Activation Kinetics of the Delayed Rectifier Potassium Current of Bullfrog Sympathetic Neurons

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Klemic, Kathryn G., Dominique M. Durand, and Stephen W. Jones. Activation kinetics of the delayed rectifier potassium current of bullfrog sympathetic neurons. J. Neurophysiol. 79: 2345–2357, 1998. We examined the activation kinetics of the delayed rectifier K+ current of bullfrog sympathetic neurons, primarily using whole cell recording. On depolarization, currents activated with a sigmoid delay but did not show a Cole-Moore shift. The time course of activation differed systematically from an exponential to a power. At most voltages, a power of 2 gave the best overall fit but a power of 3 better described the initial delay. After the delay, the time course could be fitted by a single exponential. Time constants were 15–20 ms at 0 mV and decreased to a limiting τ = 7 ms at +50 to +100 mV. Tail currents were well fitted by single exponential functions and accelerated with hyperpolarization, from τ = 15–20 ms at 0 mV to τ = 2 ms at −110 mV (e-fold for 40 mV).

Eleven kinetic models were evaluated for their ability to describe the activation kinetics of the delayed rectifier. Hodgkin-Huxley-like models did not fit the data well. A linear model where voltage sensor movement is followed by a distinct channel opening step, allosteric models based on the Monod-Wyman–Changeux model, and an unconstrained C-C-C-O model could describe whole cell data from −100 to +40 mV. After including whole cell data at +60 and +80 mV, and a maximal Popen of 0.8 from noise analysis of cell-attached patches, an allosteric model fit the data best, as the other models had difficulty describing qualitative features of the data. However, some more complex schemes (with additional free parameters) cannot be excluded. We propose the allosteric model as an empirical description of macroscopic ion currents, and as a model worth considering in future studies on the molecular mechanism of potassium channel gating.

INTRODUCTION

Voltage-dependent potassium channels have four subunits, each containing a voltage sensor (MacKinnon 1991). That has focused attention on how the four separate voltage sensors are coupled to channel opening (reviewed by Bezania and Steffani 1994; Sigworth 1993). The original Hodgkin and Huxley (1952) model for the delayed rectifier potassium conductance of squid axon proposed that the voltage sensors are identical and act independently, and the channel is open if, and only if, all four voltage sensors are in the activated position. The Hodgkin-Huxley formalism remains widely used to describe the kinetics of ionic currents for models of neuronal electrical activity.

However, studies on cloned potassium channels expressed in heterologous systems have provided ample evidence for features of gating that cannot be described by Hodgkin-Huxley models (Koren et al. 1990; Sigworth 1993; Steffani et al. 1994; Zagotta and Aldrich 1990; Zagotta et al. 1994a). In particular, channel opening appears to involve more than voltage sensor movement. Most recent models require all four voltage sensors to activate before channel opening (Sigworth 1993; Steffani et al. 1994; Zagotta et al. 1994a).

An alternative mechanism, based on the classical Monod-Wyman–Changeux (MWC) model for allosteric proteins (Monod et al. 1965), is that channels can open with any number of voltage sensors activated, but activation of each voltage sensor favors channel opening by a fixed amount of energy (Marks and Jones 1992). A physical interpretation is that voltage sensor movement is a local conformational change within one subunit, and channel opening is a concerted transition involving the entire channel protein.

It is important to test these ideas about channel gating on native channels actually expressed in neurons. We report here a study of the activation kinetics of the delayed rectifier of frog sympathetic neurons. Potassium currents have been studied extensively in these cells, and procedures for isolation of the delayed rectifier are well established (Adams et al. 1982a; Goh et al. 1989, 1992; Lancaster and Penefather 1987). These round, isopotential cells allow high-quality whole cell voltage clamp. In particular, the kinetics of the delayed rectifier can be studied accurately over a wide voltage range, as the time constants are >1 ms from −100 to +100 mV. That is important, as the behavior of rate constants at extreme voltages can be crucial for distinguishing models (Chen and Hess 1990; Koren et al. 1990).

We compared four classes of kinetic models: MWC models, Hodgkin and Huxley (1952) models, models based on Zagotta and Aldrich (1990) and Koren et al. (1990), and a C-C-C-O model with arbitrary rate constants. Objective statistical criteria (Horn 1987), in combination with some qualitative considerations, favored a version of the MWC model where the only state that actually conducts ions is the “open” state with all four voltage sensors activated (McCormack et al. 1994). Data at strongly depolarized voltages, especially the observation of a limiting Popen = 0.8 from noise analysis in cell-attached patches, were critical in discriminating among models. Many of the parameters in the favored model (here called MWC-O4) were similar to those used previously to describe calcium channel kinetics (Marks and Jones 1992), but the immediate closed-open transition was much slower for the potassium channel.

METHODS

Cells and recording conditions

Neurons were isolated from caudal paravertebral sympathetic ganglia of decapitated and pithed adult bullfrogs (Rana catesbei-
Cell-attached patches were recorded from cells bathed in a solution designed to set the resting potential to 0 mV; the solution contained (in mM) 2.5 KCl, 115 NMG-Cl, 2.5 NMG-HEPES, and 5 K-ethylene glycol-bis(β-aminoethyl ether)-N,N′,N′,N′-tetraacetic acid (EGTA). Pipettes were filled with either 10 or 100 mM K+ solution (as noted), with Na+ replaced by N-methyl-d-glucamine (NMG). The 10 mM K+ solution included (in mM) 10 KCl, 107.5 NMG-Cl, 2.5 NMG-HEPES, 2 MnCl2, pH 7.2. The 100 mM K+ solution included (in mM) 100 KCl, 107.5 NMG-Cl, 2.5 NMG-HEPES, 2 MnCl2, pH 7.2.

