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Cl^- Channels in Basolateral Renal Medullary Vesicles: V. Comparison of Basolateral mTALH Cl^- Channels with Apical Cl^- Channels from Jejunum and Trachea

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Summary. Cl^- channels from basolaterally-enriched rabbit outer renal medullary membranes are activated either by increases in intracellular Cl^- activity or by intracellular protein kinase A (PKA). Phosphorylation by PKA, however, is not obligatory for channel activity since channels can be activated by intracellular Cl^- in the absence of PKA. The PKA requirement for activation of Cl^- channels in certain secretory epithelia is, in contrast, obligatory. In the present studies, we examined the effects of PKA and intracellular Cl^- concentrations on the properties of Cl^- channels obtained either from basolaterally-enriched vesicles derived from highly purified suspensions of mouse medullary thick ascending limb (mTALH) segments, or from apical membrane vesicles obtained from two secretory epithelia, bovine trachea and rabbit small intestine. Our results indicate that the Cl^- channels from mTALH suspensions were virtually identical to those previously described from rabbit outer renal medulla. In particular, an increase in intracellular (*trans*) Cl^- concentration from 2 to 50 mM increased both channel activity (P_o) and channel conductance (g_{Cl}, pS). Likewise, *trans* PKA increased mTALH Cl^- channel activity by increasing the activity of individual channels when the *trans* solutions were 2 mM Cl. Under the latter circumstance, PKA did not activate quiescent channels, nor did it affect g_{Cl}. Moreover, when mTALH Cl^- channels were inactivated by reducing *cis* Cl^- concentrations to 50 mM, *cis* PKA addition did not affect P_o. These results are consistent with the view that these Cl^- channels originated from basolateral membranes of the mTALH.

Cl^- channels from apical vesicles from trachea and small intestine were completely insensitive to alterations in *trans* Cl^- concentrations and demonstrated markedly different responses to PKA. In the absence of PKA, tracheal Cl^- channels inactivated spontaneously after a mean time of 8 min; addition of PKA to *trans* solutions reactivated these channels. The intestinal Cl^- channels did not inactivate with time. *Trans* PKA addition activated new channels with no effect on basal channel activity. Thus the regulation of Cl^- channel activity by both intracellular Cl^- and by PKA differ in basolateral mTALH Cl^- channels compared to apical Cl^- channels from either the tracheal or small intestine.

Key Words mTALH · Cl^- channel · protein kinase A · channel activation

Introduction

This paper addresses two issues pertinent to the properties of Cl^- channels fused from epithelial vesicles into planar lipid bilayers. First, in prior studies, we utilized this technique to characterize Cl^- channels obtained from basolaterally-enriched rabbit renal medullary vesicles [3, 28, 39, 40]. These Cl^- channels, when fused into bilayers under osmotic gradient conditions, oriented themselves such that intracellular and extracellular channel apertures appeared to face *trans* (hypotonic) and *cis* (hypertonic) solutions, respectively [28, 40].

A particularly interesting characteristic of these channels was that raising *trans* Cl^- concentrations from 2 to 50 mM—that is, within the range of intracellular Cl^- activities in intact medullary (mTALH) or cortical (cTALH) thick ascending limb cells [39, 40]—increased individual channel open time probability (P_o) and increased individual channel conductance (g_{Cl}, pS) [39, 40]. ATP plus cAMP-dependent protein kinase (PKA) also activated these channels without affecting g_{Cl}, but only when *trans* Cl^- concentrations were 2 rather than 50 mM.

We interpreted these results to support our earlier contention [15, 25, 29] that antidiuretic hormone (ADH) augments simultaneously net rates of salt absorption and basolateral membrane Cl^- conductance in intact mTALH segments by increasing apical $Na^+/K^+/2Cl^-$ entry. According to this mechanism, basolateral Cl^- conductance rises because ADH increases intracellular Cl^- concentrations, rather than because the ADH-dependence increase in activity of the adenylate cyclase cascade directly activates basolateral Cl^- channels [15, 25, 29, 40]. The latter would occur only when intracellular Cl^- is

markedly depleted, e.g., with application of luminal furosemide [10, 31].

However, the Cl^- channels described above were obtained from rabbit renal medullary vesicles that were about 10-fold enriched in basolateral ($Na^+ + K^+$)-ATPase activity [3]. These studies [28, 39, 40] did not identify these channels as being explicitly of mTALH origin with respect to other outer medullary cells. Nor did our earlier studies [3, 28, 39, 40] exclude the possibility that the Cl^- channels were from intracellular organelles rather than from basolateral membranes. Thus to delineate more precisely the origin of these Cl^- channels, we prepared basolaterally-enriched membrane vesicles from very highly enriched (≈95%) suspensions of mouse mTALH segments and compared the properties of Cl^- channels obtained from such vesicles with those reported in our prior studies [28, 39, 40].

The second major purpose of the present experiments was to compare, under identical experimental conditions, the properties of basolateral mTALH Cl^- channels with apical Cl^- channels obtained from two secretory epithelia, the jejunum and trachea. The latter epithelia are, in a certain sense, mirror images of the mTALH, having basolateral $Na^+/K^+/2Cl^-$ salt entry sites and apical Cl^- channels which are activated by cAMP-dependent processes [7, 8, 13, 14, 19, 32, 34, 38].

Single channel studies in trachea, employing either the patch-clamp technique [19, 32, 38] or fusion of membrane vesicles into lipid bilayers [34], have demonstrated that the main effect of cAMP on apical tracheal Cl^- channels is to activate previously quiescent channels. It has also long been recognized that cAMP-dependent events increase apical Cl^- conductance in intestinal epithelia [7, 8, 13, 14]. In colonic cells, the mechanism for cAMP-mediated increases in apical Cl^- channel activity has been studied in detail using patch-clamp techniques [8, 13, 14] and appears to involve, at least in part, activation of previously quiescent Cl^- channels. In the small intestine, the mechanism for the enhancement of apical Cl^- conductance by cAMP has not, to our knowledge, been as clearly elucidated [7].

Furthermore, the dependence of apical membrane intestinal and tracheal Cl^- channel activity on intracellular Cl^- activity has not been reported. In our view, such a comparison between basolateral mTALH Cl^- channels and apical Cl^- channels in trachea and intestine has particular pertinence since, in cystic fibrosis [5, 23], tracheal and intestinal function are impaired, although to different degrees, while renal function, and by inference basolateral mTALH Cl^- channel activity, is consistently spared from the ravages of the disorder.

