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BURST KINETICS OF SINGLE CALCIUM-ACTIVATED POTASSIUM CHANNELS IN CULTURED RAT MUSCLE

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SUMMARY

1. Burst kinetics of single Ca-activated K channels in excised patches of surface membrane from cultured rat muscle were studied using the patch-clamp technique.

2. Channel activity was separated into bursts using a calculated gap derived from the distribution of shut intervals. Shut intervals greater than the calculated gap were taken as gaps between bursts.

3. The distribution of burst duration was described as the sum of two exponentials with mean durations of about 0·8 and 24 msec (1 μM-Ca_i, +20 mV), suggesting two classes of bursts (short and long).

4. The composition of short and long bursts was determined from comparisons of the distributions of open intervals, unit bursts (bursts of single openings), and openings/burst.

5. Short bursts consisted mainly of single openings to the open channel state of short mean lifetime. Long bursts consisted of one or more openings to the (compound) open-channel state of long mean lifetime, plus, in fewer than 70% of the long bursts, one or more openings to the short open-channel state.

6. The frequency of occurrence of bursts from each class first increased and then decreased with increasing $[Ca]_i$, with the number of long bursts increasing at a greater rate than the number of short bursts.

7. The number of openings/short burst was relatively independent of $[Ca]_i$, while the number of openings/long burst increased, often more than linearly, with increasing $[Ca]_i$. This increase arose almost entirely from an increase in openings to the long open state.

8. These results suggest that openings to the long open state typically require the binding of three or more Ca ions, and openings to the short open state typically require the binding of at least one Ca ion. This is the case whether the openings occur in isolation as bursts of single openings or in bursts composed of both types of openings. An obvious burst of channel activity would occur when the channel opens and closes several times without losing all its bound Ca.

9. The power relationship between $[Ca]_i$ and the percentage of time spent in the

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open state is accounted for in terms of the effects of $[Ca]_i$ upon mean channel open time, openings/burst, and burst rate.

10. A model is presented that describes quantitatively many features of the burst kinetics of the Ca-activated K channel for constant $[Ca]_i$.

INTRODUCTION

Single-channel recording techniques (Neher & Sakmann, 1976; Hamill, Marty, Neher, Sakmann & Sigworth, 1981) have revealed that many types of channels open and shut non-randomly under constant conditions; the openings appear grouped into apparent bursts that are separated from each other by longer periods of inactivity (Nelson & Sachs, 1979; Patlak, Gration & Usherwood, 1979; Sakmann, Patlak & Neher, 1980; Conti & Neher, 1980; Marty, 1981; Cull-Candy, Miledi & Parker, 1981; Colquhoun & Sakmann, 1981; Yellen, 1982). The observation of apparent bursts suggests that there are two or more classes of shut intervals in which at least one class has a mean shut interval considerably longer than the others. Openings separated by shut intervals of short duration would typically appear to be grouped, and this group (or burst) would typically appear to be terminated as soon as a shut interval of long duration occurred. Since the apparent bursting reflects the underlying molecular mechanism of the channel, study of bursting kinetics should provide additional information about mechanism. Bursting behaviour is predicted on the basis of models in which there is more than one closed state of the channel (Colquhoun & Hawkes, 1977, 1981; Conti & Neher, 1980; Dionne, 1981). Consistent with the apparent bursting behaviour of the Ca-activated K channel (Marty, 1981; Pallotta, Magleby & Barrett, 1981), we found in the preceding paper (Magleby & Pallotta, 1983) evidence for at least four closed-channel states.

The purpose of this paper is to examine the bursting behaviour of the Ca-activated K channel. Bursts are defined unambiguously in terms of a gap calculated from the distributions of shut intervals; all intervals greater than the calculated gap are taken as gaps between bursts. We find that the distribution of burst duration is described by two exponentials, suggesting two classes of bursts. The class of short mean burst duration is shown to consist mainly of single openings to the open-channel state of short mean open time. The class of long mean burst duration is shown to consist of one or more openings to the (compound) open-channel state of long mean open time, plus in many bursts one or more openings to the state of short mean open time. The number of openings/burst to the long open state increased with increasing $[Ca]_i$, often more than linearly, while the number of openings/burst to the short open state appeared relatively independent of $[Ca]_i$. The frequency of occurrence of both classes of bursts first increased and then decreased with increasing $[Ca]_i$, with the occurrence of long bursts increasing at a greater rate. These results are consistent with models in which openings to the long open state typically require the binding of three or more Ca ions, and openings to the short open state typically require the binding of at least one Ca ion.

METHODS

Defining bursts

A characteristic feature of the activity of many channels, including the Ca-activated K channel, is that openings seem to occur in bursts (see the Introduction). Unfortunately, it is not possible to determine except in terms of a specific model, and then only in terms of probabilities, whether a channel opening or apparent group of channel openings is a burst (Colquhoun & Hawkes, 1981). One method which is sufficient for very low levels of activity is to choose a gap to separate bursts that appears long enough to exclude most of the shut intervals that would be expected to be gaps

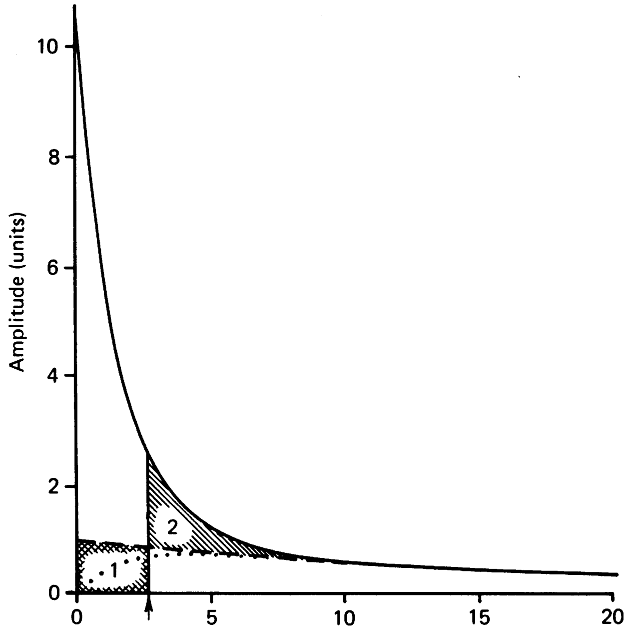

Fig. 1. Method of defining a gap to separate bursts. The continuous line plots the sum of distributions of short, intermediate and long shut intervals. The dashed line plots the distribution of long shut intervals. A gap to separate bursts is defined as the interval (indicated by arrow) where area 1 is equal to area 2. Shut intervals greater than the calculated gap are considered to be gaps between bursts; shut intervals less than or equal to the calculated gap are considered to be gaps within bursts. See text for further details.

within bursts (Colquhoun & Hawkes, 1981; Colquhoun & Sakmann, 1981). At all but the lowest levels of activity, however, the optimum gap to separate bursts might be expected to change with increasing channel activity, so that it would be convenient to have an empirical method of defining bursts. Such a method would allow a gap for separating bursts to be calculated in a manner consistent with the level of channel activity. In this section we describe a method of calculating such a gap from the distribution of shut intervals. All shut intervals greater than the calculated gap will be considered as gaps between bursts. By designating individual gaps in this manner, it is possible to partition the data into bursts to study burst kinetics over a range of channel activity.

