. If the main pathway for K transport is through the basolateral K conductance, this implies that this conductance must have decreased in the same proportion as that of the reduction in the Na-K pump activity.

Key Words potassium · Ba^{2+} · proximal tubule · electrophysiology · ion-selective microelectrode · membrane cross-talk

Introduction

Since the development of liquid ion-exchanger microelectrodes, intracellular potassium activity (a_{K_i}) has been measured by several groups in the mammalian [3, 5–7, 15] or amphibian [16, 17, 19, 20] proximal tubule. In physiological conditions, reported a_{K_i} for proximal tubules varied from 48 to 83 mM. These values are lower than the values of a_{K_i} obtained for other cell types (e.g. nerve and muscle) and can reflect a property of proximal tubules or an underestimation coming from several possible artifacts in the application of ion-selective microelectrodes to proximal tubule cells. Due to the wide distribution of membrane potential obtained from one proximal tubule to another, accurate a_{K_i} determination requires the simultaneous measurement of V_{BL} in the same cell, and therefore the use of double-barrel microelectrodes appears mandatory. However, this type of microelectrode could present several possible artifacts. For example, these somewhat larger microelectrodes may produce leaky impalements. Alternatively, the reference channel, due to some kind of contamination from the selective channel, may respond to a certain extent to the change in ionic composition of its surrounding milieu. Because of these potential artifacts, we were interested in developing an independent method to measure a_{K_i} and assess the validity of measurements obtained using double-barrel microelectrodes.

The method developed takes advantage of the presence at the basolateral membrane of K channels showing a good selectivity for K over Na [9, 27]. The direction of the K flux through these channels can be probed by recording the instantaneous effect of a K channel blocker on V_{BL}: a depolarization would indicate the blockade of a K efflux while a hyperpolarization would represent that of a K influx. By bringing V_{BL} to different levels using transepithelial current injections it is then possible to detect the value of V_{BL} at which Ba^{2+} produces no change in V_{BL}. This value of V_{BL} corresponds to the K equilibrium potential E_K.

However, as this method requires more than 1 min to determine E_K, it is not practical for the determination of a_{K_i} as a function of time. In addi-

Table 1. Composition of perfusion and bathing solutions

	Control	Glucose and alanine-free	Glucamine
NaCl	110.0	110.0	—
$NaHCO_3$	25.0	25.0	25.0
KCl	5.0	5.0	5.0
$MgCl_2$	—	—	1.2
$MgSO_4$	1.2	1.2	—
$CaCl_2$	1.8	1.8	1.8
Na_2HPO_4	3.0	3.0	—
NaH_2PO_4	1.0	1.0	—
Na acetate	4.0	4.0	—
Na_3 citrate	1.0	1.0	—
Glucose	5.5	—	—
Alanine	6.0	—	—
Mannitol	—	11.5	11.5
n-methyl-D-glucamine-Cl	—	—	110.0
Na cyclamate	—	—	14.0

Concentrations are given in mM.

tion, in conditions where the basolateral K conductance decreases, the accuracy of the method decreases dramatically. So, in such instances, the use of double-barrel microelectrodes becomes necessary in order to follow the temporal changes in a_{K_i}. Indeed, despite a possible slight underestimation, double-barrel microelectrodes allow a continuous recording of the changes in a_{K_i} and, as will be shown, are totally adequate to determine the electrochemical driving force μ_K ($V_{BL} - E_K$) acting upon K across the basolateral membrane. This will be illustrated by measuring a_{K_i} and μ_K during inhibition of Na reabsorption (J_{Na}). When J_{Na} is partially inhibited, the activity of the Na-K-ATPase is reduced and, in steady state, the K efflux through K channels is likely to be reduced in a similar proportion. A reduction in K efflux can be achieved by a reduction in μ_K or by a reduction in the basolateral membrane K conductance. A determination of μ_K is then crucial in verifying if the proximal tubule cell can down regulate its K conductance in parallel with the activity of the Na-K-ATPase [10, 12, 13, 25, 26, 30].

Materials and Methods

Luminal Perfusion

Proximal convoluted tubules were dissected from the mid-cortical region of New Zealand white female rabbit kidney at room temperature in bath containing an artificial control solution without albumin (*see* control solution, Table 1). The tubules were transferred to the perfusion chamber and perfused according to the method described previously [4, 21]. The perfusion system was equipped with a continuously flowing exchange pipette allowing a rapid exchange of the perfusate solution (5 sec) without changing the perfusion pressure or perfusion rate. The tubules were perfused at a rate greater than 100 nl min^{-1} to insure that the backflux of solutes from the peritubular solution to the lumen would not importantly influence the composition of the luminal fluid.

Bath Perfusion

Efficient peritubular perfusion and solution exchange was accomplished as described previously [21]. Briefly, the tubule was positioned near the end of two concentric glass tubes in contact with an aluminum plate thermostatically controlled by an electronic device. Each tube was connected through magnetic valves to a pressurized reservoir and a stream of solution was aimed directly at the tubule at a rate of 1 to 2 ml/min. The solution exchange time at the surface of a tubule was estimated from the time required to reach 90% of the final V_{BL} change in response to a step in K concentration from 5 to 15 mM.This time averaged 160 msec and was always less than 200 msec.

The composition of the perfusate and bathing solutions used in this study is given in Table 1. In the experiments where Ba^{2+} was used, in order to avoid precipitate formation, phosphates were replaced by cyclamate while sulfate was replaced by chloride in every bath solution used in this series. The pH of all solutions was adjusted to 7.40 after bubbling with a 95% O_2 and 5% CO_2 gas mixture. The osmolality was adjusted to 300 mOsm/kg H_2O.

Electrical Circuits

For basolateral potential measurements using single-barrel glass microelectrodes, the latter were connected to the input of a high input impedance electrometer (W.P. Instruments, model KS-700, New-Haven, CT) through a Ag/AgCl half-cell microelectrode holder filled with 1 M KCl. The circuit from bath to ground was completed with a 0.154 M NaCl agar bridge that was connected to a Ag/AgCl half-cell microelectrode holder filled with 1 M KCl. The output of the amplifier was displayed on a multichannel strip chart recorder (Brown Bovery Cervogor, model 460).

