Gating Kinetics of Four Classes of Voltage-dependent K⁺ Channels in Pheochromocytoma Cells

TOSHINORI HOSHI and RICHARD W. ALDRICH

From the Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305

ABSTRACT Clonal pheochromocytoma (PC-12) cells have four different types of voltage-dependent K⁺ channels whose activation does not require high concentrations of Ca²⁺ on the cytoplasmic side of the membrane (Hoshi, T., and R. W. Aldrich, 1988, Journal of General Physiology, 91:73-106). The durations of open and closed events of these four different types of voltage-dependent K⁺ channels were measured using the excised configuration of the patch-clamp method. The open durations of a class of K⁺ channels termed the K₂ channel, which activates rapidly and inactivates slowly in response to depolarizing pulses, had two exponential components. The closed durations of the K₂ channel had at least four exponential components. The time constants of the fastest of the two exponential components in the closed durations were very similar to those of the two exponential components present in the first-latency distribution. The first latencies of the K₂ channel decreased steeply with depolarization, contributing to the increased probability of the channel being open with depolarization. The K₂ channel also had a very slow gating process that resulted in a clustering of blank sweeps. A gating scheme containing two open states and five closed states is consistent with the observations. The Kv channel had one exponential component in the open durations and three exponential components in the closed durations. The first latencies varied greatly depending on the prepulse voltage and duration. The results were consistent with a sequential model with a large number of closed states and one open state. The Kₓ channel, which required large hyperpolarizing prepulses to remove steady state inactivation and did not show inactivation with maintained depolarization, had two exponential components in the open durations and three exponential components in the closed durations. The burst behavior of the Kₓ channel involved many more than two states. The transient Kw channel had one exponential component in the open durations and the mean open time increased with depolarization. The first latencies of the Kw channel were steeply dependent on the voltage, decreasing with depolarization.

Address reprint requests to Dr. Richard W. Aldrich, Dept. of Neurobiology, Sherman Fairchild Science Bldg., Stanford University School of Medicine, Stanford, CA 94305.
INTRODUCTION

In the preceding article (Hoshi and Aldrich, 1988), we showed that pheochromocytoma (PC-12) cells had four types of voltage-dependent K⁺ channels that differed in their unitary current amplitudes, voltage dependence of activation, time course of activation and inactivation, and other properties. These four types of channels were named the Kz, Kv, Kx, and Kw channels. Their typical chord conductance values were 12, 7, 7, and 18 pS, respectively. The Kw channels, which had the largest unitary current amplitude, were most transient in the time course of activation and were half-inactivated at a holding voltage of −70 mV. The Kz channels were less transient in time course and less dependent on the holding voltage. The Kx channels were observed most frequently. Kv and Kx channels, which had small unitary current amplitudes, did not show an appreciable amount of inactivation with maintained depolarization. In this article, we analyze the open time, closed time, and latency to the first opening of the four types of the K⁺ channels in PC-12 cells. Gating schemes consistent with the data obtained are proposed.

MATERIALS AND METHODS

Cell Culture and Electrophysiology

The cell culture and electrophysiological methods were described in Hoshi and Aldrich (1988). Unless otherwise noted, the data presented here were obtained using the outside-out configuration of the patch-clamp method (Hamill et al., 1981).

Data Analysis

Idealized sweeps were constructed as described in the preceding article (Hoshi and Aldrich, 1988). Duration histograms were constructed from the idealized sweeps. Most of the patches contained more than one K⁺ channel. A single-channel patch was infrequently obtained, making the analyses of the closed and burst durations difficult. Open-duration histograms were constructed from the events obtained from the patches that contained one to four observable channels. When two channels overlapped, the durations were randomly assigned (Aldrich et al., 1983). When more than two channels overlapped, the events were discarded. The duration data presented were obtained primarily from single-channel patches. More than three channels simultaneously opened infrequently in the voltage ranges examined. Closed- and burst-duration histograms were constructed from patches that contained only one channel of a given type. Data from many other patches were in qualitative agreement with the results presented here. First-latency histograms were corrected for the number of active channels by taking the nth root of the tail distribution function (the probability that a given first latency is greater than the time since the onset of the pulse), where n is the number of active channels. The number of active channels in a patch was determined by observing the number of simultaneously open channels. This estimate could be affected by the following three variables. (a) A low probability of the channel being open would lead to an underestimate of the total number of active channels. (b) Clustering of blank sweeps (see below) suggests that some channels were in fact inactive (not available to open) from time to time on the order of seconds to minutes, thus leading to an overestimation of the number of active channels at a given time. (c) Some channels initially present in a patch early in an experiment may cease to function later, resulting in an overestimation of the number of active channels.
Parameter estimation. The durations of the closed and open events were fitted with sums of exponentially decaying probability density functions (pdf's). The method of maximum likelihood was used to estimate the parameters. The parameters were corrected for the finite durations of the depolarizing pulses using a method similar to that described by Lawless (1982). This method, which we term the variable-censor method, maximizes the following expression:

\[ L = \prod [f(t)]^{-g} \times (1 - F(t)^g], \]

where \( L \) is the likelihood value, \( f(t) \) is the pdf, \( 1 - F(t) \) represents the probability that the measured duration is >\( t \), and \( g \) is 1 when the duration is censored (i.e., the channel dwell time was interrupted by the pulse termination) and 0 when the duration was faithfully measured. One of the differences between the variable-censor method and previous methods of analyzing durations is that this method uses the durations that are terminated by the pulse ending. For the analysis of the durations obtained from nonstationary experiments involving voltage pulses of finite durations and for the channels that inactivate, this method can be more effective than that used by Jackson (1986) and Colquhoun and Sigworth (1983), which used a constant censor duration. The effectiveness of this model is illustrated using the durations generated by a simple three-state model:

\[
\begin{align*}
\text{Closed}_1 & \xrightarrow{k_1 = 500/s} \text{Open} \xrightarrow{k_4 = 1/s} \text{Closed}_2 \\
\text{Closed}_1 & \xrightarrow{k_2 = 400/s} \text{Closed}_1 \\
\end{align*}
\]

