Biochimica et Biophysica Acta, 688 (1982) 201–210
Elsevier Biomedical Press

BBA 71177

Na^+ CHANNELS AND AMILORIDE-INDUCED NOISE IN THE MAMMALIAN COLON EPITHELIUM

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(Received October 19th, 1981)
(Revised manuscript received February 11th, 1982)

Key words: Na^+ channel; Amiloride-induced noise; (Mammalian colon)

(1) The effect of the Na^+-channel blocker, amiloride, on the short-circuit current carried by Na^+ was studied with fluctuation analysis, in rabbit descending colon epithelium. (2) In the presence of mucosal amiloride, the power spectrum of the Na^+-current noise showed a Lorentzian component. When the Na^+ current was reduced by increasing the blocker concentrations, the Lorentzian plateau decreased and corner frequency increased. Macroscopic short-circuit current and current-noise data are evidence for a two-state mechanism of the blocker interaction with the Na^+ channel. (3) On- and off-rate constants for the blocker-receptor reaction, single-channel currents and Na^+-channel density were calculated at room temperature and at 37°C. Also, the activation energy for the amiloride-receptor reaction was estimated. The microscopic parameters obtained for the Na^+ channel in the colon were similar to those found for Na^+ channels in other tight epithelia.

Introduction

Epithelia are commonly divided into two major categories [1], based on the tissue's ohmic resistance: the so-called tight epithelia, e.g., amphibian skin and urinary bladder or the distal nephron in various animal species and man, have a paracellular shunt pathway which is of high ohmic resistance when compared to apical and basolateral cell membranes. In contrast, leaky epithelia such as gall bladder, proximal kidney tubule or small intestine, have a low-resistance shunt which is the main single-barrier pathway for ionic movement. Another essential difference between tight and leaky epithelia is the mechanism of transcellular Na^+ transport [1].

There is much evidence that tight epithelia take up sodium ions passively from the mucosal solution, into the cell via an ion-selective channel, along an electrochemical gradient. On the other hand, leaky epithelia seem to be characterized rather by a co-transport uptake mechanism for Na^+ and Cl^- at the apical membrane [1]. During the last decade, the diuretic substance amiloride, a substituted pyrazinoyl-guanidine compound, has been recognized as a specific probe for Na^+ channels in apical membranes of tight epithelia [2]. At concentrations of $1 \cdot 10^{-4}$ M, amiloride selectively blocks the apical Na^+ channels in all tight epithelia as they are characterized above. The blocking mechanism in frog skin [3] and toad bladder [4] is described by noise analysis in terms of a pseudo-first-order reaction of amiloride with a receptor, probably the Na^+-channel macromolecule, on the external surface of the apical cell membrane. For instance, rate constants for association

and dissociation of amiloride at this site were obtained, as well as the single-channel current and conductance. In addition, the Na^+-channel density per unit surface area was calculated [3].

As the specific block of the apical Na^+ channels in various tight epithelia is a very characteristic feature for these tissues, we investigated the amiloride-Na^+-channel interaction in a mammalian epithelium, the descending colon of the rabbit. This epithelium is 'moderately' tight and shows an amiloride-blockable short-circuit current identical to the transcellular net Na^+ transport [5]. In this first study of amiloride kinetics in tissue from a warm-blooded animal, using noise-analysis, the results indicate that the principal characteristics of amiloride-receptor interactions in the colon are not much different from those in tissues from cold-blooded animals. These features will be discussed with respect to a general model for the Na^+-channel structure in apical cell membranes of tight epithelia. Preliminary results of this study have been reported at the spring meeting [6] of the German and Austrian Physiological Societies in Innsbruck, Austria (1981).

Methods

Colons were obtained from stunned and bled rabbits of either sex (2–2.5 kg). According to the method described by Frizzell et al. [5], the epithelium was separated from the underlying muscle layer by blunt dissection. The epithelium was then mounted in an Ussing-type lucite chamber between soft silicon rubber seals to minimize edge damage [7]. Bathing solution was (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-}/11.1 glucose. Amiloride was a gift of Merck, Sharp and Dohme. The experimental temperature (37 and 27°C) was adjusted by placing the whole chamber in a water bath connected to a thermostat. All electrical connections were fully waterproof. The short-circuit current (I_{sc}), was measured under continuous bubbling with a mixture of 95% O_2 and 5% CO_2. While the I_{sc} fluctuations were being recorded, the bubbling was stopped, and the thermostat was disconnected from the Faraday cage which contained chamber and water bath. During the noise recordings (each 2 min in duration) the bath temperature remained unchanged.

