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Differential Regulation of Membrane Potential and Conductance Via Intra- and Extracellular pH in Fused Proximal Tubular Cells of Frog Kidney

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Abstract. Intracellular pH (pH_i), membrane potential (V_m) and membrane conductance (G_m) in fused proximal tubular cells of the frog kidney, were determined at three extracellular pH (pH_o) values, 7.5, 8.5 and 6.5. Imposed changes of pH_o by ±1 pH unit induced parallel but smaller shifts of pH_i. The alkaline milieu hyperpolarized the cells and increased G_m, whereas the acid milieu depolarized and lowered G_m. We subsequently introduced a weak acid and its conjugate base (acetic acid/acetate), or a weak base and its conjugate acid (NH_3/NH_4^+), at pH_o 7.5, 8.5 and 6.5 to shift pH_i without altering pH_o, or to shift pH_i against imposed changes of pH_o. From these experiments, we observed that under some circumstances V_m varied with pH_o but without G_m or pH_i changes, whereas under other circumstances changes of G_m occurred during alterations of pH_i while pH_o and V_m remained unaltered. At $pH_i \approx 6.5$ associated with $V_m \approx -10$ mV, G_m dramatically increased to quasi-infinite values. This increase was not an artifact since G_m returned to its control value following recovery to the control solution or in the presence of hyperosmotic solution. In conclusion, we demonstrate a differential regulation whereby V_m and G_m are controlled by pH_o and pH_i: pH_o modulates mainly V_m, and pH_i modulates chiefly G_m. Furthermore, at $pH_i \approx 6.5$ and $V_m \approx -10$ mV, our data reveal a large G_m that tends towards infinite values in a reversible fashion.

Key words: Membrane potential — Conductances — pH — Acetate — NH_4Cl

Introduction

Membrane potential (V_m) and membrane conductance (G_m) as well as several other properties of cell membranes are subject to regulation through specific signals. A well-known factor that modulates membrane conductance is pH. It affects primarily the partial conductance of the membrane for potassium ions (G_K), which usually dominates G_m [1, 19], and thereby affects also V_m [4, 15, 26]. In general, high pH increases G_m and elicits membrane hyperpolarization, whereas low pH decreases G_m and produces membrane depolarization. In the present experiments we have further examined the effects of changes in extracellular pH (pH_o) and intracellular pH (pH_i) on G_m and V_m.

Several studies have measured membrane conductance in the amphibian proximal tubule by double cable analysis. However, such measurements are extremely difficult to perform and the data exhibit an enormous scatter [2, 8, 28]. For this reason, we resorted to an in vitro preparation, fused proximal tubular cells of frog kidney [11, 12]. Fused giant cells permit double impalements with microelectrodes in order to record V_m and to inject constant current pulses to monitor G_m. Our purpose was to correlate changes in V_m, in transference number (t_K) and in G_m with changes in pH_o and pH_i, in order to determine the relative importance of extra- and intracellular pH in modulating cellular parameters [6, 10]. The experimental approach of this study was: (i) to alter pH_o by ± one unit in the presence of the physiological solution; (ii) to alter pH_i by substituting 20 mM of NaCl by Na-acetate (pK = 4.7) or by NH_4Cl (pK = 9.2). The data suggest that pH_i chiefly modulates G_m, whereas pH_o mainly modulates V_m.

Materials and Methods

PCT Cells Fused into Giant Cells

All experiments were carried out on cells of proximal convoluted tubule (PCT) of frog (*Rana ridibunda*) kidney, fused into giant cells. We followed the technique originally described by Oberleithner et al. [19, 20], and more recently modified in our laboratory [7, 11]. The

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Table 1. Composite data from the parameters intracellular pH (pH_i), membrane potential (V_m), membrane conductance (G_m) and transference number for potassium (t_K), upon applying an extracellular pH (pH_o) of 7.5, 8.5, and 6.5 (series 1, $n = 8$)

pH_o	pH 7.5a	pH 7.5	pH 7.5b	pH 8.5a	pH 8.5HK	pH 8.5b
		HKa				
pH_i	7.11 ± 0.08	7.16 ± 0.08	7.10 ± 0.07	7.38 ± 0.09	7.52 ± 0.09	7.44 ± 0.10
V_m	−50.7 ± 2.4	−38.7 ± 1.2	−51.7 ± 2.5	−66.4 ± 1.4	−48.7 ± 0.6	−68.4 ± 1.5
G_m	1	1.42 ± 0.04	0.97 ± 0.03	1.61 ± 0.07	2.64 ± 0.17	1.86 ± 0.15
t_K		0.43 ± 0.05			0.63 ± 0.04	

Since some solutions recur among other solutions, discrimination is achieved by using small letters (a, b, c . . .) to denote the order of appearance of a solution, e.g., pH 7.5a, pH 7.5b. . . . HK denotes a threefold increase in extracellular potassium concentration.

animals were pithed, then the renal vasculature (aorta and renal veins) of the kidney was washed with a physiological Ringer solution to remove blood cells. Next, collagenase (IA, Sigma), 450 U/ml, was added to digest connective tissue. The composition of the physiological solution, in mM, was NaCl 82, KCl 3, $CaCl_2$ 1.8, $MgCl_2$ 1. It was buffered with 5 mM N-tris (hydroxymethyl) methyl-2-amino-ethane sulfonic acid (TES, pK 7.4), and the pH was adjusted to 7.5 by adding appropriate amounts of NaOH. The next step was to cut small fragments of the frog kidney; these fragments were bathed in a physiological solution supplemented with collagenase, Worthington, CLS 2, 3,000 U/ml, for 1 hr at 26°C. PCTs were then microdissected, collected and stored in the physiological solution at 4°C in test tubes having the inner wall covered with a thin layer albumin. Mechanical treatment reduced the fragments to an average size of 100–150 μm. Fusion of a cluster of cells into a single (giant) cell was achieved by exposure to a 15% solution of polyethylene glycol (M_r 4,000), pH 8.2, for 5 min. Following centrifugation, the pellet was resuspended in a modified Leibowitz medium (L15) as described elsewhere [7]; total calculated osmolality was 200 mOsm/liter, pH 7.6. Clusters of cells were deposited onto glass plates coated with a thin collagen layer and maintained at 4°C for 24 hr. Most clusters achieved complete fusion into giant cells and adhered firmly to the plates.

Fused cells adhering to a glass plate were placed on the stage of a microscope (Nikon, Diaphot); they were superfused with physiological solution and subsequently with any of seven different test solutions, using electronically controlled switches; thus, each cell served as its own control.

Electrophysiological Techniques

Each giant cell was impaled with two microelectrodes (a conventional and a double-barreled microelectrode) by means of two hydraulic micromanipulators (Narishige MO-103). The conventional microelectrode, filled with KCl, 1 M, was connected to an electrometer function-generator (Axoclamp 2A, Axon Instruments) delivering constant current pulses, 750 msec, 0.16 Hz, 0.5 to 2 nA, depending on the size of the cell. The second microelectrode, connected to the input of another high-impedance electrometer (WPI FD 223), was used to record the membrane potential (conventional barrel, filled with 1 M KCl), and the intracellular pH. A 1 M KCl (macro)-electrode placed downstream in the effluent served as the reverence. V_m and pH_i were continuously monitored by means of a multi-pen recorder (Linseis LS 4).

Current injection was triggered by the Axoclamp program, version 5.3, as in our previous work [11, 12]. Changes in G_m were normalized by the control value of G_m for each individual cell, using the following expression:

$$G_{m(exp)}/G_{m(ctr)} = R_{m(ctr)}/R_{m(exp)} = \Delta\Psi_{(ctr)}/\Delta\Psi_{(exp)},$$

where R_m defines membrane resistance and $\Delta\Psi$ denotes the change of V_m resulting from injection of constant current pulses. Each $\Delta\Psi_{(exp)}$ was referred to the initial $\Delta\Psi_{(ctr)}$ measured under control conditions (physiological solution, pH_o 7.5) in the same cell. Transference number of potassium, t_K, was estimated by raising $[K]_o$ concentration from 3 to 9 mM, and calculated from the resulting ΔV_m using the relationship,

$$t_K = \Delta V_m/(RT/zF)\ln(C_1/C_2),$$

where C_1 and C_2 refer to the potassium concentration before and after the step, and R, T, z and F have their usual meanings.