For the measurement of the basolateral membrane K equilibrium potential, depolarizing and hyperpolarizing current pulses of a few seconds duration supplied by a pulse generator (Physiologic Instruments, model VCC 600, San Diego, CA) were applied to the collection side of the tubule through a small inner pipette advanced in the lumen of the tubule. The latter was filled with 0.154 M NaCl and connected to a pulse generator by a fine chlorided silver wire. The current injection circuit was completed by a Ag/AgCl pellet electrode (In Vivo Metric, Healdsburg, CA) placed in the bath.

For measurements with the K-selective electrodes, the selective and the reference barrels were connected to the inputs of a high impedance Dual differential amplifier (FD223, W.P. Instruments, New Haven, CT) through Ag/AgCl half-cell microelectrode holders filled with 1 M KCl and 1 M NaCl, respectively. The circuit from bath to ground was the same as for measurements with single-barrel microelectrodes above. The output from each channel of the amplifier and the differential output were displayed on a multichannel strip chart recorder (Brown Bovery Cervogor, model 460).

Determination of E_K

The intracellular potassium activity can be evaluated by determining the basolateral membrane potassium equilibrium potential E_K. Since the K conductance of the basolateral membrane can be blocked by Ba^{2+} [3], we need only to determine the basolateral membrane potential at which Ba^{2+} no longer has any effect on V_{BL} or reverses its effect. Therefore, current pulses injected in the tubule lumen, as described above, were used to depolarize or hyperpolarize the basolateral membrane, and Ba^{2+} applications (1 mM) of 2–3 sec duration were directed at the tubule. V_{BL} was measured by impaling the tubule near the collection side (100 μm). The changes in V_{BL} produced by these Ba^{2+} pulses and measured 200 msec after the beginning of the change in V_{BL} were determined as a function of the initial V_{BL}. The potential at which these changes in V_{BL} induced by Ba^{2+} reverse direction gives directly the K equilibrium potential.

Results are given as mean ± standard error for individual measurement of V_{BL} and a_{K_i} and n is the number of impalements. A paired t test was used to obtain the statistical significance of difference between control and experimental data.

Microelectrodes

Single-barrel microelectrodes were pulled from 1 mm o.d. capillary glass tubing containing a fiber (Frederick Haer, Brunswick, ME). The electrodes were pulled on a Narishige horizontal puller (model PD-5, Narishige, Tokyo) and filled with 1 M KCl solution. Their resistance was close to 120 MΩ when dipped in control perfusate. The tip potential measured after breaking the tip of the microelectrode was usually less than 6 mV. Double-barrel potassium-selective microelectrodes were constructed from the same glass tubing following a modified version of the method of Biagi et al. [3] and Teulon and Anagnostopoulos [31]. Two 10-cm-long pieces of this glass tubing were placed side by side, the extremities off by 1 cm, twisted and pulled on a Narishige horizontal puller. The shorter stem was then smoothly bent away to facilitate filling and insure insulated electrical connections and was used as the reference barrel. The other (straight) barrel, which had a length of approximately 6 cm, was used as the selective barrel. In preliminary experiments, such microelectrodes (one barrel filled with 1 M NaCl and the other with 1 M KCl solution, without liquid ion exchanger or silanization) were tested to evaluate their ability to impale proximal tubule cells without damage to the basolateral membrane. Stable measurements of the basolateral potential (30 min) were obtained with electrodes having resistance in the order of 80–120 MΩ (mean = 90 MΩ, n = 10) when filled and immersed in 1 M KCl solution. Both barrels gave nearly identical values of basolateral membrane potential and the variations in basolateral membrane potential after omission of glucose and alanine were identical. The tip potential was less than 6 mV in control perfusate and its variation was less than 3 mV in solutions of different KCl and NaCl concentrations having the same ionic strength (150 mM). Double-barrel ion-selective microelectrodes were therefore constructed with geometrical characteristics identical to the ones used in these preliminary experiments. The selective barrel was silanized by vaporization of dichlorodimethylsilane. A small Teflon chamber of 35 cm^3 with a tight cover was constructed and 0.5 to 1 cm of the capillary of the selective barrel was introduced in this chamber through the cover of the chamber by means of a "swedge lock" fitting. The chamber was heated to 40°C and 4 or 5 drops of dichlorodimethylsilane (J.T. Baker, Phillipsburg, NJ) were introduced in the chamber. Silanization by vaporization of dichlorodimethylsilane was conducted for 5 min. Following this procedure, the electrodes were transferred to an oven and baked for 30 min at 100°C. The potassium-selective liquid ion exchanger was then introduced in the tip through a fine glass capillary (0.17 mm o.d.) up to the shoulder of the silanized barrel and allowed to migrate to the tip of the electrode. The back of this barrel was then filled with 1 M KCl solution, while the reference barrel was filled with 1 M NaCl solution. The mean resistance of the reference barrel filled with 1 M NaCl was 89 ± 3 MΩ while the resistance of the selective barrel was 24 ± 1.6 GΩ when the microelectrode was dipped in 1 M KCl solution (n = 50).

Table 2. Values of liquid junction potentials measured with Ag/AgCl electrodes at 39°C

Liquid junctions		mV
1 M KCl	1 M KCl	0
	0.1 M KCl	−0.95
	0.01 M KCl	−0.73
	0.005 M KCl	+0.55
1 M KCl	1 M NaCl	+6.51
	0.1 M NaCl	+0.35
	0.01 M NaCl	+0.01
1 M KCl	0.005 M KCl/0.145 M NaCl	+1.13
	0.010 M KCl/0.140 M NaCl	+1.13
	0.020 M KCl/0.130 M NaCl	+1.13
	0.100 M KCl/0.050 M NaCl	−0.11
1 M KCl	Control solution	+1.10

Electrode Calibration

Calibration of the double-barrel K-selective electrodes as a function of potassium activity was conducted at 39°C with an independent circuit in small thermostatically controlled 10-ml glass beakers containing the different test solutions before and after impalement.