To isolate potassium currents in whole cell recordings, Na+ was replaced by NMG and Ca2+ with Mn2+ (Block and Jones 1996). Because potassium currents can be very large in these cells (>100 nA), intracellular K+ was replaced partially by NMG to reduce the current and improve voltage control. Specifically, the electrodes were filled with (in mM) 25 KCl, 58.5 NMG-Cl, 2.5 NMG-HEPES, 10 NMG-EGTA, 5 tris(hydroxymethyl)aminomethane (Tris)-ATP, and 6 MgCl2, pH 7.2. The extracellular solution contained (in mM) 2.5 mM KCl, 115 NMG-Cl, 2.5 NMG-HEPES, and 2 MnCl2, pH 7.2. Stocks of NMG-Cl, NMG-HEPES, and NMG-EGTA were prephated to pH 7.2. NMG, Tris-ATP, EGTA, and HEPES were from Sigma (St. Louis, MO); KCl, MgCl2, and MnCl2 from Fisher (Fair Lawn, NJ); and tetraethylammonium (TEA) chloride from Eastman Kodak (Rochester, NY).

Electrodes were made from microhematocrit tubing (Fisher Scientific) or silicone elastomer (Sylgard)-coated Corning 7052 glass (Garner Glass, Indian Hills, CA). Series resistances were 2.6 ± 0.3 MΩ (n = 7, mean ± SD) before compensation (80%), with whole cell capacitances of 56 ± 14 pF, as estimated from the amplifier settings producing optimal cancellation of the capacity transients evoked by 20-mV hyperpolarizations. With 80% compensation, we estimate 0.52 ± 0.06 mV of steady-state clamp error per nA of current with τ = 30 ± 10 μs for establishment of voltage control. Given these values, the time course of the currents, including the initial delay, can be well resolved (e.g., Figs. 4 and 5, see further). For the five cells chosen for final analysis, the estimated maximal series resistance error was 6.3 ± 2.8 mV (at +80 mV). Space clamp error is minimal for isolated frog sympathetic neurons, which are essentially spherical, without dendrites. For a whole cell conductance of 140 nS (with fully activated gradients), the generalized DC space constant L is 2.4 cm (Eisenberg and Engel 1970), and the maximum voltage error due to space clamp is 0.1% (for a cell radius of 20 μm, estimated assuming a specific capacitance of 1 μF/cm2) (Jones 1987).

As the models can have different numbers of free parameters, the log error ratio LER ± AIC were resampled at 0.4 ms and restricted to 0–40 ms of activation and to 0.8–20 ms of deactivation. Therefore for each cell there were >900 data points representing activation and deactivation over a 140-mV range.

To obtain internally consistent data sets, potential artifacts including K+ accumulation and rundown were evaluated carefully (see RESULTS). To minimize the effect of K+ accumulation, we restricted the model fitting to cells with minimal observed changes in reversal potential, and only included the first 40 ms of activation and the first 20 ms of deactivation; simulations (not shown) suggest that K+ accumulation did not significantly distort activation kinetics on that time scale. A correction for accumulated inactivation was used for all tail currents and for the data from the three pulse protocol (Fig. 5A). This correction was not possible for data collected with the protocol of Fig. 4A, which was used for four of the six data sets analyzed. From the protocol in Fig. 5A, there was only ~1–5% reduction in current for these middle traces (~10 to +40 mV), so we expect little error in the uncorrected data.

As the models calculate the probability that the channel is open, the experimentally measured current traces were converted to conductances. A linear open channel I-V relation was assumed at negative voltages, and currents from +10 to +40 mV were corrected for the observed rectification using the instantaneous I-V relation for tail currents. That correction was at most a factor of 1.15 for +40 mV. The currents from steps to +60 and +80 mV were converted to conductances by first normalizing to the peak current of the trace, then multiplying by the popen value from the activation curve. For display (Figs. 12 and 14), both experimental and model conductance values were converted back to currents, assuming a linear open channel I-V relation.

Parameter estimation and evaluation of models

Models were simulated and parameters were estimated with Fortran programs on either a Sun workstation or 486/33 or higher PC. For a given parameter set, model currents were generated by numerical integration with the multistep ordinary differential equation solver LSODES (Hindmarsh 1983), which is applicable to “stiff” systems. Model parameters were estimated by minimizing the error function, defined as the sum of squared deviations between the data and model output, using an adaptive, nonlinear gradient method (NL2SOL) (Dennis et al. 1981).

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data set. The mean and standard error were then used to determine the significance from zero mean with the t distribution (Microsoft Excel).

