The Ca^{2+}-activated K^+ Channel and Its Functional Roles in Smooth Muscle Cells of Guinea Pig Taenia Coli

S. L. HU, Y. YAMAMOTO, and C. Y. KAO

From the Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203

ABSTRACT   Currents through single potassium channels were studied in cell-attached or inside-out patches from collagenase-dispersed smooth muscle cells of the guinea pig taenia coli. Under conditions mimicking the physiological state with $[K^+]_i = 135$ mM: $[K^+]_o = 5.4$ mM, three distinct types of K^+ channel were identified with conductances around 0 mV of 147, 94, and 63 pS. The activities of the 94- and 63-pS channel were observed infrequently. The 147-pS channel was most abundant. It has a reversal potential of ~ -75 mV. It is sensitive to $[Ca^{2+}]_i$ and to membrane potential. At -30 mV, the probability of a channel being open is at a minimum. At more positive voltages, the probability follows Boltzman distribution. A 10-fold change in $[Ca^{2+}]_i$ causes a 25-mV negative shift of the voltage where half of the channels are open; an 11.3-mV change in membrane potential produces an e-fold increase in the probability of the channel being open when P is low. At voltages between -30 and -50 mV, the open probability increases in an anomalous manner because of a large decrease of the channel closed time without much change in the channel open time. This anomalous activity may play a regulatory role in maintaining the resting potential. The histograms of channel open and closed time fit well, respectively, with single and double exponential distributions. Upon step depolarizations by 100-ms pulses, the 147-pS channel opens with a brief delay. The delay shortens and both the number of open channels and the open time increase with increasing positivity of the potential. The averaged currents during the step depolarizations closely resemble the delayed rectifying outward K^+ currents in whole-cell recordings.

INTRODUCTION

Until the introduction of the tight-seal patch-clamp technique (see Hamill et al., 1981), little was known about single K^+ channels in smooth muscle membranes. Among the several types of K^+ channel now known (Bolton et al., 1985; Inoue et al., 1985, 1986), a large conductance Ca^{2+}-activated K^+ channel has been studied in some detail on amphibian (Berger et al., 1984; Wong, 1985; Singer and Walsh,

1987) and mammalian smooth muscle cells (Benham et al., 1985, 1986; Inoue et al., 1985, 1986; Wolff et al., 1985; McCann and Welsh, 1986). Most of these investigations were conducted on membrane preparations exposed to symmetrical high K^+ concentrations on both sides of the membrane. Although channel openings were facilitated by such procedures, the environment was unphysiological, and the observations cannot be readily related directly to familiar physiological phenomena. A systematic investigation of K^+ channels with respect to channel types, kinetics, permeation and blockade under conditions mimicking the physiological state with an asymmetrical K^+ concentration should facilitate an understanding of the physiological roles of the single K^+ channels in cellular function.

In this paper we report our observations of the coexistence of three types of K^+ channel in the myocytes of the guinea pig taenia coli. Particular attention is focused on one of the channels with large conductance, and high sensitivity to voltage and internal Ca^{2+} concentration, its relation to the macroscopic delayed-rectifier current, and its possible regulatory role in maintaining the resting potential.

Some preliminary results have been published elsewhere (Hu et al., 1987a, b).

MATERIAL AND METHODS

Material and Solutions

Using the patch-clamp technique (Hamill et al., 1981), single-channel currents were recorded from myocytes of the guinea pig taenia coli (see Yamamoto et al., 1989a), in cell-attached or excised inside-out membrane patches. The pipette solution had the following composition (in millimolar): 136 Na^+, 5.4 K^+, 2 Ca^{2+}, 1 Mg^{2+}, 146 Cl^-, 1 $H_2PO_4^-$, 5 glucose, 10 HEPES. The pH was adjusted to 7.30 with NaOH. The solution bathing the inside of the membrane contained (in millimolar): 135 KCl, 10 HEPES, 0.6 EGTA, and varying amounts of added $CaCl_2$ to attain desired levels of $[Ca^{2+}]_i$. The pH was adjusted to 7.28 with KOH. This solution was used for both cell-attached and detached patches. Because the K^+ fully depolarized the cell membrane, the cell potential and pipette potential were the same. In the presence of 0.6 mM EGTA, the internal free Ca^{2+} concentration adjusted by the Ca-EGTA buffering system was 10^{-8} M for 0.1 mM added $CaCl_2$, 10^{-7} M for 0.4 mM, and 10^{-6} M for 0.57 mM (Imai and Takeda, 1967). Our calculated free Ca^{2+} concentrations are in complete agreement with those of Barrett et al. (1982), and ~20% lower than those by Benham et al. (1986). Most experiments on inside-out patches were done with $[Ca^{2+}]_i$ of 10^{-8} M. All experiments were performed at room temperature (22–24°C).

Experimental Procedure

Details of the experimental chamber and perfusion arrangement have been described (Yamamoto et al., 1989a). As a standard procedure, offset potential in the recording system was nulled before forming a giga-seal. The excision of a patch was usually followed by the formation of a closed vesicle at the tip of the pipette. Inside-out patches were made when such vesicles were disrupted upon passing them briefly through the air-fluid interface (Hamill and Sakmann, 1981).

Recording System and Data Analysis

Patch electrodes had diameters of 1.2–1.5 μm and resistances of ~3–4 MΩ. The recording system includes a List EPC-7 amplifier, a low-pass 8-pole Bessel filter (902; Frequency Devices Inc., Haverhill, MA) and a pulse-code modulation (PCM) data recorder with a fre-

quency response of 0–20 kHz ± 0.5 dB (DASS system, Unitrade, Dagan Corp., Minneapolis, MN). Single-channel events were first recorded on video tape with a 10-kHz filter of the amplifier, then played back after each experiment, filtered at 2 kHz (-3 dB), except where otherwise noted. The sampling rate ranged between 2.5 and 10 kHz (12-bit resolution). A window discriminator (AI 2020; Axon Instruments Inc., Burlingame, CA) was employed to detect the opening events that fell between the upper and lower thresholds of the window. An increase in current $>75\%$ of the unitary current was considered a channel opening, and a decrease in the current to $<50\%$ of the unitary current was considered a channel closing. Openings shorter than 0.1 ms were ignored. Data acquisition and analyses were all carried out with the p-Clamp software (Axon Instruments Inc.).

