Luminal Ion Channels Involved in Isotonic Secretion by Na^+-Recirculation in Exocrine Gland-acini

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Abstract

According to the novel 'Na^+-recirculation theory' isotonic secretion is accomplished by processing of the fluid in two energy-requiring steps: The first step involves laterally placed Na^+/K^+-pumps, transporting Na^+ into the lateral intercellular space (LIS) making it hypertonic with respect to the bathing solution. This drags water by osmosis from the serosal side, raising the hydrostatic pressure of the LIS, which will cause a convective flow of a hypertonic solution to pass into the lumen of the gland. The second step involves re-uptake of Na^+ by the gland cells via Na^+-selective luminal ion-channels, likewise energized by lateral Na^+/K^+-pumps, making the luminal solution isotonic. Since the junctional complex of the gland is cation-selective Cl^- flows through the cells, being secreted by apical Cl^--channels, while luminal K^+-channels maintain the apical membrane-potential at a set-point value. Here we demonstrate, for the first time, the co-existence of Na^+, Cl^-, and K^+-channels in the luminal membrane of an exocrine gland. By applying whole-cell patch clamp, we verified that Cl^--channels are activated by adrenaline, causing cell depolarization and a concomitant rise in the conductance. At the same time, the transport number for Na^+ was increased, indicating the activation of Na^+-selective channels. The existence of all three kinds of channels was verified by patch-clamp on the luminal membrane. Cl^--channels were small ATP-dependent 8 pS channels activated by cAMP, resembling the CFTR-type Cl^--channel. The K^+-channels were voltage-activated with a conductance of ~30 pS. Na^+-channels had a conductance of 5 pS at the spontaneous membrane potential and the current-voltage relationships reversed at 103 mV depolarization from the spontaneous membrane-potential. We conclude that Cl^-, K^+, as well as Na^+-channels are found in the luminal membrane of frog skin glands. The Na^+-recirculation theory constitutes a framework where the presence of these ion channels is reconciled with isotonic fluid secretion.

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Biol. Skr. Dan. Vid. Selsk. 1998, **49**:179–191

Introduction

The so-called 'leaky epithelia' share the capacity for transporting large amounts of ions and water in isotonic proportions in either absorptive or secretory direction. The concept of 'solute-coupled water transport' indicates that the transport is divided into an energy-consuming process (i.e., the vectorial transport of ions) and a dissipative process (i.e., osmosis). Models of isotonic transport generally differ where the coupling between the two processes is assumed to take place (in the lateral intercellular spaces (LIS): 'the standing gradient hypothesis', in the cell: 'transcellular osmosis', or inside specialized transport proteins: 'osmotic engines'). Here we suggest based on the novel '*Na^+-recirculation theory*' (Ussing and Eskesen, 1989; Ussing et al., 1996) that active, vectorial transport of ions may take place simultaneously in both directions (*i.e.* both inward and outward). The forward transport takes place between the cells (via the LIS) and drives water by osmosis, whereas the backward transport goes through the cells and returns part of the transported ions to the LIS. This recirculation of transported ions serves to bring the net osmolality of the otherwise hypertonic transportate back to isotonic proportions. Since both processes are active this theory states that achieving isosmolality is an active, energy-consuming process and not based on simple dissipation. Whether the net transport takes place in the inward or the outward direction is determined by properties of two membranes lining the LIS towards the serosal and mucosal side, respectively.

The Model of Na^+-recirculation in the Gland

A. Structural features of the model

The existence of a Na^+-recycling loop in the gland was suggested by Ussing and Eskesen (1989) and further developed by Ussing et al. (1996). The model is depicted in Fig. 1. The basic features of this model are:

1. The plasma membrane of the transporting cell is divided into three regions: The basal membrane, the lateral membrane facing the LIS, and the luminal membrane. Thus the so-called 'basolateral' membrane has been divided into two functionally distinct domains.

2. Two barriers between the cells are responsible for this division: The junctional complex and a hypothesized barrier at the level of the basement membrane.

3. The Na^+/K^+-pumps present in the lateral membrane energize both the forward and backward ion transport, and will thus pump Na^+ into the LIS. Parallel K^+-channels serve to recycle K^+.

4. The basal membrane is furnished with a $Na^+/K^+/2Cl^-$-cotransporter which will bring Cl^- into the cell in an electroneutral fashion, thereby raising cell-$[Cl^-]$ above electrochemical equilibrium. Basal Cl^- and K^+-channels are also present of which the last-mentioned have been identified by patch-clamping (Andersen et al., 1995).

5. As a distinctive feature of this model, the luminal membrane is supposed to posses both Cl^--, Na^+-, and K^+-selective ion channels all of which are active during secretion. Of these, the Cl^--channel serves primary Cl^--secretion, whereas the Na^+-channel is responsible for Na^+-recycling through the gland cells. The K^+-channel probably serves to stabilize the apical membrane-potential.

