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Noise Analysis Reveals K^+ Channel Conductance Fluctuations in the Apical Membrane of Rabbit Colon

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Summary. In this paper we describe current fluctuations in the mammalian epithelium, rabbit descending colon. Pieces of isolated colon epithelium bathed in Na^+ or K^+ Ringer's solutions were studied under short-circuit conditions with the current noise spectra recorded over the range of 1–200 Hz. When the epithelium was bathed on both sides with Na^+ Ringer's solution (the mucosal solution contained 50 μM amiloride), no Lorentzian components were found in the power spectrum. After imposition of a potassium gradient across the epithelium by replacement of the mucosal solution by K^+ Ringer's (containing 50 μM amiloride), a Lorentzian component appeared with an average corner frequency, $f_c = 15.6 \pm 0.91$ Hz and a mean plateau value $S_o = (7.04 \pm 2.94) \times 10^{-20}$ A^2 sec/cm^2. The Lorentzian component was enhanced by voltage clamping the colon in a direction favorable for K^+ entry across the apical membrane. Elimination of the K^+ gradient by bathing the colon on both sides with K^+ Ringer's solutions abolished the noise signal. The Lorentzian component was also depressed by mucosal addition of Cs^+ or tetraethylammonium (TEA) and by serosal addition of Ba^{2+}. The one-sided action of these K^+ channel blockers suggests a cellular location for the fluctuating channels. Addition of nystatin to the mucosal solution abolished the Lorentzian component. Serosal nystatin did not affect the Lorentzian noise. This finding indicates an apical membrane location for the fluctuating channels. The data were similar in some respects to K^+ channel fluctuations recorded from the apical membranes of amphibian epithelia such as the frog skin and toad gallbladder. The results are relevant to recent reports concerning transcellular potassium secretion in the colon and indicate that the colon possesses spontaneously fluctuating potassium channels in its apical membranes in parallel to the Na^+ transport pathway.

Key words K^+ channels · TEA · Ba^{2+} · Cs^+ · nystatin · fluctuation analysis

Introduction

Current fluctuation analysis is a relatively recent technique which has already proven to be a powerful and useful method for studying ion transport in epithelial membranes. For this reason, we applied this technique to the problem of potassium transport by the mammalian colon. The rabbit descending colon is advantageous for such studies because it has been studied extensively *in vitro* using other electrophysiological methods and radioisotopic techniques. Therefore much is known about its electrical and transport properties.

The rabbit colon possesses an amiloride-sensitive Na^+ transport system which actively absorbs Na^+ from the lumen to the serosa (Frizzell, Koch & Schultz 1976). In addition, the colon secretes K^+. The mechanism of K^+ secretion has been disputed. Some investigators found evidence for active secretion of K^+ (Yorio & Bentley, 1977; Wills & Biagi, 1982; McCabe, Cooke & Sullivan, 1982) while others reported no evidence for active transport of this ion (Frizzell et al., 1976; Frizzell & Schultz, 1978; Fromm & Schultz, 1981). Microelectrode studies indicate that the apical membrane of this epithelium possesses a significant amiloride-insensitive or "leak" ion conductance in parallel to amiloride-sensitive Na^+ channels (Wills, Lewis & Eaton, 1979*b*; Thompson, Suzuki & Schultz, 1982). Preliminary studies (Wills et al., 1979*b*; Clausen & Wills, 1981) suggest that potassium ions are responsible for at least part of this "leak" pathway. Therefore we asked the following questions: (*i*) is it possible to detect apical membrane K^+ channels in the rabbit colon by means of noise analysis, and (*ii*) if so, are the characteristics of these channels similar to those observed in amphibian epithelia such as the frog skin (Van Driessche & Zeiske, 1980*a, b*) or toad and *Necturus* gallbladder?

A portion of these results was presented at the spring, 1981, meeting of the German and Austrian Physiological Societies in Innsbruck, Austria (Zeiske, Wills & Van Driessche, 1981).

Materials and Methods

Colons were obtained from 23 domestic and 10 white New Zealand rabbits of both sexes, weighing 2–3 kg. The results

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from the two groups were similar, therefore the data have been combined. Briefly, a 10-cm length of descending colon was removed, opened as a flat sheet and rinsed free of contents. The epithelium was then separated from the underlying muscle layers using blunt dissection (Frizzell et al., 1976; Wills et al., 1979*b*) and mounted vertically between silicon rubber seals in an Ussing-type chamber which was modified to reduce edge-damage and mechanical vibrations (Van Driessche & Zeiske, 1980*a, b*). The exposed tissue area was 0.126 cm^2. The temperature of the chambers was maintained at 37° C by immersion in a temperature-controlled water bath connected to a thermistor. During recordings (approximately 2 min) the water bath was disconnected from the circulator. Both sides of the chamber were sealed off from the outside atmosphere and stirring and bubbling were discontinued to avoid mechanical noise.

The epithelium was normally bathed with a NaCl-HCO_3 Ringer's solution with the following composition (in mM): 136.2 Na^+, 7 K^+, 121 Cl^-, 2 Ca^{2+}, 1.2 Mg^{2+}, 25 HCO_3^-, 1.2 $H_2PO_4^-$, 1.2 SO_4^{2-}, and 11.1 glucose. In gluconate or potassium Ringer's, chloride and/or sodium were replaced by gluconate and/or potassium, respectively. In Ba^{2+}-containing solutions, SO_4^{2-} was replaced by Cl^- or NO_3^-. Mucosal solutions will be referred to with the index "M" and serosal solutions with the index "S". To oxygenate the tissue and maintain pH, the solutions were continuously bubbled with a mixture of 95% O_2 and 5% CO_2. Tetraethylammonium chloride (TEA) was dissolved into Ringer's solution; Ba^{2+} and Cs^+ salts were added from concentrated stock solutions in distilled water. Nystatin (mycostatin; Sigma) was prepared as a 5 mg/ml stock solution (2,923 units/ml) in methanol. Addition of methanol alone in the concentrations used in the present study had no significant effect on membrane current fluctuations. Amiloride (a generous gift of Merck, Sharp and Dohme) was added to a final concentration of 50 μM to all mucosal bathing solutions. Previous experiments (Zeiske, Wills & Van Driessche, 1982) indicated that this amount blocked Na^+ currents across the apical membrane completely. Except for nystatin, the exposure to other agents (Cs^+, TEA or Ba^{2+}) was not longer than needed for the current-noise recording periods (total time usually less than 4 min.