The inset of Fig. 1A shows some representative openings generated by computer stimulation of this model. Closed events in response to 100 test pulses of 150 ms in duration were measured. At the beginning of each depolarization, the channel was assumed to be in the Closed_1 state. The closed-duration histogram should have a sum of two exponential components with time constants of 2.0 and 1,000 ms. Their relative amplitudes, determined by \( k_2 \) and \( k_3 \), should be 0.952 and 0.048, respectively. Fig. 1A shows a duration histogram of closed events that were not terminated by the pulse ending. Conventional methods would fit the data points displayed in this histogram. Because long closed durations were often terminated by the pulse ending, no closed events longer than 100 ms were measured. Fig. 1B shows durations of the closed events that were terminated by the pulse ending. The variable-censor method would use the durations shown in both A and B. Table I shows the parameters of the exponential pdf's estimated using the following three methods: (a) maximum-likelihood fits of the events that were not censored by the pulse termination, with no censoring correction used; (b) maximum-likelihood fits of the events that were not censored by the pulse termination using the correction method shown in Jackson (1986) and Colquhoun and Sigworth (1983); the pulse duration (150 ms) was used for the right censoring limit; (c) the variable-censor method described above. The advantage of the variable-censor method is obvious. All three methods estimate the shortest component of the closed durations to be \( \sim 2.1 \) ms. However, the time constants of the long component of the closed durations estimated by methods a and b are at least 10-fold smaller than the theoretical value (1,000 ms). The variable-censor method estimates the time constant to be 1,300 ms. The fraction of the long component estimated by the variable-censor method was also closer to that generated by the model than those obtained by the two conventional methods. Even though the variable-censor method allows better estimates of long time constants than the constant-censor methods, the time constant has a relatively high variance because of the low probability of a transition before the end of the pulse. Other methods for correcting the closed durations have been used. Fukushima (1981) corrected the closed-duration pdf for the effects of elimination of the censored events but did not directly use their durations. His original formulation applied
FIGURE 1. Closed durations generated by the three-state model. (A) Durations of the closed events that were not terminated by the pulse ending. The numbers of events whose durations were equal to or greater than those indicated on the horizontal axis were plotted. The inset shows sample records produced by the model (see text). The upward deflections are the opening transitions. 100 sweeps of 150 ms in duration were generated. The channel was set to be in Closed each time the depolarizing pulse was applied. (B) Durations of the closed events that were terminated by the pulse ending. The numbers of events whose durations were equal to or greater than those indicated on the horizontal axis were plotted.

to a two-state model. The method could be used for more complex models; however, the corrections necessary depend on the underlying model. The method of Horn and Lange (1983) makes use of information from the terminated openings and closings but differs from our variable-censor method in that it determines the likelihood of an entire set of sampled points and does not treat the open and closed durations separately.

In this article, the durations of open events were fitted using a conventional method similar to that used by Jackson (1986) and by Colquhoun and Sigworth (1983), and the durations of closed events and bursts were fitted using the variable-censor method described above. The numbers of exponential components present in the durations of various events were determined by the likelihood ratio test (see Lawless, 1982; Kalbfleisch, 1985; Jackson, 1986). Only when the probability of the duration data requiring one additional exponential component was >0.95 was the additional component included.

| TABLE I | Parameters Estimated by Various Censoring Correction Methods |
|---------|-------------------------------------------------------------|
| Parameter estimation method | $\tau_1$ | $\tau_2$ |
| Model | ms | ms |
| Maximum-likelihood estimates without censor correction | 2.0 (0.959) | 1,000 (0.048) |
| Maximum-likelihood estimates with fixed-censor correction | 2.1 (0.995) | 18 (0.005) |
| Maximum-likelihood estimates with variable-censor correction | 2.1 (0.994) | 18 (0.006) |
| | 2.1 (0.96) | 1,300 (0.04) |
Solutions

The internal solution usually contained (in millimolar): 140 KCl, 2 MgCl₂, 1 CaCl₂, 11 EGTA (10 nM free Ca²⁺), 10 HEPES, adjusted to pH 7.2 with N-methylglucamine (NMG). The solution with 1 nM free Ca²⁺ was made with 0.11 mM CaCl₂ and 11 mM EGTA. The external solution contained (in millimolar): 145 NMG-Cl, 2.8 KCl, 2 MgCl₂, 10 HEPES, adjusted to pH 7.2 with HCl. In some experiments, 140 mM KCl was replaced with 140 mM K-aspartate and 145 mM NMG-Cl was replaced with 145 mM NMG-aspartate. The other solutions used are described in the figure legends. Experiments were performed at room temperature (22-24°C).

RESULTS

Gating Kinetics of the K⁺ Channels

The inset in Fig. 2A shows representative records obtained from a patch containing one K⁺ channel in response to 720-ms depolarizing pulses from -90 to +10 mV. Openings occurred in bursts and more than one burst was observed within one depolarizing epoch. As described in the preceding article, the probability of the channel being open increased rapidly upon depolarization and declined slowly.

Open durations. The durations of the open events of the K⁺ channel recorded at +10 mV are shown in Fig. 2A. The arithmetic mean open time was 12.7 ms. The open durations were fitted by a sum of two exponentials. The probability of the duration data being consistent with one exponential component was <0.001. The time constants of the two exponential components were 0.6 and 14 ms and the fractions of the openings that belonged to the short and long components were 0.26 and 0.74, respectively. In some patches, the shorter of the two components was sometimes smaller in amplitude than that shown in Fig. 2. That the open durations were fitted with a sum of two exponentials indicates that the channel had at least two kinetically distinct open states. However, the openings that belonged to the long component were responsible for the majority of the charge movement across the membrane because the time constant of the long component was an order of magnitude greater than that of the short component and because the relative amplitude of the short component was not dominant. In fact, the arithmetic mean open time of 12.7 ms was dominated by the time constant of the long component (14 ms).

