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MINI-REVIEW

Calcium-Activated Potassium Channels: Regulation by Calcium

Owen B. McManus[1]

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Abstract

A wide variety of calcium-activated K channels has been described and can be conveniently separated into three classes based on differences in single-channel conductance, voltage dependence of channel opening, and sensitivity to blockers. Large-conductance calcium-activated K channels typically require micromolar concentrations of calcium to open, and their sensitivity to calcium increases with membrane depolarization, suggesting that they may be involved in repolarization events. Small-conductance calcium-activated K channels are generally more sensitive to calcium at negative membrane potentials, but their sensitivity to calcium is independent of membrane potential, suggesting that they may be involved in regulating membrane properties near the resting potential. Intermediate-conductance calcium-activated K channels are a loosely defined group, where membership is determined because a channel does not fit in either of the other two groups. Within each broad group, variations in calcium sensitivity and single-channel conductance have been observed, suggesting that there may be families of closely related calcium-activated K channels. Kinetic studies of the gating of calcium-activated potassium channels have revealed some basic features of the mechanisms involved in activation of these channels by calcium, including the number of calcium ions participating in channel opening, the number of major conformations of the channels involved in the gating process, and the number of transition pathways between open and closed states. Methods of analysis have been developed that may allow identification of models that give accurate descriptions of the gating of these channels. Although such kinetic models are likely to be oversimplifications of the behavior of a large macromolecule, these models may provide some insight into the mechanisms that control the gating of the channel, and are subject to falsification by new data.

Key Words: Calcium-activated potassium channel; potassium channel; ion channel, channel gating.

[1] Department of Membrane Biochemistry and Biophysics, Merck Institute for Therapeutic Research, Rahway, New Jersey 07065.

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Calcium-activated potassium channels increase their permeability to potassium ions in response to increases in intracellular calcium concentration. These channels couple the membrane potential to the intracellular calcium concentration such that a rise in intracellular calcium leads to an efflux of potassium ions and a hyperpolarization of the membrane. Calcium-activated K channels provide a feedback control of the influx of calcium through voltage-dependent pathways. Because of this property, calcium-activated K channels are believed to play a role in a number of different cellular processes that depend on influx of calcium through voltage-dependent pathways, including regulated secretion and smooth muscle contraction. Calcium-activated K channels also link intracellular calcium concentration to membrane excitability and as a result are believed to play a role in regulating action potential frequency and duration in neurons and other excitable cells.

The currents flowing through single calcium-activated K channels can be measured using patch clamp and lipid bilayer techniques. These techniques allow one to directly observe the effects of intracellular calcium on the behaviour of single channel molecules in real time. These channels typically fluctuate between two discrete conductance states, open and closed, where the open state allows the passage of charged potassium ions across the membrane. Calcium acts to increase the amount of current flowing through these channels by increasing the average time the channel resides in the open state (Marty, 1981; Pallotta *et al.*, 1981; Latorre *et al.*, 1982; Adams *et al.*, 1982; Wong *et al.*, 1982; Blatz and Magleby, 1986c; Lang and Ritchie, 1987; Capoid and Ogden, 1989). In most cases, calcium does not affect the conductance of open channels, except at millimolar concentrations (Barrett *et al.*, 1982; Latorre *et al.*, 1982; Vergara and Latorre, 1983).

Use of patch clamp and lipid bilayer techniques has permitted identification of a number of different calcium-activated potassium channels. These different channels can be grouped into three broad categories based on their conductances: large-conductance calcium-activated K (BK or maxi-K) channels with single-channel conductances greater than about 150 pS, small-conductance calcium-activated K (SK) channels of less than 50 pS, and intermediate-conductance calcium-activated K (IK) channels with conductances of ~ 50–150 pS. These classifications are somewhat arbitrary, but are generally supported by pharmacological and physiological differences between these classes of channels. Maxi-K channels are typically blocked on the external side by nanomolar concentrations of charybdotoxin and submillimolar concentrations of tetraethylammonium (TEA), but not by apamin, while SK channels are typically blocked on the external side by nanomolar

concentrations of apamin, but not by charybdotoxin or submillimolar concentrations of TEA (Yellen, 1984; Blatz and Magleby, 1984, 1986c, 1987; Miller *et al.*, 1985; Lang and Ritchie, 1990). Maxi-K channels are typically less sensitive to internal calcium at negative membrane potentials than SK channels (Blatz and Magleby, 1986c; Lang and Ritchie, 1987) and thus may be less important in regulating the potassium conductance near the resting potential. In most tissues, maxi-K channels are moderately voltage dependent (*e*-fold increase in open probability per 10–15 mV, Latorre *et al.*, 1989), while, in contrast, SK channels typically show little or no voltage dependence (Latorre *et al.*, 1989) so that under depolarized conditions as occur at the peak of an action potential, the maxi-K channel is more sensitive to calcium than the SK channel (Blatz and Magleby, 1987).

In this review I would like to focus on one question. How does calcium govern the opening and closing of calcium-activated potassium channels? More results have been published on the calcium dependence of maxi-K channels than SK or IK channels, so I will give more attention to maxi-K channels. I will primarily discuss data obtained from channels studied in excised membrane patches or in lipid bilayers where the calcium concentration can be controlled.

Properties of Maxi-K Channels

Maxi-K channels are found in a wide variety of tissues and are easily identified based on their large single conductance of ~200–300 pS in 0.1–0.2 M potassium, high selectivity for potassium over sodium, and sensitivity of channel gating to both intracellular calcium and membrane voltage. These channels are ideal for kinetic studies of channel gating because of their large single conductance and because it is possible to record for extended periods from a single channel. Maxi-K channels have been extensively studied because of their wide distribution in different tissues and these favorable biophysical properties.