Electrical connections to the two barrels of the microelectrode were made as above. For completion of the circuit from the test solutions to ground, an agar bridge filled with the test solution was dipped in a common 1 M KCl beaker and the latter was connected through a 1 M KCl agar bridge to a Ag/AgCl half-cell microelectrode holder filled with 1 M KCl. The reference value for the measured potential was taken with the microelectrode replaced with a 1 M KCl agar bridge dipped in a 1 M KCl solution.

In order to obtain the accurate value of the microelectrode potential, the measured potential had to be corrected for the liquid junction potential between the tested solution and the 1 M KCl solution that makes connection to the ground Ag/AgCl half-cell. These liquid junction potentials were measured for every solution with Ag/AgCl electrodes by the method described by Laprade and Cardinal [24] and are reported in Table 2.

Ion-selective microelectrodes were calibrated in pure KCl solutions of 1, 0.1, 0.01 and 0.005 M concentrations. Electrodes were also calibrated in mixed solutions of NaCl and KCl using the following salt proportions: 100 K/50 Na; 20 K/130 Na; 10 K/140 Na; 5 K/145 Na.

The slope constant α_K was determined for each electrode in pure KCl solutions [8] and the mean selectivity coefficient of

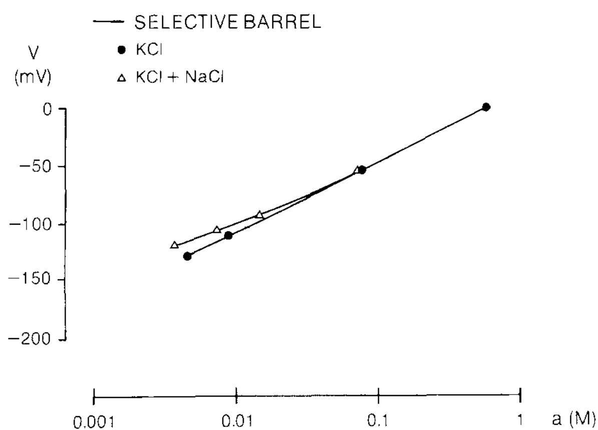

Fig. 1. Calibration curve for the K-selective channel of a typical K-selective double-barrel microelectrode in pure KCl, and mixed KCl and NaCl solutions

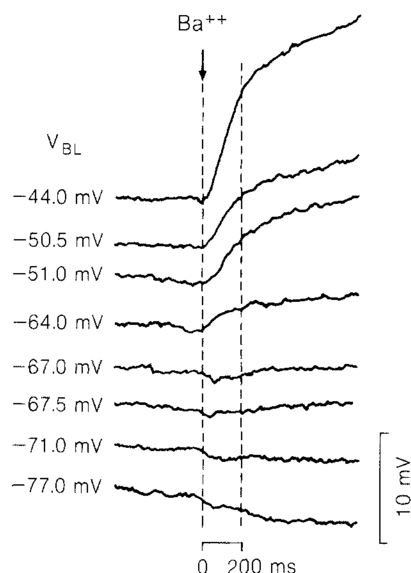

Fig. 2. Recordings of the changes in V_{BL} (ΔV_{BL}) induced by Ba^{2+} pulses (1 mM) at various initial values of V_{BL}. Depolarizations and hyperpolarizations of V_{BL} were produced by injecting constant current pulses in the tubule lumen. For this particular tubule, the control value of V_{BL} was −51 mV

sodium over potassium, K_{K-Na}, was obtained from the best fit of the following equation

$$V_K = \alpha_K \log \frac{a_{K_o} + K_{K-Na}\, a_{Na_o}}{a_{K_i}} \tag{1}$$

where V_K is the potential of the selective barrel in the mixed solution with respect to that in the 1 M KCl solution, a_{K_i} is the potassium activity in the 1 M KCl solution. a_{K_o} and a_{Na_o} are the K^+ and Na^+ activities in the test solutions.

The activity coefficient was determined from the tabulated values of $\gamma\pm$ from Robinson and Stokes [28] assuming that $\gamma+ = \gamma\pm$ and that γ_K and γ_{Na} are constant in isotonic mixtures of KCl and NaCl solutions and equal to the γ_K of pure 0.15 M KCl.

A representative calibration curve is presented in Fig. 1. The mean slope for the selective barrel in pure KCl solutions was 61.1 ± 0.1 mV per 10-fold change in a_K at 39°C ($r > 0.999$) and the selectivity coefficient K_{K-Na} determined in mixtures of KCl and NaCl was 0.0255 ± 0.0058 ($n = 50$). Considering this selectivity of our electrodes for potassium over sodium and assuming the intracellular concentration of sodium is in the order of 15–25 mM [32], no correction for the intracellular sodium activity was considered necessary. On the other hand, and in contrast with the double-barrel electrodes used in the preliminary impaling tests (no silanization and no ion exchanger), the tip potential of the reference channel was more sensitive to changes in ionic composition and was 7.8 ± 0.7 mV ($n = 50$) more positive in the 100 K/50 Na solution as compared to the 5 K/145 Na solution.

Measurement of Intracellular Potassium Activity

Proximal convoluted tubular cells were impaled using the method previously described by our group [4, 22, 23]. Punctures were in general executed near the holding pipette of the perfusion side at a 45° angle. With the procedure we have used, the intracellular K activity is read directly from the calibration curve of the selective barrel using the difference between the potential measured by the potassium-selective electrode and that of the reference electrode ($V_K - V_{BL}$). The value of V_{BI} corresponds to the difference between the potential measured by the reference electrode when impaled in the cell and that when it is immersed in the control bath solution. The value of V_K corresponds to the potential measured in the cell by the K^+-selective electrode, using for the reference the potential obtained in the control bath solution at 39°C when the ion-selective microelectrode is replaced in the circuit by a 1-M KCl bridge. This potential has to be corrected for the liquid junction potential between the bath and the 1M KCl bridge (*see* Table 2).