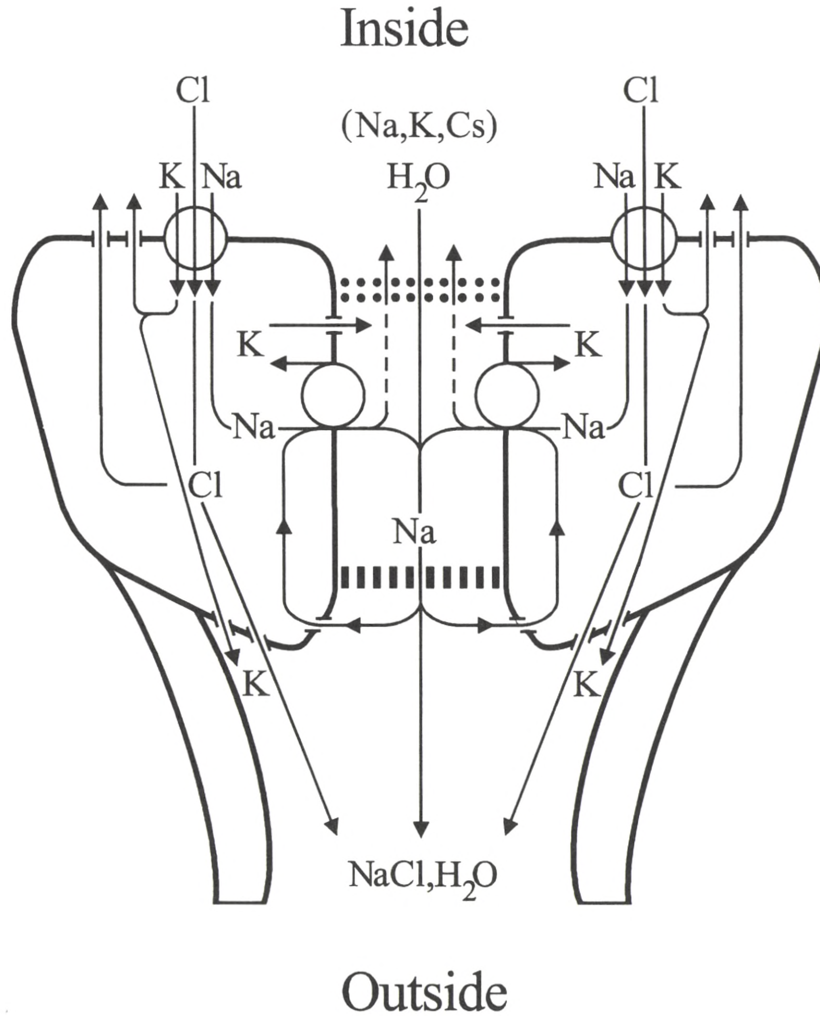

Figure 1. Model of frog skin gland maintaining a flow of isotonic fluid from 'inside' (= blood side) to 'outside' by way of Na^+-recirculation. Two identical gland cells are shown, lining the lateral intercellular space. The function of the lateral intercellular space and the concerted working of the lateral Na^+/K^+ pumps, the basal $Na^+/K^+/2Cl^-$ co-transporter, and the ion channels of the basal, lateral and apical membranes for producing an isotonic secretion are explained in detail in the text. From Ussing et al. (1996).

B. Dynamic features of the model

The frog skin glands can be stimulated to carry out stationary secretion for hours using noradrenalin (Eskesen and Ussing, 1989), the β-agonist isoproterenol (Thompson and Mills, 1983) or prostaglandin E_2 (Bjerregaard and Nielsen, 1987). According to the model, stimulation by cAMP-inducing substances will open up the luminal Cl^--channels and allow Cl^- to exit down its electrochemical gradient. For a while this will set up a transepithelial voltage-gradient, which will serve to drag Na^+ and water via the LIS. At this point of time, the gland model may agree more or less with the Silva model of the shark rectal gland (Silva et al., 1977). However, after about 30-60 minutes, the transepithelial potential gradient disappears and the glands continue to secrete in the absence of transepithelial voltage, pressure, osmotic, or chemical gradients (Ussing et al., 1996). The model explains how this may take place. Activation of the Na^+/K^+-pump will pump Na^+ into the LIS with Cl^- following probably via the basal barrier. The resulting raised NaCl-concentration of the LIS will drag water by osmosis into the LIS from the serosal bath. This allows a hydrostatic pressure build-up in the LIS, which will cause a filtrate of the LIS-solution to pass through the junctional membrane into the gland lumen by convection. An important point at this stage is to realize the conditions under which the directional flow will be set up in the outward and not the inward direction. Intuitively, if water is dragged *into* the LIS via the basement membrane and leaks *out* of the junctional membrane, the condition required is that the reflection-coefficients of the two membranes, σ^{BM} and σ^{JM}, respectively, must satisfy the condition $\sigma^{BM} > \sigma^{JM}$. Indeed, it can be shown that this is both a necessary and sufficient condition for net secretion (Larsen *et al.*, 1997).[1]

The composition of the filtrate which leaves the LIS and enters the gland lumen depends on the $[NaCl]_{LIS}$ and the σ^{JM}_{NaCl} in such a way, that $J_{NaCl}/J_v = [NaCl]_{LIS} \cdot (1 - \sigma^{JM}_{NaCl})$, where J_{NaCl} is the flux of NaCl and J_v is the water flux.[2] Since $[NaCl]_{LIS} > [NaCl]_{bath}$ due to the activity of the Na^+/K^+-pump, under a range of conditions the emergent fluid is expected to be hypertonic. This necessitates the appearance of a recirculation-loop for Na^+, where Na^+ is subjected to reuptake into the cells to recycle through the pump. The reuptake-pathway was hypothesized to be apically located Na^+-channels (Ussing and Eskesen, 1989; Ussing et al., 1996), but in principle any transport mechanism for Na^+-reuptake will do. Thus, active Na^+-secretion by the pump sets up conditions for the dragging of water via a low-resistance pathway (the LIS) into the gland lumen, where the tonicity is then regulated by returning Na^+ via a high-resistance pathway (the cells).

