STEADY STATE CURRENT NOISE FROM THE INTRINSIC GATING OF INWARD RECTIFIER CHANNELS

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ABSTRACT Steady-state current noise from the intrinsic gating of inward rectifier channels in the eggs of the marine polychaete *Neanthes arenaceodentata* was recorded under voltage clamp conditions. Lorentzian-shaped power spectra with corner frequencies near 1 Hz and zero-frequency asymptotes of $1 - 5 \times 10^{-23}$ A^2/Hz were obtained for test potentials 15–45 mV positive to E_K in solutions containing 10–40 mM K_o. The spectra are compatible with the predictions of a three-state kinetic model for inward rectification in which the single channel rectification is independent of voltage. The single channel conductance derived from this interpretation is 8 pS with 40 mM K_o.

INTRODUCTION

Inward-rectifying potassium channels have been found in a variety of tissues since their discovery by Katz (1949) in skeletal muscle. The cell membranes of cardiac muscle (Hutter and Noble, 1960), mammalian and invertebrate nerve cells (Nelson and Frank, 1967; Kandel and Tauc, 1966), oocytes (Takahashi et al., 1971; Hagiwara and Takahashi, 1974), electroplaques (Nakamura et al., 1965), and macrophages (Gallin, 1981) are all repositories of this channel. Despite this widespread distribution, remarkably little in the way of cell biology has been attributed to the inward rectifier. However, reports of modulation of inward rectification by serotonin in Aplysia neurons (Drummond et al., 1980), by internal sodium in starfish eggs (Hagiwara and Yoshii, 1979), and by calcium ions in heart muscle and tunicate eggs (Isenberg, 1977; Ohmori, 1978) suggest that purposeful variation of the level of conductance may be a general phenomena associated with this channel. An elaboration of the kinetics of gating in inward rectification might be useful in understanding the mechanisms whereby modulators exert their effects on the channel and in comprehending the role of inward rectification in cell physiology. Several papers have reported on the time-dependent kinetics of inward rectification gating in oocytes (Hagiwara et al., 1976; Gunning, 1983; Gunning and Ciani, 1983*a*) and in skeletal muscle (Almers, 1971; Hestrin, 1981; Leech and Stanfield, 1981). The results reported in this paper are meant to extend these studies into the frequency domain. A Lorentzian-shaped power spectrum, identified with conductance noise of the inward rectifier due to its intrinsic gating, has been resolved in the eggs of the marine polychaete *Neanthes arenaceodentata*. This spectrum is consistent with a three-state kinetic model for inward rectification in which the single channel conductance is independent of voltage, and is also consistent with previous estimates for the potassium dependence of the single-channel conductance. Preliminary reports of these findings have been published (Gunning and Ciani, 1983*b,c*).

METHODS

Eggs from the segmented marine worm *N. arenaceodentata* were used in these studies. A syringe inserted into the worms' coeloms was used to sluice eggs into an experimental chamber with a glass bottom to which the eggs adhered. The eggs were typically 400 μm in diameter, though eggs as small as 200 μm were occasionally found. The eggs were bathed in a saline solution containing 650 mM sucrose, 10 mM KCl, 70 mM TrisCl, and 10 mM $CaCl_2$ buffered to pH 7.6. Increased external potassium concentrations were obtained by substitution of KCl for TrisCl. Replacement of NaCl and $MgCl_2$ by sucrose reduced the low-frequency background noise and, also, the leakage current. A standard two-microelectrode, voltage clamp technique was used in these experiments. The electrodes were filled with either 3 M KCl or 4 M NaCl. Drift in the tip potentials of the voltage-sensing intracellular electrodes was a major source of low-frequency background noise. Addition of 100 mM EGTA to the electrode filling solution seemed to attenuate this noise and was routinely utilized. The electrodes generally had resistances of 5 MΩ. Experiments were done at room temperature, 22°C.

