HOW MANY CONDUCTANCE STATES DO POTASSIUM CHANNELS HAVE?

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The steady-state potassium conductance $g_K(\infty)$ of the *Rana* node of Ranvier is a sigmoid function of membrane potential which ranges from 0 to perhaps 0.15 μmho (Dodge, 1963). This voltage-dependent increase in potassium conductance could be achieved by various physical mechanisms, but two limiting cases may conveniently be distinguished: On the one hand, each individual channel might have only two conductance states (open and closed) so that total potassium conductance would reflect the number of open channels. Alternatively, individual channels might each increase their conductance in much the same way as does the total membrane. Of course, intermediate mechanisms can be envisioned in which a channel has more than two conductance states and the membrane potential determines what fraction of the time a channel spends in each of its various conductance states.

Most membrane biophysicists favor some particular mechanism, but no direct experimental support is presently available for any specific view. The only biological system for which information is available, the acetylcholine-induced conductance increase at the frog neuromuscular junction, appears to operate by altering the number of channels which open; individual channels seem to have only two conductance states, open and closed, which differ by 20–30 pmho (Anderson and Stevens, 1973). We have found, using fluctuation analysis, that frog node potassium channels may also have only two conductance states with a single channel conductance of approximately 4 pmho.

In the preliminary experiments reported here, single channel conductance γ_K was estimated by the equation

$$\gamma_K = \sigma^2/\mu(1 - f)(V - V_K) \tag{1}$$

where σ^2 is the variance in potassium current fluctuations, μ is the mean potassium current, V is membrane potential, V_K is the potassium equilibrium potential, and f is the fraction $g_K(\infty)/\bar{g}_K$. If potassium channels have only two conductance states, then γ_K calculated by Eq. 1 is the single channel conductance. If channels have multiple conductance states, then γ_K is an "average" single channel conductance whose value depends upon the various conductance states and the particular underlying probabilistic mechanism for transitions between them. Eq. 1 above is quite general and does not require that specific kinetic mechanisms be assumed (Stevens, 1972; Colquhoun et al., 1975).

If gradations in conductance are achieved by voltage-dependent alterations in the conductance of individual channels, then γ_K estimated by Eq. 1 should—except for certain specific cases—vary with depolarization. On the other hand, if g_K reflects the number of two-state channels that are open, γ_K estimated by Eq. 1 should have the same value for all membrane potentials. Our observations are consistent with this second alternative.

We have used the standard Dodge-Frankenhaeuser voltage clamp procedure to measure frog node of Ranvier potassium currents as described by Hille (1971). The sodium conductance was blocked with 100 nM tetrodotoxin and, where necessary, the potassium conductance was blocked with isotonic tetraethylammonium bromide solution (TEA). The membrane was held at -80 mV and stepped to various voltages between the holding level and $+16$ mV for approximately $\frac{1}{2}$ s for the measurement of fluctuations, and between -80 and $+80$ mV for about 40 ms to determine $g_K(\infty)$. Potassium inactivation varied, for different nodes and different clamp potentials, between about 10 and 20% at the end of the $\frac{1}{2}$ s depolarization. Mean currents were recorded at conventional gains and the g_K vs. voltage relation determined. Additionally, amplified potassium currents were led usually through a two pole-4 Hz high pass and six pole-900 (or 1,800) Hz low pass Butterworth filter to a digital computer which sampled the current record 2,048 (or 4,096) times per second. Spectral densities over the frequency range of 8 cycles to 1,000 (from 4 to 2,000 Hz in some epxeriments) were calculated with a Fast Fourier Transform routine as described earlier (Anderson and Stevens, 1973), and the variance of potassium current fluctuations was found by integrating the resulting spectrum.

Fluctuations around the mean membrane current are readily seen to be much greater when the potassium conductance is activated than when g_K is close to zero. This observation has been confirmed quantitatively in experiments in which the spectrum of fluctuations has been calculated: over the voltage range for which g_K is between 15 and 85% of its maximum value ($f = 0.15$–0.85), spectral densities of a TEA-poisoned preparation are one or more orders of magnitude less than those obtained from the untreated membrane. Thus our spectra reflect principally properties of the potassium channels.

25 estimations of γ_K have been made in eight experiments over the voltage range from -48 to $+16$ mV which gives values for f in Eq. 1 from 0.15 to 0.85. The mean of estimations made at close to 15° was 4.0 pmho ± 0.27 (standard error for 14 observations). This value was not significantly dependent on membrane potential: the linear

regression calculated between γ_K and membrane potential gave a zero voltage intercept of 4.1 pmho and a slope of 0.021 pmho/mV.

The use of Eq. 1 assumes that no appreciable contribution to the fluctuation variance σ^2 be made from frequencies outside the range investigated. The behavior of the spectral densities between 4 and 2,000 cycles suggests that this is the case. Further, conclusions from Eq. 1 are valid only if the fluctuations under investigation arise primarily from random variations in conductance of a channel associated with gating rather than from noise produced by ions moving through an "open" channel. The consistency and orderliness of our results support the first alternative. The second alternative is difficult to rule out, however, because ionic movements through membrane channels are not yet well enough understood to permit definitive predictions about the noise associated with the permeation process. If it turns out that the dominant noise source in these experiments is the transport process rather than channel gating, our measurements give an upper limit of the average single channel conductance.

Finally, it must be emphasized that, even if the dominant source for fluctuations in our experiments is potassium channel gating, our results do not necessarily imply that potassium channels are all identical or that they have only two conductance states. Specifically, we may conclude only that the average single channel conductance, as measured by Eq. 1, does not depend on membrane potential. Potassium channels may thus either have only two conductance states, or they may have multiple conductance states which are distributed in just such a way as to give a result in Eq. 1 which is constant as g_K ranges between 15 and 85% of its maximum value. We feel that the simplest interpretation is that potassium channels have only two conductance states differing by about 4 pmho.

Three other estimates for the single potassium channel conductance are available, two for the squid axon and one for the frog node. Armstrong (1975) inferred, from the kinetics of pharmacological blockage of potassium conductance, that the value of γ_K for squid is 2–3 pmho or more. Conti et al. (1975) report a γ_K of 14 pmho for squid from analysis of voltage-clamp current fluctuation currents, and Siebenga et al. (1973) obtained values ranging between 10 and 37 pmho from voltage fluctuations in frog node. Because sea water has a higher conductance than frog Ringer's, the γ_K for frog node might be expected to be somewhat smaller than that for squid.

Armstrong's estimate of γ_K depends upon the assumption that certain quaternary nitrogen compounds enter the potassium channel as readily as does potassium itself, and he feels that the actual value of γ_K should perhaps be somewhat larger than 2–3 pmho. The estimates of Conti et al. and of Siebenga et al. assume, as do the estimates we have made, that noise associated with the transport process does not contribute to the calculated γ_K; the techniques used by these authors further presuppose a specific kinetic mechanism, namely that the channel gating can be adequately approximated by a single time constant process. Possible errors arising from this assumption are difficult to assess.

Siebenga et al. (1973) reported values of γ_K obtained at potentials between −40 and

+10 mV. Their data seem to support the conclusion of a moderate, perhaps twofold, increase in single channel conductance over that voltage range. Considering the differences in experimental and theoretical techniques between their study and ours, we feel that the results are not in significant disagreement.

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