Voltage-measuring and current-passing electrodes consisted of NaCl Ringer's or Na gluconate Ringer's-filled agar bridges led to Ag-AgCl half-cells. The transepithelial resistance (R_T) was calculated (using Ohm's Law) from the open-circuit potential (V_T) and the short-circuit current (I_{sc}). Alternatively, R_T was also calculated from the current deflection produced by passing a 10-mV voltage step across the epithelium. The two methods produced equivalent results. Tissues were continuously voltage-clamped at 0 mV (short-circuit) or other specified potentials except for brief intervals (less than 30 sec) when the open-circuit transepithelial potential was monitored. Transepithelial potentials were referenced to the serosal solution.

Data Acquisition and Analysis

Details concerning the electronic equipment for low-noise amplification and recording of the short-circuit current are presented in Van Driessche and Lindemann (1978) and Van Driessche and Zeiske (1980*a, b*). Most generally the amplified current fluctuations were filtered with 48 dB/octave high pass and low pass filters (Rockland 852) with cut-off frequencies of 0.11 and 220 Hz, respectively. The filtered analog signal was then digitized at a sample interval of 2 msec, transferred to a buffer memory and stored on magnetic tape. The final spectra represent mean values of forty to sixty data blocks of 2048 data points. The data were analyzed by using either: 1) an offline computer (Digital Equipment 11–34) to average the data blocks, then calculate the power spectrum (Decus 179 fast Fourier transform routine), or 2) a recently developed online microprocesser-based system (Intel). In the latter system, three records (512 points in length) were sampled simultaneously at different sampling rates (102.4, 409.6 and 1638.4 Hz). The spectra records were superimposed into a single spectrum which covered three decades in the frequency domain. Thus, it was possible to Fourier transform the data, average them with preceeding records ($n=20$) and display the resulting power spectra during the recording. The final averaged power spectrum was then normalized to 1 cm^2 of nominal tissue area.

To evaluate the spectra, we used the following simple model for an ionic channel. In this simple case, ionic channels may fluctuate randomly between one open and one closed state with rate constants α_{01} and α_{10} as follows:

$$\text{open} \underset{\alpha_{10}}{\overset{\alpha_{01}}{\rightleftharpoons}} \text{closed}.$$

The spectrum of the current fluctuations associated with the open-close reaction of the channel can be described by a Lorentzian function of the form (*cf.* Lindemann & Van Driessche, 1977):

$$S = S_o/(1+(f/f_c)^2) \tag{1}$$

where

$$S_o = 4Mi^2 \cdot \frac{\alpha_{10}\cdot\alpha_{01}}{(\alpha_{01}+\alpha_{10})^3} \tag{2}$$

with

$$2\pi f_c = \alpha_{01}+\alpha_{10} \tag{3}$$

where S is the spectral density, f is frequency, S_o is the plateau value of the Lorentzian component, M is channel density, i is the single-channel current and f_c is the corner frequency (i.e. f at 1/2 S_o). As in previous studies (Van Driessche & Zeiske, 1980*b*; Gögelein & Van Driessche, 1981*a*), the Lorentzian plateau values were partially obscured by a high intensity, low frequency (LF) noise. For this reason the data were analyzed by simultaneously fitting a Lorentzian curve and a linear background noise component as described by Van Driessche and Zeiske (1980*b*), using the following relationship:

$$S = K_b/f^\alpha + S_o/(1+(f/f_c)^2) \tag{4}$$

where K_b/f^α is defined as the linear spectral noise component with K_b representing the background component at 1 Hz and α is its slope in a double logarithmic scale.

To fit Eq. (4) to the data, we used the same methods employed by Van Driessche and Zeiske (1980*b*). In brief, the spectra were stored and analyzed on a digital computer (PDP 11/34), equipped with a floating point processor. The curve-fitting routine utilized was the least-squares subroutine (VA05A from the Harwell Subroutine Library (Atomic Energy Establishment, Harwell, Berkshire). For further details concerning the curve-fitting procedure, *see* Van Driessche and Zeiske (1980*b*).

The slope of the linear spectral component (α) averaged 1.5 ± 0.94, similar to that reported for frog skin and *Necturus* gallbladder. Although the source of this signal was not identified, previous work suggested that it may reflect ion diffusion through parallel shunt pathways (Gögelein & Van Driessche, 1981*a*). The low frequency noise was increased by voltage clamping. At potentials larger than 50 mV, the LF noise usually exceeded the relaxation noise, preventing analysis of the spectra. Generally procedures which diminished the noise also depressed the LF component (as will be shown later in Fig. 6,

7 and 8, below). A similar observation was reported for frog skin epithelium (Van Driessche & Zeiske, 1980*a, b*).

The above features are consistent with the interpretation that the LF noise is a specific effect, such as a restricted diffusion in ionic channels, as proposed by Fishman, Moore and Poussart (1975) and Van Driessche and Zeiske (1980*a, b*). Admittedly, our selection of the fitting procedure carries with it some possibility of erroneously ascribing low frequency spectral components to specific processes. Nonetheless, we believe this simple and heuristic approach is justified as it has already proven useful in previous evaluations of K^+ current noise in other epithelia (Van Driessche & Gögelein, 1978; Van Driessche & Zeiske, 1980*a, b*; Zeiske, Van Driessche & Machen, 1980; Gögelein & Van Driessche, 1981*a, b*).

Results

Effects of Potassium Concentration and Electrical Potentials

When voltage-clamped to zero mV and bathed on both sides with Na^+ Ringer's solutions, the colon showed no spontaneous current noise as illustrated by the example in Fig. 1. The spectral density of the current signal is presented in a double logarithmic plot as a function of frequency. Under these conditions the spectral data were essentially linear between 1 and 40 Hz with a slope of about -2. In the following experiments, 50 μM amiloride was always present in the mucosal solutions.

In contrast to results obtained with Na^+ Ringer's solutions, a distinct shoulder appeared in the spectrum when a potassium gradient was imposed across the epithelium. This shoulder is illustrated by the second curve in Fig. 1 which shows the effects of replacing the mucosal Na^+ by K^+. This shoulder indicates the existence of a relaxation noise component and can be interpreted to originate from random conductance fluctuations of an ensemble of membrane channels (Verveen & DeFelice, 1974).

The inset of Fig. 1 shows the results of a simultaneous curve-fit of these data by a Lorentzian and a linear background component. The corner frequency (f_c) was 8 Hz and the plateau value (S_o) was 3.2×10^{-20} A^2 sec/cm^2 in this example. For pooled data from 10 colons, the average f_c was 15.6 ± 0.91 Hz and S_o was $(7.04 \pm 2.94) \times 10^{-20}$ A^2sec/cm^2.

The relaxation noise disappeared when K^+ Ringer's was placed on both sides of the epithelium as shown in Fig. 2. When a serosal-to-mucosal potassium gradient was imposed by replacing the mucosal solution again with Na^+ Ringer's (containing 50 μM amiloride to inhibit Na^+ transport), the Lorentzian component was re-established. Thus Lorentzian noise could be detected with either mucosally or serosally oriented K^+ gradients. The corner frequency and intensity of the noise were similar regardless of the orientation of the gradient.