Functions of Maxi-K Channels

The roles of maxi-K channels in the functions of different cells are becoming clearer due to the discovery of scorpion toxins that block this channel (Miller *et al.*, 1985; Galvez *et al.*, 1990; for reviews see Moczydlowski *et al.*, 1988; Castle *et al.*, 1989; Garcia *et al.*, this volume). In excitable cells, the maxi-K channel contributes to the repolarization phase of the action potential in GH_3 cells (Lang and Ritchie, 1990), neurons (Pennefather *et al.*, 1985; Lancaster and Nicoll, 1987), and pancreatic beta cells (Velasco and

Petersen, 1987). Block of this channel with charybdotoxin causes a broadening of the action potential. The maxi-K channel may link intracellular glucose concentration to insulin release in pancreatic beta cells through a mechanism involving inhibition of maxi-K channels by protons released during glycolysis (Cook *et al.*, 1984). In nonspiking cells, the maxi-K channel may contribute to the regulation of fluid secretion in exocrine gland cells (Petersen and Maruyama, 1984) and affect contractile tone in smooth muscle (Winquist *et al.*, 1989; Schweitz *et al.*, 1989; Jones *et al.*, 1990). More complete discussions of the role of this channel in different tissues may be found in some recent reviews (Petersen and Maruyama, 1984; Petersen, 1986; Blatz and Magleby, 1987; Moczydlowski *et al.*, 1988; Latorre *et al.*, 1989; Castle *et al.*, 1989).

Maxi-K Channels: Dose–Response Relationship for Calcium

Increasing the calcium concentration at the intracellular face of this channel leads to an increase in the open probability of the channel. The relation between calcium concentration and the steady-state open probability is usually a sigmoid function that can be empirically described by three parameters: the maximum open probability, the slope of the dose–response curve, and the midpoint of the curve. Quantitative estimation of these parameters can be obtained by fitting the dose–response data to a Hill equation (Adair, 1925).

$$P_{open} = P_{max*}[Ca]^n/(K + [Ca]^n)$$

In this equation, P_{open} is the steady-state open probability, P_{max} is the maximum open probability, n is the slope factor, and the nth root of K gives an estimate of the midpoint of the activation curve and the sensitivity of the channel to calcium. The value of n (Hill coefficient) provides a minimum estimate of the number of calcium ions involved in maximally activating the channel. Table I shows that both the midpoint and the slope of the dose–response relation for calcium activation of the maxi-K channel vary between different tissues.

Calcium Sensitivity of Maxi-K Channels

Table I presents a limited sampling of reported values for the calcium sensitivity of maxi-K channels from various tissues. The calcium concentration required to achieve an open probability of 0.5 at 0 mV ($Ca_{1/2}$) is listed as a convenient measure of calcium sensitivity. The data in Table I clearly show wide variations in the calcium sensitivity of maxi-K channels in different tissues and smaller variations in the sensitivity reported from the same tissue. Despite these variations, some general features of the calcium sensitivity in different tissues can be seen. The sensitivity of maxi-K channel gating to calcium is highest in secretory cells ($Ca_{1/2} < 1\ \mu M$), intermediate in smooth

muscle cells ($Ca_{1/2} \sim 1\ \mu M$), and lowest in skeletal muscle and neurons ($Ca_{1/2} > 1\ \mu M$).

Some of the variations in calcium sensitivity of maxi-K channels from different tissues may be due to differences in the local environment of the channel. Gating of these channels is affected by membrane lipid composition, which might vary between cell types. Moczydlowski *et al.* (1985) found that the calcium sensitivity of skeletal muscle maxi-K channels inserted into lipid bilayers was affected by membrane surface charge. Maxi-K channels opened at lower calcium concentrations when inserted into bilayers composed of a negatively charged lipid, phosphatidylserine, than in bilayers composed of a zwitterionic lipid, phosphatidylethanolamine. Membrane cholesterol content affects the gating of maxi-K channels in smooth muscle cells (Bolotina *et al.*, 1989). Elevation of cholesterol levels in the membrane leads to a decrease in membrane fluidity and a decrease in open probability of maxi-K channels at a given calcium concentration. Depletion of cholesterol content with mevinolin had opposite effects.

In addition to the wide variations in calcium sensitivity of maxi-K channels from different tissues seen in Table I, smaller variations in the calcium sensitivity of maxi-K channels from the same tissue have been reported. As first pointed out by Moczydlowski and Latorre (1983), different maxi-K channels from the same preparation can show a three- to fivefold variation in calcium sensitivity when examined under identical experimental conditions, as if each channel has its own personality. These variations could reflect different classes of maxi-K channels in the same tissue, as reported by Vaca *et al.* (1989) in the coronary artery and Reinhart *et al.* (1989) in brain vesicles. Spontaneous shifts in the gating of single maxi-K channels from skeletal and smooth muscle have been observed (Moczydlowski and Latorre, 1983; Singer and Walsh, 1987; McManus and Magleby, 1988). The ability of single maxi–K channel molecules to show large changes in gating under steady-state conditions may underlie some of the observed variations in calcium sensitivity.

The calcium sensitivity of maxi-K channels in cultured *Xenopus* neurons (Blair and Dionne, 1985) and human aortic smooth muscle cells (Bregestovski *et al.*, 1988) changes during development. The basis of the developmental change in the gating of maxi-K channels is unknown, but the differences in gating between adult and fetal human smooth muscle (Bregestovski *et al.*, 1988) closely resemble the changes in gating that occur during mode shifts between the normal and the brief open mode in cultured skeletal muscle (McManus and Magleby, 1988).

A significant portion of the variations in calcium sensitivity seen in Table I may be due to differences in experimental conditions such as membrane lipid composition, pH, and magnesium concentration. Internal pH

Table I. Calcium Sensitivity of Calcium-Activated K Channels in Different Tissues[a]

