Spontaneous and blocker-induced K^+-channel noise in frog skin (Rana Temporaria).

Willy Van Driessche and Wolfgang Zeiske

Laboratorium voor Fysiologie, Campus Gasthuisberg, B-3000 Leuven, BELGIUM.

The outer (apical, mucosal) barrier of the epithelium of frog skin is known to be mainly permeable for Na^+ ions (1). The properties of the apical Na^+ channels have been extensively studied during the last decades with various methods (2). Recently (3,4) it was found that the apical membrane of the skin of Rana Temporaria has, besides the Na^+ channels, a significant permeability for K^+ ions. All experiments discussed in this communication, performed to investigate the properties of the apical K^+ pathway, were done with NaCl Ringer's on the inner (basolateral, serosal) side. Mostly the mucosal solution had a high KCl concentration, so that a K^+ gradient directed from mucosa to serosa was established. In all experiments discussed here, the mucosal solutions contained 50 µM Amiloride to block eventual Na^+ movements across the epithelium (5). In the first section, the characteristics of the transepithelial potential difference (PD) and of the short-circuit current (SCC) will be briefly discussed. In the following section the analysis of the fluctuations of the SCC will be reviewed.

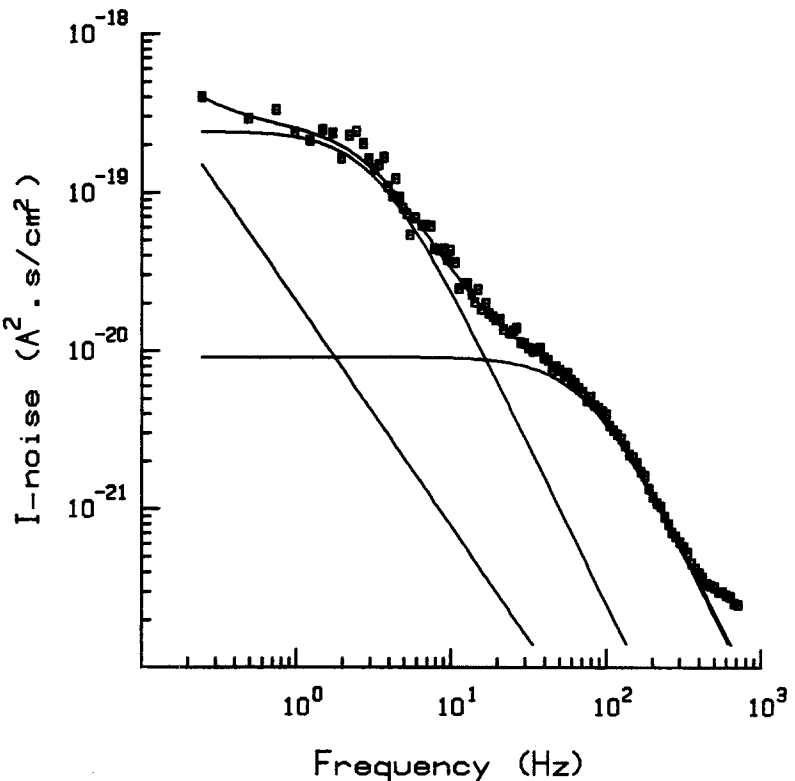
The PD recorded under open circuit conditions showed a linear correlation with the logarithm of the mucosal K^+ concentration ($(K^+)_o$). A rise of about 20-30 mV per 10-fold increase of $(K^+)_o$ was observed. However, an increase of 58 mV as would be expected from a pure K^+-selective membrane was never observed. This discrepancy may be caused by the fact that the K^+-selective structures are shunted by a less specific cellular or paracellular pathway. When the transepithelial voltage was clamped to zero, a mucosa to serosa directed short-circuit current (SCC) of about 8-20 µA/cm^2 was recorded. Increasing $(K^+)_o$ under SCC conditions with a rapid flow chamber from 2.5 to 115 mM, resulted in a fast rise of SCC. Simultaneously, the transepithelial conductance (G) increased by 100 % (from about 0.2 to about 0.4 mmho/cm^2). The relation between SCC and $(K^+)_o$ showed a saturation-like behaviour reaching a plateau at $(K^+)_o$ > 50 mM. A similar behaviour of the Na^+uptake through the apical membrane as a function of the mucosal Na^+ concentration has been studied extensively by others (6). The addition of Cs^+ (10 mM), known as a blocker for K^+ channels in other tissues (7), to the mucosal solution resulted in a sudden decrease of SCC to about 50 % of its original value and a decrease of G by a factor of four. Kinetic studies showed Cs^+ to be competitive with K^+. Among other investigated monovalent cations (Na^+, Li^+, Rb^+, NH_4^+ and protons) only mucosal Rb^+ (10 mM) and protons (2.5 mM) showed to be able to depress the K^+ current. Nagel and Hirschmann (4) investigated the K^+ permeability of the apical membrane of the skin of the same frog species with microelectrodes. Their findings agree with our results and they showed that the apical K^+ channels are blockable by mucosal Ba^{2+} ions. We could confirm the latter finding by recordings of the SCC, with the fast flow chamber as we did with Cs^+.