The voltage dependence of the time constants of the two exponential components in the open duration data pooled from six different patches is shown in Fig. 2B. The time constant of the short component was only slightly dependent on the voltage, being ~1 ms. The time constant of the long component was voltage dependent, increasing with greater depolarization. The time constant increased e-fold every 50 mV. This is equivalent to 0.5 charges moving across the entire membrane field for the sum of the closing transition rates. The relative amplitudes of the two exponential components in the open durations were quite variable among the patches examined and did not appear markedly voltage dependent in the voltage range of -10 to +60 mV. The relative amplitude of the long component in the open durations was typically 0.7-0.9. At voltages less
than −30 mV, the relative amplitude of the short component was smaller than at more positive voltages. The durations of the open events were often adequately described by a single exponential at these low voltages. The decreased amplitude of the short component in the low voltage range was probably caused in part by the lower filtering frequencies used at these voltages (typically 0.4–0.6 kHz compared with the 1–2 kHz used to obtain the data at more positive voltages) because the unitary current amplitudes were smaller. Thus, one of the factors that contributed to the increased probability of the channel being open with depolarization was the increased mean open time caused by the increase in the time constant of the long component in the open durations.

First latencies. The first-latency distributions show the probabilities that the channel has opened by a given time since the onset of the depolarizing pulse. This provides information regarding the behavior of the channel in the closed states leading to the first open state. The first-latency histogram of the openings of the K$_2$ channel elicited in response to 720-ms pulses from −90 to +10 mV is shown in Fig. 3A. The channel opened after a delay (~1 ms) on depolarization. The first-latency distribution reached the asymptotic plateau level within 10 ms.
The delay indicates that the channel had to go through more than one closed state to reach the first open state. This first-latency distribution was fitted by a sum of two exponentials with a time constant of ~0.3 ms accounting for the delay (a negative amplitude) and of 1.8 ms for the major rising component (a positive amplitude) in the distribution.

The first latencies were voltage dependent. The delay in the first-latency distribution became progressively shorter with depolarization. The voltage dependence of the time constant of the longest of the two components in the first-latency distribution is shown in Fig. 3B. This component decreased sharply with greater depolarization. In the patch shown in Fig. 3B, the time constant of the longer component decreased e-fold/10–15 mV, which corresponds to an equivalent charge movement of 1.5–2. The steeply decreasing first latency with depolarization thus contributed to the increased probability of the channel being open with depolarization. Prepulses to the voltage range of −120 to −70 mV did not markedly affect the values of the time constants in the first-latency distributions, which suggests that the prepulse voltage did not affect the initial occupancy probabilities of the channel in the resting closed states.

Closed durations. The durations of the closed events recorded at +10 mV are shown in Fig. 4. Data are plotted on a double-logarithmic scale. First latencies were not included in the closed-duration analysis. At +10 mV, closed durations, including those terminated by the pulse ending, were fitted with a sum of four exponentials. The time constants of the four components as estimated by the
variable-censor method were 0.15, 1.4, 70, and 6,000 ms. Their respective relative amplitudes were 0.80, 0.17, 0.015, and 0.015. The second-longest time constant corresponded to the interburst durations (see Fig. 2; also see below). This observation suggests that once the channel opened during a depolarizing epoch, it could enter at least four distinct closed states, one of which is the absorbing long closed state responsible for the inactivation. The relative amplitude of the fastest component in the closed durations was probably underestimated because of the filter and sampling frequencies used. The two fastest time constants in the closed durations and those in the first-latency distributions were similar in value in the patches examined. This suggests that the same gating processes were responsible for the first latencies and the closed durations, and that once the channel opened, it could go all the way back to the resting closed state it occupied before the depolarizing pulse was applied.

![Figure 4](https://jgp.rupress.org/)  
**Figure 4.** Closed durations. (A) Durations of the closed events of the K$_2$ channel at +10 mV that were not terminated by the pulse ending plotted on a double-logarithmic scale. The numbers of the closed events whose durations were equal to or greater than the durations indicated on the horizontal axis are plotted. (B) Durations of the closed events of the K$_2$ channel at +10 mV that were terminated by the pulse ending. The numbers of closed events whose durations were equal to or greater than the durations indicated on the horizontal axis are plotted.

There were not enough data available on the voltage dependence of the closed durations because we could not obtain enough single-channel patches. However, inspection of the data obtained at low voltages (-40 to -10 mV) from the patches that contained two channels, which opened simultaneously only very rarely, indicated that the closed durations became increasingly shorter with depolarization. The time constants and relative amplitudes of the second- and third-longest components in the closed durations appeared to decrease most dramatically with depolarization.

In the preceding article (Hoshi and Aldrich, 1988), we suggested that inactivation of the channel with maintained depolarization may involve two long closed states: one closed state that the channel could leave to reopen, and another very long closed state that the channel could not leave until the patch was repolarized.
HOSHI AND ALDRICH  Gating Kinetics of K⁺ Channels in PC-12 Cells 115

to a hyperpolarized voltage. Since the channel did reopen several times before entering the absorbing closed state, the entry rate into the absorbing closed state must be relatively small. Although it is possible that the second-longest time constant in the closed durations was somewhat similar to the mean dwell time in the nonabsorbing closed state, we cannot directly assign the long time constant to the mean dwell time in the nonabsorbing closed state.

Burst behavior of the K⁺ channel. Openings of the K⁺ channels occurred in bursts separated by long closed events (see inset to Fig. 2). The second-longest exponential component in the closed durations (time constant, ~70 ms; see Fig. 4) represented these interburst intervals. From the fit of the closed durations observed at +10 mV (see above), three times the time constant of the second-shortest component of the closed durations was selected as the burst criterion. Thus, the openings separated by closed events <4.5 ms were considered to have occurred within a single burst, whereas the closed events with durations >4.5 ms were considered as interburst closed durations. This criterion excluded almost 100% of the shortest component and 95% of the second-shortest closed-duration component from being erroneously included as interburst intervals. Approximately 1% of the closed events that belonged to the longest component were erroneously identified as within-burst closed events. With this criterion, the burst durations were well approximated by a sum of two exponential components with time constants of 1 and 416 ms. Their relative amplitudes were 0.03 and 0.97, respectively (Fig. 5). This result suggests that the burst behavior involves at least two states, one open state and one closed state. In the experiment shown in Fig. 5, there were, on the average, 18 openings in a single burst and there were 1.5 bursts in one sweep, provided that the depolarization elicited at least one opening.

We do not have data available on the voltage dependence of the burst durations from single-channel patches. However, data obtained from the patches with two or three channels in the low voltage range (-40 to 0 mV), using a fixed duration as the burst criterion for all the voltages, suggest that the mean burst duration was very steeply voltage dependent, increasing with depolarization when a fixed burst criterion was used. In one experiment, the mean burst durations at -40, -20, and 0 mV were ~15, 45, and 95 ms, respectively.

