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Fused Cells of Frog Proximal Tubule: I. Basic Membrane Properties

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Summary. Proximal tubular cells of the frog (*Rana esculenta*) kidney were fused within an isolated tubule portion to giant cells according to the polyethylene-glycol fusion method. Cell membrane potentials (V_m) were measured while cells were superfused with various experimental solutions. Rapid concentration step-changes of different ions allowed to calculate the respective transference numbers (t_{ion}). In some experiments the specific cell membrane resistances (R_m) were evaluated by measuring V_m induced by short current pulses injected into the cell with a second electrode. The experiments reveal: i) Fused cells of the proximal tubule exhibit a V_m of -49.5 ± 1.6 mV ($n = 65$). ii) Addition of glucose to the perfusate yields a transient depolarization, consistent with a rheogenic Na/glucose cotransport system. iii) In absence of organic substrates the whole cell membrane conductance is made up of K^+ and HCO_3^-. iv) There is a positive relationship between V_m and tK^+ and a negative relationship between V_m and $tHCO_3^-$. v) HCO_3^--induced V_m changes are attenuated or abolished when Na^+ is replaced with choline$^+$, consistent with a rheogenic Na^+/HCO_3^- cotransport system. vi) Replacement of Na^+ by choline$^+$ depolarizes V_m and increases R_m by about 50%; addition of 3 mmol/liter Ba^{2+} to the Na^+-free perfusate increases R_m by about 58% compared to the initial control value. vii) There is no measurable cell membrane Cl^- conductance. We conclude that fused cells of proximal tubule exert both luminal and peritubular membrane properties. In absence of organic substrates the cell membrane potential is determined by the HCO_3^- and K^+ transport systems.

Key Words frog kidney · proximal tubule · HCO_3^- transport · K^+ conductance · Cl^- conductance · fusion

Introduction

The evaluation of cell membrane conductances in a leaky epithelium like the proximal tubule is rendered difficult by the existence of large paracellular shunt-conductances and thus generation of intraepithelial current loops. Single cells isolated from renal tubules, however, do not tolerate intracellular impalements [20]. In an attempt to avoid these problems cells of the proximal tubule were fused to "giant cells" according the the polyethylene-glycol method [25, 26], previously described for distal tubular cells of the frog kidney [19, 20]. Here we describe the technique used to fuse single epithelial cells *within* an individual proximal tubule portion and characterize the basic cell membrane properties. The experiments show that fused proximal tubule cells display both luminal and peritubular cell membrane properties. The HCO_3^- conductance is the predominant cell membrane conductance at control conditions. The findings are consistent with a rheogenic Na^+/HCO_3^- cotransport system.

Materials and Methods

Tubule Fusion Technique

Fusion of renal epithelial cells *within* an isolated tubule portion was described recently [19]. With this fusion technique cells are initially *not* completely separated from each other but remain linked to each other (by their tight junctions) when fused. Briefly, kidneys of *Rana esculenta* were first isolated and perfused with amphibian Ringer's solution composed of (mmol/liter): 97 NaCl, 3 KCl, 5 HEPES, 1 $CaCl_2$, 1 $MgCl_2$, 5 glucose. Please note that Ca^{2+} ions have *not* been removed from the perfusate in contrast to the fusion technique published previously [20]; thus tight junctions remained intact. After the blood was rinsed off, 3 ml of the same solution containing 0.1% collagenase (Sigma, München, FRG, or Seromed, Biochrom KG, Berlin, FRG; 180 to 300 U/mg) was injected. With fine scissors, the dorsal surface of both kidneys containing primarily proximal tubules was removed in small pieces and incubated for 50 min in the collagenase-containing solution. Then, the pieces were gently sucked up and down with an Eppendorf pipette until a suspension of small tubule fragments was obtained. The tubules were centrifuged (400 × g, 2 min) and the supernatant was removed. One ml of the fusion medium was slowly added (30% polyethylene glycol, M_r 4000, dissolved in Leibovitz 15 medium,

