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Cl^- Transport in Basolateral Renal Medullary Vesicles: II. Cl^- Channels in Planar Lipid Bilayers

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Summary. The present studies examined some of the properties of Cl^- channels in renal outer medullary membrane vesicles incorporated into planar lipid bilayers. The predominant channel was anion selective having a P_{Cl}/P_K ratio of 10 and a unit conductance of 93 pS in symmetric 320 mM KCl. In asymmetric KCl solutions, the *I-V* relations conformed to the Goldman-Hodgkin-Katz equation. Channel activity was voltage-dependent with a gating charge of unity. This voltage dependence of channel activity may account, at least in part, for the striking voltage dependence of the basolateral membrane Cl^- conductance of isolated medullary thick ascending limb segments. The Cl^- channels incorporated into the planar bilayers were asymmetrical: the *trans* surface was sensitive to changes in ionized Ca^{2+} concentrations and insensitive to reducing KCl concentrations to 10 mM, while the *cis* side was insensitive to changes in ionized Ca^{2+} concentrations, but was inactivated by reducing KCl concentrations to 50 mM.

Key Words Cl^- channels/bilayers · Cl^- channels/vesicles · thick ascending limb · rectification · channel conductance

Introduction

This paper contains the results of experiments intended to characterize the properties of single Cl^- channels fused from basolaterally enriched rabbit renal medullary vesicles into planar lipid bilayer membranes. In isolated mouse mTALH segments, the increase in basolateral membrane Cl^- conductance (g^b_{Cl}) produced by ADH may be referable to a hormone-induced depolarization of basolateral membranes [11, 13]. Or put differently, g^b_{Cl} in the mammalian mTALH may be rather voltage dependent. In order to characterize this Cl^- conductive pathway further, we have utilized two approaches.

In the preceding paper [1], we provided evidence that a conductive pathway accounted for about half of $^{36}Cl^-$ uptake into basolaterally enriched renal medullary vesicles. The energetics of diffusion through this conductive $^{36}Cl^-$ pathway were similar to those in free solution. The anion selectivity sequence for the $^{36}Cl^-$ conductive pathway was $I^- > Cl^- \geqq NO_3^- \gg$ gluconate. And the Cl^- channel blocker diphenylamine-2-carboxylate (DPC) inhibited conductive $^{36}Cl^-$ flux with a K_i of 154 μM. Taken together, these data are consistent with the possibility that conductive Cl^- flux in these vesicles was channel mediated.

In the present studies, we incorporated basolaterally enriched renal medullary vesicles into planar lipid bilayers. The predominant channel activity observed was a Cl^--permselective channel having a P_{Cl}/P_K ratio of approximately 10 and a unit conductance of about 93 pS in 320 mM KCl. In symmetrical solutions, the *I-V* relation was linear, and in asymmetrical solutions, the *I-V* relation showed Goldman-Hodgkin-Katz (GHK) rectification. Channel activity, that is, fractional open time (F_o), was voltage dependent with a gating charge slightly in excess of unity. When vesicles were added to the *cis* solution, the channels were active when *cis* solutions were 320 or 150 mM KCl, and inactive with *cis* solutions containing 50 mM KCl. However, channel activity was concentration independent for *trans* solution KCl concentrations of $\geqq$15 mM. Moreover, F_o fell by reducing *trans* but not *cis* Ca^{2+} concentrations. Thus the Cl^- channels were asymmetric.

A preliminary report of these findings has appeared elsewhere [18].

Materials and Methods

The procedure for preparing basolaterally enriched vesicles from rabbit renal outer medulla, and the enzymatic characteristics of these vesicles, are described in the preceding paper [1]. For the present studies, these vesicles were suspended in 300 mM mannitol, 10 mM imidazole, 1 mM Mg gluconate and 0.1 mM Ca gluconate (pH 7.4) at a protein concentration of 10 mg/ml. The vesi-

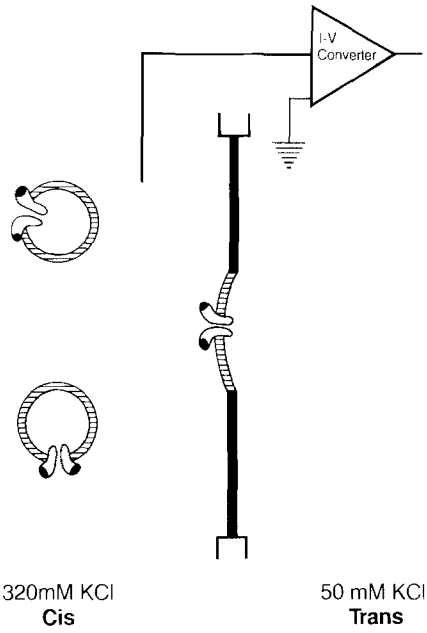

Fig. 1 Schematic illustration of the system for incorporating vesicles into bilayers. The model illustrates one of the modes of fusion, that is, with the outer surface of the vesicle facing the *cis* solution

cles were used immediately or stored on ice for no more than 24 hr prior to experiments.