Results

a_{K_i} in Control Conditions

From K *Equilibrium Potential*

In this series of experiments, hyperpolarizations and depolarizations of V_{BL} were produced by basolateral application of Ba^{2+} (1 mM) during transepithelial current injection. The average value obtained for V_{BL} in control conditions is −48.5 ± 3.2 mV (n = 16). Figure 2 represents a typical experiment and shows, for various initial basolateral membrane potentials, the variations as a function of time in V_{BL}, produced by Ba^{2+} pulses directed at the tubule. As can be best seen at V_{BL} values of −44 and −50.5 mV, the depolarization induced by Ba^{2+} occurs in two phases clearly defined by a sharp bend in the V_{BL} *vs.* time curves. The first phase is as rapid as the peritubular solution exchange, while the second

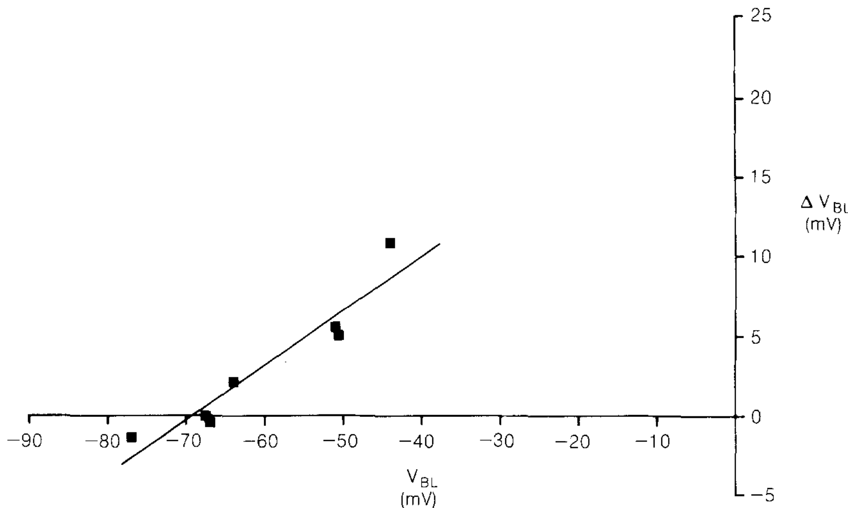

Fig. 3. Plot of the ΔV_{BL} values at 200 msec induced by 1 mM Ba^{2+} as a function of the initial V_{BL} value (from Fig. 2)

phase has a much slower time constant (3 sec). The fast time constant is most probably associated with the blockade of the membrane K conductance, while the slower one was recently shown to be inhibitable with acetazolamide and involve probably HCO_3^- efflux through the Na-$[HCO_3]_3$ cotransporter present at the basolateral membrane [2]. It can be seen that as V_{BL} hyperpolarizes, the ΔV_{BL} produced by Ba^{2+} which are positive (depolarization) decrease, reach a value close to zero at −67 mV and even become negative at −71 mV. A plot of the ΔV_{BL} values obtained 200 msec after the beginning of the change in V_{BL} as a function of initial V_{BL} is shown in Fig. 3. The choice for this value in time is motivated by the fact that it corresponds to the point of transition between the two phases of V_{BL} variation. We can observe a fairly linear relationship between V_{BL} and ΔV_{BL} with an intercept on the abscissae at −69.1 mV which corresponds to the K equilibrium potential E_K for this cell. The average value of E_K for 16 experiments was found to be −78.4 ± 4.1 mV, which gives, with a bath K activity of 3.7 mM, a value for a_{K_i} of 68.7 mM and a driving force on K ($\mu_K = V_{BL} - E_K$) of +30.1 ± 3.3 mV.

On the other hand, the fairly linear relationship observed between ΔV_{BL} induced by Ba^{2+} and initial V_{BL} is expected if the various membrane ionic conductances (both apical and basolateral) are voltage independent since the slope (m) of such a graph corresponds to $G_K/(G_o + G_A)$ (assuming that the K conductance is completely blocked), where G_K is the basolateral membrane potassium conductance, G_o the sum of the basolateral conductances other than that of K^+ and G_A, the apical membrane conductance. From this slope it is possible to obtain the apparent ratio between the basolateral membrane K conductance and the total cell membrane conductance (i.e., the K partial conductance $t_K : t_K = G_K/G_{cell} = G_K/(G_A + G_o + G_K) = m/(1 + m)$. The average value of m that was obtained in such experiments is 0.25 ± 0.04 (n = 13) from which we can calculate a value of 0.20 for t_K. Interestingly, this value is quite close to that obtained recently by Lapointe et al. [21] who found, using K steps from 5 to 15 mM, a value of t_K between 0.13 and 0.16 in similar control conditions.

Experiments aimed at measuring the K equilibrium potential and therefore a_{K_i} with Ba^{2+} pulses in conditions where transepithelial Na transport was inhibited were also conducted. Unfortunately, the uncertainty in the determination of E_K was much too large to give reliable values. Indeed, the ΔV_{BL} induced by Ba^{2+} in these conditions were much smaller so that the slope of ΔV_{BL} as a function of V_{BL} was considerably reduced most likely due to the secondary reduction in the K partial conductance (*see* Discussion). This precludes any precise determination of E_K since a slight imprecision in the values of ΔV_{BL} and consequently in the slope of the curve which was less than 0.1 would generate an important imprecision in the determination of E_K.