Intracellular pH, together with V_m, was assessed using double-barreled microelectrodes, as in previous studies [21]. Briefly, one barrel was exposed to vapors of dimethyl-trimethyl-silylamine (Fluka 41716), then the microelectrodes were baked for 2 hr at 120°C, and, a droplet of the proton exchanger (Fluka 95291) was allowed to fill the tip overnight. The next day, the nonselective barrel was filled with 1 M KCl. The shank of the selective barrel was backfilled with 67 mM NaCl solution, containing in addition 40 mM, KH_2PO_4, and 23 mM NaOH, pH 7.0. The slope of the pH microelectrodes was 50–55 mV per decade.

In this study, we devised four experimental protocols, with subsequent superfusion of six to eight solutions. Besides physiological solutions of pH_o = 6.5, 7.5 and 8.5, we applied Na-acetate (pK = 4.7) and/or NH_4Cl (pK = 9.2) solutions of the same three pH_o values. In these solutions, 20 mM of NaCl was replaced by 20 mM Na-acetate or 20 mM NH_4Cl. We used 5 mM TES (pK 7.4) to buffer solutions at pH_o = 7.5, 5 mM MES (2-[*N*-morpholino]ethane sulfonic acid, pK 6.1) to buffer solutions at pH_o = 6.5, and 5 mM TAPS (*N*-tris[hydroxymethyl] methyl--3--aminopropane-sulfonic acid, pK = 8.4) to buffer solutions at pH_o = 8.5; pH_o was adjusted with appropriate amounts of 1 M NaOH solutions. Each sequence of solutions started and ended with the respective solution at pH_o = 7.5, defining the control state.

Statistics

Results are expressed as mean ± SEM. When necessary, significance of the results was assessed using Student's *t*-test for paired or unpaired samples.

Results

Effects of pH_o, on pH_i, V_m, G_m and t_K during a Change of pH_o by ± One Unit

In this series, fused cells ($n = 8$) were routinely bathed in physiological solution buffered at pH_o 7.5. Average pH_i

pH 7.5c	pH 7.5c HKb	pH 7.5d	pH 6.5a	pH 6.5HK	pH 6.5b	pH 7.5e
7.16 ± 0.06	7.33 ± 0.07	7.16 ± 0.07	6.87 ± 0.06	6.83 ± 0.06	6.80 ± 0.06	7.10 ± 0.07
−53.6 ± 2.4	−39.5 ± 1.5	−51.1 ± 2.8	−29.1 ± 2.7	−25.1 ± 2.1	−25.5 ± 2.3	−41.9 ± 1.8
0.99 ± 0.06	1.40 ± 0.06	0.96 ± 0.06	0.72 ± 0.07	0.90 ± 0.08	0.75 ± 0.07	0.95 ± 0.06
	0.44 ± 0.05			0.14 ± 0.03		

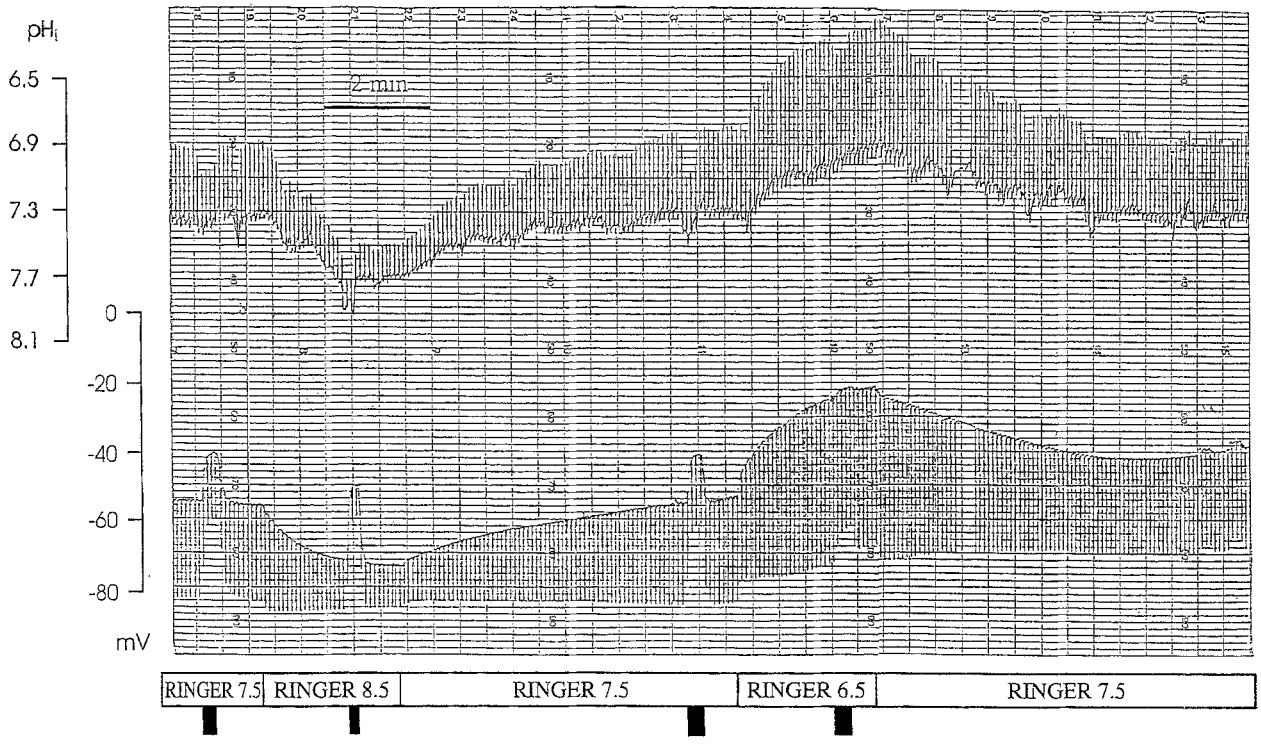

Fig. 1. Effects of changes of extracellular pH (pH$_o$) on intracellular pH (pH$_i$), membrane potential (V_m) and on membrane conductance (G_m). Original recording from series 1. (Top) Intracellular pH: capacitive transients are superimposed upon the pH$_i$ trace. (Bottom) V_m: the size of voltage transients elicited by constant current pulses is an index of G_m. In this and other tracings, black rectangles in the bottom of the main frame denote a threefold increase in $[K]_o$, which served to evaluate t_K.

in this series was 7.11 ± 0.08, the mean value of control V_m was −50.7 ± 2.4 mV, and t_K was 0.43 ± 0.05. Control G_m was 0.56 ± 0.06 mS/cm^2; for each individual cell, membrane conductance was normalized by its initial control G_m value, which then of course takes on the normalized value of 1.00.

Increasing pH$_o$ from 7.5 to 8.5 resulted in a rise of pH$_i$ to 7.44 ± 0.10. This change was associated with a hyperpolarization of −68.4 ± 1.5 mV and an increase of G_m to 1.86 ± 0.15. t_K rose to 0.63 ± 0.04.

These changes were reversible, as shown in Table 1 and Fig. 1. The alkaline pH$_o$ was lowered back to pH$_o$ 7.5, then to pH$_o$ 6.5. Corresponding pH$_i$'s were 7.16 ± 0.07 declining to 6.80 ± 0.06. Concomitantly, membrane potential changed from −51.1 ± 2.8 mV (at pH$_o$ 7.5) to −25.5 ± 2.3 mV (at pH$_o$ 6.5), whereas G_m fell from 0.96 ± 0.06 to 0.75 ± 0.07. t_K at pH$_o$ 6.5 was quite low, 0.14 ± 0.03. The value of G_m recovered entirely, following a sequence of thirteen solutions delivered one after another; however, membrane potential failed to achieve complete recovery while pH$_i$ recovered fully; t_K was not assessed at the end of the experiments.