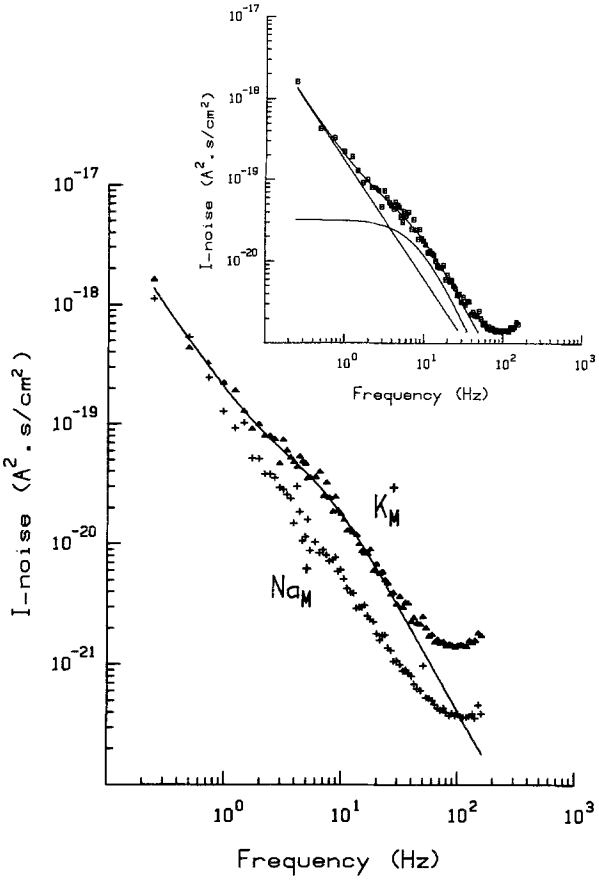

Fig. 1. Power spectra from a single colon showing the short-circuit current (I_{sc}) noise with different mucosal solutions: (1) "+" = NaCl Ringer's on both sides (Na^+_M), (2) "▲" = KCl Ringer's on the mucosa and NaCl Ringer's solution on the serosa (K^+_M). The intensity of the I_{sc} noise is greater with K^+_M than with Na^+_M. In addition a "shoulder" appears in K^+_M, indicating a Lorentzian noise component. The result of simultaneously fitting a Lorentzian curve and a linear low frequency component to the data is indicated in the inset. The corner frequency of the Lorentzian in this example was 8 Hz; S_o was 3.2×10^{-20} A^2sec/cm^2. R_T was 658 Ω cm^2 for both spectra and V_T was -13 and -12 mV for K^+_M and Na^+_M, respectively

In the absence of potassium gradients or in cases of a weak noise signal, relaxation noise could be obtained by appropriate voltage clamping of the epithelium. Figure 3 illustrates a colon which was bathed on both sides with K^+ Ringer's before and during such voltage clamping. As can be easily seen in this example, application of a small mucosa-negative potential caused the appearance of relaxation noise.

In the following experiments, we investigated more extensively the effects of electrical and chemical driving forces on the K^+ current relaxation

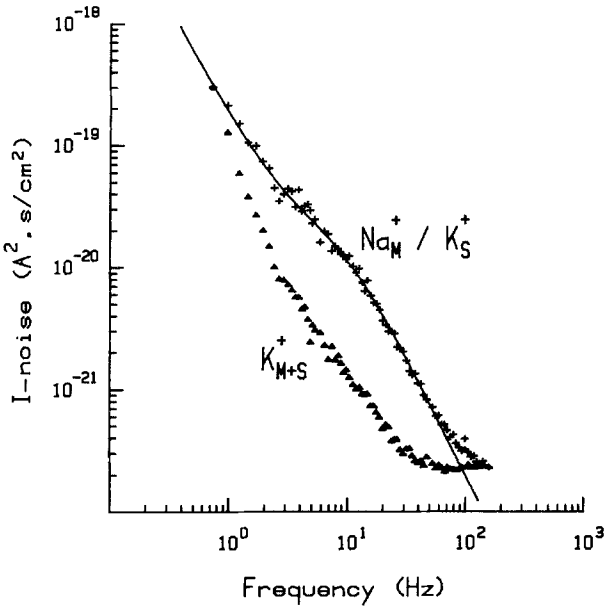

Fig. 2. The effects of potassium gradients on I_{sc}-noise spectra. "▲" = K gluconate Ringer's on both sides of the epithelium (K^+_{M+S}). No Lorentzian component was detectable. Replacement of the mucosal solution with Na gluconate Ringer's ("+" = Na^+_M/K^+_S re-established the Lorentzian noise. Amiloride (50 μM) was present in the Na^+ Ringer's solution. S_o was 2.0×10^{-20} A^2 sec/cm^2 and f_c was 9 Hz

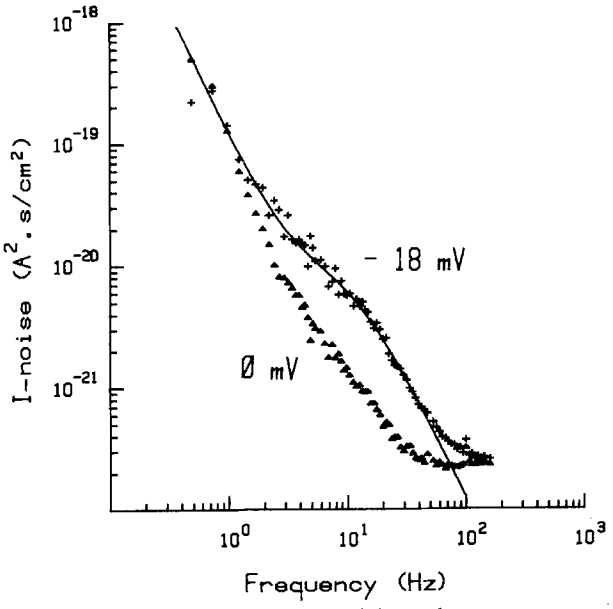

Fig. 3. The effects of clamp potential on the power spectra. In this experiment the colon was bathed on both sides with K gluconate Ringer's solution and voltage-clamped to 0 mV (▲). Application of a −18 mV (mucosa negative) potential led to the emergence of the Lorentzian component (+). S_o was 9.0×10^{-21} A^2 sec/cm^2 and f_c was 12 Hz