Tissue	G(K), pS(mM K)	$[Ca]_{1/2}$, μM	Hill	pH	[Mg], mM	Exp	Author
BK or Maxi-K							
Skeletal muscle							
Cultured rat skeletal muscle	240 (140)	~4	~2	7.2	1	P, E	Methfessel and Boheim (1982)
	218 (140)	5–10	2.7	7.2	2	P, E	Barrett *et al* (1982)
	—	—	3.6	7.2	0	P, E	McManus and Magleby (1985)
Rat plasma membrane	290 (200)	~100	1.1–1.8	7.0/7.4	0	B-n, A	Moczydlowski and Latorre (1983)
	240 (100)	45	—	7.0	0	B-n, A	Moczydlowski *et al.* (1985)
	340 (100)	4.5	—			B-c, A	
	—	50–100 (40 mV)	2	7.4	0	B-n, A	Mackinnon and Miller (1989)
Rat skeletal T-tubule	—	~85 (30 mV)	2	7.2	0	B-n, A	Golowasch *et al.* (1986)
	—	—	4.2		10		
	—	~200	2	7.0	0	B-n, A	Oberhauser *et al.* (1988)
	—	—	4		5		
	—	—	5.8		10		
Rabbit skeletal T-tubule	226 (100)	> 100	—	7.0	0	B-c, A, E	Latorre *et al.* (1982)
Smooth Muscle							
Guinea pig mesenteric artery	198 (126)	0.2–1	1.05	7.2	—	P, E	Benham *et al.* (1986)
Rabbit portan vein	273 (142)	~0.8	cal 1.3	7.2	0	P, E	Inoue *et al.* (1985)
	180 (142)	1000 (external)	—	7.2	0	P, E	Inoue *et al* (1986)
Cultured bovine aorta	266 (150)	~10 (40 mV)	—	7.3	0.25	P, E	Williams *et al* (1988)
Rabbit trachea	184 (130)	0.3	—	7.4	0	P, E	Kume *et al.* (1990)
		3	—	7.0			
Rabbit jejunum	183 (126)	0.1–1	—	7.2	—	P, E	Benham *et al.* (1986)
Rabbit colon	210 (126)	5	—	7.2	0	P, E	Mayer *et al.* (1990)
Canine colon	200 (140)	0.5–1	5.3	7.4	0	P, E	Carl and Sanders (1989)
Guinea pig taneae coli	147 (135/5)	1	cal ~1	7.28	0	P, E	Hu *et al.* (1989)
Toad stomach	250 (130)	1–10	—	7.2	2	P, E, A	Singer and Walsh (1987)
Neurons and Glia							
Rat hippocampus	220 (150)	4	1.4	7.3	0	P, E	Franciolini (1989)
Rat brain	242 (150)	~1.5	2.5	7.2	1	B-c, E	Reinhart *et al.* (1989)
	236 (150)	~8	2.1	7.2	1	B-c, E	
Astrocytoma	250 (110)	1	—	7.4	0	P, E	Pallotta *et al.* (1987)

Secretory and epithelial cells							
Mouse lacrimal gland	200 (140)	0.01	—	7.2	1.13	P, E	Findlay (1984)
Pig pancreas acinar	200 (140)	0.01–0.1	—	7.2	1.13	P, E	Maruyama *et al.* (1983)
Pig pancreas islet	250 (145)	~1	—	7.2	1.13	P, E	Findlay *et al.* (1985)
Rat pancreas beta	200 (144)	20–100	1–2	7.20–7.25	0.1–0.5	P, E	Tabcharini and Misler (1989)
Cultured rabbit kidney	141 (135/5)	~3	cal ~2.3	7.4	0	P, E	Cornejo *et al.* (1987)
Mudpuppy gallbladder	200 (100)	0.4	2.6	7.4	0	P, E	Segal and Reuss (1990)
Mudpuppy enterocytes	170 (100)	~1	1.7	7.2	1.13	P, E	Sheppard *et al.* (1988)
Frog, mudpuppy choroid plexus	224 (112)	10	—	7.4	0	P, E	Christensen and Zeuthen (1987)
IK							
Mouse olfactory neurons	130 (140)	0.5–1 (−40 mV)	—	7.4	2	P, E	Maue and Dionne (1987)
	92 (140)	~1 (−40 mV)	—	7.4	2	P, E	
Rat brain	135 (150)	—	2.2–3.2	7.2	1	B-c, E	Reinhart *et al.* (1989)
	76 (150)	—	2.2–3.2	7.2	1	B-c, E	
Cultured rat aorta	135 (142)	>1	—	7.2	0	P, E	Sadoshima *et al.* (1988)
Rabbit portan vein	92 (142)	>0.2	—	7.2	0	P, E	Inoue *et al.* (1985)
Paramecium	72 (100)	2 (−50 mV)	2.6	7.0	0	P, E	Saimi and Martinac (1989)
SK							
Cultured rat skeletal muscle	12 (140)	0.2–0.5	—	7.2	0	P, E	Blatz and Magleby (1986c)
Anterior pituitary (GH3)	11 (150)	1–3	—	7.2	2	P, E	Lang and Ritchie (1987)
Guinea pig hepatocyte	20 (150/135)	0.6–1	—	7.3	0	P, E	Capoid and Ogden (1989)
Human red blood cell	25 (140)	2	—	7.4–7.6	0	P, E	Grygorczyk *et al.* (1984)
Rat T and human B lymphocyte	34 at −80 mV (140)	>0.3	—	7.2	1	P, E	Mahamut-Smith and Schlichter (1989)
Rat sympathetic neuron	—	~1	0.8	7.3	0	W, F	Gurney *et al.* (1987)

[a] G is the single channel conductance and (K) is the potassium concentration in the experiment. When one number is given, the potassium concentration is symetrical, otherwise the concentrations are given as (in/out). $[Ca]_{1/2}$ is the internal calcium concentration that produces an open probability of 0.5 at 0 mV or, if this number was not available, at the specified membrane potential. Hill is the hill coefficient of the dose–response curve for calcium activation of the channels (see text). Cal written next to the hill coefficient indicates that this value was calculated from data in the paper. Exp gives the experimental conditions, where P represents patch clamp experiments, B is for bilayer experiments done with neutral (n) or charged (c) lipids, and W means whole cell recording. The free calcium concentration was determined with either a calcium–EGTA or calcium-BAPTA buffer system (E), direct measurement of total calcium concentration with atomic absorption spectrophotometry (A), or light-activated dissociation of calcium from photosensitive calcium buffers (F). A dash indicates that this value was not given in the paper.

affects the calcium sensitivity of maxi-K channels in some tissues. A tenfold increase in internal proton concentration caused a tenfold decrease in calcium sensitivity in pancreatic beta cells (Cook *et al.*, 1984). Maxi-K channels in smooth muscle are more sensitive to internal pH, with a threefold increase in proton concentration causing an eightfold decrease in $Ca_{1/2}$ (Kume *et al.*, 1990). Internal magnesium increases the calcium sensitivity of maxi-K channels from skeletal muscle (Golowasch *et al.*, 1986; Oberhauser *et al.*, 1988), but not smooth muscle (Toro *et al.*, 1990).