C. Flux-ratio analysis of the frog skin glands shows the presence of an 'invisible' part of the Na^+-flux

Ussing and Lind (1996) discovered that flux-ratio analysis of $^{134}Cs^+$ can be used as an accurate measure for paracellular ion-flow. This results from the fact that $^{134}Cs^+$ is irreversibly trapped in the epithelial cells upon entry. The appearing fluxes at the opposite side of the epithelium must therefore result from a stricktly paracellular route of permeation. Using the Cs^+-trapping method Ussing et al. (1996) showed that the flux-ratio of $^{134}Cs^+$ (J^{out}/J^{in}) was significantly above unity (range 3.25-11) when measured in the stationary phase of secretion under thermodynamic equilibrium conditions. The force dragging Cs^+ through the paracellular route was estimated to be 30-60 mV. This force can only be explained by solvent drag caused by a substantial directional water flow between the cells and thus reveals the secretory activity of the glands in the absence of an external

[1]In fact, by reversing the inequality, one can transform the model into an absorbing state. A similar model with the opposite inequality has been applied to the absorptive small intestine (Larsen et al., 1997).

[2]For simplicity we here consider the general case where both Na^+ and Cl^- flow through the junction, thus NaCl is treated as an uncharged species. In reality for the gland, experimental evidence suggests that the Cl^--flux proceeds via the gland cells, whereas Na^+ flows via the junction. Thus, only Na^+ is subjected to recirculation and the Cl^--flux must be regulated to match the remaining (not recirculated) part of the Na^+.

driving force. Further, Ussing et al. (1996) showed that the flux-ratio of Na^+ (J^{out}/J^{in}) was significantly less than would be expected from a purely paracellular permeation of this ion along the same route as Cs^+. The argument is that for convective ion flow, we expect that since $D_{Cs}/D_{Na} \approx 1.5$ the flux-ratios for Na^+ and Cs^+ obey

$$[J_{Na}^{out}/J_{Na}^{in}] = [J_{Cs}^{out}/J_{Cs}^{in}]^{1.5}. \quad (1)$$

Actually, if the Na^+/K^+-pumps are placed as in Fig. 1, we would even expect the inequality '>' to hold since Cs^+can not substitute for Na^+ in the pump. Regardless, Ussing and coworkers consistently found that the flux-ratio for Na^+ was significantly lower than the right side of Eq. (1). This means that a significant fraction of the Na^+-flux must return to the solution of origin, making part of the Na^+-flux in effect 'invisible'. The recirculated flux of Na^+ was estimated to be 80% of the total Na^+-flux in the LIS *i.e.* each Na^+-ion would be recirculated on average 4 times before escaping to the outside solution. Using $^{42}K^+$, Nielsen and Nielsen (1994) also showed that the flux-ratio (J^{out}/J^{in}) for K^+ was significantly larger than expected from a passive distribution of this ion. This was confirmed by comparison with the Cs^+-flux-ratio (Ussing et al., 1996). It would seem, therefore, that K^+ is subject to active secretion by the cells. Thus, Cl^-, Na^+, and K^+ channels should all be present in the luminal membrane of the gland.

Identifying Luminal Ion Channels in the Gland

The frog skin glands constitute an ideal model-epithelium for the study of isotonic secretion. The glands constitute a large acinus and a very short duct, making the final secretion very close to the primary secretion. With the Na^+-absorption of the epithelium blocked by amiloride, the activity of a large number of gland acini can be studied in isolation, using tracer-technology or electrical measurements. Furthermore, by stimulation with noradrenalin or prostaglandin E_2 the glands enter a stationary phase of secretion which can persist for hours. Recently, a preparation of isolated epithelium with intact glands *in situ* stripped of connective tissue was developed (Andersen et al., 1995), which allows patch-clamp and ion imaging technology to be applied to the glands. In the first publication using this preparation a population of 'maxi' K^+-channels was identified in the basal membrane (Andersen et al., 1995). We set out to study electrical properties of individual gland cells and the luminal ion channels involved in secretion. Sheets of epithelia with intact glands were prepared using serosal exposure to crude collagenase, followed by a hydrostatic pressure head of 10-25 cm H_2O. A piece of epithelium was mounted in a chamber with the serosal side up and perfused at the serosal, but not at the mucosal side.