The analyses of single-channel recordings comprised creating idealized records, calculating the channel mean open and mean closed times, forming the histograms for open time, closed time, and amplitude, and performing the exponential fittings for these distributions (see e.g., Colquhoun and Sigworth, 1983). The probability of a channel being in the open state (p) was calculated as the total open time divided by the total observation time (e.g., Barrett et al, 1982; Singer and Walsh, 1987). For patches with multiple channels, the total open time obtained by summing the time at each level weighted by the number of channels open at each level was used to calculate p. The probability-voltage relationship (p-V), in most cases, follows Boltzmann distribution:

$$p = [1 + \exp(-K)(V - V_h)]^{-1} \tag{1}$$

where K is the logarithmic potential sensitivity and V_h the voltage at which $p = 0.5$. From plots of $\ln[p/(1 - p)]$ vs. V, K can be obtained from the slopes of the curves, and V_h from the x-intercept where $\ln[p/(1 - p)] = 0$ (i.e., equal probability of a channel being open or closed).

Unit conductances of the channel reported throughout this paper denote the conductance at 0 mV. They were obtained as the slopes of linear regressions, fitting only those points around 0 mV (usually -20 to $+20$ mV) on single-channel current-voltage (i-V) plots, to avoid the nonlinearity over a wider range of potentials.

In determining the reversal potential, even when the entire i-V plot is available, only those points at potentials more negative than -20 mV were fitted with a linear regression line, the x-intercept of which was taken as the reversal potential. Because of some outward rectification in this voltage region, the value so determined may be slightly less negative than the actual reversal potential. In most cell-attached and excised patches, a direct experimental measurement of the reversal potential is difficult, because channel openings at hyperpolarized potentials were rare.

In experiments where single-channel events during depolarizing steps were studied, the capacitive and the leakage currents were compensated for by digitally adding the current, averaged from 10–20 current records at hyperpolarizing steps to those at depolarizing steps of the same amplitude. Summation, subtraction, and averaging were done with p-Clamp and Lotus 1-2-3 (Lotus Development Corp., Cambridge, MA) software. Data are given as the mean ± SEM, wherever it applies.

RESULTS

Coexistence of Three Types of K⁺ Channels

In excised inside-out membrane patches of taenia myocytes, three distinct levels of outward currents were observed under conditions mimicking the physiological state in which $[K^+]_o$ (K^+ concentration in the pipette) was 5.4 mM, and $[K^+]_i$ (K^+ concen-

tration in the bathing solution) was 135 mM (Fig. 1). The levels were different in the magnitude of their unitary currents and their sensitivity to membrane potential. From the direction of the currents and their sizes as related to patch potential and the reversal potential, K^+ is identified as the charge carrier of these currents.

The activity with the large unitary current predominated in virtually every patch observed, and it was strongly enhanced by increasing depolarization. The openings with the smaller currents appeared infrequently, and did not show any evident voltage dependence. The latter probably represented two additional types of channels rather than two sublevels of the large channel because of the following observations. (a) In most cases, the unitary currents of smaller sizes arose from and returned to zero level (see Fig. 1, middle and right panels). They stood by themselves and were
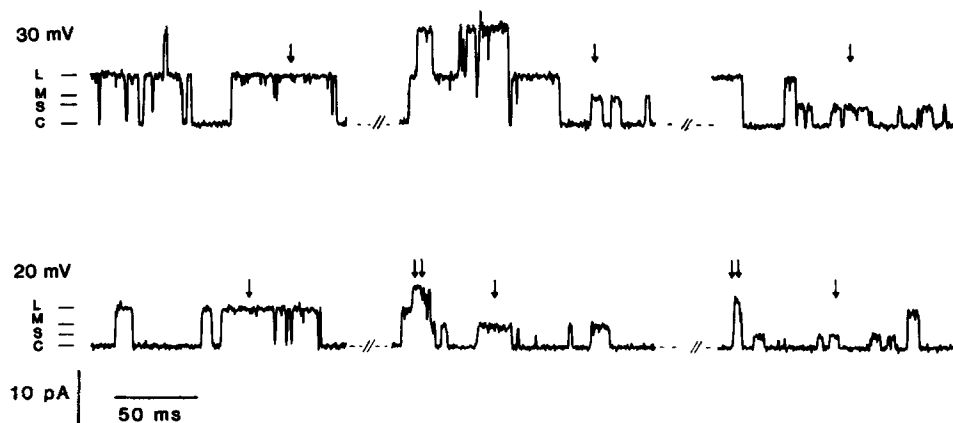

FIGURE 1.   Presence of three distinct types of K^+ channel in inside-out membrane patch of smooth muscle cell of guinea pig taenia coli. $[K^+]_i/[K^+]_o = 135/5.4$. Single-channel currents (upward deflection denotes outward current) in top or bottom row are segments chosen from extended recordings at clamped patch potential of 30 or 20 mV. Currents of large, medium, and small sizes are presented in left, middle, and right panels, respectively, indicated by the single arrow on top of the traces. Short bars on left side mark the level of zero current (C), and current levels for small (S), medium (M), and large (L) channels. Double arrows in lower row indicate simultaneous opening of medium and large channel (middle), of small and large channel (right). $[Ca^{2+}]_i = 10^{-8}$ M.

independent of the large openings. Direct transitions between the large and the medium, or between the medium and the small levels were not seen in our experiments. (b) In some records, the unitary currents of the large and the medium or the large and the small appeared additively (Fig. 1), indicating that two types of channels opened simultaneously, rather than two large channels, one to its full size and the other to a sublevel size.