RESULTS

Cell-attached recording

In the few cases where channel activity was low enough to observe individual channel gating, the transitions were not resolved sufficiently for kinetic analysis (not shown). Most patches contained many channels and exhibited macroscopic currents (Fig. 1). Currents were voltage dependent and activated with a clear delay, as expected from previous studies of whole cell delayed rectifier currents in frog sympathetic neurons (Adams et al. 1982a; Lancaster and Pennefather 1987). Half-maximal activation was at $-2.8 \pm 3.6$ mV ($n = 3$). Nonstationary noise analysis (Sigworth 1980) was used to estimate the single channel current and channel open probability (Fig. 2). Fits of the variance versus mean relationship (Fig. 2, C and D) yielded a slope conductance of 9.1 pS (Fig. 2E). The maximal $p_{\text{open}}$ was $0.75 \pm 0.09$ ($n = 4$) at $+40$ mV and $0.79 \pm 0.06$ at $+80$ mV ($n = 3$). A saturating $p_{\text{open}} < 1$ at strongly depolarized voltages also has been observed for the Shaker K+ channel, after removal of fast inactivation (maximum $p_{\text{open}} = 0.8$) (Hoshi et al. 1994), and for Kv2.1 (maximum $p_{\text{open}} = 0.9$) (Shieh et al. 1997).

Isolation and measurement of whole cell delayed rectifier current

Channel gating produced a substantial amount of noise in the cell-attached macropatch currents, which interfered with analysis of the time course of the macroscopic current. Thus whole cell recording was used to examine the activation kinetics of the delayed rectifier in more detail. Whole cell recording also allowed the use of pharmacological criteria for current isolation.

Our recording conditions should prevent current through all of the known voltage-dependent channels of these cells, except for the delayed rectifier and M-type potassium currents (Adams et al. 1982a,b; Block and Jones 1996). The M current, which is much smaller than the delayed rectifier above $-30$ mV, is relatively resistant to TEA (Adams et al. 1982b). Figure 3A illustrates outward currents, and their blockade by TEA. Ninety to 95% of the current was blocked by high mM extracellular TEA (Fig. 3, B and C), and the dose-response relation was well described by an IC50 of 1.8 mM comparable with previous estimates for the delayed rectifier of frog sympathetic neurons (Adams et al. 1982b; Block and Jones 1997; Pennefather et al. 1985). The IC50 for TEA did not depend on voltage (Fig. 3D) but the amount of TEA-resistant current was slightly larger at strongly depolarized voltages (see also Block and Jones 1997). TEA did not modify the time course of the current, as we found no difference in activation or deactivation time constants for the total current compared with difference currents $\pm 1$ mM or 10 mM TEA (see Fig. 9C). That suggests that extracellular TEA acts as a fast, low-affinity blocker of a single population of TEA-sensitive channels.

Although most of the total current recorded in our conditions is the TEA-sensitive delayed rectifier, contamination from the residual 5–10% of unidentified current could affect our kinetic analysis. Therefore we isolated the delayed rectifier more fully as the difference current $\pm 10$ mM TEA, in cells where the leakage current was stable ($12 \pm 130$ pA at $-60$ mV in the 7 cells analyzed in detail here). In addition to reducing contamination from M current or other TEA-resistant ionic currents (Block and Jones 1997; Zhu and Ikeda 1993), subtraction removed the rapid (<1 ms) TEA-resistant outward current (Fig. 3A), which we previously speculated might be a gating current (Jones 1987).

Two basic voltage protocols were used. First, activation kinetics were examined using depolarizing steps to different voltages, in 10-mV increments (Fig. 4A). Also, deactivation kinetics were examined from tail currents at various voltages, following voltage steps to $+40$ mV (Fig. 4B). "Steady-state" activation curves (Fig. 4C) were measured from the initial amplitudes of tail currents after partial repolarization to $-20$ mV with the protocol of Fig. 4A. From an empirical fit to a Boltzmann function, the delayed rectifier was half-activated at $+1.9 \pm 2.8$ mV ($n = 5$).

Further kinetic analysis of delayed rectifier currents required correction for two effects. First, although the currents appear to inactivate little during a 70-ms depolarization, the current decreased from pulse to pulse within a train (see prepulse of Fig. 4B), probably due to inactivation from "partially activated" closed states along the activation pathway (J. Xie, K. G. Klemic, and S. W. Jones, unpublished results; see Aldrich 1981; Aldrich et al. 1979; Marom and Levitan 1994).

Second, the currents at strongly depolarized voltages did not increase as expected from the increased driving force. Note that the records at $+60$ and $+80$ mV nearly superimpose in Fig. 4A. That was not due to inactivation, as it also was observed when voltage steps were given in reverse order (i.e., most positive voltages first), and it was not associated with a decrease in tail current amplitude (Fig. 4A). Instead, that effect was related to a nonlinear instantaneous I-V relation, with inward rectification at strongly depolarized voltages (Fig. 5B). The rectification results from block by intra-
cellular NMG, an effect shared by other impermeant and weakly permeant cations (Block and Jones 1997). The rectification was not apparent in the data from cell-attached patches (Fig. 1B). The low affinity of NMG block suggests a fast, operationally time-independent block, which would not affect our measurements of the time course of channel activation. More directly, tail currents were monoexponential, without the ‘hook’ expected for a voltage-dependent blocker if the kinetics of block were slow enough to interact with the time course of gating. Furthermore, the time course of activation was very similar between cell-attached patches (with no NMG) and whole cell recordings.

The protocol illustrated in Fig. 5A was used to correct for these effects. That protocol includes both depolarizing and hyperpolarizing voltage steps in each record, so it contains all of the information in the protocols of Fig. 4, A and B. To correct for accumulated inactivation, currents were normalized to the value at the end of a step to +40 mV, which was given in the middle of each record. However, the longer depolarization in the triple pulse protocol uncovered another complication, the accumulation of extracellular K⁺.