Consider the schematic diagram of superimposed overlapping distributions of shut intervals shown in Fig. 1. Let the continuous line represent the sum of the distributions of short, intermediate and long shut intervals (see Magleby & Pallotta, 1983), and the dashed line represent only the distribution of long shut intervals. The calculated gap (arrow) will be defined as the gap where the number of intervals in the distribution of long shut intervals with durations less than the gap (area

1) is equal to the number of intervals in the distributions of intermediate and short shut intervals with durations greater than the gap (area 2). Thus, the calculated gap is that interval where the number of overlapping intervals between the long distribution and the combined short and intermediate distributions is equal and offsetting. If the distribution of long shut intervals is composed mainly of gaps between bursts (see restrictions below), then, using the calculated gap to separate bursts could give reasonable approximations of the correct number of bursts and number of openings/burst.

The calculated gap (T) was determined by solving the following equation for T with numerical methods:

$$\tau_S M_S(0)\, e^{-T/\tau_S} + \tau_I M_I(0)\, e^{-T/\tau_I} = \tau_L M_L(0)\,(1 - e^{-T/\tau_L}),$$

where $M_S(0)$, $M_I(0)$, and $M_L(0)$ are the initial magnitudes of the distributions of short and intermediate, and long shut intervals, and τ is the mean shut interval (time constant) of the indicated distributions. (The product of a magnitude and time constant gives the area of an exponential distribution.) In using this equation, the intercepts of the distributions were normalized for a bin size of 1 msec and τ was expressed in msec. Gaps calculated with this equation were typically about 3–4 times the mean duration of the distribution of intermediate shut intervals.

Errors introduced by defining a gap to separate bursts in this manner would depend on the underlying model and its rate constants. For a four-state model of the type considered by Colquhoun & Hawkes (1981) and for the models described by schemes (1) and (2) in the previous paper (Magleby & Pallotta, 1983), the distribution of gaps between bursts for the schematic diagram in Fig. 1 would be more closely approximated by the dotted line. It can be seen, then, that a gap calculated to set area 1 equal to area 2 would tend to be too brief for models of this type. A gap that was too brief would tend to separate groups of openings into two or more bursts when they were one burst. This would lead to an underestimation in the number of openings/burst. However, if the mean duration of the distribution of long shut intervals is long compared to the mean duration of the short and intermediate distributions of shut intervals, which would be the case for low to moderate levels of activity, then the underestimation using a gap calculated by setting area 1 equal to area 2 would be negligible. As the mean duration of the long shut intervals decreases, as occurs with increasing $[Ca]_i$ (Magleby & Pallotta, 1983), the underestimation could become significant, as is the case for Fig. 1. The magnitudes and effects of such errors on the interpretation of the data will be discussed later.

Preparation and experimental conditions

Single-channel currents from Ca-activated K channels were recorded with the patch-clamp technique (Neher & Sakmann, 1976; Hamill *et al.* 1981) from patches of surface membrane excised from cultured rat skeletal muscle. Experimental details are the same as in the preceding paper (Magleby & Pallotta, 1983), with the exception that most of the experiments were performed typically with the patch clamped to +20 mV instead of +30 mV (normal intracellular side positive). The more negative membrane potential decreased channel activity over that observed at +30 mV (Barrett, Magleby & Pallotta, 1982) making it easier to separate bursts.

Estimating openings/burst from a geometric distribution

Unlike the durations of open and shut intervals which are continuous random variables, the number of openings in a burst is a discrete random variable, which for certain models can be described by the sum of one or more geometric distributions (Colquhoun & Hawkes, 1981). A geometric distribution is a discrete equivalent of a continuous exponential distribution.

Histograms of distributions of the number of openings/burst are described in this paper by the sum of two geometric distributions, each with the form:

$$N(r) = N(1)\, b^{r-1},$$

where $N(r)$ is the number of bursts with r openings, $N(1)$ is the number of bursts with one opening, and b is a constant. A semilogarithmic plot of such a geometric distribution yields discrete values (plotted at $1, 2, 3, \ldots, n$ openings/burst) which define a straight line with a slope of $\log b$ and which intercepts the ordinate at $N(1)$; b is thus equal to 10^m, where m is the slope.

The mean number of openings/burst in each geometric distribution is given by the total number of openings in that distribution divided by the number of bursts in that distribution:

$$\text{mean openings/burst} = \frac{\sum_{r=1}^{\infty} rN(r)}{\sum_{r=1}^{\infty} N(r)}.$$

Substituting $N(1)\,b^{r-1}$ for $N(r)$ and simplifying gives

$$\text{mean openings/burst} = 1/(1-b).$$

The total number of bursts in each distribution simplifies to

$$\text{number of bursts} = N(1)\,[1/(1-b)],$$

which is the product of the intercept and the mean for the distribution. The total number of openings in each distribution is given by the product of the number of bursts and mean openings/burst.

Terminology

The distribution of all open intervals of the Ca-activated K channel is described by two exponential distributions with short and long mean open times (Magleby & Pallotta, 1983). The distribution of long mean open time is consistent with an underlying compound open-channel state whose lifetime increases with increasing $[Ca]_i$, and the distribution of short mean open time appears to be dominated by an open-channel state whose lifetime is brief and independent of $[Ca]_i$. In order to simplify the terminology in this paper, openings in the long open distribution will be referred to as openings to the long open state, and openings in the short open distribution will be referred to as openings to the short open state. It should be kept in mind, however, that the underlying composition of the distributions is not firmly established, and that 'state' may refer to several open states or a compound state.

RESULTS

In order to facilitate direct comparisons between Figures, all the data in Figs. 2–6 in this paper were obtained from a single excised membrane patch-clamped to +20 mV. Although the patch contained two Ca-activated K channels, the levels of activity were sufficiently low in all but the highest $[Ca]_i$ (1 μM) for typically only one channel to be open at one time. Consequently, estimates of activity during bursts would be little affected by the two-channel patch. Results similar to those presented in the Figures were found in two or more additional patches, including two containing only a single Ca-activated K channel.

