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Regulation of Basolateral Membrane Potential After Stimulation of Na^+ Transport in Proximal Tubules

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Summary. We have previously shown that stimulation of apical Na-coupled glucose and alanine transport produces a transient depolarization of basolateral membrane potential (V_{bl}) in rabbit proximal convoluted tubule (PCT, S1 segment). The present study is aimed at understanding the origin of the membrane repolarization following the initial effect of addition of luminal cotransported solutes. Luminal addition of 10–15 mM L-alanine produced a rapid and highly significant depolarization of V_{bl} (20.3 ± 1.1 mV, n = 15) which was transient and associated with an increase in the fractional K^+ conductance of the basolateral membrane (t_K) from 8 to 29% ($P < 0.01$, n = 6). Despite the significant increase in t_K, the repolarization was only slightly reduced by the presence of basolateral Ba^{2+} (2 mM, n = 6) or quinine (0.5 mM, n = 5). The repolarization was greatly reduced in the presence of 0.1 mM 4-acetamino-4′isothiocyamostilbene-2,2′-disulfonic acid (SITS) and blunted by bicarbonate-free solutions. Intracellular pH (pH_i) determined with the fluorescent dye 2′,7′-*bis*-2-carboxyethyl-5(and -6)-carboxyfluorescein (BCECF), averaged 7.39 ± 0.02 in control solution (n = 9) and increased to 7.50 ± 0.03 in the first 15 sec after the luminal application of alanine. This was followed by a significant acidification averaging 0.16 ± 0.01 pH unit in the next 3 min. In conclusion, we believe that, contrary to other leaky epithelia, rabbit PCT can regulate its basolateral membrane potential not only through an increase in K^+ conductance but also through a cellular acidification reducing the basolateral HCO_3^- exit through the electrogenic Na-3(HCO_3) cotransport mechanism.

Key Words electrophysiology · potassium conductance · intracellular pH · bicarbonate transport · Na-coupled transport

Introduction

Over the last 10 years, it has become clear that the membrane permeabilities (or ionic conductances) of different tight and leaky epithelia can change as a function of the amplitude of transepithelial transport performed by the tissue [7, 19]. For example, addition of Na^+ cotransported solutes to the apical solution stimulates the transepithelial Na^+ flux (J_{Na}) in a number of leaky epithelia. Contrary to the predictions based on a simple Koefoed-Johnsen-Ussing model for transepithelial Na^+ transport, some studies reported that intracellular Na^+ concentration ($[Na]_i$) increases only transiently while stimulation of the basolateral pumping rate is sustained [11, 18]. In parallel with this stimulation of Na-K-ATPase activity, the basolateral membrane K^+ conductance increases dramatically which brings the cell to a highly active state: increased apical Na^+ permeability, increased basolateral pumping rate and increased basolateral K^+conductance [2, 8, 12, 13]. It was shown in several epithelia that these changes in membrane properties generate a repolarization of the basolateral membrane potential. Indeed, for a variety of epithelia, the initial depolarization associated with a sudden increase in the apical Na-cotransport rate is followed by a significant recovery in approximately 4–10 min [2, 3, 6, 8, 12, 13, 24]. The origin of this secondary repolarization is considered to be the increase in the basolateral membrane K^+ conductance because the repolarization is completely sensitive to basolateral application of barium for some tissues [12, 16]. The present study is aimed at understanding the origin of the membrane repolarization following luminal addition of Na-cotransported solutes in rabbit PCT perfused in vitro.

Materials and Methods

Tubule Perfusion

PCT were dissected from the midcortex of New Zealand white rabbit kidneys at room temperature and were identified as S1 segments by the presence of the glomerulus at less than 1 mm from the segment used. Tubules were cannulated and perfused at a high flow rate (greater than 100 nl/min) as described previously [13] with a system allowing the exchange of luminal solution in 0.5 sec and the exchange of peritubular solutions in 0.15 sec. The control solution (S_c) had the following composition (in mM): NaCl 102, KCl 5, $NaHCO_3$25, $Na_2HPO_4$3, NaH_2PO_4 1, $MgSO_4$ 1, $CaCl_2$