A. Whole-cell properties of secretory acinar cells

As a starting point, we performed patch-clamp in the whole-cell configuration to study the secretory behaviour of the entire cell (Sørensen and Larsen, 1997a). By using permeabilization of the patch by the poreforming antibiotic nystatin, we were able to study the membrane potential, V_c (by clamping the pipette-current to 0) and the conductance (by clamping the pipette-voltage to values between -90 and +90 mV in 10 mV steps) in cells with a relatively unperturbed intracellular environment. The unstimulated (resting) gland cells had membrane-potentials of -65 mV, governed by the equilibrium potential for K^+, as shown by substitution of K^+ for Na^+ in the bath, which yielded a linear dependence of V_c on log(bath-[K^+]) with a slope of 31 mV/decade (*i.e.* the transport-number for K^+ would be 31/58 = 0.53). Perfusion of the bath with a low-[Cl^-] (8 mM) or a low-[Na^+] (3 mM) solution hardly changed the membrane-potential (transport-numbers 0.01 and 0.05, respectively).

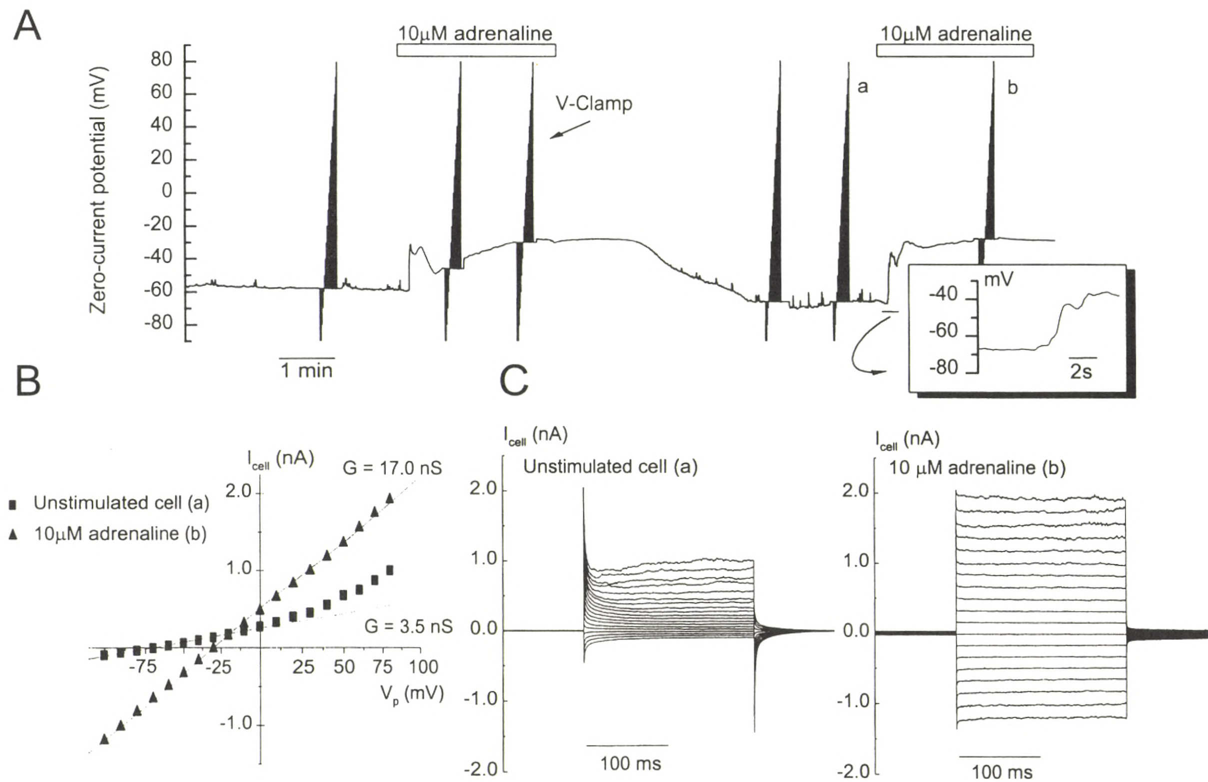

Figure 2. Nystatin-permeabilized whole-cell experiment on gland cell. The glands were perfused with NaCl-Ringer (containing, in mM: 110 NaCl, 3.7 KCl, 3 Na-acetate, 10 glucose, 5 HEPES, 1 $CaCl_2$, 1 $MgCl_2$, pH=7.4) and patched with a high-K^+ pipette-solution (containing, in mM: 30 KCl, 10 NaCl, 90 K-gluconate, 5 TES, 1 EGTA, 10 glucose, 1 $MgCl_2$, 0.38 $CaCl_2$, 0.1 mg/ml nystatin, pH=7.2 with KOH, total $[K^+]$=126 mM). *A.* Voltage-trace from a whole-cell experiment. The pipette was clamped to zero current by the amplifier (EPC-9) in order to monitor the voltage. Periodically, the pipette-voltage was clamped at values between -90 and +90 mV in 10 mV steps ('V-Clamp') from the spontaneous membrane-potential for measurement of the current-voltage relationship. Application of 10 μM adrenaline caused a very rapid (note inset) depolarization, which was fully reversible. *B.* Steady-state current-voltage relationships obtained at markers a and b (see *A*). The conductance (G) was found as the slope of the regression-line for points at $\leq$ 0 mV for the unstimulated condition and for all points after stimulation. *C.* Individual current-traces for the voltage-pulses at a and b (see *A*). Note the activation at depolarized potential by the unstimulated cell. All voltages were corrected for the liquid junction potential (10 mV referenced to the pipette) between pipette and bath.

