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Ba^{2+}-Induced Conductance Fluctuations of Spontaneously Fluctuating K^+ Channels in the Apical Membrane of Frog Skin (*Rana temporaria*)

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Summary. We studied the influence of mucosal Ba^{2+} ions on the recently described (Zeiske & Van Driessche, 1979*a*, *J. Membrane Biol.* **47**:77) transepithelial, mucosa towards serosa directed K^+ transport in the skin of *Rana temporaria*. The transport parameters G (conductance), PD (potential difference), I_{sc} (short-circuit current, "K^+ current"), as well as the noise of I_{sc} were recorded. Addition of millimolar concentrations of Ba^{2+} to the mucosal K^+-containing solution resulted in a sudden but quickly reversible drop in I_{sc}. G and I_{sc} decreased continuously with increasing Ba^{2+} concentration, $(Ba^{2+})_o$. The apparent Michaelis constant of the inhibition by Ba^{2+} lies within the range 40–80 μM. The apical membrane seems to remain permselective for K^+ up to 500 μM $(Ba^{2+})_o$. Higher $(Ba^{2+})_o$, however, appears to induce a shunt (PD falls, G increases). This finding made an accurate determination of the nature of the inhibition difficult but our results tend to suggest a K^+-channel block by K^+-Ba^{2+} competition.

In the presence of Ba^{2+}, the power spectrum of the K^+ current shows a second Lorentzian component in the low-frequency range, in addition to the high-frequency Lorentzian caused by spontaneous K^+-channel fluctuations (Van Driessche & Zeiske, 1980). Both Lorentzian components are only present with mucosal K^+ and can be depressed by addition of Cs^+ ions, thus indicating that Ba^{2+} ions induce K^+-channel fluctuations. The dependence of the parameters of the induced Lorentzian on $(Ba^{2+})_o$ shows a rise in the plateau values to a maximum around 60 μM $(Ba^{2+})_o$, followed by a sharp and progressive decrease to very low values. The corner frequency which reflects the rate of the Ba^{2+}-induced fluctuations, however, increases quasi-linearly up to 1 mM $(Ba^{2+})_o$ with a tendency to saturate at higher $(Ba^{2+})_o$. Based on a three-state model for the K^+ channel (having one open state, one closed by the spontaneous fluctuation and one blocked by Ba^{2+}) computer calculations compared favorably with our results. The effect of Ba^{2+} could be explained by assuming reversible binding at the outer side of the apical K^+ channel, thereby blocking the open channel in competition with K^+. The association-dissociation of Ba^{2+} at its receptor site is thought to cause a chopping of the K^+ current, resulting in modulated current fluctuations.

Membrane-bound ionic channels can often be blocked by inorganic ions with a chemical character similar to that of the transported ion. It has been established, for example, that K^+ ions inhibit Na^+ permeation through pores in the apical frog skin membrane (Gebhardt, Fuchs & Lindemann, 1972; Mandel & Curran, 1973), that Cs^+ and Ba^{2+} ions block K^+ fluxes through excitable membranes (Isenberg, 1976; Sperelakis, Schneider & Harris, 1967), and that Ca^{2+} channels are blocked by La^{3+} in muscle (Mayer, van Breemen & Casteels, 1972). Some cation selective channels can be occluded very specifically by certain organic molecules. These blockers are thought to act in their cationic form as was demonstrated for the Na^+-channel blocker tetrodotoxin (Hille, 1966), the K^+-channel blocker tetraethylammonium (Hille, 1967) in excitable tissues, and for Na^+-pore blockers in frog skin, like 2,4,6,-triaminopyrimidine (Zeiske, 1975) and amiloride (Ehrlich & Crabbé, 1968). Whatever the mechanism of a channel block may be, it is plausible that a reversible blocking mechanism causes conduction noise by randomly chopping the ionic current through the pore. This was demonstrated for the first time by Fishman, Moore and Poussart (1975*a*, 1977) and Moore, Fishman and Poussart (1979) who observed K^+-conductance noise induced by tetraethylammonium (TEA) and its C10-derivative in squid axon. Fishman

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et al. (1977) also described a K^+-conduction noise induced by 4-aminopyridine (4AP). An amiloride-induced Na^+-channel noise was found by Lindemann and Van Driessche (1977). For small inhibitory inorganic ions it has been suggested that the blocking mechanism might be too fast to be recorded (Van Driessche & Zeiske, 1980). TEA and amiloride induced conductance fluctuations of higher frequencies than those contained in the current noise of the nonblocked ionic channels. However, 4AP caused conductance fluctuations of lower frequencies than those observed in the noise of the nonblocked K^+ channel. In all cases, the "spontaneous" conductance-noise component was depressed in the presence of the blocking agents.

Recently K^+-selective channels in the outer skin border of the frog species *Rana temporaria* were described for the first time (Zeiske & Van Driessche, 1978). These channels are localized in the apical membranes of the epithelium (Hirschmann & Nagel, 1978). We found a Lorentzian component in the K^+-current noise (Van Driessche & Zeiske, 1978) and interpreted our results in terms of spontaneously occurring K^+-channel fluctuations between one open and one closed state (two-state model). The inhibitory effect of protons, Rb^+ and Cs^+ ions on the K^+ current (Zeiske & Van Driessche, 1979*a*) was studied in detail with noise analysis, and we found blocker-dependent shifts in the corner frequency of the Lorentzian component in the spontaneous K^+-current noise to lower frequencies (Van Driessche & Zeiske, 1980). Using microelectrode techniques, Hirschmann and Nagel (1978) showed that the K^+ channels in the apical membrane of *R. temporaria* can be blocked by Ba^{2+} ions. In this paper we describe in detail the effect of mucosal Ba^{2+} ions on (i) transepithelial electrical parameters which characterize the K^+ transport and (ii) the effect of Ba^{2+} on the K^+-current noise. It will be shown that the blocking action of Ba^{2+} is similar to the action of amiloride on the Na^+ channel. Ba^{2+} ions reduce the (high frequency) spontaneous K^+-current noise, but they induce an additional (low frequency) Lorentzian component. The data suggest that, in the open state during the spontaneous fluctuations, Ba^{2+} ions reversibly bind to the channel and cause a blockage. The relaxation time of this process is appreciably longer than that of the spontaneous fluctuations and strongly depends on the blocker concentration. The experimental results can, at least qualitatively, be described by a three-state kinetic model. A similar kinetic reaction scheme was used by Coronado and Miller (1979) to describe Cs^+-induced conductance fluctuations of sarcoplasmic K^+-currents. Some of the results have been reported at the Autumn Meeting of the German Physiological Society (Zeiske & Van Driessche, 1979*c*).

Materials and Methods

Rapid Flow Experiments

A conventional Ussing-type Lucite chamber as described previously (Zeiske & Van Driessche, 1979*a*) was used to study the time course of the effect of Ba^{2+} ions on transepithelial parameters. Rapid solution changes at the mucosal side of the abdominal skin (3 cm^2) of the frog species *Rana temporaria* were effected by a syringe. (For details, *see* Zeiske & Van Driessche, 1979*a*.)

Noise Experiments

For the recording of the K^+-current noise a different chamber was used where the skin (0.126 cm^2) was mounted with minimal edge damage between soft silicone rubber rings. Details of the experimental procedures and the processing of the data can be found in two previous papers (Van Driessche & Lindemann, 1978; Van Driessche & Zeiske, 1980). However, we had to introduce two major alterations. The first concerns the recording of the current noise. The Ba^{2+}-induced current fluctuations showed a Lorentzian component in the power spectrum. In the concentration range from 1 to 4000 μM $(Ba^{2+})_o$, the corner frequency of this Lorentzian shifted from about 3 to more than 100 Hz. Moreover, at small Ba^{2+} concentrations, the spontaneous Lorentzian component having a corner frequency of about 70–100 Hz could also be seen. To guarantee a sufficiently fine spectral resolution, we usually analyzed the current-noise signal in two different frequency ranges. The lower one was digitized at 2-msec intervals (40 data blocks of 2048 points, averaged after the calculation of the spectra). The higher one was sampled at 0.5-msec intervals (60 data blocks of 2048 points, averaged after the calculation of the spectra). Filtering was done with an 8-pole Butterworth filter adjusted to 220 or 880 Hz for the low- or high-frequency range, respectively. Spectral values at frequencies above 182 Hz in the low- and 730 Hz in the high-frequency range were discarded. The second modification was the fitting procedure. The averaged low- and high-frequency spectra were displayed in the same plot on the monitor of the display processor. In case the power spectrum contained only the spontaneous Lorentzian component, the data-fit "by eye" with the computer-generated Lorentzian curve was sufficiently accurate (*cf.* Van Driessche & Zeiske, 1978; Van Driessche & Zeiske, 1980). However, where for small Ba^{2+}-concentrations the spectra were composed of two Lorentzian components, the fitting procedure by eye became erroneous. As will be shown in *Results* (Fig. 5*a*), the Ba^{2+}-induced second Lorentzian dominates the spectrum at high Ba^{2+}-concentrations where the "spontaneous" K^+-current noise is no longer observable. There is, however, a large background noise which, at frequencies smaller than the corner frequency of the induced Lorentzian, overlaps the Lorentzian plateau so much that the fit by eye cannot be correct. To make up for these disadvantages, we fitted the spectral points with a sum $S(f)$ of one or two Lorentzians ($S^{(1)}$ and $S^{(2)}$) of the general form $S = S_o/(1+(f/f_c)^2)$, and a background-noise component S_b of the form K_b/f^α.