1.5, *n*-methyl-D-glucamine (-HCl) 10, Na-lactate 1, Na-pyruvate 0.1, glutamine 0.5, mannitol 17. Alanine was added to this solution in replacement for mannitol. After bubbling with 5% CO_2 95% O_2, pH was adjusted to 7.4 with small quantities of HCl or NaOH, and the osmolality was increased to 300 mOsm/kg by adding a small amount of mannitol. In most of the experiments, the S_c solution was used as the bathing solution and a 1–2 ml/min perfusion rate at 39°C was achieve. In experiments where peritubular K^+ concentration was increased from 5 to 15 mM, 10 mM KCl replaced *n*-methyl-D-glucamine (HCl) contained in S_c solution. When the effect of basolateral Ba^{2+} was tested, the bathing solution was replaced throughout the experiment by a solution where all the phosphate and sulfate were replaced by chloride.

Electrophysiology

Basolateral membrane potential (V_{bl}) was measured with single-barrel microelectrodes as described in a previous paper [13]. Measurements of transepithelial potential (V_t) were performed with a voltage-current-clamp system (Physiologic Instruments, model VCC 600, San Diego, CA) using a single-barrelled perfusion pipette as a bridge in contact with the tubule lumen. V_t and V_{bl} measurements were displayed on a multichannel strip-chart recorder, converted to video signals (Neuro-Data, model DR-384, New York, NY) and stored on video tapes. Recorded signals were later digitized and analyzed on an IBM AT microcomputer using commercial data acquisition software (Computerscope, R.C. Electronics, Santa Barbara, CA).

Apparent ratio of basolateral K^+ conductance to total membrane conductance (or transport number for K^+, t_K) was assessed by calculating the ratio of the observed change in V_{bl} occurring when the bath K^+ concentration was changed from 5 to 15 mM (*n*-methyl-D-glucamine replacement) and the calculated change in the Nernst potential for K^+. This calculation assumes that the conductance of the paracellular pathway is at least one order of magnitude higher than the conductance of apical membrane [14] and that the change in bath K^+ concentration does not modify K^+ conductance significantly.

Fluorescence Measurements

Intracellular pH (pH_i) was measured with the fluorescent dye 2',7'-*bis*-2-carboxyethyl-5(and-6)-carboxyfluorescein (BCECF) using dual-excitation wavelength fluorescence microscopy (Spex CM III). Microdissected proximal convoluted tubules were transferred in a bath attached to the stage of an inverted microscope (Zeiss IM 35) equipped for fluorescence measurements. The tubule was cannulated and perfused with the S_c solution and the acetoxymethyl form of the fluorescent probe (BCECF-AM) was added to the nonperfusing bath solution (final concentration 6 μM). Temperature was raised to 39°C, and the fluorescence at a wavelength of 530 nm was measured while the excitation was alternated from 500 to 450 nm (integration period = 0.5 sec). The system contained a back illuminated continuously variable rectangular diaphragm that could be placed over a portion of the proximal tubule. Photon counting was performed within a rectangle slightly wider than the tubule diameter and 70 μm in length. As the BCECF-AM entering the cell was de-esterified, the photomultiplier reading was continuously monitored and the loading was stopped when 1 to 2 × 10^6 cps were recorded using 500 nm excitation wavelength. The perfusion of the bath was then started using S_c solution, and a stabilization period of 10 min was observed. At the end of the experiment, luminal perfusion was stopped and bath solution was exchanged for a high K^+ (120 mM) imidazole-HEPES buffer containing 4 μM nigericin. Fluorescence ratio was measured at pH 7.25, 7.50 and 7.75 and used as a calibration for the experiment performed on the same tubule. Background to tubule fluorescence at both 500 and 450 nm was lower than 1 : 100 and was not subtracted.

Statistics

Results are reported as the mean ± standard error of the mean (SEM) where "*n*" refers to different experiments (different cell punctures or different tubules for pH_i measurements). Differences between experimental and control values were compared using the paired or unpaired Student's *t* test as appropriate. A *P* value lower than 0.05 was required to reach statistical significance.