Lipid bilayer membranes were formed by painting a lipid solution over a 0.2–0.3 mm aperture in the wall of a polystyrene Mueller-Rudin cup [14]. The solutions used to form bilayers were a 1:1 mixture of phosphatidylserine and phosphatidylethanolamine in decane (20 mg lipid/ml). The aqueous phases initially contained 3 ml of 50 mM KCl, 1 mM $CaCl_2$, 20 mM Tris, 32 mM HEPES (pH 7.4) solutions in both the *cis* and *trans* chambers. Formation and thinning of the bilayer were monitored electrically.

The bilayers were voltage clamped using a patch-clamp amplifier (Dagan 8900) connected to the bilayer chambers by Ag-AgCl electrodes in 3 M KCl agar bridges. The *trans* chamber was grounded. The output of the clamp amplifier was digitized at 44 kHz, recorded on VHS tape (PCM-2, Medical Systems), and simultaneously displayed on an oscilloscope (Hitachi VC6020). Records were replayed, filtered by an 8-pole Bessel filter (Model 802LPF, Frequency Devices), digitized (System 570, Keithley) and analyzed by computer using "Analysis" software written by Dr. Hubert Affolter (kindly provided by Dr. R. Coronado, Baylor College of Medicine). Records were filtered at 1 kHz (−3 dB cutoff) and sampled at 3 kHz.

In order to fuse membrane vesicles to bilayer membranes, we adopted the techniques described by Coronado [5] and by others [3]. Namely, we added aliquots of vesicles to the *cis* chamber and created an osmotic gradient for volume flow from *trans* to *cis* chambers by making the *cis* chamber hypertonic to the *trans* chamber. Based on the observations of other workers [3–5, 16], we presume that osmotic volume flow across the bilayers produced osmotic lysis and fusion of vesicles adjacent to the planar bilayers with the latter.

Figure 1 is a schematic illustration of the system used in our experiments. After formation of the bilayer, the *cis* chamber was made hypertonic by the addition of 300 μl of 3 M KCl (final KCl = 320 mM). Then 10–20 μl of a vesicle suspension were added to the *cis* chamber with stirring. Fusion of a channel-containing vesicle with the bilayer was detected as the appearance of gated currents across the bilayer. Figure 1 indicates schematically one of the ways in which a Cl^- channel might be incorporated into a bilayer, that is, with the outer surface of the vesicle oriented toward the *cis* solution.

We considered that fusion of Cl^- channels into the bilayers had occurred when we observed current-voltage relations indicating channels selective for Cl^- rather than K^+ (*see* Fig. 2, Results). We observed such fusion in about 15% of our attempts. In the remaining trials, we either observed no fusion or, on infrequent occasions, current-voltage relations consistent with K^+ channels. This paper provides no further information about the latter.

After detection of a Cl^- channel in the bilayer, additional changes in the solutions were made depending on the experimental protocol. These maneuvers are described in detail in Results. The calcium activity of the *cis* or *trans* chamber was varied by the addition of EGTA, and the free calcium activity was calculated as described by Pershadsingh and McDonald [17]. Solution exchanges were accomplished by perfusing the chamber with 30 ml of the appropriate solution. DPC was added from a 30 mM stock solution in DMSO in volumes of 10–20 μl; the DPC was added slowly and with stirring to avoid precipitation. Addition of these volumes of DMSO without DPC had no effect on either native bilayer conductance or Cl^- channel conductance.

The data in this paper are presented using the following conventions. The bilayer voltages are referenced to the *trans* chamber, which was grounded. Movement of chloride from the *cis* to *trans* chamber is indicated by a negative current and appears as a downward deflection in current traces. All results are expressed as mean values ± SEM for the indicated number of experiments. A single bilayer was taken to be $n = 1$.

Results

Identification of Cl^- Channels

Figure 2 presents a typical tracing indicating that a Cl^- channel had been incorporated into a bilayer membrane. For the protocol illustrated in Fig. 1, that is, 320 and 50 mM KCl in *cis* and *trans* chambers, respectively, E_{Cl} was approximately 42 mV, with the *trans* chamber at ground. Thus the direction and magnitude of the transmembrane currents at the V_H values shown in Fig. 2 indicate a Cl^- current through the channel. As indicated in Materials and Methods, the protocol shown in Fig. 2 was used to identify Cl^- channels in each experiment prior to subsequent maneuvers.