The unit conductances of the channels around 0 mV are 146.9 ± 3.3 pS (n = 72), 93.5 ± 6.1 pS (n = 5), and 63.3 ± 2.8 pS (n = 14). The presence of all three types of channel in the same patch, as in the example shown in Fig. 1, was rare. Accompanying the activity of the large channel, which occurred in every patch, the activity of the 63-pS channel occurred in ~25% of the patches observed, and that of the

94-pS channel in <10%. In cell-attached patches, the 94-pS channel was never detected even at very depolarized potentials. For these reasons, we shall focus our attention on the voltage-gated K^+ channel with a conductance of ~150 pS.

Voltage Dependence and Calcium Dependence of Channel Activity

General features. Fig. 2 *A* presents current records of a 150-pS K^+ channel from an inside-out patch at various voltages. In this patch, the potential at which substantial opening first became evident was ~ −60 mV. In 25 excised patches where $[Ca^{2+}]_i = 10^{-8}$ M, the average potential at which substantial openings were first observed was −36 ± 4 mV. In cell-attached patches from healthy cells, no
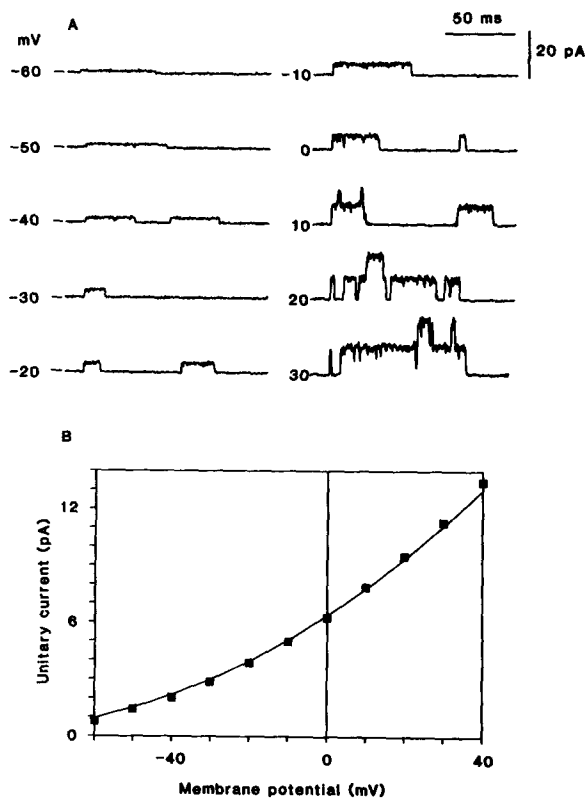

FIGURE 2. Current of K^+ channel with large conductance. (*A*) Single-channel currents at various membrane potentials. Inside-out patch. Short bars on left side of traces indicate channel closed level. Note longer open dwell time at −40, −50, or −60 mV than at −20 or −30 mV. (*B*) *i-V* relationship for data recorded in *A*. Points (■) represent observed data, continuous line is theoretical curve calculated with Goldman-Hodgkin-Katz constant-field equation under asymmetrical K^+ condition of 135/5.4. Unit conductance (~0 mV), 132 pS; reversal potential, −78 mV (see Methods for measuring these values).

opening was observed at cell membrane potentials negative to 0 mV, suggesting that the $[Ca^{2+}]_i$ in an intact cell was lower than 10^{-8} M (see Fig. 4 *A*). In the condition of $[K^+]_i$ 135:$[K^+]_o$ 5.4, no inward channel openings with measurable dwell times and unitary currents were seen even at very negative potentials (to −120 mV), except some brief inward flickerings.

Fig. 2 *B* gives the single-channel *i-V* relationship. The continuous line is drawn according to the Goldman-Hodgkin-Katz constant field equation (Goldman, 1943; Hodgkin and Katz, 1949) for a K^+ current with $[K^+]_i$ 135:$[K^+]_o$ 5.4. The *i-V* curve is nonlinear, displaying outward rectification. The slope conductance is 67 pS between

-60 and -30 mV, 132 pS between -20 and 10 mV, and 186 pS between 10 and 40 mV. The mean observed E_K, -75 mV, is probably less negative than the real E_K (see Methods). However, it is in general agreement with that predicted by the Nernst equation for a K^+-selective channel at $[K^+]_i$ 135:$[K^+]_o$ 5.4. Thus, contributions to channel current by ions other than K^+ are small.

OPEN PROBABILITY AND ANOMALOUS ACTIVITY. The dependence of the overall channel activity on membrane potential is illustrated in Fig. 3. With the i-V and p-V curves shown together (Fig. 3 A), it is evident that p is minimum at -30 mV, and is larger at both more and less negative voltages. At the same voltages from -60 to
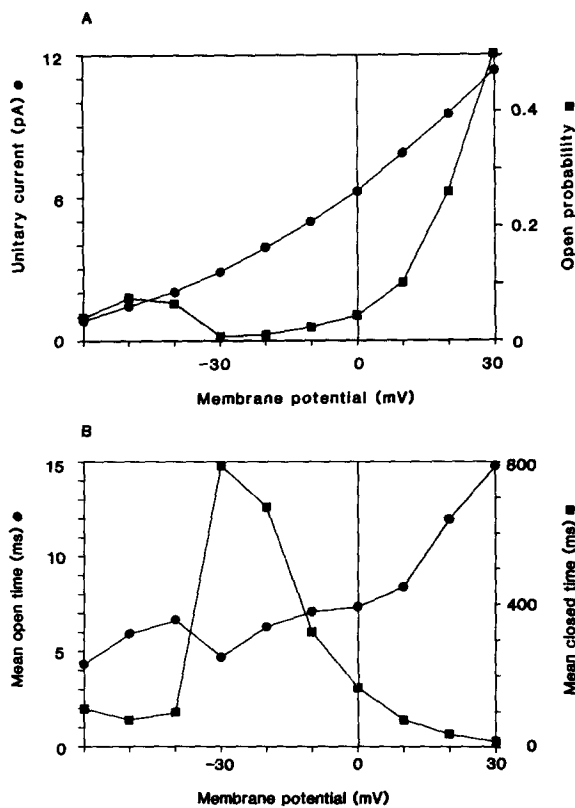

FIGURE 3. Voltage sensitivity of overall channel activity. Results measured from same single-channel recording as shown in Fig. 2. (A) Single-channel i-V (●) and p-V (■) relationship. Ordinate on left side for current, on right side for probability. i-V curve is the same as in Fig. 2; note its monotonic nature from -60 to $+30$ mV. P-V curve has minimum at -30 mV, and follows Boltzman relation from -30 to $+30$ mV. From -30 to -50 mV, p-V relation shows unexpected increased open probability (anomalous activity region). (B) Mean open time (●) and mean closed time (■) as functions of membrane voltage. Ordinate on left side for open time, on right side for closed time. All lines drawn by connecting adjacent points. Note precipitous shortening of mean closed time from -30 to -40 mV.