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

K_b represents the amplitude of the background component at 1 Hz and α its slope in the double-logarithmic scale. In most spectra the slope was significantly different from one.

To fit Eq. (1) to the experimental data, we used subroutine VA05A from the Harwell Subroutine Library (Atomic Energy Research Establishment, Harwell, Berkshire). This method is a compromise between three different algorithms for minimizing a sum of squares, namely Newton-Raphson, Steepest Descent, and Marquardt. The linear parameters K_b, $S_o^{(1)}$ and $S_o^{(2)}$ were calculated by linear regression after each iteration where VA05A calls the user-defined subroutine to evaluate the function value of Eq. (1). This specific feature of our method decreases the number of iterations to determine the nonlinear parameters α, $f_c^{(1)}$ and $f_c^{(2)}$. A similar procedure was used by Droogmans, Raeymaekers and Casteels (1977) to fit a combination of two exponential functions. All computations were done on a PDP11/34 computer equipped with a floating point processor. Mean values are given ± SEM.

Solutions

If not otherwise stated, the serosal solution was air-bubbled NaCl-Ringer with 2.5 mM $KHCO_3$, 1 mM $CaCl_2$ and 115 mM NaCl, pH 8.4. In the chamber used for noise measurements, the bubbling was stopped during recording. The mucosal solution contained 115 mM KCl, 5 mM Tris-HCl buffer (pH 7.4). Ba^{2+} was added as $BaCl_2$ and Cs^+ as CsCl. The solution in the mucosal chamber half was exchanged by syringe. In the conventional chamber the Ba^{2+}-induced changes of the transepithelial electrical parameters were nearly instantaneous, but they took about 30 sec in the chamber used for noise experiments due to delayed solution mixing in front of the epithelium. After Ba^{2+} experiments, especially at millimolar Ba^{2+} concentrations, the cleaning of the noise chamber became quite a problem. The K^+ current recorded with the rapid-flow chamber recovered relatively quickly after Ba^{2+} treatment. Despite intensive washing with Ba^{2+}-free Ringer even after using low $(Ba^{2+})_o$, the noise chamber was so contaminated that we still observed a Ba^{2+}-induced Lorentzian in the spectra. Before starting a series of experiments, it therefore became necessary to clean the chamber with the following solutions: (i) a detergent, (ii) a 10 mM EDTA solution, and (iii) a solution of 100 mM Na_2SO_4, in H_2O. If we wanted to use a skin repeatedly for several experimental series, we had to wash away the Ba^{2+} with a sulfate Ringer solution, thereby cleaning the chamber and storage sites on the outer skin surface from Ba^{2+} ions. Solutions with a special composition will be mentioned in the text or in the figure legends. Amiloride was a gift of Merck, Sharp and Dohme, Ltd.

Results

Steady-State Kinetics of the Ba^{2+} Inhibition

In a previous paper (Zeiske & Van Driessche, 1979*a*) we described an inward, mucosa-to-serosa directed short-circuit current (I_{sc}) carried by K^+ ions. This current directly depends on both the presence of mucosal K^+ and the existence of a transepithelial K^+-concentration gradient in the short-circuited state. The K^+ current could rapidly and reversibly be blocked by the addition of Cs^+ to the K^+ containing solution. Figure 1 shows a similar experiment where $BaCl_2$ is added to the mucosal KCl-Ringer solution, with NaCl-Ringer at the serosal side. To prevent Na^+ uptake which can eventually be caused by a back-leak, amiloride (50 μM), which blocks Na^+ channels in the apical membrane (Ehrlich & Crabbé, 1968), was added to the mucosal solution.

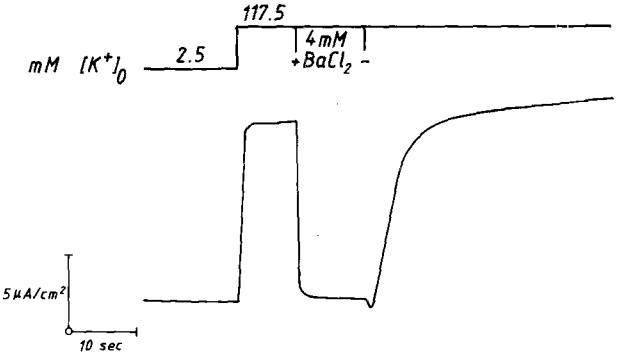

Fig. 1. Time course of the short-circuit current after a sudden increase in the mucosal K^+ concentration from 2.5 to 117.5 mM (KCl was substituted for NaCl). Then $BaCl_2$ (4 mM) was added to the outer solution and removed again. All mucosal solutions contained 50 μM amiloride

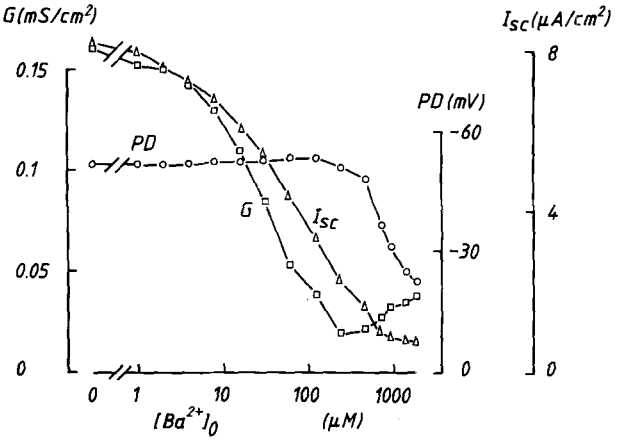

Fig. 2. The influence of Ba^{2+} in the mucosal KCl-solution (50 μM amiloride) on short-circuit current (I_{sc}, Δ), transepithelial conductance (G, □) and potential difference (PD, ○)

The short-circuit current, which we may also refer to as K^+-current, drops almost instantaneously after $BaCl_2$ addition, from 13.4 to 2.0 μA/cm². The current decrease cannot be due to the addition of chloride ions since their concentration is hardly altered, and experiments with other chlorides in place of $BaCl_2$ are without effect on the current (Zeiske & Van Driessche, 1979*a*). Thus the observed effect is exerted by Ba^{2+} ions. The rapid onset of the inhibitory Ba^{2+} effect tends to suggest a blockage of the K^+ channels at the apical skin border. After removal of Ba^{2+} from the mucosal solution I_{sc} returned to its original value within 10 sec.

Figure 2 demonstrates the influence of Ba^{2+} ions on the transepithelial electrical parameters, i.e., the K^+ current, I_{sc}, the K^+ dependent potential difference, PD (outside negative), and the conductance, G.

The conductance was calculated as the ratio of the current deflections caused by a brief voltage-clamp pulse of 10 mV. In this experiment, Ba^{2+} ions were added to the mucosal solution before the onset of the so-called "long-time effect" which is thought to induce a time- and $(K^+)_o$-dependent transition of K^+ channels into less selective ionic pathways (Zeiske & Van Driessche, 1979*a*). I_{sc} and G decreased continuously as the $(Ba^{2+})_o$ was increased from 1 to 500 μM. At 500 μM $(Ba^{2+})_o$, I_{sc} and G reached about 10% of their values in Ba^{2+}-free solution. From 1–200 μM $(Ba^{2+})_o$, PD remains practically unchanged but decreases at higher concentrations. At $(Ba^{2+})_o > 1$ mM, I_{sc} tends to reach a minimum while G increases again quite considerably. In the high $(Ba^{2+})_o$ range, PD and G change inversely and relatively symmetrically. Such typical effects of high $(Ba^{2+})_o$ were seen with all skins. Though it seems that shunt pathways open at high $(Ba^{2+})_o$, this Ba^{2+} effect which becomes dominant when practically all K^+ channels are blocked, still cannot be satisfactorily explained. Nevertheless we may conclude that, in the lower $(Ba^{2+})_o$ range, the change in I_{sc} reflects the decrease in the K^+-channel conductance caused by the Ba^{2+} block. Though the halfmaximal values of I_{sc} and G are within the same order of magnitude, they are still different by a factor of about two. The reason for this discrepancy remains obscure. When the (trans- and/or paracellular) shunt conductance becomes comparable to the K^+ conductance, the PD decreases as one would expect from the Goldman equation (Goldman, 1943). The additional effect of high $(Ba^{2+})_o$ on the shunt then seems to dominate the PD course. Preliminary experiments with identical K^+-free solutions on both sides indicate that Ba^{2+} induces small outward currents even in the absence of any chemical or electrical gradient (own *unpublished observation*). For these reasons, an exact determination of a shunt current could not be made. The apparent Michaelis constant of the Ba^{2+} block, K_{Ba}, derived from the course of I_{sc} or G, is within the range 40–80 μM. Because of the poorly defined shunt current or conductance, K_{Ba} could be estimated only roughly from the points of inflection of the S-shaped curves.