From K-*Selective Electrodes*

Figure 4 (control period) represents a typical experiment showing the simultaneous measurement of V_{BL} and V_K after cell penetration. V_{BL} shows an abrupt negative deflection following cell puncture and a stable value in the control period. V_K in all experiments shows a rapid positive deflection and remains stable in the control period. The following criteria were used to accept successful impalements: an abrupt deflection in V_{BL} and V_K after cell im-

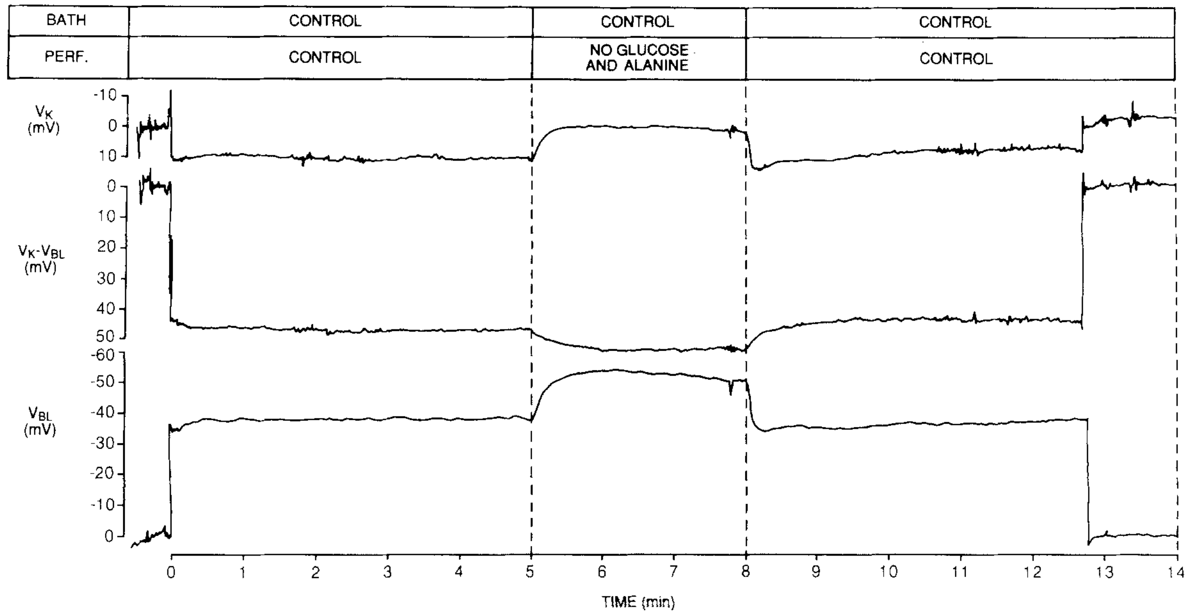

Fig. 4. Typical recording of the effect of glucose and alanine-free luminal solution on V_{BL} and a_{K_i} (as monitored by $V_K - V_{BL}$)

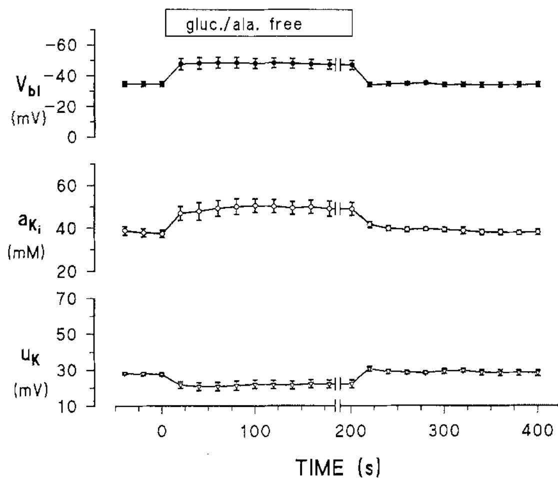

Fig. 5. Same as Fig. 4 but average of five tubules. Vertical bars represent SEM

palement; stability of these two parameters for a 5-min control period; a rapid return of V_{BL} and V_K to their baseline value upon withdrawal of the microelectrode from the cell; a hyperpolarization of the basolateral potential after removal of glucose and alanine from the lumen; an unaltered response of the selective microelectrode after experimentation on the tubule, i.e., a mean slope of more than 59 mV per 10-fold change in a_K at 39°C. The mean basolateral potential difference measured with the reference barrel was -36.6 ± 1.1 mV, and the mean intracellular potassium activity was 40.6 ± 1.7 mM ($n = 19$). The equilibrium potential E_K calculated from this intracellular activity and a bath potassium activity of 3.7 mM gives a value of -64.0 ± 1.1 mV ($n = 19$). μ_K, the electrochemical potential difference for potassium which can be calculated from ($V_{BL} - E_K$) gives $+27.6 \pm 1.8$ mV and favors the exit of potassium from the cell.

Effect of Inhibition of J_{Na} on a_{K_i}

As seen in Figs. 4 and 5, when glucose and alanine are replaced in the tubule lumen by mannitol, V_{BL} hyperpolarizes rapidly (30 sec) and remains fairly constant over the experimental period. This behavior is very similar to what we have previously reported in these conditions [4, 22]. After 3 min (Fig. 5), the average V_{BL} hyperpolarization is 12.6 ± 1.7 mV ($n = 5$). On the other hand, the intracellular potassium activity increases rapidly in the first minute and then plateaus to a new steady-state value. After 3 min, the mean increase in a_{K_i} is 12.2 ± 2.7 mM ($n = 5$). The increase in intracellular potassium activity after luminal glucose and alanine substitution was present in all the experiments and was therefore highly significant ($P < 0.001$). Readdition of glucose and alanine to the luminal solution in the post-control period produces a fast depolarization of the basolateral membrane potential accompanied by a decrease in intracellular potassium activity with a return to control values. The Nernst equilibrium potential E_K for potassium after 3 min of omission of glucose and alanine in the lumen is more negative