Conductance Properties in Symmetrical Solutions

Figure 3 shows the *I-V* relations for Cl^- channels in five different bilayer membranes exposed to symmetrical 320 mM KCl solutions. The experimental

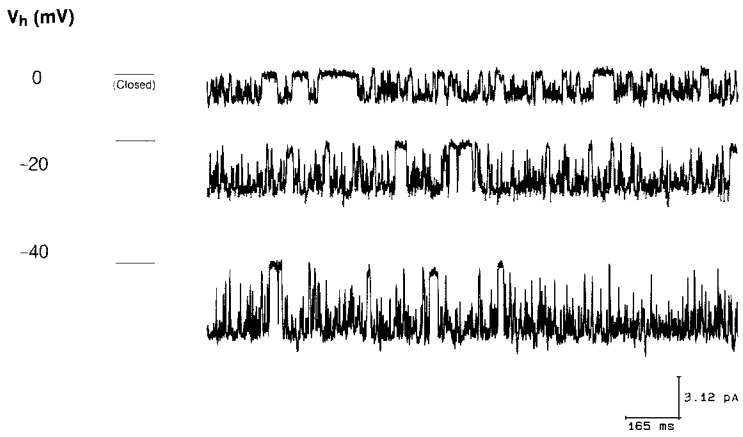

Fig. 2. Single Cl^- channel recordings with a medullary basolateral membrane vesicle incorporated into a planar bilayer. In this experiment, the *cis* chamber contained 320 mM KCl and the *trans* chamber contained 50 mM KCl. The *trans* chamber was grounded. Channel openings are represented as downward deflections, that is, negative currents. The direction and magnitude of currents at the indicated voltages show that the channel is Cl^- selective

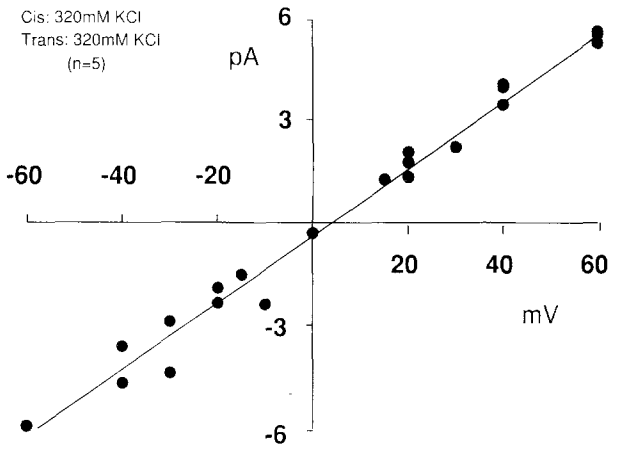

Fig. 3. Current-voltage relations for five different Cl^- channels, each in a different bilayer, all exposed to symmetrical 320 mM KCl solutions. The *I-V* relation was linear ($r = 0.99$). The mean conductance of the five channels under these conditions was 93 ± 4.5 pS

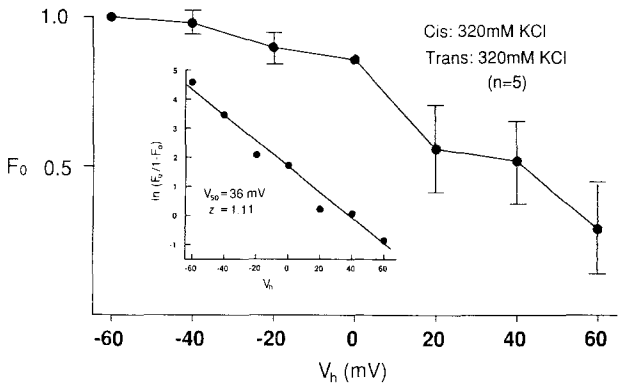

Fig. 4. The relation between fractional open time (F_o) and holding voltage (V_H) for Cl^- channels in the five bilayer membranes shown in Fig. 3, that is, with 320 mM KCl in both aqueous phases. The results are expressed as mean values ± SEM for the five bilayers indicated in Fig. 3. The inset shows the data for the relation between F_o and V_H plotted according to a Boltzmann distribution (Eq. (1)) where Z is the gating charge required for channel opening

data could be fit by a linear regression ($r = 0.99$) having nearly a zero-voltage intercept. The Cl^- conductance of the channels in the five bilayers was 93.0 ± 4.5 pS.

Figure 4 illustrates the relations between fractional open time (F_o) and holding voltage (V_H) for the five Cl^- channels bathed in symmetrical 320 mM KCl solutions and reported in Fig. 3. The data presented in Fig. 4 show clearly that F_o was voltage dependent, varying from an F_o of nearly unity at $V_H = -60$ mV to an F_o of approximately 0.3 at $V_H = 60$ mV. The inset in Fig. 4 expresses the relation between F_o and V_H according to a Boltzmann distribution for a simple two-state model for the Cl^- channels, that is:

$$\ln \frac{F_o}{1 - F_o} = \frac{ZF}{RT} V_H - \Delta G_i \qquad (1)$$

where Z is the apparent gating charge required for channel opening and ΔG_i is the voltage-independent free energy change for channel opening [2]. The inset in Fig. 4 shows that $\ln F_o/1 - F_o$ was a linear function of V_H. The slope of this relation yielded $Z = 1.11$; the V_H at which 50% of the channels were open was 36 mV.

