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pH_i-Dependent Membrane Conductance of Proximal Tubule Cells in Culture (OK): Differential Effects on K^+- and Na^+-Conductive Channels

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Summary. Confluent monolayers of the established opossum kidney cell line were exposed to NH_4Cl pulses (20 mmol/liter) during continuous intracellular measurements of pH, membrane potential (PD_m) and membrane resistance (R'_m) in bicarbonate-free Ringer. The removal of extracellular NH_4Cl leads to an intracellular acidification from a control value of 7.33 ± 0.08 to 6.47 ± 0.03 (n = 7). This inhibits the absolute K conductance (g_{K^+}), reflected by a decrease of K^+ transference number from 71 ± 3% (n = 28) to 26 ± 6% (n = 5), a 2.6 ± 0.2-fold rise of R'_m, and a depolarization by 24.2 ± 1.5 mV (n = 52). In contrast, intracellular acidification during a block of g_{K^+} by 3 mmol/liter $BaCl_2$ enhances the total membrane conductance, being shown by R'_m decrease to 68 ± 7% of control and cell membrane depolarization by 9.8 ± 2.8 mV (n = 17). Conversely, intracellular alkalinization under barium elevates R'_m and hyperpolarizes PD_m. The replacement of extracellular sodium by choline in the presence of $BaCl_2$ significantly hyperpolarizes PD_m and increases R'_m, indicating the presence of a sodium conductance. This conductance is not inhibited by 10^{-4} mol/liter amiloride (n = 7). Patch-clamp studies at the apical membrane (excised inside-out configuration) revealed two Na^+-conductive channels with 18.8 ± 1.4 pS (n = 10) and 146 pS single-channel conductance. Both channels are inwardly rectifying and highly selective towards Cl^-. The low-conductive channel is 4.8 times more permeable for Na^+ than for K^+. Its open probability rises at depolarizing potentials and is dependent on the pH of the membrane inside (higher at pH 6.5 than at pH 7.8).

Key Words intracellular pH · K^+ conductance · Na^+ conductance · OK cells

Introduction

The net reabsorption of Na^+, water, and solutes in the proximal renal tubule is paralleled by an acid extrusion into the lumen via different sodium-dependent and -independent mechanisms [18]. There has been ample evidence for the involvement of apical Na^+/H^+ exchanger and basolateral Na^+/HCO_3^- cotransport in this parallel Na^+ reabsorption and H^+ secretion along the proximal part of the nephron [1, 23].

Na^+-driven amino acid transport across the apical membrane of proximal tubule cells changes both the intracellular sodium concentration and intracellular pH (pH_i) [14]. This again influences Na^+ and H^+ fluxes due to changes of their electrochemical driving forces. Thus, intracellular homeostasis of inorganic ions and pH have to be closely linked to prevent excessive changes of intracellular sodium content and volume as well as of pH_i. There has been some evidence for the existence of Na^+ and K^+ channels in the apical membrane of proximal tubules [6, 13], but their physiological role is yet unclear.

The established opossum kidney cell line (OK) [11] possesses, among many other similarities to the proximal tubule, stretch-activated potassium channels [26] that mediate regulatory volume decrease during osmotic stress [10] as occurs in the proximal kidney tubule by large solute uptake. The regulation of the intracellular pH of OK cells is mediated by PTH- and ANF-regulated Na^+/H^+ exchange [16, 17, 22] that can be blocked by amiloride. We recently showed that monolayers of OK cells resemble a leaky epithelium [25] like the proximal tubule. Therefore, a confluent monolayer of OK cells serves as a suitable model for the study of pH_i-dependent electrical properties in the proximal renal tubule.

In the present study, we propose a model that shows how the intracellular pH may regulate Na^+ and H^+ homeostasis. This hypothesis would imply a pH dependence of ionic conductances in the physiological pH_i range of proximal tubule cells for which we tested our model system.

Materials and Methods

Cell culture and microelectrode measurements have been described elsewhere [25]. In brief, OK cells from passages 98 to 110 were maintained in bicarbonate-buffered Minimal Essential

Medium (MEM) at pH 7.4. For experiments, cells were grown on 3 cm ϕ plastic petri dishes for one week after having reached confluency. The apical surface was constantly superfused with prewarmed Ringer solution (37°C) containing (in mmol/liter) NaCl 122.5, KCl 5.4, $MgCl_2$ 0.8, $CaCl_2$ 1.2, Na_2HPO_4 0.8, NaH_2PO_4 0.2, HEPES 10 (titrated to pH 7.4 by NaOH 1 mol/liter). In some experiments, NaCl was replaced by choline chloride or sodium gluconate, respectively. In the case of gluconate-containing solutions, Ca^{2+} activity was monitored by an ion-selective electrode and titrated to control level by $CaCl_2$.

Microelectrodes were pulled to an input resistance of 2–5 × 10^7 Ω (1 mol/liter KCl filling solution). Cell membrane potential (PD_m) was measured against an Ag/AgCl reference electrode (in 3 mol/liter KCl). Cell membrane resistance (R'_m) and current-voltage curves were measured by the voltage deflection after the injection of current pulses and corrected for the microelectrode resistance. The current pulses were delivered by a high-impedance injection unit (Biologic, F-38130 Echirolles, France) and triggered by a computer. Apparent transference numbers were determined by extracellular step changes of the respective ion (actual membrane potential change divided by Nernst'ian potential change).