Open durations immediately preceding and following the interburst durations. If the channel entered the closed states responsible for the interburst intervals and inactivation preferentially from one of the two open states, the duration histogram of the openings that were immediately followed by these long closed events should be different from the duration histogram of all the openings (see Fig. 2A). Fig. 6A shows the durations of the openings that were immediately followed by closed events whose durations were ≥4.5 ms, the burst criterion used earlier (see Fig. 5). The durations of the open events immediately before these long closed events were fitted by a sum of two exponentials with time constants of 0.6 and 14 ms. Their relative amplitudes were 0.18 and 0.82. The probability of the durations being consistent with a single-exponential distribution was <0.001. The values of these open-duration parameters were similar to those of the duration of all the open events. According to the Kolmogorov-Smirnov test, there was no statistically significant difference between the distributions of the durations of the open events that were followed by the closed durations ≥4.5 ms.
FIGURE 5. Burst durations of the $K_z$ channel. (A) Durations of the burst events of the $K_z$ channel at $+10$ mV that were not terminated by the pulse ending. The burst criterion was $4.5$ ms, approximately three times the time constant of the second-shortest component in the closed durations. The numbers of the burst events whose durations were equal to or greater than the durations indicated on the horizontal axis are plotted. (B) Durations of the burst events of the $K_z$ channel at $+10$ mV that were terminated by the pulse ending. The numbers of the burst events whose durations were equal to or greater than the durations indicated on the horizontal axis are plotted.

and of the events that were followed by the closed durations <4.5 ms. No statistically significant difference was found in these two types of open-time distributions when the criterion duration of the immediately following closed event was 2 or 8 ms.

FIGURE 6. Openings that were immediately followed by long closed events and openings that were preceded by long closed events. (A) Durations of the open events that were immediately followed by the closed events $\geq 4.5$ ms, approximately three times the second-shortest time constant in the closed durations. The data were fitted with a sum of two exponentials with time constants of 0.6 and 14 ms, and relative amplitudes of 0.18 and 0.82. $n = 23$. (B) Durations of the open events that immediately followed the closed events whose durations were $\geq 4.5$ ms. The data were fitted with a sum of two exponentials with time constants of 0.54 and 15 ms and relative amplitudes of 0.18 and 0.82, respectively. $n = 12$. 
Similarly, the durations of the open events that immediately followed long closed events could be examined to see if the channel preferentially returned to one of the two open states from the long closed states (Fig. 6B). These open durations were fitted with a sum of two exponentials with time constants of \(0.54\) and \(\sim 15\) ms. Their relative amplitudes were \(0.18\) and \(0.82\), respectively. The probability that the durations were consistent with one exponential component was 0.025. According to the Kolmogorov-Smirnov test, there was no statistical difference between the distributions of the openings that immediately preceded closed events \(>4.5\) ms and of those that immediately followed closed events \(>4.5\) ms. These results suggest that the channel can enter the closed states that contribute to the interburst closed events from either of the two open-state components and then go back to either of the two open states. Pailotta (1985) carried out similar analyses on the inactivation mechanism of the large \(Ca^{++}\)-dependent \(K^+\) channels in rat muscle cells, which had at least two open states. He found that these large \(Ca^{++}\)-dependent \(K^+\) channels entered the long closed states preferentially from the short-lived open state. We did not have enough events to determine the relative possibilities of the channel entering the long closed states from the two open states or those of the channel returning to the two open states from the long closed states.

**Clustering of blank sweeps.** One of the striking properties of the \(K_2\) channel observed in excised patches is that blank sweeps (sweeps without any opening) were observed clustered together in time from one depolarizing epoch to the next. Fig. 7A plots the probability of the channel being open during a pulse against the sweep number. A probability of zero represents a failure of the channel to open during the 720-ms depolarizing epoch. Blank sweeps were obviously clustered. Runs analysis was used to determine the probability that this clustering of the blank sweeps occurred purely by chance (Horn et al., 1984; Standen et al., 1985). This analysis of the data, including the leading and the last runs, gave a \(Z\) value of 6.8. This \(Z\) value indicates that there was virtually no possibility that this pattern occurred by chance. Although the blank sweeps were observed clustered in time, when the channel did open, the average open duration in one sweep did not vary greatly from one sweep to the next. In most of the patches examined, we did not find any clear and consistent relationship between the frequency of stimulation and the degree of clustering of blank sweeps when the pulse repetition interval was between 3 and 20 s. We did not test a pulse repetition interval \(>20\) s.

This observation suggests that there must be a very slow inactivation process that generates the clustering of blank sweeps. For the experiment shown in Fig. 7A, there were \(\sim 6.5\) sweeps in the closed runs and there were \(\sim 5.4\) sweeps in one active run. Assuming that the depolarizing pulses did not affect the gating processes that generated the clustering of blank sweeps markedly, the results suggest that the channel stayed in the long closed state for \(\sim 30-40\) s, and once the channel entered the active mode of gating, where the channel was available to open, it stayed in this gating mode for \(\sim 30\) s.

Fig. 7B is a contingency table, which shows the frequency of observing blank sweeps depending on whether the channel was open when the immediately preceding pulse ended, given that the channel opened at least once in the
FIGURE 7. Clustering of blank sweeps. (A) The probability of the channel being open during a 720-ms depolarizing epoch from -90 to +10 mV is plotted against the sweep number. The pulses were applied every 5 s. A probability of 1 means that the channel was open all the time and a probability of 0 (no bar) indicates that the channel failed to open during the pulse. The patch contained only one active channel. (B) Contingency table showing the correlation between observing an opening at the end of one depolarizing epoch and observing an opening during the next depolarizing epoch. Each sweep with at least one channel opening (non-blank sweep) was classified depending on (a) whether the channel was open at the end of the pulse and (b) whether the immediately following sweep had at least one opening. The same patch as in A. (C) Voltage dependence of the probability that the clustering of blank sweeps occurred purely by chance. Openings of two K\(_2\) channels were recorded at +30 mV after 500-ms prepulses to -50, -70, and -90 mV. The pulses were applied every 5 s. The data were then analyzed as shown in A and the Z values were calculated and then converted into their corresponding probabilities. The probability values were plotted against the prepulse voltage. Since this patch contained two active channels, these are underestimates of how non-random the clustering is.
preceding pulse. The null hypothesis, that there was not a negative correlation between the channel being open at the end of the pulse and the next sweep being a blank sweep, was tested using the phi coefficient method (Conover, 1980). The null hypothesis was rejected at the critical level of 0.0001 (one-way test). The results indicated that when the channel was open at the end of the pulse, the channel almost always opened during the next depolarizing pulse. This observation suggests that the channel entered the long closed state responsible for the clustering of blank sweeps not at the prepulse voltage but at the command voltage. One possibility is that the long closed state responsible for the inactivation of the channel with maintained depolarization is also responsible for the clustering of blank sweeps.