The analysis of the fluctuations of the K^+ current around its macroscopic mean value, were done under SCC conditions (transepithelial voltage clamped to zero) in the frequency range from 1 to 800 Hz with a low-noise voltage clamp circuit (8). The power spectrum of these fluctuations recorded with $(K^+)_o$ = 117.5 mM, revealed a spontaneous relaxation noise component (9), described by a Lorentzian function: $S(f) = S_o/(1 + (f/f_c)^2)$. The mean plateau value was S_o = (1.50 ± 0.05) x 10^{-20} A^2.s/cm^2 and the corner frequency f_c = (81.0 ± 3.4) Hz (n = 14). The existence of this relaxation noise com-

ponent in the power spectrum suggests that the above described apical K^+ selective channels switch randomly between an open and closed state. Arguments for the fact that the relaxation noise component is caused by K^+ movements through apical K^+ channels are : The relaxation noise component was only in the spectrum when the mucosal K^+ concentration was sufficiently large (> 40 mM). If choline was used as main cation in the mucosal solution the relaxation noise component disappeared. Substitution of Cl^- in the mucosal and serosal solutions by other anions did not alter the relaxation noise component. Cs^+ ions (10 mM) in the mucosal solution rapidly depress the relaxation noise component to the level below the amplifier noise. As the mucosal Cs^+ concentration was increased gradually from 0 to 5 mM, a shift of f_c to lower frequencies was observed. The shift of f_c may be explained on the basis of a Cs^+ - K^+ competition with a three state model as was used for the Amiloride-sodium competition (10). In this case the relaxation time of the Cs^+-receptor binding reaction must be assumed to be much smaller than the relaxation time for the spontaneous transitions between the open and closed state. Among the other tested alkali cations, Rb^+ was the only blocker, through less potent than Cs^+. Using the Lorentzian parameters, S_o and f_c, and the values obtained for the value of the macroscopic K^+ current (I_K, assumed to be equal to SCC), estimations for the single channel current (i) and the K^+ channel density (M) may be made with following equations based on a simple two-state model, having one open (o) and one closed (1) state (11): $S_o = 4\ Mi^2\ P_o P_1\ \tau$, in which the relaxation time $\tau = 1/2\pi f_c$; $I_K = M\ i\ P_o$. P_o and P_1 represent the occupational probabilities for a channel being in the open or closed state, respectively. In this model τ is related to the mean time open (θ_o) and the mean time closed (θ_1) by : $1/\tau = 1/\theta_o + 1/\theta_1$. The evaluation of M and i is hampered by the fact that arbitrary values for P_o and P_1 have to be assumed. Speculative assumptions for the P-values may be avoided if the channel parameters (M and i) are calculated from drug-induced relaxation noise components, as was done for the apical Na^+ channel with the Amiloride method (11,12).