Results

Basic Observations

As described previously [2, 14], stimulation of the transepithelial Na^+ transport (J_{Na}) by luminal addition of a Na-cotransported solute depolarizes V_{bl} transiently. In the present case, a saturating concentration of alanine (10–15 mM) depolarized V_{bl} by 20.3 ± 1.1 mV (n = 15, V_{bl} starting values averaged −51.2 ± 2.1 mV), and the cell repolarized to a value equal to its initial value within 4 min (−50.2 ± 3.0 mV, averaged repolarization[1] = 19.4 ± 2.0 mV, *see* Fig. 1). During this period the transepithelial potential hyperpolarized from 0 to approximately −2 mV with a time course similar to the typical result shown in Fig. 1. t_K was measured in six experiments during the stimulation of J_{Na} by luminal alanine. Figure 1 shows a typical example of the effect of repeated increases in the bath K^+ concentration from 5 to 15 mM on V_{bl} and on V_t. The average values are shown in Fig. 2. t_K averaged 0.17 ± 0.04 in the control condition and decreased instantaneously to 0.08 ± 0.03 ($P < 0.05$) after the luminal addition of alanine. In the next 4 min, t_K increased progressively to 0.29 ± 0.04 ($P < 0.01$). When alanine was removed from the lumen in the post-experimental period, t_K first increased to 0.41 ± 0.08 and decreased to a stable value of 0.22 ± 0.07 which was not significantly different from the initial values obtained for the four paired tubules.

[1] All along the present study, the term repolarization will be used to describe specifically the basolateral membrane potential shift toward more negative values in the period (≈4 min) following luminal addition of alanine. Hyperpolarization and depolarization have their usual meaning.

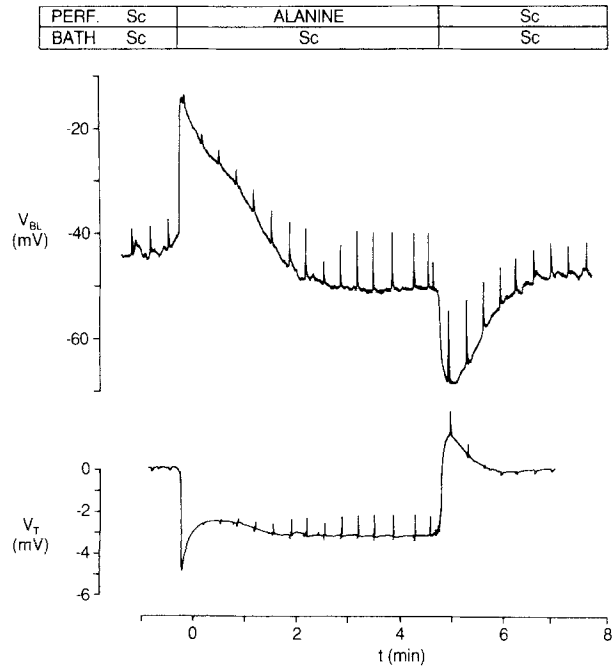

Fig. 1. Recording of basolateral membrane potential (V_{bl}) and transepithelial potential (V_t) during stimulation of Na-coupled alanine transport. The pulses on the recordings result from rapid increments of the basolateral K^+ concentration from 5 to 15 mM (1-sec duration)

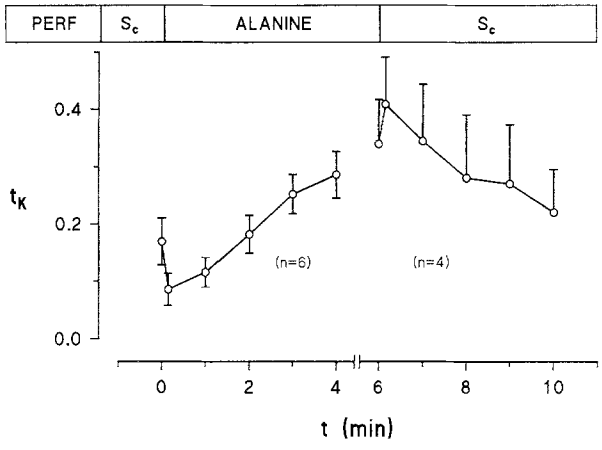

Fig. 2. Changes in the average fractional K^+ conductances (t_K) during stimulation of the transepithelial Na^+-coupled alanine transport. These numbers correspond to the ratio of the apparent basolateral K^+ conductance divided by the total cellular conductance