The low-noise voltage clamp apparatus has been described previously [8]. However, compared to our earlier method of noise analysis [7,9], we now used on-line fluctuation analysis of the short-circuit current with the following modifications, as compared to [7,9]. Two CPUs * were used in order to enable real-time analysis of the noise data. The data were sampled under control of an SBC 80/30 (Intel) CPU board, while in the meantime the calculations were done with an SBC 86/12 CPU board. Four records 512 points in length could be sampled simultaneously with different sample rates. The ratio of the different sample rates was 1:4:16:64. In this way the superimposed spectra cover four decades in the frequency domain. In the present study, however, only three channels were used with sample rates: 102.4, 409.6 and 1638.4 Hz. The corresponding fundamental frequencies were 0.2, 0.8 and 3.2 Hz. To avoid aliasing, the analog signal of each channel was filtered with a 36 dB/octave low-pass filter. The cut-off frequencies were 45, 180 and 720 Hz. The spectra of the three records were calculated with a Fast Fourier Transform routine written in integer arithmetics. The spectra of 20 records of each channel were averaged and finally plotted together in a double-logarithmic plot. For clarity, only the mean value of the spectral values of the frequency lines in an interval of 1/8 of an octave was plotted. The final averaged spectra were stored on disk in a PDP 11/34 computer. The transmission of the data was done via a serial interface. Curve fitting and plotting of data were done as previously described [9]. Means values are given ±S.E.

Results

With NaCl-Ringer solution as mucosal and serosal bathing medium, the short-circuited epithelium exhibits a net inward Na^+ movement, which can be totally blocked by $1 \cdot 10^{-4}$ M amiloride in the mucosal solution [5]. Under normal conditions I_{sc} is nearly identical to the transcellular net Na^+ transport (Na^+ current, I_{Na}). Fig. 1a shows how a stepwise increase in the

* CPU, Central Processing Unit.

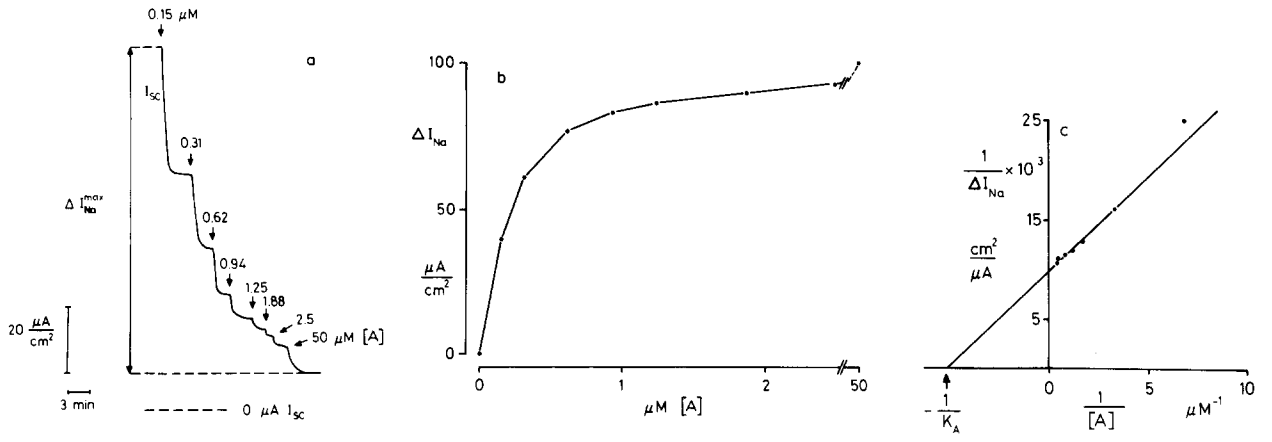

Fig. 1. (a) Time course of the short-circuit current (I_{sc}) when the mucosal amiloride concentration [A] is increased stepwise. Correction of I_{sc} for the shunt current (I_{sc} at 50 μM [A]) gives the Na^+ current I_{Na}, and its decrease ΔI_{Na}. (b) Dependence of the Na^+-current decrease, ΔI_{Na}, on the amiloride concentration. (c) Double-reciprocal plot of ΔI_{Na} vs. [A]. K_A is the apparent Michaelis constant of the amiloride blocking effect.

mucosal amiloride concentration quickly reduces I_{sc}. In this experiment a maximal response was obtained with more than 10 μM amiloride. The remaining I_{sc} is probably due to residual net ionic movements [5]. If we define the amiloride-blockable I_{sc} as the Na^+ current, I_{Na} [5], we find that the amiloride-induced decrease of I_{Na} (ΔI_{Na}) shows a hyperbolic dose-response relationship (Fig. 1b). That the steady-state kinetics of the amiloride block are of the Michaelis-Menten type can be concluded from the linearity of the dose-response relation in a double-reciprocal plot (Fig. 1c). For this representative experiment the amiloride concentration for the half-maximal effect on I_{Na} (apparent Michaelis constant K_A) is 0.2 μM.