Table 1 displays details of thirteen means ± SEM for pH$_i$, V_m and G_m, plus four t_K values. Figure 2 schematically represents the main steps of this protocol.

Results from the first series indicate that both V_m

and G_m are sensitive to pH_o/pH_i. G_m is more sensitive to alkalinization (pH_o and/or pH_i), by contrast, V_m is more sensitive to acidification (pH_o and/or pH_i).

Effects of a Moderate Load of Na-Acetate at pH_o 7.5 and 6.5

In the present series we studied the effects of a moderate acetate load, 20 mM, at pH_o 7.5 and 6.5. Proximal convoluted cells are endowed with transporters, namely, the monocarboxylate-OH exchanger [5] and the Na-organic anion cotransport [3, 16]. Such transporters are good candidates to mediate acetate entry into and exit out of the cells.

In this batch of cells (n = 6), at the physiological pH_o, pH_i was 7.31 ± 0.03, V_m was −54.2 ± 3.0 mV and, as previously, all conductances were normalized by the initial control G_m. A threefold increase in $[K]_o$ elicited an 11.4 ± 1.2 mV depolarization, giving a calculated t_K of 0.41 ± 0.04. On exposure to the Na-acetate solution of pH_o = 7.5, pH_i rose, though the rise was not statistically significant. These data suggest that the permeability of the anion acetate is slightly higher than that of the undissociated acetic acid. Membrane potential, after a transient depolarization, reached a mean value of −61.6 ± 6.5 mV, not significantly different from control, and the same was true for G_m, whose value was 1.65 ± 0.31. A threefold increase of $[K]_o$ resulted in a depolarization of 11.6 ± 1.2 mV, corresponding to a t_K of 0.42 ± 0.04, a value similar to that observed in the initial control (acetate-free) solution. Withdrawal of the acetate solution alkalinized the cell even more: this delayed alkalinization suggests that an intracellular excess of acetate anions was gradually disposed of over a few minutes; during this alkaline overshoot (pH_i peaked at 8.05 ± 0.06), V_m was unchanged (−62.5 ± 6.0 mV), while G_m peaked at 3.40 ± 0.93. Then, pH_i relaxed gradually to 7.47 ± 0.05, close to the initial control pH_i, V_m recovered to its initial value, −56.8 ± 4.3 mV, and G_m relaxed towards the initial control value, finally stabilizing at 1.18 ± 0.04.

Lowering pH_o from 7.5 to 6.5 resulted in a fall of pH_i from 7.47 ± 0.05 to 7.03 ± 0.07 over a few minutes. Concomitantly, membrane potential decreased from −56.8 ± 4.3 to −31.8 ± 4.9 mV. Subsequent exposure to the acetate solution at pH_o = 6.5 strongly acidified the cells to pH_i 6.52 ± 0.09, and further depolarized V_m to −13.0 ± 3.7 mV; t_K was 0.03 ± 0.01. Changes in G_m were very irregular. In three out of the six cells, normalized G_m fell to 0.56 ± 0.05 (Fig. 3), but in one cell it increased to 9.80 (Fig. 4) and in two other cells, it increased to (quasi-) infinite values. However, the latter did not indicate irreversible damage, since after reintroducing the physiological solution, the cells returned to a final G_m of 1.26 ± 0.25, V_m of −44.9 ± 3.7 mV and pH_i of 7.38 ± 0.04.

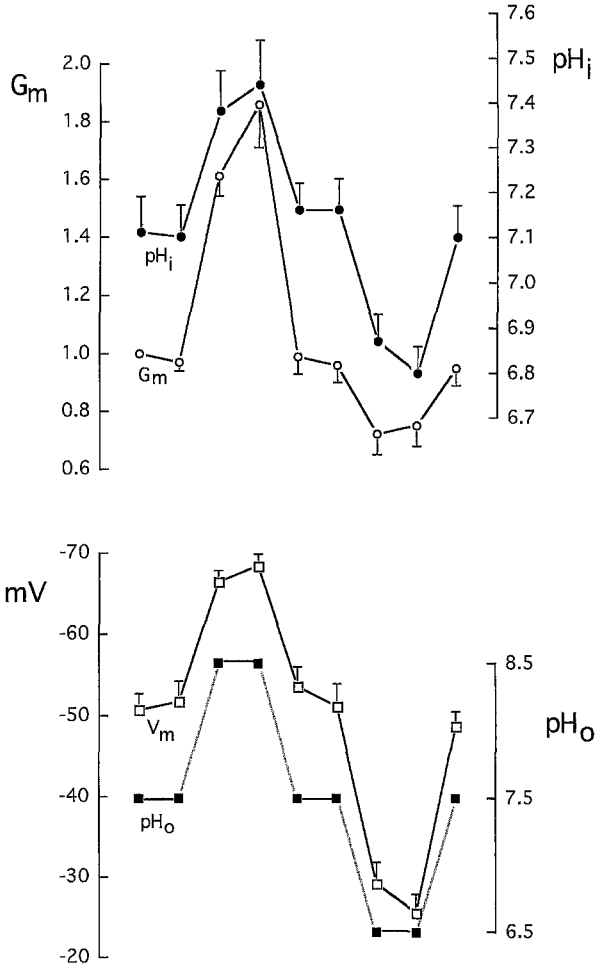

Fig. 2. Schematic representation of the main steps composing protocol 1. Four broken lines represent, from top to bottom, pH_i (●), G_m (○), V_m (□) and pH_o (■). Half-tint lines (pH_o) indicate displacements of ±1 pH unit.

The main results of this series are summarized in Fig. 5 and Table 2. Results from this series indicate, for one thing, that the behavior of V_m and G_m can be dissociated, since, for instance, Na-acetate withdrawal of pH_o 7.5 increases membrane conductance without altering membrane potential. Second, the tremendous increase of G_m observed during Na-acetate perfusion at pH_o 6.5 suggests activation of some ionic conductance(s).

Effects of a Moderate load of NH_4Cl at pH_o 7.5 and 8.5

In these experiments, NH_4Cl was applied at pH_o 7.5 and 8.5. The weak base NH_3 is known to enter proximal cells [27]. In this series (n = 7), control V_m was −53.9 ± 4.1 mV and control pH_i was 7.21 ± 0.04; t_K was 0.36 ± 0.06. As before, control G_m was given the value of 1.00. Exposure to the NH_4Cl solution, 20 mM, failed to alter