$+30$ mV, the unitary current increases monotonically with increasing depolarization. The p-V curve between -30 and $+30$ mV obeys Boltzmann relationship, as is supported by the excellent linearity of $\ln[p/(1 - p)]$ vs. V curves (not shown), with an inverse slope, K^{-1}, of 11.6 mV. The pattern of activity on depolarization is characterized by increased frequency of opening (shortened closed time) and prolonged open time (Fig. 3 B).

The probability of channel openings between -30 and -60 mV deviates surprisingly from what is expected. We refer to this region as the anomalous activity region, in which the pattern of activity is characterized by a high frequency of open-

ing (short closed time) and a relatively constant and short open time (as contrasted to that at depolarized voltage levels, Fig. 3 *B*). Most isolated patches were quiescent at very negative potentials, possibly because the anomalous activity was lost through patch excision. Although anomalous activity was observed in only a fraction of the excised patches, its occurrence demonstrates that the channel is capable of being active at ~ -50 mV, the usual resting potential of taenia myocytes (Inomata and Kao, 1985; Yamamoto et al., 1989*a*).

OPEN TIME AND CLOSED TIME. The channel mean open time and mean closed time as functions of potential are presented in Fig. 3 *B*. The mean closed time reaches its maximum at ~ -30 mV, and falls sharply at more negative potentials. The mean open time changes relatively little at negative membrane potentials. Thus, the higher open probability at ~ -50 mV than at -30 mV results from a large decrease in long closed time rather than a small increase in mean open time. These observations may imply an involvement of this channel in maintaining the natural resting potential at ~ -50 mV.

Sensitivity to voltage. Because the 150-pS channel is sensitive to both membrane voltage and to $[Ca^{2+}]_i$, the influence of membrane voltage on the channel activity needs to be specified at fixed $[Ca^{2+}]_i$. Fig. 4 *A* shows *p-V* curves of the same cell at $[Ca^{2+}]_i$ of 10^{-6}, 10^{-7}, and 10^{-8} M. For each individual curve at a constant $[Ca^{2+}]_i$, *p* is obviously voltage dependent. The channel openings were first detected at ~ -30 mV with $[Ca^{2+}]_i$ of 10^{-8} or 10^{-7} M and ~ -70 mV at 10^{-6} M. In 10^{-6} M, *p* tends to saturate or decline at potentials more positive than 30 mV (see also Benham et al., 1986), a phenomenon which might be attributed to a Ca^{2+} blockade of K⁺ channel (Vergara and Latorre, 1983). All *p-V* curves in the potential range from -30 to $+40$ mV follow the Boltzmann relationship (Eq. 1), and are also plotted in the $\ln[p/(1-p)]$ vs. *V* format (Fig. 4 *B*), from which *K* and V_h are obtained (see Methods).

The results show that an *e*-fold increase of *p* takes place with changes of 11.2, 8.6, and 13.7 mV in membrane potential at $[Ca^{2+}]_i$ of 10^{-8}, 10^{-7}, and 10^{-6} M, respectively. An average value of 11.3 mV for K^{-1} is obtained with $[Ca^{2+}]_i$ up to 10^{-6} M, which is well within the range of the voltage sensitivity of the Ca^{2+}-activated K⁺ channel in a number of tissues (Marty, 1983; Singer and Walsh, 1987).

To determine the source of the voltage dependence of *p* at low *p* values, we studied the voltage sensitivity of the channel mean open time (T_o) and mean closed time (T_c) at $[Ca^{2+}]_i$ of 10^{-8} M (Fig. 5). From the plot of the natural logarithm of mean open time and mean closed time against membrane potential, inverse slope of -14.4 mV for T_c and 57.2 mV for T_o are obtained (see also Fig. 10 of Benham et al., 1986; Fig. 10 of Singer and Walsh, 1987). By comparing these values with K^{-1} of 11.2 mV (at 10^{-8} M $[Ca^{2+}]_i$) for *p*, we conclude that at low values of *p* the influence of membrane voltage on channel gating is predominantly on the rate constants determining the mean closed time. However, as *p* increases, the predominance of one factor becomes less as both open time and closed time begin to exert an influence. Thus, with $[Ca^{2+}]_i$ of 10^{-7} M when *p* is higher, an inverse slope for T_c is -22.6 mV, and one for T_o 47 mV, neither being close to the K^{-1} of 8.4 mV.