The Ba^{2+}-dependent shunt problem became considerably important when we tried to find out whether Ba^{2+} is a competitive K^+-channel blocker, as had been shown for the K^+-current inhibition by Cs^+ ions (Zeiske & Van Driessche, 1979*a*). To avoid the long-time effect, it was necessary to perform the experiments with the rapid-flow chamber which was used in the case of Cs^+. A successive substitution of K^+ for Na^+ in the mucosal solution (containing 50 μM amiloride) gives a saturable I_{sc}-$(K^+)_o$ relationship (Zeiske & Van Driessche, 1979*a*). Figure 3

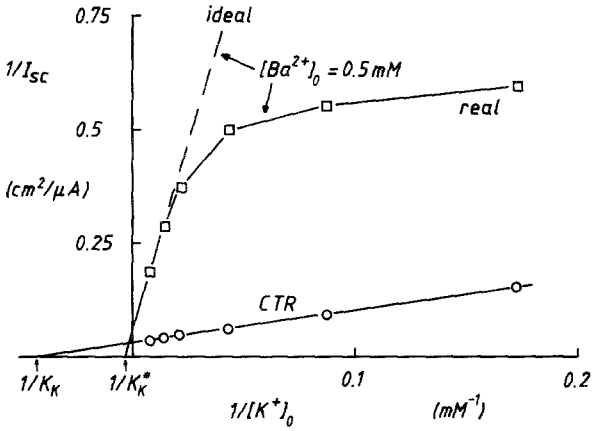

Fig. 3. Double-reciprocal plot of the short-circuit current (I_{sc}) *vs.* the mucosal K^+ concentration, $(K^+)_o$, in the absence (*CTR*) and presence of 0.5 mM $BaCl_2$ in the K^+-containing solution. K^+ was substituted for Na^+ (concentration sum 115 mM; 50 μM amiloride). The apparent Michaelis constants (negative reciprocal value of abscissa intersections) are denoted K_K for the *CTR*-case, and K_K^* in presence of Ba^{2+}

shows, in a double-reciprocal diagram, a linear control line. In the presence of 500 μM $(Ba^{2+})_o$, the K^+ current is much depressed. At high $(K^+)_o$, a linear extrapolation of the curve seems to have the ordinate intercept in common with the control line. This would be expected for competitive inhibition by Ba^{2+}. At small $(K^+)_o$, Ba^{2+} induces a large deviation from the ideal straight line (dashed) to an almost horizontal course. It would seem that a shunt current dominates the plot at low $(K^+)_o$. In this $(K^+)_o$ range, the values of $1/I_{sc}$ do not represent the reciprocal K^+ current. If we tentatively assume a 1:1 competition between Ba^{2+} and K^+, we obtain from the apparent Michaelis constants of the current saturation (i.e., the reciprocal negative abscissa intersection), K_K (control) and K_K^* (with Ba^{2+}), the apparent Michaelis constant K_{Ba} of the Ba^{2+} block according to

$$K_K^* = K_K \left(1 + \frac{(Ba^{2+})_o}{K_{Ba}}\right). \tag{2}$$

With $K_K = 11$ mM and $K_K^* = 200$ mM, we get $K_{Ba} = 29$ μM. This is in the same order of magnitude as the apparent Michaelis constants obtained from the course of I_{sc} and G in Fig. 2. In terms of a $K^+ - Ba^{2+}$ competition, the results from Fig. 3 must nevertheless be interpreted with care because they are based on only a few data points in the range of a sufficiently high $(K^+)_o$.

Noise Experiments

The double-Lorentzian phenomenon. In earlier publications we reported the existence of a Lorentzian component in the power spectrum of the K^+-current

noise (Van Driessche & Zeiske, 1978; Van Driessche & Zeiske, 1980; Zeiske & Van Driessche, 1979*b*). We also investigated in detail the blocking effects of H^+, Rb^+, Cs^+ and some polyvalent metal ions on the K^+ noise (Van Driessche & Zeiske, 1980; Zeiske & Van Driessche, *manuscript submitted*). A common feature of the inhibition was that the K^+-current decrease was paralleled by a depression of the K^+-dependent Lorentzian, and accompanied by a more or less marked leftward shift of the corner frequency. With Ba^{2+} we found an additional phenomenon, namely, the induction of a second Lorentzian component in the power spectrum.

Figure 4*A* shows, in a double-logarithmic diagram, the power density spectrum of the K^+-current noise. In the control case (curve *1*) NaCl-Ringer was used as serosal and KCl-Ringer as mucosal medium. The Lorentzian component is clearly marked by a high-frequency slope of -2 and a short plateau in the middle frequency range. At the left side, low frequency background noise masks the plateau. The deviation from the Lorentzian shape at the highest frequencies is caused by the increasing amplifier noise (Fishman, Poussart & Moore, 1975*b*). A fit of the control-spectrum by a single Lorentzian plus a $1/f^\alpha$ component (*see Materials and Methods*) yielded a plateau value $S_o^{(1)} = 12.4 \times 10^{-21}\,A^2\,sec/cm^2$ and a corner frequency of $f_c^{(1)} = 73.7\,Hz$. This Lorentzian component will be referred to as the "spontaneous" Lorentzian. We concluded (Van Driessche & Zeiske, 1978; 1980) that these fluctuations are due to an open-close mechanism of K^+ channels in the apical membrane. According to a two-state model of fluctuating channels, $2\pi f_c$ is the chemical rate of the channel kinetics (Lindemann & Van Driessche, 1977). After addition of 7.8 μM $BaCl_2$ to the mucosal solution, we observe a clearly pronounced second Lorentzian in the lower frequency range. Simultaneously, the K^+ current was depressed from 4.05 to 2.98 $\mu A/cm^2$ with a paralleled decrease in the plateau of the spontaneous Lorentzian and a small increase in the corner frequency $f_c^{(1)}$. The induced (left) Lorentzian had a much higher plateau of $S_o^{(2)} = 242 \times 10^{-21}\,A^2 sec/cm^2$, and a corner frequency $f_c^{(2)} = 3.2\,Hz$. These values were obtained from a fit of a double Lorentzian plus $1/f^\alpha$ to the data. In most experiments, α varied between 0.7 and 1.5. In this experiment α was 1.18 for the control and 1.42 with Ba^{2+}.

Experiments with K^+-free mucosal solution showed no Ba^{2+}-induced Lorentzian in the spectrum, which indicates that this component was strictly dependent on the presence of K^+ ions. Further evidence, that the site of action of Ba^{2+} is the spontaneously fluctuating K^+ channel, comes from the observation (Fig. 4*B*) that 10 mM CsCl in the outer

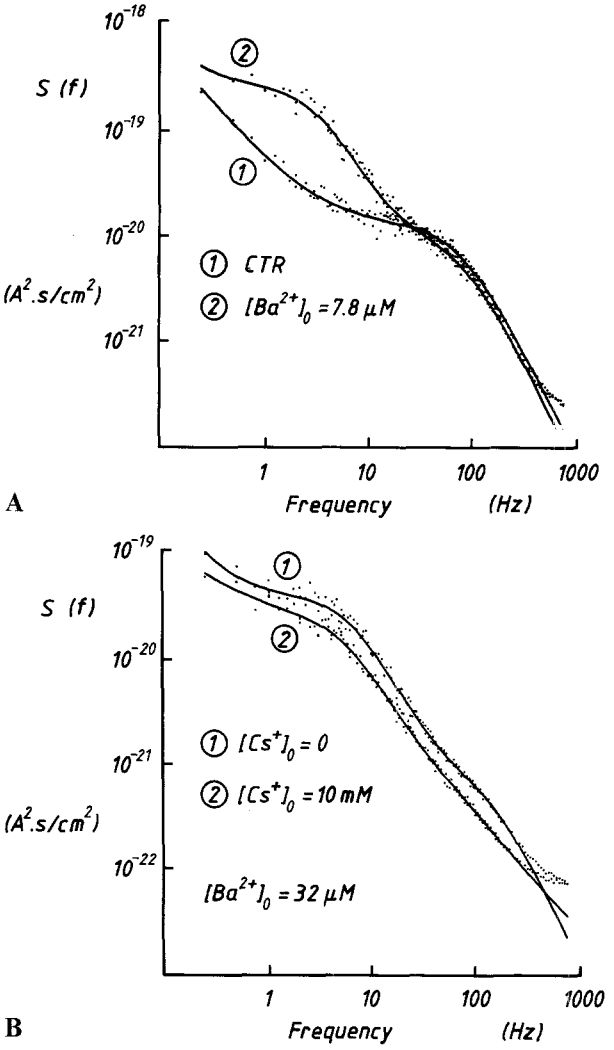

Fig. 4. (*A*): Power spectrum of the short-circuit current noise without (curve *1*) and with 7.8 μM $BaCl_2$ (curve *2*) in the mucosal KCl-solution (containing 50 μM amiloride). The single spectra (dots) were fitted (*solid lines:* curve *1*, one Lorentzian plus $S_b(f) = K_b/f^\alpha$ component; curve *2*, two Lorentzians plus $S_b(f)$ component), displayed together on the screen and photographed. Fit-parameters for curve *1*:
$S_o^{(1)} = 12.4 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(1)} = 73.7\,Hz$;
$S_b(1) = 44.7 \times 10^{-21}\,A^2\,sec/cm^2$; $\alpha = 1.18$.