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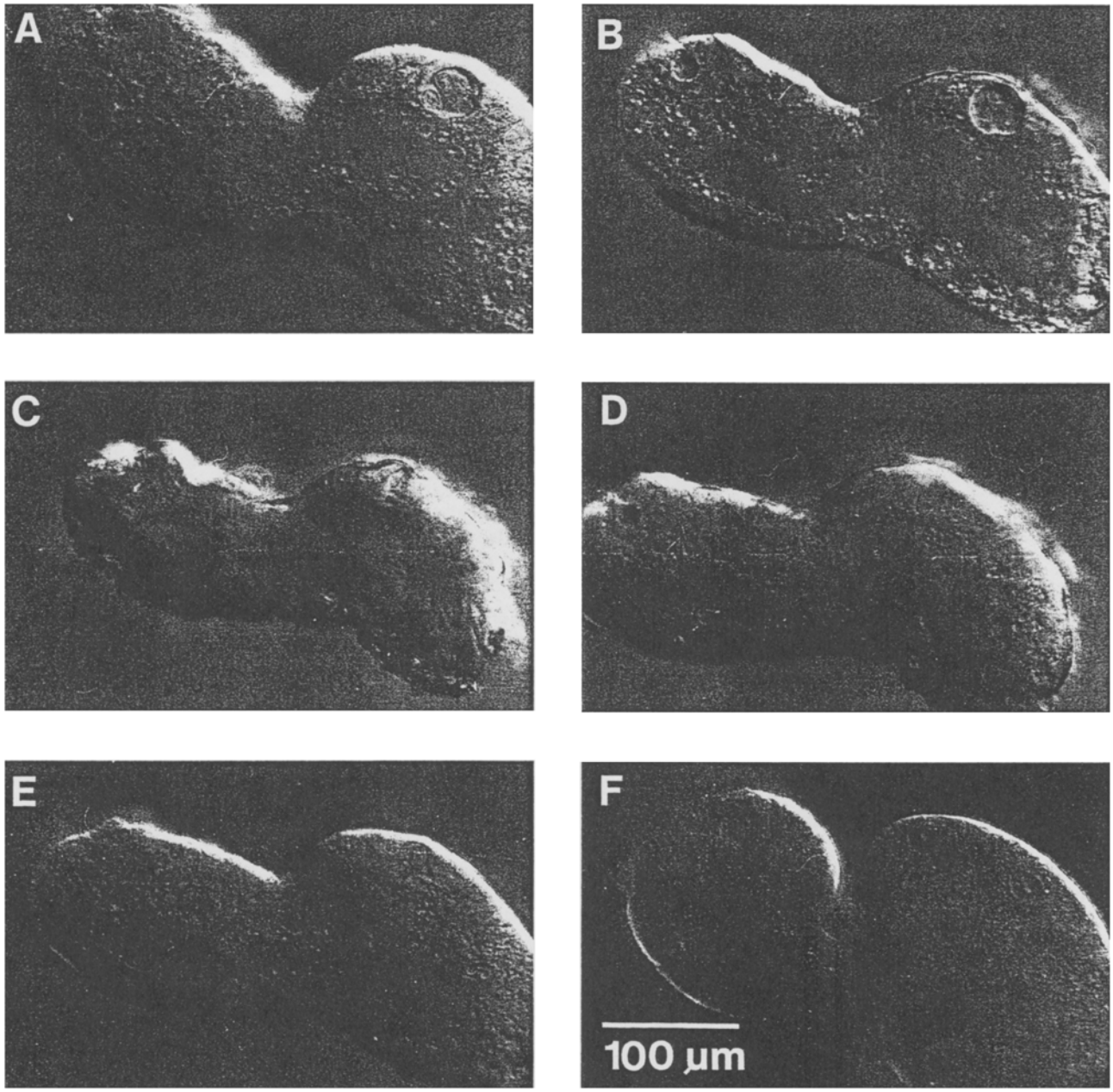

Fig. 1. The various steps of cell fusion within an individual proximal tubule segment. (*A*) Tubule in culture medium (Leibovitz- 15) after isolation from the collagenase-treated frog kidney (for details *see* ref. 19). (*B*) Superfusion with fusion medium containing 30% polyethylene glycol (PEG). (*C*) Dehydrated tubule 3 min after exposure to PEG. (*D*) Omission of PEG and rehydration. (*E*) Tubule a few hours after PEG treatment. (*F*) Fused tubule after PEG treatment kept in culture medium at 6°C for 48 hr. Please note, that the tubule approaches spherical shape

diluted to 170 mosmol/liter, pH 8.2), and the cells were again centrifuged (400 × *g*, 2 min). Finally, they were suspended in 1 ml of Leibovitz 15 medium (200 mosmol/liter, pH 7.8) and transferred onto a thin microscope coverslip pretreated with poly-L-lysine (0.1 g/liter; Serva, Heidelberg, FRG). At this time most tubule fractions still had a clearly visible lumen and a cylindrical shape. Cell borders were well visible (630-fold magnification, Normarsky optics). Then, they were incubated for 24 to 48 hr at 6°C. Over this time period membrane fusion proceeded until some of the tubules were homogeneous and often round-shaped. The individual steps of the tubule fusion technique are shown in Fig. 1. Then, the fused tubules were ready for electrophysiological studies using differential interference contrast microscopy (inverted microscope, IM 35, Fa. Zeiss, Oberkochen, FRG, objective 63/1.4, oil).

In order to test whether fusion between cells was incomplete or complete we used intracellular iontophoretic injections of the fluorescence dye lucifer yellow. This method is described

Table. Composition of the perfusion solutions[a]

Parameter	I	II	III	IV	V	VI	VII	VIII
NaCl	90	78	80	48			92.5	
KCl	3	15	3	3	3	3	3	3
$MgCl_2$	1	1	1	1	1	1	1	1
$CaCl_2$	1	1	1	1	1	1	1	1
$NaHCO_3$	10	10	20	10				
Na gluconate				42				
Choline Cl					90	80		
Choline HCO_3				10	20			10
Mannitol								165
HEPES							5	
pCO_2 (%)	1	1	1	1	1	1		1
pH	7.6	7.6	8.0	7.6	7.6	8.0	7.6	7.6

[a] Parameters are given in mmol/liter if not otherwise indicated. Glucose (5.5 mmol/liter) was used as the organic substrate and applied in solution I between the experimental periods. Solution IV was titrated with $CaCl_2$ (Ca^{2+} binds to gluconate) to obtain a Ca^{2+} activity (measured with a Ca^{2+} macroelectrode) identical to that of the other solutions. If applicable $BaCl_2$ (3 mmol/liter) was added to the perfusates (exchanged for NaCl).