The eggs were originally voltage clamped to their resting potentials. Positive voltage steps were then delivered by a computer through a digital-to-analogue converter. The output of the current-to-voltage (I-V) converter was amplified and passed through a 20-pole, elliptic, low-pass, anti-aliasing filter (Unigon Industries Inc., Mount Vernon, NY, model LP 120) with a corner frequency of 200 Hz. 20 records of 2,048 points each were digitized at 2.44 ms/point (0.2 Hz frequency spacing), alternating with recovery periods at the resting potential to prevent accumulation and depletion problems. After digitally subtracting the mean values and applying cosine taper windows to the data, the power spectra were calculated via an FFT program. Power spectra such as those shown in Fig. 1 have been recorded at potentials between 10 and 45 mV positive to the resting potential in ten separate experiments.

RESULTS

The low-frequency power spectrum of inward rectifier gating (Fig. 1 *A,B,C*) has a Lorentzian form $S_I(f) = [S_o/1 + (f/f_c)^2]$, where S_o is the low-frequency asymptote and f_c is the corner frequency. Above 10 Hz, background noise obscures the conductance noise. These power spectra can be identified with the inward rectifier based on several observations. (*a*) Lorentzians are observed at potentials where inward rectifier conductance noise is expected to be appreciable, but not at potentials where the fraction of channels or the driving force on K^+ is negligible (Fig. 1 *E,F*). (*b*) The corner frequencies of the Lorentzian spectra approximate 1 Hz, which matches well with the time constant of current relaxation (Fig. 2 *D*) observed in the real-time data ($f_c = 1/[2\pi\tau]$). (*c*) The single-channel conductance calculated from the power spectra is consistent with both the voltage and potassium dependence of the macroscopic currents (Fig. 3) and is in good agreement with single-channel recordings of the inward rectifier channel measured at large hyperpolarizations. (*d*) There are no other significant ionic conductances in the potential range of this study.