A source of apparent variation in calcium sensitivities reported for maxi-K channels is the use of different binding constants when calculating the free calcium concentration in a calcium–EGTA buffer system. Failure to account for the effects of temperature, pH, ionic strength, other divalent cations, and the purity of EGTA samples can result in large errors in estimating free calcium concentrations in such buffer systems (Miller and Smith, 1984).

The sensitivity of some maxi-K channels to calcium in intact cells may be modulated by a number of factors, including c-AMP dependent protein kinase (Ewald *et al.*, 1985), GMP (Williams *et al.*, 1988) and G-proteins (Cole and Sanders, 1989; Toro *et al.*, 1990). Changes in the activity of these factors could lead to even wider variations in the calcium sensitivity of maxi-K channels in intact cells than are seen in Table I. Such variations in calcium sensitivity in different cells may allow this channel to play a role in diverse processes in different cells.

The variations in calcium sensitivity of maxi-K channels seen in Table I are probably too great to be entirely explained by the experimental differences noted above. The basis of these variations in calcium sensitivity may be understood with the help of peptide toxins to characterize the properties of these channels in different tissues, or by examination of maxi-K channels from different tissues under identical defined conditions (such as a lipid bilayer).

Hill Coefficients Suggest Four or More Calcium Binding Sites

The Hill coefficient n measures the slope of the dose–response relation between calcium and open probability of the channel. It is obtained by fitting the channel open probability measured at different calcium concentrations with the equation shown above. This value gives an estimate of the minimum number of functional ligand binding sites on a receptor (Adair, 1925). The observed Hill coefficient depends on the number of binding sites, the interactions between the binding sites, and the relationship between occupancy of binding sites and the experimentally measured parameter (open probability in this case).

The Hill coefficients listed in Table I range from about 1 to almost 6. Most values in Table I are close to 2 or 3. Most of the quantitative data was obtained in skeletal muscle where the Hill coefficients are typically ~ 2–4 in the absence of magnesium and 4–6 in the presence of millimolar magnesium (Table I). The simplest interpretation of this data is that the maxi-K channel in skeletal muscle has at least four to six binding sites for calcium that are functionally involved in the gating of the channel.

Magnesium alone does not directly activate the channel, suggesting that magnesium may bind to sites on the channel separate from the calcium binding sites involved in activation of the channel (Oberhauser *et al.*, 1988). The increase in the Hill coefficient induced by magnesium may be explained by a number of possible mechanisms. Magnesium could expose previously inaccessible calcium binding sites, change the interactions between the calcium binding sites, or affect the coupling between occupancy of the calcium sites and opening of the channel (Golowasch *et al.*, 1986). Magnesium probably does not affect the Hill coefficient through a surface charge effect because changes in membrane potential (Golowasch, *et al.*, 1986), surface charge of the lipids (Reinhart *et al.*, 1989), or changes in the external surface charge of the channel protein induced by trimethyloxonium modification (MacKinnon and Miller, 1989) do not affect the Hill coefficient.

Properties of the Calcium Binding Site

Maxi-K channels from skeletal muscle can be activated by a number of divalent cations, although calcium is the most effective (McManus and Magleby, 1984; Oberhauser *et al.*, 1988). Oberhauser *et al.* (1988) found the following selectivity sequence for channel activation by divalent cations: $Ca^{2+} > Cd^{2+} > Sr^{2+} > Mn^{2+} > Fe^{2+} > Co^{2+}$. Mg^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, and Ba^{2+} did not measurably activate the channel. This sequence for activation nearly matches the sequences for binding affinities of divalent ions to calmodulin and troponin C(see references in Oberhauser *et al.*, 1988). The ability of divalent ions to activate the channel depends on ionic radius, as only divalent cations with radii between 0.072 and 0.113 nm could activate the channel (Oberhauser *et al.*, 1988). In addition to ionic size, details of the bonding interactions between cations and the channel determine the ability of cations to activate the channel because cations with similar radii (Ca^{2+} and Cd^{2+}) had a ~ 100-fold difference in potency, and trivalent cations (Tb^{3+} and Eu^{3+}) with ionic radii similar to calcium did not activate the channel. Some divalent ions do not activate the channel possibly because they do not bind to the activation site or because the channel does not make the conformational changes leading to channel opening after these ions have bound (Oberhauser *et al.*, 1988).

Inhibition of Maxi-K Channels by Intracellular Calcium

High concentrations of intracellular calcium can cause a decrease in the open probability of maxi-K channels in skeletal muscle (Methfessel and Boheim, 1982; Moczydlowski and Latorre, 1983; Vergara and Latorre, 1983), smooth muscle (Benham *et al.*, 1986; Mayer *et al.*, 1990), secretory cells (Marty *et al.*, 1984; Findlay, *et al.*, 1985; Jia *et al.*, 1988), and epithelial cells (Kolb *et al.*, 1986). The inhibitory effect of intracellular calcium is enhanced at depolarized membrane potentials (Methfessel and Boheim, 1982; Vergara and Latorre, 1983; Marty *et al.*, 1984; Benham *et al.*, 1986; Findlay *et al.*, 1985; Kolb *et al.*, 1986). This inhibition can occur at micromolar calcium concentrations in secretory cells that are activated by submicromolar concentrations of calcium (Marty *et al.*, 1984; Findlay *et al.*, 1985; Jia *et al.*, 1988). Inhibition by intracellular calcium occurs at millimolar calcium concentrations in skeletal (Vergara and Latorre, 1983) and smooth muscle (Mayer *et al.*, 1990). Maxi-K channels from skeletal muscle display a time- and voltage-dependent inactivation in response to step depolarizations, due to entry into a long-lifetime closed state from a brief-duration open state (Pallotta, 1985).

Kinetic Studies of Maxi-K Channels Reveal the Expected Number of States

Single-channel recording allows the expeimenter to observe, in real time, transitions between open and closed conformations of a single-channel molecule. The durations of the open and closed events reflect the time spent in different conformational states of the channel involved in gating. Statistical analysis of open and closed durations can provide information about the number of conformations of the channel involved in gating and about the relationships between these conformations. For a channel with discrete states, each different open conformation will give rise to an exponential component in the distribution of open-interval durations under steady-state conditions, although not all exponential components may be detected (Colquhoun and Hawkes, 1977, 1981). The same rules apply to closed intervals. In order to obtain a minimum estimate of the number of channel states involved in gating, one can simply count the number of exponential components in the distributions of open and closed interval durations.