Sensitivity to $[Ca^{2+}]_i$. When the membrane potential was held constant, the probability of the channel being open rose steeply with increasing $[Ca^{2+}]_i$. The results in Fig. 4 *A* give *p* at 0 mV of 0.02, 0.08, and 0.52 for $[Ca^{2+}]_i$ of 10^{-8}, 10^{-7},
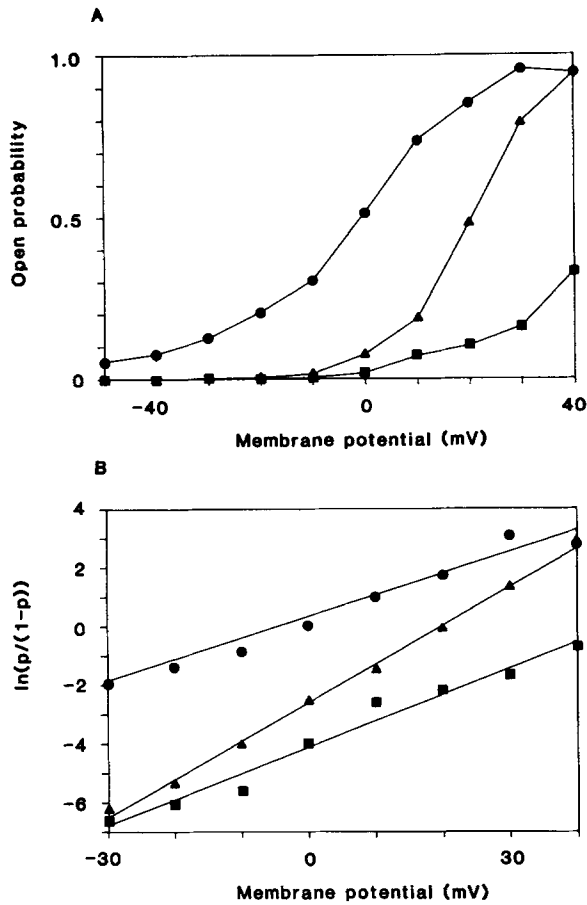
A

B

FIGURE 4. Dependence of probability of channel opening (p) on membrane potential and $[Ca^{2+}]_i$. One inside-out patch under different conditions. (A) p-V plot. Points denote observed data in solution with $[Ca^{2+}]_i$ of 10^{-8} (■), 10^{-7} (▲), and 10^{-6} M (●). (B) Data from A are plotted with $\ln[p/(1 - p)]$ as ordinate. Symbols have same meaning as in A. Continuous lines are least-square linear regression fittings.

and 10^{-6}, respectively. Thus, the $[Ca^{2+}]_i$ needed to produce half-activation of the channel at 0 mV is $\sim 10^{-6}$ M, which is in close agreement with the results found in other smooth muscle cells: 9×10^{-7} M for rabbit portal vein cell (estimated from Fig. 2 B, Inoue et al., 1985), 5×10^{-7} M for rabbit jejunal cell (Benham et al., 1986), and 2×10^{-6} M for toad stomach cell (estimated from Fig. 6, A and B, Singer
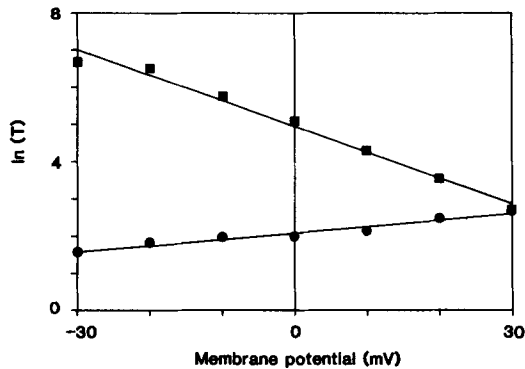

FIGURE 5. Voltage dependence of mean open time and mean closed time. Same inside-out patch as shown in Fig. 3. Mean open time (T_o, ●) and mean closed time (T_c, ■) in a natural logarithmic scale are plotted against membrane potential. Straight lines are least-square linear regressions fittings.

and Walsh, 1987). The average values of V_h ($n = 3$), obtained from data plotted in Fig. 4 B, are 44.1 ± 7.6, 19.2 ± 5.9, and -4.8 ± 3.9 mV for $[Ca^{2+}]_i$ of 10^{-8}, 10^{-7}, and 10^{-6} M, respectively. Hence, a 10-fold increase of $[Ca^{2+}]_i$ produces an ~25 mV negative shift of the potential where p is 0.5.

The change in probability with different $[Ca^{2+}]_i$ results mainly from changes in the mean closed time. By increasing $[Ca^{2+}]_i$ from 10^{-8} to 10^{-7} M, the mean closed time at 0 mV shortened sixfold, whereas the mean open time only doubled. At the same time, the maximum number of channels opened simultaneously rose from one to four.

The unit conductance was practically unaffected by changes in intracellular calcium concentration. The average change in unit conductance from a total of six inside-out patches was <3% as $[Ca^{2+}]_i$ increased 100-fold, from 10^{-8} to 10^{-6} M (see also Barrett et al., 1982; Wong et al., 1982).

Insensitivity to $[Ca^{2+}]_o$. We also examined the relationship between extracellular calcium concentration and the channel activity in inside-out patches. Ideally, such experiments should be done with different pipette solutions on the same patch. However, as we were unable to change pipette solutions without compromising the giga-seal, these experiments were done by making comparisons between a pair of patches from one cell, or from adjacent cells in the same experimental dish. Detailed studies were not carried out because of the lack of a real control. In three pairs of inside-out membrane patches, no significant change in conductance or channel activity occurred with a 10-fold increase of $[Ca^{2+}]_o$, but there was a positive shift of the i-V relations. For instance, for one pair the i-V curves in 2 mM and 20 mM of $[Ca^{2+}]_o$ were parallel. The unit conductance was 134 pS in 2 mM and 129 pS in 20 mM. There was also a positive voltage shift of ~15 mV. The latter is consistent with our observation in whole-cell recordings (Yamamoto et al., 1988b), where a 10–20 mV positive shift of i-V curve occurred for a 10-fold increase of $[Ca^{2+}]_o$. This shift may be explained by the effect of Ca^{2+} on the density of surface negative charges (Frankenhaeuser and Hodgkin, 1957).

Channel Kinetics

From seven patches, the open and closed times of all observed openings in extended recordings were measured and plotted as histograms. The distributions of open time and closed time, and their exponential fittings from an inside-out patch held at 0 mV, are shown in Fig. 6. The open-time distribution (Fig. 6 A) is adequately fitted by a single exponential, but the closed-time distribution (Fig. 6 B) can be described only as the sum of two exponentials. The time constants of these exponentials varied from patch to patch. For the data shown in Fig. 6, the time constant is 8.30 ms for open-time distribution, and 2.6 and 47.0 ms for closed-time distribution. The channel mean open time (T_o) and mean closed time (T_c) are calculated to be 11.6 and 38.4 ms, respectively. These results suggest the occurrence of a minimum of one open-channel state and two closed-channel states for this channel.