The interaction of amiloride with the apical Na^+ channel in frog skin has been studied in great detail [2]. Application of fluctuation analysis to the short-circuit current revealed a Lorentzian component in the power spectrum of the current noise when amiloride was present [3,10]. The Lorentzian noise is thought to arise from a pseudo -first-order blocking reaction of the Na^+ channel by amiloride. Fig. 2 shows the power spectrum of the short-circuit current fluctuations in the absence of (CTR), and with 1 μM amiloride in the mucosal Na^+-Ringer solution. For the control spectrum, we obtain a $1/f^{\alpha}$ spectrum with a slope which tends to flatten in the high-frequency range. This latter phenomenon is due to the rise in amplifier noise at high frequencies [11]. The spectral values in presence of amiloride are clearly larger than for the control and a distinct shoulder can be seen in the middle frequency range.

In addition to the curve fitted by the computer to the data, the two fit components are shown: the

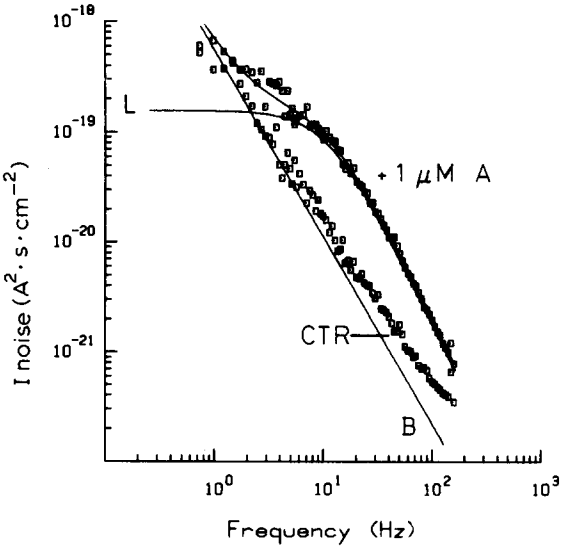

Fig. 2. Power spectrum of the short-circuit current fluctuations in the absence (CTR=control) (I_{Na}=34.1 μA/cm²) and in presence of 1μM amiloride (A) (I_{Na}=19.7 μA/cm²) in the mucosal NaCl-Ringer solution. Also shown is the fit for the amiloride-induced noise, a sum of Lorentzian (L) and background component (B).

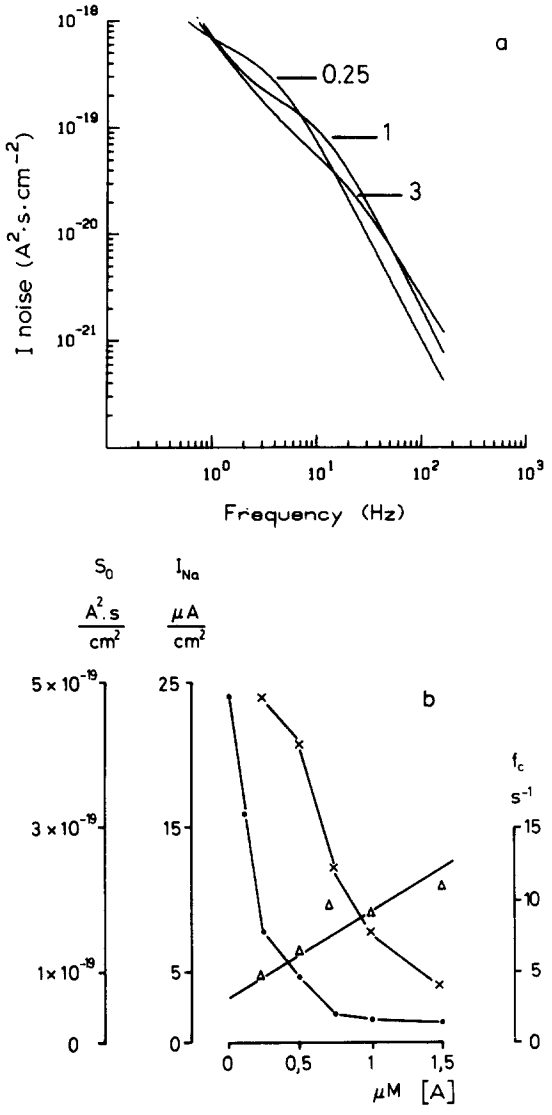

Fig. 3. (a) Power spectrum at three different mucosal amiloride concentrations (figures on the lines (μM)). For clarity, only the resultant line from the fit is shown. I_{Na} (0.25 μM amiloride) = 25.3 $\mu A/cm^2$; I_{Na} (1 μM amiloride) = 19.7 $\mu A/cm^2$; I_{Na} (2 μM amiloride) = 11.7 $\mu A/cm^2$. (b) Changes in Na^+ current (I_{Na}, O), the Lorentzian plateau (S_0, ×) and corner frequency (f_c, △) as a function of the amiloride concentration [A]), for a representative colon epithelium.

straight line represents a low-frequency background (B) noise assumed to be equal to K_b/f^α (compare also Ref. 9). In this case, $K_b = 5.6 \cdot 10^{-19}$ $A^2 \cdot s \cdot cm^{-2}$ and $\alpha = 1.7$. The Lorentzian (L) component is defined by a low-frequency plateau value, S_0, and the half-maximal 'corner frequency', f_c (f at $S(f) = S_0/2$). At $f > f_c$, the slope of L in the double-logarithmic diagram is -2.