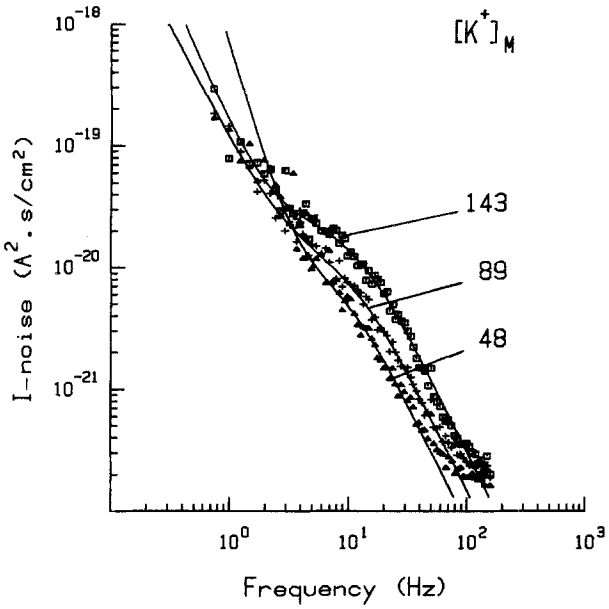

Fig. 4. The effects of potassium concentration gradient $[K^+]$ on K^+-current fluctuations. As spontaneous Lorentzian noise could not be detected at $[K^+]_M$ below 50 mM when the epithelium was clamped at 0 mV, this figure displays spectra recorded at +18 mV (mucosa positive). Increasing $[K^+]_M$ above this value increased the intensity of the noise (S_o). $[K^+]_M$ and S_o values ($S_o \times 10^{+20}$ A^2 sec/cm^2) for each of the spectra were as follows: (1) ▲, 48 mM and 1.0, (2) +, 89 mM and 1.1, and (3) □, 143 mM and 2.3. f_c was not significantly affected by $[K^+]_M$ (9, 11 and 12 Hz, respectively). All solutions contained gluconate as the major anion. S = Na gluconate Ringer's solution. M solutions also contained 50 μM amiloride

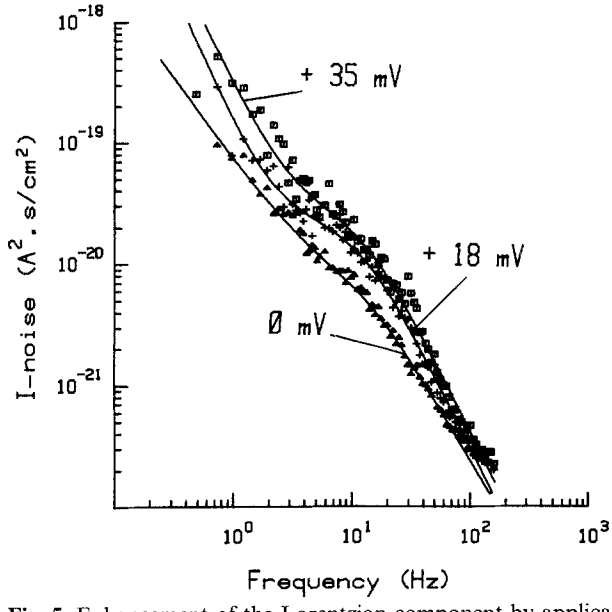

Fig. 5. Enhancement of the Lorentzian component by application of a mucosa-positive electrical driving force. In this experiment a serosally directed potassium gradient was imposed across the epithelium. Clamp potentials and S_o values ($S_o \times 10^{21}$ A^2 sec/cm^2) were as follows: (1) ▲, 0 mV and 0.5, (2) +, +18 mV and 2.3, and (3) □, +35 mV and 2.7. f_c values were 14, 12 and 12 Hz, respectively

noise. In these studies potassium gradients were imposed across the epithelium, usually directed from mucosa to serosa. In general, the epithelium tolerated this procedure more easily than prolonged exposure to high potassium levels in the serosal solution. Figure 4 illustrates the effects of increasing mucosal potassium concentration on the current noise. In this example, because spontaneous Lorentzian noise could not be detected with $[K^+]_m$ below 50 mM when the epithelium was clamped at 0 mV, the experiment was done at a clamp PD of +18 mV (mucosa positive). The intensity of the current noise was increased as a function of increasing potassium concentration, whereas the corner frequency remained essentially constant, as found for the reversed gradient experiments above.

The effects of electrical driving forces on the current relaxation noise are more clearly illustrated in Fig. 5. In this experiment, a transepithelial potassium gradient was present as described above. As shown in this example, the current as well as the Lorentzian noise increased when the clamp potential was increased in a mucosa-positive direction. Again, f_c was not significantly affected by clamp potential, as summarized in Table 1.

Effects of Potassium Channel Blocking Agents

Because of the dependence of the relaxation noise on potassium and electrical gradients described above, the effects of potassium channel blockers were examined. Substances such as TEA, Cs^+ and Ba^{2+} are known to interfere with spontaneously fluctuating potassium channels in the apical membranes of other epithelia (Van Driessche & Gögelein, 1978; Van Driessche & Zeiske 1980*a, b*). Therefore the action of each of these ions was tested in the presence of a mucosa-to-serosa gradient of potassium as described above.

TEA. TEA is known to block potassium channels both in excitable membranes such as squid axon (Hille, 1967) and in epithelial membranes such as the gallbladder (Van Driessche & Gögelein, 1978). Addition of 10–30 mM TEA to the mucosal bathing solution of the colon decreased the current fluctuation intensity by 33–50% ($n=6$). However, f_c was not significantly affected (*see* Fig. 6). Control experiments (with 30 mM NaCl and 50 μM amiloride substituted for the TEA-Cl) indicated that a small part of this effect (<5%) was not due to TEA alone but rather was caused by the addition of permeable anions (in this case Cl^-) as shown in Fig. 6. The effects of TEA were rapid

Table 1. Effect of voltage clamp steps on I_{sc} noise characteristics

V_T* (mV)	$S_o \times 10^{20}$ (A^2sec/cm^2)	f_c (Hz)
0	3.9±1.5	16±0.7
+18	10.9±4.6	16±1.8
+35	15.9±6.7	16±2.5

M = K gluconate Ringer's solution and 50 μM amiloride. S = Na gluconate Ringer's solution.
n(no. of colons) = 5.
$X \pm$ SEM
* Mucosa positive.