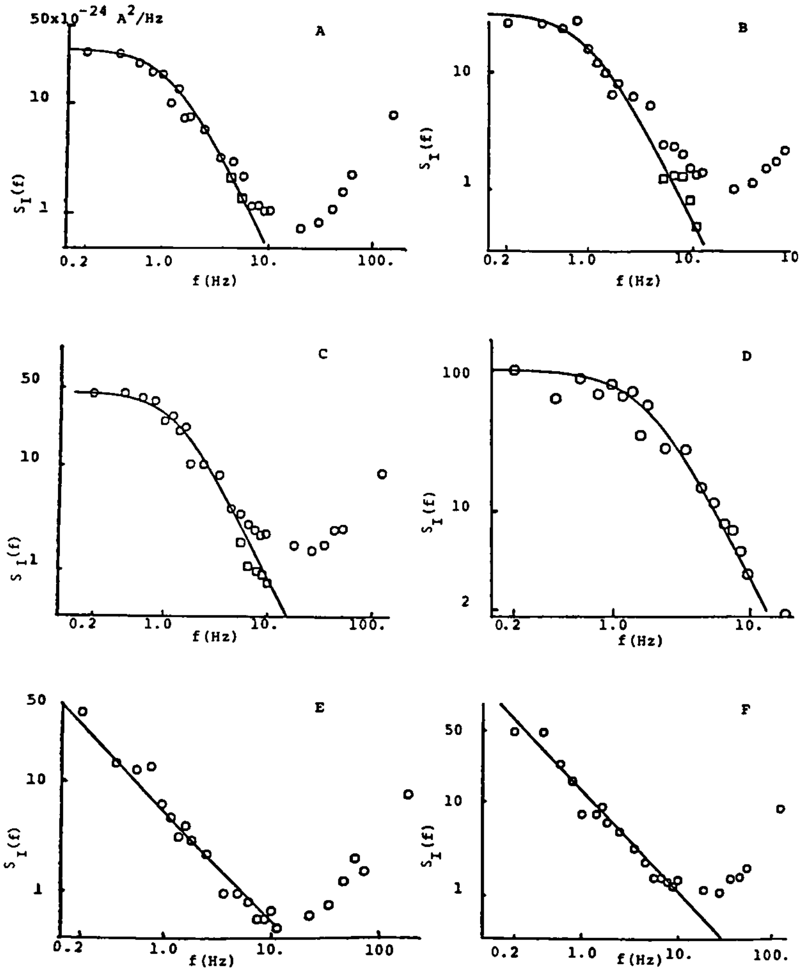

FIGURE 1 Power spectra of inward rectifier steady-state currents. *A,B,* and *C;* power spectra due to intrinsic gating of the channel. *A,* 10 mM K_o. $\Delta V = +16$mV. Holding potential, V_H, equals -84 mV. The solid curve (~) is drawn to the equation $S_I(f) = 2.8 \times 10^{-23}/[1 + (f/1.2)^2]$ A^2/Hz. *B,* 20 mM K_o. $\Delta V = +21$ mV. $V_H = -67$ mV. The solid curve is drawn to $S_I(f) = 2.9 \times 10^{-23}/[1 + (f/1.4)^2]$ A^2/Hz. *C,* 40 mM K_o. $\Delta V = +26$ mV. $V_H = -50$ mV. The solid curve is drawn to $S_I(f) = 4.7 \times 10^{-23}/[1 + (f/1.3)^2]$ A^2/Hz. *D,* channel gating at hyperpolarizing potentials in the presence of external barium. 20 mM K_o. 0.5 mM Ba_o. $\Delta V = -30$ mV. $V_H = -67$ mV. Barium is known to block the inward rectifier channel (Hagiwara et al., 1978; Standen and Stanfield, 1978). The solid curve is drawn to $S_I(f) = 1 \times 10^{-22}/[1 + (f/1.8)^2]$ A^2/Hz. In the absence of barium and other blocking ions, only 1/f noise is seen at hyperpolarizing potentials. *E,F,* control spectra. *E,* 20mM K_o. $\Delta V = 0$ mV. $V_H = -67$ mV. Because $V_H = E_K$ in *Neanthes* eggs under these experimental conditions, no conductance noise is expected at the resting potential. The solid line has a slope of -1. *F,* 40mM K_o. $\Delta V = +55$ mV. $V_H = -50$ mV. The open-channel probability is <0.01 at this potential. The solid line has a slope of -1. In all of these spectra, circles represent raw spectral points and squares represent difference spectra between the raw and control spectra. All spectra are from the same egg.

To calculate a single-channel conductance from these power spectra, it is first necessary to postulate a kinetic model for the inward rectifier. It has been demonstrated elsewhere (Gunning, 1983) that the three-state model

$$0 \underset{y}{\overset{z}{\rightleftharpoons}} C \underset{x}{\overset{w}{\rightleftharpoons}} 1$$

where 0 is the only open state, the single-channel conductance is voltage independent, the rate constants x and w are independent of both voltage and external potassium, and $y + z \gg x + w$ can describe the steady state conductance, the apparent instantaneous conductance, and the time constant of current relaxation observed in voltage clamp experiments on *Neanthes* eggs. The apparent "instantaneous" conductance is, according to this model, the result of a transition between 0 and the closed state C that is too fast for resolution by a two-microelectrode voltage clamp. The solid lines through the data in Fig. 2 *B,C,D* are drawn to this kinetic scheme. This model predicts that the power spectrum of the steady-state current fluctuations will be the composite of two Lorentzians. Due to the large background noise at high frequencies originating in the thermal noise of the voltage-sensing microelectrode, a Lorentzian corresponding to the hypothesized fast gating process between 0 and C cannot be determined. However, the model can still be tested against the experimental power spectra for consistency, and the model-dependent, single-channel conductance can still be calculated. In this analysis, the fraction of the total variance (the area beneath the power spectrum) expected to be subtended by the low-frequency Lorentzian is calculated and compared with the area under the experimental Lorentzians. The steady state power spectrum associated with the above three-state kinetic model is (Gunning, 1982; Conti and Wanke, 1975)

$$S_1(f) = 4\,\mathrm{VAR}\left[\frac{\tau_1 - \tau_s}{\tau_1 - \tau_2} \cdot \frac{\tau_1}{1 + (2\pi f \tau_1)^2} + \frac{\tau_s - \tau_2}{\tau_1 - \tau_2} \cdot \frac{\tau_2}{1 + (2\pi f \tau_2)^2}\right],$$