It has been shown for blocker-induced Lorentzian noise that the values of S_0 and f_c depend, in a characteristic way, on the blocker concentration [3,9]. Fig. 3a clearly demonstrates that a rise in amiloride concentration produces a shift of the Lorentzian component in the amiloride-induced noise to higher frequencies but lower plateau values. In a plot of the Lorentzian parameters S_0 and f_c, together with the Na^+ current I_{Na}, as a function of the amiloride concentration (Fig. 3b), certain characteristic features become obvious: the decrease in the Lorentzian plateau value parallels the drop in current. Above 2.5 μM amiloride, the Lorentzian disappears into the background noise. At the same time, I_{Na} becomes very small and approaches zero. Within the concentration range over which the amiloride-induced noise can be observed, the corner frequency, f_c, increases, roughly, as a linear function of the blocker concentration. The changes in Lorentzian parameters described so far are identical to the changes in noise characteristics reported for the amiloride blockage of Na^+ channels [3], as well as for the Ba^{2+}-blockage of K^+ channels [9], both in the apical cell membrane of frog skin. Consequently, we analyzed the amiloride interaction with the colon's apical Na^+ channel using a common theory [3,9].

The association of the amiloride cation (A) with a membrane-bound receptor (R) near to or in the mouth of the Na^+ channel is assumed to be a pseudo-first-order reaction, proceeding at a rate α_{01}, which is proportional to the bulk amiloride concentration, [A], with the 'apparent' rate constant, k'_{01}, being the proportionality factor.

The 'apparent' rate constant, k'_{01}, is thought to be the product of the 'true' rate constant, k_{01}, and the partition coefficient ($\beta > 1$) for the amiloride concentration in front of the reactive membrane site [3].

$$\underset{\text{open}}{A + R} \underset{\alpha_{10} = k_{10}}{\overset{\alpha_{01} = k'_{01}[A]}{\rightleftharpoons}} \underset{\text{closed}}{AR} \qquad (1)$$

The formation of AR is supposed to lead, directly or indirectly, to a closure of the Na^+ channel. Reopening is thought to occur when AR dis-

sociates with a rate, α_{10}, assumed to be equal to the rate constant, k_{10}, of this process. The random interaction of amiloride with the site R is supposed to result in small fluctuations in the number of open Na^+ channels, thereby producing fluctuations of the macroscopic Na^+ current around a mean value I_{Na}, at a fixed amiloride concentration. Following further this model first developed by Lindemann and Van Driessche [3], we adopt also the following relations:

$$I_{Na} = M \cdot i \cdot P_0 \tag{2}$$

The mean Na^+-current density, I_{Na}, equals the product of the total (open + closed) Na^+-channel number per cm^2 (M), the probability of finding the channels open (P_0), and the Na^+ current carried by a single channel (i). With a constant M, the hyperbolic I_{Na} decrease when [A] is raised (cf. Fig. 1), indicates that the product $i \cdot P_0$ depends hyperbolically on [A]. The single-channel current is a function of (1) the intrinsic Na^+ permeability of the channel, (2) the Na^+-concentration gradient, and (3) the electrical gradient across the apical cell membrane, V_0. It has been shown that V_0 hyperpolarizes — though only slightly — in the short-circuited colon, after addition of amiloride [12]. In the descending colon epithelium the ratio of the apical over basolateral resistance increased from 4:1 to 8:1 with $1 \cdot 10^{-4}$ M amiloride [13]. Taking these facts into account, together with the notion that we are able to see amiloride-induced noise only with rather high amiloride doses, we might assume that the negative intracellular potential in the short-circuited state, V_0, does not change very much in the presently studied amiloride-concentration range. A change will even be less detectable with regard to the recently discovered apical 'leak' conductance [13] of about the same magnitude as the Na^+ conductance. For these reasons we think it justified to explain the hyperbolical I_{Na} decrease (Fig. 1) upon raising [A], by a decrease in P_0. We assume that the following relationship is obeyed:

$$P_0 = K_A/(K_A + [A]) \tag{3}$$

where [A] is the amiloride concentration in the bulk solution, and K_A a (Michaelis-type) constant.