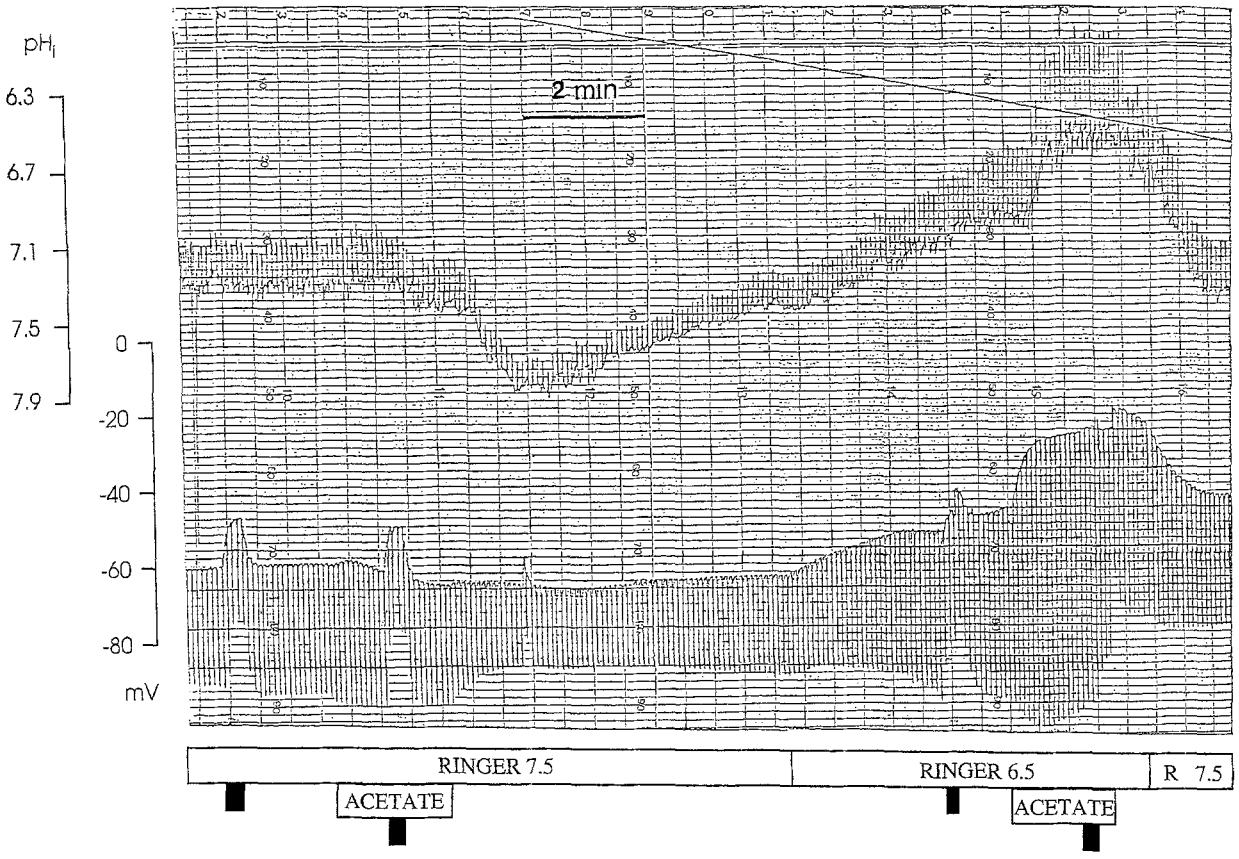

Fig. 3. Effects of adding 20 mM Na-acetate, at pH_o 7.5 and 6.5 on pH_i, V_m and G_m. Original recording from series 2. (Top) pH_i; (Bottom) V_m.

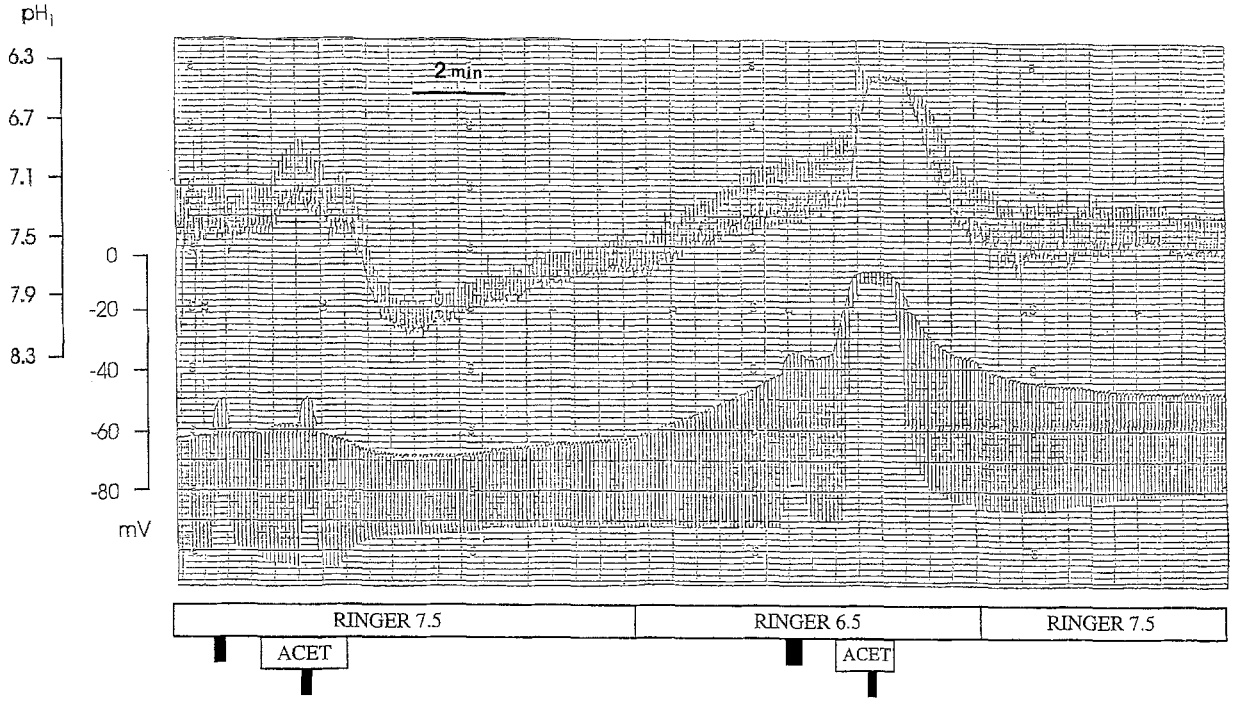

Fig. 4. As in Fig. 3, effects of adding 20 mM Na-acetate, at pH_o 7.5 and 6.5, on pH_i, V_m and G_m. Original recording from series 2. (Top) pH_i; (Bottom) V_m. Contrasting with Fig. 3, during exposure to Na-acetate solution (20 mM), pH_o 6.5, G_m increased to 9.8 with regard to the paired control. Return to the physiological solution yields V_m and G_m close to their respective initial controls.

V_m significantly (peak V_m was −57.6 ± 2.6 mV); however, pH_i increased from 7.22 ± 0.04 to 7.84 ± 0.04 and then relaxed gradually to 7.78 ± 0.05. Concomitantly, G_m increased by a factor of 1.68 ± 0.09, while t_K increased only slightly (to 0.50 ± 0.04). Removal of the NH_4Cl solution inverted the alkalinization to an acidification (pH_i shifted from 7.78 ± 0.05 to 6.88 ± 0.03), after which pH_i returned slowly towards control. These changes were associated with a significant depolarization, from −57.2 ± 3.5 mV (under NH_4Cl) to −40.9 ± 3.6 mV (after NH_4Cl removal) which gradually relaxed to −47.7 ± 3.6 mV; G_m decreased transiently to 0.70 ± 0.07, and then also recovered towards the initial control value, 0.91 ± 0.07.

Next, pH_o was set to 8.5 in a physiological solution. pH_i rose gradually to a plateau of 7.51 ± 0.08; concomitantly, V_m increased to −64.9 ± 2.5 mV, and G_m rose to 1.50 ± 0.20 of control. Exposure to NH_4Cl, at pH_o 8.5, increased pH_i to 8.66 ± 0.09 but failed to alter V_m (−62.7 ± 1.5 mV), whereas G_m rose significantly to 2.85 ± 0.39. t_K was 0.60 ± 0.03. Withdrawal of the NH_4Cl from the alkaline solution strongly acidified the cells from pH_i of 8.60 ± 0.11 to 7.47 ± 0.10, but it failed to significantly alter V_m (−56.4 ± 4.0 mV), while G_m fell from 2.53 ± 0.40 to 1.24 ± 0.11. The final control values for pH_i, V_m and G_m were indistinguishable from their initial counterparts, despite a sequential exposure of the cells to a total of 16 artificial solutions one after another. Figure 6 illustrates this experimental series, and Fig. 7 and Table 3 summarize the main observations of this protocol.

As was the case in the second series of experiments, the changes in pH_i and in G_m may occur without concomitant changes in V_m.