It was pertinent to evaluate whether the Cl^- channel conductance of 93 pS in 320 mM KCl (Fig. 2) represented a unit conductance for these Cl^- channels. Thus we evaluated a frequency histogram of current amplitudes for Cl^- channels in the bilayers reported in Fig. 3 at a V_H of 40 mV where, from Fig. 4, F_o is approximately 0.5. These results are shown in Fig. 5. In each of three Cl^- channels in three different bilayers, the major open state corresponded to a current of about 4 pA, and thus to a conductance of about 100 pS, in accord with the

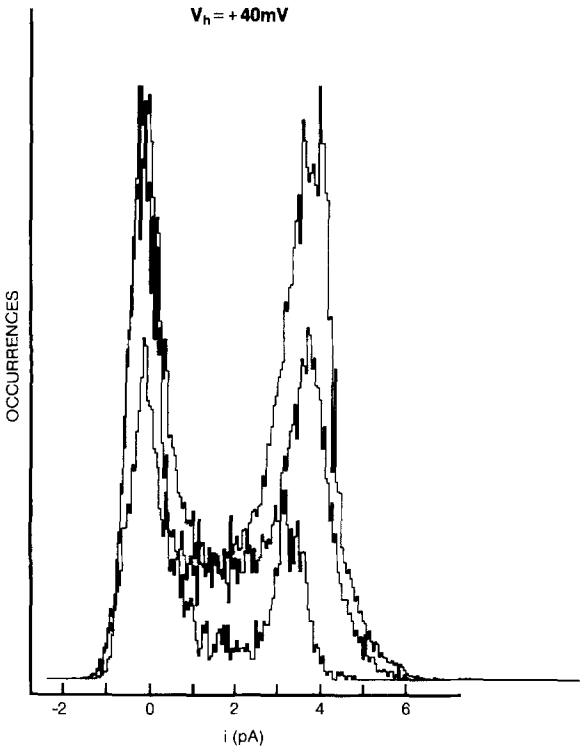

Fig. 5. A frequency histogram of current recordings for three of the bilayers indicated in Fig. 3 at a holding voltage of 40 mV. There were two major peaks of occurrences in each of the three bilayers, one at approximately zero current, that is, the closed state, and one at approximately 4 pA, that is, at a conductance of about 100 pS

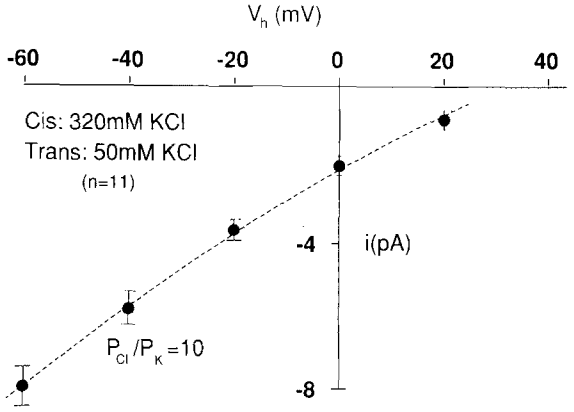

Fig. 6. Current-voltage relations of Cl^- channels in asymmetrical solutions. The *I-V* relations of 11 Cl^- channels in 11 different bilayers were determined under the same conditions described in Figs. 1 and 2, i.e., 320 mM KCl *cis*, 50 mM KCl *trans*. In each Cl^- channel in each bilayer, current measurements were made at each of the indicated holding voltages. Thus all measurements were paired. The results are expressed as mean values ± SEM. The dashed line was calculated from the Goldman-Hodgkin-Katz equation using a Cl^- : K^+ permeability ratio of 10 : 1

overall results in Fig. 3. The results in Fig. 5 also show that there was a second major peak of activity at zero current, that is, for a closed state.

Thus when taken together, the results in Figs. 3–5 indicate that, in symmetrical 320 mM KCl solutions, the major Cl^- conductance activity was a single Cl^- channel having a 93 pS conductance. The channels existed predominantly in two major states, open and closed, depending on V_H. We note as well that, in some instances, we also observed small subconductance states for these Cl^- channels. These smaller subconductances occurred infrequently, that is, about 10–15% of the time, and are not considered further in this paper.

Conductance Properties in Asymmetrical Solutions

Figure 6 illustrates the results of experiments in 11 bilayers in which we evaluated the *I-V* relations of these Cl^- channels in asymmetrical solutions, that is, 320 and 50 mM KCl in *cis* and *trans* solutions, respectively. For each channel in each bilayer, we measured currents at each of the V_H values indicated in Fig. 6. Thus all measurements in each bilayer were paired. F_o was below 0.2 at V_H values greater than 20 mV (*see* Fig. 7), and current amplitudes were rather small for V_H greater than 20 mV. Consequently, the V_H values for these paired measurements were restricted to the range −60 to 20 mV.

The results presented in Fig. 6 show clearly that, in asymmetrical solutions, the *I-V* relations for these channels were nonlinear. Rather, the *I-V* relation could be expressed in terms of the Goldman-Hodgkin-Katz equation and a P_{Cl}/P_K ratio of 10, indicated by the dashed line in Fig. 6. At a V_H of −60 mV, the limiting slope conductance was 106 pS, in good agreement with the single-channel Cl^- conductance of 93 pS obtained in symmetrical 320 mM KCl solutions (Fig. 3).