K^+ Channel Blockers

In order to see if the increase in t_K was responsible for the membrane repolarization after luminal addition of alanine, stimulation of J_{Na} was done in the presence of K^+ channel blockers. The efficiency of quinine and Ba^{2+} as K^+ channel blockers was verified in a preliminary series of experiments. In the continuous presence of 10 mM alanine in the luminal solution, a dose-response curve was first obtained for the quinine-induced membrane depolarization as measured 2 sec after the rapid addition of quinine to the bath solution. The maximum depolarization was 14.1 ± 0.6 mV, and the inhibition constant (K_i) was 0.50 ± 0.06 mM (n = 5, *data not shown*). A quinine concentration of 0.5 mM was used in the following experiments because higher concentrations showed poor reversibility after long application periods ($t >$ 2 min). The characterization of the effect of quinine was pursued by adding 2 mM Ba^{2+} in a basolateral solution containing 0.5 mM quinine. In this series of experiments, quinine produced an initial depolarization of 20 ± 2.3 mV (n = 4) while the subsequent addition of Ba^{2+} further depolarized the cell by 11 ± 3.0 mV (*see* Fig. 3). In another series of experiments Ba^{2+} was added before quinine. The initial depolarization caused by Ba^{2+} averaged 17 ± 2.4 mV (n = 4) while the subsequent application of quinine depolarized the cell by only 3.4 ± 0.9 mV (*see* Fig. 3). It appears that a large fraction of quinine-sensitive channels can be blocked by Ba^{2+} (very small effect of quinine in the presence of Ba^{2+}) while neither one of these two blockers can be considered as a total inhibitor of the basolateral K^+ conductance. To determine to what extent these blockers can affect the basolateral K^+ conductance, K^+ concentration pulses (from 5 to 15 mM) were performed in the basolateral solution in the presence of 0.5 mM quinine and 0.5 mM quinine + 2 mM Ba^{2+} in the basolateral solution (10 mM alanine was present in the luminal solution). The depolarization induced by the K^+ pulses always disappeared (n = 4) upon addition of quinine and, in most of the cases, the pulses started to produce hyperpolarization of 2–5 mV in the presence of quinine and even more in the presence of quinine + Ba^{2+}. The calculated t_K are shown in Fig. 3. Similar K^+-induced hyperpolarizations have been reported by others [10, 12] and are thought to originate from the stimulation of the Na-K-ATPase current in the presence of a more resistive basolateral membrane. We conclude that the K^+ channel blockers have reduced the basolateral K^+ conductance dramatically to the point where the K^+ stimulation of the Na-K-ATPase becomes dominant with respect to the effect coming from the K^+ channels.

To test the effect of Ba^{2+} on the repolarization that follows the stimulation of J_{Na}, six tubules were perfused with the usual S_c solution and bathed in a solution where phosphate and sulfate have been replaced by chloride. The mean starting V_{bl} prior to

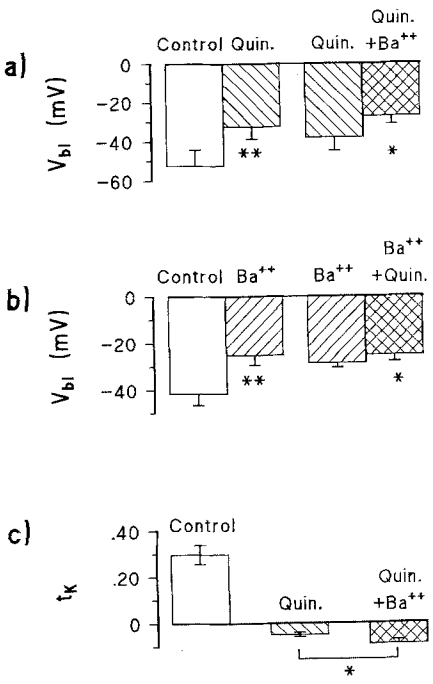

Fig. 3. Effect of basolateral application of 0.5 mM quinine and 2 mM Ba^{2+} on the basolateral membrane potential (V_{bl}). (*a*) Quinine was added first and, after a stabilization period of 2 to 5 min, the effect of Ba^{2+} was tested in the continuous presence of quinine ($n = 4$). (*b*) Ba^{2+} was added first and then quinine was added in the presence of Ba^{2+} ($n = 4$). (*c*) The effect of quinine and quinine + Ba^{2+} was measured on the transport number for K (t_K) as calculated from the change in V_{bl} produced by an increase from 5 to 15 mM in the basolateral K concentration ($n = 4$). $^*P < 0.05$; $^{**}P < 0.01$