According to the 'two-state model' for the amiloride-receptor interaction [3], the corner frequency (f_c) of the Lorentzian noise component is related to the reaction rates:

$$2\pi f_c = \alpha_{01} + \alpha_{10} = k'_{01}[A] + k_{10} \tag{4}$$

The ratio of the constants k_{10}/k'_{01} is supposed to represent the apparent Michaelis constant K_A of the kinetics of the P_0 ($= I_{Na}$) decrease. K_A might therefore be interpreted as the dissociation constant of the AR complex. In the frame of our model, the plateau value of the Lorentzian function normalized to 1 cm^2 epithelial surface, is given by (from Ref. 3):

$$S_0 = \frac{k'_{01}}{\pi^2} \cdot \frac{I_{Na} \cdot [A]}{f_c^2} \cdot i \tag{5}$$

Insertion of the experimental parameters I_{Na}, k'_{01}, S_0 and f_c for a particular amiloride concentration [A] into Eqn. 5 allows calculation of the single-channel current, i. Moreover, inserting i into Eqns. 2 and 3 lets us compute the Na^+-channel density, M. To determine the rate constants k'_{01} and k_{10}, we pooled the data from different preparations (Table I, Fig. 4). According to Eqn. 4, the rate constants were obtained from a linear regression analysis of the relation between corner frequency and amiloride concentration (see also Fig. 4). From the slope of the regression line (correlation coefficient 0.91) we calculate the mean association rate constant (±S.D.) for the amiloride-receptor reaction, k'_{01}, to be (68.4 ± 5.5) $\mu M^{-1} \cdot s^{-1}$; from the

TABLE I

COMPARISON OF MACROSCOPIC AND MICROSCOPIC KINETIC PARAMETERS AT 37°C

Colon	k'_{01} ($\mu M^{-1} \cdot s^{-1}$)	k_{10} (s^{-1})	K_A^{noise} (μM)	$K_A(I_{Na})$ (μM)
3	59.6	6.3	0.155	1.25
4	39.6	18.9	0.477	0.18
9	96.8	2.5	0.026	0.31
13	87.4	8.8	0.101	0.26
15	50.9	10.7	0.210	0.20
14	64.1	9.4	0.147	0.19

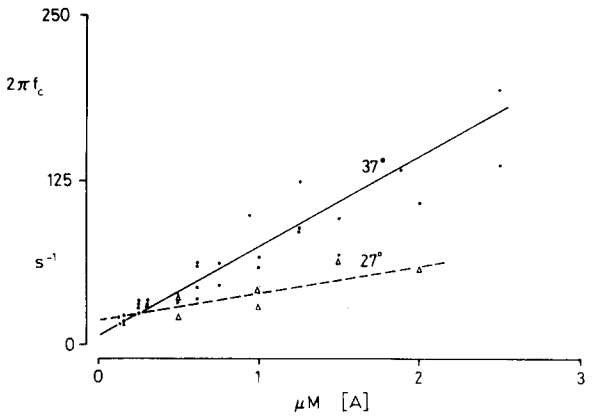

Fig. 4. Reaction rate $2\pi f_c$ as a function of the amiloride concentration ([A]), pooled from seven colons at 37°C (○) and two colons at 27°C (△). A linear regression yields the two straight lines. At 27°C: $y=(20.42\pm6.13)x+(18.9\pm8.27)$; At 37°C: $y=(68.45\pm5.46)x+(7.42\pm6.08)$.

ordinate intercept, the dissociation rate constant, k_{10}, is (7.42 ± 6.08) s^{-1}, both values being for a temperature of 37°C. Their ratio, the apparent dissociation constant K_A of the amiloride-receptor complex is then 0.109 μM which is very close to the Michaelis constant for the half-maximal amiloride effect, obtained, for example, from the I_{Na} dose-response curve in Fig. 1 or 3b.

In a set of preliminary experiments we investigated the influence of temperature on the amiloride-receptor kinetics. The corner frequencies at a temperature of 27°C (Fig. 4, triangles) were always clearly smaller than at 37°C (dots). A tentative linear regression fit permits the calculation of the rate constant at the lower temperature as $k'_{01}=(20.4\pm6.13)$ $\mu M^{-1}\cdot s^{-1}$ and $k_{10}=(18.9\pm8.27)$ s^{-1}, with a correlation coefficient of 0.83 for the regression line. Clearly, k'_{01} at 27°C is about one-third of that at 37°C, whereas the k_{10} values for both temperatures appear comparable, within the experimental error. A general increase in rate constants with temperature can be expected on the basis of the chemical kinetics. Fig. 5 shows, for a single experiment, that not only the corner frequencies but also the Lorentzian plateau decreases when the action of amiloride (0.5 μM) is studied at 27°C instead of 37°C. In this and all similar experiments, I_{Na} was greater at 37°C than at 27°C.