The experimental results in this paper show clearly that Cl^- channels obtained from basolateral vesicles prepared from suspensions of mouse mTALH segments have virtually the same properties as those described previously [28, 39, 40] for Cl^- channels obtained from basolaterally enriched rabbit outer medullary homogenates. Second, dependence of Cl^- channel activity on *trans* Cl^- concentrations was a property unique to basolateral mTALH Cl^- channels with respect to apical Cl^- channels from tracheal or jejunal vesicles. Lastly, in each of the three different types of Cl^- channels, (ATP + PKA) produced channel activation by different processes.

Materials and Methods

The procedures for the incorporation of membrane vesicles into lipid bilayers, and the subsequent analysis of channel currents, have been described previously [28]. In the present studies, lipid bilayers were formed from a 1 : 1 mixture of phosphatidylserine and phosphatidylethanolamine in decane (20 mg lipid/ml). The *cis* and *trans* chambers uniformly contained 1 mM $CaCl_2$ and 5 mM HEPES, pH 7.4. The KCl concentrations in *cis* and *trans* solutions in each experiment are indicated in Results. Membrane vesicles were added to the *cis* chamber. All voltages were referenced to the *trans* chamber, which was ground. Changes in the Cl^- concentrations of *cis* and *trans* chambers were carried out as described previously [28, 39, 40].

PKA Effects

The catalytic subunit of protein kinase A (PKA) used in the present experiments was identical to that described previously [40]. When present, the aqueous concentrations of ATP and PKA were 1 mM Mg ATP and 1 μg/ml PKA. To evaluate explicitly the effects of (ATP + PKA) on Cl^- channels already incorporated in bilayers, we perfused *cis* chambers with 25 ml of buffer after obtaining Cl^- channel activity in a bilayer. This maneuver removed residual vesicles from *cis* chambers, thus excluding the possibility that observed (ATP + PKA) effects were due to fusion of added vesicles from *cis* chambers into bilayers, rather than to (ATP + PKA) effects on Cl^- channels already incorporated into bilayers.

As in prior experiments [28, 39, 40], experimental maneuvers were preceded by a control period such that each channel served as its own control, and data were recorded for 5–10 min before and after the addition of different agents. Data were filtered at 200 Hz and sampled at 2 kHz for computer analysis. Openings and closings of the channel were defined by a 50% threshold discriminator. The open time probability (P_o) of an individual Cl^- channel was related to the number of channels (N) present in a given bilayer as

$$P_o = P_oN/N$$

where P_oN is the cumulative probability of channel activity.

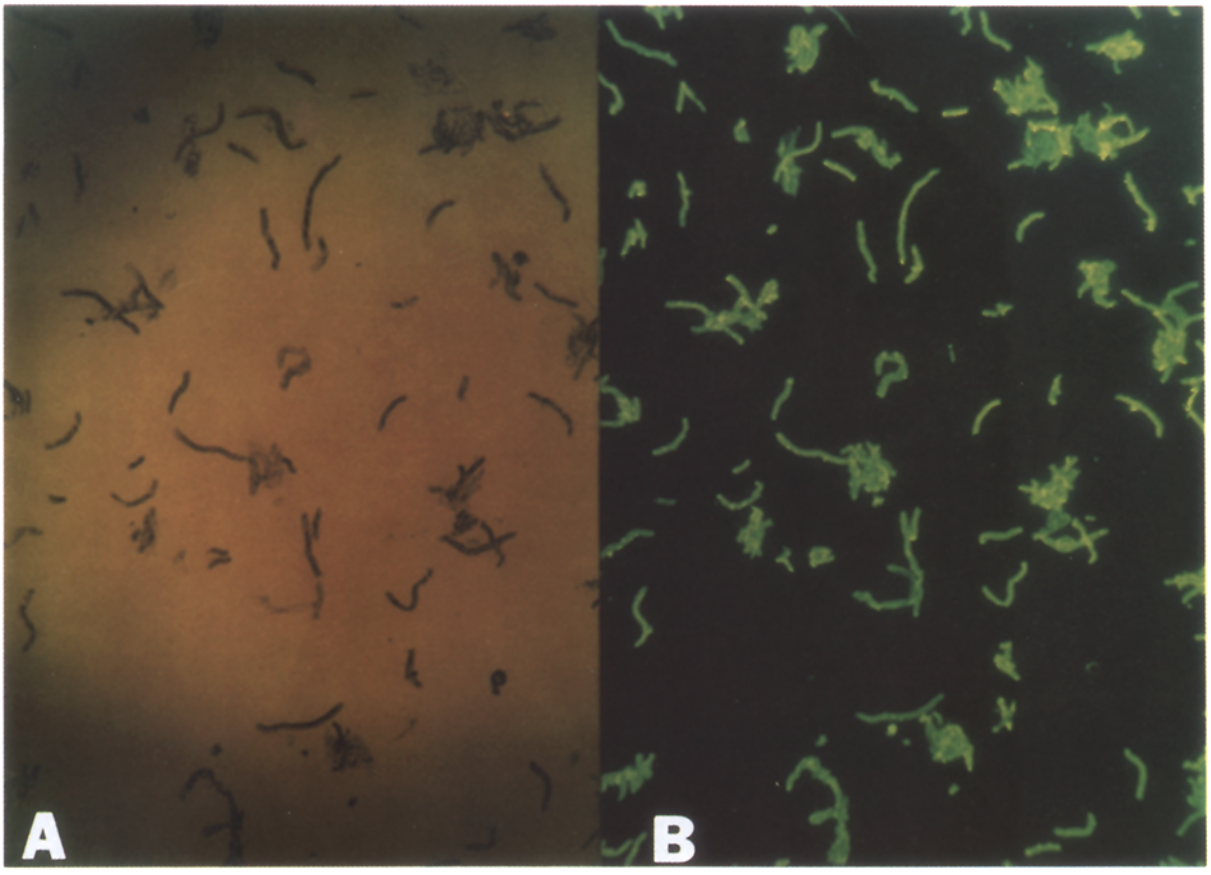

Fig. 1. (*A*) A representative photomicrograph of a suspension of mouse mTALH segments prepared according to the method of Trinh-Trang-Tan et al. [33] as modified by Kikeri et al. [21]. (*B*) a photomicrograph of the same field as in *A* showing virtually uniform staining of these tubule segments with anti-Tamm-Horsfall antibody.