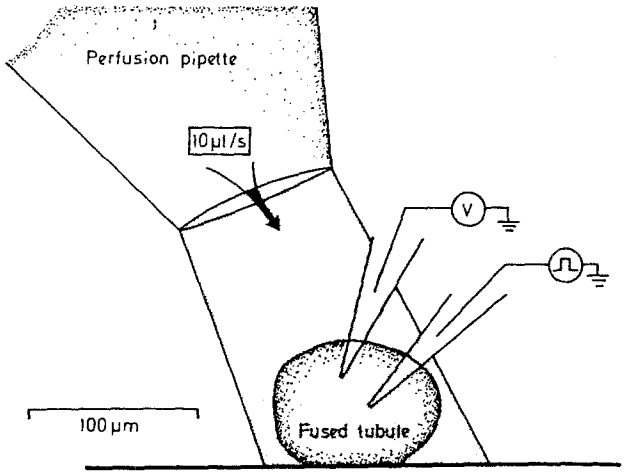

Fig. 2. Experimental technique. The fused tubule is attached to the glass surface of the perfusion chamber. Cell voltage is measured by one microelectrode while another microelectrode delivers short current pulses. The tubule under study is superfused by means of a multi-channel perfusion system. The mouth of the perfusion pipette is located close to the tubule in order to perform rapid changes of the extracellular fluid

in detail elsewhere [19]. By using the high resolution Nomarsky-optics we were able to disclose any cell membranes left in the intracellular compartments of incomplete cell fusions. Those cells were not used for the experiments.

Perfusion System and Perfusion Solutions

Cells were superfused via a multichannel pipette, its mouth being placed some 100 µm away from the cell under study (Fig. 2). Cells were superfused with high perfusion rates (intact cells stick firmly to the glass surface) and complete changes of the perfusion solution were achieved within less than a second. The control and the experimental perfusion solutions are summarized in the Table.

Measurement of Cell Membrane Potential (V_m) and Selection of Cells

V_m was measured using conventional microelectrodes filled with 1 mol/liter KCl, connected to a high-impedance electrometer (Fa. Frankenberger, Germering, FRG). Tip diameter was <1 µm, the resistance between 60 and 120 MΩ. Cells (i.e. fused tubules) were used for electrophysiological evaluation that appeared completely fused (vide supra). Optical control of the cell morphology, on the one hand, and electrical control by the V_m measurement, on the other hand, revealed that most cells with a $V_m < 35$ mV were damaged (cytoplasmic vascuoles, swelling of nuclei, etc.). Therefore, only stable cell membrane recordings with a V_m of at least −35 mV or more were used for our study.

Calculation of Transference Numbers

The transference number of an ion (t_{ion}) was calculated according to the equation:

$$t_{ion} = \Delta V_m/(RT/zF)\ \ln(c_1/c_2)$$

where V_m is the change of the electromotive force of the cell membrane upon a concentration step of an individual ion. c_1 and c_2 refer to the ion concentration before and after the step. R, T, z and F have their usual meanings.

It is of paramount importance that the concentration step is performed fast to avoid secondary phenomena (e.g. changes of intracellular electrolytes) that occur during the V_m measurement. In our case, concentration steps were completed within fractions of a second.

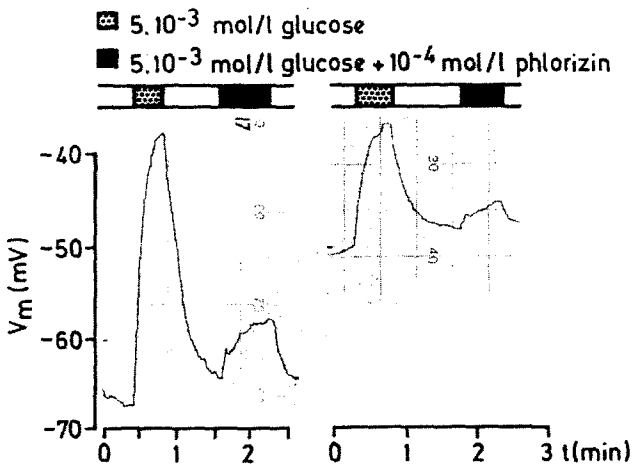

Fig. 3. Original registration of V_m in two different cells. Note the depolarization upon addition of 5.5 mmol/liter glucose and its inhibition during application of 0.1 mmol/liter phlorizin