Association of a Moderate Acetate Load at Alkaline pH_o Followed by a Moderate NH_4Cl Load in the Presence of an Acid pH_o

Thus far, we studied the effects of a primarily acidifying solution (acetate) at control and acid pH_o and the effects

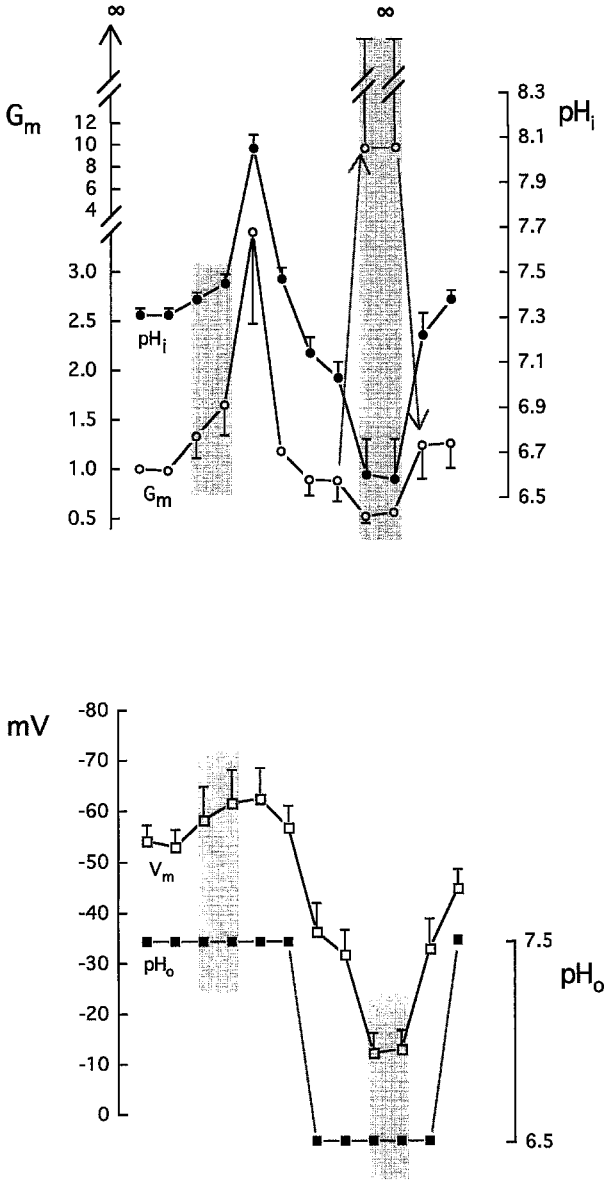

Fig. 5. Schematic representation of the main steps associated with the acetate solution, 20 mM, pH_o 7.5 and 6.5. Light shaded areas represent perfusion with acetate solution.

Table 2. Parameters pH_i, V_m, G_m and t_K assessed at pH_o 7.5 and 6.5*

pH_o	pH 7.5a	pH 7.5HK	pH 7.5b	pH 7.5 acet	pH 7.5 acet HK	pH 7.5 acet b	pH 7.5c	pH 7.5d
pH_i	7.31 ± 0.03	7.33 ± 0.04	7.31 ± 0.03	7.38 ± 0.03	7.43 ± 0.04	7.45 ± 0.04	8.05 ± 0.06	7.47 ± 0.05
V_m	−54.2 ± 3.0	−42.7 ± 2.3	−53.0 ± 3.3	−58.2 ± 6.6	−46.6 ± 5.6	−61.6 ± 6.5	−62.5 ± 6.0	−56.8 ± 4.3
G_m	1	1.27 ± 0.03	0.98 ± 0.02	1.33 ± 0.22	1.99 ± 0.36	1.65 ± 0.31	3.40 ± 0.93	1.18 ± 0.04
t_K		0.41 ± 0.04			0.42 ± 0.04			

* Acetate for Cl substitution, 20 mM, was applied at both pH_os (series 2, $n = 6$). The notation "n INF" at the bottom of the table indicates that n cells of the group had infinite conductance; in these cases, cells displaying infinite conductance recovered after solution change.

of a primarily alkalinizing solution (NH_4Cl) at control and alkaline pH_o. We next considered the effects of acetate at alkaline pH_o and the effects of NH_4Cl at acid pH_o.

Figure 8 shows a representative recording from this series ($n = 7$ cells). At physiological pH_o, pH_i was 7.23 ± 0.03, average V_m was −45.7 ± 1.3 mV and control G_m was assigned the value of 1.00. Increasing K concentration by a factor of three depolarized V_m by 7.7 ± 0.63 mV, yielding a t_K of 0.28 ± 0.02. Raising pH_o from 7.5 to 8.5 resulted in cell alkalinization (pH_i went up to 7.63 ± 0.05) and hyperpolarization (to −61.4 ± 2.6 mV) while G_m increased by a factor of 1.73 ± 0.18. Exposure to the Na-acetate solution, 20 mM, at pH_o 8.5, alkalinized to a peak pH_i of 8.01 ± 0.05, yet V_m, after a transient depolarization remained constant (−56.9 ± 4.6 mV), although G_m increased, 3.26 ± 0.24-fold. t_K rose to 0.56 ± 0.04. Removing the acetate solution at pH_o 8.5 caused a pronounced alkalinization to a peak of pH_i 8.40 ± 0.07, V_m hyperpolarized to −64.1 ± 3.6 mV and G_m increased to 4.71 ± 0.29 with regard to control.

Upon lowering pH_o stepwise to 7.5 and then to 6.5, pH_i returned to 7.48 ± 0.14 and 6.91 ± 0.11, respectively, whereas V_m depolarized to −47.5 ± 4.9 mV at $pH_o = 7.5$,

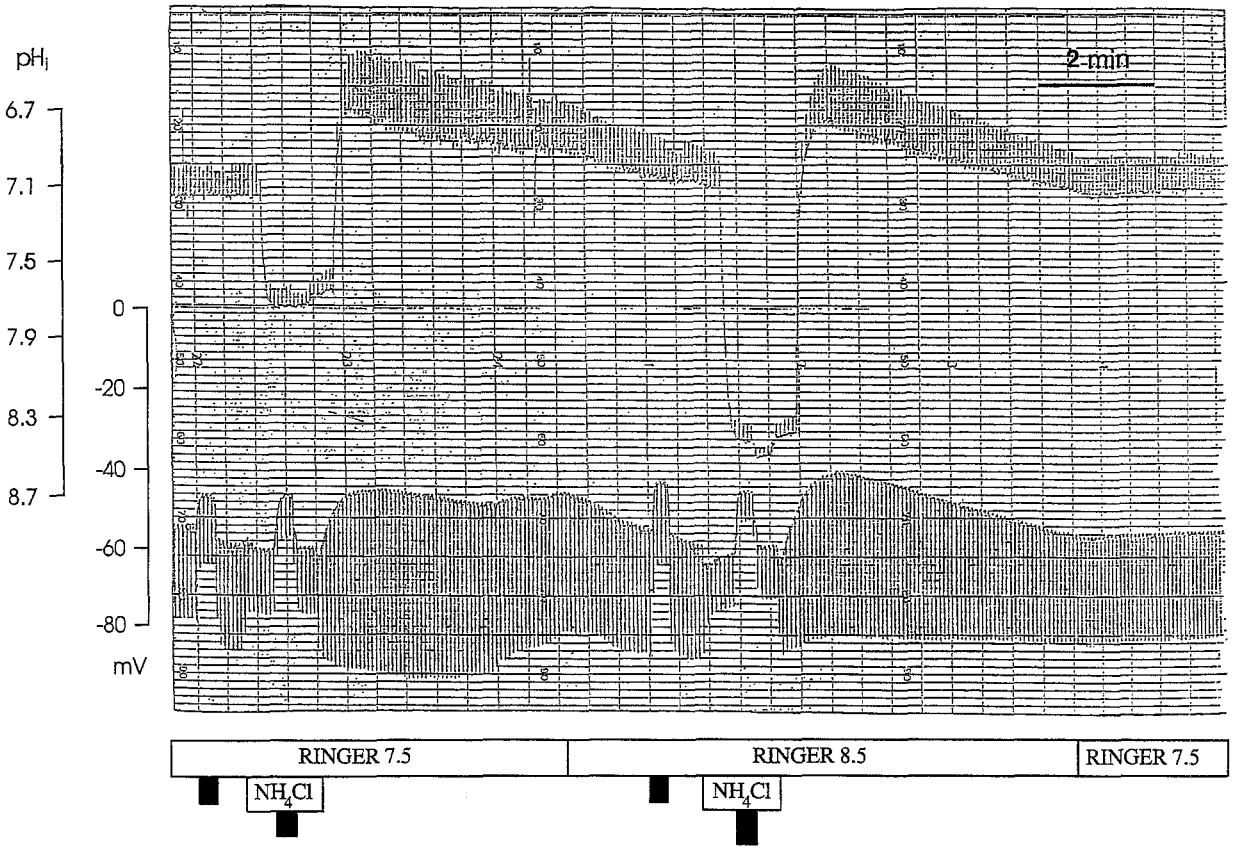

Fig. 6. Effects of adding 20 mM NH_4Cl, at pH_o 7.5 and 8.5, on pH_i, V_m and G_m. Original recording from series 3. Symbols as in other figures.