The patch-clamp technique [8] was applied to the apical membrane of OK cells. All experiments were carried out in the cell-excised inside-out configuration. Data were recorded with a L/M EPC-5 patch-clamp amplifier (List, Darmstadt, FRG) and stored on a video recorder after the signal had been digitized with a pulse code modulator (PCM 501, Sony, Köln, FRG). After low-pass filtering with an 8-pole Bessel filter (corner frequency 0.4 kHz) the data analysis was done with the help of a PC computer and data acquisitation and analysis software (Pclamp; Axon Instruments, Burlingame, CA). For complete analysis, only patch experiments with one channel activity were used. Open probability (P_o) was calculated by the equation

$$P_o = \frac{\Sigma t_{op}}{t_{tot}}$$

where Σt_{op} is the sum of all times when the channel is in an open state and t_{tot} is the whole time of channel analysis. In the single channel current tracings, an upward-current deflection corresponds to the movement of positive charge from bath to pipette. All patch experiments were performed at room temperature, with symmetrical solutions both in the bath and in the pipette containing (in mmol/liter) Na-gluconate 123, HEPES 10, NaCl 1, NaOH 7 (pH 7.4). The selectivity for sodium over potassium (P_{Na^+}/P_{K^+}) was determined according to the Goldman-Hodgkin-Katz equation from the shift of the reversal potential of single channel I/V-curves that is observed when asymmetrical solutions were applied in the bath and the pipette. The ionic compositions of the bath was (in mmol/liter): Na^+ 13, K^+ 117, gluconate$^-$ 119, HEPES 10, Cl^- 1 (pH 7.4), and that of the pipette filling solution: Na^+ 117, K^+ 13, gluconate$^-$ 119, HEPES 10, Cl^- 1 (pH 7.4).

For the measurement of intracellular pH we used neutral carrier-based ion exchanger (Fluka, Buchs, Switzerland) microelectrodes with an input resistance of about 2–4 × 10^{11} Ω. A pH-selective and a conventional microelectrode were impaled into the same cell to measure PD_m and E_{H^+}. The pH signal was amplified by an electrometer (Frankenberger, D-8034 Germering, FRG) with 10^{15} Ω input resistance.

Data are usually given as the arithmetic means ± standard error (SEM). Significance was tested by Student's t test.

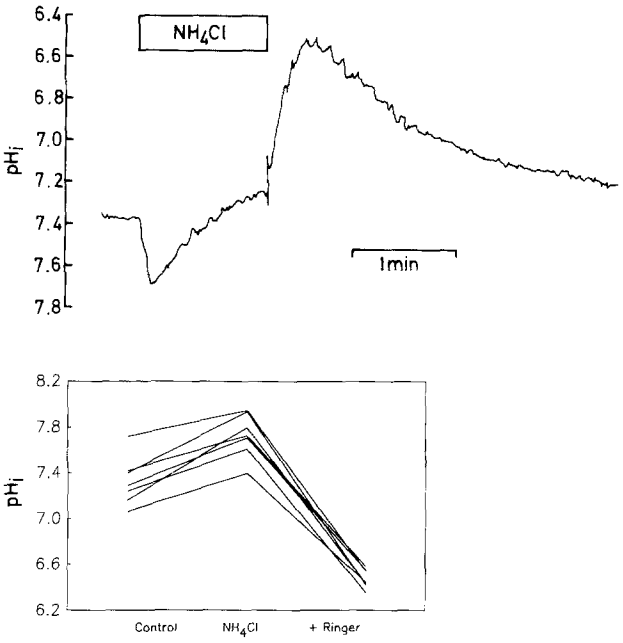

Fig. 1. Intracellular pH (pH_i) in response to extracellular NH_4Cl (20 mmol/liter) in bicarbonate-free Ringer (redrawn from an original recording). The maximal pH_i-changes of seven similar experiments are depicted below

Results

The ammonia prepulse technique [2] was used to alter the intracellular pH (pH_i) of OK cells. Figure 1 shows that pH_i is nearly identical to that of the bath solution under control conditions (7.33 ± 0.08; n = 7). A superfusion with 20 mmol/liter NH_4Cl causes a transient alkalinization with a maximum at pH 7.73 ± 0.07 as the result of NH_3 diffusion. The slow decrease of pH_i during continuous NH_4Cl superfusion is most probably due to NH_4^+ influx through conductive elements (e.g., K^+ channels). This NH_4^+ influx is reflected electrically by a significant reduction of the transmembrane resistance (R'_m) to 66 ± 6% (n = 70) of the control value together with a depolarization by 3.2 ± 0.7 mV (n = 72). Removal of extracellular NH_4Cl causes a marked intracellular acidosis to a minimum of 6.46 ± 0.03 (n = 7 paired experiments), followed by a slow recovery phase that is most likely due to the action of the Na^+/H^+ antiport.