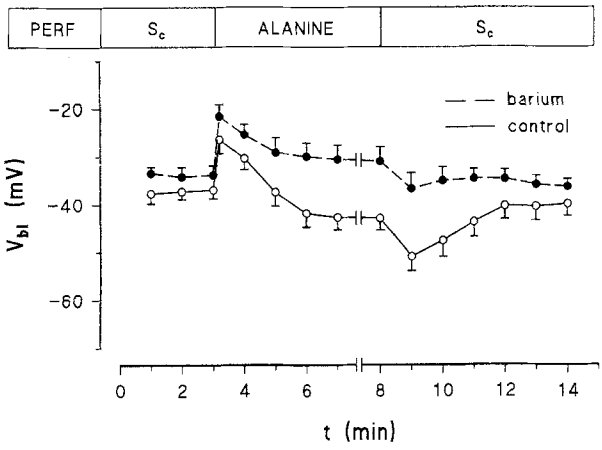

Fig. 4. Time course of V_{bl} during 10 mM alanine addition to the luminal solution in control and in the presence of 2 mM Ba^{2+} in the bathing solution. The relative repolarization in the presence of basolateral Ba^{2+} is significantly smaller than the control relative repolarization ($P < 0.01$)

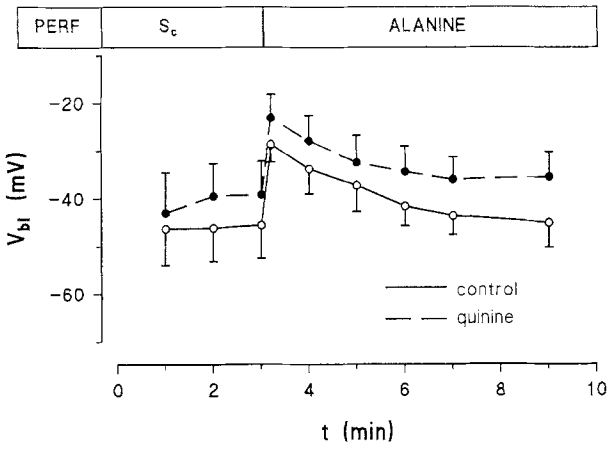

Fig. 5. Absence of effect of basolateral quinine (0.5 mM) on the repolarization of the basolateral membrane potential (V_{bl}) following luminal addition of alanine

Ba^{2+} addition was -39.2 ± 1.1 mV and depolarized to -28.0 ± 1.4 mV immediately after addition of 2 mM Ba^{2+} to the bathing solution. After a period varying from 4 to 9 min, V_{bl} became stable [1] and 10 mM alanine could be added to the luminal solution. Luminal alanine produced a partially transient V_{bl} depolarization as shown in Fig. 4. The initial depolarization following alanine application averaged 12.2 ± 1.3 mV and was followed by a repolarization averaging 9.0 ± 2.4 mV. After removing alanine from the luminal perfusate, Ba^{2+} was removed from the bath solution and V_{bl} was allowed to stabilize for a period of 4 to 10 min. Alanine was then added to the luminal solution, and a second transient depolarization was recorded. The initial depolarization averaged 10.6 ± 1.2 mV and was followed by a repolarization averaging 16.3 ± 0.7 mV. This last repolarization averaged $163 \pm 17\%$ of the initial depolarization which is significantly different ($P < 0.01$) from the average repolarization of $72 \pm 13\%$ recorded in the presence of Ba^{2+} for the same cells.