This approach is conceptually straightforward, and is best used with large amounts of stable data from a single channel. The methods used in this approach are described in Colquhoun and Sigworth (1983) and Horn and Lange (1983) and briefly consist of fitting the distributions of all interval durations with an increasing number of exponential components with maximum likelihood techniques until a significant improvement in the likelihood is not longer obtained. The number of significant components supported by

the data gives a minimum estimate of the number of kinetic states involved in gating.

The most extensive kinetic data for maxi-K channels are available in skeletal muscle where six to eight closed and three to four open states were observed over a wide range of channel open probability (McManus and Magleby, 1988). At least one of these closed states is likely a long-lifetime inactivated state of the channel (Barrett *et al.*, 1982; Pallotta, 1985) that is not involved in activation of the channel and may be due to block of the channel by calcium (Vergara and Latorre, 1983). Six or more components were observed at low calcium levels (McManus and Magleby, 1988) where calcium block would be negligible (Vergara and Latorre, 1983), suggesting that at least six closed states are involved in activation of the channel by calcium.

The Hill coefficients for the maxi-K channel in this tissue (Table I) suggest that the gating of the channel may be affected by the binding of four or more calcium ions. Binding of each calcium ion would be expected to create an additional chemical state of the channel, so one might expect to see a limited number of kinetic states that correspond to the channel with different numbers of calcium ions bound to it. In the simplest physical model where calcium binds to four identical activating sites when the channel is closed, as many as five distinct closed states might be seen. If the calcium binding sites are not identical or binding of calcium causes conformational changes in the closed channel that affect binding of additional calcium ions, then additional kinetic states might be expected. For example, if the channel has two pairs of different calcium binding sites that are always accessible, then nine different closed states would be expected that correspond to occupancy of the various sites by calcium. If the channel has four different calcium binding sites that are always accessible to calcium, then as many as 16 different closed states could exist. The kinetic experiments mentioned above have found evidence for about six closed states of the channel involved in activation by calcium, which suggests that either many of the binding sites for calcium are functionally similar, or that if they are different, calcium must bind to the different sites in a specific sequence. Thus the observation of at least six closed kinetic states of the channel is consistent with the minimum number expected for a channel whose gating is affected by the binding of four or more calcium ions.

Two to four kinetic open states of the maxi-K channel in skeletal muscle have been reported (Barrett *et al.*, 1982; Magleby and Pallotta, 1983a; McManus and Magleby, 1988). The observation of fewer open than closed states is consistent with a model for the channel where the channel cannot open unless one or more calcium ions have bound to the channel. Magleby and Pallotta (1983a) observed that the frequency of observing brief open intervals increased approximately linearly and then declined somewhat as

calcium was raised, while the frequency of observing long openings increased with the second power of calcium concentration. No openings were observed in the absence of calcium. Although it is often unrealistic to identify kinetic states with specific components in a distribution of interval durations, these results suggest that the channel can open for brief times with one calcium ion bound and for longer times with two or more calcium ions bound and does not open when no calcium ions are bound to the channel. Thus the different open states may reflect differences in the number of calcium ions bound to the channel. Alternatively, the observation of fewer open than closed states may have a trivial explanation. The durations of the open intervals fall within a much narrower range than the shut durations, making it more difficult to separate components with small areas or similar time constants.

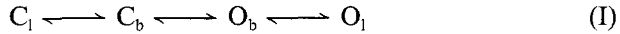
Fewer kinetic states were resolved in studies of maxi-K channels in smooth muscle (Benham *et al.*, 1986; Hu *et al.*, 1989; Carl and Sanders, 1989; Kume *et al.*, 1990) and epithelial cells (Kolb *et al.*, 1986; Cornejo *et al.*, 1987; Sheppard *et al.*, 1988) than in the skeletal muscle data described above. This difference may be related to the lower Hill coefficients reported in these tissues (Table I).

Transition Pathways between Open and Closed States

The distributions of interval durations suggest that the maxi-K channel can enter a number of kinetic states. A basic question about the mechanisms controlling channel gating involves the sequence of transitions between these states. The sequence of transitions between the various states is reflected, in a complex way, in the observed sequence of open and closed intervals. Analysis of the sequence of events can provide information about the structure of the gating model.

One can distinguish between gating mechanisms that allow a single or multiple transition pathways between open and closed states by examining the durations of adjacent open and closed intervals. If the transition rates between the states remain constant over time under steady-state conditions, then the time spent in any state will be independent of which states were previously entered. As a result, the durations of adjacent open and closed intervals will be independent of each other for models with a single transition pathway between open and closed states. For example, schemes I and II both contain a brief (C_b) and a long (C_l) lifetime closed state, and a brief (O_b) and a long (O_l) lifetime open state, and could thus generate similar distributions of interval durations. These schemes differ in that scheme I has a single transition pathway between open and closed states while scheme II has two pathways. Scheme I predicts that the durations of adjacent open and closed intervals must be independent, while scheme II permits a nonrandom relation-

ship between the durations of sequential open and closed times where long open times may preferentially occur next to brief times


(I)

(II)

McManus *et al.* (1985) found an inverse relationship between the durations of adjacent open and closed events for the maxi-K and fast Cl channels in skeletal muscle. These results suggest multiple transition pathways between open and closed states for the maxi-K channel. In addition, these results confirm the existence of multiple open and closed states of the maxi-K channel.

These results are consistent with the data of Magleby and Pallotta (1983a) which suggested that the maxi-K channel from skeletal muscle can open to different open states depending on the number of calcium ions bound to the channel.

The same information can be expressed in a more quantitative form as the cross correlation function between open and closed times (Colquhoun and Hawkes, 1987; Ball *et al.*, 1988). Similar information can also be obtained from the autocorrelation function of the durations of successive open durations or closed durations (Fredkin *et al.*, 1985; Colquhoun and Hawkes, 1987). For examples, if the channel makes repeated transitions between the brief closed state and the long open state in scheme II (because the transition rate between C_b and O_l is much greater than between C_b and C_l), then a sequence of longer than average openings would be expected. The autocorrelation function measures the tendency of open times (or closed times) to occur in such clumps. Analysis of the distributions of cumulative probabilities associated with a sequence of open or closed times may be a more sensitive measure of nonrandom grouping of dwell times than autocorrelation functions (Ramanan and Brink, 1990).