With the recording solutions used (see METHODS), the equilibrium potential for K⁺ should be −60 mV, but the

![FIG. 2. Nonstationary noise analysis of currents from a cell-attached patch. A and B: sample records of mean current (top) and variance (bottom), for 16 depolarizations to +40 mV (A) or +80 mV (B) from the holding potential of −60 mV. Mean currents are leak subtracted, but the variance was calculated from the unsubtracted records. C and D: plots of variance versus mean current, for the data of A and B. Smooth curves are fits to the parabolic relation Var = i²I/N, where i is the single channel current, I is the mean current, and N is the number of channels in the patch. Maximal current (if all channels were open) is I_max = iN, with i and N determined from the fit to the parabola. p_open was calculated by dividing the steady-state current (I_ss) by I_max. For this patch, values were i ≈ 0.51 pA, n ≈ 15 channels, I_ss ≈ 5.37 pA, p_open = 0.69, at +40 mV; and i ≈ 0.79 pA, n ≈ 21.3 channels, I_ss = 13.3 pA, p_open = 0.79, at +80 mV. E: single channel current-voltage relations from 4 patches, all with 100 mM K⁺ in the recording pipette.](http://jn.physiology.org/)

![FIG. 3. Blockade of whole cell delayed rectifier currents by tetraethylammonium (TEA). A: leak-subtracted currents, with digital Gaussian filtering at 1 kHz, recorded before, during, and after recovery from bath application of 10 mM TEA. B: dose-response relations for TEA from 4 cells. Values were calculated from the initial amplitudes of tail currents at −20 mV after steps to +30 and +40 mV, with the average of values before and after TEA application as the control value. Data were fitted to the law of mass action with IC50 = 2.2 mM (• − •) or IC50 = 1.8 mM with 5% of the total current resistant to TEA (□ − □). C: block by TEA at different voltages, for the indicated TEA concentrations (1−100 mM). Peak currents were measured from the protocols of Figs. 4 and 5. Straight lines are linear regression fits to the mean values at each concentration. D: dose-response relations for TEA, measured from peak currents at +20 mV (•) and +100 mV (□). ——, averaged fits to the law of mass action, with a fraction of the current resistant to TEA (f_res). At +20 mV, IC50 = 1.73 mM and f_res = 0.04; at +100 mV, IC50 = 1.66 mM and f_res = 0.11. Values in C and D are means ± SE, n = 4.](http://jn.physiology.org/)
observed reversal potential was \(-47.8 \pm 6.3\) mV (\(n = 7\), Fig. 4B protocol). To test for K\(^+\) accumulation, we measured the instantaneous tail currents after depolarizations of different amplitude (0 to +80 mV) and duration (10–200 ms). There was a current-dependent shift in reversal potential of 5–10 mV, with \(\tau \approx 90\) ms at +40 mV (Fig. 6B). It is worth noting that this occurred despite the use of spherical, isolated cells in a continuously superfused recording chamber.

**Time course of delayed rectifier activation**

The delay in activation of voltage-dependent channels is explained generally by a series of voltage-dependent transitions among closed states before channel opening. Probably the simplest such model includes \(\sim n\) identical and independent voltage sensors, each of which follows first-order kinetics, as in the Hodgkin and Huxley (1952) model for the delayed rectifier of the squid giant axon. In that case, the analytic solution for the time course of the change in conductance (\(g_K\)) during a voltage step is Eq. 11 of Hodgkin and Huxley (1952)

\[
g_K = g_{K_0}(1 - e^{-\tau/\tau})^n
\]

where \(g_{K_0}\) is the steady-state \(g_K\), \(g_{K_0}\) is \(g_K\) at the previous voltage, \(n\) is the number of voltage sensors, and \(\tau\) is the time constant for movement of a voltage sensor. If \(g_{K_0} = 0\), this reduces to a more familiar form

Can we assume that \(g_{K_0} = 0\), for our holding potential of \(-60\) mV? If some voltage sensors are already open at \(-60\) mV, then brief hyperpolarization should close them, increasing the delay (Cole and Moore 1960). We did not observe that effect (Fig. 7). Therefore if channel opening depends solely on the movement of \(n\) identical and independent subunits, the time course of current activation should be described by Eq. 2.

Currents on depolarization to voltages between –10 and +40 mV are shown in Fig. 8 together with superimposed fits assuming different values of \(n\). At first glance, the currents appear well fit with \(n = 2\), but on closer examination, it is clear that the initial delay was not well described (Fig. 8B). At most voltages, the delay could be described with \(n = 3\), but currents at later times were not fitted well, especially at more positive voltages. Fits were uniformly poor with \(n = 1\) (no delay at all) or \(n = 4\) (which gave an exaggerated delay, compared with the time course of the remainder of the current).

As an empirical description of the time course of activation at different voltages, currents were fitted to a single exponential function, ignoring the initial delay, without constraining the current at time 0 to be \(0\) (Fig. 9). That gave a good description of activation kinetics at late times (Fig. 9B) (see Zagotta et al. 1994b). Time constants measured...
in this way peaked at 15–20 ms, near the half-maximal activation voltage (see Fig. 4C), and reached a limiting \( \tau = 7 \) ms above \(+40\) mV. The time course of deactivation, measured from the protocol of Fig. 4B or Fig. 5A, was fitted well by a single exponential at all voltages. Deactivation did not reach a limiting time constant at negative voltages, at least above \(-120\) mV (Fig. 9A). Channels could close three- to fourfold more rapidly than the limiting rate for channel opening. The time constant for deactivation changed \( e \)-fold for \( 40.1 \pm 5.4 \) mV \((n = 7)\). These features do not depend on the use of TEA subtraction (Fig. 9C). In cell-attached patches with \( 100 \) mM \( K_0^+ \), activation rates were similar, and deactivation was slower but with a similar voltage dependence (Fig. 9D). High \( K_0^+ \) is known to slow channel closing introduced by Hodgkin and Huxley (1952) in these (Block and Jones 1997) and many other potassium channels (e.g., Swenson and Armstrong 1981).