Stimulation of the gland by $10\,\mu M$ adrenaline caused a fast (seconds) depolarization to $V_c = -32$ mV (Fig. 2) which was sustained in the presence of agonist. This was accompanied by an increase of cell conductance by a factor of 2-15. The current-voltage relationship in the stimulated cell rectified at most slightly in the outward direction, whereas non-stimulated cells often had a strongly outwardly rectifying current-voltage curve due to the presence of voltage-activated K^+-channels.[3]. In the stimulated state, the transport number for Cl^- now increased to 0.27, shown by a substantial (but very variable) depolarization upon lowering of bath-$[Cl^-]$, whereas transport numbers for K^+ and Na^+ were 0.15 and 0.11, respectively. Thus upon stimulation, the gland activated Cl^--channels (Fig. 1). This increase in the Cl^--conductance caused a secondary decrease in the transport-number for K^+. The increase in the transport-number for Na^+ from 0.05 to 0.11 in face of the increased total conductance is interesting, since this could be taken to indicate that Na^+-channels had also been activated substantially, an important and discriminatory property of the Na^+-recirculation model. However, this interpretation would be premature, since the change in V_c upon Na^+-removal could be secondary to effects on Na^+-dependent co- and counter-transporters ($Na^+/Ca^{2+}-$, Na^+/H^+-exchangers and $Na^+/Cl^-/(K^+)$-cotransporters of differing stochiometry). We therefore tested the Na^+-channel blocker amiloride on activated cells, which caused a hyperpolarization in 2/3 of the cells investigated. This hyperpolarization could be interpreted as representing blocking of Na^+-selective channels, but again the results were not conclusive, since we had to use high concentrations of amiloride ($100\,\mu$M) for the effect to appear. This overlaps with the sensitivity of the Na^+/H^+-exchanger for amiloride and could therefore again be explained by secondary effects of amiloride. Using the flourescent dye Fura-2 for measurement of intracellular Ca^{2+} and the pH-sensitive dye BCECF, we were able to monitor effects on these parameters by perturbation of bath-Na^+. These (unpublished) results showed that stimulation by adrenaline caused a transient increase in cell-Ca^{2+} from 75 nM to a peak of 540 nM followed by a plateau-level of 160 nM, but left the pH relatively unaffected (pH=7.55 before and pH=7.50 after stimulation). Na^+-removal was ineffective in raising cell-Ca^{2+} in both stimulated and unstimulated cells, whereas it resulted in a slight acidification (from pH=7.50 to pH=7.37) under stimulated conditions. However, the application of $100\,\mu$M amiloride did not affect cell-pH[4] under either condition. These negative results together with the increase in the Na^+-transport number argue for the appearance of a Na^+-conductance in stimulated cells.

B. Single-channel properties of luminal ion-channels

To verify directly the presence of the suggested ion channels in the luminal membrane, we undertook the task of patching the luminal membrane. This was possible by slitting up the acinus using a discarded patch-clamp pipette. A fresh pipette could then be used for going through the slit and forming a patch on the apical membrane.

In *cell-attached* patches, application of $2\,\mu$M isoproterenol, $12.5\,\mu$M forskolin, or 0.5 mM dibutyryl-cAMP and 0.1 mM 3-isobutyl-1-methyl-xanthine (IBMX) activated small Cl^--channels with a conductance of 8.3 pS at the membrane potential (Sørensen and Larsen, 1997b). The current-voltage (i/V-) relationship for the channel followed the Goldman-Hodgkin-Katz equation for passive electrodiffusion with a reversal potential very close to 0 mV pipette potential. This means that following maximal activation using cAMP-inducing substances, the equilibrium potential for Cl^- coincided with the membrane potential (within the error of measurement). This was also the conclusion of whole-cell studies.

[3]The presence of these channels can explain why the transport numbers in this study did not add to unity: A perturbation of the membrane potential in either direction would be antagonized by the activation/inactivation of K^+-channels.

[4]That the dye was able to measure changes in cell-pH was shown by applying an ammonium-prepulse to the loaded cells.

