SINGLE POTASSIUM CHANNEL CONDUCTANCE IN THE FROG NODE OF RANVIER

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ABSTRACT The single K^+-channel conductance was calculated from the variance of the spontaneous potassium noise currents in voltage clamped frog node. Essential for this calculation is the mean potassium conductance during the noise measurement. So far this quantity has been underestimated, apparently due to K^+-ion accumulation. With the proper values, the single K^+-channel conductance is an increasing function of membrane voltage.

Information concerning the ion conduction mechanism in nerve membrane can be obtained from noise analysis (1). Using this technique, Begenisich and Stevens (2) first presented estimates of the single potassium channel conductance (γ_K) obtained from voltage clamped frog node. These estimates did not change significantly with membrane voltage. Similar results were reported by Van den Berg et al. (3) and by Neumcke et al. (4).

A complication in estimating γ_K arises from the accumulation of K^+ ions at the external surface of the nodal membrane, resulting in a changing potassium reversal potential, E_K (4, 5, 6). Our observations of these effects during noise analysis (this paper) have led us to reexamine the voltage dependency of γ_K. The possible existence of different populations of potassium channels, as proposed by Ilyin et al. (7), appears inconsistent with the analysis by De Bruin of both the potassium conductance kinetics (6) and the current fluctuations (8). Therefore, we assumed the presence of a single population of identical channels, as in the previous reports on potassium current fluctuations in the frog node (2–4).

Experiments were performed on single nerve fibers from the sciatic nerve of the frog *Rana esculenta,* which were functionally identified as motor fibers. The voltage clamp procedures, which allow accurate calibration of the membrane currents, have been described elsewhere (9). Ringer's solution (115 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 0.50 mM $MgCl_2$, 5.0 mM Tris; pH = 7.4), containing 300 nM tetrodotoxin (TTX) to block the sodium currents, continuously superfused the node. The temperature, measured close to the node, remained constant at 15 or 19°C (±0.2°C).

Potassium current records and spectral estimates of the noise currents were obtained at 5-s intervals during sequences of ~40 voltage steps, then averaged and stored on disk. A 2-s hyperpolarizing prepulse towards −95 mV preceded each voltage step to eliminate the inactivation of the K^+ conductance, present at the holding voltage (−75 mV). The spectral density was obtained in the successive voltage steps as follows. An analogue subtracting device, containing a sample-hold circuit, was triggered at the peak of the potassium current. This effectively eliminated the DC component of the membrane current during each voltage step. As the potassium current started to decrease after its peak value, the remaining signal was high-pass filtered at 5 Hz (second-order Butterworth filter) and amplified. Inspection of this signal revealed an initial transient, which had decayed completely within 100–150 ms after the onset of the voltage step. Therefore, noise current samples were taken from 200 to 550 ms with a frequency of 8.333 and 0.833 kHz, respectively. Increasing the waiting time before sampling of the noise currents did not decrease the apparent contribution of 1/f noise to the spectra.

Aliasing in the spectral density was prevented by eighth order low-pass filtering. Spectral estimates were calculated by fast Fourier transform (FFT), respectively, from 66–5,000 Hz and from 6.6–500 Hz and were averaged during the successive steps. The final spectrum (from 6.6–5,000 Hz) was corrected for the transfer function of the filters.

As the exact mechanism of K^+-ion conduction still is unknown (3, 4, 6, 7, 10), the description of the spectral density is not straightforward. Analysis of the spectra according to the different models so far applied (3, 4, 10), using a weighted least-squares curve fitting procedure,

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indicated that a 1/f noise component was indispensable at test voltages between -50 and $+20$ mV. Outside this voltage range, only 1/f noise was found (3, 9). Adding a Lorentzian (3) or a diffusionlike component (4) proved to be insufficient for an adequate description of all spectra. Excellent fits were obtained with a model containing a 1/f noise component and two Lorentzian functions, S_1 and S_2 (Fig. 1 *a*). These Lorentzian components were each characterized by a low-frequency asymptote and a cutoff frequency that differed by approximately one order of magnitude (8). At membrane voltages between -45 and $+15$ mV, the S_2 component could not be identified in ~30% of the spectra (Fig. 1 *b*), mainly due to a relatively large contribution of background noise to the spectral density at high frequencies. Yet, in these cases the same voltage dependency of S_1 was found as before. The S_2 component is an extension to the model used by Van den Berg et al. (3) and has been reported in the preliminary observations by Stevens (10). Because the three noise components in excess of the background noise level were eliminated following the application of internal TEA and Cs^+ (11), they are apparently associated with the potassium conducting system.