The relative potassium conductance (t'_{K^+}) was measured by quick step changes of the extracellular potassium concentration from 5.4 to 40 mmol/liter during intracellular acidification. Figure 2 shows that t'_{K^+} sharply decreases after ending the NH_4Cl superfusion. Concomitantly, PD_m depolarizes by 24.2 ± 1.5 mV and R'_m rises 2.6 ± 0.2-fold (n = 52). t'_{K^+} falls from a control level of 71 ± 3% (n = 28) to 26

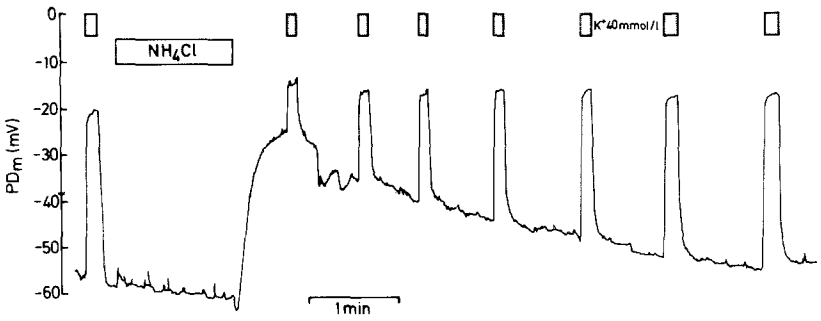

Fig. 2. Changes of K^+ conductance in response to intracellular acidification. t'_{K^+} was calculated from a step increase of extracellular K^+ from 5.4 to 40 mmol/liter (redrawn from an original recording)

± 6% (n = 5) immediately after having finished the ammonia prepulse. During the recovery phase from intracellular acidosis, t'_{K^+} rises up to control values and the membrane hyperpolarizes. Under these conditions, t'_{K^+} and PD_m are linearly correlated. The respective correlation coefficient of five individual experiments is -0.993 ± 0.002. Thus, the absolute K^+ conductance (g_{K^+}) is markedly blocked by an intracellular pH decrease from 7.4 to 6.5. In order to evaluate the influence of NH_4^+ outflow on the relative K^+ conductance, we measured the relationship between t_{K^+} and PD_m in control cells and after prepulse acidification. Figure 3 shows that there is no significant difference between control and acidotic cells. If the outflow of NH_4^+ ions markedly contributed to the overall cell membrane conductance, it would generate an inside negative membrane potential on its own so that t'_{K^+} should be less at acidosis than under control conditions (nonionic NH_3 diffusion is electroneutral and therefore undetectable by PD_m measurements). From the correlation of PD_m and t'_{K^+} under control conditions we can obtain an estimate for the intracellular potassium concentration. Assuming a linear relationship between PD_m and t'_{K^+}, 100% t'_{K^+} would yield a PD_m of about −94 mV, and so the intracellular K^+ concentration can be calculated to be 113 mmol/liter.

We previously reported [25] that the K^+ conductance of OK cells is nearly completely blocked by 3 mmol/liter $BaCl_2$ in the extracellular fluid. Current-clamp experiments revealed that barium is more effective at negative PD_m than at positive membrane potential with respect to the reduction of the overall cell membrane conductance (Fig. 4), indicating that Ba^{2+} blocks K^+ channels from the extracellular membrane side.

We performed a NH_4Cl-prepulse experiment under 3 mmol/liter $BaCl_2$ in order to test for the presence of a K^+-independent pH-regulated conductance that is normally masked by the predominant g_{K^+}. Figure 5 is an original recording of such an experiment. The vertical bars reflect input resistance after correction for microelectrode resistance (R'_m).

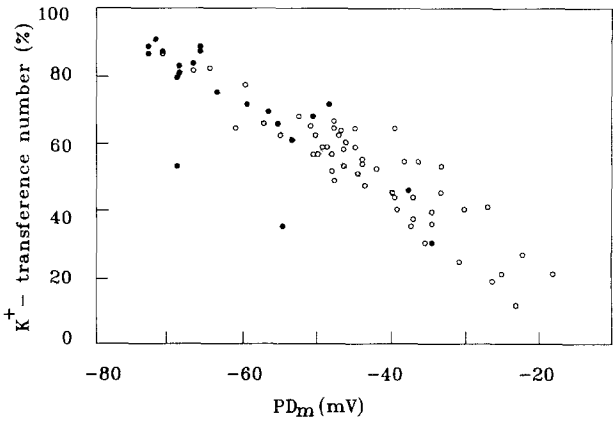

Fig. 3. Relationship between K^+-transference number and PD_m under control conditions (filled circles, solid line) and after intracellular acidification (20 mmol/liter NH_4Cl, open circles, dashed line). The regression lines represent linear least squares analysis; the difference between both regressions is not significant. t'_{K^+} were calculated from K^+ step increases from 5.4 to 40 mmol/liter (n = 23 control; n = 67 NH_4Cl)

$BaCl_2$ superfusion depolarizes PD_m by 24.2 ± 1.2 mV (n = 48). R'_m rises 7.5 ± 1.5-fold above control level (n = 25), indicating the closure of K^+ channels. During NH_4Cl superfusion (intracellular alkalosis), there is a slight hyperpolarization and R'_m increase ($\Delta PD_m = -2.1 \pm 1.1$ mV; R'_m/R'_m (control) = 1.34 ± 0.12; n = 20), whereas after finishing the ammonia pulse PD_m depolarizes and R'_m decreases. (ΔPD_m = 9.8 ± 2.8; R'_m/R'_m (control) = 0.68 ± 0.07; n = 17). The slope of the respective I/V curve increases at negative PD_m (Fig. 4). Recovery from this decreased R'_m generally occurs at a slower rate than the recovery from R'_m increase after finishing an ammonia pulse without barium, but is very variable from cell to cell. These data are obviously in contrast to the PD_m changes and R'_m changes without barium (Fig. 6), where intracellular acidosis causes a R'_m increase. Hence, there must be a second pH-regulated ion conductance in OK cells that is inhibited by intracellular alkalosis and stimulated by intracellular acidosis. These changes of PD_m and R_m cannot be attrib-