Kinetic Models for Activation of Maxi-K Channels by Calcium

The duration of open and closed intervals recorded from maxi-K channels can cover a wide time range, which suggests that the channel can enter a number of different open and closed states. Unfortunately, transitions between states with the same conductance cannot be directly observed at the single channel level, although, in some cases, charge movement associated

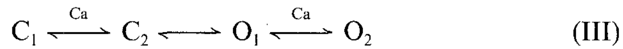
with these transitions can be measured from a population of channels as gating currents. Because the transitions within an aggregate of open or closed states cannot be directly observed, the properties of the states within an aggregate have been the subject of some speculation. At the two extremes, the states may be considered as a smooth continuum of a very large number of states (see, for example, Levitt, 1989) or as a small collection of discrete states. The attraction of some models based on a continuum of states is that a model containing a small number of parameters can generate data over a wide dynamic range, although, if some simplifying assumptions are allowed, discrete state models with few parameters can also predict complex single-channel behavior (see, for example, Zagotta and Aldrich, 1990). The relative merits of any two models for a particular ion channel may be evaluated by statistical comparisons with data from that channel (Horn, 1987). I would like to present arguments for the use of discrete state models over continuum models for the gating of maxi-K channels and not dwell on statistical comparisons between specific models.

Maxi-K channels open in response to intracellular calcium, presumably due to binding of a finite number of calcium ions to the channel. Binding of a finite number of calcium ions would give rise to a finite number of distinct chemical states of the channel. Interestingly, the number of kinetic states observed for this channel are consistent with the number expected for a channel that binds four or more calcium ions (see above discussion).

McManus and Magleby (1989) tested one prediction of discrete state Markov models and found that gating of maxi-K channels in skeletal muscle was consistent with this prediction. For a Markov model under steady-state conditions, the time spent in any state is independent of the pathway by which the state was entered. Therefore the behavior of the system at any moment is independent of previous history and is memoryless. The lifetimes of the kinetic states will be reflected, in a complex way, in the time constants of the distributions of open and closed times. It follows that the time constants in the conditional distributions of open or closed times should be independent of the durations of the adjacent events used to select the conditional distributions (Fredkin *et al.*, 1985). McManus and Magleby (1989) found that the time constants in the conditional distributions of open and of closed times were independent of adjacent interval duration and hence of pevious channel activity. The areas of some components of the conditional distributions changed as a function of the adjacent dwell times, showing that the kinetics of the channel changed in the different conditional distributions. These results suggest that the gating of the maxi-K is independent of previous activity, as predicted for a Markov process. These arguments support the use of discrete-state models for the gating of maxi-K channels as a simple and reasonable starting point in efforts to understand the gating of these channels.

Previous Models for Gating of Maxi-K Channels

A number of determined efforts have been made to develop a model that can quantitatively describe the gating of the maxi-K channel. Moczydlowski and Latorre (1983) presented a four-state model (scheme III) with two calcium binding steps that gave an excellent description of the open probability of the maxi-K channel from skeletal muscle as a function of calcium and membrane potential.



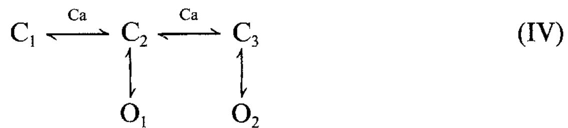
$$C_1 \overset{Ca}{\rightleftharpoons} C_2 \rightleftharpoons O_1 \overset{Ca}{\rightleftharpoons} O_2 \quad \text{(III)}$$

In this model, the voltage dependence of the gating of the channel was determined by the voltage dependence of calcium binding to the channel. The binding of calcium to the open and closed states was assumed to be very fast, so that the two open states would be in rapid equilibrium, as would the two closed states. This assumption of fast binding explains their observation of single kinetic components in the distributions of open and closed times. This model is consistent with other data. The relationship between calcium and open probability was fit with a slope of 1.2 to 2 on Hill plots, suggesting that two or more calcium ions bound to activate the channel. The time constants of both the open and closed time distributions changed linearly as a function of calcium concentration, suggesting that a calcium ion could bind to both open and closed states of the channel. At low calcium concentrations, the mean open times converged to a single value at all voltages and, at high calcium concentrations, the mean closed times also converged to a single value at all voltages. This observation suggested that the transition between open and closed states was not very voltage dependent.

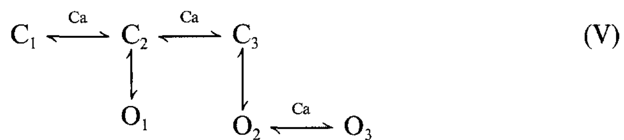
This framework allowed Moczydlowski and Latorre to estimate the location of the calcium binding sites within the membrane field and the affinity of the two sites for calcium, although it should be remembered that these estimates are model dependent. The affinity of calcium for the closed state was weaker than for the open state. Interestingly, the estimates of the location of the two binding sites within the membrane field differed, with the binding site for the closed channel state sensing ~75% of the voltage drop across the membrane and the binding site for the open channel sensing ~95%. This suggests that the two binding sites may be different and that calcium binds to the two sites in a specific sequence, perhaps because the binding site for the open state is not accessible unless the channel is open. Alternatively, the binding sites may be identical, but when the channel opens, the local voltage gradient at the binding sites changes, leading to an apparent shift in the location of the binding site within the membrane field. These results suggest that the calcium binding sites are close to the outside face of the channel, but are only accessible to calcium ions from the inside.

Methfessel and Boheim (1982) presented an activation-blockade model for the gating of the maxi-K channel in rat myotubes. They proposed a high-affinity binding site for calcium on an "activation" gate; binding of calcium to this site leads to opening of the channel. They also proposed a second low-affinity site for calcium on a negatively charged blocking particle. This particle blocks the channel in a voltage-dependent manner; binding of calcium to this gating particle neutralizes its charge and prevents the gating particle from blocking the channel. In this model, the voltage dependence of gating results from the blocking reaction, which, in most cases, represents the open–closed transition. This model qualitatively accounts for the increase in open times and decrease in closed times observed as internal calcium was raised. If one assumes that activation and blockade are strictly coupled with activation preceding the blocking reaction, then the model of Methfessel and Boheim is equivalent to the four-state model of Moczydlowski and Latorre (1983). However, the voltage dependence of gating in the model of Methfessel and Boheim lies in the open–closed transition, while in the model of Moczydlowski and Latorre, the voltage dependence is due to the voltage dependence of calcium binding.