Preparation of Mouse mTALH Segments

Suspensions of mouse mTALH segments were prepared according to the method of Trinh-Trang-Tan et al. [33] as modified by Kikeri et al. [21]. This method yields a tubule suspension containing ≈95% mTALH segments [21, 33]. Figure 1*A* shows a photomicrograph of the dispersed segments prepared in our laboratory, and Fig. 1*B* shows the same field of these dispersed segments stained with fluorescein-labeled anti-Tamm-Horsfall antibody using a previously described method [21]. As reported previously by these two laboratories [21, 33], there was virtually uniform staining of these dispersed segments with antibody, indicating that the trouble segment population contained at least 95% mouse mTALH segments. In accord with this view, we found that O_2 consumption by these tubule suspensions, measured using established techniques [21, 33], was inhibited 65.0 ± 9.1% (n = 3; SEM) by 1 mM furosemide.

Preparation of Membrane Vesicles

mTALH suspensions obtained from 6 to 8 mice were homogenized in a small glass-glass homogenizer by hand, and basolaterally-enriched membrane vesicles were prepared by differential centrifugation as described previously for whole rabbit outer medulla [3, 28]. Membrane vesicles from rabbit small intestine and from bovine tracheal epithelium were prepared by differential centrifugation and divalent cation precipitation according to the methods of Maenz and Forsyth [24] and Langridge-Smith et al. [22], respectively. The final vesicle pellets were resuspended in 250 mM sucrose, 30 mM histidine (pH 7.4) to a concentration of 3–5 mg protein/ml.

Table 1 summarizes the enrichment of the three different membrane vesicles preparations for several marker enzymes using standard techniques [3, 18]. The membranes obtained from the mouse medullary tubule suspensions were enriched nearly tenfold in basolateral membrane ($Na^+ + K^+$)-ATPase, a result virtually identical to that reported by us in membranes prepared from whole rabbit outer medulla [3]. The enzymatic enrichments of the small bowel membranes were also virtually identical to those reported by Maenz and Forsyth [24], and indicated more than a 15-fold enrichment in apical membranes. The enrichment in alkaline phosphatase which we obtained in bovine tracheal membranes (sevenfold) was lower than the 19-fold enrichment reported by Langridge-Smith et al. [22]. However, we achieved greater de-enrichment in ($Na^+ + K^+$)-ATPase than did the latter group. Thus the relative enrichment in apical with respect to basolateral membrane markers was quantitatively similar for both preparations.

Table 1. Vesicle purification data

Tissue	Fraction	Alkaline phosphatase	$(Na^+ + K^+)$-ATPase	Acid phosphatase	Glucose-6 phosphatase
		(nM/min · mg protein)			
Mouse mTALH	homogenate (*H*)	6.03	7.32	2.37	1.54
	vesicle (*V*)	8.78	61.88	6.54	1.89
	V/H	1.46	8.45	2.76	1.23
Rabbit small bowel	*H*	3.56	0.43	2.78	0.59
	V	56.50	0.81	7.77	1.18
	V/H	16.80	1.88	2.79	2.00
Bovine trachea	*H*	4.56	2.47	2.06	0.64
	V	32.22	0.60	8.65	2.70
	V/H	7.1	0.25	4.20	4.15

Materials

Bovine brain lipids were obtained from Avanti Polar Lipids. The catalytic subunit of protein kinase A was purified from bovine heart using the method of Reimann and Beham [30]. All other chemicals were purchased from Sigma.

Results

Basolateral mTALH Vesicles from Mouse mTALH Segments

The *I/V* relations over the range from −60 to 60 mV in symmetrical 270 mM KCl solutions for Cl^- channels from basolateral mTALH vesicles were linear with a zero-voltage intercept of -0.3 ± 0.2 (SEM) pA, which was not significantly different from zero ($n = 4$). The single channel conductance (g_{Cl}, pS) of 89 ± 6 pS ($n = 4$) determined from the slope of the *I/V* relations was virtually identical to that reported previously by us for individual Cl^- channels using basolaterally-enriched rabbit medullary vesicles under comparable experimental conditions [28, 39, 40]. In addition, as in rabbit medullary vesicles [28, 39, 40], the Cl^- channels in the present study demonstrated voltage-dependence of open probability which could be described by the Boltzmann relation.

Interactions Among cis and trans Cl^- and/or PKA

Figure 2 shows a continuous representative tracing illustrating the effects of varying *trans* Cl^- concentrations on P_o and P_oN in a single bilayer at a single holding voltage of −20 mV. Specifically, the results presented in Fig. 2 indicate that increasing *trans* Cl^- concentrations increased P_oN primarily because of an increase in P_o. An analysis of channel activity using the Boltzmann relation (Fig. 3) shows that varying *trans* Cl^- concentrations had, in six paired experiments, no effect on gating charge (*Z*); rather, the effect on channel activity was mediated via chemical, or voltage-independent (ΔG), determinants of channel activity. Finally, in these experiments, the slope conductances from −40 to 0 mV were 63.0 ± 1.1 pS and 86.0 ± 6.0 pS at *trans* Cl^- concentrations of 2 and 50 mM, respectively ($n = 6$; $P < 0.03$). Thus raising the *trans* Cl^- concentration increased $\bar{g}_{Cl}$, the time-average conductance of these Cl^- channels, by increasing both P_o and g_{Cl} simultaneously. These results are virtually identical to those reported previously for Cl^- channels obtained from rabbit medullary vesicles [28, 39, 40].

In prior studies with rabbit medullary vesicles [40], we found that *trans* (ATP + PKA) addition restored channel activity when the latter had been reduced by lowering *trans* Cl^- concentrations to 2 mM. We also observed that, with 270 mM *cis* Cl^-, *cis* (ATP + PKA) addition had no effect on channel activity. However, as noted previously [40], the latter experiments did not exclude the possibility that, if *cis* Cl^- concentrations were reduced sufficiently to completely inactivate Cl^- channels, that is, to 50 mM *cis* Cl^- [39, 40], addition of *cis* (PKA + ATP) might reactivate such channels.

The paired experiments illustrated in Fig. 4 addressed this question. In accord with our earlier data with rabbit medullary vesicles [39], reducing *cis* Cl^- concentrations to 50 mM produced complete Cl^- channel inactivation which was not affected by *cis* (ATP + PKA) addition but was reversed when *cis* Cl^- concentrations were restored to 270 mM. In the same Cl^- channels, adding *trans* (ATP + PKA) to solutions containing 2 mM Cl^- produced a P_o increase similar to that described in Fig. 3.