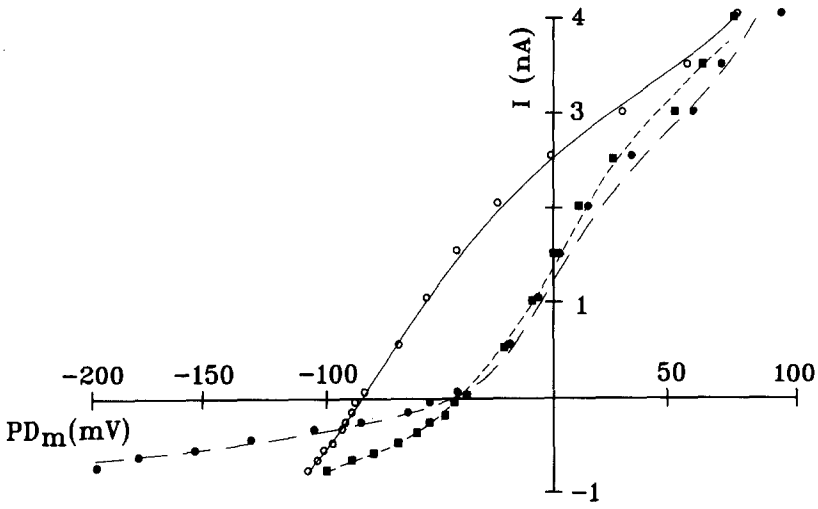

Fig. 4. Current/voltage (I/V) curves derived from single electrode current-clamp experiments in one cell (control: open circles, solid line). Total membrane conductance is markedly diminished in the presence of 3 mmol/liter $BaCl_2$ (filled circles, dashed line) at negative PD_m. Intracellular acidification under barium (filled squares, dotted line) elevates membrane conductance again. Similar results were obtained in another three experiments

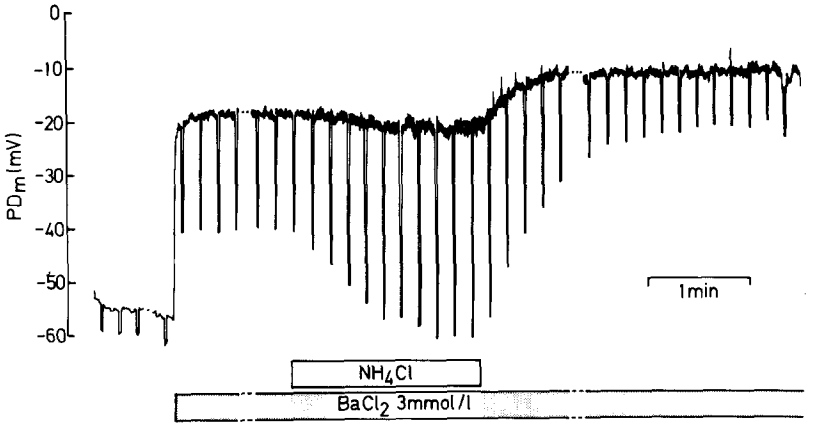

Fig. 5. Membrane potential (PD_m) and cell membrane resistance (R'_m) in response to intracellular acidification in the presence of 3 mmol/liter $BaCl_2$. When extracellular NH_4Cl is present, PD_m slightly hyperpolarizes and R'_m increases. After the washout of NH_4Cl, the opposite phenomena are observed

uted to the influx and efflux of NH_4^+ because the resulting PD_m changes would go into the opposite direction: NH_4^+ influx would depolarize the membrane during alkalosis and NH_4^+ efflux would hyperpolarize the membrane during acidosis. Obviously, barium is capable of blocking both K^+ and NH_4^+ conductive pathways, suggesting that NH_4^+ and K^+ use the same channels also in OK cells.

Acidosis-stimulated depolarization and R'_m decrease can be due to the outward movement of an anion or the inward movement of a cation. Therefore, we tested for the presence of Cl^-- and Na^+-conductive pathways under $BaCl_2$.

A decrease of extracellular Cl^- concentration from 130 to 18 mmol/liter leads to a small decrease of R'_m ($R'_m/R'_{m(\text{control})} = 0.8 \pm 0.1$; $n = 7$) and no change in PD_m after correction for liquid junction potentials. Neither DIDS (10^{-4} mol/liter) nor anthracic acid (10^{-4} mol/liter) affect PD_m or R'_m under barium ($n = 4$) whether there has a been a preceding NH_4Cl pulse or not. Thus, Cl^- movement is very unlikely to account for the observed acidosis-activated conductance.

A decrease of extracellular Na^+ from 130 to 7 mmol/liter under barium, however, elevates R'_m 1.4 $\pm$ 0.1 times above control ($n = 12$). PD_m hyperpolarizes by 11.2 $\pm$ 1.7 mV. After a NH_4Cl prepulse R'_m rises 1.6 $\pm$ 0.1-fold above control due to the same Na^+-step decrease ($n = 8$). These results strongly suggest the presence of a minor conductive pathway for sodium. However, amiloride (10^{-4} mol/liter) did not affect PD_m and R'_m when it was superfused after an ammonia prepulse under barium ($n = 7$).