Fit-parameters for curve *2*:
$S_o^{(1)} = 9.1 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(1)} = 77.3\,Hz$;
$S_o^{(2)} = 242 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(2)} = 3.2\,Hz$;
$S_b(1) = 20.2 \times 10^{-21}\,A^2\,sec/cm^2$; $\alpha = 1.42$.
(*B*): Influence of mucosal CsCl on the spontaneous and Ba^{2+}-induced K^+-current noise. Curve *1* denotes the fit in absence, curve *2* denotes the fit in presence of 10 mM CsCl in the mucosal KCl solution containing 32 μM $BaCl_2$ and 50 μM amiloride. Photographed from the screen as described in *a*. Fit-parameters for curve *1*:
$S_o^{(1)} = 0.70 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(1)} = 121\,Hz$;
$S_o^{(2)} = 37.1 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(2)} = 6.3\,Hz$;
$S_b(1) = 6.4 \times 10^{-21}\,A^2\,sec/cm^2$; $\alpha = 1.6$

Fit-parameters for curve *2*:
$S_o^{(1)} = 0.17 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(1)} = 84\,Hz$;
$S_o^{(2)} = 20.6 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(2)} = 5.5\,Hz$;
$S_b(1) = 11.7 \times 10^{-21}\,A^2\,sec/cm^2$; $\alpha = 0.9$

solution depressed both the spontaneous and the Ba^{2+}-induced Lorentzian. A quantitative interpretation of the effect of Cs^+ on spontaneous and Ba^{2+}-induced noise could possibly be obtained with a four-state model for a Cs^+-Ba^{2+}-K^+ competition. Thus Ba^{2+} ions cause an additional low-frequency fluctuation pattern superimposed on the spontaneous K^+-channel noise. Contrary to the amiloride-induced fluctuations (Lindemann & Van Driessche, 1977), the Ba^{2+}-induced current noise contains frequencies much smaller than the spontaneous noise, and is therefore similar to the K^+-channel noise induced by 4-aminopyridine in squid axon (Fishman et al., 1977).

Characteristics of the Ba^{2+}-induced K^+ noise. The rise of the Ba^{2+}-induced Lorentzian can already be observed at micromolar Ba^{2+} concentrations where the K^+ current is only slightly changed. Figure 5*a* shows the influence of increasing $(Ba^{2+})_o$ on spontaneous and Ba^{2+}-induced current noise. With 7.8 μM $(Ba^{2+})_o$, we observe a clear increase in the induced Lorentzian while the "spontaneous" Lorentzian is little changed. With 500 μM $(Ba^{2+})_o$ a shift to the right is seen, and the plateau $S_o^{(2)}$ decreases below that for 7.8 μM $(Ba^{2+})_o$. The spontaneous K^+ noise which is expected to decrease (Ba^{2+} blocks like Cs^+) will be progressively masked by the shift to the right of the induced Lorentzian. With 500 and 1500 μM $(Ba^{2+})_o$ only the latter one can be observed.

The change in the parameters of the Ba^{2+}-induced Lorentzian, over a $(Ba^{2+})_o$ range from 1 μM up to 2 mM, is shown, for a representative skin, in Fig. 5*b*. $S_o^{(2)}$ is depicted as a function of log $(Ba^{2+})_o$, whereas $2\pi f_c^{(2)}$, the rate for the Ba^{2+}-dependent open-close mechanism, is represented as a function of $(Ba^{2+})_o$. $S_o^{(2)}$ decreases continuously and disappears below the background noise at $(Ba^{2+})_o > 2$ mM. It is evident that the maximum value of $S_o^{(2)}$ lies around the same $(Ba^{2+})_o$ as the point of inflection of the I_{sc}-curve in Fig. 2.

It was found for the amiloride-induced conductance noise of the Na^+ channel that the corner frequencies of the induced Lorentzian increased linearly with increasing amiloride concentration (Lindemann & Van Driessche, 1977). If the spontaneous conductance fluctuations do not significantly alter the blocker-induced noise, such a plot allows the determination of the rate constants for the blocker-receptor reaction.

$$Ba + R \underset{k_{20}}{\overset{k_{02}}{\rightleftharpoons}} BaR$$

open ① blocked ②

We therefore looked to see whether we would obtain a similar result with the Ba^{2+}-induced K^+ noise. Indeed we found a practically linear increase in

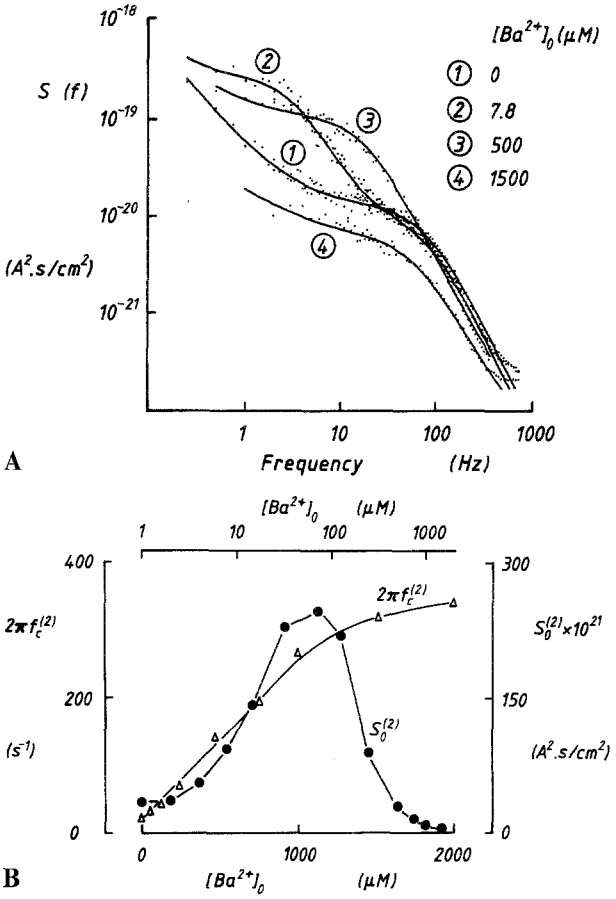

Fig. 5. (*A*): Power spectra of the K^+-current noise at different mucosal Ba^{2+} concentrations, $(Ba^{2+})_o$. The curves were obtained as described in Fig. 4. The mucosal KCl solutions contained 50 μM

The fit-parameters for curve *1* (single Lorentzian plus $S_b(f) = K_b/f^\alpha$) are

$S_o^{(1)} = 12.4 \times 10^{-21}\,A^2\,sec/cm^2$, $f^{(1)} = 73.7$ Hz,
$S_b(1) = 44.7 \times 10^{-21}\,A^2\,sec/cm^2$ and $\alpha = 1.18$;

for curve *2* (double-Lorentzian plus K_b/f^α):

$S_o^{(1)} = 9.1 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(1)} = 77.3$ Hz;
$S_o^{(2)} = 242 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(2)} = 3.2$ Hz;
$S_b(1) = 20.2 \times 10^{-21}\,A^2\,s/cm^2$; $\alpha = 1.42$;

for curve *3* (single Lorentzian plus $S_b(f)$):

$S_o^{(2)} = 98.1 \times 10^{-21}\,A^2\,sec/cm^2$, $f_c^{(2)} = 17.6$ Hz;
$S_b(1) = 53.6 \times 10^{-21}\,A^2\,sec/cm^2$; $\alpha = 1.0$;

for curve *4* (single Lorentzian plus $S_b(f)$):

$S_o^{(2)} = 5.1 \times 10^{-21}\,A^2\,sec/cm^2$; $f_c^{(2)} = 59.8$ Hz;
$S_b(1) = 13.6 \times 10^{-21}\,A^2\,sec/cm^2$; $\alpha = 0.81$.