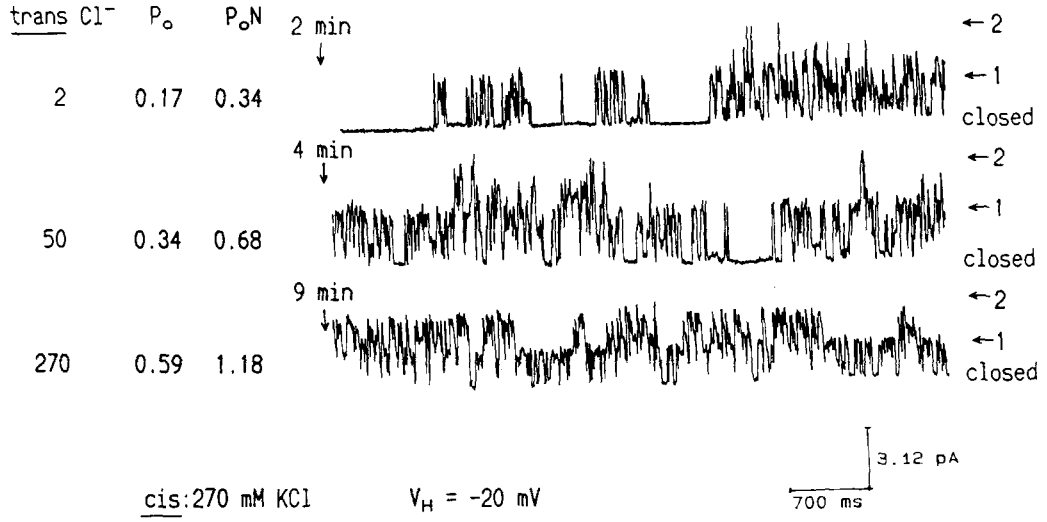

Fig. 2. A representative tracing illustrating the effect of varying *trans* Cl^- concentrations on P_o in vesicles from basolaterally-enriched suspensions of mouse mTALH segments.

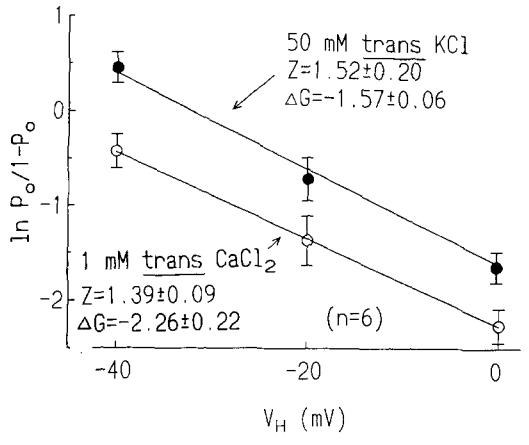

Fig. 3. A Boltzmann plot of paired experiments in six bilayers, illustrating the effects of *trans* Cl^- concentrations of 2 and 50 mM on P_o, Z and ΔG.

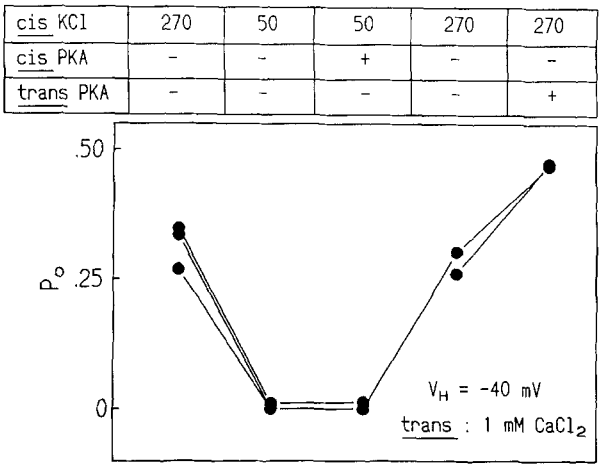

Fig. 4. The interplay of varying *cis* and *trans* (ATP + PKA) and *cis* KCl solutions on P_o in Cl^- channels from basolaterally-enriched vesicles prepared from mouse mTALH segments. The lines connect measurements in individual bilayers.

Finally, Fig. 5 shows a representative experiment indicating that, in a Cl^- channel from these basolaterally-enriched mouse mTALH vesicles, as in channels from rabbit medullary vesicles [40], the combination of *trans* (ATP + PKA) increased P_o (*see* Fig. 4) by increasing open time probability for an individual channel rather than by increasing channel number. Although the data are not shown in Figs. 4 and 5, Boltzmann plots indicated that, in the experiments using 2 mM *trans* Cl^-, *trans* (ATP + PKA) increased P_o by affecting ΔG but not Z. These results are identical to those obtained with Cl^- channels from rabbit medullary vesicles [40].

Two other factors pertinent to the (ATP + PKA) effect on these Cl^- channels are noteworthy. First, in the Cl^- channels illustrated in Figs. 4 and 5, adding (ATP + PKA) to the *trans* solution uniformly increased P_o but did not affect the unit channel conductance. The mean values for the latter, calculated from slope conductances from −40 to 0 mV, were 62.5 and 65.0 without and with *trans* (ATP + PKA), respectively.

Second, the augmentation of P_o by *trans* (ATP + PKA) addition was observed in each of the three Cl^- channels shown in Figs. 4 and 5. Likewise, in our earlier studies with rabbit medullary vesicles, *trans* (ATP + PKA) addition increased P_o when

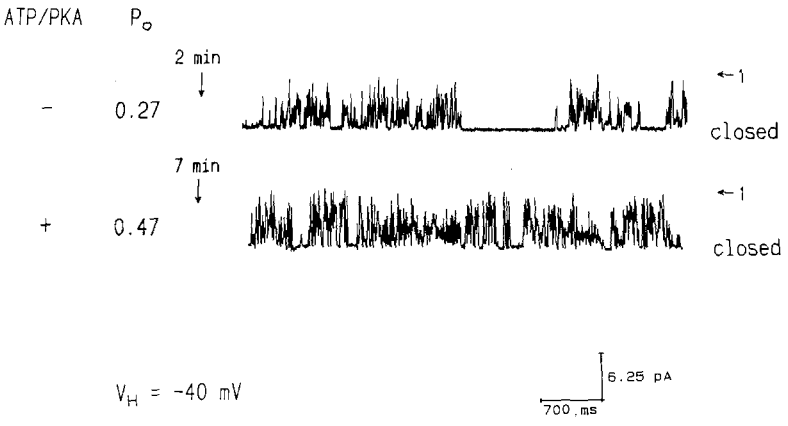

Fig. 5. A representative experiment illustrating the effects of (ATP + PKA) on P_o in Cl^- channels from basolaterally-enriched vesicles obtained from mouse mTALH tubule segments. The *cis* and *trans* solutions contained 270 mM Cl^- and 2 mM Cl^-, respectively.

trans Cl^- concentrations were 2 mM in eight out of eight experiments (*see* Fig. 5 in Ref. 40). In fact, a review of all of (ATP + PKA) additions to *trans* solutions containing 2 mM Cl^- in our laboratory indicates that, in a total of 18 bilayers (including published [40] and unpublished studies), *trans* (ATP + PKA) increased P_o in 15 bilayers. All of these experiments involved paired observations on Cl^- channels from rabbit medullary vesicles. In other words, the (ATP + PKA) effect in these Cl^- channels was quite reproducible.