**Kinetic models for the delayed rectifier**

A kinetic model for the delayed rectifier should reproduce several crucial qualitative features of the data: a sigmoidal activation curve with maximal \( p_{\text{open}} \approx 0.8 \), activation kinetics imperfectly fit by a power law but with \( n = 2 \) or 3 better than \( n = 1 \) or 4, monoeXponential tail currents, and an asymmetrical dependence of \( \tau \) on voltage. Based on these considerations, we considered four classes of model. Some of the models seemed likely to have sufficient complexity to describe the data, while others did not. The number of free parameters was kept to a minimum. We attempted to use objective statistical criteria to discriminate models (Horn 1987).

**CLASS N MODELS.** We began by considering models based on Hodgkin and Huxley (1952), where the channel is open if, and only if, a fixed number of identical and independent voltage sensors are activated. As such models produce currents with a time course described by a power law, we knew that such models could not fit our data perfectly, but models with \( n = 2 \) and \( n = 3 \) might come close. In the simplest version of such models, the rate constants for voltage sensor activation and deactivation would depend exponentially on voltage (Sigworth 1993; Stevens 1978; Tsien and Noble 1969). But the observation of a limiting time constant for activation at positive voltages suggested that nonexponential dependence of rate constants on voltage would be required. We thus considered all three forms of voltage dependence introduced by Hodgkin and Huxley (1952)

\[
E: \quad k = k_0 e^{V/V_a}
\]

\[
S: \quad k = k_0 (e^{-(V-V_a)/a} + 1)
\]

\[
L: \quad k = k_0 (V-V_a)/(e^{-(V-V_a)/a} - 1)
\]

We refer to these three forms as “E” (for exponential), “S” (for sigmoid), and “L” (for a voltage dependence approaching linear at extreme voltages). For a fixed \( n \) there are four free parameters for a class N \(_E\) model \((k_0, a, \text{ and } V_a)\), for activation and deactivation rates; \( a = z F / RT \), where \( z \) is the apparent amount of charge moved between a state and a transition state), and six free parameters for N \(_S\) and N \(_L\) models \((k_0, a, \text{ and } V_a; \text{ each for activation and deactivation})\). Six versions of class N models were considered, three with \( n = 2 \) (N\(_2E\), N\(_2S\), and N\(_2L\)), and three with \( n = 3\).
CLASS C MODELS. These were based on Zagotta and Aldrich (1990) and Koren et al. (1990), and are illustrated in Fig. 10A. Based on the molecular biology, it was assumed that there are four identical voltage sensors. The subscripts on the states indicate the number of voltage sensors in the position favoring channel opening. The rate constants for movement of each voltage sensor (here, $k_\text{V}$ or $k_{-\text{V}}$) are identical except for statistical factors, and a separate channel opening process ($k_\text{o}$ and $k_{-\text{o}}$) follows voltage sensor movement.

If the channel opening step is independent of voltage (Koren et al. 1990; Zagotta and Aldrich 1990), this model predicts a limiting time constant for channel opening at large positive voltages, $\tau = 1/(k_\text{o} + k_{-\text{o}})$. But there also would be an even slower limiting time constant for channel closing at negative voltages ($\tau = 1/k_{-\text{o}}$), which is not consistent with our data. We thus considered both the original version of this model, with voltage-independent $k_\text{o}$ and $k_{-\text{o}}$ (C5O model), and a version with a voltage-dependent $k_{-\text{o}}$ (C5Ov). For both, it was assumed that any voltage-dependent rate constants depend exponentially on voltage. The C5O model has six free parameters, four rate constants ($k_\text{V}, k_{-\text{V}}, k_\text{o},$ and $k_{-\text{o}}$) and the amount of charge associated with

FIG. 8. Time course of activation, fitted to exponentials raised to a power. A: currents from 1 cell (thicker lines) during the first 40 ms of depolarization to voltages from −10 to +40 mV, in 10-mV increments, were fitted to Eq. 2 with different values of $n$ (thinner lines). B: expanded view of data from A to examine the initial delay for $n = 2$ and $n = 3$.
sensor is likely to deactivate before the global conformational change, and therefore deactivation can be voltage dependent and rapid. Figure 11B illustrates the movement of the channel through the various states, in response to a depolarizing step. The MWC model behaves similarly, except that the other open states (O0-O3) also are considered to be conducting channels.

In principle, one testable prediction of the MWC model is that the limiting $p_{\text{open}}$ at negative voltages should reach a constant value, defined by the $C_0$-$O_0$ equilibrium, rather than continuing to decrease logarithmically (as for the other models tested here, including MWC-O4). The MWC model is an interesting exception to the rule that the limiting slope of $\ln(p_{\text{open}})$ versus voltage can be used to estimate the amount of charge moved during channel activation (Sigg and Bezanilla 1997). However, for the best-fit parameters here, the limiting $p_{\text{open}}$ is $10^{-8}$ for the MWC model, well below the detectable range.