Burst duration described by two exponentials

Fig. 2*A* presents single-channel currents recorded from the membrane patch with 1 μM-Ca_i. Analysis of the distribution of shut intervals from 195 sec of data (12,355 intervals) indicated that the calculated gap to separate bursts for these data was 6·46 msec (details in Methods). The data were then separated into bursts by classifying shut intervals greater than 6·46 msec as gaps between bursts, and shut intervals less than or equal to 6·46 msec as gaps within bursts.

The durations of all bursts defined in this manner are presented as a histogram in Fig. 2*B*. Burst duration was described by the sum of two exponentials as shown by the semilogarithmic plot of the data in Fig. 2*C* and *D*. The longer distribution of burst duration had a mean of 24 msec and the shorter a mean of 0·83 msec.

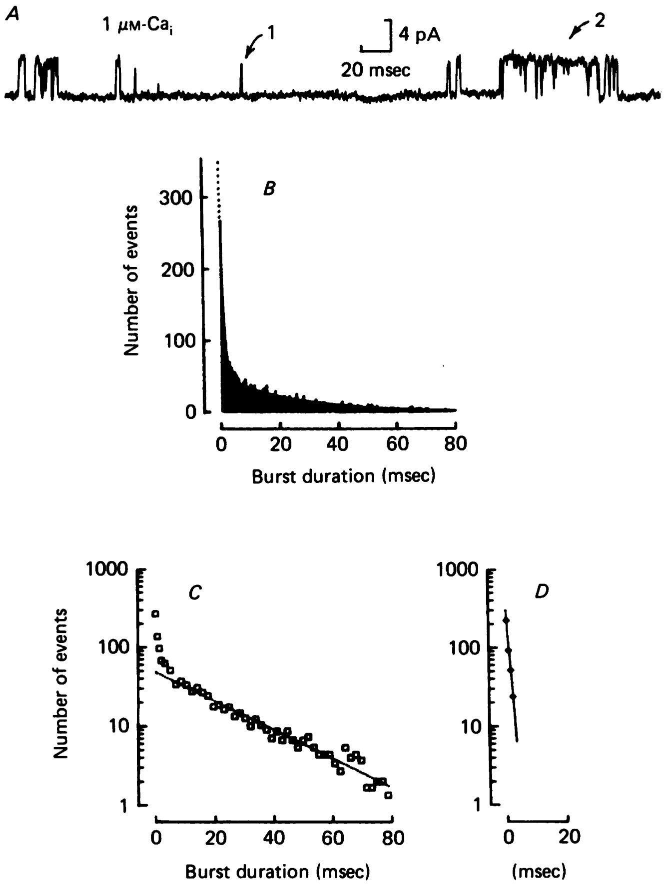

Fig. 2. Burst duration is described by the sum of two exponentials. *A*, currents recorded from an excised membrane patch. The concentration of Ca at the normal intracellular surface was 1 μM. Upward (outward) current indicates channel opening. Arrows are explained in text. *B*, histogram of the durations of all bursts during a continuous 196 sec record. The data were separated into bursts using a gap of 6·46 msec, calculated from the distribution of shut intervals as shown in Fig. 1 and described in Methods. The line, which is mainly obscured by the data, indicates the sum of the two distributions of burst duration. Bin size of 0·6 msec. *C*, data in *B* plotted on semilogarithmic co-ordinates. Bin size for the first five points was 0·6 msec; thereafter each point represents the average of three consecutive 0·6 msec bins. The line indicates the distribution of long-burst duration. The number of bursts in this distribution is: 47 bursts (intercept) × 24 msec (time constant)/0·6 msec (bin size) = 1880 bursts. *D*, distribution of short-burst duration, obtained by subtracting the distribution of long-burst duration from the distribution of all bursts. 415 bursts, +20 mV.

Dividing the sum of these two exponentials by the total number of bursts normalizes the area of the distribution to 1 and yields the probability density function (p.d.f.) of burst duration. For Fig. 2 (1 μM-Ca_i, +20 mV) this was

$$\text{p.d.f.}_{\text{burst duration}} = 0{\cdot}22e^{-t/0{\cdot}83\ \text{msec}} + 0{\cdot}034e^{-t/24\ \text{msec}}.$$

Calculations from this p.d.f. indicate that there were about 4·5 bursts from the distribution of long mean burst duration for every burst from the distribution of short mean burst duration. Arrow 1 indicates a burst with a 76 % chance of being from the distribution of short-burst duration. Arrow 2 indicates a burst with over a 99·99 % chance of being from the distribution of long-burst duration.

Two distributions of burst duration were observed in every set of data (over 20) analysed in this manner. It seems unlikely that the two distributions arose through an error introduced by using the calculated gap to define bursts, since two

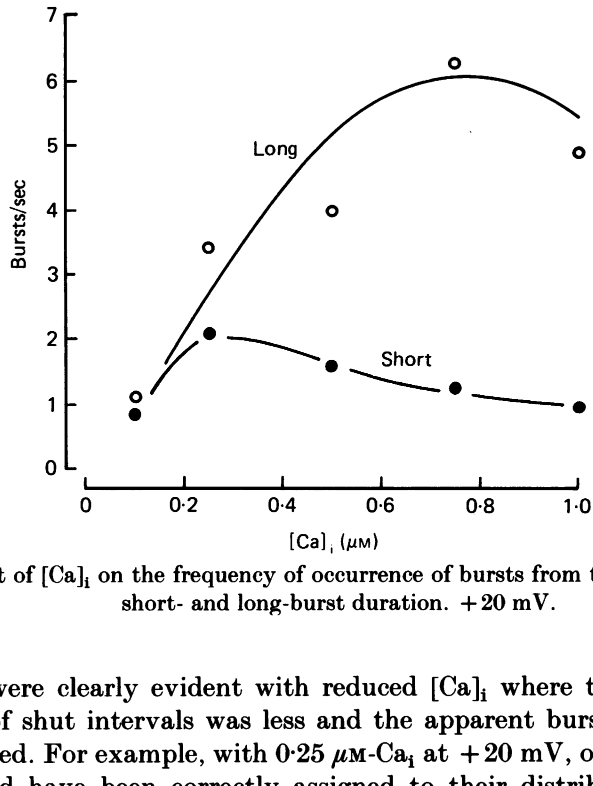

Fig. 3. Effect of [Ca]$_i$ on the frequency of occurrence of bursts from the distributions of short- and long-burst duration. +20 mV.

distributions were clearly evident with reduced [Ca]$_i$ where the overlap between distributions of shut intervals was less and the apparent bursts were much more clearly separated. For example, with 0·25 μM-Ca$_i$ at +20 mV, over 98 % of the shut intervals would have been correctly assigned to their distributions, and a two-component distribution of burst duration was still clearly evident, with a p.d.f. of $0{\cdot}71e^{-t/0{\cdot}53\,\mathrm{msec}} + 0.12e^{-t/5{\cdot}2\,\mathrm{msec}}$. Colquhoun & Sakmann (1981) have found that acetylcholine-activated ion channels exhibit bursts whose duration is described by more than one exponential.