Apical Vesicles from Rabbit Small Intestine

The *I/V* relations in symmetrical 320 mM KCl solutions for Cl^- channels from apical membranes of rabbit small intestine yielded a single channel conductance of 124 ± 12 pS (n = 3); the zero-voltage intercept was −0.6 ± 0.4 pA, which was not significantly different from zero. The channels demonstrated voltage-dependent kinetics with P_o increasing upon depolarization and little if any rectification, judging from the near linear fit of the experimental data (r = 0.997). The individual channel conductance of 124 pS was more than twofold greater than that observed by Giraldez et al. [8] for Cl^- channels in isolated *Necturus* enterocytes using patch-clamp techniques.

Effects of Varying trans Cl^-

Figure 6 shows a representative experiment illustrating the effects of varying *trans* Cl^- concentrations on P_o. In this representative tracing, increasing the *trans* Cl^- from 2 to 320 mM had no effect on P_o in a bilayer containing a single Cl^- channel at a holding voltage of −40 mV. Similarly, the Boltzmann distribution of the results of paired experiments with V_H in the range −40 to 0 mV in six bilayers showed that, either at 2 or 50 mM *trans* Cl^-, both Z (1.69 ± 0.15 *vs.* 1.69 ± 0.20, mean paired difference = NS) or ΔG (−2.36 ± 0.38 *vs.* −2.08 ± 0.23, mean paired difference = NS) were virtually identical, as was P_o over the range of V_H values tested.

Effects of trans (ATP + PKA)

Figures 7 and 8 and Table 2 illustrate the effects of paired experiments involving (ATP + PKA) addition to *trans* solutions on Cl^- channel properties. The representative tracing presented in Fig. 7 shows clearly that the major effect of *trans* (ATP + PKA) addition was to increase channel number within a given bilayer rather than to increase the open time probability of an already open channel. The frequency histogram presented in Fig. 8, obtained from the tracing shown in Fig. 7, illustrates this point clearly. When (PKA + ATP) were added to *trans* solutions, there occurred a second current peak, approximately twice as great in current magnitude as that observed under control conditions.

Table 2 presents a quantitative summary, for six different bilayers, of paired experiments comparable to the representative experiment show in Figs. 7 and 8. The results in Table 2 show clearly that *trans* (ATP + PKA) addition activated approximately one previously inert Cl^- channel in each bilayer, but did not affect the activity of the control Cl^- channel, nor increase the unit channel conductance. In other words, it is likely that the Cl^- channels activated by (ATP + PKA) were similar, and possibly identical, to those observed in the control state. The results in Table 2 also indicate that, in these Cl^- channels from apical small intestine, a fraction of channels retained constant activity with time in the absence of (ATP + PKA). The data shown in Fig. 7 and Table

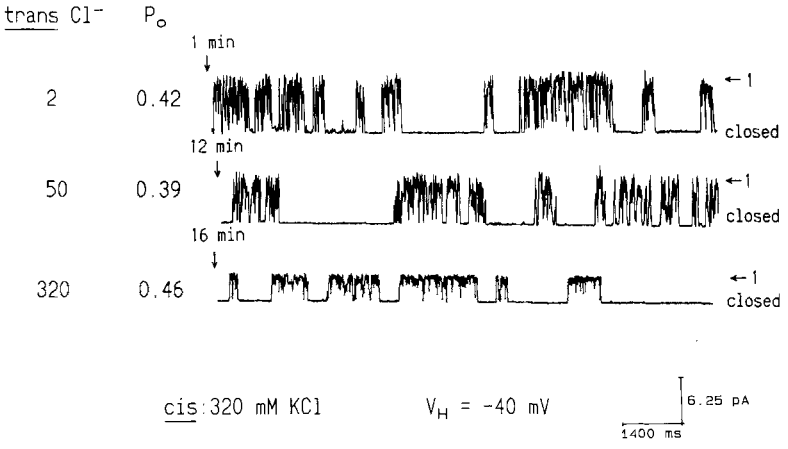

Fig. 6. A representative experiment illustrating the effects of varying *trans* Cl^- concentrations on P_o in a Cl^- channel from apically-enriched vesicles from rabbit small intestine.

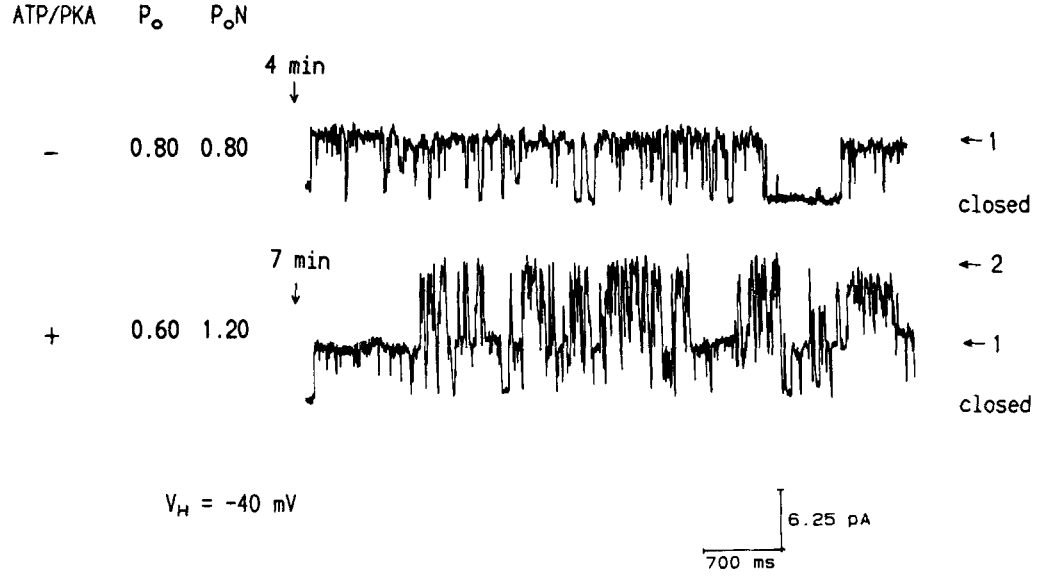

Fig. 7. A representative experiment illustrating the effects of *trans* (ATP + PKA) on P_o and P_oN in Cl^- channels from apically-enriched vesicles from rabbit small intestine. The *cis* and *trans* solutions contained 320 mM Cl^- and 2 mM Cl^-, respectively.