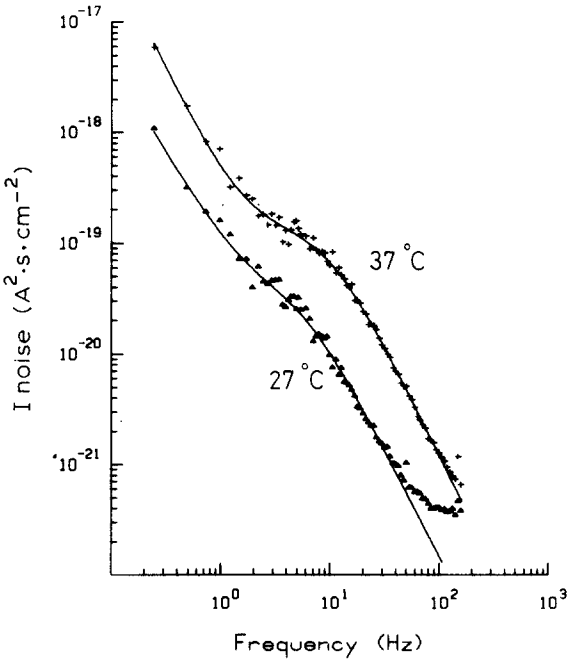

Fig. 5. (0.5 μM) amiloride-induced noise power at two different temperatures. At 27°C (△): $I_{Na}=4.8$ $\mu A/cm^2$, $S_0=3.27\cdot10^{-20}$ $A^2\cdot s\cdot cm^{-2}$, $f_c=5.8$ s^{-1}. At 37°C (+): $I_{Na}=5.3$ $\mu A/cm^2$, $S_0=1.32\cdot10^{-19}$ $A^2\cdot s\cdot cm^{-2}$, $f_c=9.5$ s^{-1}.

Discussion

1. Source and interpretation of the spectral noise components

In two previous papers [9,15] we pointed out the difficulties in interpreting parameters from Lorentzian spectra, as they might be attenuated by series and parallel elements in the epithelial equivalent circuit. There is no doubt that the amiloride-induced Na^+-current fluctuations arise from interactions of the blocker with the apical epithelial membranes; the fastness and reversibility of the Na^+-current block by amiloride are clear evidence for this assumption. As already stated by Van Driessche and Gögelein [16], the time constant of the parallel apical resistance-capacitance (RC) element can cause deviations from the Lorentzian shape if the noise source resides in the same membrane. Such a deviation was, however, never observed in our experiments. An attenuation of the apical noise by the basolateral series resistance can be neglected since, even in the absence of amiloride, the ratio of apical over basolateral resistance is

about 4:1 [13]. Also, an attenuation by possibly too large a ratio of series (solutions between voltage electrodes + mucous layer + connective tissue + remaining musculature) over shunt resistance, being about 0.1 (our unpublished data and Ref. 17) should not exist. Therefore it seems that, especially in the amiloride concentration range studied ($[A] > K_A$), our voltage clamp was mainly across the apical membrane. Consequently, the current fluctuations analysed in terms of Lorentzian noise can be assumed to reflect non-attenuated conductance fluctuations in the apical cell membranes.

The kinetics of the amiloride effect look identical to those observed in other tight Na^+-transporting tissues [2,3,4,18]. From Fig. 1 we can see that the dose-response curve is of the Michaelis-Menten type. A pseudo-first-order reaction of the amiloride cation with its receptor predicts a linear increase of the Lorentzian corner frequency when [A] is raised; this prediction was fulfilled as shown in Figs. 3b and 4. The coincidence of the macroscopially (from I_{Na} data) and microscopically (from noise experiments) obtained K_A values is sometimes striking (e.g., Fig. 3; Table I); in a minority of cases there is, however, a large disagreement which cannot be explained to date.

The mechanism of the amiloride-blockage of the Na^+ channel is still obscure. For frog skin, a modifier-site action near the entrance of the Na^+ channel [19], as well as a direct cork-like action [18] have been proposed, both mechanisms in competition with Na^+. We cannot make a decisive statement about this issue. We should, however, stress that, at least for frog skin, the action of the blocker strongly depends upon the availability of several important reactive positions in the amiloride molecule [19,20]. This has recently been supported by using noise analysis to study the effect of amiloride analogues in frog skin [21]. In all amiloride-sensitive epithelia investigated so far, the amiloride-receptor interaction may be described by pseudo-first-order kinetics. This indicates that the kinetics, as revealed by noise-analysis, reflect just the rate-determining step having first-order kinetics within a probably multi-step interaction of the blocker with its receptor. In this context the description of the blockage in terms of a simple 'two-state model' is justified.