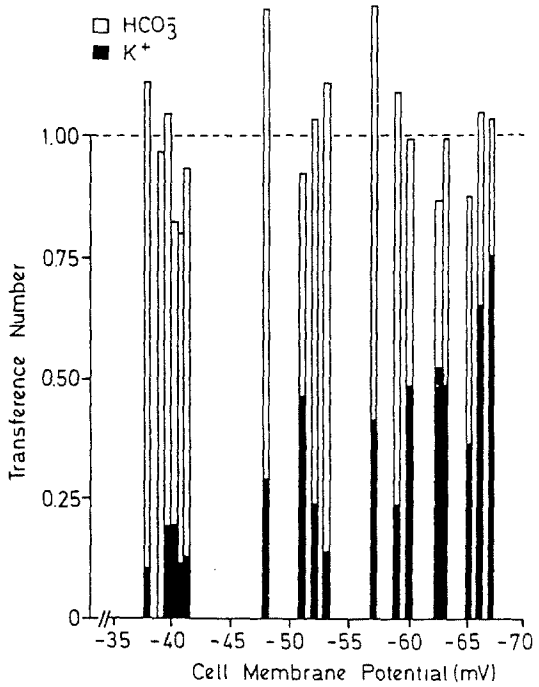

Fig. 4. Paired measurements of K^+ and HCO_3^- transference numbers (tK^+, $tHCO_3^-$) in individual fused cells. The dark part of each column represents tK^+, the light part represents $tHCO_3^-$. Note that the sum of both approaches unity, regardless of the magnitude of V_m

Measurement of the Cell Membrane Resistance (R_m)

R_m was measured by means of two microelectrodes inserted into the fused cell. With one electrode current pulses (10^{-9} to 10^{-8} A, 0.5 sec) were injected while V_m was recorded with the other electrode. The cell input resistance was calculated according to Ohm's law:

$$R_m = \Delta V_m / I$$

where V_m is the cell membrane potential deflection in response to the injected current pulse (I).

Then R_m was related to the cell surface (S):

$$R_m\ (\Omega\ \text{cm}^2) = R_m\ (\Omega) \cdot S\ (\text{cm}^2).$$

Since fused cells were approximately spherical, S was calculated as the surface of a sphere:

$$S = 4\pi r^2$$

where r (measured optically) is the radius of the sphere.

Statistics

Statistical analyses were performed using paired or unpaired Students' t-tests. Results are given as means ± standard error of the mean (M ± SEM). Differences were considered statistically significant when P was less than 0.05.

Results

Cell membrane potentials were measured successfully in 65 fused cells. The mean value of V_m was −49.5 ± 1.6 mV. The criteria of reliability were: (i) spontaneous V_m of at least −35 mV, obtained in solution I, and (ii) no visible morphological changes while recording V_m (cytoplasmic vacuoles, membrane blebs, cell swelling).

Rheogenic Glucose Transport

It was of interest whether fused cells express both luminal and peritubular membrane properties. Therefore, we tested for the existence of a classical luminal transport system, namely Na^+-coupled glucose transport. As shown in Fig. 3 (original registrations), the cell membrane depolarizes by 9.5 ± 2.7 mV (n = 10) upon addition of 5.5 mmol/liter glucose to the glucose-free perfusate (solution I). This V_m change indicates a rheogenic glucose entry (i.e. glucose coupled to Na^+) [13]. It is largely suppressed by 10^{-4} mol/liter phlorizin (ΔV_m = 2.1 ± 0.8 mV, n = 6), a specific blocker of the Na^+ glucose cotransport system [5, 9, 23]. We conclude that fused cells exhibit luminal membrane properties.

K^+ and HCO_3^- Transport

Proximal tubules are characterized by luminal [11] and peritubular [2, 15–18] K^+ conductances and by