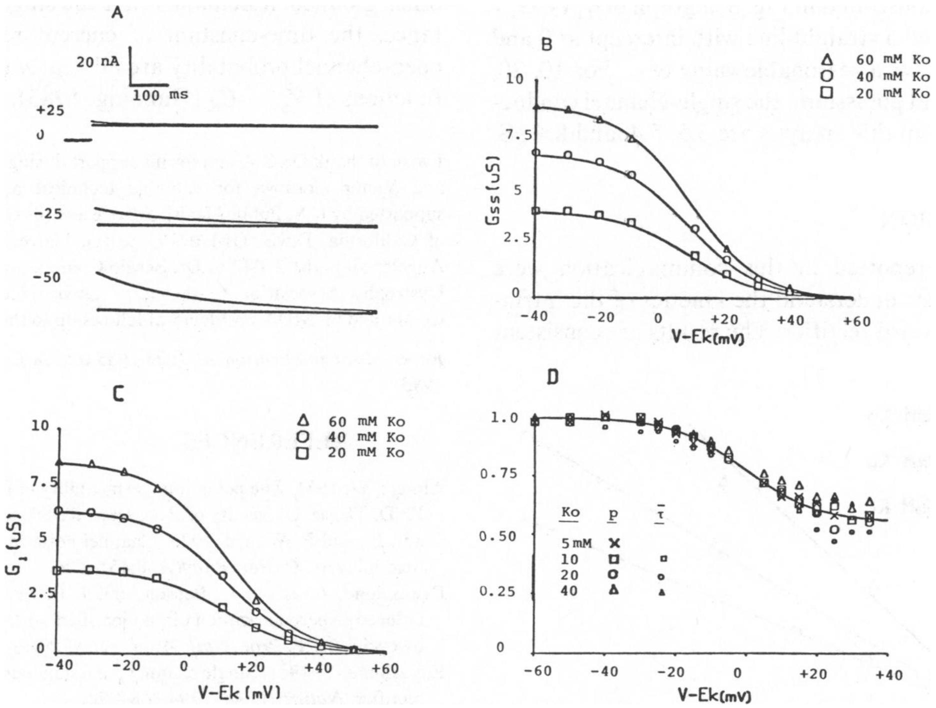

FIGURE 2 Dependence of conductance on voltage, external potassium, and time. *A*, current relaxation in response to voltage steps of +25, −25, and −50 mV. K_o = 40 mM. $V_H = -50$ mV. Hyperpolarizing pulses elicit an instantaneous inward current followed by a time-dependent exponential current increase. Depolarizing pulses cause an instantaneous outward current, smaller than the response to the symmetrical hyperpolarizing pulse, and a slow exponential decrease in current. (Steady-state current levels have been drawn in beneath the current relaxations for reference.) Due to the capacitive transients, the current records do not settle until 1 ms after the beginning of the voltage step. *B*, steady-state conductance, G_{ss}, as a function of ΔV for three concentrations of external potassium. The solid lines are drawn to the equation $G_{ss} = 0.41[K_o]^{0.75}/\{1 + \exp[(\Delta V - 5)/11]\}$. *C*, "instantaneous" conductance, G_I, as a function of ΔV for three concentrations of external potassium. The conductance plotted in this figure is the conductance 1 ms after the beginning of the voltage pulse. G_I may represent the result of a fast gating transition between open and closed states. The solid lines are drawn to the equation $G_I = 0.39[K_o]^{0.75}/\{1 + \exp[(\Delta V - 14)/11]\}$. *D*, the time-constant of current relaxation ($\bar{\tau}$) and the ratio of the steady-state and instantaneous conductances (p) normalized to their maximum values. The maximum limiting values of the time constants in 10, 20, and 40 mM K_o are 223, 219, and 237 ms, respectively. The solid line is drawn to the equation $p = \bar{\tau} = 0.55 + 0.45/[1 + \exp(\Delta V/10)]$. The quantatative agreement between p and $\bar{\tau}$ is predicted by the three-state model considered in the text.