Effect of [Ca]$_i$ on frequency of bursts

The relative numbers of bursts from the distributions of short- and long-burst duration changed with [Ca]$_i$. This is shown in Fig. 3. With 0·1 μM-Ca$_i$ there were about equal numbers of short and long bursts. The frequency of occurrence of both short and long bursts then increased and decreased with increasing [Ca]$_i$, with frequency of occurrence of long bursts being more Ca-sensitive than that of short bursts. This suggests that, on the average, the binding of more Ca ions might be required to generate long bursts than short bursts.

Unit-burst duration described by two exponentials

The Ca-activated K channel typically opens to both short and long open 'states' (see Terminology in the Methods; Barrett *et al.* 1982; Magleby & Pallotta, 1983). Since the duration of short bursts appears similar to the lifetime of the short open state, a possible explanation for the distribution of short-burst duration is that it results mainly from isolated openings to the short open state. If this is the case, then the distribution of short-burst duration should be mainly composed of single openings whose mean duration is similar to that of the short open state.

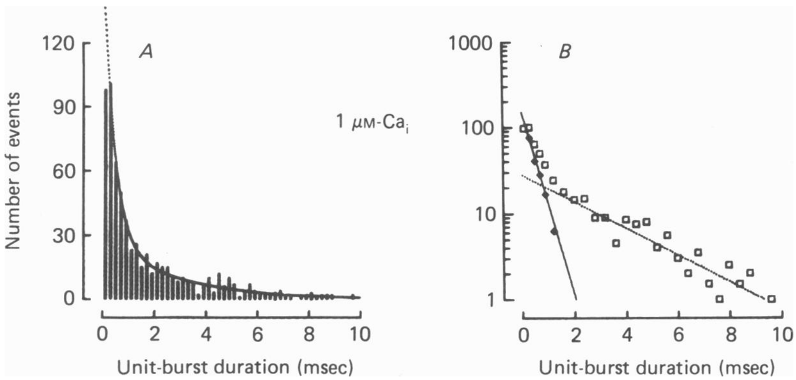

Fig. 4. Unit-burst duration is described by the sum of two exponentials. *A*, histogram of all bursts of single openings. The line is the sum of the two exponentials determined in *B*. Bin size of 0·2 msec. In *B*, the open squares plot the data from *A* on semilogarithmic co-ordinates. Bin size was 0·2 msec for the first five points; thereafter each point represents the average of two consecutive 0·2 msec bins. The line through the open squares indicates the distribution of long unit-burst duration (392 bursts). The filled diamonds plot the difference between the distribution of long unit-burst duration and the distribution of all unit bursts. The line through the filled diamonds indicates the distribution of short unit-burst duration (308 bursts). 1 μM-Ca$_i$, +20 mV.

To explore this possibility we examined the distribution of all bursts consisting of single openings (unit bursts). Such a histogram is presented in Fig. 4*A*. Unit-burst duration was described as the sum of two exponentials as shown by the semilogarithmic plot of the data in Fig. 4*B*. The shorter distribution had a mean of 0·41 msec, the longer a mean of 2·8 msec. These values are similar to the mean lifetimes of the short and long open-channel states in this experiment, which were 0·47 and 3·3 msec respectively.

In eight comparisons of this type over a range of $[Ca]_i$ the mean duration of short unit bursts ($0{\cdot}43 \pm 0{\cdot}10$ msec) was not significantly different from the mean lifetime of the short open state ($0{\cdot}42 \pm 0{\cdot}05$ (S.D.) msec). The mean lifetime of the short open state appeared independent of $[Ca]_i$, as described previously (Magleby & Pallotta, 1983). The mean duration of short unit bursts also appeared independent of $[Ca]_i$. The similarity in mean duration of short unit bursts and mean lifetime of the short open state and the lack of Ca sensitivity of the duration and lifetime of these events suggests that the distribution of short unit bursts arises mainly from isolated openings to the short open state.

In contrast to the short open state, the lifetime of the long open state has been found to increase with $[Ca]_i$ (Magleby & Pallotta, 1983), and the duration of long unit bursts also increased. This increase in long unit-burst duration was similar to the increase in the lifetime of the long open state. For example, in one experiment at +30 mV the mean lifetime of the long open state increased from 1·3 to 2·5 to 4·2 msec as $[Ca]_i$ was increased from 0·1 to 0·25 to 0·5 μM-Ca_i, and the mean duration of long unit bursts increased in a parallel manner from 1·2 to 2·5 to 4·5 msec. The similarity in mean duration and Ca sensitivity of duration of long unit bursts when compared to the lifetime of the long open state suggests that the distribution of long unit-burst duration arises mainly from isolated single openings to the long open state. For the experiment in Fig. 4, over half of all the unit bursts were isolated openings to the long open state.

Comparison of distributions of short-burst duration and short unit-burst duration

To determine whether the distribution of short-burst duration might be composed of short unit bursts, we compared the two distributions. For the experiment shown in Figs. 2 and 4 there were 415 short bursts with a mean duration of 0·83 msec (Fig. 2*D*) compared to 308 short unit bursts with mean duration of 0·41 msec (Fig. 4*B*). In eight comparisons of this type the mean duration of short bursts (0·54 ± 0·13 (S.D.) msec) was not significantly different from the mean duration of short unit bursts (0·49 ± 0·16 msec). For these same eight comparisons there were 1·17 ± 0·23 bursts in the distribution of short-burst duration for every burst in the distribution of short unit-burst duration. The similarities in the mean durations and total number of short bursts when compared to short unit bursts suggests that short bursts are mainly composed of short unit bursts. Since short unit bursts appear to arise mainly from isolated single openings to the short open state (previous sections), then short bursts would also arise mainly from isolated single openings to the short open state.

Composition of long bursts

If the distribution of short-burst duration (Fig. 2) is composed of openings to the short open state, then most if not all of the openings to the long open state must be in the distribution of long-burst duration. Thus, each long burst would contain one or more openings to the long open state, and the distribution of long unit bursts would be contained in the distribution of long bursts. A simple calculation shows that some long bursts must also contain one or more openings to the short open state. For the data presented in Figs. 2 and 4, 2168 (18 %) of the openings were to the short open state and 10423 (82 %) were to the long open state (1 μM-Ca_i), as determined from the distribution of open intervals (not shown). Since only about 415 (19 %) of the openings to the short open state would be needed to generate the observed bursts of short duration, then 2168 minus 415 or 1753 (81 %) of the openings to the short open state must have been included in bursts of long duration. Thus, under these experimental conditions, about one out of every seven openings (1753/(1753 + 10423)) in the bursts of long duration were openings to the short open state; the others were to the long open state.