(*B*): Parameters of the Ba^{2+}-induced Lorentzian as function of the Ba^{2+} concentration in mucosal KCl Ringer (50 μM amiloride). The reaction rate of the Ba^{2+}-K^+ channel interaction, $2\pi f_c^{(2)}$ (triangles), is displayed as a linear function of $(Ba^{2+})_o$ (left ordinate −lower abscissa); the plateau value, $S_o^{(2)}$ (dots), is represented in a semilogarithmic way (right ordinate − upper abscissa)

$f_c^{(2)}$ with increasing $(Ba^{2+})_o$, although only up to Ba^{2+} concentrations of about 1 mM (Fig. 5*b*). At larger $(Ba^{2+})_o$, the $f_c^{(2)}$ increase was subproportional. To settle whether this finding was significant, we pooled

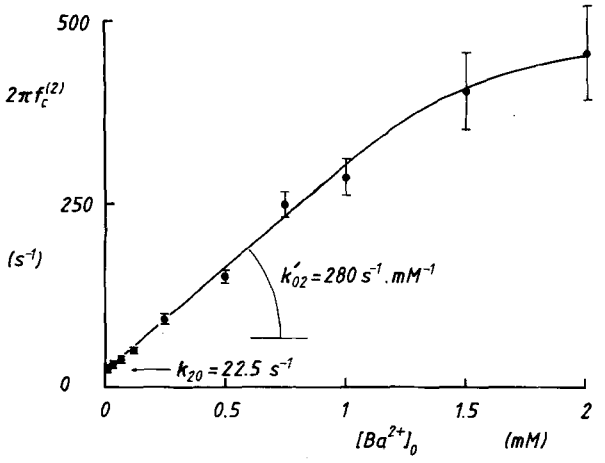

Fig. 6. The reaction rate of the Ba^{2+}-induced current fluctuations, $2\pi f_c^{(2)}$, as a function of the $BaCl_2$-concentration in the mucosal KCl solution (50 μM amiloride). The data are pooled from 13 experiments (±SEM indicated by vertical bars). From the slope of the linear part the association rate constant k'_{02} of the Ba^{2+}-receptor reaction is obtained, from the ordinate intercept the dissociation rate constant k_{20}. The mean value of $2\pi f_c^{(1)}$ measured in the absence of Ba^{2+} was in this series of experiments:

$\overline{2\pi f_c^{(1)}} = (496 \pm 22)\,\text{sec}^{-1}$

data from 13 different skins. Figure 6 clearly demonstrates that we have a quasi-linear f_c increase at lower $(Ba^{2+})_o$ with a saturation-like course at $(Ba)_o > 1$ mM. If the two-state model is applicable at low $(Ba^{2+})_o$, where the $2\pi f_c^{(2)} - (Ba^{2+})_o$ relationship is linear, the apparent rate constant for the association reaction (k'_{02}) can be determined from the slope of the straight line and is found to be $280\,\text{sec}^{-1} \cdot \text{mM}^{-1}$. The rate constant for the dissociation reaction can be determined from the ordinate intercept, $k_{20} = 22.5\,\text{sec}^{-1}$. The apparent equilibrium constant for the Ba^{2+}-receptor reaction, calculated as the ratio k_{20}/k'_{02}, is $K'_{Ba} = 80.4$ μM. The close agreement of this value with the K_{Ba}-value determined from Fig. 2, and also the quasi-linear increase of $2\pi f_c^{(2)}$ with $(Ba^{2+})_o$ suggest that, at low $(Ba^{2+})_o$, the pseudo two-state model with pseudo first-order kinetics is applicable. Thus it seems that, in this concentration range, the spontaneous open-close mechanism has little effect on the Ba^{2+}-receptor kinetics.

So far it seems that at low $(Ba^{2+})_o$, the action of Ba^{2+} on the K^+ channel is very similar to that of amiloride on the Na^+ channel. The interpretation of the amiloride-induced noise was based on a model of competitive blockage of Na^+ channels by amiloride (Lindemann & Van Driessche, 1977). In the discussion section we shall present a similar kinetic model which describes the saturation-like course of the $2\pi f_c^{(2)} - (Ba^{2+})_o$ relationship and the dependence of $S_o^{(2)}$ on log $(Ba^{2+})_o$ as depicted in Fig. 5*b*.

Discussion

Steady-State Kinetics

Like Cs^+ or H^+, muscosal Ba^{2+} also blocks the K^+-dependent short-circuit current. The onset of the Ba^{2+}-induced changes in the K^+ current is very rapid and reversible. This finding suggests an action at the external surface of the apical membranes, especially since the cell membranes generally show little passive permeability towards divalent cations. Furthermore, microelectrode studies in K^+-permeable skins (Hirschmann & Nagel, 1978) clearly showed that Ba^{2+} ions block K^+ channels from the apical side. It has also been demonstrated that basolateral K^+ channels in frog skin can be blocked by Ba^{2+} in the serosal bathing solution (Nagel, 1978). With regard to the moulting cycle of the frog, it is not unlikely that the here described apical K^+ channels are "remainders" after the transformation of former serosal to new mucosal membranes during the moult (*see also* Zeiske & Van Driessche, 1979*a*). The Ba^{2+} inhibition, however, does not necessarily indicate a common origin of apical and basolateral K^+ channels. As is already known for H^+ and Cs^+, Ba^{2+} ions are also reported to be blockers of K^+ channels in other tissues (*cf.*, e.g., Sperelakis et al., 1967). Comparison of the chemistry and of the molecular shape of the more or less hydrated K^+ and Ba^{2+} ions gives rise to the idea that Ba^{2+} may compete with K^+ for negative sites near or in the K^+ channels. Interaction with this site could facilitate K^+ transfer, whereas Ba^{2+} would adhere and thus block the K^+ pathway. For many ionic blockers this concept of competitive inhibition has been assumed. The mathematical description resembles the competition concept in enzyme kinetics. It has been shown by analyzing Lineweaver-Burk plots that amiloride is a competitive Na^+ blocker (Zeiske & Lindemann, 1974) and that Cs^+ ions competitively inhibit the K^+ passage through the apical frog skin membrane (Zeiske & Van Driessche, 1979*a*). We tried to settle the question of the K^+-Ba^{2+} competition with steady-state kinetics, but obtained poor results. The main difficulty was a Ba^{2+}-induced shunt current, the origin of which remains unclear. This effect is apparent for $(Ba^{2+})_o > 500$ μM, where the transepithelial conductance increases (*see* Fig. 2). The simultaneously rising shunt-current component dominates the I_{sc} (*see* Fig. 3) which no longer represents the K^+ current. Thus in the Lineweaver-Burk diagram in Fig. 3 the most reliable data points must lie near the origin. Considering the data in the literature, we might speculate that Ba^{2+} is a competitive blocker of the K^+ current.

Noise Experiments

As it has already been pointed out, the noise experiments suffered seriously from the poor reversibility of the Ba^{2+} effect. However, it must be stressed that the microscopic reversibility of the Ba^{2+}-induced fluctuation is not in question, but rather the extreme difficulty and slowness of the washout of Ba^{2+}. It seems that, even after removal of Ba^{2+}, storing sites are able to supply the epithelium with enough Ba^{2+} to evoke the second Lorentzian. To overcome this difficulty, we used the washing procedure described in *Materials and Methods*.

The interpretation of noise data obtained from multicellular systems is complicated because a "true" voltage clamp across the membrane, which contains the fluctuating channels, is difficult to achieve. A "true" voltage clamp across the apical membrane of frog skin was realized by Lindemann and Van Driessche (1977) after depolarizing the basolateral membranes with K^+-Ringer. This treatment reduces the basolateral membrane resistance such that its contribution to the transepithelial resistance can be neglected. This procedure cannot be used in our experiments because K^+ current (Zeiske & Van Driessche, 1979*a*) and K^+-current fluctuations (Van Driessche & Zeiske, 1980) depend on the existence of a mucosa-to-serosa directed K^+ gradient. However, even without depolarization of the serosal membranes, the apical membrane resistance is about 2–3 times larger than that of the serosal membrane, especially if Na^+ is replaced by K^+ in the outer bathing solution (Nagel & Hirschmann, 1980). The ratio of apical to serosal membrane resistance becomes even larger in the presence of mucosal Ba^{2+}. We therefore believe that, under our experimental conditions, an "almost true" voltage clamp across the apical membrane is achieved, at least at the lower frequency limit of current fluctuations. Consequently, the transepithelially recorded fluctuations will be attenuated only moderately at low frequencies. However, due to possible alterations of the membrane impedance ratio at higher frequencies, the shape of the transepithelially recorded spectra may be different from spectra of fluctuations in the apical K^+ conductance. We never observed deformations of the Lorentzian shape under various experimental conditions (*cf. also* Van Driessche & Zeiske, 1980). In consequence it is likely that the measured Lorentzian parameters are not significantly different from those expected from a "true" voltage clamp across a single membrane.