pH 6.5a	pH 6.5HK	pH 6.5b	pH 6.5 acet a	pH 6.5 acet HK	pH 6.5 acet b	pH 6.5c	pH 7.5e
7.14 ± 0.07	7.08 ± 0.04	7.03 ± 0.07	6.55 ± 0.08	6.53 ± 0.07	6.52 ± 0.09	7.22 ± 0.10	7.38 ± 0.04
−36.3 ± 5.7	−32.4 ± 4.2	−31.8 ± 4.9	−12.2 ± 4.0	−12.3 ± 3.4	−13.0 ± 3.7	−33.0 ± 5.9	−44.9 ± 3.7
0.89 ± 0.16	1.08 ± 0.20	0.88 ± 0.21	2.68 ± 1.80	3.67 ± 1.95	2.87 ± 2.30	1.24 ± 0.34	1.26 ± 0.25
	0.24 ± 0.10			0.03 ± 0.01			
			1 INF	1 INF	2 INF		

to finally reach a mean value of −23.8 ± 1.2 mV at pH_o = 6.5. G_m also reverted to 1.77 ± 0.76 (pH_o = 7.5) and decreased to 0.96 ± 0.10 at pH_o = 6.5.

Exposure to NH_4Cl at pH_o 6.5 failed to significantly affect pH_i (6.92 ± 0.11). Simultaneously, V_m fell to −17.7 ± 1.4 mV, t_K fell to 0.05 ± 0.01, and G_m was 1.16 ± 0.10. Upon withdrawal of the NH_4Cl solution from the acid solution, the cells acidified further to 6.46 ± 0.09 and V_m depolarized further to −11.7 ± 2.0 mV, while in some experiments G_m slightly dropped and in others G_m tremendously increased: its average value was 15.1 ± 8.9. Upon reintroduction of the control solution, pH_i recovered to 7.27 ± 0.07, compared to the initial control of 7.23 ± 0.03; however, V_m only reached −29.2 ± 3.4 mV compared to −45.7 ± 1.3 mV initially. G_m recovered to 1.36 ± 0.08, although in two out of seven cells, G_m became transiently infinite.

Figure 9 and Table 4 collect pertinent data from this series of experiments. This last series includes two subsets: (i) An acetate solution, 20 mM, buffered at pH_o 8.5, led to an increase of pH_i, 8.01 ± 0.05 and activated G_m, whose peak was 3.26 ± 0.24; as in the previous series (series 2) acetate withdrawal was followed by an alkaline pH_i overshoot and a high G_m; (ii) The second phase of this series focused on the effects of an NH_4Cl solution of pH_o 6.5: upon withdrawal of NH_4Cl, pH_i fell to a nadir, 6.46, V_m decreased to −11.7 mV and G_m covered a range of values from 0.94 and up to almost infinite values.

It was confirmed that at pH_o and/or $pH_i \approx 6.5$ (as shown in protocol 2) a number of cells attained a V_m close to zero and a (quasi-) infinite conductance, reversible upon return to the physiological solution. This point will be further considered in the Discussion.

Dependence of t_K on V_m

During the above four protocols, t_K values were assessed (Tables 1, 2, 3 and 4). Figure 10 shows that t_Ks lie along a single regression, -0.011×0.149, $r^2 = 0.947$ with regard to V_m.

Discussion

In this study, we looked for the effects of pH on three electrophysiological parameters of the proximal renal cell: V_m, G_m and t_K. Whereas pH_o may be set at will, pH_i is variable and was measured continuously during alterations of pH_o (±1 pH U) and in the absence or presence of a weak acid or a weak base and their respective con-

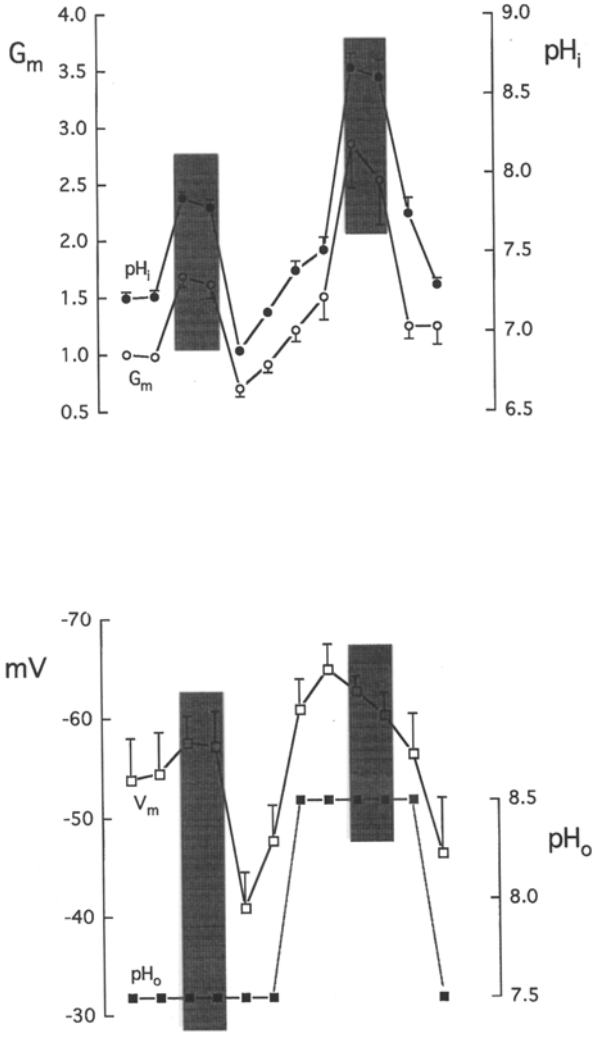

Fig. 7. Schematic representation of the main steps of the third protocol. pH_o values are 7.5 and 8.5, in the presence of the control solution and of a solution containing NH_4Cl, 20 mM, at the expense of NaCl. Heavy shaded areas represent NH_4Cl solution, 20 mM.

Table 3. pH_i, V_m, G_m and t_K, at pH_o 7.5 and 8.5*

pH_o	pH 7.5a	pH 7.5HK	pH 7.5b	pH 7.5 NH_4a	pH 7.5 NH_4 HK	pH 7.5 NH_4b	pH 7.5c	pH 7.5d
pH_i	7.21 ± 0.04	7.23 ± 0.05	7.22 ± 0.04	7.84 ± 0.04	7.84 ± 0.05	7.78 ± 0.05	6.88 ± 0.03	7.12 ± 0.03
V_m	−53.9 ± 4.1	−44.0 ± 2.9	−54.5 ± 4.1	−57.6 ± 2.6	−43.7 ± 1.9	−57.2 ± 3.5	−40.9 ± 3.6	−47.7 ± 3.6
G_m	1	1.34 ± 0.02	0.98 ± 0.04	1.68 ± 0.09	2.36 ± 0.15	1.61 ± 0.12	0.70 ± 0.07	0.91 ± 0.07
t_K		0.36 ± 0.06			0.50 ± 0.04			

* (Series 3, n = 7). NH_4Cl for NaCl substitution, 20 mM, was applied.

jugates, preferential diffusion of nonionic species should lead to homologous variation of pH_i (i.e., alkalinization upon exposure to a weak base, acidification upon exposure to a weak acid) [6, 24]. Nominally CO_2/HCO_3-free media were used to avoid activation of HCO_3-dependent membrane transporters, especially the Na/HCO_3 cotransporter, whose conductance may overwhelm G_K [22].