In another set of experiments we investigated the sodium conductance of OK cells more directly by applying the patch-clamp technique in the excised inside-out configuration. Under those conditions, we found two types of channels. Figures 7 and 8 show the original single-channel currents for both types in relation to different clamp voltages. It is obvious that the conductivity of both channels is different; the reversal potential, however, is identi-

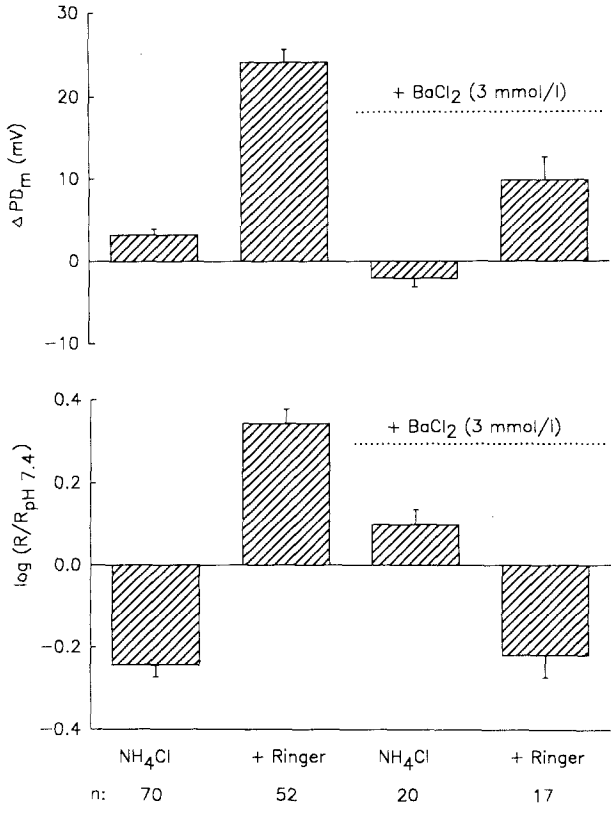

Fig. 6. Changes in cell membrane potential (ΔPD_m, top) and relative cell membrane resistance (as compared with control at pH 7.4, below) in response to intracellular pH changes (control Ringer and under 3 mmol/liter $BaCl_2$). Relative cell membrane resistance is depicted logarithmically because in this presentation a rise or fall of R'_m by the same factor produces equal changes of relative cell membrane resistance

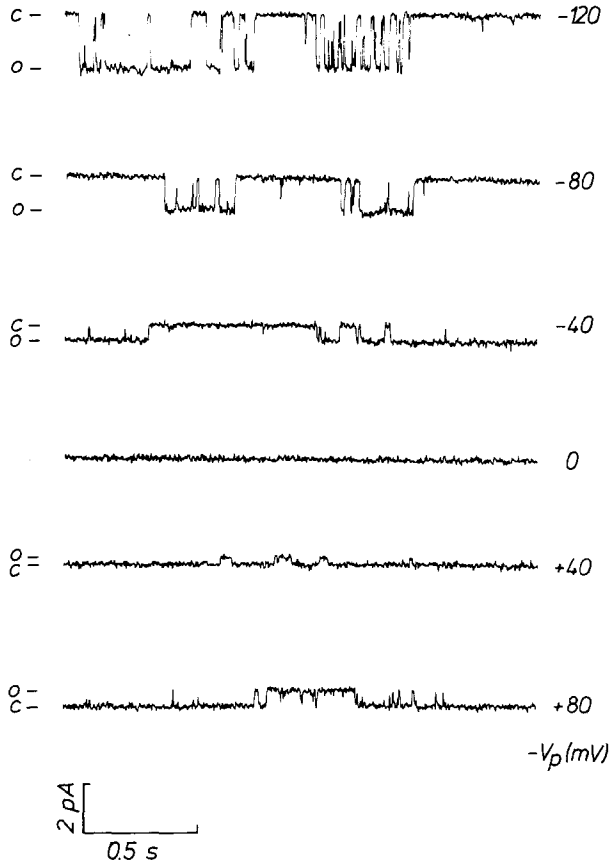

Fig. 7. Single-channel current recordings at different clamp potentials (excised patch, inside-out configuration): The patch contains one single, low-conductive sodium channel. *c* denotes the closed and *o* the open state, and V_p the clamp potential of the pipette

cal (0 mV). Both channels are slightly inward rectifying, i.e., have a reduced single-channel conductance at depolarizing potentials (Fig. 9). The conductivities—calculated at the physiological voltage range (−20 to −80 mV) when sodium is the only permeable ion present—are 18.8 ± 1.4 pS (n = 10) and 146 pS, respectively. Figure 8 demonstrates that the high-conductive channel shows more flickering characteristics between the open and closed states compared to the longer open and closed times of the low-conductive channel. Neither channel is permeable to chloride, because the reversal potential is not affected by elevating the chloride concentration in the bath from 0 to 130 mmol/liter (n = 4). The high-conductive channel does not show a selectivity for sodium over potassium and can be elicited by membrane stretch (*data not shown*). In contrast, the low-conductance channel is about five times more selective for sodium than for potassium (P_{Na^+}/P_{K^+} = 4.8). Its open probability is very low in the physiological voltage range (P_o < 0.5%) but dramatically rises at depolarizing potentials (n = 5; Fig. 10). When the bath pH—that corresponds to the intracellular pH in the inside-out configuration—is lowered from 7.8 to 6.5, the open probability rises, but the voltage dependence is nevertheless maintained (n = 3). This shows that the low-conductance channel is moderately sodium selective and voltage and pH sensitive.