Effect of $[Ca]_i$ on mean burst duration

The mean duration of the distribution of long bursts was found to increase with increasing $[Ca]_i$. For example, in one experiment at +30 mV mean long-burst duration increased from 2·8 to 11·9 to 46·7 msec as $[Ca]_i$ increased from 0·1 to 0·25 to 0·5 μM. The visually observed increase in apparent burst duration with increasing $[Ca]_i$ (see Fig. 4*A*, Magleby & Pallotta, 1983) would then result from both the increase in the mean duration of long bursts described above and the increase in the relative number of long to short bursts shown in Fig. 3. Burst duration is determined by many factors including changes in mean open lifetimes, so that it is difficult to interpret in terms of mechanism. The number of openings/burst, on the other hand, gives more direct information about mechanism and will be examined in the following sections.

Distribution of number of openings/burst

The number of openings/burst were counted and plotted both as histograms and on semilogarithmic co-ordinates. Results are shown in Fig. 5 for 0·1 and 1 μM-Ca_i. The distribution of openings/burst was described by two components in each case: a distribution of few openings/burst (mean of about 1·2 openings/burst) that appeared relatively Ca-independent, and a longer Ca-dependent distribution of many openings/burst, with a mean of 1·86 openings/burst in 0·1 μM-Ca_i and 5·39 openings/burst in 1 μM-Ca_i.

To determine whether the distribution of few openings/burst might consist of the same openings as the distribution of short-burst duration, we compared the number of events in these two distributions. With 1 μM-Ca_i the distribution of few openings/burst consisted of 339 bursts of about one opening each (Fig. 5*D*), the distribution of short-burst duration consisted of 415 bursts of about one opening each (Fig. 2*D*, same 196 sec of data in each case). With 0·1 μM-Ca_i the distribution of few openings/burst consisted of 547 bursts of about one opening each (Fig. 5*B*); the distribution of short-burst duration consisted of 545 bursts of about one opening each (same 635 sec of data in each case). In eight comparisons of this type over a range of $[Ca]_i$ there were 1·27 ± 0·84 (S.D.) bursts in the distribution of few openings/burst for every burst in the distribution of short-burst duration. Excluding one somewhat deviant point gave 1·01 ± 0·44. The bursts in each of these distributions were of about one opening each. The similarities between the two distributions in number of bursts and the number of openings/burst suggests that the distribution of few openings/burst consists mainly of the same openings as the distribution of short-burst duration. It follows then that the distribution of many openings/burst would consist of the same openings as the distribution of long-burst duration.

Since the distribution of short-burst duration consists mainly of isolated single openings to the short open state (see previous sections), then the distribution of few openings/burst must also consist mainly of single isolated openings to the short open state. Since the distribution of long-burst duration contains openings to both open states, then the distribution of many openings/burst would also contain openings to both open states. In these experiments the mean number of openings/burst, mainly in the distribution of many openings/burst, would be underestimated somewhat due to failure to detect very brief shut intervals (see Magleby & Pallotta, 1983).

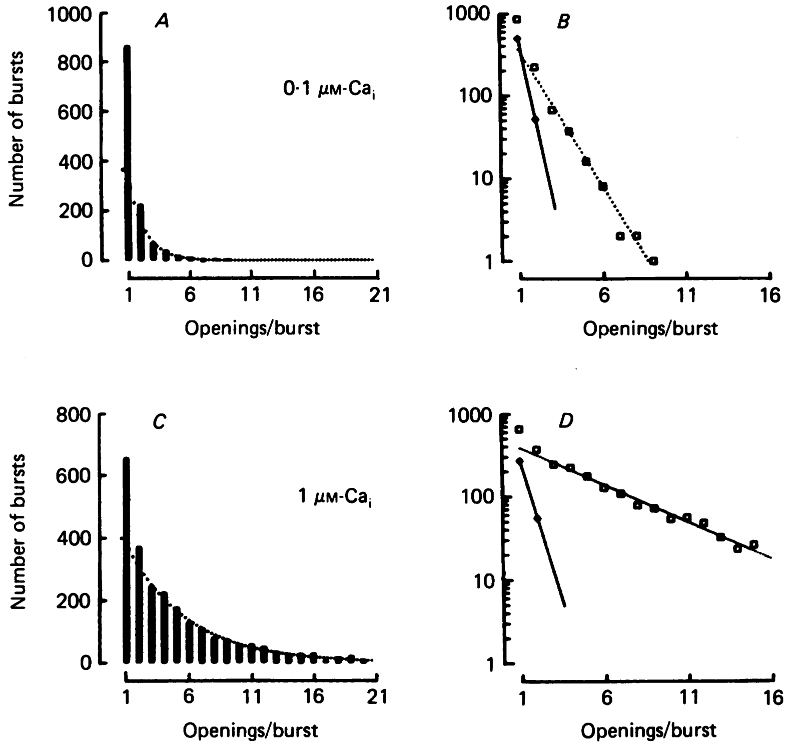

Fig. 5. Number of openings/burst is described by the sum of two geometric distributions. *A* and *C*, histograms of the number of openings/burst determined from 635 sec of data in 0·1 μM-Ca$_i$ and 196 sec of data in 1 μM-Ca$_i$. The lines indicate the component of many openings/burst. Data above this line gives rise to the component of few openings/burst. In *B* and *D* the open squares plot the data from *A* and *C*, respectively, on semilogarithmic co-ordinates. The lines through the open squares indicate the distribution of many openings/burst which had 683 bursts of 1·86 mean openings/burst in *B* and 2086 bursts of 5·39 mean openings/burst in *D*. The filled diamonds plot the difference between the distribution of many openings/burst and the distribution of openings/burst for all bursts. The lines through the filled diamonds indicates the distribution of few openings/burst which had 547 bursts of 1·1 mean openings/burst in *B* and 339 bursts of 1·26 mean openings/burst in *D*. +20 mV.

Summary of relationships among the various distributions

The preceding results suggest that the distributions of short-burst duration, short unit bursts, and few openings/burst each contain mainly the same set of openings plotted in a different manner. These openings are to the short open state. The distributions of long-burst duration and many openings/burst, which plot mainly the same set of openings in terms of either duration or number, each contain essentially all openings to the long open state plus some openings to the short open state.

Effect of [Ca]$_i$ on number of openings/burst

Fig. 6*A* plots the effect of [Ca]$_i$ on the mean number of openings/burst for the distributions of few and many openings/burst. Changes in [Ca]$_i$ had little effect on the number of openings/burst for the distribution of few openings/burst (open circles). In contrast, openings/burst increased with increasing $[Ca]_i$ for the distribution of many openings/burst (filled circles).

Resolving the distribution of many openings/burst into openings to the short and long open states

It was concluded in a previous section that bursts from the distribution of many openings/burst can contain openings to both the short and long open states. It is of interest, therefore, to determine whether the Ca-induced increase in the number of openings/burst for the distribution of many openings/burst arose from an increase in the number of openings to the short or long open states.