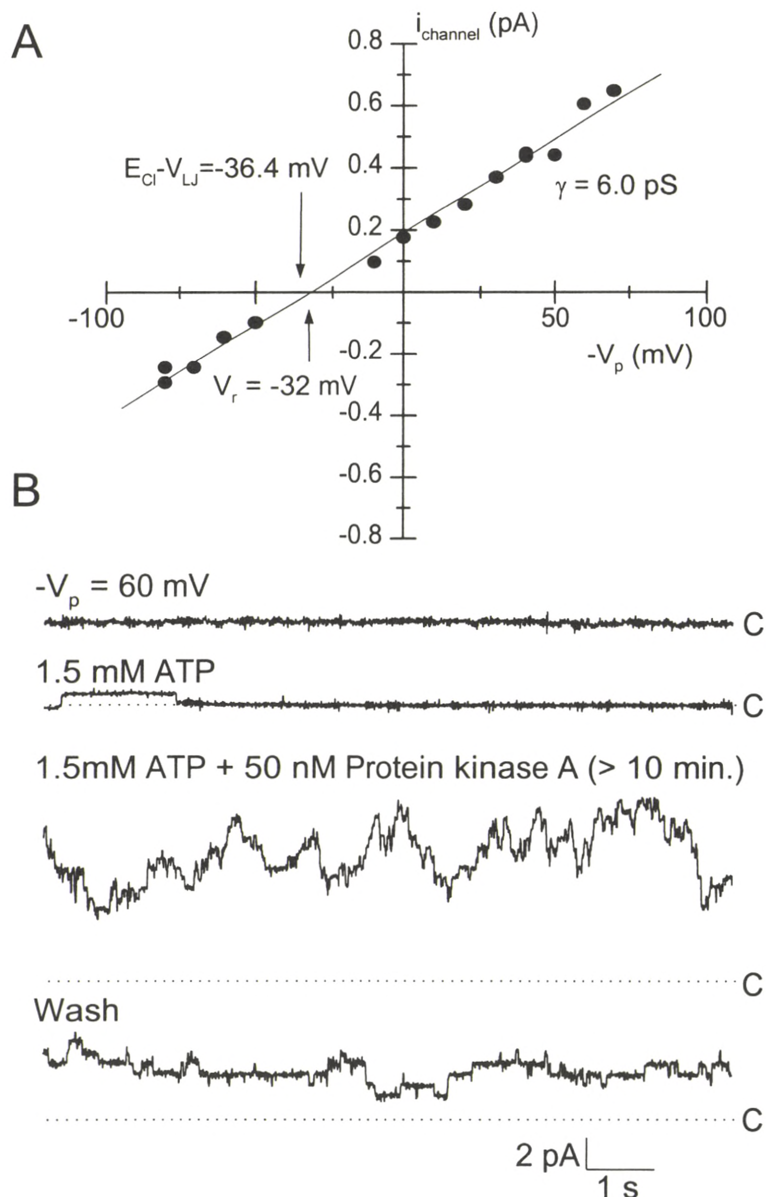

Figure 3. Excised inside-out patch from the luminal membrane of the gland patched with NaCl-Ringer in the pipette (see legend to Fig 2) and exposed to an intracellular solution containing 25 mM Cl^- on the cytoplasmic side (composition, in mM: 15 NaCl, 105 gluconic acid, 10 TRIS-HCl, 105 TRIS(hydroxymethyl)aminomethane, 5 HEPES, 1 EGTA, 0.371 $CaCl_2$, 1.1 $MgCl_2$, pH=7.2). *A.* Current-voltage relationship for the channel. The conductance (γ) and the reversal-potential (V_r) were identified by linear regression analysis. The equilibrium potential for Cl^- corrected for the junction potential at the reference electrode ($E_{Cl} - V_{LJ}$) compares well with V_r, confirming Cl^--selectivity of the channel. Voltages are given as the negative of the pipette-potential (V_p), *i.e.* as -V_p. *B.* Current traces for the patch held at 60 mV and exposed to intracellular solution alone, or after the sequential addition of 1.5 mM ATP and 50 nM catalytic subunit of cAMP-dependent protein kinase. Finally, the patch was washed with intracellular solution alone. 'C' denotes the current with all channels closed.

The channel was also studied in *excised inside-out* patches, where it exhibited fast run-down in the absence of ATP. Fig. 3 shows a patch where application of ATP induced a single channel, whereas incubation with the catalytic subunit of cAMP-dependent protein kinase activated several channels. The activation could be reversed by removing ATP. The i/V-relationship shows the Cl^--selectivity of these ~ 6 pS channels in this case. The activation in cell-attached or inside-out configuration by cAMP and the ATP-dependence of the channel in the phosphorylated state together with the low single-channel conductance constitute a hallmark for the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^--channel. The message for CFTR as well as the protein itself has been localized to frog skin glands in *Xenopus* by Engelhardt et al. (1994). Thus CFTR Cl^--channels constitute the route of cAMP-dependent Cl^--secretion in glands of frog skin, resemblant of the shark rectal gland (Gögelein et al., 1987, Marshall et al., 1991).

In cell-attached or inside-out patches (stimulated or resting) K^+-channels were also often noted - often in the same patches as the small Cl^--channel. The K^+-channel was voltage-dependent and activated by membrane depolarisation from the spontaneous membrane potential (corresponding to 0 mV pipette potential). The channel in Fig. 4 had a chord conductance of 28 pS at 50 mV (voltage referenced to the pipette), which clearly distinguishes it from the basal 'maxi' K^+-channel, which has a conductance of ~ 200 pS (Andersen et al., 1996). Due to the voltage-dependence, only points at ≥ 0 mV could be obtained on the i/V-curve. This voltage-dependent activation would make the channel ideally suited for maintaining the apical membrane-potential at some set-point value.

In resting cells, Na^+-channels were frequently found in the cell-attached configuration (Fig. 5, and Sørensen and Larsen, 1997b). These had a relatively fast kinetics with openings lasting in the order of 10-100 ms, which distinguish them from the slowly gating heterologous expressed α,β,γ-ENaC (*E*pithelial *Na* *C*hannel)-type channel (Canessa et al., 1994). The mean conductance at 0 mV pipette potential was 5.1 pS and the i/V-relationship reversed at 103 mV depolarization with respect to the spontaneous V_c. The study of these channels was hampered by a fast run-down in cell-attached patches. The Na^+-channels were found separately, or together with K^+- and/or Cl^--channels.