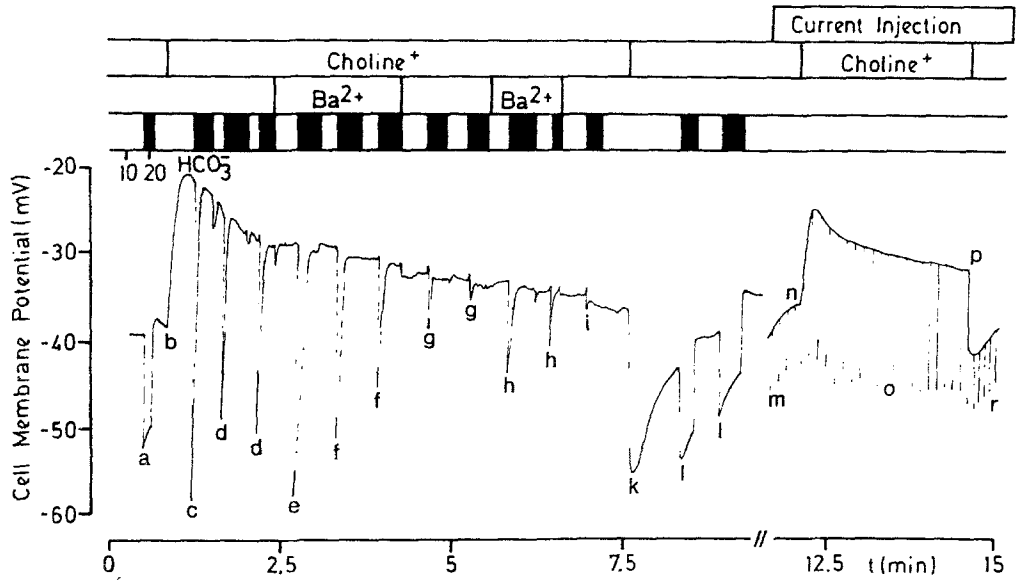

Fig. 5. Original registration of V_m. Note the decline of the V_m induced by HCO_3 concentration steps as a function of time. Ba^{2+} (3 mmol/liter) magnifies the HCO_3-induced ΔV_m, consistent with its inhibitory action on K^+ conductance. Choline (substituted for Na^+) increases R_m indicated by the larger current-induced voltage deviations. (*a*) HCO_3 concentration step change in presence of Na^+; (*b*) removal of Na^+; (*c*) first HCO_3 step in Na^+-free solution; (*d*) two additional HCO_3 steps; (*e*) first HCO_3 step in presence of Ba^{2+}; (*f*) two additional HCO_3 steps in presence of Ba^{2+}; (*g*) experimental condition identical to (*d*), however, the cell was exposed already for about 3 min to the choline$^+$ (Na^+-free) perfusate; (*h*) experimental condition as in (*f*) but with prolonged exposure to the Na^+-free perfusate; (*i*) HCO_3 step after removal of Ba^{2+}; (*k*) readdition of Na^+; (*l*) two subsequent HCO_3 steps; (*m*) current-induced voltage-changes in presence of Na^+; (*n*) removal of external Na^+; (*o*) current-induced voltage changes in absence of Na^+ (substituted by choline$^+$); (*p*) readdition of Na^+; (*r*) identical to (*m*)

a peritubular HCO_3^- transport system. We asked the following question: How are K^+ and HCO_3^- conductance related to each other and to what extent do they contribute to the maintenance of V_m? Such a relationship is documented in Fig. 4. Conductances are expressed in terms of transference numbers. K^+ and HCO_3^- transference numbers were both evaluated in one individual fused cell. It is obvious that (i) the sum of tK^+ and $tHCO_3^-$ approaches unity in each individual cell, irrespective of V_m and that (ii) there is a positive relationship between V_m and tK^+ and an inverse relationship between V_m and $tHCO_3^-$. In absence of organic substrates, both K^+ and HCO_3^- conductances are the major and the only (vide infra) conductances, irrespective of V_m.

Na^+ Dependence of the HCO_3^- Conductance

There is accumulating evidence that HCO_3^- transport is coupled to Na^+ [3, 4, 6, 10, 27]. Such a coupling was examined in our preparation by performing ion substitution experiments in presence and absence of the respective counter-ion. Choline$^+$ served as the Na^+ substitute; HEPES-buffered solutions served as HCO_3^--free perfusates.

a) HCO_3^- Concentration Step Changes in Presence and Absence of Na^+

During control conditions (in presence of Na^+) a rapid HCO_3^- concentration step from 10 to 20 mmol/liter (i.e. from sol. II to III) results in a hyperpolarization of -12.5 ± 0.9 mV ($n = 19$). This corresponds to a $tHCO_3^-$ of 0.7 ± 0.05 ($n = 19$). This hyperpolarization is a transient one, although its steady-state value (following the peak value) is also far above the control ($V_m = -8.3 \pm 1.5$ mV, $n = 14$). When Na^+ in the perfusate is substituted by choline$^+$ (sol. II is substituted by sol. V) both the shape as well as the magnitude of the same HCO_3^- step is drastically altered (*see* Figs. 5 and 6). Initially, after Na^+ removal the same HCO_3^- step produces a much higher hyperpolarization as compared to control conditions. The peak is transient and V_m returns either completely (Fig. 5) or partially (Fig. 6) to the initial value. In the course of 10 to 20 min (performing HCO_3^- steps repeatedly) this peak decreases continuously in amplitude and finally disappears completely (*see* Fig. 5) or remains at a (constant) reduced amplitude (*see* Fig. 6). Since the first HCO_3^- step after Na^+ replacement exhibits a "hyper-Nernstian" response ($\Delta V_m = -27 \pm 3.7$ mV, n