Additional fluctuations of the K^+ current could be induced with mucosal Ba^{2+} ions (see figure). The Ba^{2+}-induced fluctuations were seen as a second Lorentzian component in the power spectrum (analysis from 0.25 up to 800 Hz). The Lorentzian parameters of the induced component (S_o^* and f_c^*) could be investigated over a mucosal Ba^{2+} concentration range from 1 µM up to 2 mM. f_c^* increased quasi-linearly from about 2 Hz ($(Ba^{2+})_o$ = 1 µM) up to 50 Hz at $(Ba^{2+})_o$ = 1 mM. At higher Ba^{2+} concentrations f_c^* showed a tendency to saturate. S_o increased up to $(Ba^{2+})_o$ = 60 µM where a maximum was reached. Increasing $(Ba^{2+})_o$ further caused S_o^* to decrease to very small values. The spectra recorded in the presence of Ba^{2+}, composed of two Lorentzian components, can be described by a three state model (13) consisting of: one open state (o), a spontaneously closed state (1) and a Ba^{2+}-blocked state (2). At low $(Ba^{2+})_o$ the Lorentzian parameters of the induced component can be described by transitions between the open and the Ba^{2+}-blocked state and seem not to be influenced by the spontaneous transitions between the open and the spontaneous closed state. From the linear part of the f_c^* - $(Ba^{2+})_o$ relation the rate constants for the association (k_{02}) and the dissociation (k_{20}) step can be calculated as : k_{02} = 280 $s^{-1}.mM^{-1}$ and k_{20} = 22.5 s^{-1}. Using these rate constants the occupational probabilities (P_o, P_2) may be calculated. Using the above described equations the single channel current was calculated as being 1 pA. The number of Ba^{2+}-blockable channels was evaluated to be 1 channel per 5 µm^2. From the saturation-like behavour of f_c^* at high $(Ba^{2+})_o$ we were able to get a rough estimation for the rate constants (k_{01} and k_{10}) of the transitions between the open and spontaneously closed state. Model calculations with a three state model showed

that the saturation of f_c^* at $(Ba^{2+})_o > 1$ mM can only be obtained if $k_{10} >> k_{01}$. Consequently, it can be concluded 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. Moreover, the number of Ba^{2+}-blockable channels, determined with small $(Ba^{2+})_o$ should not differ significantly from the total number of K^+ channels. With microelectrodes it was shown that the electrochemical gradient across the apical membrane is not significantly influenced by small Ba^{2+} concentrations (4). Consequently it may be assumed that the single channel current (i) is not significantly altered at small $(Ba^{2+})_o$. Using the values of i obtained from the Ba^{2+}-induced relaxation noise we calculated the occupational probability for a channel being spontaneously closed in the absence of Ba^{2+}. For these calculations we used S_o, f_c and I_K-values measured in the absence of Ba^{2+} and the equation :
$P_1 = S_o/4\ I_K.i.\tau$. We found $P_1 = 0.051$. This result confirms that the change to find a channel in the closed state is rather small.



Legend: Spectrum of current fluctuations recorded with $(Ba^{2+})_o$ = 7.8 µM. The data points were fitted with a sum of two Lorentzians and a $1/f^{\alpha}$ component. The latter only contributes significantly to the total noise power at lower frequencies. Diffusion of K^+ ions through open channels or through structures in series with these channels, may be the origin of this noise component. The corner frequency of the spontaneous Lorentzian component is 77.3 Hz and that of the Ba^{2+}-induced component 3.2 Hz.

1. Koefoed-Johnsen, V. and Ussing, H.H. (1958) Acta physiol. scand. 42, 298-308.
2. Lindemann, B. and Voûte, C. (1976) in Frog Neurobiology. R. Llinas and W. Pracht, editors. Springer Verlag Berlin, Heidelberg, New York.
3. Zeiske, W. and Van Driessche, W. (1979) J. Membrane Biol. 47, 77-96.
4. Nagel, W. and Hirschmann, W. (1980) J. Membrane Biol. 52, 107-113.
5. Ehrlich, E.N. and Crabbé, J. (1968) Pfluegers Arch. 302, 79-96.
6. Biber, T.U.L. and Curran, P. (1970) J. Gen. Physiol. 56, 83-99.
7. Carmeliet, E. (1980) Pfluegers Arch. 383, 143-150.
8. Van Driessche, W. and Lindemann, B. (1978) Rev. Sci. Instrum. 49, 52-57.
9. Van Driessche, W. and Zeiske, W. (1980) J. Physiol. 299, 101-116.
10. Lindemann, B. and Van Driessche, W. (1978) in Membrane Transport Processes, Vol. 1. J.F. Hoffman, editor. Raven Press, New York.
11. Lindemann, B. and Van Driessche, W. (1977) Science, N.Y. 195, 292-294.
12. Van Driessche, W. and Lindemann, B. (1979) Nature (London) 282, 519-520.
13. Van Driessche, W. and Zeiske, W. (1980) J. Membrane Biol., in press.