While the number of openings to each open state in the distribution of many openings/burst can be calculated in several different ways, the following method typically gave the most consistent results. The total number of openings in the combined distributions of few and many openings/burst was first calculated as described in the Methods. Since these two distributions contain all openings, the number of openings to the short open state in the combined distributions was obtained by multiplying the total number of openings by the percentage of openings to the short open state, determined from the probability density function of open intervals (see Magleby & Pallotta, 1983). The number of openings to the short open state in the distribution of many openings/burst was then estimated by subtracting the number of short unit bursts (or the number of bursts in the distribution of short-burst duration) from the total number of openings to the short open state. The number of openings to the long open state in the distribution of many openings/burst was then obtained from the total number of openings in this distribution minus the number of openings to the short open state in this distribution. Dividing the numbers of openings to the short and long open states in the distribution of many openings/burst by the number of bursts in this distribution then gave the mean number of openings to each state in the distribution of many openings/burst.

The filled squares in Fig. 6*B* plot the mean number of openings to the long open state in an average burst from the distribution of many openings/burst. They increased from about 1·5 to 4·6 for a 10-fold increase in $[Ca]_i$. The open squares plot the mean number of openings to the short open state in an average burst also drawn from the distribution of many openings/burst. The mean number, about 0·7, appeared relatively independent of $[Ca]_i$, although the variation in data points in experiments of this type could have obscured a small effect. Since some bursts in the distribution of many openings/burst might be expected to have more than one opening to the short open state, then the actual number of bursts in this distribution with openings to the short open state would be fewer than 70% of the long bursts.

Fig. 6 shows, then, that the number of openings/burst to the short open state (open symbols), whether occurring in relative isolation, as would be the case for the bursts from the distribution of few openings/burst, or whether associated with openings to the long open state, as would be the case for bursts from the distribution of many openings/burst, was relatively Ca-independent. In contrast, openings/burst to the long open state increased with increasing $[Ca]_i$, and this increase often appeared to inflect upward, as shown by the filled squares in Fig. 6*B*. No increase in openings/burst with increasing $[Ca]_i$ would be consistent with binding one Ca ion for channel opening, while a linear increase would suggest binding of two Ca ions for channel opening (Colquhoun & Hawkes, 1981), and an upward inflexion would suggest the binding of at least three Ca ions for opening. The findings in Fig. 6 are thus consistent with

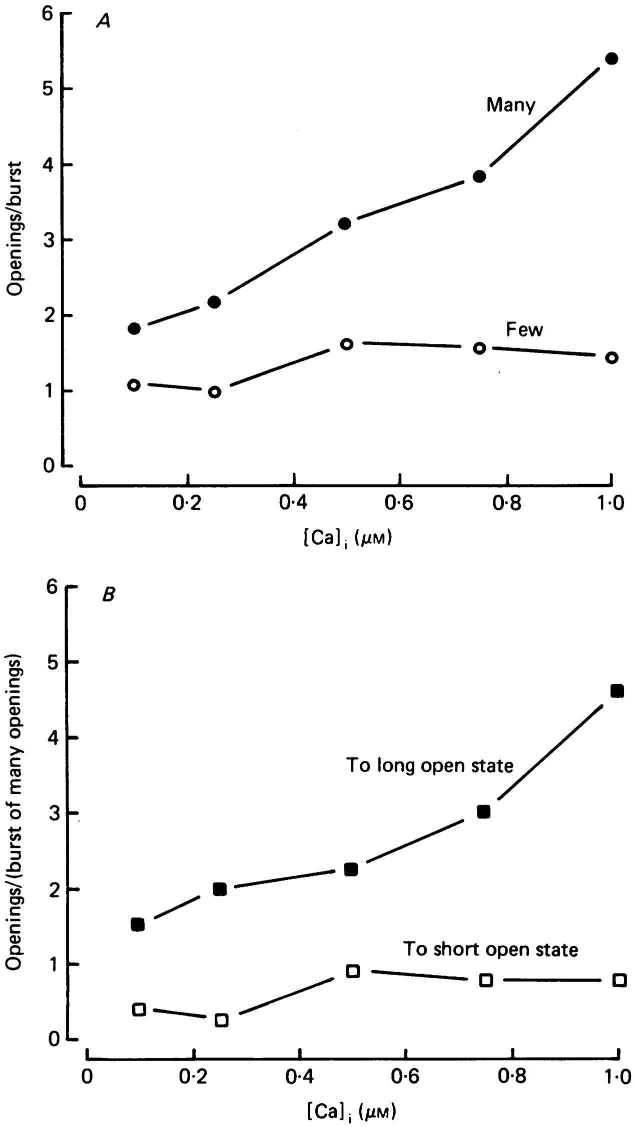

Fig. 6. Effect of $[Ca]_i$ on the composition of bursts. *A*, plot of the mean number of openings/burst for the distributions of few and many openings/burst. *B*, plot of the number of openings/burst to the short and long open-channel states for bursts drawn only from the distribution of many openings/burst. +20 mV.

models in which openings to the long open state require the binding of three or more Ca ions, and openings to the short open state require the binding of perhaps one Ca ion.

Factors underlying the effect of $[Ca]_i$ on percentage of time open

The percentage of time that a channel is open increases as a steep function of $[Ca]_i$ (Barrett *et al.* 1982). Fig. 7 shows that this effect can be accounted for in terms of the