A Physiological Role Deduced from Averaged Currents

Among the physiological functions of K^+ channel, an important one is repolarizing the membrane after it has been depolarized. Such a role has been postulated for

Ca^{2+}-activated K^+ channels in smooth muscle cell (e.g., Bolton et al., 1985; Benham et al., 1986; Singer and Walsh, 1987), but direct supporting evidence has been lacking. To substantiate such a role, it is essential to have some idea of how rapidly currents through the Ca^{2+}-activated K^+ channel develop. In place of observing channel functions at steady holding potentials, patches were subjected to step depolarizations of 100 ms duration from a fixed holding potential (Fig. 7 A). The unit conductance of the channel illustrated in the figure was 151 pS. In response to depolarizing steps, the Ca^{2+}-activated K^+ channel characteristically opened with some initial delay. As the membrane potential became more positive, the delay shortened markedly. At the same time, the channel open time lengthened, and the number of open channels increased.
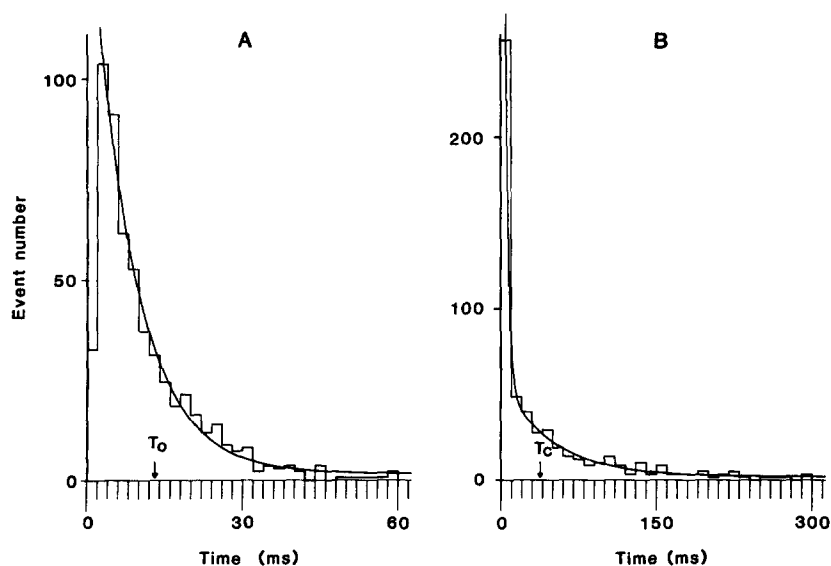

FIGURE 6. Frequency distribution of channel open and closed time at 0 mV. Inside-out patch containing one Ca^{2+}-activated K^+ channel. $[Ca^{2+}]_i = 10^{-8}$ M. Total number of events, 566. (A) Open-time histogram and its single exponential fitting shown as smooth line: 148.0 exp $(-t/8.3)$ + 1.65. Bin width, 2 ms. Mean open time (T_o), 11.6 ms. (B) Closed-time histogram and its double exponential fitting, 1,366.6 exp $(-t_1/2.6)$ + 58.7 exp $(-t_2/47.0)$ + 2.1. Bin width, 10 ms. Mean closed time (T_c), 38.4 ms.

For each depolarizing step, repetitive pulses at intervals of 3 s were imposed on the patches. Summing and averaging 80–100 consecutive single sweeps at each step depolarization produced currents shown in Fig. 7 B. These traces exhibit the typical features of the macroscopic K^+ currents seen in whole-cell recordings: a gradual onset, a maintained magnitude as well as an acceleration of the activation kinetics, and an outward rectification with increasing positivity of the potential (compare with records in Yamamoto et al., 1989b). The averaged currents can be approximately fitted with n^2 curves, with time constants of 16.5, 15.8, 15.3, 14.6, 12.8, and 10.7 ms for depolarizing steps to 20, 30, 40, 50, 60, and 70 mV, respectively. If more single sweeps had been used for the averaged current, the traces would

undoubtedly be smoother, and the kinetic fit better. However, the time frame observed is compatible with the whole-cell outward currents recorded at 33°C (Yamamoto et al., 1989*b*), and with the duration of the action potential of taenia myocyte at room temperature (Yamamoto et al., 1989*a*). In seven experiments of this type, the activities of the 150-pS channel were consistently observed. Up to five channels open simultaneously within the 100-ms depolarizing steps. The openings
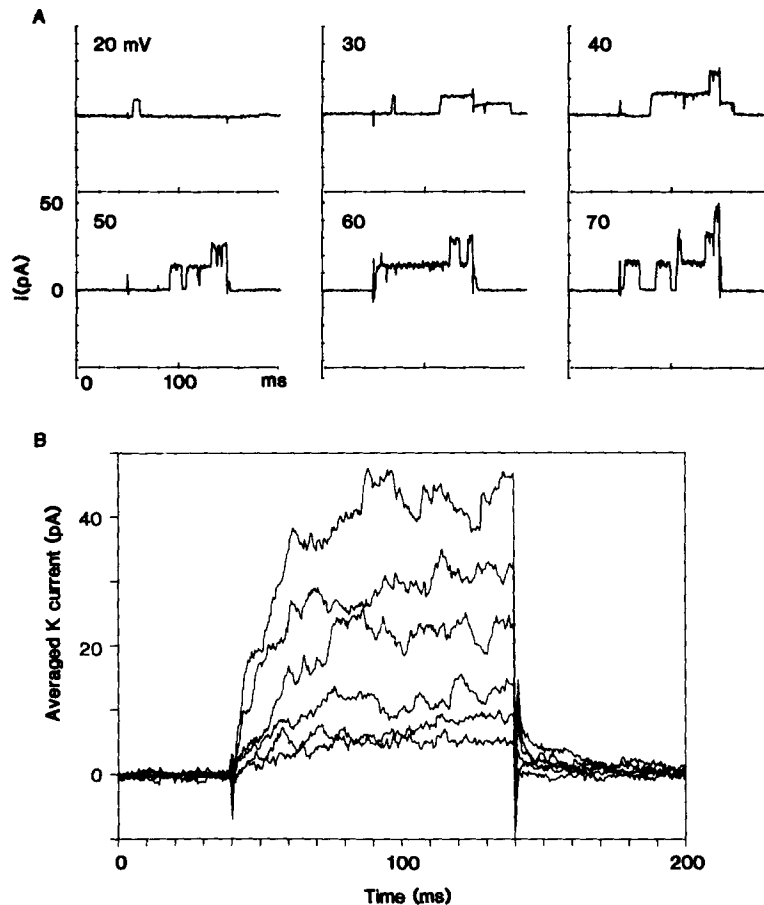