A similar series of experiments were performed with 0.5 mM quinine as K^+ channel blocker. The depolarization due to the luminal application of alanine (10 mM) averaged 17.0 ± 3.3 mV in control experiments and did not change significantly in the presence of 0.5 mM quinine in the basolateral solution (16.2 ± 2.5 mV, $n = 5$). Similarly, neither was the repolarization observed in the 4 min following luminal application of alanine affected by the presence of quinine (15.0 ± 0.6 mV *vs.* 13.0 ± 1.7 mV, control *vs.* quinine, NS; $n = 5$). Figure 5 shows a graph of the average values of V_{bl} during addition of alanine in control conditions and in the presence of quinine. Quinine failed to affect both the absolute and the relative value of the repolarization compared

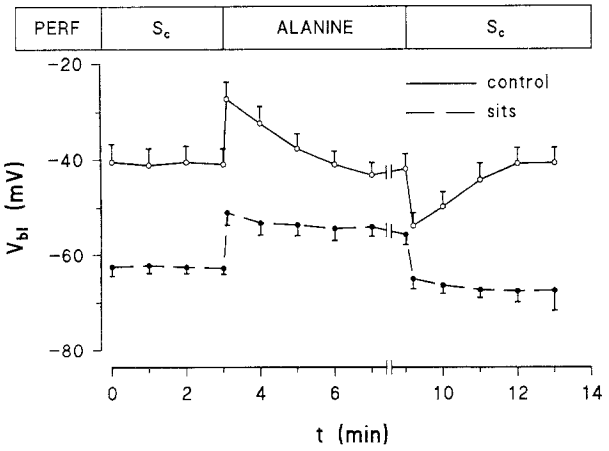

Fig. 6. Effect of SITS of the basolateral membrane potential (V_{bl}) repolarization following luminal addition of alanine

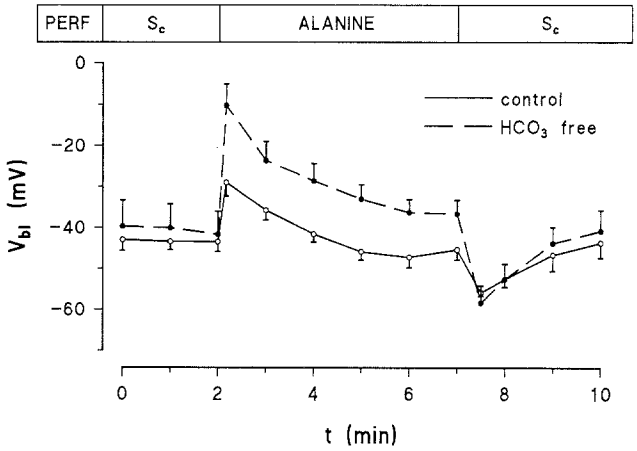

Fig. 7. Effect of replacement of CO_2-HCO_3 on the basolateral membrane potential (V_{bl}) response to luminal addition of alanine. The relative repolarization in bicarbonate-free solution is significantly smaller than the control relative repolarization ($P < 0.04$)

to the initial depolarization (89 ± 20% with quinine *vs.* 91 ± 18% in control conditions).

Effect of SITS and HCO_3-Free Solutions

An important part of the basolateral membrane conductance originates from the Na-3HCO_3 cotransporter which can be irreversibly inhibited by stilbene disulphonates (e.g., 4-acetamino-4′isothiocyamostilbene-2,2′-disulfonic acid, SITS) [4, 5]. A series of experiments were conducted to see if the membrane repolarization following luminal application of alanine was sensitive to this blocker. In this series of experiments the effect of alanine was recorded before and during the basolateral application of SITS (0.1 mM). As for the other tubules studied, luminal alanine addition (10 mM) depolarized V_{bl} by 13.0 ± 0.7 mV before eliciting a strong repolarization by 14.6 ± 1.9 mV ($n = 7$). Contrary to the reported observations on proximal straight tubules where a simple hyperpolarization was observed with SITS [4], addition of SITS to the bath slightly depolarized the cell by 7.6 ± 2.3 mV in the first minute before producing a slow hyperpolarization by 30.3 ± 3.5 mV in the next 10–20 min (final $V_{bl} = -62.0 \pm 1.7$ mV, $n = 5$). In the presence of SITS, luminal application of alanine (10 mM) rapidly depolarized V_{bl} by 11.4 ± 2.3 mV and reduced the secondary repolarization to 3.9 ± 1.3 mV. As represented in Fig. 6, while the initial effect of luminal alanine was not significantly different from the initial depolarization observed in control conditions (13.0 mV in control *vs.* 11.4 mV in the presence of SITS), the subsequent repolarization was significantly blunted by SITS (13.6 mV in control *vs.* 3.9 mV in the presence of SITS, $P < 0.01$, $n = 7$).