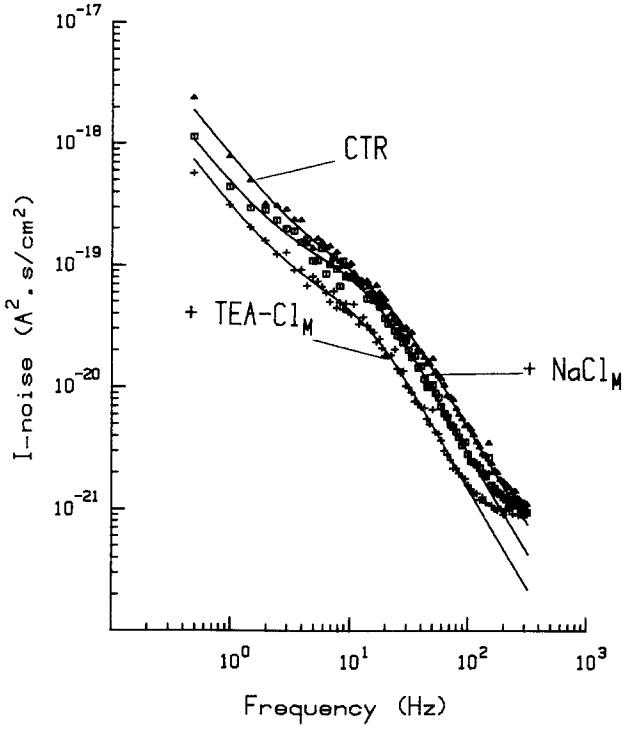

Fig. 6. Reduction of I_{sc} noise by mucosal application of TEA. ▲ = control spectrum with M = K gluconate Ringer's solution and S = Na gluconate Ringer's (CTR). S_o was 6.4×10^{-20} A^2 sec/cm^2 and f_c was 20 Hz. + = spectrum after addition of 10 mM mucosal TEA Cl (+TEA-Cl_M). S_o decreased to 4.4×10^{-20} A^2 sec/cm^2 and f_c was not significantly changed (14 Hz). □ = as in CTR except with addition of 10 mM NaCl (and 50 μM amiloride). A small influence on the noise intensity is shown. $S_o = 8.1 \times 10^{-20}$ A^2 sec/cm^2 and $f_c = 15$ Hz. $NaNO_3$ showed similar effects

(less than 2 min) and completely reversible. Serosal addition of TEA, in contrast, had no effect.

Cs^+. Cs^+ is known to block potassium channels in the basolateral membrane of the colon in a voltage-dependent manner (Wills, Eaton, Lewis & Ifshin, 1979*a*). Addition of this cation to the mucosal solution in the present experiments diminished the

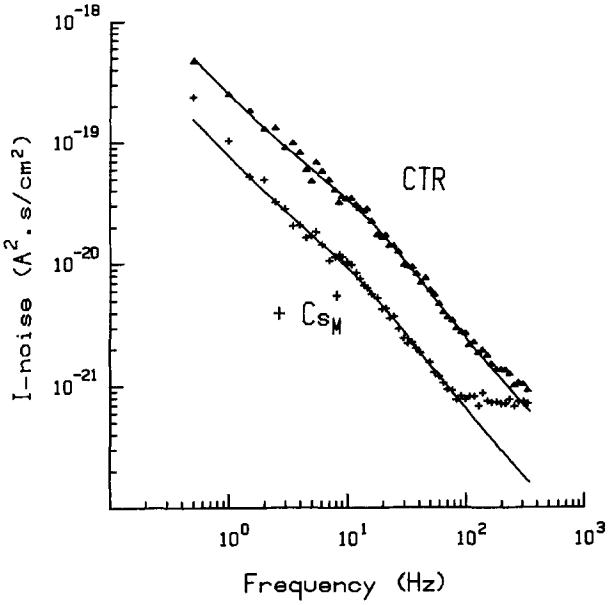

Fig. 7. Effects of mucosal addition of Cs^+ on I_{sc} noise. Conditions in this experiment were the same as in Fig. 6. (▲ = CTR) and (+ = Cs^+_M) show the spectra before and after addition of 10 mM $CsNO_3$. S_o values were 16.4×10^{-21} and 4.3×10^{-21} A^2 sec/cm^2 and f_c's were 16 and 14 Hz before and after Cs^+ addition, respectively. The decrease in the I_{sc} noise was greater than the same amount of $NaNO_3$ (*see also* Fig. 6)

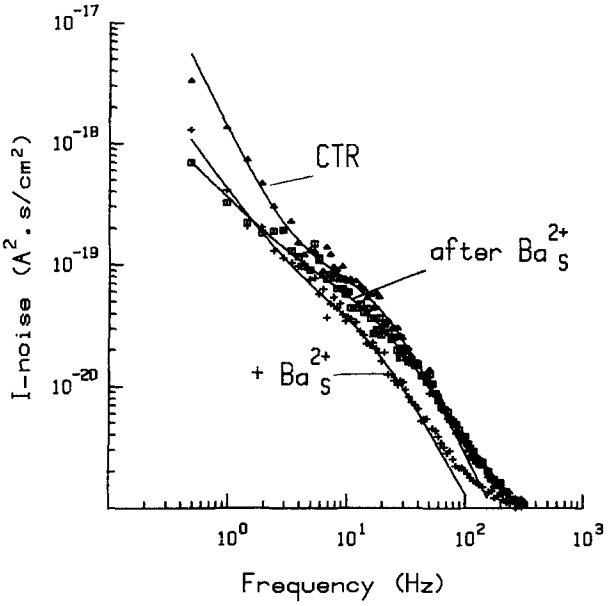

Fig. 8. Effects of serosal Ba^{2+}. Experimental conditions were similar to Fig. 6. In this example the I_{sc} noise was suppressed by serosal application of 5 mM Ba^{2+}. S_o before Ba^{2+} was 8.1×10^{-20} A^2 sec/cm^2 and f_c was 19 Hz. During Ba^{2+}, S_o was 3.0×10^{-20} A^2 sec/cm^2 and f_c was 15 Hz. The effect was poorly reversible and there was a small reduction of the transepithelial resistance (≃13%) after several minutes exposure to Ba^{2+}. S_o after Ba^{2+} was 3.5×10^{-20} A^2 sec/cm^2 and f_c was 18 Hz

relaxation noise in a reversible manner ($\Delta S_o = 15$–75%; *see* Fig. 7). As with TEA, the reduction of the current fluctuations was larger than could be accounted for by the addition of small amounts of permeable anions such as Cl^- or NO_3^-. Serosal Cs^+ had no effect over a voltage-clamp potential range of +30 to −50 mV

Ba^{2+}. In contrast to Cs^+ and TEA, mucosal addition of Ba^{2+} did not affect the Lorentzian component. Addition of Ba^{2+} (5 mM) to the serosal solution, however, reduced the relaxation noise by at least 50%. Again, the corner frequency was not significantly affected (*see* Fig. 8). The effects of serosal Ba^{2+} were poorly reversible and the resistance of the colon was slightly decreased after prolonged exposure (approximately 20 min) to the agent ($R_T = 359 \pm 34.2$ and 313 ± 26.8 Ω cm^2 before and after Ba^{2+}, respectively; $n = 3$).