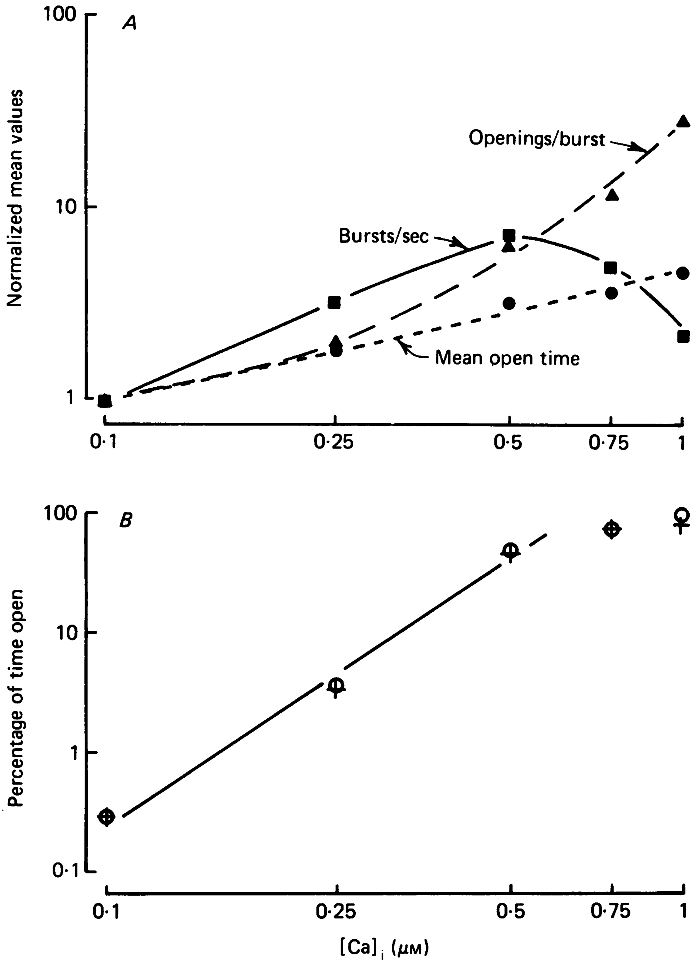

Fig. 7. Factors underlying the Ca-induced increase in the percentage of time that the channel spends open. *A*, effect of $[Ca]_i$ on observed mean channel open time, observed mean number of openings/burst, and observed mean number of bursts/sec. Note that the plotted parameters represent the means of two distributions in each case. *B*, plot of percentage of time open *vs.* $[Ca]_i$. Open circles are experimental data. The plus symbols plot the product of the three factors in *A* times the percentage of time open in 0·1 μM-Ca_i. Note logarithmic co-ordinates in *A* and *B*. +30 mV, same experiments as in Figs. 1–5 of Magleby & Pallotta (1983).

Ca dependence of the underlying channel kinetics (+30 mV, different experiment from previous Figures). Fig. 7*A* plots on semilogarithmic co-ordinates the normalized values of the mean number of bursts/sec (squares), the mean number of openings/burst (triangles), and the mean open-channel lifetime (circles); the data were normalized to 1 at 0·1 μM-Ca_i. The open circles in Fig. 7*B* plot the observed percentage of time that the channel was open; the continuous line has a slope of 2·9. The plus symbols show that the product of the three Ca-dependent factors can account for the percentage of time open. While this Figure shows the internal consistency of the data by presenting an overview of the general action of $[Ca]_i$ on mean channel kinetics, it should be remembered that the plotted underlying factors are means of both Ca-dependent and Ca-independent factors, as shown in the previous Figures.

DISCUSSION

This paper examines burst kinetics of the Ca-activated K channel in excised membrane patches. Bursts were defined in terms of a calculated gap derived from the distribution of shut intervals. Any shut interval greater than the calculated gap was considered a gap between bursts; any shut interval equal to or less than the calculated gap was considered a gap within bursts.

Using a calculated gap to separate bursts, the distribution of burst duration was composed of two apparent exponential distributions (Fig. 2). The shorter distribution (short bursts) was composed of bursts of mainly single openings (unit bursts) to the open-channel 'state' of short mean lifetime (see *Terminology* in the Methods). The longer distribution (long bursts) was composed of bursts of one or more openings to the long open-channel 'state', plus, in fewer than 70 % of the long bursts, one or more openings to the short open-channel state. The number of openings/burst in the distribution of short bursts appeared relatively independent of $[Ca]_i$. In contrast, the number of openings/burst in the distribution of long bursts increased with $[Ca]_i$. This increase arose almost entirely from an increase in the number of openings/burst to the long open state.

The observation of two distributions of number of openings/burst (Fig. 5) is consistent with a minimum of two readily occurring open-channel states (Colquhoun & Hawkes, 1982), as concluded previously from the two exponential distributions of open intervals (Barrett *et al.* 1982; Magleby & Pallotta, 1983). Note that the distribution of openings/burst is obtained entirely from the distribution of shut intervals without regard to the durations of the open intervals.

In the preceding paper (Magleby & Pallotta, 1983) it was found that a simple model with three closed and two open states could account quantitatively for the distributions of open and shut intervals for constant $[Ca]_i$. This model was

$$\begin{array}{ccccc} R & \underset{k_{-1}}{\overset{k_1 Ca}{\rightleftharpoons}} & CaR & \underset{k_{-2}}{\overset{k_2 Ca}{\rightleftharpoons}} & Ca_2R \\ & & \alpha' \updownarrow \beta' & & \alpha \updownarrow \beta \\ & & CaR^* & & Ca_2R^* \end{array} \qquad (1)$$

where R is the unbound closed channel, CaR and Ca_2R are bound closed-channel states, CaR^* is the open state of short mean lifetime and Ca_2R^* the open-channel state of long mean lifetime.

Using the generalized numerical methods detailed by Colquhoun & Hawkes (1977, 1981, 1982) we determined whether scheme (1) could account for the burst kinetics of the Ca-activated K channel. Rate constants that described the observed distributions of open and shut intervals at a given $[Ca]_i$ were first determined as described by Magleby & Pallotta (1983). These rate constants were then used to calculate the distributions of burst duration and openings/burst predicted on the basis of scheme (1). For 1 μM-Ca_i (+20 mV) rate constants that described the distributions of open and shut intervals were: $k_1 = 25 \times 10^6$ M^{-1} sec^{-1}, $k_{-1} = 150$ sec^{-1}, $k_2 = 310 \times 10^6$ M^{-1} sec^{-1}, $k_{-2} = 1000$ sec^{-1}, $\alpha' = 2130$ sec^{-1}, $\beta' = 85$ sec^{-1}, $\alpha = 303$ sec^{-1}, $\beta = 1400$ sec^{-1}. (Since there were two channels in the patch, k_1 represents a composite forward rate constant. Calculations indicated that the actual value of k_1 for each channel would be less than half of the listed composite value. For the low levels of channel activity in these experiments only one channel was typically bursting at any time. Consequently, the measured activity during each burst was from a single channel.)

The calculated distribution of burst duration with these rate constants was

$$\text{p.d.f.}_{\text{burst duration}} = 0{\cdot}24\text{e}^{-t/0{\cdot}45\text{ msec}} + 0{\cdot}036\text{e}^{-t/25\text{ msec}} + 0{\cdot}0025\text{e}^{-t/0{\cdot}36\text{ msec}} + 0{\cdot}00019\text{e}^{-t/2{\cdot}5\text{ msec}},$$

which is in reasonable agreement with the experimentally observed distribution presented in Fig. 1 and the Results. Since only 0·14 % of the bursts would be contained in the third and fourth components of this distribution, these components would be too small to be observed experimentally.

The calculated distribution of openings/burst was the sum of two geometric distributions, with 5·66 and 1·17 mean openings/burst. The calculated values are in good agreement with the experimentally observed values of 5·39 and 1·26 mean openings/burst (Fig. 5*D*).