Clustering of the blank sweeps was also highly dependent on the prepulse voltage. Fig. 7C plots the probability that the clustering of the blank sweeps occurred by chance against the holding voltage. Since this particular patch contained two active channels, this is an underestimate of how non-random the clustering was. When the holding voltage was sufficiently negative, little clustering of blank sweeps was observed. Any clustering of blank sweeps observed with prepulses to very negative voltages occurred by chance. With more positive prepulses, more blank sweeps were observed and these blank sweeps were observed clustered together as indicated by larger Z values. In a single-channel patch such as that shown in Fig. 7A, prepulses to voltages more negative than -110 mV are required to prevent such clustering of blank sweeps. Thus, a very slow voltage-dependent gating process operating in the holding voltage range is at least partly responsible for clustering of blank sweeps. This result, along with the observation that the immediately following sweep was not likely to be a blank sweep when the channel was open at the end of the pulse, suggest that the channel enters a very long closed state at the command voltage and leaving that state is facilitated by very negative prepulses. This voltage dependence of the clustering of blank sweeps probably accounted in part for the dependence of the probability of the channel being open on the prepulse voltage (see Hoshi and Aldrich, 1988).

It appeared that the clustering of blank sweeps was more marked at more positive test voltages. In the low voltage range of -40 to -10 mV, where there were more apparent blank sweeps, these blank sweeps appeared randomly placed. At more positive voltages (>0 mV), there were often fewer blank sweeps, but the blank sweeps that did occur appeared clustered together. Clustering of blank sweeps appeared more prominent in excised patches than in cell-attached patches. We also observed a very small number of K_2 channels in excised patches that did not display clustering of blank sweeps at all.

The results just presented suggest that the K_2 channel has at least two open states and that the open state with the longest dwell time is more frequently visited. The channel appeared to have a number of closed states, and the results suggest at least four. On depolarization, the channel goes through a series of closed states to reach the long open state. The channel then exhibits burst behavior involving the two open states and two closed states. The burst could be terminated by the channel entering into a long closed state. Once in this long
closed state, the channel then can re-enter the burst mode or enter an extremely long closed state, which the channel rarely leaves until the transmembrane voltage is changed to a negative level. This absorbing state is probably responsible for the clustering of blank sweeps. The increased probability of the channel being open with greater depolarization is brought about by the shortened first latency, increased mean burst duration, increased mean open time, and the decrease in some components of the closed durations. A scheme consistent with the results is presented in the Discussion.

Kv Channels

The inset in Fig. 8A shows representative openings of a Kv channel recorded at +40 mV. As shown in the preceding article, the Kv channels do not show much inactivation with maintained depolarization. The peak probability of the channel being open is not markedly dependent on the holding voltage.

Open durations. The durations of the openings of a Kv channel recorded at +30 mV are plotted in Fig. 8A. The open durations were fitted by one exponential component with a time constant of 4.3 ms. The voltage dependence of the mean open time is shown in Fig. 8B. The mean open times were 2-4 ms, increasing only slightly with depolarization. This represents an e-fold increase in mean open time per 100 mV, corresponding to 0.25 charges moving across the entire membrane voltage field for the sum of the closing rates.

First latencies. The preceding article (Hoshi and Aldrich, 1988) showed that the time course of activation of the ensemble average of Kv channel currents was drastically slowed by more negative prepulses. This slowing of the activation by hyperpolarizing pulses is also illustrated in the first-latency histograms in Fig. 9A. First-latency distributions of the openings recorded at +40 mV after prepulses to -100 mV for 2 s and of those recorded at +40 mV without the prepulses are compared. The holding voltage was -50 mV. After the prepulses to -100 mV, the first latency was noticeably longer. The median of the first latencies without the prepulses was ~0.2-0.3 ms. With the prepulses, the median of the first latencies was ~44 ms, ~100-200 times greater than that without the prepulse. Prepulses to -100 mV also lengthened the initial delay in the channel opening.

For prepulses to the same voltage, longer prepulses resulted in longer first latencies. Fig. 9B compares the first latencies of the events recorded at +40 mV after prepulses to -100 mV for durations of 0.25, 1, and 2 s. The holding voltage was -50 mV. Clearly, the longer prepulses led to longer first latencies. The medians of the first latencies recorded after 0.25-, 1-, and 2-s prepulses to -100 mV were 6, 18, and 44 ms, respectively. That the median of the first latencies varied 100-fold depending on the prepulse parameters indicates that on depolarization the channel has a very large number of closed states and that the initial distribution among the closed states is voltage dependent. Similar observations have been made for the K+ channels in squid axon (Cole and Moore, 1960; White and Bezanilla, 1985).