In natural systems a double-Lorentzian noise has been found for the fluctuating activation and inactivation gates in the Na^+ channel of the node of Ranvier (Conti et al., 1979). A drug-induced Lorentzian, in addition to a spontaneous channel noise, was shown for the inhibitory effect of amiloride in apical Na^+ channels in frog skin (Lindemann & Van Driessche, 1977) and toad bladder (Van Driessche & Hegel, 1978), and also for the K^+ channel block in nerve axon by TEA (Fishman et al., 1975*a*; Moore et al., 1979). In these cases the induced noise contained higher frequencies than the spontaneous one, but 4-aminopyridine (Fishman et al., 1977) shows low-frequency induced noise like the phenomenon described here. At low $(Ba^{2+})_o$, the Ba^{2+}-induced noise contains lower frequencies than the spontaneous noise. At $(Ba^{2+})_o > 100\,\mu M$, the spontaneous component was masked by the induced fluctuations. Besides these Lorentzian components, a low-frequency background noise was observed. We describe its frequency dependence by a K_b/f^α-function (*see also Materials and Methods*). Other investigators found α varying between 0.5 and 1.5 and therefore classified the background noise as a *1/f* type, describing a rather common noise form in nature thought to be due to ionic diffusion in open channels (Fishman et al., 1975*a*). Since in many cases our spectra could not be fitted with sufficient accuracy by a *1/f* background-noise component, we decided to fit with $1/f^\alpha$. It turned out that α varied between 0.7 and 1.5, depending on the experimental conditions. At low $(Ba^{2+})_o$, where two Lorentzians could be fitted to the experimental data, the $1/f^\alpha$ component was significantly smaller at 1 Hz than $S_o^{(2)}$. In this case the same results for the parameters $S_o^{(2)}$ and $f_c^{(2)}$ can be obtained using only the sum of two Lorentzians to fit the experimental data, omitting the most left spectral values. At higher $(Ba^{2+})_o$, as well as in Ba^{2+}-free controls, the K_b/f^α component made a significant contribution to the power density only at frequencies well below f_c. Consequently, we could neglect the influence of the $1/f^\alpha$ component on the computation of S_o- and f_c-values. As shown in the figures, the fits with $1/f^\alpha$ as background component yielded very satisfying results. At present we do not know the origin of the background noise, but we might speculate that it is largely dependent on the K^+ uptake from the mucosal solution. Evidence for this assumption comes from the observation that the intensity of the background noise strongly depends on the K^+-concentration gradient across the epithelium (Van Driessche & Zeiske, 1980, Figs. 2*a* and 5*a*). Moreover, Cs^+ ions are able to reduce the low-frequency background noise (*ibid.*, Fig. 4*a*), thus indicating the selective K^+ pathway as a possible source of the background noise. Similarly Ba^{2+} reduces the background fluctuations (spectra 1 and 4 in Fig. 5*a*). The background component may also be related to ionic

movements through other "shunt" pathways, the conductance of which is obviously strongly influenced by Ba^{2+} ions (*see* Fig. 2).

A Three-State Model

Lorentzian components in power spectra of current noise are generally accepted to be caused by fluctuations of ionic channels between conducting and nonconducting states. In our previous work (Van Driessche & Zeiske, 1980) we used the two-state model (Stevens, 1972; Hill & Chen, 1972) to describe spontaneous fluctuations of K^+ channels in the apical cell membrane of the frog skin (*R. temporaria*). For the spontaneous transition between one open and one closed channel state, Eqs. (3) and (4) describe the interrelations between the Lorentzian parameters S_o and f_c, the total number of channels M, the single channel current i, the probabilities to find a channel open (P_o) or closed ($P_1 = 1 - P_o$), and the macroscopic relaxation time τ of the microscopic fluctuation process:

$$S_o = 4Mi^2 P_o P_1 \tau \tag{3}$$

$$2\pi f_c^{(1)} = \frac{1}{\tau} = \alpha_{01} + \alpha_{10}. \tag{4}$$

The inverse of τ is the chemical rate of the fluctuation and equal to $2\pi f_c$ (Stevens, 1972; Hill & Chen, 1972). The reaction rate is the sum of the transition probabilities α_{01} and α_{10} in the following reaction scheme which describes the K^+-channel fluctuation between two states:

$$R \underset{\alpha_{10}}{\overset{\alpha_{01}}{\rightleftharpoons}} R^*$$

open ⓪ closed ①

The occupational probabilities P_o and P_1 are related to α_{01} and α_{10} through the mass-equilibrium law as

$$P_0 = \frac{\alpha_{10}}{\alpha_{01} + \alpha_{10}} \quad \text{and} \quad P_1 = \frac{\alpha_{01}}{\alpha_{01} + \alpha_{10}}. \tag{5}$$

For the spontaneous fluctuations we assume α_{01} and α_{10} to be equal to the rate constants k_{01} and k_{10}, respectively.

Regardless of the exact mechanism of K^+-conductance blockage, the interaction of the K^+ channels with Ba^{2+} ions obviously leads to additional random conductance fluctuations of the K^+ channel. If we assume that the Ba^{2+}-induced channel block occurs only in the open channel state, we may formulate the following three-state model:

$$\begin{array}{ccccc} & & Ba^{2+} & & \\ & & \overset{\alpha_{02}}{\searrow} & & \\ BaR & \underset{\alpha_{20}}{\overset{\alpha_{02}}{\rightleftharpoons}} & R & \underset{\alpha_{10}}{\overset{\alpha_{01}}{\rightleftharpoons}} & R^* \end{array}$$

blocked ② open ⓪ closed ①

Here, R denotes the open K^+-channel, R^* its closed state caused by the spontaneous fluctuation, and BaR another closed state after combining with Ba^{2+} ions. A similar three-state model was already assumed for the inhibitory Cs^+ effect on the K^+ conductance of cation channels from muscle membranes, incorporated in a phospholipid bilayer (Coronado & Miller, 1979). Such a model predicts the existence of a double-Lorentzian in the power spectrum. Using the method described by Chen and Hill (1973) we derived the equations for the plateau value and the corner frequency of the spontaneous and the Ba^{2+}-induced Lorentzian (*see Appendix*). These equations are similar to those derived for the amiloride-induced Na^+-current noise in frog skin (Lindemann & Van Driessche, 1978).

As pointed out before, we assume the Lorentzian parameters obtained from transepithelial noise measurements to be reliable. A further test for this assumption is the comparison of the experimentally found dependence of $S_o^{(2)}$ and $f_c^{(2)}$ on $(Ba^{2+})_o$ with the theoretical calculations. We used Eqs. (A3), and (A5)–(A11) to simulate the experimentally obtained dependence of $f_c^{(2)}$ and $S_o^{(2)}$ on $(Ba^{2+})_o$ as shown in Fig. 5*b*. In *Results* we suggested that, at low $(Ba^{2+})_o$, the Ba^{2+}-receptor reaction seems to be little affected by the spontaneous open-close mechanism, and we determined the rate constants k'_{02} and k_{20}. For the computer simulation we also need the values of k_{01} and k_{10}. The sum of these rate constants is equal to $2\pi f_c^{(1)} = 496\ \text{sec}^{-1}$, measured in the absence of Ba^{2+}. However, the individual values are unknown. We tested different sets of rate constants, the sum of which was equal to $2\pi f_c^{(1)}$. Only for $k_{10} \gg k_{01}$, could the saturation-like behavior of the $2\pi f_c^{(2)} - (Ba^{2+})_o$ relationship, as found experimentally at $(Ba^{2+})_o > 1$ mM, be obtained. If this condition was not fulfilled the subproportional increase of $2\pi f_c^{(2)}$ occurred at even smaller $(Ba^{2+})_o$ values, and a much lower saturation level of $2\pi f_c^{(2)}$ (at high $(Ba^{2+})_o$) than in the experiments was obtained.

Plateau values and corner frequencies of the Ba^{2+}-induced K^+-current fluctuations were then calculated. With the values of the kinetic parameters indicated in the legend of Fig. 7, the calculated $S_o^{(2)}$ are displayed in Fig. 7 as function of $\log (Ba^{2+})_o$. We obtained a very good qualitative agreement with our results shown in Fig. 5. With our assumption $k_{10} \gg k_{01}$, Eq. (A7) reduces to Eq. (3) for

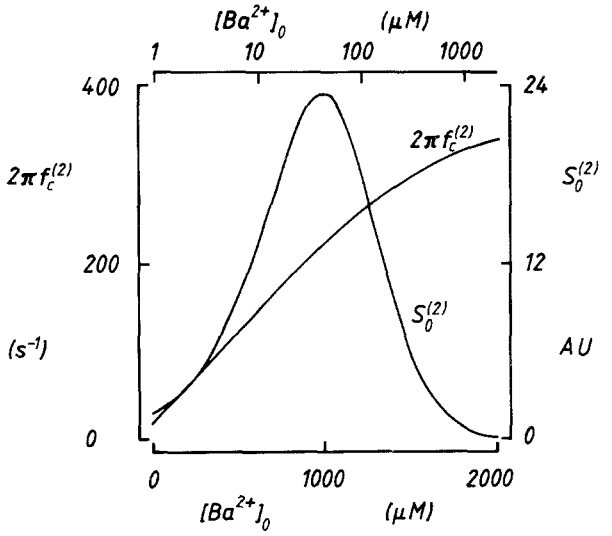

Fig. 7. Result of the computer simulation with a three-state model. Coordinate system as in Fig. 5*B*. The solution is approximative only for the course of $2\pi f_c^{(2)}$; the plateau values, $S_o^{(2)}$, are given in arbitrary units, AU. The curves were calculated with the following parameters for the three-state model: $k_{01}=26\,\text{sec}^{-1}$; $k_{10}=470\,\text{sec}^{-1}$; $k'_{02}=280\,\text{sec}^{-1}\cdot\text{mM}^{-1}$; $k_{20}=22.5\,\text{sec}^{-1}$

$(Ba^{2+})_o \leqq K'_{Ba}$. Then a maximum of $S_o^{(2)}$ occurs when the product $P_o P_1 \tau^{(2)}$ is maximal. The $(Ba^{2+})_o$-dependence of this product predicts a $S_o^{(2)}$-maximum near $(Ba^{2+})_o = K'_{Ba}$. The $2\pi f_c^{(2)}$ values are plotted against $(Ba^{2+})_o$ to compare the computer simulation to our results from Fig. 6. We indeed obtain a similar course, and we see that, for small $(Ba^{2+})_o$, $2\pi f_c^{(2)}$ depends almost linearly on $(Ba^{2+})_o$. Computer calculations for very high $(Ba^{2+})_o$ reveal that the saturation value of $2\pi f_c^{(2)}$ is equal to k_{10}. A most important feature of this model is that it predicts the order of magnitude of the quasi-saturating $2\pi f_c^{(2)}$ for high $(Ba^{2+})_o$ only for the case $k_{10} \gg k_{01}$. We chose the values $k_{10}=470\,\text{sec}^{-1}$ and $k_{01}=26\,\text{sec}^{-1}$. The consequence of our findings is that, in the absence of Ba^{2+}, the mean open time of the K^+ channel is considerably longer than the mean time of channel closure.