2 differ qualitatively from those obtained in Cl^- channels from basolateral mouse mTALH vesicles (Fig. 5) or basolateral rabbit medullary vesicles, where *trans* (ATP + PKA) increased P_oN by increasing the P_o in channels having baseline activity rather than by increasing channel number.

Finally, we should emphasize that, in contrast to *trans* (ATP + PKA) effects on basolateral mTALH Cl^- channels (*see above*), the effects of adding these agents to *trans* solutions on apical Cl^- channels from rabbit jejunum were relatively inconstant. Thus in addition to the six bilayer experiments summarized in Table 2, there were 12 other bilayer experiments carried out under identical experimental conditions where *trans* (ATP + PKA) addition did not activate new channels. Furthermore, in those 12 bilayers, (ATP + PKA) had no effect on the activity of the channels present in the control bilayers.

Apical Vesicles from Bovine Trachea

The *I/V* relations for Cl^- channels from bovine trachea in symmetrical 270 mM Cl^- solutions demonstrated a single channel conductance of 113 ± 10 pS (n = 5); the zero-voltage intercept was −0.2 ± 0.1 pA, which did not differ significantly from zero. Furthermore, the Cl^- channel also displayed a similar voltage-dependence of open probability to that of the Cl^- channels from the small intestine and mTAL. While the *I/V* data could be described by a

single linear regression ($r = 0.994$), it appears that the I/V relation in symmetric solutions may exhibit a small degree of outward rectification such that the conductance determined for negative voltages was 128.7 ± 5.9 and for positive voltages was 88.1 ± 3.6 pS ($n = 5$).

Effects of Varying trans Cl^-; Self-Inactivation with Time

The respresentative experiment presented in Fig. 9 together with other paired experiments ($n = 5$), were expressed according to a Boltzman distribution. The Boltzmann distributions over the voltage range −40 to 0 mV were linear with either 2 mM ($r = 0.98$) or 50 mM ($r = 0.97$) *trans* Cl^-. The results showed that variations in *trans* Cl^- concentrations had no effect on the determinants of channel activity [$Z = 2.22 \pm 0.11$ *vs.* 2.26 ± 0.10 (mean paired difference = NS)]; [$\Delta G = 3.30 \pm 0.14$ *vs.* 3.56 ± 0.14 (mean paired difference = NS)]. Likewise, either at 2 or 50 mM *trans* Cl^-, P_o was the same at each of the V_H values examined.

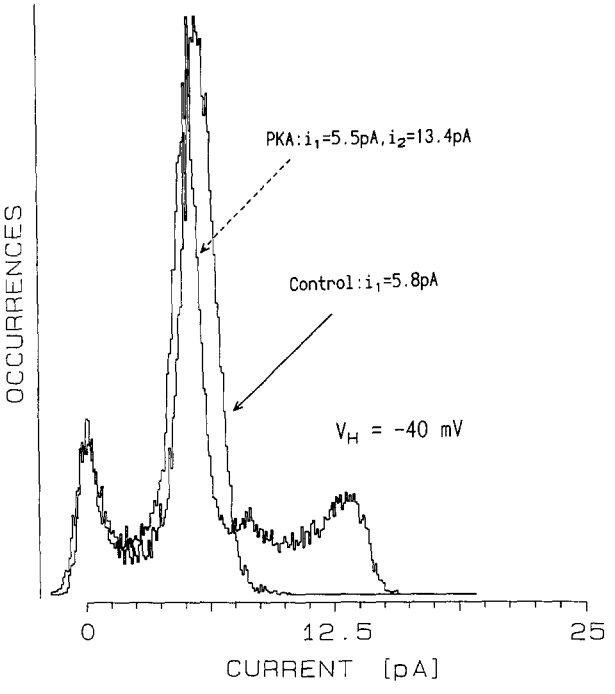

Fig. 8. A frequency histogram of the data from Fig. 10.

The results shown in Fig. 9 also illustrate that, in accord with earlier observations on Cl^- channels from apical tracheal membranes, studied either by osmotic fusion techniques [34] or by patch-clamp techniques [32], the tracheal apical Cl^- channels self-inactivated with time. We observed this effect in each bilayer studied in the present experiments ($n = 12$; mean time for channel inactivation = 8 ± 2 min [SEM]) (Figs. 10 and 11). In contrast, this inactivation of Cl^- channels with time has not occurred in over 75 Cl^- channels studied previously which were fused from rabbit renal medulla into bilayers (including published [28, 39, 40] and unpublished observations in our laboratory), not in the mouse basolateral mTALH Cl^- channels ($n = 11$) or rabbit apical jejunal channels ($n = 24$) studied in the present experiments.

It is pertinent in this regard to compare our observations with tracheal Cl^- channels to those reported by others. Using fusion techniques, Valdivia et al. [34] observed a Cl^- channel from tracheal epithelium that had a conductance of 71 pS in symmetrical 150 mM Cl^- solutions and a conductance of 130 pS with *cis* and *trans* Cl^- concentrations of 410 and 160 mM, respectively. These channels spontaneously inactivated with time. In patch-clamp studies, Schoumacher et al. [32] also observed a gradual inactivation of a 45 pS Cl^- channel in human apical tracheal membranes. Finally, in patch-clamp studies with apical trachea, complete channel inactivity has been observed unless the channels are activated either by the

Table 2. Effects of *trans* (ATP + PKA) on properties of jejunal apical Cl^- channels

(ATP + PKA)		N	P_o	P_oN	g_{Cl}
					(pS)
−		1.2 ± 0.2	0.53 ± 0.08	0.63 ± 0.15	122.9 ± 7.5
+		2.2 ± 0.2	0.49 ± 0.06	1.07 ± 0.15	115.4 ± 4.7
	Δ =	1 ± 0.0	0.03 ± 0.04	0.54 ± 0.09	7.5 ± 3.5
	p =	$<10^{-4}$	NS	0.001	NS
			($n = 6$)		

A summary of the effects of *trans* (ATP + PKA) addition on apical jejunal vesicles on the indicated variables. The experimental protocol was identical to that shown in Fig. 10. The data are for the six bilayers in which (ATP + PKA) augmented P_oN. As indicated in the text, there were 12 bilayers in which *trans* (ATP + PKA) addition had no effect on P_oN.