The spectra presented in the figures show that the fitting procedure is sufficiently accurate to approximate the spectral density data. The very steep low-frequency background noise was surprising. Several hypotheses have been forwarded to interpret low-frequency noise. For slopes (in the double-logarithmic power spectra) around -1, a 'restricted diffusion' in ionic pathways was thought to cause this type of noise [22]. Occasionally, this '$1/f$ noise' could be attributed to the existence of transepithelial ionic diffusion through K^+-specific channels [7,9]. In colon, we observed in some preparations a steep low-frequency noise with a slope of about -2, even when all Na^+ transport was abolished at high mucosal [A] (our unpublished data). We do not know at this point what the responsible noise source might be, nor what significance this observation has. Among other explanations, the existence of a second Lorentzian-type noise at lower frequencies, caused by some interaction of Na^+ with the channel molecule, could explain the steep slope of our spectra. A similar hypothesis was discussed for Na^+-induced Na^+-channel noise in frog skin [18].

2. A tentative evaluation of kinetic and single-channel parameters

2.1. Experiments at 37°C. The statistical evaluation (Fig. 4, Tables I and II) of our data in terms of the 'two-state model' raises some problems. The first derives from the determination of k'_{01} and k_{10}. As can be seen from Fig. 4 (37°C), the deviation of the linear regression line from the experimental data is not large; in fact, the correlation coefficient of 0.914 states an excellent fit. The standard devia-

TABLE II

SINGLE-CHANNEL CURRENT (i) AND CHANNEL DENSITY (M) AT 37°C

n	colon	i (pA)	M (μm^{-2})	I_{Na} ([A]=0) ($\mu A \cdot cm^{-2}$)
6	3	0.14±0.01	10.77±1.58	148
5	4	1.07±0.06	0.25±0.05	269
5	9	0.17±0.03	8.33±0.91	142
3	13	0.12±0.02	8.22±1.24	98
4	14	0.57±0.06	1.73±0.43	98
7	15	0.31±0.03	6.30±0.94	194

tion of the slope is relatively small with 8% (k'_{01} = (68.44 ± 5.46) $\mu M^{-1} \cdot s^{-1}$. On the other hand, the standard deviation of the ordinate intercept (k_{10}) is 82% of the mean k_{10} value of (7.42 ± 6.08) s^{-1}. It is clear that, for the calculation of the single-channel current (i) and the Na^+-channel density (M), we have to take these errors into account. The single-channel current, i, is easy to obtain by rearranging Eqn. 5 to Eqn. 7 when the experimental parameters I_{Na}, S_0 and f_c for a particular [A] value are used, and when the mean value for k'_{01} is taken from the pooled data in Fig. 4.

With i once calculated, M is obtained from a rearranged form (Eqn. 6) of Eqn. 2, with k_{10} equally taken from Fig. 4. As expected from the discussion of the dependence of i on V_0 (see Results), for a series of different amiloride concentrations in a particular preparation, the single values of i did not vary systematically. Therefore we are allowed to average the i values and M values from different experiments. For six different colons, the mean values of i and M so obtained are given in Table II. As can be seen, the i values for colons 3, 9, 13, 15 are very close. The interdependence of i and M, by the way they are calculated, is obvious. The number M was calculated using the equation

$$M = \frac{2\pi}{k_{10}} \cdot \frac{I_{Na} \cdot f_c}{i} \tag{6}$$

while i was calculated from

$$i = \frac{\pi^2 f_c^2 S_0}{k'_{01} \cdot [A] \cdot I_{Na}} \tag{7}$$

In Eqn. 7, S_0 and I_{Na} are both normalized to 1 cm^2. If the 'true' investigated area of Na^+-transporting cells (depending on the individual cell heterogeneity, the mucous-producing crypt regions, and the actual stretching of the epithelial preparation) is not identical to the macroscopically measured tissue area exposed to the solution, this effect will cancel out for the determination of i (S_0/I_{Na} in Eqn. 6). Here, only the partition coefficient which is contained in k'_{01} is an individual variable, perhaps depending on mucous secretion activity or other diffusional hindrance. Larger variability of i in different tissues may also be produced by the actual value of the negative intracellular cell potential, V_0 (= apical membrane potential) in the short-circuited state. As already discussed, V_0 is supposed to become more negative with increasing [A], but this change may be more or less obscured (see Ref. 12) by the existence of 'leak' pathways [13] in parallel to the Na^+ channels. In this context we have to consider that recent studies have revealed an apical K^+ channel in the colon epithelium which shows significant conductance and might be the candidate for the leak path [6,14,23]. In Eqn. 6, however, the possible error in 'true' cell surface area, is introduced by the value of I_{Na}, so that the resulting values for M should be contaminated by this source of uncertainty. The M values for colons 3, 9, 13 and 15 are quite similar. Possibly the error in surface area does not play too important a role. The most serious source of error for the calculation of M is the uncertainty in the determination of k_{10}. The S.D. for the mean value of k_{10} is almost of the same magnitude as k_{10} itself. Consequently, in our further analysis, we should only consider the order of magnitude for the Na^+ channel density. For all determinations ($n = 30$) of i and M in six colons, at 37°C, the final mean values are $i(37) = (0.39 \pm 0.06)$ pA and $M(37) = (6.1 \pm 0.8)$ μm^{-2}.