Magleby and Pallotta (1983a, b) proposed two kinetic schemes for the calcium-dependent gating of maxi-K channels in skeletal muscle,



(IV)



(V)

where C and O represent closed and open states, respectively. Scheme IV gave an excellent quantitative description of the observed distributions of open and closed interval durations (Magleby and Pallotta, 1983a) and of the distribution of burst durations and number of openings per burst (Magleby and Pallotta, 1983b) at a single calcium concentration. However, scheme IV was unable to predict the calcium-dependent changes in the open and closed time distributions. Scheme V contains an additional open state and was also able to fit the distributions of open and closed times at a single calcium concentration and could predict the calcium-dependent changes in the time constants of the open-time distribution. However, scheme V did not account for other effects of calcium on the gating of the channel.

Identifiability of a Model for the Maxi-K Channel

One goal of kinetic analysis of the maxi-K channel is to obtain the best quantitative model of channel gating. It might be asked whether this is possible. Simply, can the correct model be identified? This question has been examined in detail in a number of recent papers (Fredkin *et al.*, 1985; Fredkin and Rice, 1986; Bauer *et al.*, 1987; Ball and Sansom, 1989; Kienkar, 1989). The general question of identifiability of models may be divided into two more specific questions. (1) For any given model and data set, is there a unique combination of rate constants that provides the best solution? (2) Can the single best model be identified?

In order to obtain a unique best set of rate constants for a given model, the data must contain enough information to specify all of the rates in the model. To determine whether this is possible, we must estimate how much kinetic information is contained in the single-channel data, and how much information is necessary to specify the rates. I would like to address these questions by first discussing some specific examples and then considering the general case.

The simplest model contains an open and a closed state and two rates. These two parameters can be determined from the time constants of the distributions of open and closed times. Scheme II is a four-state model with six rates that determine the behavior of the system. The distribution of open times generated by this model would be expected to contain two exponential components as would the closed time distribution. Each component of these distributions would specify two parameters, a time constant and an area, with the restriction that all of the areas must sum to one for each distribution. This data can specify up to $2(N_o + N_c - 1) = 6$ parameters, where N_o is the number of open states and N_c is the number of closed states. Both the model and the data contain six parameters and one might hope that this would be sufficient to specify a unique set of best rates for this model. However, Blatz and Magleby (1986b) have shown that, for a slightly more complicated model, a unique set of rate constants could not be found when the number of parameters in the distributions equalled the number of parameters in the model. Thus, for many models, the distributions of open and closed times obtained at a single steady-state condition are not sufficient to identify a unique set of best rates.

Two approaches may yield additional kinetic information from single-channel data. The simplest method is to collect data under a number of different experimental conditions. For an agonist-activated channel, such as the maxi-K channel, data collected at different calcium concentrations should all be predicted by the same single set of rates. This requirement that the model account for calcium-dependent changes in the open and closed time

distributions places great restrictions on the rates and increases the chances of identifying a unique best solution.

Analysis of the sequence of occurrence of the events can provide additional information that is not contained in the distributions of open and close times. Three methods can be used to obtain estimates of the rates based on the information contained in the sequence of events.

In the first method, the information obtained from autocorrelation (Fredkin *et al.*, 1985; Colquhoun and Hawkes, 1987; Ball and Sansom, 1988) or cross-correlation functions (Steinberg, 1987; Ball *et al.*, 1988) fit to the data are compared with the predictions of a given model and set of rates, and this information is used to help distinguish solution sets that give identical open and closed time distributions. This approach was used by Blatz and Magleby (1989) to distinguish among a set of models for the fast Cl^- channel from skeletal muscle that could not be distinguished based on the open and closed time distributions alone. A difficulty with this approach is that one has to make arbitrary decisions about how to weight the fits to the event time distributions and the autocorrelation functions.

The other two approaches avoid the problem of how to weight the fits to different types of data by fitting *all* of the data to the model in one operation. The second approach was developed by Horn and Lange (1983) and applied to sodium channel currents by Horn and Vanderberg (1984). The probability of observing the complete sequence of events in the data is calculated for a given model and set of rates. The rates are then changed until those with the greatest likelihood given the data are found. The advantage of this method is that all of the information in the data is used. This method can be computationally demanding because of the large number of conditional probabilities that are evaluated.

In the third method, a model is fit to pairs of sequential open and closed times (Fredkin *et al.*, 1985; Ball and Sansom, 1989). These pairs of open and closed times can be plotted in so-called two-dimensional distributions where the open time of the pair is plotted in one dimension, the closed time of the pair is plotted in the second dimension, and the density of pairs of particular durations is plotted in the third dimension. The advantages of this method are that all of the kinetic information is contained in these two-dimensional distributions (Fredkin *et al.*, 1985; Fredkin and Rice, 1986; Bauer *et al.*, 1987) and that the data can be compressed into a small number of binned open and closed time pairs. It should be pointed out that the two-dimensional distributions contain the same information as the standard open and closed time distributions for models with a single transition pathway between open and closed states (Fredkin *et al.*, 1985).

How much kinetic information can be obtained from single-channel data using these techniques? For a channel with two conductance levels, the

two-dimensional distributions contain enough information to specify up to $2(N_o^* N_c)$ parameters (Fredkin *et al.*, 1985; Bauer *et al.*, 1987). If the gating of the channel is assumed to be at thermodynamic equilibrium so that the gating is statistically identical when viewed forwards or backwards in time, then the two-dimensional distributions can specify up to $(N_o^* N_c + N_o + N_c - 1)$ parameters (Bates *et al.*, 1990). When data are collected under many different experimental conditions, such as agonist concentrations, more parameters can be determined.

The number of possible transition rates between states can be as high as $n(n - 1)$, where n is the total number of open and closed states (Bauer *et al.*, 1987) if transitions are allowed between all states. It seems unlikely that transitions occur between all states of the maxi-K channel because then the channel would have to bind multiple calcium ions and open in a single step. A more plausible model might allow sequential binding of calcium ions, which would reduce the number of possible transitions. Thus the number of rates in a model of the gating of the maxi-K channel can be less than the number of parameters that can be specified by the data. This means that it may be possible to obtain a unique best set of rates for a particular model, but tests of uniqueness would be necessary for each specific case.