Under selected experimental conditions, our data indicate that pH_o modulates mainly V_m, whereas pH_i chiefly modulates G_m. In a first experimental series, pH_o was raised or lowered by ± 1 pH U relative to the physiological value, pH_o 7.5. Changes of pH_o were attended by similar, though smaller, changes of pH_i by ≈ 0.3 pH U from the control state. At pH_o 7.5 and 8.5, pH_i was lower than the respective pH_o, whereas at pH_o 6.5, $pH_i \geqslant pH_o$. From measured V_m and pH_i values (Table 1), we assessed the electromotive force for proton entry into the cell at pH_o 8.5, at pH_o 7.5 and pH_o 6.5, i.e., ≈1, 28 and 50 mV, respectively. Thus, if fused giant proximal cells were endowed with a genuine H^+ conductance, as observed in OK cells [14], it could have deleterious effects on pH_i homeostasis, especially when the cells are put in an acid milieu and have a low membrane potential. The second point is that an increase of pH_o (and pH_i) leads to membrane hyperpolarization, increase in t_K and rise of G_m; conversely the fall of pH_o (and therefore, also pH_i) conveys membrane depolarization, fall of G_m and decrease of t_K. The dependence of t_K on pH_o has been reported elsewhere [26]. However, it is important to stress that changes of t_K were not necessarily proportional to changes in G_m: alterations of G_m are not solely related to fluctuations of G_K. Figure 2 suggests that G_m is more sensitive to alkaline pH_o than to acid pH_o. However, since pH_i varies in the same direction as pH_o, it is not yet clear which of the two is more important for G_m.

In a second protocol, we delivered acetic acid-

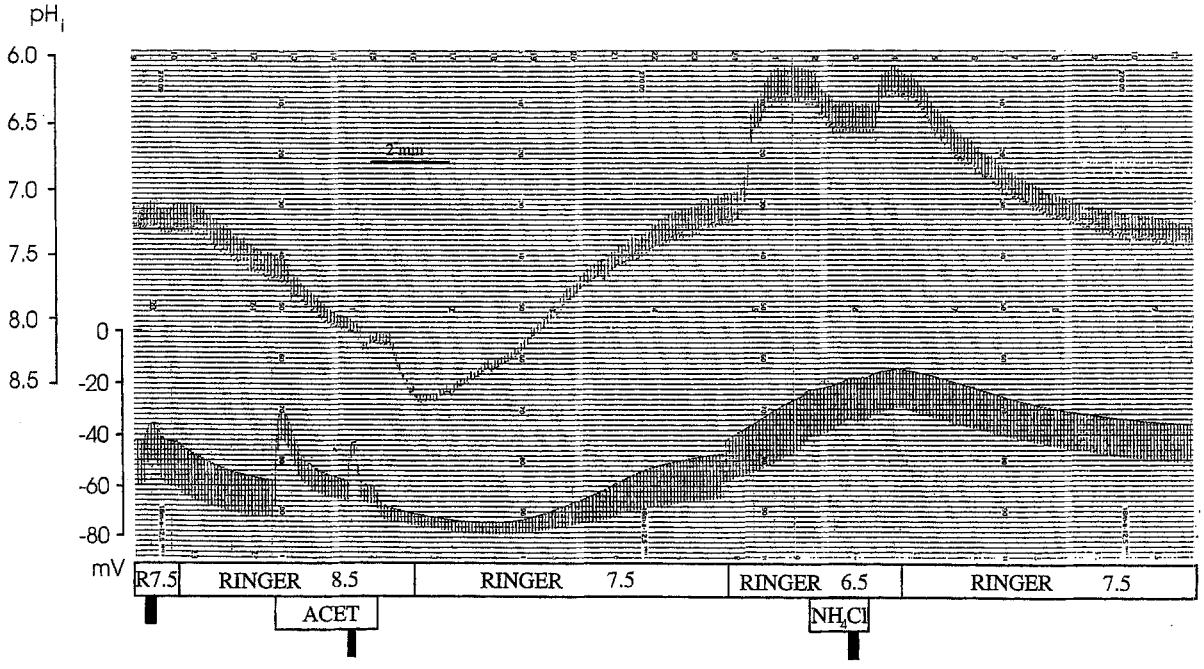

Fig. 8. Effects of adding 20 mM Na-acetate, at pH_o 8.5, and 20 mM NH_4Cl, at pH_o 6.5 on pH_i, V_m and G_m. Original recording from series 4. Symbols as in other figures.

pH 8.5a	pH 8.5HK	pH 8.5b	pH 8.5 NH_4a	pH 8.5 NH_4HK	pH 8.5 NH_4b	pH 8.5c	pH 7.5e
7.38 ± 0.06	7.45 ± 0.07	7.51 ± 0.08	8.66 ± 0.09	8.61 ± 0.05	8.60 ± 0.11	7.47 ± 0.10	7.29 ± 0.04
−60.9 ± 3.0	−47.2 ± 1.5	−64.9 ± 2.5	−62.7 ± 1.5	−46.1 ± 1.2	−60.3 ± 2.2	−56.4 ± 4.0	−46.4 ± 5.6
1.21 ± 0.10	1.88 ± 0.19	1.50 ± 0.20	2.85 ± 0.39	4.05 ± 0.44	2.53 ± 0.40	1.24 ± 0.11	1.24 ± 0.16
	0.49 ± 0.06			0.60 ± 0.03			

acetate-containing solutions at physiological and at acid pH_o. Whereas we anticipated cytoplasmic acidification in the presence of the acetate solution, we observed an increase in pH_i at pH_o 7.5; similar findings were reported in the rabbit [13] and also in the amphibian PCT, where lactate elicits an alkalinization [25]. In both cases, the rise of pH_i is likely mediated by entry of the conjugate pair *via* the Na-monocarboxylate transport system. Changes in V_m were only transient.

However, at pH_o 6.5, exposure to an acetate solution led to significant and sustained depolarization and cell acidification, achieving iso-pH across the cell membrane. Under these conditions, G_m does not behave consistently: in a single preparation, G_m was finite, and/or genuinely infinite, in a reversible fashion. Two mechanisms may contribute to generate the low pH_i associated with a very high G_m: (i) at pH_o 6.5, the concentration of the nonionic form (weak acid) is increased 10-fold with regard to pH_o 7.5, the diffusion of this nonionic form may contribute to the observed acidification; (ii) the activation of a voltage-dependent cationic conductance [11] may account for the large depolarization. Alternatively, acidification, as well as depolarization, could account for a greater "leak" for protons.

At this stage, two issues emerge. First, at pH_o 7.5, withdrawal of an acetate solution increases G_m without altering V_m; the increase of G_m appears to be related to the cytoplasmic alkalinization. The data from the first series already underlined the sensitivity of G_m to pH, yet discrimination between pH_i *vs.* pH_o was not possible. It now seems clear that G_m is sensitive to pH_i rather than to pH_o. Second, at pH_o 6.5, addition of acetate leads to a steep acidification, whereby $pH_i \approx pH_o \approx 6.5$, to a sharp depolarization ($V_m \approx -12$ mV) and to a large increase in G_m. To get insight into the mechanism(s) underlying the activation of membrane conductance in the presence of the Na-acetate solution at pH_o 6.5, two cells exhibiting an infinite conductance were further studied: G_m fell to a measurable value when 80 mM sucrose was added on top of the saline solution (*data not shown*). This suggests that the increase of G_m was related to cell distension subsequent to an influx of acetic acid/acetate. It is noteworthy that amphibian proximal cells are endowed with stretch-activated channels [9]. Activation of a cationic, stretch-sensitive conductance may contribute to the observed depolarization (V_m drifts towards E_{cat}), especially during addition of acetate at pH_o 6.5, and this could be a means to preserve cellular homeostasis [11].