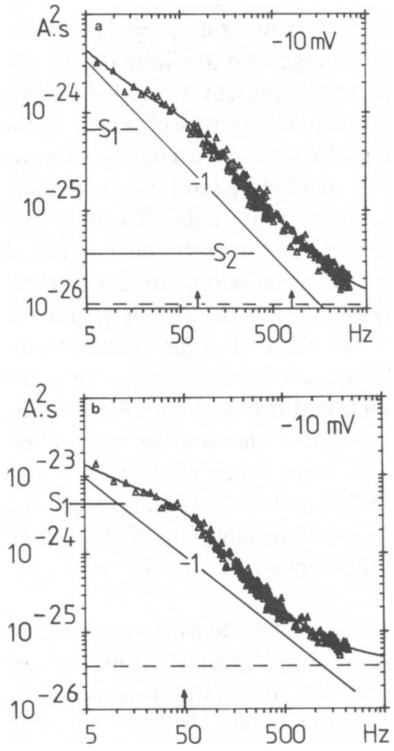

FIGURE 1 (*a*) An example of the spectral density of the potassium current fluctuations at -10 mV at 19° C. The line with slope -1 represents the estimated contribution of the 1/f noise. The horizontal lines (S_1 and S_2) indicate the low-frequency asymptotes of either Lorentzian, while estimates of cutoff frequencies are indicated with arrows. Estimated background noise is shown in this fit as an interrupted line. (*b*) A spectrum containing 1/f noise and a single Lorentzian, obtained from a different node. Test voltage, temperature, and symbols are the same as in *a*.

From the parameter estimates for S_1 and S_2, the variance (σ^2) of the Lorentzian noise was calculated. Then, the single channel conductance has been obtained according to (12, 13)

$$\gamma_K = (g_K \sigma^2)/[\langle I_K \rangle^2 (1 - g_K/\bar{g}_K)] \qquad (1)$$

with mean potassium current $\langle I_K \rangle$. To obtain both the potassium conductance, g_K, during the noise measurement and the maximal potassium conductance, $\bar{g}_K$, uncontaminated by the changes in E_K, a double pulse method (5, 6) was used (see legend Fig. 2 *c*). This revealed that the K^+-conductance remains practically constant within the interval used for noise analysis (Fig. 2 *c*), whereas the potassium current during these depolarizing voltage steps decreases with 5 to 30% after its peak value at 20–40 ms (Fig. 2 *a*, *b*). This decrease in I_K has been interpreted as inactivation of the potassium conductance, since K^+ accumulation was assumed to have reached an equilibrium within 20–40 ms (4, 5). As the decrease in I_K after its peak value is not parallelled by a similar decrease in g_K, accumulation of K^+-ions apparently does not reach an equilibrium before 200–400 ms.

Calculation of γ_K through Eq. 1 revealed that the single potassium channel conductance estimates increased from 1 pS at -45 to ~6 pS at $+10$ mV (Fig. 3 *a*). In previous reports, where the K^+-channel conductances were estimated from noise analysis, changes in E_K were considered negligible (2, 3) or corrected for short term changes only (<20–40 ms [4]). When we considered, likewise, the decrease in I_K as conductance inactivation, calculations led to nearly voltage independent estimates of γ_K as well. A mean value of 1.7 pS (with SEM = 0.14, 66 estimates) was obtained in this way (Fig. 3 *b*).

Because estimates of the variance, and therefore of γ_K, depend on the model used to describe the spectral density (3, 4, 10), we also calculated σ^2 by numerical integration of the spectra (2, 4). This led to variance estimates, that were on the average ~90% larger than the total variances of S_1 and S_2. Assuming short term (<20–40 ms) changes only in E_K, the resulting single channel conductance estimate did not depend significantly on membrane voltage and had a mean value of 3.1 pS (with SEM = 0.27, 40 estimates), close to previously reported values (2, 4). However, with the proper corrections for the accumulation effects, estimates of γ_K now increased from almost 3 to 8 pS.

Direct effects of the K^+-ion concentration outside the node ($[K^+]_0$) on the potassium conductance are known (14). Therefore, conductance values obtained with the double pulse method and used to calculate γ_K (see Eq. 1), may have been biased by changes in $[K^+]_0$. However, potassium conductance measurements in K^+-Ringer's solution showed that the maximal potassium conductance, $\bar{g}_K$, hardly increased in 115 mM $[K^+]_0$ compared with normal Ringer's solution. In addition, it has been suggested that such concentration effects develop very slowly, i.e., in the order of seconds (6).