where

$$\tau_1 = \frac{y+z}{yx + z(x+w)}, \quad \tau_s = \frac{1}{x+w},$$

$$\tau_2 = \frac{1}{y+z}, \quad \text{and} \quad VAR = Npq\gamma^2(\Delta V)^2$$

where N is the number of inward rectifier channels, p is the open-channel probability, $q = 1 - p$, γ is the single-channel conductance, and ΔV is the displacement of the holding potential from the equilibrium potential of potassium. A necessary requirement for the experimental data to be consistent with the three-state model is that

$$\gamma_1/\gamma = \frac{\tau_1 - \tau_s}{\tau_1 - \tau_2} = \frac{y}{y+z} \cdot \frac{w}{x+w} = \overline{G}_1 \cdot \frac{w}{x+w}$$

where γ_1 is that fraction of γ obtained by considering only the low-frequency Lorentzian, $\overline{G}_1$ is the normalized "instantaneous" conductance (Fig. 2 *C*), and $1 - w/(x+w)$ is the ratio of the normalized steady-state and instantaneous conductances in the limit of large depolarizing pulses (Fig. 2 *D*). As demonstrated in Fig. 3, a graph of γ_1 vs. $\overline{G}_1 \cdot w/(x+w)$ is indeed a straight line with intercept at 0 and slope equal to $1/\gamma$ for a reasonable value of γ. For 10, 20, and 40 mM external potassium, the single-channel conductances obtained from this analysis are 3.5, 5.4, and 8.4 pS, respectively.

DISCUSSION

The experiments reported in this communication were undertaken to better understand the kinetics of the intrinsic gating of the inward rectifier. The results are consistent with a three-kinetic-state model in which the single-channel conductance does not rectify with voltage. The calculated single-channel conductances are proportional to $K_o^{.63}$ which conforms well with the potassium dependence of the macroscopic maximum limiting conductance (Hagiwara and Takahashi, 1974; Fig. 2 *B*, this paper), which, in *Neanthes*, equals K_o^a, where $0.55 < a < 0.75$. Also, the single-channel conductance is in good agreement with the results found in Na-block noise analysis experiments on tunicate egg inward rectifier (Ohmori, 1978, 1980, 1981; Fukushima, 1982), in single-channel experiments of the block of inward rectifier channels in tunicate eggs by sodium and rat myotubes by barium (Fukushima, 1981; Ohmori et al., 1981), and by Ba-block noise analysis experiments in these *Neanthes* eggs (Fig. 1 *D*). Although the power spectra reported here are consistent with the three-state kinetic model by which they have been interpreted, it should be mentioned that they also show consistency when interpreted according to a two-state kinetic scheme where the single-channel conductance rectifies as a function of voltage. However, this model seems unlikely on other grounds: it demands that the single-channel conductance, the time-constant of current relaxation, and the open-channel probability are all nearly identical sigmoidal functions of $V_m - E_K$ (Gunning, 1983).

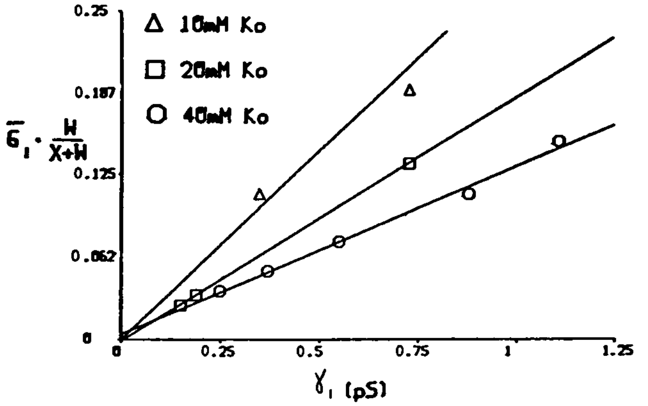

FIGURE 3 A plot of $\overline{G}_1 \cdot w/(x+w)$ vs. γ_1 for three concentrations of external potassium. $\gamma_1 = VAR/[\bar{i}\Delta Vq]$ where VAR is the area under the experimental power spectrum, $\bar{i}$ is the steady-state current, and q is the model-dependent closed-channel probability. $\overline{G}_1$ and $w/(x+w)$ are derived from real-time data (Fig. 2). The straight lines are drawn to $\overline{G}_1(w/x+w) = +0.007 + 1/(8.4\ \text{pS})$ for $K_o = 40$ mM (○); $\overline{G}_1(w/x+w) = -0.001 + 1/(5.4\ \text{pS})$ for $K_o = 20$ mM (□); and $\overline{G}_1(w/x+w) = 0 + 1/(3.5\ \text{pS})$ for $K_o = 10$ mM (△). The lines through the 40 and 20 mM K_o data were fit by the least-squares method. The line through the 10 mM K_o data has been forced through the origin.

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