For 0·1 μM-Ca$_i$ (+20 mV) the rate constants that described the distributions of open and shut intervals were: $k_1 = 275 \times 10^6$ M^{-1} $k_{-1} = 415$ sec^{-1}, $k_2 = 495 \times 10^6$ M^{-1} sec^{-1}, $k_{-2} = 2200$ sec^{-1}, $\alpha' = 2500$ sec^{-1}, $\beta' = 20$ sec^{-1}, $\alpha = 606$ sec^{-1}, $\beta = 1200$ sec^{-1}. The calculated distribution of openings/burst was two geometrics with 1·61 and 1·04 mean openings/burst, in good agreement with the observed distributions of 1·86 and 1·1 mean openings/burst (Fig. 5*B*).

Scheme (1), and scheme (2) considered in Magleby & Pallotta (1983), also gave reasonable descriptions of burst kinetics for additional experiments including some with single-channel patches. Thus, schemes (1) and (2) may be considered as starting points to study the burst kinetics of the Ca-activated K channel. However, as discussed by Magleby & Pallotta (1983), the actual molecular scheme of the channel is most likely to be more complex, as different rate constants are required to describe the channel properties for each [Ca]$_i$.

In terms of scheme (1) and the terminology in Colquhoun & Hawkes (1981), a gap *within* bursts would consist of a sojourn in {CaR, Ca$_2$R} starting with an exit from either open state and terminating in either open state. Gaps *between* bursts would consist of the sum of (a) a sojourn in {CaR, Ca$_2$R} starting with an exit from either open state and terminating in R and (b) a sojourn in {R, CaR, Ca$_2$R} starting in R and terminating in either open state. Thus, gaps *within* bursts have no underlying transitions to R, and gaps *between* bursts would have one or more underlying transitions to R. Openings would typically appear to be grouped into bursts when the channel closes and reopens without a transition to R (without losing all of its bound Ca), as these shut periods would tend to be brief compared to shut periods associated with one or more transitions to R. Further discussion of how apparent bursting activity is generated by models with intermediate closed states may be found in the papers of Colquhoun & Hawkes (1977, 1981) and Dionne (1981).

In terms of scheme (1), with 0·1 μM-Ca_i at +20 mV an average burst from the distribution of short bursts would consist typically of a single opening to CaR*, and an average burst from the distribution of long bursts would consist of about two openings to Ca_2R* plus, in fewer than 70 % of the long bursts, one or more openings to CaR*. The number of short and long bursts would be about equal. With 1 μM-Ca_i (+20 mV) an average short burst would still consist typically of a single opening to CaR*, but an average long burst would now consist of about five openings to Ca_2R*, plus, in fewer than 70 % of the long bursts, as was the case in 0·1 μM Ca_i, one or more openings to CaR*. Long bursts would now outnumber short bursts by about 5 to 1 (Figs. 2 and 5).

The observation that long bursts can contain openings to both the short and long open states excludes models for the channel in which the short and long open states are separated by an unbound state of the channel. In models of this type, openings to both the short and long states would never be contained in the same burst, since the obligatory transition through the unbound state to go from one open state to the other would terminate a burst.

Our method of determining the gap to separate bursts would lead to an underestimation of the mean number of openings/burst if scheme (1) is the correct molecular scheme. Re-analysis of the data using a gap to separate bursts more consistent with scheme (1) indicated that the underestimation of openings/bursts in terms of this scheme was negligible for the lower levels of $[Ca]_i$ examined and that the underestimation increased to only about 3–10 % with 1 μM-Ca_i. Errors of this magnitude would have little effect on the results presented in this paper, but they would act to reduce the upward inflexion in the number of openings/burst to the long open state somewhat. An upward inflexion, which was often much more pronounced than shown in Fig. 6*A*, is consistent with models in which three or more Ca ions bind for openings to the long open state. Thus, an additional state would have to be added to scheme (1) to account for this observation. Scheme (2) of Magleby & Pallotta (1983) requires that three Ca ions bind for openings to the long open state, but scheme (2) is still not sufficient to account for the effect of $[Ca]_i$ on channel kinetics without a change in rate constants.

Although many questions remain unanswered, the results obtained in this, the previous paper (Magleby & Pallotta, 1983) and that of Barrett *et al.* (1982) suggest a general overview of how the Ca-activated K channel functions. The channel has two exponential distributions of open intervals of different mean open time but similar conductance, suggesting two readily occurring open-channel states. The distribution of short mean open time is probably dominated by an open-channel state whose lifetime is brief (about 0·5 msec) and independent of $[Ca]_i$ (short open-channel state). In contrast, the distribution of long mean open time is probably dominated by a compound open-channel state whose lifetime increases with $[Ca]_i$, from 1·2 msec in 0·1 μM-Ca_i to 5·5 msec in 1 μM-Ca_i at +30 mV (long open-channel state). The three observed distributions of shut intervals suggest a minimum of three readily occurring closed-channel states involved in typical channel activity, and kinetic analysis of the data suggests that there may be more. The channel also infrequently enters an open state of reduced conductance and an inactivated closed-channel state.

Channel activity typically occurs in bursts of two types: short bursts of typically single openings to the short open state, and long bursts of one or more openings to the long open state, plus, in many long bursts, one or more openings to the short open state. At very low $[Ca]_i$ (0·01 μM, +30 mV), short bursts dominate so that openings typically occur in isolation to the short open state. Because the openings are brief and widely separated, the channel is open less than 1 % of the time. As $[Ca]_i$ is raised (0·5 μM, +30 mV): (1) the interval between bursts decreases, (2) the increase in the number of long bursts far exceeds the increase in the number of short bursts, (3) the number of openings in each long burst increases (the increase is due to an increase in openings to the long open state) and (4) the observed mean lifetime of the long open state increases. Channel activity now occurs in obvious bursts of typically many openings, but there are still occasional bursts of one opening which may be to either open state. Prominent gaps between bursts are still apparent, however, so that the channel is open less than 50 % of the time. At high levels of $[Ca]_i$ (1 μM, +30 mV) the gaps between bursts are brief, and the long bursts become even longer due to a further increase in the number of openings/burst and in the observed lifetime of the long open state. Long bursts now far outnumber short bursts, and consequently most of the openings are to the long open state. Since the channel opens soon after each closing, it is open over 80 % of the time.

In addition to the bursting behaviour of the Ca-activated K channel, bursts of channel activity have been observed for voltage-activated K channels in squid axons (Conti & Neher, 1980), glutamate-activated channels in locust (Cull-Candy *et al.* 1981; Cull-Candy & Parker, 1982), and acetylcholine-activated channels in cultured chick muscle (Nelson & Sachs, 1979), embryonic rat muscle (Jackson & Lecar, 1979), and frog muscle (Sakmann *et al.* 1980; Colquhoun & Sakmann, 1981). Thus, bursting behaviour appears to be a common feature of at least four different types of channels.

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