Effects of Nystatin

Previous microelectrode experiments indicated that mucosal application of the polyene antibiotic nystatin effectively abolishes the apical membrane resistance of the colon within seconds by increasing its permeability to small monovalent ions (Wills et al., 1979*b*). In contrast to the reduction in the apical membrane resistance, mucosal nystatin did not significantly reduce the basolateral membrane and paracellular resistances so long as intracellular ionic composition was not severely disrupted. To lessen disruption of the intracellular ionic contents, the mucosal solution contained no Na^+ nor Cl^- and its K^+ activity approximated the intracellular K^+ activity (for further details, *see* Wills et al., 1979*b*). More recent work has demonstrated that *serosal* application of nystatin depolarizes the basolateral membrane potential and increases the resistance ratio, indicating that nystatin is capable of reducing basolateral membrane resistance and altering its permeability properties (Wills, *unpublished observations*).

Figure 9 illustrates the effects of nystatin on the spontaneous relaxation noise. When added to the mucosal solution (K gluconate Ringer's), nystatin (40 units/ml) increased the transepithelial potential and current ($\Delta V_T = 440\%$ and $\Delta I_{sc} = 710\%$; *see* Table 2) and decreased the transepithelial resistance ($\Delta R_T = 35\%$). While these effects were qualitatively similar to our previous results with this drug on the colon (Wills et al., 1979*b*), they were not as large. In the present experiments, a lower dose of nystatin was employed. Therefore it is

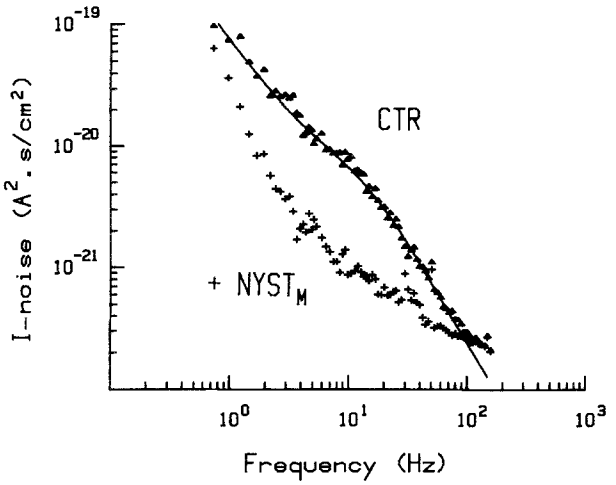

Fig. 9. Application of nystatin (40 units/ml) to the mucosal solution abolished the Lorentzian noise component. ▲ (CTR) = control where M = K gluconate Ringer's solution and S = Na gluconate Ringer's solution. S_o was 5.4×10^{-20} A^2 sec/cm^2 and f_c was 14 Hz. + (+NYST$_M$) = with nystatin

Table 2. Electrical properties of the colon and the effects of nystatin

	V_T (mV)	R_T (Ω cm^2)	I_{sc} (μA/cm^2)
Control	-5.9 ± 0.87	434 ± 181.0	26 ± 4.0
Nystatin mucosa (40 units/ml)	-25.1 ± 3.37	145 ± 6.8	158 ± 21.1

M = K gluconate Ringer's solution
S = Na gluconate Ringer's solution
$n = 8$.

likely that the apical membrane resistance was reduced but not completely abolished by this procedure. The most dramatic effect of the drug was a complete suppression of the relaxation noise. Serosal application of nystatin had no significant effect on the noise signal. Thus reduction of the apical membrane resistance was associated with an abolition of the potassium noise, whereas a reduction of the basolateral membrane resistance had no significant effect.

Discussion

The present results describe for the first time a spontaneous current relaxation noise signal arising from a mammalian "tight" epithelium. The appearance of this current fluctuation noise was dependent on the presence of $[K^+]$ gradients across the epithelium or the imposition of transepithelial electrical potentials. Disruption of the native permeability properties of the apical membrane through the use of the polyene antibiotic nystatin abolished the signal. In the following sections we will first discuss the evidence that the source of the noise signal is localized to the apical membrane, next identify the ion responsible for the current, and lastly, compare these findings to channel fluctuations in other epithelia. Before beginning, however, it is useful to examine the electrical properties and equivalent circuit of this epithelium.

The Influence of Epithelial Equivalent Circuit Parameters on Channel Relaxation Noise

From Eq. (2), it is obvious that the relaxation noise plateau (S_o) depends on the channel transition kinetics (α_{01}, α_{10}), as well as on the single-channel current (i) and channel density (M). In the colon, contrary to observations in other epithelia (Gögelein & Van Driessche, 1981*b*; Zeiske & Van Driessche, 1981), the transition rate constants were not affected by the experimental manipulations, as indicated by the lack of change in f_c. Therefore changes in S_o elicited by changing the ionic or electrical gradients, or by applying K^+ channel blockers, must be accounted for by changes in M or i. One would expect that the application of channel blockers would directly decrease the number of channels available for spontaneous fluctuations (M), thus decreasing S_o. S_o will also decrease when a series resistance element (such as the basolateral membrane resistance in the case of apical membrane channels) is increased, resulting in signal attenuation (Van Driessche & Gögelein, 1980).

The single-channel current (i) for the case of apical membrane potassium channels is described by Eq. (5) below:

$$i = (E_K - V_m) \cdot \gamma \tag{5}$$

where (E_K) is defined as the Nernst potential for K^+ diffusion across the apical membrane, V_m is the apical membrane potential under short-circuit conditions, and γ is the single-channel conductance.

Before interpreting the experimental results, we will briefly discuss the effects predicted from Eq. (5) on the single-channel currents when the electrochemical driving force for K^+ is altered. Three such manipulations were used including: 1) a serosally directed $[K^+]$ gradient (i.e., mucosal replacement of Na^+ by K^+), 2) a mucosally directed $[K^+]$ gradient (serosal Na^+ replacement by K^+), and 3) serosal Ba^{2+} addition. The effects of these manipulations on paracellular currents will be addressed separately.

Serosally Directed $[K^+]$ Gradient. Normally, a small net electrochemical driving force (approximately -17 mV) favors potassium exit when the epithelium is voltage-clamped to 0 mV (Wills & Biagi, 1982). An increase in $[K^+]_m$ will reduce the chemical driving force across the apical membrane. As for the effect on V_m, previous microelectrode measurements of the apical membrane potential (Wills et al., 1979*b*) predict that only a small change may occur in this potential when the mucosal Na^+ is replaced by K^+. The apical membrane potential is usually about -53 mV, cell interior negative, under short-circuit conditions in the absence of Na^+ transport (Schultz, Frizzell & Nellans, 1977). Since the chemical driving force is now reduced or eliminated, the net electrochemical driving force will now reflect only V_m and will favor the inward movement of potassium ions. Therefore in terms of Eq. (5) above, after replacement of the mucosal bath with a high $[K^+]$ Ringer's, the single-channel current i should increase and change direction because of the larger driving force ($E_K - V_m$) now favoring potassium entry. From constant field considerations (Goldman, 1943), one might also expect an elevation in $[K^+]_m$ to increase γ, also leading to an increase in i.