**FIG. 10.** Models for delayed rectifier activation kinetics. A: model of Zagotta and Aldrich (1990) and Koren et al. (1990). This is the basis of the class C models in the text. B: model of Marks and Jones (1992), based on Monod et al. (1965). Two interpretations of this model are considered, where all of the “O” states are open and have equal conductance (MWC), and where only the $O_0$ state can conduct current (MWC-O4).

**CLASS M MODELS.** A natural way to produce asymmetrical voltage dependence is to allow channels to open and close via different pathways. One possibility is a model (Fig. 10B) proposed previously for activation of calcium channels (Marks and Jones 1992). It is formally identical to the MWC model (Monod et al. 1965) for allosteric proteins, with voltage sensor movement corresponding to ligand binding, and channel opening corresponding to the transition to the active (high-affinity) state. In principle, the channel can open with any number of voltage sensors moved, but with the allosteric factor $f < 1$, activation of each voltage sensor stabilizes the open state of the channel by a fixed amount. Although it appears complex, because of microscopic reversibility, the MWC model has the same number of free parameters as the C5Ov model.

We also considered a variant of the MWC model where only the last open state ($O_4$) actually can conduct ions (McCormack et al. 1994). Physically, this would mean that the channel cannot pass current until all the voltage sensors have moved and the global conformational change has occurred. This model, which we will refer to as MWC-O4, has the same free parameters as does the MWC model.

To illustrate how the MWC-O4 model works, Fig. 11A shows rate constants for two different voltages (from the parameters of Table 1). On depolarization to positive voltages, activation of the voltage sensors is rapid, contributing a delay but affecting the subsequent time course little, as the microscopic channel opening step is rate limiting. Generally, all four voltage sensors activate before the global conformational change. On repolarization, at least one voltage sensor is likely to deactivate before the global conformational change, and therefore deactivation can be voltage dependent and rapid. Figure 11B illustrates the movement of the channel through the various states, in response to a depolarizing step. The MWC model behaves similarly, except that the other open states (O0-O3) also are considered to be conducting channels.

In principle, one testable prediction of the MWC model is that the limiting $p_{\text{open}}$ at negative voltages should reach a constant value, defined by the $C_0$-$O_0$ equilibrium, rather than continuing to decrease logarithmically (as for the other models tested here, including MWC-O4). The MWC model is an interesting exception to the rule that the limiting slope of $\ln(p_{\text{open}})$ versus voltage can be used to estimate the amount of charge moved during channel activation (Sigg and Bezanilla 1997). However, for the best-fit parameters here, the limiting $p_{\text{open}}$ is $10^{-8}$ for the MWC model, well below the detectable range.

**FIG. 11.** Behavior of the MWC-O4 model. A and B: rate constants ($s^{-1}$) for the MWC-O4 model, calculated from the average best-fit parameters (Table 1), to illustrate activation (A) and deactivation (B). Primary pathways for channel opening and closing are indicated with bold type and thicker arrows. C: simulated movement of the channel among the various states for steps between the voltages of A and B. “Open” states are shown with thicker lines. Open states with <3 voltage sensors activated were not significantly populated.
All of the class N models and the C5O model were significantly worse than MWC-O4 (Fig. 13A). This analysis could not discriminate between the MWC and MWC-O4 models, but the C5Ov and UC3O model fits were statistically better than the MWC-O4 model.

Effect of data above +40 mV

At this stage, we examined how well the models described the qualitative features of the data. One problem became apparent with the MWC model. As the primary pathway for deactivation is via O-O transitions followed by channel closing (see Fig. 11B), the MWC model predicts a hook in tail currents that is not observed in the data (Fig. 12B). This did not produce a statistically poor fit, as the initial part of the tail currents was not heavily weighted. Because of the failure of the MWC model to produce monoexponential tail currents,

### TABLE 1. Model parameters

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWC-O4</td>
<td>( k_{o} )</td>
<td>242 ± 70</td>
<td>( z_{V} )</td>
<td>0.82 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>( k_{o} )</td>
<td>141 ± 42</td>
<td>( z_{-V} )</td>
<td>−0.58 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>( k_{o} )</td>
<td>133 ± 37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( k_{o} )</td>
<td>35 ± 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 1/f )</td>
<td>12 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5Ov</td>
<td>( k_{o} )</td>
<td>160 ± 40</td>
<td>( z_{V} )</td>
<td>1.34 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>( k_{o} )</td>
<td>40 ± 13</td>
<td>( z_{-V} )</td>
<td>−0.42 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>( k_{o} )</td>
<td>95 ± 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( k_{o} )</td>
<td>59 ± 9</td>
<td></td>
<td>−0.48 ± 0.06</td>
</tr>
<tr>
<td>UC3O</td>
<td>( k_{12} )</td>
<td>280 ± 140</td>
<td>( z_{12} )</td>
<td>1.07 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>( k_{21} )</td>
<td>2800 ± 2000</td>
<td>( z_{21} )</td>
<td>−0.54 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>( k_{21} )</td>
<td>1300 ± 2300</td>
<td>( z_{23} )</td>
<td>0.99 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>( k_{50} )</td>
<td>38 ± 34</td>
<td>( z_{52} )</td>
<td>−1.21 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>( k_{00} )</td>
<td>112 ± 23</td>
<td>( z_{50} )</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>( k_{00} )</td>
<td>76 ± 10</td>
<td>( z_{03} )</td>
<td>−0.39 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD for fits to 5 data sets, including data from −100 to +80 mV, with maximal \( p_{o} \) = 0.8. Rate constants (at 0 mV) have units \( s^{-1} \); values are in electronic charge units, and the allosteric factor \( f \) is unitless.