Figure 7 illustrates the relation between F_o and V_H for the 11 bilayers reported in Fig. 6. The results presented in Fig. 7 show that F_o fell appreciably as V_H became positive. Finally, the inset in Fig. 7 shows that the logarithmic ratio of open to closed states in asymmetrical solutions, that is, $\ln F_o/1 - F_o$, could be accounted for in terms of Eq. (1) and a gating charge of 1.15, which is virtually identical to the comparable value obtained in symmetrical solutions (Fig. 4).

Concentration Dependence of F_o

The results presented in Figs. 4 and 7 were obtained not only at varying voltages but also with different KCl concentrations in the *trans* solutions. Thus it

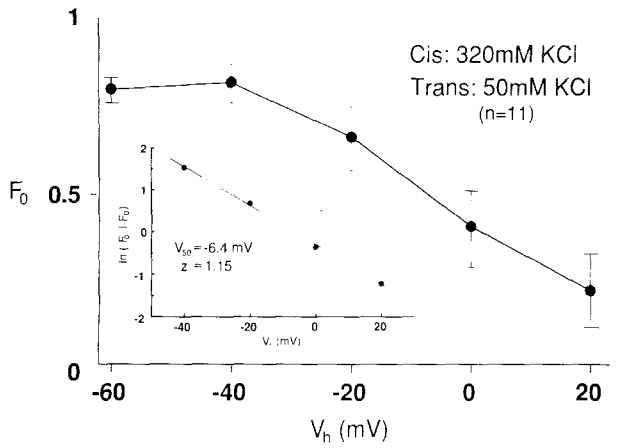

Fig. 7. Fractional open time of Cl^- channels (F_o) as a function of holding voltage (V_H). The fractional open time (F_o) of the same 11 channels depicted in Fig. 6 was determined over the voltage range −60 to +20 mV. As indicated in Fig. 6, the *cis* solution was 320 mM KCl and the *trans* solution was 50 mM KCl. The results are expressed as mean values ± SEM. The inset shows the data for the relation between F_o and V_H plotted according to a Boltzmann distribution (Eq. (1))

was pertinent to evaluate the variation of F_o with changes in the salt concentrations in the aqueous phases.

Figure 8 shows the results of an experiment in which the KCl solution was varied in the *trans* solution in the same bilayer membrane. The results presented in Fig. 8 show that, at a V_H of −40 mV (where, from Fig. 7, F_o is at a maximum), channel activity remained relatively constant when the *trans* KCl concentration was either 35 or 160 mM KCl. In other experiments using the format shown in Fig. 8, reducing the *trans* KCl concentrations from 50 to 10 mM KCl had no effect on F_o. Thus from the data in Fig. 8, as well as in Figs. 6 and 7, channel activity was consistently expressed with relatively low (≧10 mM) KCl concentrations in *trans* solutions.

Figure 9 shows the results of reducing *cis* solution KCl concentration on channel activity. A Cl^- channel was incorporated into a bilayer using the protocol shown in Fig. 2. Then the *cis* KCl concentration was reduced to 50 mM KCl. A shown in the recording in Fig. 9, channel activity was nearly completely absent when the *cis* KCl concentration was reduced from 320 to 50 mM KCl. Finally, as shown in the lower tracing in Fig. 9, we raised the *cis* and *trans* KCl concentrations to 320 mM KCl to exclude the possibility that the channel had been permanently inactivated, and channel activity reappeared.

These data indicate that the Cl^- channels were asymmetrical. On the *trans* side, channel activity occurred with ≧10 mM KCl solutions (Figs. 6–8). However, when the *cis* solution KCl concentration was reduced from 320 to 50 mM KCl, the channels were inactivated (Fig. 9). We have also noted, in experiments similar to those shown in Figs. 8 and 9, that Cl^- channel activity was only slightly reduced (≅20%) by reducing the *cis* KCl concentration to 150 mM.

Effect of Varying Ionized Ca^{2+}

Cl^- channels in some epithelia are calcium activated [6]. To test the effect of ionized Ca^{2+} on Cl^- channels in these basolateral membrane vesicles, we carried out paired experiments on the effects of EGTA addition to either the *cis* or *trans* solutions. Cl^- channels were incorporated into bilayers using the protocol described in Figs. 1 and 2, that is, 320 and 50 mM KCl in *cis* and *trans* solutions, respectively, with 1 mM Ca^{2+} in both *cis* and *trans* solutions. Then varying amounts of EGTA were added to either *cis* or *trans* solutions while channel activity was monitored at V_H values of −20 to −40 mV where, from Fig. 7, F_o was greater than 0.5.

The results of these experiments are shown in Fig. 10. It is evident from these results that, in a given bilayer, reducing the *trans* ionized Ca^{2+} concentration to less than 50 nM produced graded reductions in F_o. Although there was considerable variation among individual bilayers, it is reasonable to conclude from the data shown in Fig. 10 that reducing the *trans* concentration of ionized Ca^{2+} to less than 30 nM produced a significant suppression of Cl^- channel activity. In contrast, the data presented in Fig. 10 also showed that, in four different bilayers, reducing the *cis* solution ionized Ca^{2+} to 15 nM had no effect on channel activity.