FIGURE 7. Activity of Ca^{2+}-activated K^+ channel evoked by 100-ms steps depolarizing to 20, 30, 40, 50, 60, and 70 mV. Holding potential, -40 mV. Inside-out patch. $[Ca^{2+}]_i = 10^{-8}$ M. (A) Selected sweeps showing single-channel openings at depolarizing pulse to potentials marked in upper-left corner of each panel. Voltage step occurs between 40 and 140 ms, recognizable in these current traces by small residual artifacts at beginning and end of steps. Note: (*a*) clear opening of potassium outward tail at the end of pulses to 30 and 40 mV; (*b*) some delay in channel openings except at 60 and 70 mV where delay is very brief. Calibrations for record of 50 mV applies to all others. (*B*) Family of averaged currents from single sweeps at corresponding potentials (as indicated in *A*) show gradual onset, maintained amplitude as well as accelerating ON kinetics, and increasing outward rectification with increasing positivity. Capacitive and leakage current were compensated digitally.

of the other two types of channel were extremely rare during such step depolarizations, though they did appear infrequently in patches at constant holding potential. This observation tends to exclude the possibility of these two channels being components of the peak outward K^+ current.

DISCUSSION

Our experiments have shown the coexistence of three types of K^+ channel in the guinea pig taenia myocyte. In an asymmetrical condition of $[K^+]_i$ 135:$[K^+]_o$ 5.4, they have conductances of 147, 94, and 63 pS. Direct proof for any physiological functions of the channels with intermediate and small conductances remains to be obtained. The large K^+ channel is by far the predominant channel in this preparation. The combination of a large conductance, a strong Ca^{2+} dependence, a voltage dependence, and a high selectivity to K^+ is typical of the Ca^{2+}-activated K^+ channel identified in a variety of cell types.

Channel Density

The density of the large Ca^{2+}-activated K^+ channel can be estimated by using the method proposed by Singer and Walsh (1987). The patch pipettes used in our experiments have resistances ranging between 3 and 4 MΩ, which would produce patches of ~4 μm^2 (Sakmann and Neher, 1983). In those patches an average of three channels were observed when $[Ca^{2+}]_i = 10^{-8}$ M. The mean surface area of the isolated taenia myocyte is 4,359 ± 211 μm^2 (Yamamoto et al., 1989a). These data yield a total of ~3.3 × 10^3 channels per cell, and a density of 0.75 channel per μm^2. Such a density is in agreement with that in smooth muscle cell from the toad stomach (Singer and Walsh, 1987). However, we believe that this density is an underestimate. When $[Ca^{2+}]_i$ is as low as 10^{-8} M, there is a low probability for multiple channels to open simultaneously, thus easily causing an underestimation of the total number of channels in one patch. We did not attempt to calculate the channel density by dividing the macroscopic peak outward current by the appropriate unitary current, because the macroscopic currents we obtained from whole-cell recording were of relatively small sizes, owing to an even lower $[Ca^{2+}]_i$ (~10^{-10} M in 1 mM EGTA with no added Ca^{2+}, Yamamoto et al., 1989b).

Physiological Roles of the Channel

Delayed rectification and repolarization. The activity of the large Ca^{2+}-activated K^+ channel is a preeminent feature in the taenia myocyte membrane, suggesting some significant contribution to cell function. The results in Fig. 7 show that the averaged currents through the Ca^{2+}-activated K^+ channel develop exponentially with voltage-dependent time constants that are compatible with the time course of the repolarization phase of an action potential at room temperature (22–24°C). In our whole-cell recording of the taenia myocyte, the peak K^+ currents, which have activation time constants ranging from 1.7 to 10.6 ms at positive potentials up to 70 mV (32–34°C), are considered important for repolarization (Yamamoto et al., 1989b). The qualitative resemblance between the averaged single-channel currents and the macroscopic delayed-rectifier currents strongly suggest that the 150-pS

Ca^{2+}-activated K$^+$ channel in smooth muscle cell is the single-channel basis of the high K$^+$ permeability that governs the repolarization phase of an action potential.

Anomalous activity and resting potential. The results illustrated in Fig. 3 *A* reveal an unexpected feature of the Ca^{2+}-activated K$^+$ channel. The probability of a channel being open at negative voltages around the resting potential (-50 mV) deviates from the Boltzman distribution, which it obeys well at potentials more positive than -30 mV. This phenomenon may be explained either by the emergence of a new type of channel, or by a change in the voltage dependence of the activity of the large-conductance Ca^{2+}-activated K$^+$ channel. The former possibility is rendered unlikely by the monotonic continuity of the single-channel current-voltage relationship, which obeys the Goldman-Hodgkin-Katz constant-field relation through the entire range of potentials (Fig. 3 *A*). The channel activity in the anomalous region is apparently associated with the latter possibility. It has a high open frequency (short mean closed time) and a relatively short mean open time. This pattern is different from that at more depolarized potentials where the channel displays a high open frequency and a long mean open time (Fig. 3 *B*). Possibly, the anomalous activity is linked to another physiological role, that of maintaining the resting potential. However, in cell-attached patches, channel openings at these potentials are very rare, in part because of the very low intracellular Ca^{2+} concentration. Another possibility is that there might be some cellular repressor factor which modulates the channel activity, and that such modulating influence is removed on patch excision.