2.2. Experiments at 27°C. In an attempt to obtain some idea of kinetics and microscopic channel parameters at lower temperatures, we evaluated eight experiments done at 27°C. For this condition, we calculated $i(27) = (0.09 \pm 0.03)$ pA, and $M(27) = (3.2 \pm 1.6)$ μm^{-2} using the parameters k'_{01} and k_{10} from Fig. 4. Inspection of Fig. 4 allows the rough estimation that—at 37°C—k'_{01} is about 3-times larger than at 27°C, whereas k_{10} is not significantly changed. It is therefore clear that the apparent Michaelis constant, K_A, must decrease with a rise in temperature, which causes a temperature dependence of P_0 (Eqn. 4). While the single-channel current can be reliably calculated with the parameter k'_{01} from the regression line, the above mentioned uncertainty in the determination of M still remains due to the error in k_{10}. A true difference between $M(37)$ and $M(27)$ is therefore questionable and should not be subject to speculation. The single channel current could be expected to be somewhat larger at higher temperatures since a more active electrogenic [24] Na^+/K^+ pump would lower the negative V_0 even more, thus

increasing the driving force for Na^+ across the apical membrane. The comparison of $i(37)$ and $i(27)$ favors this interpretation. The combined effects of temperature on i and P_0 must therefore result in a not easily predictable change in I_{Na} when amiloride is present. For the experiment in Fig. 5, I_{Na} at 37°C was higher than I_{Na} at 27°C, which would reveal the temperature effect on i to be important (compare Eqn. 2). Since i^2 dominates the expression for S_0 (Eqn. 5), it is not surprising that S_0 at 37°C is larger than at 27°C (Fig. 5). I_{Na} should be larger at 37°C than at 27°C also for amiloride-free preparations ($P_0 = 1$). Indeed, this was generally the case.

No significant change in k_{10} is observed when the temperature is increased from 27 to 37°C. However, from the temperature dependence of k'_{01} we can calculate a Q_{10} value of 3.3 and, according to the Arrhenius relationship, the activation energy for the amiloride-receptor association, $E_a = 90.0\ kJ \cdot M^{-1}$. These values are comparable to those found for the Ba^{2+} block of K^+ channels [25], or the TTX block of Na^+ channels [26] in the squid axon.

3. Comparison with data from non-mammalian epithelia. In summary, the following picture of the Na^+-channel-amiloride interaction emerges from our experiments. The association-dissociation at the receptor site can be described by pseudo-first-order reaction kinetics. We can compare the final results from the colon with those obtained by noise analysis from frog skin and toad bladder (Table III). For 27°C, which is about room temperature, the values for k'_{01} are very similar for the three tissues. k_{10} is more than an order of magnitude smaller in toad bladder. Within the limits of our analysis, also the values for i and M are of the same order of magnitude in these three tissues. For this reason, the K_A values from colon and frog skin compare favorably, but K_A is much smaller for the bladder. So far, there seems no major difference in microscopic properties of the Na^+ permeability in the apical membranes of these tight epithelia. It is even likely that the basic concept of the Na^+ channel is identical in tissues from warm- and cold-blooded animals. For a variety of tight Na^+-transporting tissues, there is good evidence for this notion on the basis of steady-state kinetic studies [2].

Our results show that the colon's Na^+ channel might be equivalent and equally well explorable as those in frog skin and toad bladder. Then this epithelium may provide a mammalian model for understanding the microscopic features of Na^+ uptake in distal kidney membranes, as they are not yet accessible for fluctuation analysis. Amiloride-induced Na^+-current fluctuations might be the right tool for assessment of problems in colon literature, such as, for example, the effect of aldosterone on Na^+ absorption/K^+ secretion [27], or for investigation of possible effects of toxic heavy metal ions on the colon's Na^+-transport properties [28].

Acknowledgements

The authors would like to thank Mrs. L. De Handschutter-Janssens for perfect technical assis-

TABLE III

COMPARISON OF NOISE ANALYSIS RESULTS FOR THREE Na^+-TRANSPORTING TISSUES

	Colon		Frog skin (*Rana esculenta*) [a]	Toad bladder (*Bufo marinus*) [b]
	37°C	27°C		
k'_{01} ($s^{-1} \cdot \mu M^{-1}$)	68.4	20.5	13	18.4
k_{10} (s^{-1})	7.5	18.98	12	0.55
i (pA)	0.4	0.1	0.3	0.18
M (μm^{-2})	6.1	3.2	0.7–2	0.8
K_A (μM)	0.1	0.9	1	0.03

[a] From Refs. 3, 10, 18; at room temperature.

[b] From Ref. 4; at room temperature.

tance. In addition we are indebted to Dr. T. Hoshiko for use of his linear regression analysis program and his helpfulness. One of us (N.K.W.) was supported by N.I.H. grant No. AM 29962.

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