Rate constants for the MWC-O4 and C5Ov models are defined as in Fig. 10. States in the UC3O model are identified as C1-C2-C3-O; e.g., \( k_{00} \) is the rate constant from O to C1 at 0 mV, and \( z_{03} \) is the associated charge.

**CLASS U MODEL.** For comparison, we wished to test a more empirical model, with fewer built-in assumptions about how ion channels work. For a current that shows a delay on activation, with monoexponential deactivation, a linear scheme with multiple closed states and a single open state seems plausible. With a delay approximated by \( n = 2 \) or higher, a minimal model would be C-C-C-O. [When rate constants are not constrained, the sigmoidicity generally decreases compared with a strict power law model (Zagotta et al. 1994a.)] That model has six rate constants. We assumed that each could depend exponentially on voltage, giving 12 free parameters. Note that this model appears simpler than the class C and M models, based on the number of states, but it actually has considerably more free parameters. This model will be referred to as UC3O (‘‘U’’ for unconstrained).

**Initial evaluation of kinetic models**

We estimated parameters for the 11 models introduced above, by fitting the time course of activation and deactivation recorded from −100 to +40 mV in each of six cells. The data sets were chosen to minimize the effects of K+ accumulation and other potential artifacts (see METHODS). The fits are illustrated for one data set in Fig. 12. The residual error in fitting the data to each model, relative to the MWC-O4 model, is shown in Fig. 13A. For this analysis, we did not constrain the maximal \( p_{o} \). Instead, an arbitrary scaling factor was included to convert \( p_{o} \) to conductance, effectively introducing an additional free parameter into each model.

As expected, the class N models gave significantly poorer fits, especially for \( n = 3 \) (not shown in Fig. 12) or with simple exponential voltage dependence (N2E). For the N2S and N2L models, deviations from the data were less dramatic, but the initial delay was described poorly (as expected from the power law fits, Fig. 8). The C5O model described the initial delay poorly near 0 mV, and it described the later time course poorly at more positive voltages (Fig. 12A).

**FIG. 12.** Experimental data (−110 to +40 mV) fitted by different models. A: 8 models were fitted (thinner lines) to the first 40 ms of activation. Currents (thicker lines) are from Fig. 8, for voltage steps in 10-mV increments between −10 and +40 mV. B: fits of the M-class models to tail currents, in 20-mV increments from +30 to −110 mV, after repolarization from +40 mV, for the same cell.
Calculating the LER-AIC values, the UC3O model was no longer statistically different from the MWC-O₄, and the fit to the C5Ov model was just barely statistically better ($P = 0.049$). The voltage dependence of the time constants now was predicted correctly by each model. Also, each model accurately predicted the activation curve. However, at positive voltages, the predicted limiting $p_{\text{open}}$ was 0.78 for the MWC-O₄ model, versus 0.98–0.99 for the other models, compared with the value of 0.8 obtained from noise analysis. So as a more stringent test, we examined the ability of the MWC-O₄, UC3O, and C5Ov models to fit the data (including records at +60 and +80 mV), with the steady-state activation curve normalized to give a saturating $p_{\text{open}} = 0.8$, rather than allowing an arbitrary scaling factor between $p_{\text{open}}$ and conductance. We also included the C5O model in the analysis as it easily can give a limiting $p_{\text{open}} < 1$.

When this expanded range of data was included, the MWC-O₄ model was superior to the UC3O, C5Ov, and C5O models (Figs. 14 and 15). The quality of the fit decreased at all voltages for the class C models (compare Fig. 14 with 12). Visually, the currents were best fit by the MWC-O₄ model. By contrast, the C5O model predicted activation time constants that continued to decrease with depolarization, and the C5Ov and UC3O models predicted time constants that were slower than observed above +40 mV. The activation curve (Fig. 15B) was predicted accurately by the C5O model, but $p_{\text{open}}$ increased beyond 0.8 for the C5Ov and UC3O models. Several of the parameters for the UC3O model varied widely from cell to cell (Table 1), suggesting that they were not well defined by the data. Finally, the statistical analysis using the LER-AIC value (Fig. 13B) confirmed that the fit was significantly better for the MWC-O₄ model. Although this analysis required the assumption that the saturating $p_{\text{open}}$ value was the same for cell-attached macropatches and whole cell data, it supports the
conclusion that the MWC-O4 model describes the data better than other models of comparable complexity.

**Discussion**

**Activation kinetics of the delayed rectifier**

The kinetics of the delayed rectifier have been studied previously in these cells with two-microelectrode voltage clamp on neurons in the acutely excised ganglion (Adams et al. 1982a; Lancaster and Pennefather 1987). Those studies also reported that tail currents deactivate with a simple exponential time course, with time constants depending exponentially on voltage, varying e-fold for 30 mV (Adams et al. 1982a) or 24 mV (Lancaster and Pennefather 1987) compared with our value of 40.1 ± 5.4 mV (mean ± SD, n = 7).