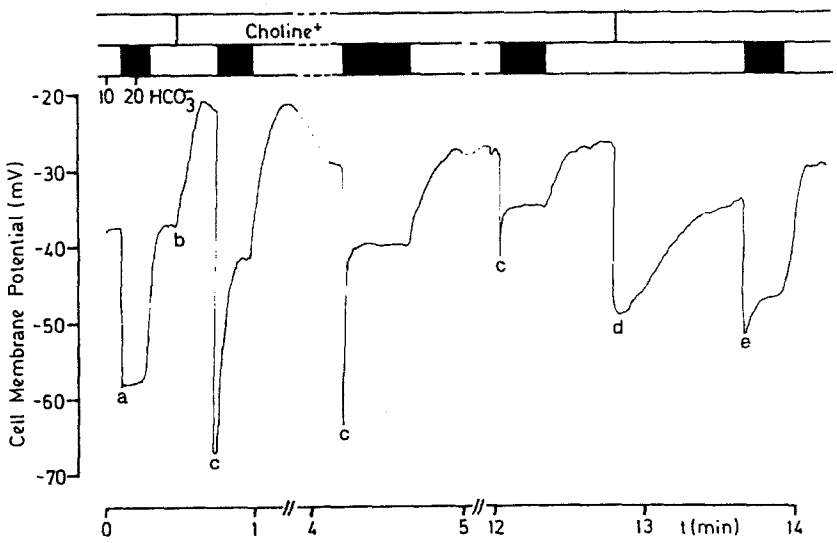

Fig. 6. Original registration of V_m. Extracellular HCO_3^- concentration steps (from 10 to 20 mmol/liter) induce large transient hyperpolarizations. Choline$^+$ (substituted for Na^+) depolarizes V_m. The HCO_3^--induced ΔV_m in the early phase of choline treatment is increased (*c*) compared to control conditions (*a*) but then decreases with time. (*a*) HCO_3^- concentration step change in presence of Na^+; (*b*) removal of extracellular Na^+; (*c*) HCO_3^- steps in absence of Na^+; (*d*) readdition of Na^+; (*e*) HCO_3^- step as in (*a*)

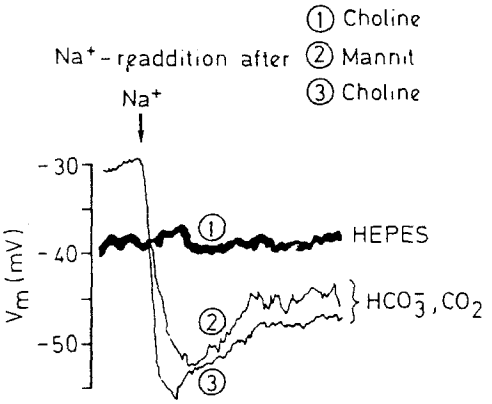

Fig. 7. Original registrations of V_m in an individual fused cell. Note the different response upon Na^+ readdition to the Na^+-free solution in presence and absence of HCO_3^-

= 12), we assumed at first sight that a secondary effect was involved. In order to exclude a possible increase of V_m due to intracellular alkalinization and thus increase of the K^+ conductance [2, 12] during the HCO_3^- step, 3 mmol/liter Ba^{2+} was added to the perfusate. Figure 5 shows that Ba^{2+} cannot reduce the magnitude of the transient hyperpolarization. This led to the conclusion that the HCO_3^- step itself (and not a putative activation of the K^+ conductance by intracellular alkalinization) is responsible for the phenomenon and that it was not appropriate to consider simple Nernstian response when dealing obviously with a HCO_3^- transport system involving a charged carrier.

Nevertheless, the disappearance of the HCO_3^--induced hyperpolarization with time in Na^+ free solutions is consistent with rheogenic Na^+-coupled HCO_3^- transport as initially described by the elegant studies of Boron and Boulpaep [4].

b) Na^+ Removal and Na^+ Readdition in Presence and Absence of HCO_3^-

As shown in Figs. 5 and 6, Na^+ replacement by choline$^+$ consistently results in a depolarization (ΔV_m = 14.2 ± 1.3 mV, n = 14) applying HCO_3^--containing solutions. This depolarization is more or less transient, and sometimes slowly recovers to the control V_m within 5 to 15 min. Conversely, Na^+ readdition results in a hyperpolarization that is transient exhibiting a peak ΔV_m of −22.5 ± 3.4 mV, n = 14 (Figs. 5, 6, and 7). Figure 7 demonstrates that readdition of Na^+ to the perfusate hyperpolarizes V_m independently whether Cl^- is present or not (sol. VII). However, the hyperpolarization is virtually absent with HCO_3^--free (HEPES-buffered) perfusates. Na^+ readdition only yields a ΔV_m of −1.8 ± 1 mV, n = 5, in HEPES-buffered solutions. This is indeed strong evidence for a negatively charged Na^+/HCO_3^- cotransport system.

c) R_m in Control Conditions and During Choline$^+$ (± Ba^{2+}) Exposure

As illustrated in Fig. 5 addition of 3 mmol/liter Ba^{2+} to the choline$^+$-containing solution has no further marked effect on V_m. Ba^{2+} does, however, depolarize the fused cells by 14.3 ± 2.6 mV (n = 5) in control conditions (sol. I). This leads to the conclusion that the K^+ conductance is severely diminished by choline$^+$ exposure. In order to test this assumption the specific cell membrane resistances (R_m) were measured during control conditions (sol. I), during choline$^+$ exposure (sol. V) and during choline$^+$ plus Ba^{2+} exposure. The fused cell was im-