How will changing the electrical driving force across the colon affect single-channel currents? Because the apical membrane resistance (R_a) is nearly 8 times larger than the basolateral membrane resistance (R_{bl}) (Schultz et al., 1977; Wills et al., 1979*b*), voltage-clamping the epithelium will mainly affect the apical membrane potential. Thus increasing the electrical driving force by appropriate voltage-clamping will result in an increased single-channel current in the case of a mucosal $[K^+]$ solution as described above.

Mucosally Directed $[K^+]$ Gradient. How does the application of a mucosally-directed potassium gradient across the colon affect the electrical parameters of the colon? Wills et al. (1979*b*) demonstrated that the basolateral membrane potential is essentially a potassium diffusion potential, attenuated by small permeabilities to Na^+ and Cl^-. Elevations of the serosal $[K^+]$, therefore would be expected to depolarize the basolateral membrane potential (V_{bl}) as well as reduce the chemical driving force for K^+ across this membrane. The intracellular potential under short-circuit conditions will be reduced when a high $[K^+]$ Ringer's solution is the serosal bath. Consequently, the apical membrane electrical driving force (favoring potassium entry) will be reduced. Since a chemical gradient for K^+ exit is present across the apical membrane, the electrochemical potential favoring potassium exit from the cell across the apical membrane will be increased. As a result, the single-channel current should increase. With high potassium on both sides of the epithelium, the chemical driving force is also abolished such that no significant net driving force for K^+ entry or exit will exist. The electrical driving force across the apical membrane and the single-channel current should be restored by voltage-clamping the epithelium.

Serosal Ba^{2+} Addition. The basolateral membrane can also be depolarized by serosal addition of Ba^{2+}. Addition of 5 mM Ba^{2+} to the serosal bath depolarized V_{bl} by nearly 80% (Wills, 1981; and *unpublished observations*). Preliminary results suggest that Ba^{2+} reduces the potassium conductance of this membrane similar to its effects in other tight epithelia (Nagel, 1979). Consequently, serosal Ba^{2+} should act to reduce the electrical driving force across the apical membrane, similar to the effects of serosal K^+ Ringer's solutions. In our experiments, serosal Ba^{2+} was added to epithelia bathed on the mucosal side with K^+ Ringer's solution (i.e. a serosally directed $[K^+]$ gradient). In this condition the single-channel current should be reduced because of the reduction in the electrical driving force favoring potassium entry. The effects of serosal addition of other potassium blocking agents on the electrical driving force across the apical membrane are more difficult to predict. Wills et al. (1979*a*) found that Cs^+ blocked the potassium conductance of the basolateral membrane at large negative potentials beyond those used in the present study. As yet no studies have determined the effects of TEA on this membrane.

Location of the Signal Source

Several factors suggest that the spontaneous current fluctuations arise from the apical membrane. First, it is unlikely that the basolateral membrane could generate such a large signal since the resistance of the basolateral membrane is much lower than the apical membrane under the conditions employed in these experiments (Wills et al., 1979*b*, *see above*). Therefore any basolateral membrane signal should be too attenuated to be detectable (Van Driessche & Gögelein, 1980). Second, it is similarly unlikely that the noise source is in the paracellular pathway. For a paracellular path, one might expect a symmetrical access for serosally or mucosally applied agents. If true, the difference in efficacy of the well known K^+ channel blockers Ba^{2+} (which acted only from the serosal side),

Cs^+, and TEA (which acted only from the mucosal side) may suggest that the fluctuating channels are situated in the cellular pathway. Lastly, the known action of mucosal nystatin in reducing the apical membrane resistance (*see below*) and its abolition of the noise source also argue for a cellular channel source of the relaxation noise rather than a paracellular origin.

In the present study, it was not possible to resolve the location of the potassium noise source by using amiloride. The method was successfully used in previous studies of the frog skin (Van Driessche & Zeiske, 1980*a*) where a small amount of Na^+ was present in the mucosal solution. When amiloride is added to the short-circuited frog skin a large hyperpolarization is produced in the intracellular potential (Helman & Fischer, 1977) and thus the net electrochemical driving force for K^+ movement is altered. By comparison, the expected change in the intracellular potential in the colon is only a few millivolts (Schultz et al., 1977) and was too small to have a measurable effect on the relaxation noise. Perhaps the most striking effect of the present results was the elimination of the noise signal by mucosal, but not serosal, nystatin addition. This is strong evidence that the spontaneous current fluctuations arise from the apical membrane because nystatin acts to reduce the net electrical and chemical driving forces (*see* Eq. 5) across the apical membrane (Wills et al., 1979*b*). In contrast, the reduction of the noise signal seen after serosal Ba^{2+} can be interpreted in two ways. First, Ba^{2+} increases the basolateral membrane resistance in series with the noise source. Second, serosal Ba^{2+} acts indirectly to depolarize the short-circuit apical membrane potential as described above.

Source of the Relaxation Noise

The dependence of the apical membrane relaxation noise on potassium gradients and its response to imposed transepithelial electrical potentials suggest that the relaxation noise is due to potassium channels. Other findings that indicate the spontaneously fluctuating channels are potassium channels include the inhibitory effects of potassium channel blocking agents on the relaxation noise. Unfortunately, none of the blocking agents employed in these experiments was able to totally suppress the transepithelial current or relaxation noise. In addition, the current was not always correlated with decreases in the relaxation noise. Since no specific transcellular K^+ current could be extracted from the I_{sc} measurements, it was not possible to estimate single-channel conductances or channel density from the present data. Nonetheless, the fluctuation analysis technique has permitted us to observe the characteristics of a conductive pathway which is not readily accessible by other transepithelial electrical methods.

Because the relaxation noise was rapidly and reversibly decreased by mucosal Cs^+ or TEA, it is likely that these agents act directly by blocking potassium channels. Neither of these substances was effective from the serosal side. In contrast Ba^{2+} had no effect from the mucosal side but serosal addition in concentration ranges of 4–15 mM depressed the current fluctuations. As described above, this effect may be secondary to the effects of Ba^{2+} on the basolateral membrane potential.

The failure of mucosal Ba^{2+} to block the relaxation noise is unexplained. This may be related to the use of high mucosal K^+ solutions which were necessary to see the relaxation noise. It is possible that Ba^{2+} and K^+ may compete for binding sites at the potassium channel. If true, then it is possible that either 1) TEA or Cs^+ act on the potassium channels at a different site, or 2) that the K_m for Ba^{2+} binding is much higher than the K_m for TEA or Cs^+.