Our observation may also explain some aspects of the penetration-induced hyperpolarization reported by Walsh and Singer (1980). They showed that the high K$^+$ permeability caused by the penetration of a capillary microelectrode into toad stomach myocytes with a low resting potential resulted from a transient increase of the activity of a Ca^{2+}-activated K$^+$ channel. Even if [Ca^{2+}]$_i$ was elevated to 10^{-6} M after penetration, without anomalous activity, the probability of a channel being open would be ~4% at -50 mV in taenia myocytes (Fig. 4 *A*). Since the [Ca^{2+}]$_i$ sensitivity of the toad stomach myocyte is very similar (see page 840, and Fig. 6 of Singer and Walsh, 1987), such a probability would be virtually nil at -40 mV, the resting potential of the impaled cells. These probabilities cannot account for any prolonged sizable hyperpolarization. Instead, the anomalous activity at negative potentials, as described herein, could be a more realistic alternative mechanism.

REFERENCES

Barrett, J. N., K. L. Magleby, and B. S. Pallotta. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. *Journal of Physiology.* 331:211–230.

Benham, C. D., T. B. Bolton, R. J. Lang, and T. Takewaki. 1985. The mechanism of action of Ba^{2+} and TEA on single Ca^{2+}-activated K$^+$ channels in arterial and intestinal smooth muscle cell membranes. *Pflügers Archiv.* 403:120–127.

Benham, C. D., T. B. Bolton, R. J. Lang, and T. Takewaki. 1986. Calcium-activated potassium channels in single smooth muscle cells of rabbit jejunum and guinea-pig mesenteric artery. *Journal of Physiology.* 371:45–67.

Berger, W., R. Grygorcyk, and W. Schwarz. 1984. Single K$^+$ channels in membrane evaginations of smooth muscle cells. *Pflügers Archiv.* 402:18–23.

Bolton, T. B., R. J. Lang, T. Takewaki, and C. Benham. 1985. Patch and whole-cell voltage clamp of single mammalian visceral and vascular smooth muscle cells. *Experientia.* 41:887–894.

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 191–264.

Frankenhaeuser, B., and A. L. Hodgkin. 1957. The action of calcium on the electrical properties of squid axons. *Journal of Physiology.* 137:218–244.

Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *Journal of General Physiology.* 27:37–60.

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

Hamill, O. P., and B. Sakmann. 1981. A cell-free method for recording single channel currents from biological membranes. *Journal of Physiology.* 312:41–42P.

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of giant axon of the squid. *Journal of Physiology.* 108:37–77.

Hu, S. L., Y. Yamamoto, and C. Y. Kao. 1987a. Single channel basis of delayed rectification in dispersed cells of guinea pig taenia coli. *Biophysical Journal.* 51:200a. (Abstr.)

Hu, S. L., Y. Yamamoto, and C. Y. Kao. 1987b. Selectivity of the single delayed rectifier channel in smooth muscle cells of the guinea pig taenia coli. *Federation Proceedings.* 46:507. (Abstr.)

Imai, S., and K. Takeda. 1967. Calcium and contraction of heart and smooth muscle. *Nature.* 213:1044–1045.

Inomata, H., and C. Y. Kao. 1985. Action of Ba^{++} on ionic currents of the guinea-pig taenia coli. *Journal of Pharmacology and Experimental Therapeutics.* 233:112–124.

Inoue, R., K. Kitamura, and H. Kuriyama. 1985. Two Ca-dependent K channels classified by the application of tetraethylammonium distribute to smooth muscle membranes of the rabbit portal vein. *Pflügers Archiv.* 405:173–179.

Inoue, R., K. Okabe, K. Kitamura, and H. Kuriyama. 1986. A newly identified Ca^{2+}-dependent K$^+$ channel in the smooth muscle membrane of single cells dispersed from the rabbit portal vein. *Pflügers Archiv.* 406:138–143.

Marty, A. 1983. Ca^{2+}-activated K$^+$ channels with large unitary conductance. *Trends in Neurosciences.* 6:262–265.

McCann, J. D., and M. J. Welsh. 1986. Calcium-activated potassium channels in canine airway smooth muscle. *Journal of Physiology.* 372:113–127.

Sakmann, B., and E. Neher. 1983. Geometric parameters of pipettes and membrane patches. *In* Single Channel Recording. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 37–51.

Singer, J. J., and J. V. Walsh, Jr. 1987. Characterization of calcium-activated potassium channels in single smooth muscle cells using the patch-clamp technique. *Pflügers Archiv.* 408:98–111.

Vergara, C., and R. Latorre. 1983. Kinetics of Ca^{2+}-activated K$^+$ channels from rabbit muscle incorporated into planar bilayers. *Journal of General Physiology.* 82:543–568.

Walsh, J. V., Jr., and J. J. Singer. 1980. Penetration-induced hyperpolarization as evidence for Ca^{2+} activation of K$^+$ conductance in isolated smooth muscle cells. *American Journal of Physiology.* 239:C182–189.

Wolff, D., X. Cecchi, D. Naranjo, O. Alvarez, R. Latorre. 1985. Cation selectivity and Cs$^+$ blockade in a Ca^{2+}-activated K$^+$ channel from rabbit intestinal smooth muscle. *Biophysical Journal.* 47:136a. (Abstr.)

Wong, B. S., H. Lecar, and M. Adler. 1982. Single calcium-dependent potassium channels in clonal anterior pituitary cells. *Biophysical Journal.* 39:313–317.

Wong, B. S. 1985. Single calcium-dependent potassium channels from amphibian stomach smooth muscle cells. *Biophysical Journal.* 47:136a. (Abstr.)

Yamamoto, Y., S. L. Hu, and C. Y. Kao. 1989a. The inward current in single smooth muscle cells of the guinea pig taenia coli. *Journal of General Physiology.* 93:521–550.

Yamamoto, Y., S. L. Hu, and C. Y. Kao. 1989b. The outward current in single smooth muscle cells of the guinea pig taenia coli. *Journal of General Physiology.* 93:551–564.