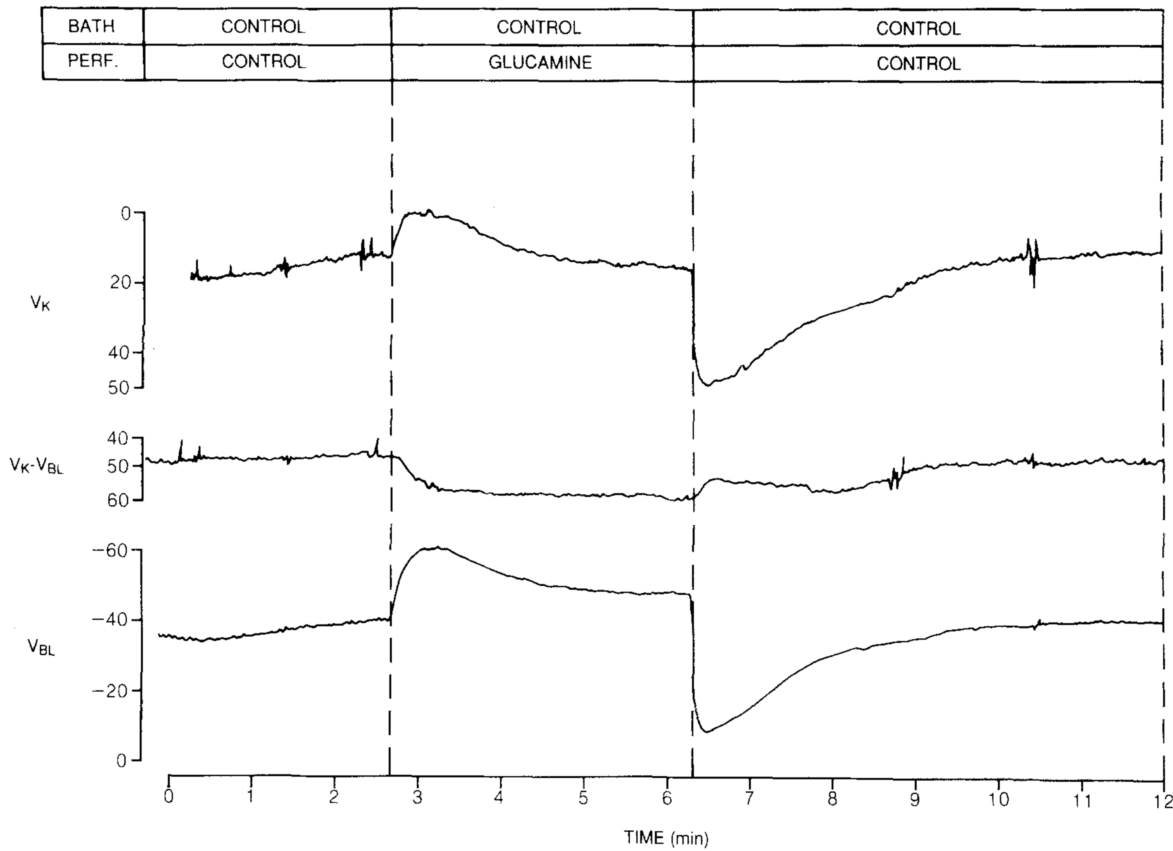

Fig. 6. Typical recording of the effect of removal of glucose, alanine, 110 mM Na and Na-cotransported anions (glucamine solution, *see* Table 1) on V_{BL} and a_{K_i} as monitored by $(V_K - V_{BL})$

by 7.2 ± 1.3 mV as compared to its value in the control period. However, this increase in the absolute value of E_K is less than the simultaneous hyperpolarization of V_{BL} (12.6 mV) and results in a reduction of 5.4 ± 2.0 mV in the driving force for K^+ exit (μ_K) (Fig. 5).

The aim of the following experiments was to further reduce the transtubular transport of sodium by replacing, in addition to glucose and alanine from the lumen, all anions cotransported with sodium by cyclamate and by reducing the luminal concentration of sodium from 150 to 40 mM. This solution was called the glucamine solution (Table 1). Sodium was replaced by *n*-methyl-D-glucamine in these experiments and not by choline as previously done [4] because we observed in preliminary experiments that choline can enter the cell and interfere severely with the K-selective electrodes. There was no influence of glucamine on the behavior of the K electrodes in vitro.

As shown in Figs. 6 and 7, when the control solution was replaced by the glucamine solution, V_{BL} hyperpolarized rapidly by 21.4 ± 1.6 mV (n = 5). This hyperpolarization is comparable to the one

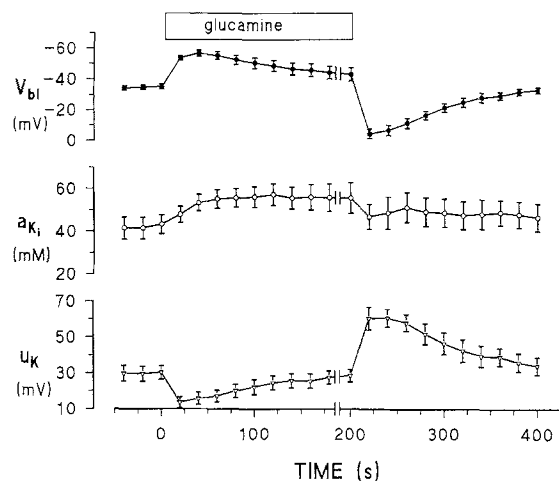

Fig. 7. Same as Fig. 6 but average of five tubules. Vertical bars represent SEM

reported by our group using a similar solution containing choline as the Na substituting ion (ΔV_{BL} = −20.5 mV) [23]. The cell hyperpolarization was transient with the basolateral potential coming back

close to the control value in approximately 4 min. Returning to the control solution produced a dramatic depolarization of the cell with the basolateral potential reaching on average a very low value of -4.6 ± 3.1 mV ($n = 5$). However, this depolarization was also transient, and the basolateral potential returned to the control value in less than 5 min.

Figure 6 and 7 show that the glucamine solution produced an increase in the intracellular activity of potassium. This increase was rapid (10.6 ± 1.6 mM ($n = 5$) after 40 sec) and reached a stable value of 14.1 ± 4.4 mM ($n = 5$) after 200 sec. When returning to the control solution, the intracellular cell potassium activity gradually returned to the control value.