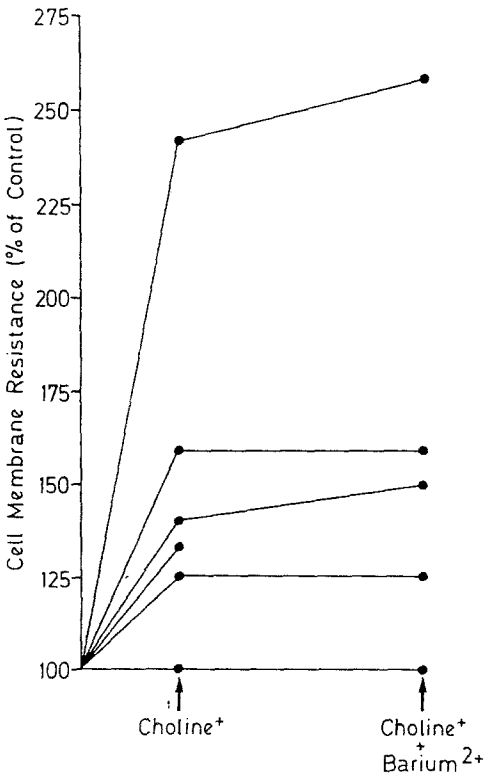

Fig. 8. Cell membranes resistances related to their individual control values in presence of choline$^+$ (± 3 mmol/liter Ba^{2+}). Please note that Ba^{2+} does not enhance R_m to a significant extent

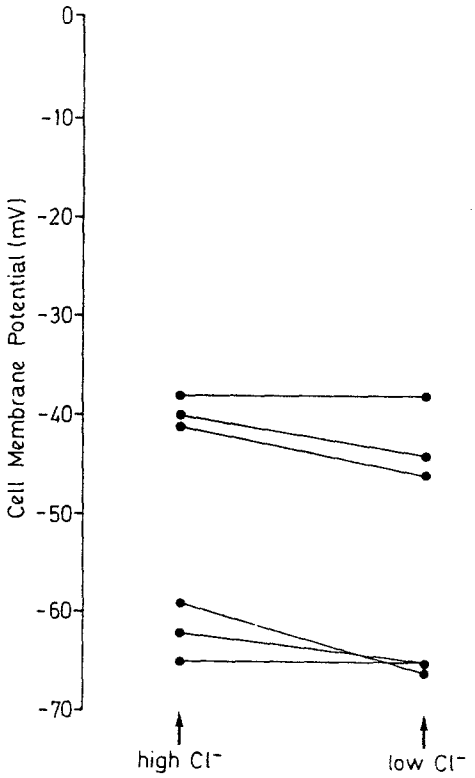

Fig. 9. Cell membrane potential (V_m) during control (high Cl^-) conditions (104 mmol/liter Cl^-) and after replacement of 42 mmol/liter Cl^- by gluconate$^-$ (low Cl^-). The values are corrected for liquid junction potentials

paled with two microelectrodes (Fig. 2) and R_m was evaluated at the various conditions. Six successful experiments were performed. Figure 8 shows the individual resistance changes. There is a consistent (with the exception of one experiment) increase of R_m when Na^+ is substituted by choline in the bath solution. The fact that the addition of Ba^{2+} could not further increase R_m to a significant extent leads to the conclusion that the cell membrane K^+ conductance is already severely diminished in choline-containing solutions.

Cl^- Conductance

In order to evaluate a Cl^--conductive pathway in fused proximal cells, Cl^- concentration steps were performed in the bath solution by substituting 42 mmol/liter NaCl by Na gluconate (sol. IV). This should, in case of a Cl^- conductance, yield a transient *depolarization,* as shown in fused cells of the diluting segment [24]. Figure 9 demonstrates, however, that fused proximal cells respond rather with a small *hyperpolarization* after reduction of extracellular Cl^- concentration. This argues against a significant Cl^- conductance in proximal tubule cells.

Discussion

Cell Fusion within Intact Tubules

The introduction of the cell fusion technique for the electrophysiological investigation of epithelial transport raised the question whether transport systems of *both sides* of the initially polarized epithelium are still present and in function. Now we have evidence, that fused cells indeed exhibit both luminal and peritubular cell membrane properties. The rheogenic Na^+/glucose cotransport system and its inhibition by phlorizin can be clearly attributed to the luminal cell membrane [9]. The HCO_3^- transport system is a characteristic transport property of the peritubular membrane [4, 15, 27]. Instead of isolating the cells first and then (after separation) fusing them to large cells, proximal cells were fused directly *within* short tubule fragments. The only (but important) difference between this technique and the fusion technique published previously [20] is that the tight junctions are left intact. Thus linkage between cells is still present whereas collagenase-treatment enzymatically denudates the cell surfaces, the most important parameter for successful

cell-to-cell fusion. This method [19] yields a more homogeneous preparation since contamination with other cell types (distal tubule, collecting duct, etc.) is avoided. Although the size of a "fused tubule" allows simultaneous intracellular impalements with two or more microelectrodes, the polarity has obviously vanished. Therefore, the direction of transport cannot be studied with this model.