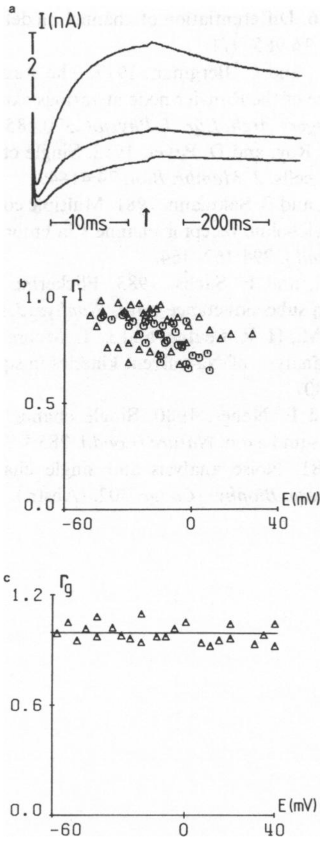

FIGURE 2 (*a*) During depolarizing voltage steps potassium currents reach a peak value within 40 ms followed by a voltage-dependent decrease (note the change in the time scale indicated by the arrow). Membrane currents were obtained at −40, −30, and −20 mV (from bottom to top). (*b*) On the vertical axis the ratio r_I of the potassium current at $t = 375$ and its peak value at 20–40 ms is plotted vs. membrane voltage. This ratio is a decreasing function of membrane voltage, as the decrease in potassium current preceding and during fluctuation analysis is more pronounced in larger depolarizations (*a*). Effects of temperature (Δ = 15°C, ⊙ = 19°C) apparently are of minor importance as compared with the variation within the nodes. (*c*) Similar to *b* the ratio r_g of the potassium conductance at $t = 375$ ms and its value at 20–40 ms is plotted as function of membrane voltage. Potassium conductance estimates (5 nodes; 15–17°C) were obtained independent of E_K by means of a double pulse method (5, 6). With this method, the potassium conductance has been obtained at the end of a test pulse, E_1, by the application of a voltage step towards E_2. From the instantaneous change in the membrane current, ΔI, the potassium conductance g_K at the end of E_1 then can be obtained: $g_K = \Delta I/(E_1 - E_2) - g_L$. The voltage independent leakage conductance has been obtained similarly, with hyperpolarizing test pulses E_3 and E_4, where $g_K = 0$, and therefore, $g_L = \Delta I/(E_3 - E_4)$.

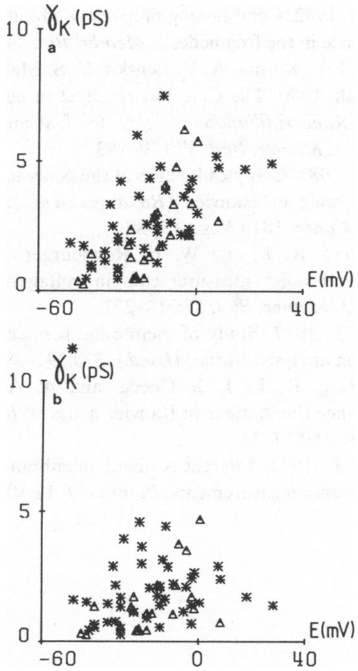

FIGURE 3 (*a*) Single channel conductance, γ_K, as function of membrane voltage was calculated through Eq. 1 from the variance of S_1 and S_2 (∗). In case S_2 could not be detected, γ_K was estimated from the variance of S_1 only (Δ). A dependency of γ_K on temperature (15 or 19°C) was not found. The increase in γ_K with membrane voltage is apparent. (*b*) As in *a* a single channel conductance estimate, γ_K^*, was obtained with g_K (Eq. 1), erroneously, corrected for the decrease in I_K (Fig. 2 *b*), as if this reflected a decreased conductance at $t = 375$ ms. The voltage dependency originally found (*a*) is nearly completely abolished by this procedure.

Therefore, different conducting (open) states within a single population of potassium channels are proposed, comparable with potassium channels in HeLa cells (15), acetylcholine channels in embryonic muscle (16, 17), and sodium channels in squid axon (18). In frog node, the underlying conductance mechanism apparently contains at least a relatively slow and a fast step, as indicated by the occurrence of S_1 and S_2. Observations on single potassium channels in squid axon indicated that the open channel is in fast equilibrium with a second closed state (19). As this phenomenon is parallelled by the presence of an additional fast relaxation component in the spectra (20), comparable with S_2, similar fast transitions are suggested for the frog node.

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