Concluding Remarks

The model in Fig. 1 is considered a working hypothesis at this time. Distinguishing properties of the model (e.g. the lateral placing of Na^+/K^+-pumps, the two barriers between the cells, the apical Na^+-entry pathways, the elevated NaCl-concentration of the LIS during secretion) constitute solid, testable predictions which can be addressed using established methods. The preparation of glands stripped of connective tissue, but still attached to the epithelium, seems an ideal preparation for investigating an isotonic secreting epithelium at all levels of organisation. So far, the patch-clamp experiments on the glands have placed the presupposed ion channels of Fig. 1 in the luminal membrane. Thus both Cl^--, Na^+- and K^+-channels are present in the luminal membrane of a secretory epithelium. Curiously, this means that similar ion-channels are present in a secretory epithelium and in several absorptive epithelia, such as the toad skin epithelium (Larsen et al., 1987, Harvey and Larsen, 1993, Sørensen and Larsen, 1996) and the airways (Haws et al., 1992, Russo et al., 1992, Voilley et al., 1994, Burch et al., 1995) which also express both CFTR-like Cl^--channels and Na^+-channels. In the absorptive airway-epithelium the function of the secretory Cl^--channel is generally unknown, even though severe dysfunction results from its absence (cystic fibrosis). Instead, it is supposed to function in the regulation of apical Na^+-channels in an anta-

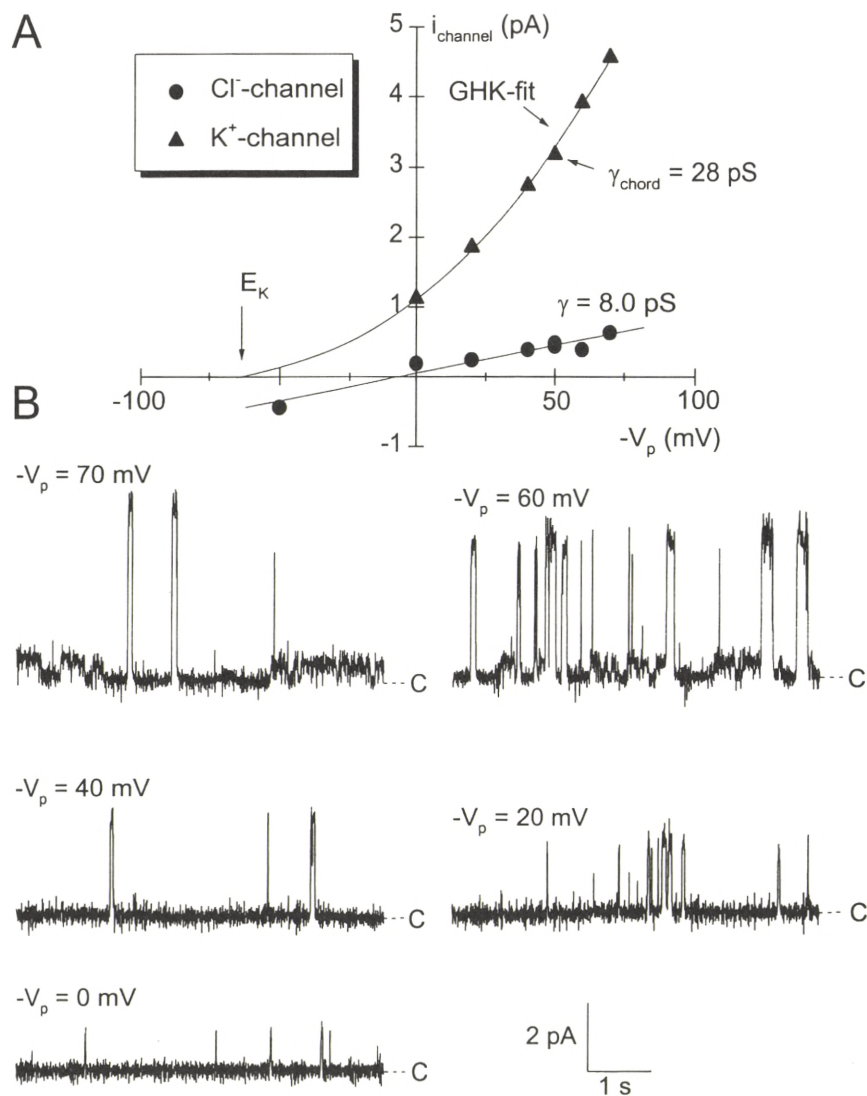

Figure 4. Cell-attached patch on the luminal membrane of a gland cell stimulated by $2\mu M$ isoproterenol. NaCl-Ringer in the pipette. *A.* Current-voltage relationship for two different types of channels in the same patch. Filled circles show currents through 8 pS Cl^--channels of the same type as shown in Fig. 3. Filled triangles show currents through a K^+-channel, which was only noted at $-V_p \geq 0$mV. The i/V-relationship for the Cl^--channel was fitted by linear regression, whereas the i/V-relationship for the K^+-channel was fitted by the Goldman-Hodgkin-Katz equation for passive electrodiffusion of K^+:

$$i_K = P_K \frac{(V_c - V_P)F^2\,([K]_c - [K]_o \exp(-F(V_c - V_p)/RT))}{RT\ \ (1 - \exp(-F(V_c - V_p)/RT))}$$

where we fixed the cellular and outer K^+-concentrations $[K]_c$ and $[K]_o$ to 140 and 3.7 mM, respectively, and fitted with the permeability for K^+ (P_K) and the spontaneous membrane-potential (V_c) as free parameters. The best fit (line) yielded $P_K = 1.64 \times 10^{-13}\text{cm}^3 s^{-1}$ and V_c=-28.2 mV. This yields a reversal potential for K^+ currents at $-V_p = -63$ mV. The chord conductance at 50 mV was calculated using this value. *B.* Current traces at different potentials. Note the small Cl^--channels at 60 and 70 mV.