As, at low $(Ba^{2+})_o$, the two-state model adequately describes the Ba^{2+}-induced fluctuations, we tentatively calculated the single-channel current i with the following expression derived from Eq. (3), with $I_k = M \cdot i \cdot P_o$ and index 2 substituted for 1:

$$i = \frac{\pi^2}{k'_{02}\cdot(Ba^{2+})_o} \cdot \frac{(f_c^{(2)})^2 \cdot S_o^{(2)}}{I_k}. \tag{6}$$

To derive the expressions for the probabilities P_o and P_2 we assumed $\alpha_{02} = k'_{02}\cdot(Ba^{2+})_o$ and $\alpha_{20} = k_{20}$. The number of Ba^{2+}-blockable K^+ channels, M_{Ba}, was calculated from

$$M_{Ba} = \frac{I_k}{P_o \cdot i}. \tag{7}$$

For $(Ba^{2+})_o = 15.6\,\mu\text{M}$, the calculations of i and M_{Ba} were done using the experimental values I_k (assumed to be equal to I_{sc}), $S_o^{(2)}$ and $f_c^{(2)}$ obtained from 6 different skins. The mean values were $\bar{I}_k = (14.4 \pm 1.4)\ \mu\text{A/cm}^2$, $\bar{f}_c^{(2)} = (4.2 \pm 0.3)\,\text{Hz}$ and $\bar{S}_o^{(2)} = (389 \pm 78) \times 10^{-21}\ \text{A}^2\,\text{sec/cm}^2$. With k'_{02} from the $2\pi f_c^{(2)} - (Ba^{2+})_o$ relation in Fig. 6, we obtained the mean values for the single-channel current and the channel density, $\bar{i} = (1.00 \pm 0.14)\,\text{pA}$ and $\bar{M}_{Ba} = (0.18 \pm 0.02)\ \mu\text{m}^{-2}$, respectively.

In a previous paper (Van Driessche & Zeiske, 1980) we tentatively assumed equal probabilities to find the K^+ channel in the open or closed state. We computed from the spontaneous Lorentzian $\bar{i} = (0.37 \pm 0.05)\,\text{pA}$ and $\bar{M} = (0.53 \pm 0.08)\ \mu\text{m}^{-2}$. The discrepancy between the formerly and the presently obtained values for the single-channel current must be due to the arbitrary assumption of equal rate constants of the open-close reaction. The electrochemical gradient for K^+ across the apical membrane will not be altered significantly by such small Ba^{2+} concentrations as 15.6 μM, because the negative intracellular potential in the short-circuited state remains practically constant at this $(Ba^{2+})_o$ (Hirschmann & Nagel, 1978). Therefore, the values of i obtained without Ba^{2+} and with 15.6 μM $(Ba^{2+})_o$ should be approximately equal. If this is true we may calculate the probability for a channel to be spontaneously closed (P_1), in the absence of Ba^{2+}, by rearranging Eq. (3) to:

$$P_1 = \frac{2\pi f_c^{(1)} S_o^{(1)}}{4 I_k i}. \tag{8}$$

We used the values of $f_c^{(1)}$, $S_o^{(1)}$ and I_k (assumed to be equal to I_{sc}) which were recorded in absence of Ba^{2+} in the same series of skins that served for the computation of i from the Ba^{2+} experiments: $\bar{I}_k = (20.0 \pm 2.6)\,\mu\text{A/cm}^2$, $\bar{S}_o^{(1)} = (8.0 \pm 2.2) \times 10^{-21}\ \text{A}^2\,\text{sec/cm}^2$ and $\bar{f}_c^{(1)} = (86.9 \pm 7.6)\,\text{Hz}$. The mean value for P_1 obtained by this procedure was $\bar{P}_1 = (0.051 \pm 0.006)$. This result confirms our conclusion from the model calculations with the three-state model, namely $k_{10} \gg k_{01}$ or $P_o \gg P_1$.

$\bar{M}_{Ba}$ may differ from $\bar{M}$, the total channel density, because the Ba^{2+}-method only permits one to determine the number of Ba^{2+}-blockable K^+ channels. This is, according to our model, the number of open and Ba^{2+}-blocked channels. The above-discussed fact that $k_{10} \gg k_{01}$ and $P_o \gg P_1$ means that the fraction of spontaneously closed channels must be very small. Consequently $\bar{M}_{Ba}$ will not differ much from $\bar{M}$. The K^+-channel density calculated with the Ba^{2+}-method is astonishingly small compared to the Na^+-channel density in the apical membrane (Lindemann & Van Driessche, 1977) though the observed K^+ currents are not that much smaller than the usually recorded

Na^+ currents. We already mentioned the possibility that the apical K^+ channels may originate from an incomplete "serosal to mucosal" membrane transformation during the moult. This might have occurred in each or most of the transformed cells. Assuming a uniform K^+-channel distribution over the apical surface of the cells of the outermost stratum granulosum layer, we find one K^+ channel per 5 μm^2 surface area. However, we easily could come to much higher local channel densities if the arbitrary assumption of a uniform channel distribution is discarded. In this case, the new outer living cell layer would contain a large population of mainly untransformed "inner" cells with highly K^+-permeable membranes.

Though our kinetic data and the description of our noise results would favor the assumption of a simple $K^+ - Ba^{2+}$ competition, we do not know whether they actually compete for the same site. We only know that interaction with one ionic species makes the transport molecule unavailable for the interaction with the other ionic species. However, comparison of the ionic radii of K^+ and Ba^{2+} would not exclude a simple cork-like blocking action of Ba^{2+}. In such a case it would be similar to the hypothetical action of other cation-channel blockers like Cs^+ (Adelman, 1971), TTX (Smythies et al., 1974) or TEA (Adelman, 1971). It would be different from the kind of blocking Na^+ channels in frog skin by Na^+ which have been discussed to induce channel closure by an indirect mechanism (Lindemann & Voûte, 1976; Zeiske, 1979).

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Appendix

Corner Frequencies and Plateau Values Calculated for the Three-State Model

We consider an ensemble of M independent and equivalent channels, each of which can be in one of the three states of our model. In its conducting state (o) each channel can carry the single-channel current (i). We assume the conductances of the closed (1) and blocked (2) state to be zero. The transition probabilities between any two states (from i to j) are denoted by α_{ij}. Making the assumption of pseudo first-order kinetics for the Ba^{2+} block and of a 1:1 complex of Ba^{2+} with its reaction site, the probabilities for the transition between open and blocked channel state are $\alpha_{02} = k_{02} \cdot (Ba^{2+})_m$ and $\alpha_{20} = k_{20} \cdot (Ba^{2+})_m$ represents the Ba^{2+} concentration just in front of the reaction site of Ba^{2+}. k_{02} and k_{20} are the rate constants for the association and dissociation steps, respectively. $(Ba^{2+})_m$ is related to the bulk concentration $(Ba^{2+})_o$ by the partition coefficient $\beta = (Ba^{2+})_m/(Ba^{2+})_o$, which has previously been used to describe amiloride-induced Na^+-channel noise (Lindemann & Van Driessche, 1977). If the membrane carries negative fixed charges on its outer surface, β will be >1. Consequently we must write $\alpha_{02} = k_{02} \cdot \beta \cdot (Ba^{2+})_o = k'_{02} \cdot (Ba^{2+})_o$, where k'_{02} is the apparent rate constant of the association step. As in the two-state model, the probabilities for the transitions between open and closed state are $\alpha_{01} = k_{01}$ and $\alpha_{10} = k_{10}$.