The first latencies of the Kv channel in response to depolarizing pulses to different test voltages in the range of -10 to +50 mV were not noticeably dependent on the test voltage. The median of the first latency ranged from ~5
FIGURE 8. Open durations of the K_v channel. (A) Durations of the openings of the K_v channel recorded at +30 mV. The data were fitted with a single exponential with a time constant of 4.3 ms. The inset shows representative openings of the K_v channel recorded at +40 mV. Scale bars: 1.6 pA, 20 ms. n = 64. (B) Voltage dependence of the mean time of the K_v channel. Depolarizing pulses from a holding voltage of -50 mV to the voltages indicated were applied. The durations of the openings were fitted with a single exponential. Data are from the same patch as that shown in A and Figs. 9 and 10.

to 8 ms when the 250-ms prepulses to -100 mV were applied from a holding voltage of -50 mV. Without the prepulses, from a holding voltage of -50 mV, the median first latencies in the range of -10 to 50 mV were ~0.2-1 ms. Most of the voltage-dependent gating behavior of the channel that directly caused the changes in the probability of the channel being open took place at voltages more

FIGURE 9. First latencies of the K_v channel. (A) First-latency distributions of the openings of the K_v channel recorded at +40 mV after prepulses to -100 mV for 2 s (b) and without the prepulses (a). The holding voltage was -50 mV. This patch contained only one active K_v channel. (B) First-latency distributions of the openings of the K_v channel recorded at +40 mV after prepulses to -100 mV for 250 (a), 1,000 (b), and 2,000 (c) ms. The holding voltage was -50 mV.
negative than $-10$ mV (Hoshi and Aldrich, 1988). At these lower voltages, the unitary current amplitudes of these channels were too small to study.

**Closed durations.** The closed-time histograms of the events recorded at $+30$ mV are shown in Fig. 10. The closed durations were fitted with a sum of three

---

**FIGURE 10.** Closed durations of the Kv channel. (A) Durations of the closed events recorded at $+30$ mV that were not terminated by the pulse ending. The numbers of the closed events whose durations are equal to or greater than the durations indicated on the horizontal axis are plotted. The data are presented in the semilogarithmic scale. The pulse duration was 120 ms. (B) Durations of the closed events at $+30$ mV that were terminated by the pulse ending. The numbers of the closed events whose durations are equal to or greater than the durations indicated on the horizontal axis are plotted. The data shown in A and B were fitted with a sum of three exponentials with time constants of 0.28, 3.9, and 12 ms and relative amplitudes of 0.7, 0.19, and 0.11, respectively. (C) Voltage dependence of the time constants of the three exponential components in the closed durations. The patch was depolarized from $-50$ mV to the voltages indicated. (D) Voltage dependence of the relative amplitudes of the three exponential components in the closed durations. The patch was depolarized from $-50$ mV to the voltages indicated. The squares represent the shortest component, the diamonds represent the second-largest component, and the triangles represent the longest of the three exponential components.
exponentials with time constants of 0.28, 3.9, and 12 ms. Their relative amplitudes were 0.70, 0.19, and 0.11, respectively. The voltage dependence of the time constants of the three exponential components in the closed durations is shown in Fig. 10C and relative amplitudes of the three components are shown in Fig. 10D. The shortest two components were mostly independent of the voltage in the range of −10 to +60 mV. The time constant of the longest component had a slight bell-shaped dependence on the voltage. Its time constant was the smallest around +20 to +50 mV and was greater at more positive and negative voltages. None of the time constants of the closed durations were as long as those found in the first latencies recorded at the same voltage after large negative prepulses (see Fig. 9, A and B). Thus, once the Kv channel opens, it does not go back all the way to the closed states that are responsible for the first-latency behavior. The closed durations recorded at one test voltage were not noticeably affected by the prepulse voltage or prepulse duration. The Kv channels did not show a noticeable degree of clustering of blank sweeps.

**Kx Channels**

Kx channels do not inactivate appreciably during maintained depolarization and are extremely sensitive to the holding voltage. Prepulses to voltages more negative than −100 mV are required in order to remove inactivation. Representative records of the Kx channel obtained at +50 mV after 1-s prepulses to −130 mV are shown in the inset to Fig. 11.

**Open durations.** The durations of the openings of the Kx channels at +50 mV are shown in Fig. 11. The durations were fitted with a sum of two exponentials with time constants of 1.0 and 8.2 ms and relative amplitudes of 0.1 and 0.9. The inset shows representative openings of a Kx channel recorded at +50 mV after 1-s prepulses to −130 mV. n = 357. Scale bars: 1.6 pA, 120 ms.

**First latencies.** The first-latency distribution of the Kx channel obtained at +50 mV after 1-s prepulses to −130 mV are shown in Fig. 12. Even though the
FIGURE 12. First-latency distribution of the K₇ channel. The probability that the channel opened by the time indicated on the horizontal axis is plotted (circles). The patch, which contained only one active channel, was depolarized to +50 mV for 480 ms after 1-s prepulses to −130 mV. The patch was stimulated every 5 s. 243 depolarizing epochs were applied. The distribution was fitted with a sum of two exponential components with time constants of 1.2 ms, accounting for the initial delay, and 30 ms, accounting for the major rising phase (smooth line).

Data were taken at a moderately positive voltage (+50 mV), first latencies as long as 100 ms were observed. As with other channels discussed in this article, the greatest slope of the first-latency distribution function was not found at $t = 0$ ms, which suggests that the channel enters at least two closed states before the first open state. The final plateau level reached by the distribution was not unity, presumably because the channel was somewhat inactivated at a prepulse voltage of −130 mV. The first-latency distribution at +50 mV was fitted with a sum of two exponential functions. The fast component, with a time constant of 1.2 ms, accounted for the initial delay and the slower component, with a time constant...
of 30 ms, was responsible for the major rising phase in the first-latency distribution.

**Closed durations.** Closed-duration histograms of the Kx channel are shown in Fig. 13. The closed durations were fitted with a sum of three exponential functions with time constants of 0.5, 16, and 400 ms. The relative amplitudes of these three components were 0.80, 0.19, and 0.01, respectively. The results suggest that the channel can enter at least three distinct closed states. In the voltage range of +50 to +80 mV, the time constants of all three components in the closed durations decreased with depolarization. The shortest two time constants in the closed durations (0.5 and 16 ms) did not match those in the first-latency distributions (1.2 and 30 ms). The results thus suggest that once the channel opens, it does not go back to the closed states involved in the transitions from the resting closed state to the first open state.

**Burst behavior.** As shown in the inset to Fig. 11 (also see Fig. 13A in Hoshi and Aldrich, 1988), the openings of the Kx channel were observed in groups separated by short closures. The burst criterion was three times the time constant of the shortest of the three closed-time components. Closed durations \( \geq 1.8 \) ms represented the interburst intervals. The burst durations are shown in Fig. 14. Using the variable-censor method, the durations were fitted by a sum of two exponentials with time constants of 29 and 57 ms. Their relative amplitudes were 0.64 and 0.39, respectively. The arithmetic mean burst duration was 39 ms. Each burst was composed of 4.8 openings on the average. The frequency histogram of the number of openings in a given burst was described by a monotonically decreasing function. However, it did not resemble a simple geometric distribution, which suggests that the burst behavior involves a large number of states.