It should also be noted that the suppression of Cl^- channel activity by reducing *trans* ionized Ca^{2+} concentrations was reversible. Thus in four of the Cl^- channels indicated in Fig. 10 where F_o had been suppressed by adding EGTA to *trans* solutions, raising the *trans* solution ionized Ca^{2+} concentrations to greater than 6 μM by subsequent Ca^{2+} addition restored channel activity to within 15–20% of control values.

Effect of DPC

As indicated in the preceding paper [1], the Cl^- channel blocker diphenylamine-2-carboxylate (DPC) suppressed Cl^- conductance in basolateral renal medullary vesicles. To test the effects of DPC on Cl^- channels, we incorporated the latter into bilayers according to the protocol shown in Figs. 1 and 2. Then DPC was added to both aqueous phases, so that measurements were paired within a bilayer.

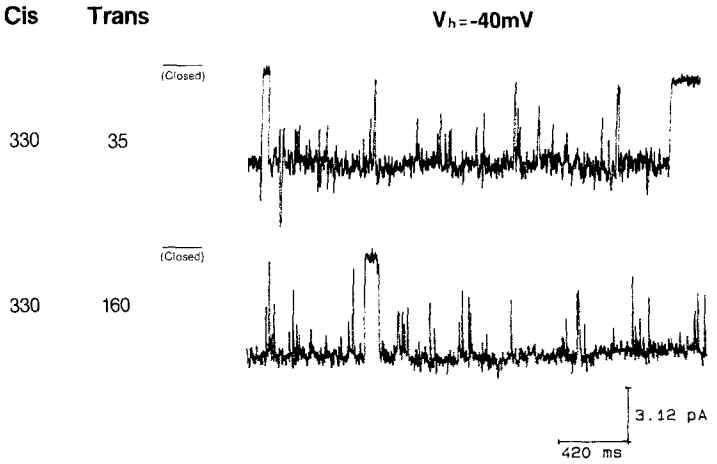

Fig. 8. A continuous tracing on the same Cl^- channel in the same bilayer showing that relatively high salt concentrations are not required on the *trans* side for channel activity. Vesicles were fused into a bilayer using the protocol illustrated in Figs. 1 and 2. Then the *cis* and *trans* sides were made 330 and 35 mM KCl, respectively. Channel activity was present and raising the *trans* KCl concentration to 160 mM had no perceptible effect on channel activity

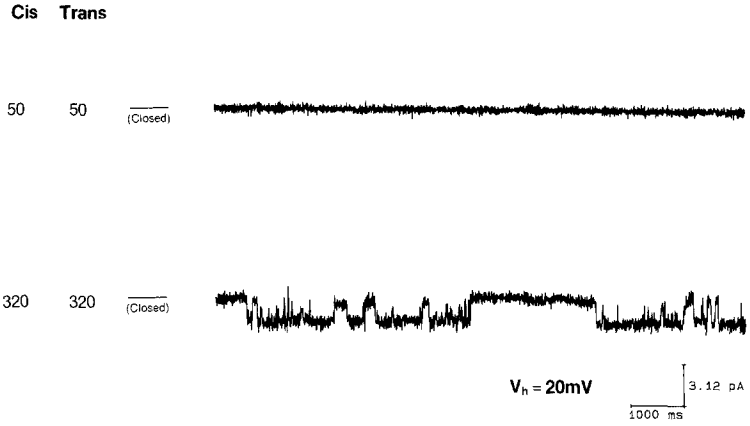

Fig. 9. A continuous tracing on the same Cl^- channel in the same bilayer illustrating the effect on channel open time of varying external salt concentrations symmetrically. Vesicles were fused into a bilayer using the protocol illustrated in Figs. 1 and 2, and Cl^- channel activity was detected using the protocol shown in Fig. 2. Then the *cis* solution as well as the *trans* solution was made 50 mM KCl. Finally, to verify that channel activity was reversible, both aqueous phases were made 320 mM KCl

In paired observations on six different bilayers, 0.1 mM DPC reduced F_o from 0.56 ± 0.09 to 0.32 ± 0.14 (Δ = 0.24 ± 0.07; P = 0.016). Figure 11 illustrates the mechanism for DPC inactivation of Cl^- conductance. After DPC addition, channel activity was reduced because of the introduction of long, closed intervals. Channel activity during open bursts was not affected by DPC. Finally, although the data are not shown in Fig. 11, DPC also resulted in a 20% decrease in the open-channel current. Duration-amplitude plots indicated that the decrease in channel current was not due to attenuation of fast openings. DPC has also been reported to decrease the single-channel amplitude in canine tracheal cells [23].