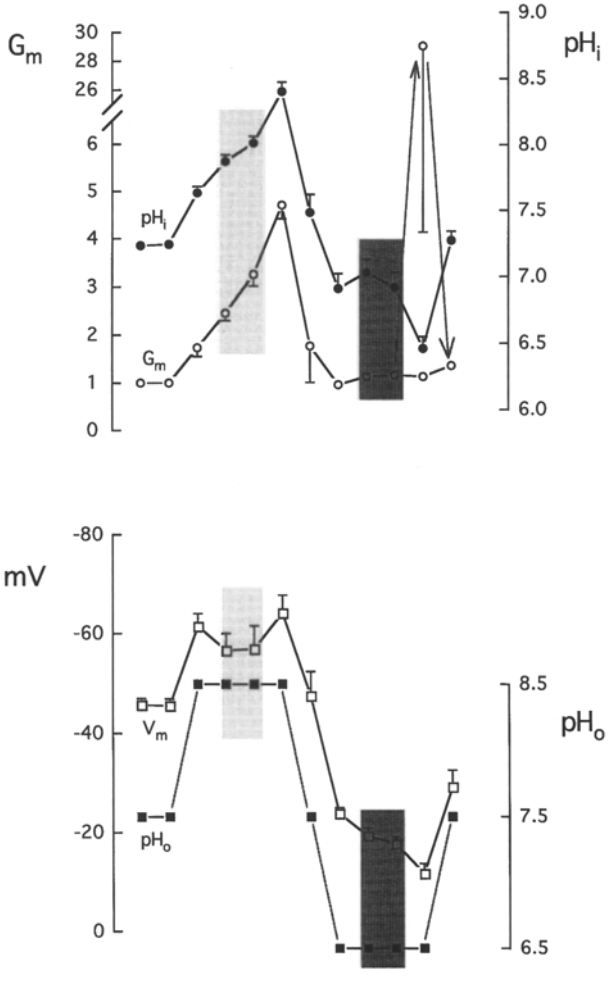

Fig. 9. Representation of the principal steps of the fourth series of this study. Note a large increase in conductance upon withdrawal of the NH_4Cl solution. Light and heavy rectangles, correspond to solutions Na-acetate and NH_4Cl (20 mM each), respectively.

Table 4. pH_i, V_m, G_m and t_K as a function of pH_o

pH_o	pH 7.5a	pH 7.5	pH 7.5b	pH 8.5a	pH 8.5	pH 8.5	pH 8.5
		HK			acet a	acet HK	acet b
pH_i	7.23 ± 0.03	7.26 ± 0.03	7.24 ± 0.03	7.63 ± 0.05	7.87 ± 0.05	7.96 ± 0.06	8.01 ± 0.05
V_m	−45.7 ± 1.3	−38.0 ± 1.1	−45.5 ± 1.4	−61.4 ± 2.6	−56.6 ± 3.4	−41.0 ± 2.5	−56.9 ± 4.6
G_m	1.00	1.35 ± 0.03	1.00 ± 0.01	1.73 ± 0.18	2.45 ± 0.15	4.59 ± 0.41	3.26 ± 0.24
t_K		0.28 ± 0.02				0.56 ± 0.04	

Acetate, 20 mM, was introduced into an alkaline solution, whereas NH_4Cl, 20 mM, was bathed in acid media. (Series 4, $n = 7$, except in columns 10, 14 and 15, $n = 6$).

In the third protocol, we introduced the couple NH_3/NH_4^+ at physiological or alkaline pH_o (+1 pH U). The use of an ammonium pulse is a routine procedure [18, 23] and has already been used in our experimental model [12]: as expected, we initially observed cell alkalinization, followed by acidification upon withdrawal of the NH_3/NH_4 solution. In this protocol, as well as in series 1 and 2, the pH_i increase was associated with a G_m rise. At pH_o 7.5, withdrawal of NH_3/NH_4, lowered pH_i to 6.88 and G_m fell to 70% of control; similar values for pH_i, 6.87 and G_m, 72% of control, were obtained in protocol 1 during exposure to cells at pH_o 6.5. The major difference between these two states resides on the value of V_m (−40.9 ± 3.6 *vs.* −29.1 ± 2.7 mV), suggesting that V_m is sensitive to pH_o rather than to pH_i. Up to this point, it appears that G_m is modulated by pH_i whereas V_m is sensitive to pH_o. These conclusions are reinforced by the data obtained with the fourth experimental protocol.

In the last experimental series we used a physiological solution, containing Na-acetate at pH_o 8.5 and NH_4Cl at pH_o 6.5. The highest alkalinization in this protocol was 8.40. Upon withdrawal of the Na-acetate solution, we observed the highest G_m (G_m = 4.71); recovering to the control solution, at pH_o 8.5, pH_i was 7.63 and G_m 1.73, yet once again, V_m was not significantly different from the paired control (−61.4 ± 2.6 *vs.* −64.1 ± 3.6 mV). This observation confirms that pH_i influences G_m without a major effect on V_m. The deepest intracellular acidification (pH_i 6.46) was obtained upon withdrawal of NH_4Cl. As in protocol 2, where V_m was deeply depolarized, we witnessed a quasi-equality of pH_o and $pH_i \approx 6.5$ associated with a large increase of G_m, whereas V_m was steeply depolarized to −11.7 mV. The actual nature of the signal that activates a membrane conductance is presently under investigation.

From the composite data, it appears that V_m is mainly modulated by pH_o, with high sensitivity at acid pH_o, relative to alkaline pH_o, whereas G_m is chiefly modulated by pH_i. Few studies have been devoted to differential effects between pH_i and pH_o. A recent study on glomus cells [17], showed that V_m is sensitive to pH_i rather than pH_o; this suggests a different regulation in that preparation. A second piece of evidence emerges: as shown in Fig. 10, t_K is strongly potential dependent. Last, it appears that the modulation of G_m by pH_i (increase of G_m at alkaline pH_i, fall of G_m at acid pH_i) may be possibly overwhelmed to preserve cell homeostasis: indeed, we observed the activation of a conductance triggered beyond a breakpoint, apparently defined by a range of V_m values of ≈−10 to 0 mV and of a pH_i of ≈6.5.

In conclusion, our study shows differential regulation of V_m and G_m by pH_o and pH_i. This differential regulation could allow a subtle modulation among rheogenic transporters of the proximal cell during pathophysiological states, during metabolic acidosis and alkalosis.

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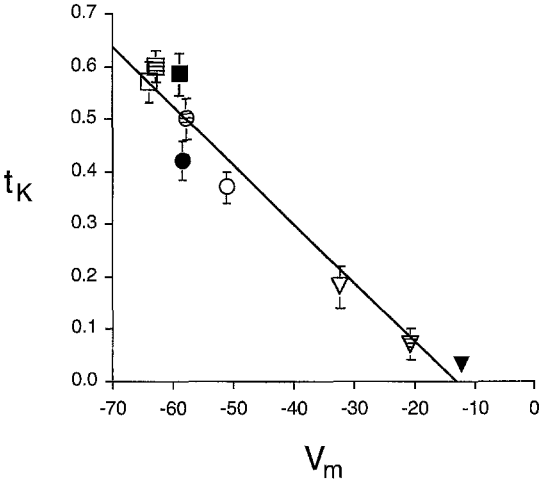

Fig. 10. t_K dependence as a function of V_m. The regression line is defined by the equation $t_K = -0.011\ V_m - 0.149$, with $r^2 = 0.947$. Open symbols: physiological solution; filled symbols: acetate; horizontal bars: NH_4Cl; circles: pH_o 7.5; squares: pH_o 8.5; triangles: pH_o 6.5.

pH 8.5b	pH 7.5c	pH 6.5a	pH 6.5a	pH 6.5	pH 6.5	pH 6.5b	pH 7.5d
			NH_4	NH_4HK	NH_4b		
8.40 ± 0.07	7.48 ± 0.14	6.91 ± 0.11	7.03 ± 0.10	7.02 ± 0.10	6.92 ± 0.11	6.46 ± 0.09	7.27 ± 0.07
−64.1 ± 3.6	−47.5 ± 4.9	−23.8 ± 1.2	−19.3 ± 1.6	−18.0 ± 1.5	−17.7 ± 1.4	−11.7 ± 2.0	−29.2 ± 3.4
4.71 ± 0.29	1.77 ± 0.76	0.96 ± 0.10	1.13 ± 0.09	1.22 ± 0.10	1.16 ± 0.10	15.1 ± 8.9	1.36 ± 0.08
				0.05 ± 0.01			

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