As can be seen in Fig. 7, at the end of the experimental period, μ_K is not different from the value obtained in the control period (mean change of -1.7 ± 2.1 mV ($n = 5$), NS), which suggests that the increase in the intracellular potassium activity, with the glucamine solution, cannot be due to a reduction in the driving force for K^+ exit.

Discussion

a_{K_i} in Control Conditions

In control conditions, the mean value of a_{K_i} obtained from E_K determinations with single microelectrodes is 68.7 ± 3.5 mM. This value is significantly higher ($P < 0.0001$) than the one we have obtained with ion-selective microelectrodes (40.6 ± 1.7 mM). On the other hand, this value of a_{K_i} obtained with double-barrel ion-selective microelectrodes is only slightly lower than the one obtained with similar microelectrodes by Biagi et al. (48.6 ± 2.27 mM) [3] and by Sasaki (59.9 ± 2.0 mM) [29] for pars recta. However, as V_{BL} values obtained in pars recta are consistently more negative than the one obtained in proximal convoluted tubules [3, 18], a higher a_{K_i} can be expected in pars recta. Nevertheless, our results point to a possible significant underestimation of a_{K_i} using double-barrel ion-selective microelectrodes.

Among the possible factors that may lead to an underestimation of a_{K_i} with double-barrel microelectrodes, the first is the presence of a larger leak around a double-barrel electrode than around a conventional microelectrode. This would lead to an underestimation of V_{BL}, a decrease in the transmembrane K gradient and quite likely to a reduction in the electrochemical gradient. As a matter of fact, the average V_{BL} reported in this paper is less negative for double-barrel than for single-barrel microelectrodes (-36.6 ± 1.1 mV *vs.* -48.5 ± 3.2 mV). In our previous studies, in control conditions with single-barrel microelectrodes, V_{BL} was -42.5 ± 3.2 mV [21] and -42.4 ± 1.4 mV [23]; although, to a lesser extent, these values are still higher than the ones obtained here with double-barrel microelectrodes. This phenomenon was also observed by Biagi et al. [3] who found a difference of 7 mV, V_{BL} obtained with double-barrel ion-selective microelectrodes giving an average of -37.8 ± 3.5 mV, while V_{BL} with single-barrel electrodes gave -43.6 ± 3.2 mV. The presence of a leaky impalement is, however, challenged by the fact that the initial hyperpolarization after omission of glucose and alanine from the lumen was identical with the one previously reported by our group [4] using conventional microelectrodes (14.1 from ref. 4 *vs.* 14.0 in the present study). Indeed, another possibility for the underestimation of V_{BL} and consequently a_{K_i} is the nonindifferent behavior of the reference channel due to the use of NaCl as the filling electrolyte and/or by the partial contamination of the reference channel by the silanization procedure and eventually by the ion-selective resin. Indeed, as mentioned in Materials and Methods, contrary to the double-barrel microelectrodes without treatment (neither silanization nor resin) that showed less than $+3$ mV change in tip potential from a solution containing 5 mM K/145 mM Na to a solution containing 100 mM K/50 mM Na, which likely mimics the change in ionic composition seen by the electrode upon impalement, the reference channel of the ion-selective microelectrodes showed an average change of 7.8 ± 0.7 mV ($n = 50$) in the positive direction for the same solutions. This effect is partially due both to the silanization procedure and to the resin since the mere silanization of the channel that normally contains the resin induced in the reference channel a corresponding change in tip potential of $+5.3 \pm 0.5$ mV ($n = 12$). Therefore, if we correct our V_{BL} values for this estimated change in tip potential of the reference electrode upon impalement, we would obtain a value of -44.4 mV, which approaches the values of -48.5 ± 3.2 mV obtained with single-barrel microelectrodes in the present study and compares well with our values of -42.4 ± 1.4 and -42.5 ± 3.2 obtained in previous studies [21, 23]. This change in tip potential of the reference electrode leads to an underestimation of V_{BL} that would reduce a_{K_i} by approximately 35%, which would bring a_{K_i} in control condition to 55 mM, thus significantly closer to the value of 68.7 mM determined from the K equilibrium potential. However, it should be pointed out that the above two types of studies were not conducted on the same tubules so that the higher values of V_{BL} obtained with single-barrel microelectrodes in the present study might very well reflect a higher cell K activity in these experiments as will be seen below.

Nevertheless, besides these artifacts inherent to the design of a double-barrel ion-selective microelectrode, one may also consider the localization of the K measured by the two techniques used: ion-selective microelectrodes measure a_{K_i} in the vicinity of the tip of the microelectrode while from E_K determination a_{K_i} is calculated from the potassium concentration close to the inner surface of the basolateral membrane. Compartmentalization of K or variation of its activity coefficient might also contribute to differences observed using these two different techniques.

An interesting observation with both techniques is that intracellular K is maintained higher than its electrochemical equilibrium by roughly 30 mV. In the series of experiments using double-barrel microelectrodes the driving force for K efflux (μ_K) was 27.6 ± 1.8 mV (n = 19), and a similar value of 30.1 ± 3.3 mV (n = 16) was obtained with the single-barrel microelectrode method. In addition, pooling data from the two techniques, a significant correlation was observed between V_{BL} and E_K displaying a slope of 0.79 and a displacement along the E_K axis confirming the average driving force of 30 mV for K efflux (*data not shown*). So, despite an *ab initio* underestimation of a_{K_i} by double-barrel microelectrodes, this technique agrees with another completely different one on the value of the net driving force on K ions, which would indicate an adequate functioning of the selective barrel as well as negligible leakage induced by the double-barrel microelectrode. This finding would therefore strongly support the possibility discussed above that the underestimation of V_{BL} and a_{K_i} with double-barrel ion-selective microelectrodes is largely due to the change in tip potential of the reference electrode upon impalement since this correction, which affects both V_{BL} and E_K, cancels out when we calculate μ_K.