Discussion

Net Cl^- absorption in the mTALH involves primarily conductive Cl^- flux across basolateral membranes [11, 13]. In the preceding paper [1], we described some of the characteristics of conductive Cl^- flux in basolaterally enriched renal medullary vesicles. The present experiments, involving vesicles fused with planar bilayers, indicate that at least one of the Cl^--conductive pathways in these basolaterally enriched renal medullary vesicles is a Cl^- channel.

We note in this connection that, as pointed out in the preceding paper [1], the vesicles used in these experiments, while basolaterally enriched, also contain an admixture of apical membranes. However, in the mTALH, Cl^- conductances are limited to basolateral membranes [11]. Thus it is reasonable to argue that the Cl^- channels studied in the present experiments were of basolateral origin.

The results in Figs. 3–5 show that, in symmetrical 320 mM KCl solutions, the Cl^- channels incorporated in these bilayers existed in two major states, open and closed, and that channel activity, that is, F_o, was voltage dependent. This voltage dependence of F_o is similar to that observed in Cl^- channels from trachea [22] and from a colonic cell line [9]. In the open state, the single-channel conductance was about 93 pS. It seems unlikely, given

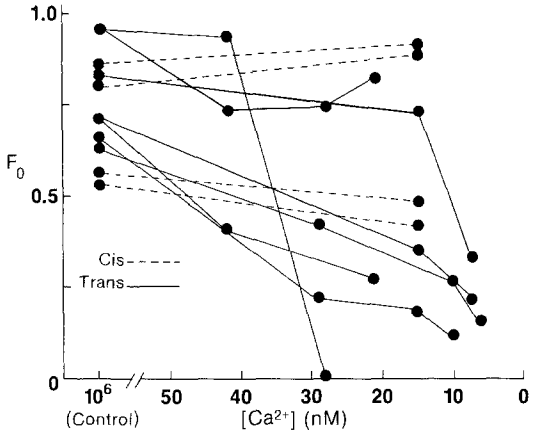

Fig. 10. Effect of varying ionized Ca^{2+} on F_o. Cl^- channels were incorporated into bilayers with *cis* and *trans* solutions containing 320 and 50 mM KCl, respectively, and 1 mM Ca^{2+} in both aqueous phases (Fig. 1). Then EGTA was added either to *cis* solutions or to *trans* solutions to reduce ionized Ca^{2+} concentrations, as indicated in Materials and Methods, to the indicated values. The lines connect measurements in individual bilayers. The solid lines indicate EGTA addition to *trans* solutions. The dashed lines indicate EGTA addition to *cis* solutions

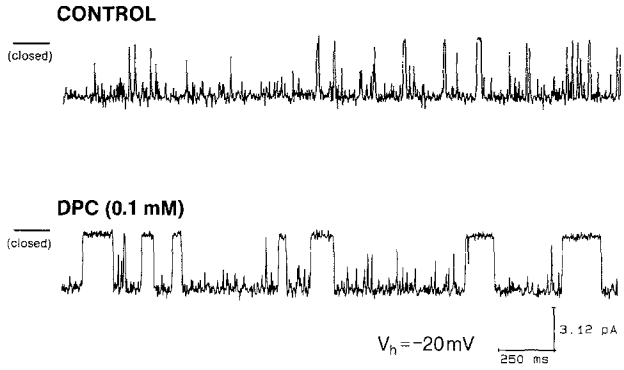

Fig. 11. Effects of DPC on channel activity. Channel activity before (upper tracing) and after (lower tracing) the addition of 0.1 mm DPC to the *cis* chamber. Both tracings were obtained from the same channel at $V_H = -20$ mV with 320 mM KCl in the *cis* chamber, 50 mM *trans*

the results in Fig. 4, that this 93-pS conductance was the sum of smaller channel conductances. The Cl^- conductance observed in our experiments was larger than in Cl^- channels from mammalian trachea [7, 12, 20–23], shark rectal gland [7, 8], rat colon [19] or T84 colonic cells [9]. It was less than the conductance of Cl^- channels in apical membranes of A6 epithelial cells [15] or some of the Cl^- channels in basolateral membranes of rabbit urinary bladder epithelium [10].

In asymmetrical KCl solutions, the *I-V* relation was nonlinear and conformed to the Goldman-Hodgkin-Katz equation (Fig. 6). The slope conductance from these experiments at $V_H = -60$ mV was 106 pS, in accord with the results shown in Fig. 3. The chloride-to-potassium permeability ratio of 10 calculated from these data (Fig. 6) is similar to that reported for Cl^- channels in rat colon enterocytes [19] and A6 cells [15] and is slightly higher than that reported for canine tracheal epithelial cells [21, 23].

At least two lines of evidence indicate that these channels were asymmetrical. As shown in Fig. 10, Cl^- channel activity was markedly suppressed when the ionized Ca^{2+} concentration in the *trans* solution was reduced to values less than 30 nM, but F_o was unaffected when the ionized Ca^{2+} concentrations of the *cis* solutions were reduced to 15 nM. Both in T84 cells cultured from mammalian colon and in human trachea, Cl^- channel inactivation occurs when intracellular ionized Ca^{2+} concentrations are reduced to values in the range of 20 nM [6]. Moreover, such ionized Ca^{2+} concentrations are characteristic of intracellular rather than extracellular fluids. Thus it is reasonable to conclude, using the model illustrated in Fig. 1, that the vesicles which fused with bilayer membranes were predominantly right side out, and that those vesicles fused with bilayers so that the intracellular side of the Cl^- channels faced the *trans* solution.