The Kx channel showed some clustering of blank sweeps, although not as
much as that displayed by the $K_z$ channel. When the channel did open, the arithmetic mean open time did not vary much from one sweep to the next. The results suggest that the $K_x$ channel has five closed states. Two of the five closed states are involved in the transitions leading to the first open state and the other three are involved in the transitions that the channel makes once it opens. We could not readily determine whether the two open states of the $K_x$ channel communicate directly with each other.

$K_w$ Channels

In response to depolarizing pulses, the $K_w$ channel activated quickly and inactivated with a time constant of $\sim 40-80$ ms. The inset to Fig. 15A shows representative openings of a $K_w$ channel recorded at $-10$ mV.

**Figure 15.** Open durations of the $K_w$ channel. (A) Open-time histogram of the $K_w$ channel recorded at $-10$ mV. The patch was depolarized to $-10$ mV following a 250-ms prepulse to $-100$ mV. The data were fitted with a single exponential with a time constant of 3.3 ms (smooth line). $n = 46$. The inset shows representative openings of the $K_w$ channel recorded at $-10$ mV. Scale bars: 1.6 pA, 40 ms. (B) Voltage dependence of the mean open time of the $K_w$ channel. The patch was depolarized to the voltages indicated on the horizontal axis. The smooth line is an exponential curve with an equivalent charge movement of $-0.6$ (fitted by eye).

*Open durations.* An open-time histogram of the $K_w$ channel recorded at $-10$ mV is shown in Fig. 15A. The open durations were fitted by one exponential with a time constant of 3.3 ms. Open durations were also adequately described by one exponential at other voltages. This suggests that the $K_w$ channel has only one open state under the experimental conditions. The voltage dependence of the time constant is shown in Fig. 15B. The mean open duration increased slightly with greater depolarization. The mean open time increased e-fold/40–50 mV.

*First latencies.* The first-latency distributions of the $K_w$ channel recorded at $-20$, $-10$, and 0 mV are shown in Fig. 15A. The channels opened faster and faster with greater depolarization. The voltage dependence of the first latencies
was measured using the time for the first-latency distribution to reach 50% of the plateau level (the median first latency). The patch was depolarized to the voltages indicated after 250-ms prepulses to -100 mV. The first latencies decreased steeply in the range of -20 to +10 mV. The time to reach 50% of the plateau level decreased by e-fold/10-15 mV. This was the voltage range where the overall probability of the channel being open increased most steeply (see Fig. 19B in Hoshi and Aldrich, 1988). The very steep voltage-dependent first latencies thus contributed to the increased probability of the channel being open with depolarization.

*Closed and burst durations.* We could not obtain enough data from single-channel patches to have detailed information on the closed-time properties of the K_w channels. However, we do know that the K_w channel openings occur in bursts with a mean duration of ~10 ms at -30 mV. More than one burst did occur within a single depolarizing epoch. The apparent mean burst duration increased with further depolarization.

**DISCUSSION**

*Characteristics of Four Types of Voltage-dependent K⁺ Channels*

Table II summarizes the main characteristics of the four types of voltage-gated K⁺ channels found in PC-12 cells described here and in the preceding article (Hoshi and Aldrich, 1988). The K_w channels have the most transient kinetics, followed by the K_z, and then by the K_v and K_x channels.
TABLE II
Summary of Properties of Four Types of Voltage-dependent K⁺ Channels in PC-12 Cells

|                      | Kₓ channel | Kᵧ channel | Kz channel | Kw channel |
|----------------------|------------|------------|------------|------------|
| Open-channel i(V), −50 to +100 mV range | Sublinear  | Sublinear  | Supralinear | Sublinear  |
| Chord conductance, 0 to +40 mV range | 14−18 pS   | 11−14 pS   | 5−9 pS     | 5−9 pS     |
| Inactivation time course | Fast (40−80 ms) | Moderate (100−500 ms) | Slow       | Slow       |
| Prepulse inactivation | Yes        | Yes        | No         | Yes        |
| Components in open durations | 1          | 2          | 1          | 2          |
| Components in closed durations | ?          | 4          | 3          | 3          |
| Clustering of blank sweeps | No         | Yes        | No         | Yes        |
| Blocked by | TEA, 4-AP | TEA, 4-AP (variable), quinidine | TEA, quinidine | TEA, quinidine |
| Not blocked by | CTX, apamine | CTX        | CTX        | CTX        |

Kz Channels

Model of gating for the Kz channel. We have the most information on the gating properties of the Kz channels. We propose the following scheme for the gating of the Kz channel:

\[
\begin{align*}
\text{Closed}_1 & \xrightarrow{k_1} \text{Closed}_2 \xrightarrow{k_5} \text{Open}_1 \xrightarrow{k_5} \text{Closed}_4 \xrightarrow{k_7} \text{Closed}_5 \\
\xrightarrow{k_6} & \text{Closed}_5 \xrightarrow{k_{15}} \text{Closed}_5 \xrightarrow{k_{14}} \text{Open}_2
\end{align*}
\]

Although there may be other models that could explain the data, this model concisely summarizes the results. The channel is assumed to be in the Closed₁ state when the depolarizing pulse is applied. The channel then enters the first open state. These transitions generate first latencies with a sum of two exponential components. Once the channel opens, the channel can go back all the way to the resting closed state (Closed₅). This property is responsible for the observation that the time constants in the first-latency distributions are very similar to the two fastest time constants in the closed durations. We propose that the channel first enters the open state with a long dwell time (Open₁) and that the two open states of the channel do not communicate with each other. Therefore, according to our model, the distribution of the first open durations should be described by one exponential, whereas the distribution of all open durations should be described by the sum of two exponentials. Our preliminary experiments suggest that this is indeed the case. According to the model, the closed-duration distri-
Hoshi and Aldrich  Gating Kinetics of K⁺ Channels in PC-12 Cells

Bution should have five exponential components, one more than found in the data shown in Fig. 4. We assume that the dwell time in Closed₅ is very similar to the shortest time constant in the closed durations and did not appear as a separate component. Two long closed states, Closed₄ and Closed₅, account for the inactivation of the channel. Closed₄ accounts for the interburst behavior of the channel. Closed₅ is probably an absorbing state at the voltages where channel activation takes place. Since the longest time constant in the first latencies was the most voltage-dependent parameter, we suggest that the voltage dependence of the K⁺ channel comes predominantly from the voltage dependence of rate constant $k₁$.