Discussion

The present study investigates the regulation of ionic conductances in OK cells by changes of the intracellular pH in bicarbonate-free solution. We recently reported that pH_i under HCO_3^-/CO_2-buffered condition (5% CO_2, pH 7.4) is about 0.4 pH units lower than without bicarbonate [25]. Thus, our acidification experiments start at an "unphysiologically" alkaline pH of 7.3 (intact cells) or 7.8 (excised patch) and cover the whole

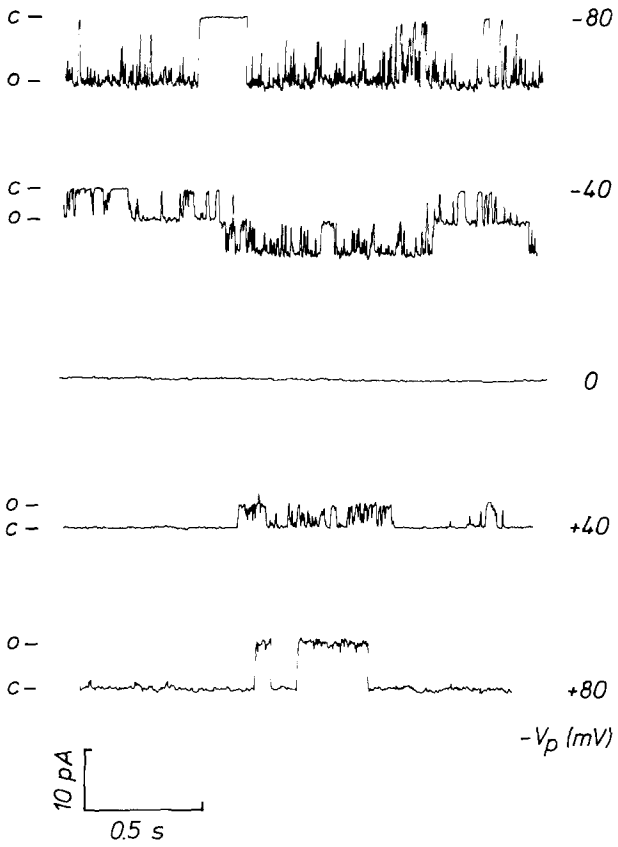

Fig. 8. Original tracing of two single, high-conductive channels (excised patch, inside-out configuration). For symbols, *see* legend of Fig. 7

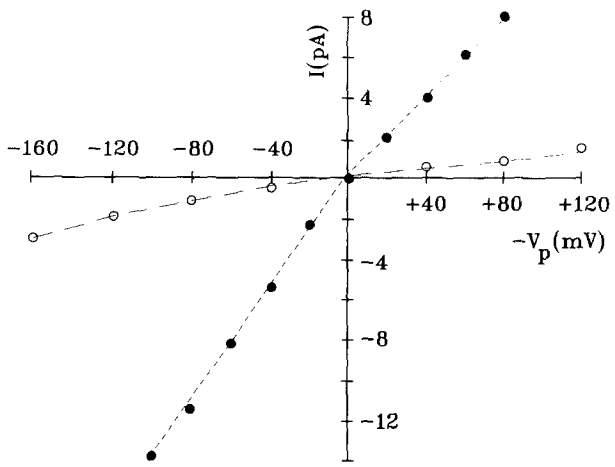

Fig. 9. Current/voltage (I/V) relationship of the low- (open circles) and the high-conductive channels (filled circles) depicted in Figs. 7 and 8. The conductivities are 17.5 and 146 pS, respectively, between −20 and −80 mV. I denotes the single-channel current, V_p the clamp potential of the pipette (similar results were obtained in nine other experiments)

range up to moderate intracellular acidosis (pH 6.5).

Method

The ammonia prepulse technique implies the disadvantage that ammonium not only passes the membrane by nonionic diffusion, but NH_4^+ ions can, however to a smaller extent, directly penetrate the cell membrane via K^+ channels. Thus, changes of membrane potential and membrane resistance can be produced both by NH_4^+ fluxes and a regulation of ionic channels. Therefore, we measured the actual relative K^+ and Na^+ conductances by step changes of the extracellular ion concentrations. We found that the correlation between PD_m^* and t'_{K^+} is not influenced by NH_4Cl-induced intracellular acidosis. So, either the NH_4^+ efflux after the finishing of the ammonia superfusion is too small to be measured, or NH_4^+ behaves just the same as K^+ with respect to its transmembrane permeation. Probably NH_4^+ ions leave the cell much slower than they enter the intracellular space, because the interior negative membrane potential reduces the NH_4^+ efflux. Thus, measurement of K^+ conductance at low pH_i is not significantly biased by NH_4^+ fluxes. Interestingly, in the presence of barium we did not observe any NH_4^+-mediated currents, neither during nor after NH_4Cl superfusion. This confirms our assumption that $BaCl_2$-blockable K^+ channels are the main entry pathway for NH_4^+ ions.