According to this view, the asymmetrical dependence of channel activity on aqueous salt concentrations (Figs. 8 and 9) indicates that Cl^- channel activity requires as little as 10 mM KCl in *trans* solutions, that is, in solutions facing the intracellular aspect of these Cl^- channels. In contrast, when *cis* solutions, presumably facing the extracellular aspect of these Cl^- channels, had 50 mM KCl concentrations, channel activity was virtually abolished (Fig. 9). Moreover, as indicated in connection with Fig. 9, channel activity was present when *cis* KCl concentrations were 150 mM. Thus the intracellular and extracellular faces of these Cl^- channels have differing salt requirements, presumably Cl^- concentration requirements, for conformations resulting in an open state. The present studies provide no information, however, about the structural bases for these requirements.

Finally, it is pertinent to consider the present data in the context of results in intact mTALH segments. In the latter, a depolarization of basolateral membranes from approximately −50 to −39 mV produced by antidiuretic hormone (ADH) is accompanied by a rise in the calculated value for basolateral Cl^- conductance (g^b_{Cl}) from about 65 mS cm^{-2} to about 142 mS cm^{-2} [13]. We have proposed that this rise in basolateral Cl^- conductance is the consequence of the basolateral depolarization produced by a rise in cell Cl^- activity, since luminal furo-

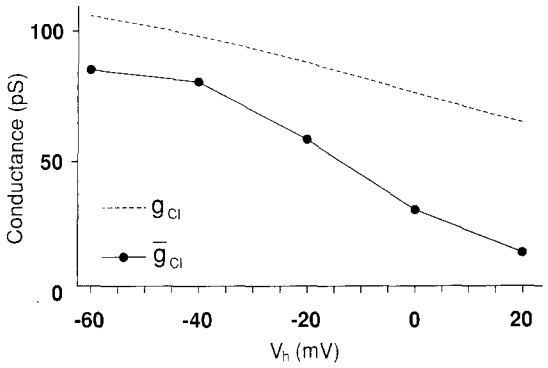

Fig. 12. Effect of voltage on time-averaged single-channel conductance. The upper dashed line represents the slope Cl^- conductance (g_{Cl}) derived from the GHK equation for a $Cl^-:K^+$ selectivity ratio of 10 (Fig. 6). The lower solid line represents the effective, or time-averaged, Cl^- conductance ($\bar{g}_{Cl}$) calculated as the product of g_{Cl} at a given V_H, using the data in Fig. 6, and the fractional open time (F_o) for that voltage, using the data in Fig. 7

semide blocks the ADH-induced rise in g^b_{Cl} [13]. However, such an increase in g^b_{Cl} is greater, in quantitative terms, than expected for GHK rectification, although in qualitative terms, this voltage dependence of g^b_{Cl} is in the direction expected from GHK formalism [11, 13].

The present experiments provide an insight into a mechanism by which a relatively small depolarization of basolateral membranes might produce an appreciable increase in g^b_{Cl}. Specifically, $\bar{g}_{Cl}$ (pS), the time-averaged single-channel Cl^- conductance may be expressed as the product:

$$\bar{g}_{Cl} = g_{Cl}F_o \qquad (2)$$

where g_{Cl} is the single-channel conductance at a given V_H (Fig. 6), and F_o is the fractional open time at varying values of V_H (Fig. 7). For asymmetrical solutions where the *cis* (extracellular) and *trans* (intracellular) aspects of these Cl^- channels face 320 and 50 mM KCl, respectively, the results in Figs. 6 and 7 indicate that the variation of F_o with V_H will produce significant voltage dependence for $\bar{g}_{Cl}$. That is, the results in Fig. 7 show that F_o increases as V_H becomes less positive. Thus for a Cl^- channel having an intracellular aspect facing the *trans* solution, F_o increases as the intracellular face is depolarized.

Figure 12 presents a quantitative illustration of this view. The dashed line for g_{Cl} in Fig. 12 was obtained from slope conductances of the data in Fig. 6. The solid line in Fig. 12, for $\bar{g}_{Cl}$, was obtained by multiplying these slope conductances by the F_o values indicated in Fig. 7 for varying values of V_H. The results presented in Fig. 12 show that, for V_H in the range of -20 to 20 mV and an E_{Cl} of 42 mV, the variation in $\bar{g}_{Cl}$ with V_H within 30 mV of E_{Cl} is appreciably greater than expected from GHK relations, because F_o is voltage dependent. It is therefore plausible to consider the possibility that, in intact mTALH segments, a comparable variation of F_o with basolateral voltage may account, at least in part, for the variation of g^b_{Cl} with basolateral voltage [11, 13].

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