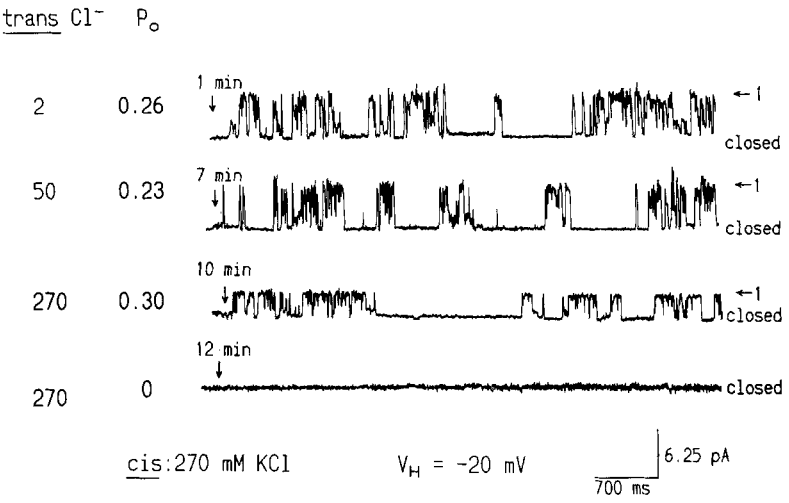

Fig. 9. A representative tracing illustrating, in Cl^- channels from apical tracheal membranes, that varying *trans* Cl^- concentrations had no effect on P_o and that, in accord with earlier observations of others [32, 34], these channels self-inactivated with time to a P_o of virtually zero when (ATP + PKA) were absent.

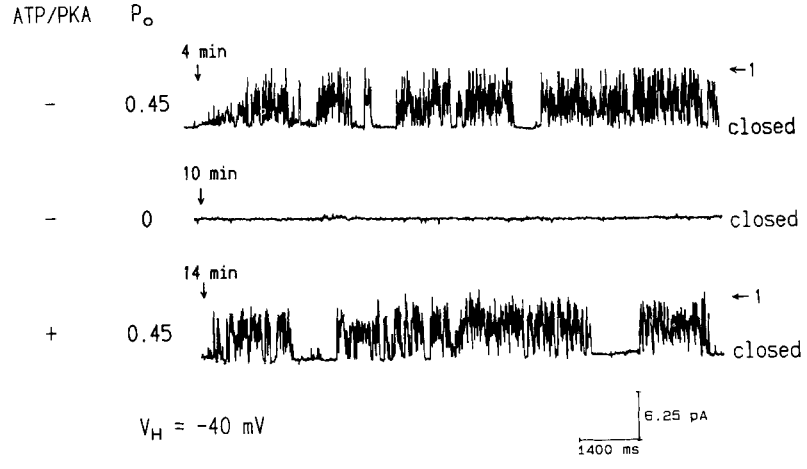

Fig. 10. A representative experiment with Cl^- channels from apical tracheal vesicles illustrating that, following self-inactivation, (ATP + PKA) restored channel activity. The *cis* and *trans* solutions contained 270 and 2 mM KCl, respectively.

adenylate cyclase cascade [19, 32, 38] or by other mechanisms [19, 32, 37, 38].

Effect of trans (ATP + PKA)

A representative experiment illustrating the effects of *trans* (ATP + PKA) on channel activity is shown in Fig. 10. Figure 11 presents the results of a number of such experiments carried out with *trans* Cl^- concentrations of either 2 or 270 mM. The results shown in Figs. 10 and 11 confirm clearly, in these Cl^- channels from bovine trachea, that *trans* (ATP + PKA) reactivated Cl^- channels which had inactivated with time, a result which has been widely reported for Cl^- channels from mammalian apical tracheal membranes [19, 32, 34, 38], but which fails to occur with activation of the adenylate cyclase cascade in apical tracheal Cl^- channels from humans with cystic fibrosis [19, 32, 36]. The data presented in Fig. 11 indicate that, in our experiments, such reactivation of Cl^- channels with (ATP + PKA) occurred in four of seven bilayers tested.

Discussion

One of the primary purposes of the experiments reported in this paper was to identify the origin of Cl^- channels described previously by us [28, 39, 40]

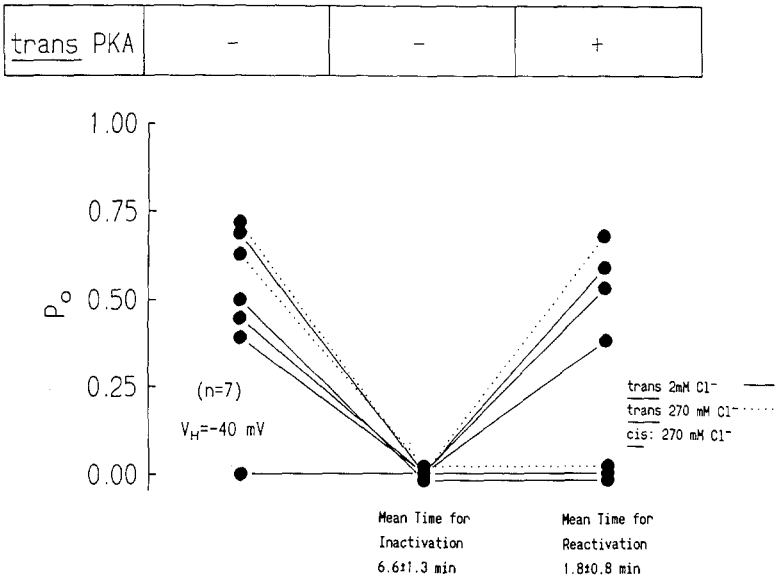

Fig. 11. Paired observations in seven bilayers on the reactivation of self-inactivated Cl^- channels from apical vesicles from trachea by (PKA + ATP).

which were fused from basolaterally-enriched rabbit renal medullary vesicles into bilayers. The results show that the Cl^- channels obtained from highly enriched suspensions of basolaterally-enriched mouse mTALH segments (Fig. 1; [21, 33]) had virtually identical properties to those obtained from basolaterally-enriched rabbit medullary vesicles.