In contrast to serosal Ba^{2+} effects, serosal addition of Cs^+ or TEA was ineffective. One possible explanation for this lack of effect is a voltage dependence of the blocking action, at least for Cs^+. Potential-dependent effects of Cs^+ on the basolateral membrane potassium conductance have been previously found from current-voltage measurements in the rabbit colon (Wills et al., 1979*b*). As above, a second explanation could be that the K_m values for K^+ channel blockage by serosal Cs^+ or TEA are much larger than for serosal Ba^{2+}.

Comparison to K^+ Fluctuations in Other Epithelia

The relaxation noise described here shows some similarities to potassium relaxation noise recorded from the apical membranes of other epithelia such as the frog skin and stomach (Van Driessche & Zeiske, 1980*a, b*; and Zeiske, Van Driessche & Machen, 1980, respectively), toad gallbladder (Van Driessche & Gögelein, 1978) and *Necturus* gallbladder (Gögelein & Van Driessche, 1981*a, b*). Like the frog skin and unlike gallbladder, the relaxation noise was usually present only when a potassium gradient was present across the epithelium. However, unlike frog skin, the relaxation noise in the colon did not depend on the orientation of the concentration gradient. As in the case of all the other epithelia, voltage-clamping in a direction

enhancing the electrochemical gradient increased the intensity of the current noise (S_o). However, this procedure had no effect on the corner frequency (f_c), in contrast to the gallbladder and the frog skin, where f_c was shifted to lower frequencies as S_o increased with mucosa-positive potentials (Gögelein & Van Driessche, 1981*b*; Zeiske & Van Driessche, 1981).

The corner frequency (mean $f_c = 15.5 \pm 0.91$ Hz) was equal to that found in frog gastric mucosa (Zeiske, *unpublished results*), larger than *Necturus* gallbladder (2–6 Hz; Gögelein & Van Driessche, 1981*a*) and smaller than in frog skin (81 Hz; Van Driessche & Zeiske, 1980*a*).

The major difference between the various epithelia with respect to potassium channel noise was the action of blocking agents. In frog skin (Van Driessche & Zeiske, 1980*a, b*) mucosal Ba^{2+} and Cs^+ were effective in suppressing relaxation noise while TEA was ineffective. In contrast, in *Necturus* gallbladder, mucosal Cs^+ was ineffective while Ba^{2+} and TEA inhibited the signal. Thus rabbit colon presents yet another pattern for the array of agents which are able to block potassium channels in epithelial membranes.

Comparison of Apical and Basolateral Potassium Channels

Unlike the apical membrane, which now appears to have potassium channels in addition to its more well-known Na^+ permeability, little is known about the channel properties of the basolateral membrane although this membrane is primarily permeable to K^+ (Wills et al., 1979*a, b*). In preliminary experiments to be published elsewhere (Van Driessche, Wills, Hillyard & Zeiske, *in preparation*) we were able to access the basolateral membrane by adding a maximum dose (200 units/ml) of nystatin to the mucosal solution. Nystatin at this level virtually abolishes the apical membrane resistance (Wills et al., 1979*b*). Consequently, with this method, one can voltage-clamp across the basolateral membrane. In these experiments, the spectra were recorded in a frequency range of 1–400 Hz. A novel Lorentzian component emerged in the power spectra with an average corner frequency of 210 ± 59 Hz and a plateau of 3.1 $(\pm 1.40) \times 10^{-21} A^2$ sec/cm^2 (f_c and S_o values are averaged for 3 colons). The Lorentzian component was abolished after addition of 5 mM serosal Ba^{2+}. It is possible that this signal may arise from basolateral membrane potassium channels since microelectrode experiments (Wills, *unpublished observation*; *see* Results) demonstrated a blockage of basolateral membrane K^+ conductance by Ba^{2+}. The same nystatin method has also been employed in the frog skin and tadpole skin with similar results (Van Driessche, Wills, Hillyard & Zeiske, *in press*). This technique may now allow comparison of apical and basolateral membrane channel properties. One intriguing aspect of these data is the difference in corner frequency between the basolateral membrane and apical membrane K^+ channels. It would be interesting to determine whether the biochemical structure of the channels or some feature of their membrane micro-environments may account for this difference.

Implications for the Electrical Properties of the Rabbit Colon

Our present results confirm and extend our previous observations concerning the electrical properties of the rabbit descending colon. The findings are consistent with an apical membrane potassium conductance and thus support the results of previous microelectrode and impedance studies of this epithelium (Wills et al., 1979*b*; Clausen & Wills, 1981). These studies showed that a significant conductance was present in the apical membrane even in the absence of Na^+ and Cl^- in the mucosal bathing solution. The present results indicate that at least part of this conductance may be through fluctuating K^+ channels. In addition, the findings are relevant to our recent radioisotopic investigations which demonstrated active potassium secretion by the colon (Wills & Biagi, 1982). The presence of potassium ion conductance noise suggests that potassium secretion may include exit of potassium from the cell via conductive channels in the apical membrane. It remains to be determined whether such net potassium fluxes can be blocked by the same agents which blocked the potassium noise, particularly mucosal Cs^+ or TEA.

In this regard it is notable that some authors (Frizzell et al., 1976; Frizzell & Schultz, 1978; Frizzell & Turnheim, 1978; Fromm & Schultz, 1981) do not observe net potassium transport by the colon. It is possible that this difference may be related to differences in the apical membrane potassium permeability. Thus it would be interesting to determine how the potassium noise which we have observed may be modified by different conditions of K^+ transport. Recently, Fromm and Schultz (1981) have proposed that the paracellular pathway in the colon is highly selective for potassium and that the permeability to potassium decreases as the ambient K^+ concentration is increased. An investigation of this notion was beyond the scope

of this paper. Further experiments evaluating the potassium conductance of the paracellular route are necessary to resolve this issue.

In summary, we have observed a spontaneous relaxation current noise from the isolated rabbit descending colon. The relaxation noise responds to potassium and electrical potential gradients across the epithelium and is reduced by potassium channel blocking agents, suggesting that the signal arises from spontaneously fluctuating potassium channels. Reduction of the apical membrane resistance by mucosal application of the polyene antibiotic nystatin abolished the signal. Therefore the noise appears to arise from K^+ channels in the apical membrane. These findings are consistent with previous microelectrode and impedance measurements which demonstrated a potassium conductance in the apical membrane. Lastly, the spontaneously fluctuating potassium channels observed in the colon resemble in some aspects those located in excitable membranes and the apical membranes of other epithelia such as the frog skin, gallbladder and gastric mucosa.

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