Another way to reduce the Na-3HCO_3 transport rate at the basolateral membrane is to omit CO_2-HCO_3 in the luminal and basolateral perfusate. In this series of experiments, 25 mM $NaHCO_3$ was replaced by 25 mM HEPES and enough mannitol to bring the osmolarity to 300 mosM/kg (the solution was bubbled with 100% O_2). In each experiment, the effect of luminal alanine was recorded in S_c solution and after 3–6 min in the HEPES solution. When post-control recordings were available they were pooled with the initial recordings in S_c solution (*see* Fig. 7). Addition of alanine in the control period produces a transient depolarization on V_{bl} (average depolarization of 14.1 ± 1.2 mV followed by an average repolarization of 16.3 ± 2.2 mV, $n = 5$). The removal of CO_2-HCO_3 produced a rapid but transient depolarization of V_{bl} from a starting value of −46.3 ± 3.7 mV to −12.8 ± 6.7 mV which was followed by a slower hyperpolarization to −41.7 ± 5.7 mV. Luminal addition of alanine in the absence of bicarbonate in the solutions produced a stronger initial depolarization of 31.5 ± 3.2 mV followed by a slow and incomplete repolarization of 23.8 ± 2.1 mV ($n = 5$) which was, in absolute value, significantly smaller than the initial depolarization ($P < 0.04$, $n = 5$). In agreement with this observation, the percentage of recovery after the initial depolarization was significantly reduced (114 ± 10% in control solutions and 77 ± 6% in bicarbonate-free solutions, $P < 0.04$, $n = 5$).

pH_i Measurements

The fact that the repolarization phenomena was inhibitable with SITS and sensitive to the presence of CO_2-HCO_3 in the solution prompted us to measure

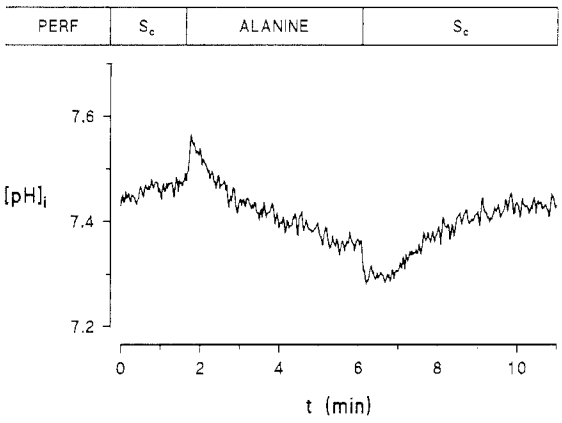

Fig. 8. Effect of luminal addition of alanine on the intracellular pH (pH_i) as measured by the fluorescent probe BCECF

pH_i during the stimulation of J_{Na} by luminal alanine. pH_i was measured with BCECF in nine different tubules. Figure 8 shows a representative example of this experimental series. The average starting pH_i was 7.39 ± 0.02 and increased to 7.50 ± 0.03 (n = 9) in the first 15 sec following the luminal addition of alanine (10 mM). pH_i decreased by 0.16 ± 0.01 and reached a stable value of 7.34 ± 0.02 after 3 min of alanine exposure. This stable value of pH_i is significantly more acidic than the starting value of pH_i in S_c solutions ($P < 0.05$). When alanine was replaced in the luminal solution, pH_i decreased initially to 7.27 ± 0.02 and returned to 7.36 ± 0.02 after 3–4 min.

Discussion

A transient depolarization is very commonly observed when J_{Na} is stimulated in a leaky epithelium. In the case of small intestine and proximal tubule [2, 8, 12, 13], an increase in the absolute or relative K^+ conductance at the basolateral membrane was also observed concomitantly to the repolarization phenomena. The observation that Ba^{2+} can block the repolarization in the case of *Necturus* small intestine [16] and in frog proximal tubule [12] support the contention that an increase in t_K is at the origin of the cellular repolarization. The experiments presented here indicate that in the case of rabbit PCT (S1 segment), the increase in t_K is not the only factor in the cellular repolarization following alanine addition to the luminal solution.