K^+ Conductance

A decrease of pH_i from 7.4 to 6.5 reduces t'_{K^+} from 71 to 26% accompanied by the respective R'_m increase. This shows that K^+ conductance is regulated by pH_i in the physiological pH range. We have preliminary data from t'_{K^+} measurements in OK cell monolayers grown on permeable filters that are perfused separately from the apical and basolateral surface. The greatest part of barium-blockable K^+ conductance seems to be located basolaterally, but there is still a significant apical K^+ conductance left (*data not shown*). This is in agreement with the findings of Merot et al. [13], who distinguished apical, acidosis-inactivated K^+ channels in primary cultures of rabbit proximal tubules. There are obviously important differences in the polar localization of K^+ channels

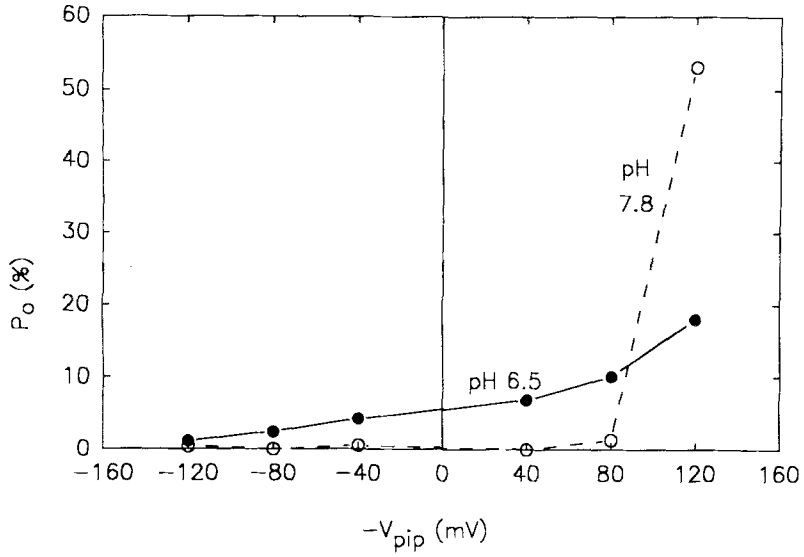

Fig. 10. Dependence of the open probability (P_o) of the low-conductive channel on pipette potential (V_{pip}) at two different bath pH (inside-out configuration; similar results were obtained in another two experiments)

between cultured cells and the intact tubule, where apical K^+ conductance amounts to only 4% of total apical conductance [12]. We found that barium blocks K^+ conductance preferably at negative injected current. This corresponds to the reported voltage-dependent decrease of K^+ channel open probability in primary cultured proximal tubule cells [13]. It must be emphasized, however, that the applied single electrode current-clamp method is not suitable to resolve quick changes of R_m. Preliminary dual electrode current-clamp experiments revealed a delayed increase of R_m at positive currents, so that the data obtained with injection of positive current must be interpreted rather reluctantly.

Messner et al. [14, 15] showed that Na^+ driven amino acid reabsorption in the proximal tubule of frog kidney transiently alkalinizes the cytoplasm and elevates K^+ conductance. Also, an apical Na^+ influx enhances the driving force for the basolateral Na^+/K^+-ATPase and thus tends to elevate the intracellular K^+ concentration. A stimulation of this K^+ conductance by intracellular alkalosis may allow rapid K^+ recirculation during Na^+/solute cotransport even before the intracellular K^+ concentration rises and thus prevent the membrane potential from depolarizing too much.

K^+ channels also play an important role in the maintenance of osmotic homeostasis in heavily transporting epithelia. Being responsible for the regulatory volume decrease during hyposmotic stress, K^+ channels are also involved in cell volume regulation of OK cells [10]. K^+ channels of the proximal renal tubule and of OK cells are stretch activated [24, 26]. It is, however, not yet known if mechanical membrane stretch is really involved in cell volume maintenance in the proximal tubule in vivo. An alternative stimulus for K^+ channel opening would be a transient cell alkalinization, similarly leading to a K^+ efflux.

Na^+ Conductance

In the presence of barium, intracellular alkalinization causes a significant increase of cell membrane resistance and a hyperpolarization of PD_m. This can only be explained by the reduction of some rheogenic pathway. The responsible ion is surely not potassium, since a reduction of outward K^+ flux would depolarize the membrane. Vice versa, intracellular acidosis results in the opening of a conductive pathway (decrease of R'_m) and a depolarization of PD_m under barium). The most probable candidates that could contribute to this conductance—Na^+ and Cl^-—were tested, and a significant, though small, Na^+ conductance was found. This Na^+ conductance was slightly higher in the state of acidosis compared to controls under barium; the difference is, however, not significant. Interestingly, amiloride (10^{-4} mol/liter) was not able to block this Na^+ conductance.