When taken together, these observations (Figs. 1–5; and Fig. 10 in Ref. [28]) are consistent with the view that the Cl^- channels described in Figs. 2–5 most likely originated in plasma membranes of mouse mTALH, and the Cl^- channels described previously [27, 39, 40] were from plasma membranes of rabbit mTALH. In our view, the data presented in Fig. 4 provide evidence in support of the view that these Cl^- channels came from plasma membranes rather than from intracellular organelles. More specifically, the intracellular effector system (ATP + PKA) had explicitly sided effects—that is, activation of P_o from the *trans* side only. By way of contrast, if these channels were from intracellular organelles, it is reasonable to infer that (ATP + PKA) would have augmented P_o when added either to *cis* or *trans* solutions in the experiments shown in Fig. 4. Our prior observations [28] on the effects of varying *cis* and *trans* ionized Ca^{2+} activity on P_o are also consistent with this view.

Now there is general agreement that, in intact mouse [15, 17, 26, 31], rabbit [12, 35] and hamster [41] mTALH segments, Cl^- provides the dominant basolateral membrane conductance, while the Cl^- transference number of apical membranes is undetectably low [11, 16, 17]. Thus we conclude that the Cl^- channels described in Figs. 2–5, as well as those reported in earlier studies [28, 39, 40], originated in basolateral membranes of mouse and rabbit mTALH, respectively.

The second major purpose of the present studies was to compare the characteristics of basolateral mTALH Cl^- channels with Cl^- channels from apical small intestine and trachea. The results in Figs. 4, 5, 7, 8, 10 and 11 and Table 2 illustrate that P_oN activation, albeit occurring via different means, occurred in each of the three types of Cl^- channels when the intracellular effector system (PKA + ATP) was added to *trans* solutions. Thus we conclude that each of the three Cl^- channels reported in the present study oriented the same way in bilayers, that is, with intracellular apertures facing *trans* solutions. There were, however, at least two cardinal sets of differences among these three types of Cl^- channels.

First, an obligate monotonic relation between *trans* Cl^- concentrations and P_o, the activity of a given Cl^- channel, occurred only with basolateral mTALH Cl^- channels (Figs. 2 and 3; [39]) and not with apical Cl^- channels from either small intestine (Fig. 6) or trachea (Fig. 9). We stress in this regard that, during net Cl^- absorption in intact rabbit [10] or mouse [25] mTALH segments, the calculated [25] or measured [10] intracellular Cl^- activities are in the range 8–25 mM, while the $K_{1/2}$ for maximal activation of rabbit mTALH Cl^- channels fused into bilayers is 10 mM [39]. In other words, the dependence of $\bar{g}_{Cl}$ for basolateral mTALH channels fused into bilayers on *trans* Cl^- concentrations is consistent with the view that, in intact mTALH segments, intracellular Cl^- activity may be a cardinal determinant of basolateral Cl^- conductance, and hence of net rates of NaCl absorption by that nephron segment. These data with Cl^- channels from basolateral

mTALH membranes contrast sharply with the results in Cl^- channels from apical membranes of jejunum and trachea. In the latter two Cl^- channels, variation in *trans* Cl^- concentrations had no effects on channel activity (Figs. 6 and 9) while (ATP + PKA) were cardinal determinants of channel activity (Figs. 7 and 8, Table 2; Figs. 10 and 11).

The present results with Cl^- channels from small intestine and trachea are quantitatively similar to those reported by others in colonic T84 cells [13] and trachea using either patch clamp [19, 32, 38] or vesicle fusion technqiues [34]. Namely, the cAMP effector system activated previously quiescent Cl^- channels (Figs. 2, 10 and 11), without altering the conductance or activity of already active channels. Our results also confirm previous reports [32, 34] of the tendency of tracheal Cl^- channels to "run down" spontaneously with time, a phenomenon which could be reversed with (PKA + ATP) (Fig. 11). In contrast, a fraction of intestinal Cl^- channels were active, and remained active, in the absence of (ATP + PKA).

Two types of Cl^- channels have been implicated as putative secretary channels for airway and intestinal epithelia: an outward rectifying voltage-dependent 20–80 pS [13, 19, 32, 34] Cl^- channel; and a linear voltage-independent, small conductance (5–25 pS) channel [6, 9, 21]. recent studies involving the expression of the CFTR gene have provided strong evidence that the small, linear Cl^- channel may be the cystic fibrosis channel [1, 4, 20]. The role of the outward rectifying channel in Cl^- secretion is not certain, although it may mediate calcium-stimulated Cl^- secretion [2, 6]. The CFTR channel has not been studied in a reconstituted system. Nonetheless, it appears that the channel we have described from apical membrane tracheal vesicles in this paper more closely resembles, among the two channel types noted above, the outward-rectifying channel rather than the CFTR channel [2, 6, 36]. Clearly, however, added data will be required to establish the relations between the apical Cl^- channels from trachea and small intestine described in this paper and those involved in Cl^- secretion by intact trachea and small intestine.

However, to our knowledge, the available data in mammalian mTALH segments [10, 12, 15, 16, 25, 31, 35] indicate that the cardinal, and possibly sole, function of basolateral Cl^- channels in mTALH segments is in net Cl^- absorption. Thus the characteristics of the mTALH Cl^- channels studies in the present experiments, if extrapolated to the in vivo case, may provide an insight into why cystic fibrosis has no significant effect on renal function [5, 23]. Consider, for example, the fact that diluting segments of mammalian nephrons, that is, mTALH plus cTALH, absorb approximately 25–30% of the filtered NaCl [27], and that the principal mode of Cl^- efflux from cells to blood is via a basolateral Cl^- conductance [12, 17, 41]. Clearly, then, even a modest impairment in basolateral Cl^- channel activity would produce striking renal losses of NaCl, a phenomenon not described in cystic fibrosis [5, 23].

In this regard, the results of our prior studies [40] with rabbit mTALH basolateral membrane Cl^- channels showed that (ATP + PKA) increased P_o in individual Cl^- channels (*see* Fig. 5) when the *trans* Cl^- concentrations were 2 mM, but not when the latter were 50 mM. Now an intracellular Cl^- concentration of 2 mM in intact mTALH or cTALH segments of either mouse or rabbit is appreciably less than that observed even when apical salt entry is abolished with furosemide or related agents [10, 25]. Thus it may be that, for intact mTALH segments, a (PKA + ATP) effect on basolateral Cl^- channels has a vestigial and possibly redundant mechanism for regulating P_o while having no effect on g_{Cl} (*see* Results). Rather, intracellular Cl^- activity may be the cardinal determinant of both the conductance and open time probability (*see* Fig. 3) of basolateral Cl^- channels. This postulate provides, at a minimum, a reasonable frame of reference for explaining the fact that cystic fibrosis has no significant effect on renal function, namely, that direct activation of basolateral Cl^- channels by the adenylate cytclase cascade is not a necessary condition for full expression of activity in these channels, as it is in apical membranes of trachea and small intestine [5, 23, 38].

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