Effect of Luminal Replacement of Glucose and Alanine on a_{K_i}

A reduction of J_{Na} is expected to reduce the basolateral Na-K-ATPase activity responsible for maintaining intracellular K higher than its thermodynamical equilibrium. Removal of luminal glucose and alanine is known to inhibit roughly one third of Na reabsorption in rabbit proximal convoluted tubule [14]. Blockade of luminal entry of glucose in rabbit PCT was reported to reduce the ouabain-sensitive oxygen consumption by 23% [11]. Therefore, it seems reasonable to assume that removal of both glucose and alanine decreases the Na-K-ATPase activity by approximately one fourth.

a_{K_i} determination through the measurement of E_K as described above with conventional microelectrodes would require more than 1 min and hence cannot be used to obtain the time course of a_{K_i} after replacement of luminal glucose and alanine. In addition, this maneuver is expected to significantly decrease the ratio between the basolateral K conductance and the total cellular conductance [21]. In this situation the slope of the relationship between V_{BL} and the Ba^{2+}-induced changes in V_{BL} (Fig. 3) decreases and the determination of E_K becomes inaccurate. From the discussion above, double-barrel K-selective microelectrodes appear quite adequate to measure μ_K as a function of time and, despite a possible slight underestimation, certainly allow a good estimate of the changes in a_{K_i}. Indeed, for the observed changes in a_{K_i} and the estimated variations of the tip potential of the reference channel, the underestimation for the extreme changes in a_{K_i} would be of the order of 1 mM. In the reported experiments, we detected a time-dependent increase in a_{K_i} averaging 12.2 ± 2.7 mM, 3 min after glucose and alanine replacement in the luminal perfusate. During this time period, V_{BL} hyperpolarized by 12.0 ± 2.3 mV, which results in a 20% reduction in the electrochemical driving force for K efflux across basolateral membrane. This reduction in μ_K is somewhat smaller than the expected reduction in the Na-K-ATPase activity. If this is indeed the case, the basolateral K conductance is expected to have decreased slightly in order to lead to a steady state between Na-K-ATPase uptake and passive K efflux through the K conductance.

Effect of Glucamine Solution

If other Na-cotransported solutes (sulphate, phosphate) and 110 mM Na are replaced in addition to alanine and glucose, the Na reabsorption rate must be reduced drastically. Indeed, most of the known permeation pathways for sodium entry into the cell are then blocked or largely inhibited. The only pathway left for the sodium entry would be the Na-H antiporter since it has a low K_M (6–13 mM) for luminal Na [1]. In this situation, a_{K_i} was shown to increase by 14.1 ± 4.4 mM while V_{BL} hyperpolarized only transiently and stabilized to a value only 7.8 ± 3.6 mV more negative than the control V_{BL}. The electrochemical driving force for K efflux across the basolateral membrane was 30.4 ± 3.9 mV in control conditions and 28.6 ± 3.5 mV 200 sec after changing luminal perfusate to the glucamine solution. These two values are not significantly different, while the Na-K-ATPase activity must be reduced to a very large extent. This result is very similar to the earlier findings of Grasset et al. [10], who reported that the

electrochemical potential for K across the basolateral membrane of *Necturus* small intestine is not affected by a three- to fourfold increase in transcellular Na transport. If passive K transport across the basolateral membrane is mostly conductive, as the driving force for K efflux is unchanged, the basolateral K conductance must have been down-regulated to achieve a steady-state situation where the Na-K-ATPase mediated K influx equals the passive K efflux through the K conductance. In fact, this downregulation in K conductance is very likely to participate in the V_{BL} depolarization occurring immediately after the initial hyperpolarization due to the sudden reduction in the apical Na^+ entry. It is probably responsible also for the very depolarized value of V_{BL} reached when J_{Na} is re-established in the postexperimental period. In average, V_{BL} reached -4.6 ± 3.1 mV immediately after the entry of the control perfusate in the lumen. This low V_{BL} value is, most likely, reached because of a combination of factors, including a high basolateral resistance, a low K conductance and a high Na influx when Na and several cotransported solutes are added simultaneously [21]. Within the same framework, the increase in K activity observed after inhibition of transepithelial transport and which is maintained after a steady-state regime is established at the end of the experimental period can be explained by the hyperpolarization of the basolateral membrane, which persists all along this experimental period. Indeed, this hyperpolarization, which is induced by the blockade of luminal entry of Na through the cotransport systems, tends to slow down the exit of K from the cell and contributes to increase the intracellular K content. Another factor that may contribute to the initial increase of intracellular K is the reduction in cellular volume, which has been reported in earlier studies to persist throughout the experimental period [4, 21]. However, this decrease in cell volume, which may accelerate the increase in cellular K content initially, cannot be responsible for the higher steady-state K activity observed at the end of the experimental period, since the latter depends solely on the pump activity and the various cell ionic conductances and emf's. In the present case, the decrease in the apical cotransport emf's which causes the basolateral hyperpolarization would be at the origin of the increased intracellular K activity.

Conclusion

Using conventional microelectrodes and the properties of the basolateral membrane K channels, the intracellular K activity and the basolateral membrane potential in control conditions were found to be 68.5 mM and -48.5 mV, respectively. Comparison of these values with those obtained with double-barrel ion-selective microelectrodes would indicate an underestimation of the latter. However, consideration of the variation of the tip potential of the reference barrel of the double-barrel microelectrode with different ionic composition upon impalement corrects, in a large proportion, this underestimation. Although the absolute values obtained for a_{K_i} appears to depend on the technique used, the two techniques agree on the K electrochemical driving force, as an underestimation of V_{BL} leads to an identical underestimation of E_K with double-barrel microelectrodes. Using double-barrel microelectrodes, it was shown that a_{K_i} increases, while the driving force on K remains unchanged when J_{Na} is drastically reduced. This strongly suggests that the decrease in the fractional K conductance at the basolateral membrane that has been measured in these conditions [21] comes from a downregulation of the absolute basolateral K conductance that parallels a reduced Na-K-ATPase activity.

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