Adams et al. (1982a) fitted the time course of activation to Eq. 2 with n = 2, although they noted that either n = 2 or n = 3 could fit the data approximately but not exactly (see their Fig. 14D). Time constants for activation, measured assuming n = 2, increased linearly with voltage between +50 and +90 mV in apparent contrast to our results using n = 1 (Fig. 8). But if we fit our data to n = 2–4, we also find that the measured time constant decreases slightly in that region. Our measure (a fit to n = 1, ignoring the delay) is particularly sensitive to steps late in the activation pathway, and thus should be a good method to detect a rate-limiting voltage-independent rate constant.

The molecular basis of the delayed rectifier of frog sympathetic neurons is not known, but it seems likely that it is a member of the superfamily of Kv-type K⁺ channels. This delayed rectifier is sensitive to millimolar concentrations of TEA (Adams et al. 1982b) and 3,4-diaminopyridine (Goh et al. 1989) and is resistant to charybotoxin (Goh et al. 1992). The quantitative analysis here used difference currents ± TEA to isolate the delayed rectifier, but the crucial features of the data were also visible in the total leak-subtracted currents: fast tail currents at negative voltages, a limiting time constant for activation above +40 mV and a sigmoid time course for activation not well described by an exponential raised to a power (Fig. 9C, and analysis not shown). Given the large number of potassium channels cloned to date, we cannot be certain that the macroscopic delayed rectifier current results from the activity of a single homogeneous population of channels. However, kinetically distinct currents of significant amplitude could produce multiexponential decay of tail currents, which was not observed.

**Models for the delayed rectifier**

When all of the available data were considered, we concluded that the MWC-O4 model was superior to the other models tested. That was based both on quantitative evaluation of the models (Fig. 13B), and on qualitative considerations. Specifically, only the MWC-O4 model reproduced all of the main qualitative features of the data, including a sigmoidal activation curve with a limiting p_{open} of 0.8, monoeponential voltage-dependent tail currents, and a limiting time constant for activation at positive voltages.

When only part of the data were considered (from −100 to +40 mV), the rank order of models was different (Fig. 13A). That is consistent with previous reports that data at extreme voltages are important for distinguishing models (Chen and Hess 1990; Koren et al. 1990), by revealing weakly voltage-dependent steps that can become rate limiting. This also demonstrates that quantitative tests of models can be misleading if the data chosen for fitting do not fully reflect the qualitative features of the experimental results.

It is noteworthy that considerable effort was necessary to produce internally consistent data, including correction for cumulative inactivation and for a nonlinear instantaneous I-V relation. Without correction, these seemingly minor problems produced systematic errors, which interfered with estimation of model parameters and with quantitative comparison of models.

The limiting p_{open} of 0.8 at strongly depolarized voltages was an important factor in distinguishing models. This assumes that the p_{open} value obtained from cell-attached macro-patches is applicable to whole cell data. That assumption appears reasonable, given the similar kinetics observed in the two recording conditions (Fig. 9D). If channel gating is fast, noise analysis can underestimate the single channel conductance and overestimate p_{open} (Silberber and Magleby 1993). If p_{open} is less than what we estimate, that would accentuate the difficulty that class C and U models had in producing a limiting p_{open} value.

Although the MWC-O4 model provided the best description of the data, some discrepancies remain. In particular, that model predicts that the delay in channel opening should become very brief at strongly depolarized voltages (Fig. 14A), as the rate constant for voltage sensor movement increases exponentially with voltage. The model could reproduce accurately the observed delay in activation at 0 mV (6 ms, measured as the time to reach 1% of the steady-state conductance), but at +80 mV, the model predicted a delay of 0.8 ms compared with the observed 1.7 ms.
The allosteric nature of the MWC-O₄ model contributes to its ability to fit the data, notably by allowing channels to open and close by different pathways, with different voltage dependence. However, that explanation is not unique. Addition of an additional state to the C₅O₄ model, either a blocked closed state after the open state (as in Zagotta et al. 1994a) or an additional closed state between C₄ and O₄ voltages. Finally, the delayed rectifier does not show a Cole-Moore shift at strongly hyperpolarized voltages (Fig. 7) unlike Shaker (Bezanilla et al. 1994; Stefani et al. 1994; Zagotta et al. 1994b). Explanations for a Cole-Moore shift, such as multiple voltage-dependent transitions per subunit (Zagotta et al. 1994a), add complexity to a model. We conclude that the differences between our findings and those for Shaker channels primarily reflect differences in the gating of the two channel types.

Can the Shaker models describe gating of the frog delayed rectifier? We were unable to obtain good fits with the Zagotta et al. (1994a) model, which does not include a voltage-independent step at positive voltages. In particular, the many voltage-dependent transitions in that model produced greater sigmoidicity than observed (analyzed as in Fig. 16), and the sigmoidicity increased with depolarization (as observed for Shaker, but not for the delayed rectifier). The Bezanilla et al. (1994) model could describe our data well, but the parameters were not well defined for some transitions. In particular, the equilibrium for the first C-C step in that model was far to the right, suggesting that transition was unnecessary for our data. Our evaluation of that model should be considered preliminary, as our fitting procedure was sensitive to the initial parameter estimates, implying that true global error minima were not reached. On the other hand, that difficulty reflects the ability of different parameter sets to fit the data, suggesting that our data do not fully determine the parameters.

One striking result, both in our study and for Shaker family channels, is that the data cannot be accurately described by Hodgkin-Huxley models (Bezanilla et al. 1994; Koren et al. 1990; Zagotta et al. 1994b). That is also true for voltage dependent Na⁺ channels (Armstrong and Bezanilla).
REFERENCES