A quantitative evaluation of this sodium conductance is difficult. Under control conditions (potassium channels open, PD_m about −60 mV) there is no detectable PD_m change when sodium is completely replaced by choline [25]. Thus, sodium conductance is likely to be elicited by a cell depolarization. This hypothesis matches the observed increase of the single channel open probability of the low-conductive channel described in the present paper, supporting the notion that this channel is responsible for the overall sodium conductance. Unfortunately, with our methods we could not alter PD_m deliberately

in the intact cell when K^+ channels were blocked. Furthermore, recovery of PD_m and R_m from the acidosis-induced changes in the presence of barium is mostly incomplete and generally slower than the respective recovery without barium. This is likely to be due to an impairement of intracellular K^+ homeostasis at blocked K^+ channels and/or changes of the electrical driving force because of the barium-induced depolarization.

Patch-Clamp Experiments

In order to get a more positive evidence for the presence of Na^+ channels in the apical membrane of OK cells, we used the patch-clamp technique in the excised inside-out configuration. In these experiments we found two types of sodium transporting channels with different single-channel conductances. Especially the low conductive channel shows long open and closed characteristics typical for epithelial sodium channels [5]. Both channels are inwardly rectifying; i.e., their single channel conductivities decrease at positive clamp voltages. The low-conductive channel shows a 5 : 1 selectivity of sodium over potassium and is activated both by depolarization and an acidification of the intracellular side of the patch that is in the same order of magnitude as the pH_i changes applied by the ammonia prepulse technique in intact cells. The open probability of this channel is low, but the channel density is high, for we identified at least one low-conductive channel in all but one successfully excised patches.

In all experiments, the channel activity increases by excising the patch. Mostly, there is no channel activity at all in the cell-attached mode. This observation contrasts to the behavior of OK cell K^+ channels that are most active in the cell-attached configuration [21]. Obviously, there is a sodium conductive channel in the apical membrane of OK cells with some similarities to that observed by Gögelein and Greger [6] in the apical membrane of rabbit pars recta proximal tubules. The single channel conductance of these Na^+ channels is in the same range as that of our low-conductance channels (12 *vs.* 19 pS). Also, the amiloride sensitivity is lower in both preparations than typical [3].

In conclusion, we propose the following hypothetical model for the physiological role of a pH_i-dependent apical Na^+ channel (Fig. 11). When H^+ is transported into the tubule lumen by Na^+/H^+ antiport, pH_i rises and therefore Na^+ conductance is reduced. This protects the cell from a too heavy Na^+ influx and allows basolateral Na^+/K^+-ATPase to cope with Na^+/H^+-mediated sodium influx. The same situation occurs when there is active sodium-driven solute reabsorption that increases pH_i. On the other hand, in the absence of H^+ secretion due to a low Na^+/H^+-exchange activity, there is a cytoplasmic acidification and Na^+ can enter the tubule cell independently through acidosis-stimulated channels. Such a de-coupling of H^+ from Na^+ fluxes is useful if Na^+/H^+ exchange activity is regulated itself. This regulation has been shown in many preparations, best in lymphocytes, where Na^+/H^+ exchange can be very rapidly upregulated by phorbol esters [7]. In OK cells, Na^+/H^+ activity is inhibited by parathyroid hormone, atrial natriuretic peptide and cyclic nucleotides [16].

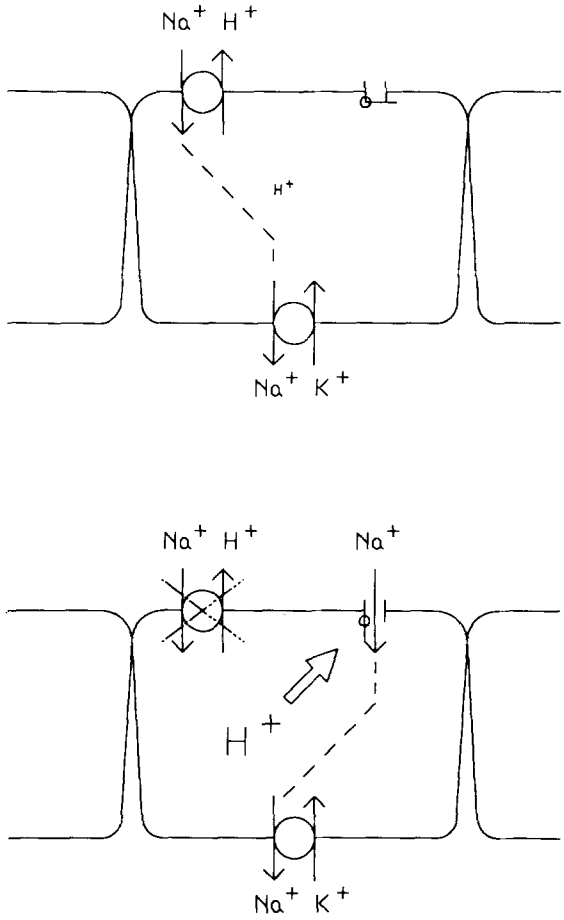

Fig. 11. A model for the regulation of transepithelial H^+ and sodium transport. Normally (top), Na^+ is partly imported via the apical Na^+/H^+ antiport. When this antiport is blocked (bottom), pH_i falls and opens an apical Na^+ conductance, leading to a de-coupling of H^+ secretion and Na^+ reabsorption

It is an interesting fact that the K^+ conductance of epithelial cells is rather uniformly stimulated by intracellular alkalosis [9, 19, 20], whereas Na^+ channels are either blocked [4] or stimulated [9] by alkalo-

sis. This different pH sensitivity may allow different tissues to regulate different sodium transport pathways by pH_i changes.

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