$tHCO_3^-$ and tK^+ Determine V_m

There is a positive relationship between the magnitude of V_m and tK^+ (this was also found by Biagi and Sohtell in the rabbit proximal tubule [3]) and a negative relationship between V_m and $tHCO_3^-$. This could, of course, reflect a difference in the cell membrane equipment concerning K^+ channels and HCO_3^- transport proteins. Then, this relationship should determine V_m. There is, however, also evidence for the assumption, that the magnitude of V_m itself determines the K^+ conductance. This was already demonstrated by Messner et al. [17, 18]: Depolarization induced either by phenylalanine, by ouabain, or by current injection into the epithelium results in an increase of the cell membrane resistance. One of our observations supports this assumption: During choline$^+$ exposure (depolarization) Ba^{2+} has no marked effect on V_m.

The large $tHCO_3^-$ (mean = 0.7 ± 0.05, n = 19) of fused cells suggests that the rheogenic HCO_3^- transport system is the dominant conductive pathway of the proximal tubule and thus an important determinant of V_m. High $tHCO_3^-$ and small tK^+ have been reported already for the basolateral membrane of *Necturus* [15]. Also Messner et al. presented a low tK^+ of 0.3 in the frog proximal tubule [17]. It was shown recently that there is a functional heterogeneity along the amphibian proximal tubule [14]. Cells of the early portions are characterized by HCO_3^- transport whereas K^+ transport is dominant in the late portion of the proximal nephron. This is consistent with the highly variable ratios of $tHCO_3^-/tK^+$ found in this study.

Na^+-Dependent HCO_3^- Transport

There is strong evidence for a Na^+-coupled rheogenic (i.e. negatively charged) HCO_3^- transport system: (i) The cell membrane potential depolarizes when Na^+ is replaced by choline and hyperpolarizes, when Na^+ is readded. These effects are not evoked by choline$^+$ itself because mannitol, used as a substitute for NaCl does the same. From these observations, we can also exclude a significant Na^+ conductance: in this case Na^+ removal or Na^+ readdition should yield the opposite effects. As there were also no organic substrates present in the solutions, HCO_3^- is the only anion (sol. VII) that can be cotransported. ii) The cell membrane voltage changes, produced by HCO_3^- concentration steps from 10 to 20 mmol/liter are abolished (after some minutes) or diminished when Na^+ is replaced by choline$^+$. Nevertheless there remain at least two questions concerning the shape and amplitude of the HCO_3^--induced V_m changes in Na^+-free solutions: The slow disappearance of the HCO_3^--induced V_m changes (in virtually Na^+-free perfusates) can be interpreted by a high affinity of the transport carrier to Na^+ and a rather slow removal of extracellular Na^+ (e.g. unstirred layers close to the cell membrane). The increase of the peak hyperpolarization (compared to control) induced by increasing extracellular HCO_3^- from 10 to 20 mmol/liter could be interpreted as an increase of the K^+ conductance during the HCO_3^- step in absence of Na^+. The failure to inhibit this peak by Ba^{2+} clearly argues against this possibility. Derived from the present data we now interpret it as the consequence of the R_m increase during choline$^+$ exposure. The mechanism of the choline-induced loss of the cell membrane K^+ conductance is still unclear. Assuming that the K^+ channels are voltage-sensitive and their open probability is reduced at depolarized cell membrane potentials it is tempting to speculate that the choline-induced decrease of the K^+ conductance is caused by the drop in cell voltage. The R_m of 4.9 kΩ cm^2 in control conditions correlates well with cable analysis data: This specific cell membrane resistance obtained for the first time in an isolated proximal tubule cell is between values measured for the apical and basolateral cell membrane of *Necturus* by Guggino et al. [7] and is almost identical to the apical R_m of *Necturus* given by Matsumura et al. [16]. Choline$^+$ plus Ba^{2+} increase R_m only by about a factor of 1.5. This observation is consistent with the relatively small K^+ conductance of the cell membrane during control conditions. It also corresponds well to data obtained for the basolateral cell membrane of *Necturus* (by Planelles et al. [21]): This study reports an increase of R_m by the addition of Ba^{2+} from 3.5 kΩ cm^2 to 4.6 kΩ cm^2.

Lack of Cl^- Conductance

Hyperpolarization of V_m induced by extracellular reduction of Cl^- in proximal tubule was already reported for the proximal tubule by other investigators [1, 8, 22]. Such a hyperpolarization could be explained by the partial inhibition of a Cl^-/HCO_3^- exchange system (by reduction of the chemical

driving force for Cl^- to drive the carrier) and subsequent intracellular accumulation of HCO_3^-. This in turn would alkalinize the cytoplasm and increase the pH-sensitive K^+ conductance. Nevertheless, the hyperpolarization argues against a significant Cl^- conductance in the proximal tubule. Thus, transcellular Cl^- transport, if present at all, must be accomplished by an electroneutral transport mechanism.

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