Quantitatively, one can divide the cellular conductance in two parts: the K^+ conductance of the basolateral membrane and the conductance of all the other apical and basolateral mechanisms lumped together. When these conductances are used with their respective reversal potentials in an equivalent circuit of the entire cell (neglecting the resistance of the paracellular pathway) one obtains the following equation for the basolateral potential of the cell.

$$V_{bl} = t_K (E_K) + (1 - t_K) (E_L).$$

The K^+ transport number has been estimated in the present study and increases from 0.08 to 0.29 during the repolarization process. The K^+ reversal potential has been recently estimated by us [15] and is expected to change from −83 to −78 mV when transepithelial Na transport is stimulated. Calculation of the lumped reversal potential for the other mechanisms (E_L) including the Na-HCO_3 basolateral cotransporter gives a value of −27.2 mV at the peak of the depolarization caused by addition of luminal alanine and a value of −38.8 mV at the end of the repolarization process. The difference between these two values is only 11.6 mV and can be accounted for by the change in the reversal potential associated with the Na-HCO_3 cotransporter and the time-dependent change in the emf of the Na-alanine cotransporter itself as intracellular alanine concentration increases with time. If E_L would happen to stay constant during the repolarization process, the repolarization would be reduced to 47% of the observed value.

In agreement with this analysis, the repolarization process was more sensitive to SITS than it was to Ba^{2+} and quinine and affected by CO_2-HCO_3 free medium. There are two ways the repolarization may be accounted for by the basolateral Na-HCO_3 transport mechanism: (*i*) the primary effect can be an inhibition of the transporter which produces a decrease in the bicarbonate exit (the cell becoming more negative) and an alkalinization, or (*ii*) the primary effect is a reduction in the intracellular bicarbonate concentration (cellular acidification) which produces a decrease in the bicarbonate exit (a cellular hyperpolarization). The measurement of intracellular pH confirms the second possibility.

The change in pH_i is biphasic during the experimental period: in the first 15 sec the cell alkalinizes by 0.11 pH unit, reaches a maximum and undergoes a slow acidification which is, in absolute values, significantly larger than the pH change observed initially (0.16 *vs.* 0.11 pH unit). This observation is different from the one reported by Messner, Koller and Lang, [17] for frog PCT. In their case the secondary acidification was not significantly different from the initial alkalinization. They conclude that the transient change in pH_i was due to the effect of the transient change in V_{bl}. Because the initial change

of V_{bl} and pH_i have the same time course, we believe as Messner et al. and others [17, 20–22] that the initial change in pH_i is due to the effect of the basolateral membrane potential on the bicarbonate transporter. Since the Na-3HCO_3 transporter is electrogenic [4, 23], a change in membrane potential is expected to influence the transport rate at least for some given voltage range. This relation between membrane potential and bicarbonate transport rate has recently been described for the *Ambystoma* proximal tubule [20, 21]. It is unlikely however that the changes in pH_i during the whole experimental period is simply a "passive" effect of V_{bl} on pH_i. In average, the initial membrane depolarization and the secondary membrane repolarization have the same amplitude in absolute values while the secondary acidification is significantly larger than the initial rapid alkalinization ($P < 0.02$). We believe that the cell is responding to the rapid stimulation of J_{Na} by a progressive acidification. The mechanism of this cellular acidification is not known. This pH_i regulation of V_{bl} is in agreement with a recent paper by Beck and Potts [1] where transient changes in V_{bl} caused by different maneuvers could be blocked by acetazolamide. Although the transport mechanisms and their regulation may be quite different, it is interesting to note that, in a tight epithelium, pH_i has been shown to affect both the apical and the basolateral conductance [9]. pH_i was suggested as the intracellular messenger balancing the apical and basolateral conductance.

In summary, contrary to other leaky epithelia, the repolarization of V_{bl} following luminal addition of alanine is neither abolished by Ba^{2+} nor by quinine. In rabbit PCT, the mechanism by which the cell repolarizes after addition of luminal alanine is SITS sensitive and requires the presence of CO_2-HCO_3. In the S1 portion of the rabbit PCT, pH_i seems to be very sensitive to changes in the basolateral membrane potential and vice versa. The most likely mechanisms at the origin of the secondary repolarization are a cellular acidification affecting the basolateral Na-3(HCO_3) cotransporter together with an upregulation of the basolateral K^+ conductance.

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