Thus the A-matrix of the differential equations governing the time dependence of occupation probabilities (Chen & Hill, 1973) is

$$A = \begin{pmatrix} -(\alpha_{01}+\alpha_{02}) & \alpha_{10} & \alpha_{20} \\ \alpha_{01} & -\alpha_{10} & 0 \\ \alpha_{02} & 0 & -\alpha_{20} \end{pmatrix}. \tag{A1}$$

The corner frequencies are, except for the sign, equal to the eigenvalues (λ_1 and λ_2) of A:

$$2\pi f_c^{(1)} = r_1 + \rho \tag{A2}$$

$$2\pi f_c^{(2)} = r_2 - \rho. \tag{A3}$$

Here the reaction rates of the noncoupled (spontaneous and Ba^{2+}-induced) fluctuations are identical to the ones in the two-state model:

$$r_1 = k_{01} + k_{10} \tag{A4}$$

$$r_2 = k'_{02} \cdot (Ba^{2+})_o + k_{20}. \tag{A5}$$

Coupling of both reactions, as required by the three-state model, causes a symmetrical shift ρ of the original rates:

$$\rho = \tfrac{1}{2} \cdot ((r_2 - r_1) + \sqrt{(r_1 - r_2)^2 + 4 k_{01} k'_{02} \cdot (Ba^{2+})_o}). \tag{A6}$$

Following the method of Chen & Hill (1973), the plateau values $S_o^{(1)}$ and $S_o^{(2)}$, corresponding to the corner frequencies $f_c^{(1)}$ and $f_c^{(2)}$, may be obtained from the normalized eigenfunctions of the S-matrix obtained by transforming A to a real symmetrical matrix:

$$S_o^{(k)} = 4 \cdot M \cdot i^2 P_o / (Q \cdot 2\pi f_c^{(k)}) \tag{A7}$$

where

$$Q = 1 + \frac{\alpha_{01}\alpha_{10}}{(\alpha_{10} - 2\pi f_c^{(k)})^2} + \frac{\alpha_{02}\alpha_{20}}{(\alpha_{20} - 2\pi f_c^{(k)})^2}. \tag{A8}$$

P_o, the steady-state probability of finding a channel in the open state, may be calculated from the mass-equilibrium law:

$$P_o = \frac{K'_{Ba}}{K^*_{Ba} + (Ba^{2+})_o} \tag{A9}$$

with

$$K^*_{Ba} = K'_{Ba} \cdot \left(1 + \frac{k_{01}}{k_{10}}\right) \quad \text{(A10)}$$

and the apparent equilibrium constant

$$K'_{Ba} = k_{20}/k'_{02}. \quad \text{(A11)}$$

References

Adelman, W.J., Jr. 1971. Electrical studies of internally perfused squid axons. *In:* Biophysics and Physiology of Excitable Membranes. W.J. Adelman, editor. p. 274. Van Nostrand Reinhold, New York – Cincinnati – Toronto – London – Melbourne

Chen, Y., Hill, T.L. 1973. Fluctuations and noise in kinetic systems. Application to K^+ channels in the squid axon. *Biophys. J.* **13**:1276

Conti, F., Neumcke, B., Nonner, W., Stämpfli, R. 1979. Low frequency fluctuations of Na current in myelinated nerve. *Pfluegers Arch.* **379**:*R*40

Coronado, R., Miller, C. 1979. Voltage-dependent cesium blockade of a cation channel from fragmented sarcoplasmic reticulum. *Nature* (London) **280**:807

Droogmans, G., Raeymaekers, L., Casteels, R. 1977. Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *J. Gen. Physiol.* **70**:129

Ehrlich, E.N., Crabbé, J. 1968. The mechanism of action of amipramizide. *Pfluegers Arch.* **302**:79

Fishman, H.M., Moore, L.E., Poussart, D.J.M. 1975*a*. Potassium-ion conduction noise in squid axon membrane. *J. Membrane Biol.* **24**:305

Fishman, H.M., Moore, L.E., Poussart, D.J.M. 1977. Ion movements and kinetics in squid axon: II. Spontaneous electrical fluctuations. *In:* Electrical Properties of Biological Polymers. S. Takashima and H.M. Fishman, Editors. *Ann. N.Y. Acad. Sci.* **303**:399

Fishman, H.M., Poussart, D.J.M., Moore, L.E. 1975*b*. Noise measurements in squid axon membrane. *J. Membrane Biol.* **24**:281

Gebhardt, U., Fuchs, W., Lindemann, B. 1972. Resistance response of frog skin to brief and long lasting changes of $(Na)_o$ and $(K)_o$. *In:* Role of Membranes in Secretory Process. L. Bolis, R.D. Keynes, and W. Wilbrandt, editors. p. 284. North-Holland, Amsterdam

Goldman, D.E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37

Hill, T.L., Chen, Y.D. 1972. On the theory of ion transport across the nerve membrane. V. Two models for the Cole-Moore K^+-hyperpolarization delay. *Biophys. J.* **12**:960

Hille, B. 1966. The common mode of action of three agents that decrease the transient change in sodium permeability in nerves. *Nature* (*London*) **210**:1220

Hille, B. 1967. The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. Gen. Physiol.* **50**:1287

Hirschmann, W., Nagel, W. 1978. The outer membrane of frog skin: Impermeable to K^+? *Pfluegers Arch.* **373**:*R*48

Isenberg, G. 1976. Cardiac Purkinje fibers: Cesium as tool to block inward rectifying potassium currents. *Pfluegers Arch.* **365**:99

Lindemann, B., Van Driessche, W. 1977. Sodium-specific membrane channels of frog skin are pores: Current fluctuations reveal high turnover. *Science* **195**:292

Lindemann, B., Van Driessche, W. 1978. The mechanism of Na-uptake through Na-selective channels in the epithelium of frog skin. *In:* Membrane transport processes. Vol. 1, p. 155. J.F. Hoffman, editor. Raven Press, New York

Lindemann, B., Voûte, C. 1976. Structure and function of the epidermis. *In:* Frog Neurobiology. R. Llinas and W. Precht, editors. p. 169. Springer-Verlag, Berlin – Heidelberg

Mandel, L.J., Curran, P.F. 1973. Response of the frog skin to steady-state voltage clamping. II. The active pathway. *J. Gen. Physiol.* **62**:1

Mayer, C.J., van Breemen, C., Casteels, R. 1972. The action of lanthanum and D-600 on the calcium exchange in the smooth muscle cells of the guinea-pig *taenia coli*. *Pfluegers Arch.* **337**:333

Moore, L.E., Fishman, H.M., Poussart, D.J.M. 1979. Chemically induced K^+ conduction noise in squid axon. *J. Membrane Biol.* **47**:99

Nagel, W. 1978. Ba^{++} decreases G_K in frog skin. *Fed. Proc.* **37** (**3**):1869

Nagel, W., Hirschmann, W. 1980. K^+-permeability of the outer border of the frog skin (*R. temporaria*). *J. Membrane Biol.* **52**:107

Smythies, J.R., Benington, F., Bradley, R.J., Bridgers, W.F., Morin, R.D. 1974. The molecular structure of the sodium channel. *J. Theor. Biol.* **43**:29

Sperelakis, N., Schneider, M.F., Harris, E.J. 1967. Decreased K^+ conductance produced by Ba^{++} in frog sartorius fibers. *J. Gen. Physiol.* **50**:1565

Stevens, C.F. 1972. Inferences about membrane properties from electrical noise measurements. *Biophys. J.* **12**:1028

Van Driessche, W., Hegel, U. 1978. Amiloride induced fluctuations of short circuit current through toad urinary bladder. *In:* Sixth International Biophysics Congress Abstracts. p. 215, Kyoto

Van Driessche, W., Lindemann, B. 1978. Low-noise amplification of voltage and current fluctuations arising in epithelia. *Rev. Sci. Instrum.* **49**:52

Van Driessche, W., Zeiske, W. 1978. Fluctuations of the K^+-current in the frog skin (*Rana temporaria*). *Arch. Int. Physiol. Biochem* **86**:684

Van Driessche, W., Zeiske, W. 1980. Spontaneous fluctuations of potassium channels in the apical membrane of frog skin. *J. Physiol.* (*London*) **299**:101

Zeiske, W. 1975. The influence of 2.4.6-triaminopyrimidine on Na-transport in frog skin. *Pfluegers Arch.* **359**:*R*127

Zeiske, W. 1979. Ph.D. Thesis. University of the Saarland, Saarbrücken

Zeiske, W., Lindemann, B. 1974. Chemical stimulation of Na-current through the outer surface of frog skin epithelium. *Biochim. Biophys. Acta* **352**:323

Zeiske, W., Van Driessche, W. 1978. K^+-uptake across the outer border of frog skin (*R. temp.*) and its inhibition by Cs^+-ions. *Pfluegers Arch.* **373**:*R*48

Zeiske, W., Van Driessche, W. 1979*a*. Saturable K^+ pathway across the outer border of frog skin (*Rana Temporaria*): Kinetics and inhibition by Cs^+ and other cations. *J. Membrane Biol.* **47**:77

Zeiske, W., Van Driessche, W. 1979*b*. Kinetics of K^+ channels in the apical membrane of frog skin: Control by voltage, pH and polyvalent cations. *Arch. Int. Physiol. Biochem.* **87**:331

Zeiske, W., Van Driessche, W. 1979*c*. Influence of Ba^{2+} on K^+-current noise in frog skin. *Pfluegers Arch.* **382**:*R*23

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