The task of finding a unique set of rates for a model becomes more difficult when the time constants in the distributions are similar and when the necessary filtering of the data causes brief events to be missed. Additional equally likely solutions can occur in the presence of missed events, although the artifactual solutions can be identified by comparing the solutions obtained at different levels of filtering (Blatz and Magleby, 1986a; Yeo *et al.*, 1988).

A more difficult problem of identifiability involves finding the correct model among a large group of models. Some models may be excluded from consideration because they are unable to describe the distributions of open and closed times, as was done by McManus *et al.* (1988) and Korn and Horn (1988) for fractal type models of the maxi-K channel. Other models can sometimes be eliminated if they are unable to predict the dose–response curve for calcium or the observed relationship between adjacent interval durations. For instance, McManus *et al.* (1985) were able to eliminate all Markov models for the maxi-K channel in skeletal muscle that contained only a single transition pathway between open and closed states by examining durations of adjacent interval durations. A more difficult exercise is to distinguish between models that, under some circumstances, can give reasonable fits to the data. Blatz and Magleby (1989) were able to distinguish between a number of models that gave equally good descriptions of the distributions of event durations for the fast Cl channel in skeletal muscle by comparing the predictions of the models with the observed relationships between adjacent interval durations.

A more general and objective way to distinguish between models is to statistically compare the ability of different models to fit the data. The ability to distinguish between different models will be enhanced by using all of the information contained in the data and obtaining data under different experimental conditions as discussed above. A problem with this approach is that one has to obtain the "best" parameters for each model for the comparisons to be valid.

Kienkar (1989) proposed a formal way to examine the identifiability of models using similarity transforms. He has shown that if the matrices of transition rates for two models can be related by a similarity transform that does not combine states with different conductances, then these two different models can predict the same single-channel behavior and are therefore not identifiable. It becomes more difficult to relate different models through similarity transformations when data are collected under several experimental conditions, such as agonist concentrations.

Properties of SK and IK Channels

SK channels can be differentiated from maxi-K channels based on differences in single-channel conductance, sensitivity to calcium and voltage, and pharmacology (see recent reviews by Blatz and Magleby, 1987 and Latorre *et al.*, 1989). Briefly, SK channels have a smaller single-channel conductance of ~10–20 pS, their gating is less voltage dependent and more sensitive to calcium than maxi-K channels from the same tissue at negative membrane potentials, and they are typically blocked by apamin, but not by charybdotoxin or submillimolar concentrations of external tetraethylamonium.

IK channels are a loosely defined group and show a less clear pharmacological profile than maxi-K or SK channels. For instance, IK channels in rat brain are blocked by submillimolar TEA (Reinhart *et al.*, 1989), while other IK channels in rabbit portal vein are not (Inoue *et al.*, 1985). IK channels also share pharmacological similarities with some maxi-K and SK channels found in the same tissues. For instance, IK channels and one type of maxi-K channel found in rat brain synaptosomes are blocked by charybdotoxin (Reinhart *et al.*, 1989).

Functions of SK and IK Channels

SK channels are generally more sensitive to calcium at negative membrane potentials than maxi-K channels from the same tissue (Blatz and Magleby, 1987; Latorre *et al.*, 1989) and therefore may be involved in

controlling membrane excitability near the resting potential. In excitable cells, the SK channel contributes to the slow after-hyperpolarization following the action potential in GH_3 cells (Lang and Ritchie, 1990), neurons (Pennefather *et al.*, 1985), and cultured skeletal muscle cells (Romey and Lazdunski, 1984). The SK channel may regulate action potential frequency in some cells. Block of this channel in GH_3 cells increased the rate of spontaneous action potentials in some GH_3 cells (Lang and Ritchie, 1990), and the aberrant appearance of this channel in skeletal muscle cells of patients suffering from myotonic muscular dystrophy (Renaud *et al.*, 1986) may contribute to the abnormal trains of action potentials observed in response to electrical stimulation, and the impaired ability to relax after voluntary contractions. In nonspiking cells, the SK channel may be involved in the response to hormonal stimulation in hepatocytes (Banks *et al.*, 1979) and GH_3 cells (Lang and Ritchie, 1987).

In a detailed study of the behavior of IK channels *in situ*, Gola *et al.* (1990) found this channel contributes to spike repolarization and frequency-dependent firing blockade in a snail neuron.

Calcium Sensitivity of SK and IK Channels

Less is known about the gating of SK and IK channels than is known about maxi-K channels. Examination of Table I suggests that SK channels are more sensitive to calcium than maxi-K channels in skeletal muscle. When recording from maxi-K and SK channels in the same membrane patch excised from skeletal muscle, Blatz and Magleby (1986c) found that SK channels were activated at a tenfold lower calcium concentration than was required to activate maxi-K channels. The calcium sensitivity of SK channels (0.1–1 μM causes half maximal activation) is comparable to the calcium sensitivity of maxi-K channels found in some smooth muscle and secretory cell preparations. The calcium sensitivities reported in Table 1 for 1K channels (half activation at $\sim 1\ \mu$M) are comparable to the calcium sensitivities of maxi-K channels from smooth muscle. Examination of the steepness of the calcium dose–response curves for SK channels from cultured skeletal muscle (Blatz and Magleby, 1986c) and guinea pig hepatocytes (Capoid and Ogden, 1989) suggests that more than one calcium ion may be involved in activating the SK channel.

Future Prospects

Peptide toxins that block specific types of calcium-activated K channels have been useful tools in efforts to define the roles of these channels in

different tissues. Scorpions are a rich source of toxins that block K channels (see review by Garcia *et al.*), and the discovery of additional toxins should aid attempts to understand the functional roles of calcium-activated K channels.

These toxins may also serve as biochemical markers for these channels, and may be useful in attempts to isolate these channels. Structure–function studies with a purified or cloned channel may allow tests of some predictions based on kinetic studies, including the number of binding sites for calcium on a channel. The results of the kinetic studies discussed in this review may serve as a framework for mutagenesis studies on the gating properties of these channels.

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