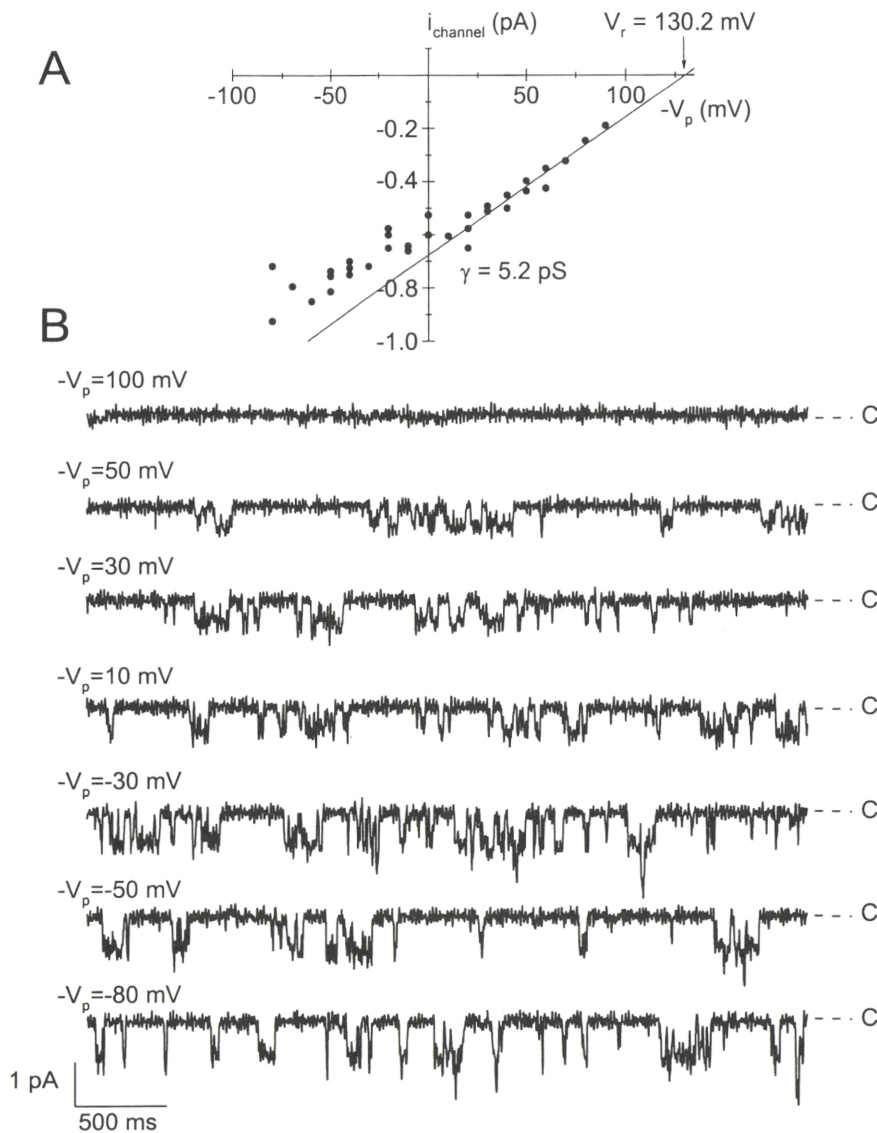

Figure 5. Cell-attached patch on the luminal membrane of an unstimulated gland cell with NaCl-Ringer in the pipette. *A*. Current-voltage relationship of Na^+-selective channel. Each point shows the amplitude of one full opening. Line shows linear regression to points $-V_p > 0\,\mathrm{mV}$ and yields the single-channel conductance (γ) and the reversal-potential (V_r). *B*. Current-traces of Na^+-channels at different potentials. Note the relatively fast kinetics.

gonistic fashion: Upregulation of CFTR Cl^--channel-activity results in diminished Na^+-channel activity (Knowles et al., 1983, Willumsen and Boucher, 1991; Stutts et al., 1995). The frog skin glands present the interesting possibility of investigating the same type of interaction in a secretory epithelium, where the function of Cl^--channels is well understood, but the function of a Na^+-channel unassigned in all previous secretory models. A prediction from Fig. 1 is that both channels should be active simultaneously, indicating an opposite mode of interaction (if any). So far, this question is still open, since the luminal ion channels have only been studied in the resting and acutely stimulated state, under conditions where the large current flowing through Cl^--channels would probably obscure Na^+-channel activity. Thus, upregulation of Na^+-channels may appear in the long run as the epithelium enters the state of stationary secretion under equilibrium conditions.

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