We suggest that the voltage dependence of the entry and exit rate constants of the Closed₅ state, $k₇$ and $k₈$, are responsible for the clustering of the blank sweeps. Clustering of blank sweeps could be explained as follows. At the test voltage, the channel opens and then eventually enters Closed₅. The entry rate constant for Closed₄, $k₇$, is voltage dependent, increasing with depolarization. Thus, the channel does not enter Closed₅ at resting voltages. The exit rate constant from Closed₅, $k₈$, is also voltage dependent. However, the rate constant decreases with depolarization. At the voltages where activation is significant, this exit rate constant is negligible, trapping the channel in this state. The exit rate constant, $k₈$, does not become noticeable until the patch is hyperpolarized to a very negative voltage. If the holding voltage is not negative enough, the channel stays trapped in Closed₅ and is unavailable to open when the next depolarizing pulse is applied (see Fig. 7 C). According to this scheme, clustering of blank sweeps is simply an inactivation process and should be eliminated by a very long pulse repetition interval. In our experiments, we tried repetition intervals up to 20 s and did not see any consistent results.

Physiologically, the short open state may not play an important role. Since the mean dwell time of the short open state and its frequency of occurrence are much smaller than those of the long open state, most of the charge across the membrane is carried through the long open state. If the short open state is disregarded, the proposed model then reduces to:

$$\text{Closed}_1 \xrightarrow{k_1} \text{Closed}_2 \xrightarrow{k_3} \text{Open} \xrightarrow{k_5} \text{Closed}_4 \xrightarrow{k_7} \text{Closed}_5$$

Standen et al. (1985) presented an analysis of single K⁺ channels presumed to underlie delayed rectifier K⁺ currents in frog skeletal muscle cells. These delayed rectifier channels, whose single-channel current amplitudes are ~15 pS, were also modeled by a scheme similar to that shown above.

**Kᵥ Channels**

The most interesting property of the Kᵥ channel is the dependence of the first latencies of the openings on the prepulse voltage and duration. This property is similar to the effect of holding voltage on macroscopic K⁺ currents first described by Cole and Moore (1960) and investigated by White and Bezanilla (1985) using the gating current method. The fact that the first latencies of the openings recorded at the same voltage could vary 100-fold depending on the prepulse
pulse (see Fig. 9) suggests that the channel has a very large number of closed states and that the prepulses affect the distributions of the channel in these closed states. Once the channel opens upon depolarization, it can enter at least three different closed states.

**$K_x$ Channels and $K_w$ Channels**

The time course of the probability of the $K_x$ channel being open resembles that predicted by a sequential three-state model with one open state (Closed $\rightarrow$ Closed $\rightarrow$ Open) in that it follows a sigmoidal activation time course and that the open probability does not decline with maintained depolarization. However, distributions of the open durations, closed durations, and first latencies of the $K_x$ channel have two, three, and two exponential components, respectively. The three-state model predicts one exponential component in the open-duration distribution and two exponential components in the closed-duration distribution. Since we do not have information on the closed durations of the $K_w$ channel, we cannot propose a gating scheme. However, we know that the first latencies that were very steeply voltage dependent contributed at least partly to the increased probability of the channel being open.

We thank Drs. D. Baylor and D. O'Dowd for comments on the manuscript.

This work was supported by the U.S. Public Health Service grant NS-23294 and by a Searle Scholars Program/Chicago Community Trust grant to R. W. Aldrich. T. Hoshi was supported by postdoctoral fellowships from the American Heart Association, California Chapter, and the National Institutes of Health.

*Original version received 30 July 1987 and accepted version received 7 October 1987.*

**REFERENCES**

Aldrich, R. W., D. P. Corey, and C. F. Stevens. 1983. A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature.* 306:436–441.

Cole, K. S., and W. J. Moore. 1960. Potassium ion current in the squid giant axon; dynamic characteristics. *Biophysical Journal.* 1:1–14.

Coquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single channel records. In *Single Channel Recording.* B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 191–263.

Conover, W. J. 1980. *Practical Nonparametric Statistics.* John Wiley & Sons, New York. 184–189.

Fukushima, Y. 1981. Single channel potassium currents of the anomalous rectifier. *Nature.* 294:368–371.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.

Horn, R., and K. Lange. 1983. Estimating kinetic constants from single channel data. *Biophysical Journal.* 43:207–223.

Horn, R., C. A. Vandenberg, and K. Lange. 1984. Statistical analysis of single sodium channels: effects of $N$-bromoacetamide. *Biophysical Journal.* 45:323–335.

Hoshi, T., and R. W. Aldrich. 1988. Voltage-dependent whole-cell $K^+$ currents and underlying single $K^+$ channels in pheochromocytoma cells. *Journal of General Physiology.* 91:73–106.
Jackson, M. B. 1986. Kinetics of unliganded acetylcholine receptor channel gating. *Biophysical Journal*. 49:663–672.

Kalbfleisch, J. G. 1985. Probability and Statistical Inference. Vol. II. Springer-Verlag, New York. 360 pp.

Lawless, J. F. 1982. Statistical Models and Methods for Lifetime Data. John Wiley & Sons, New York. 580 pp.

Pallotta, B. S. 1985. Calcium-activated potassium channels in rat muscle inactivate from a short-duration open state. *Journal of Physiology*. 363:501–516.

Standen, N. B., P. R. Stanfield, and T. A. Ward. 1985. Properties of single potassium channels in vesicles formed from the sarcolemma of frog skeletal muscle. *Journal of Physiology*. 364:339–358.

White, M. M., and F. Bezanilla. 1985. Activation of squid axon K⁺ channels: ionic and gating studies. *Journal of General Physiology*. 85:539–554.