Gating Properties of Single SK Channels in Hippocampal CA1 Pyramidal Neurons

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ABSTRACT The activation of small-conductance calcium-activated potassium channels (SK) has a profound effect on membrane excitability. In hippocampal pyramidal neurons, SK channel activation by Ca^{2+} entry from a preceding burst of action potentials generates the slow afterhyperpolarization (AHP). Stimulation of a number of receptor types suppresses the slow AHP, inhibiting spike frequency adaptation and causing these neurons to fire tonically. Little is known of the gating properties of native SK channels in CNS neurons. By using excised inside-out patches, a small-amplitude channel has been resolved that was half-activated by ~0.6 μM Ca^{2+} in a voltage-independent manner. The channel possessed a slope conductance of 10 pS and exhibited nonstationary gating. These properties are in accord with those of cloned SK channels. The measured Ca^{2+} sensitivity of hippocampal SK channels suggests that the slow AHP is generated by activation of SK channels from a local rise of intracellular Ca^{2+}.

INTRODUCTION

The slow afterhyperpolarization (AHP) in hippocampal neurons has an important influence on membrane excitability. It is activated after a burst of action potentials and underlies spike frequency adaptation, terminating burst firing (Madison and Nicoll, 1984). The slow AHP is generated by activation of small conductance calcium-activated potassium channels (SK), resulting from the entry of Ca^{2+} through voltage-gated Ca^{2+} channels (Lancaster and Nicoll, 1987). It has been assumed that multiple SK channels may exist because the AHP in some cell types is sensitive to the bee venom toxin, apamin (e.g., bullfrog sympathetic neurons, Pennefather et al., 1985) and is not in others (e.g., rat hippocampal CA1 pyramidal neurons, Lancaster and Adams, 1986). This assumption has been confirmed by the cloning of three distinct members of the SK channel family, SK1–3 (Köhler et al., 1996; Joiner et al., 1997). Both the apamin-insensitive SK1 and the apamin-sensitive SK2 channels exhibited a single channel conductance of ~10 pS in isotonic potassium and were half-activated by 0.6–0.7 μM Ca^{2+} (Köhler et al., 1996; Hirschberg et al., 1998). These data are in agreement with the single SK channel properties observed in GH_3 anterior pituitary cells (Lang and Ritchie, 1987), T lymphocytes (Grissmer et al., 1992), and adrenal chromaffin cells (Park, 1994). However, the only study of single SK channels in hippocampal pyramidal neurons reported a larger single channel conductance of 18–20 pS and a slightly lower sensitivity to Ca^{2+} [open probability (P_o) 0.5 with 1 μM Ca^{2+}; designated P(o) in figures] (Lancaster et al., 1991).

A significantly greater Ca^{2+} sensitivity of SK channels might be expected from intracellular Ca^{2+} imaging studies in hippocampal neurons, since it has been shown that during the slow AHP the somatic Ca^{2+} concentration increases only to 0.1 μM (Knöpfel et al., 1990). However, the P_o of SK channels at the peak of the slow AHP has been estimated to be 0.4–0.6 (Sah and Issacson, 1995; Valiante et al., 1997), predicting an intracellular Ca^{2+} concentration of 0.5–1 μM. It is possible that neither the channel described previously in hippocampal neurons (Lancaster et al., 1991) nor a homolog of the recombinant SK1–3 underlies the slow AHP in hippocampal neurons. Alternatively, hippocampal SK channels may only be activated by local increases in Ca^{2+} that are not reflected by the somatic measurements.

We have examined the gating properties of the hippocampal SK channel. By using inside-out membrane patches excised from acutely isolated hippocampal CA1 pyramidal neurons, we have determined that hippocampal SK channels exhibit a conductance of 10 pS (in symmetrical potassium solutions), are half-activated by ~0.6 μM Ca^{2+}, and display voltage-independent gating. In addition, hippocampal SK channels display nonstationary kinetics. These properties are very similar to cloned rSK2 and hSK1 channels (Köhler et al., 1996; Hirschberg et al., 1998) and suggest that activation of SK channels underlying generation of the slow AHP occurs by a local rise of submembrane Ca^{2+} to levels higher than those measured in the soma.

MATERIALS AND METHODS

Acutely dissociated hippocampal CA1 neurons were obtained as described previously (Cloues et al., 1997). Briefly, Sprague-Dawley rats (9–14 days old) were anesthetized with halothane and decapitated. Hippocampi were rapidly dissected and cut into 300–400-μm slices. Slices were incubated at 37°C in a dissociation solution containing (in mM): Na_2SO_4, 82; K_2SO_4, 30; HEPES, 10; $MgCl_2$, 5; ethylene glycol bis (b-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 1 (pH 7.4), with added protease type

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XXIII (3 mg/ml) for 7–8 min and bubbled with O_2. Tissue slices were then transferred to a solution containing trypsin inhibitor (1 mg/ml) and bovine serum albumin (BSA) (1 mg/ml) for 1 min and finally rinsed in dissociation solution containing no enzyme. The CA1 region was microdissected and triturated into Falcon Primaria dishes as needed.

Cells were washed and superfused (15 ml min^{-1}) with an external solution containing (in mM): $KMeSO_4$, 125; KCl, 35; HEPES(Na), 10; EGTA, 10; $CaCl_2$, 5.64 (to give an estimated free concentration of 0.06 μM (Fabiato and Fabiato, 1979) (pH 7.4 with KOH). Cells in this solution had ~0 mV membrane potential. All potentials are expressed as the negative of the potential imposed on the pipette. Membrane patches were first excised to the inside-out patch configuration into the Mg^{2+}-free superfusion solution containing 0.1 μM Ca^{2+}. The free Ca^{2+} concentration was raised up to 3 μM (Fabiato and Fabiato, 1979) once the patch had stabilized. Changes in solutions containing different concentrations of free Ca^{2+} were achieved by bath superfusion and were complete within several seconds.

Excised inside-out patch recordings (Hamill et al., 1981) were made using thick-walled (1.5 mm O.D., 0.5 mm I.D.) quartz electrodes (7–10 MΩ) containing the external solution described above, supplemented with 100 nM charybdotoxin to prevent contamination by BK channel openings. Voltage-dependent potassium channel activity was prevented by including 4-aminopyridine (1 mM), 3,4-diaminopyridine (1 mM), α-dendrotoxin (200 nM), and β-dendrotoxin (200 nM) in the pipette solution. Single channel currents were recorded with an Axopatch 200 amplifier using a CV201A headstage (Axon Instruments, Foster City, CA), filtered at 1–4 kHz with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) and acquired at 100-μs intervals for analysis using Pulse (Heka, dist. by Instrutech Corp., New York, NY) onto a Quadra 650 (Apple Computer, Cupertino, CA). No differences in open and closed times were seen when data were filtered either at 1 kHz or 4 kHz. Single channels were analyzed using MacTAC (Bruxton Corp., Seattle, WA, dist. by Instrutech Corp., NY). The "50% threshold" technique was used to estimate event amplitudes and durations, with each transition inspected visually before being accepted. Open and closed duration histograms were constructed with MacTacfit (Bruxton Corp., distributed by Instrutech Corp.), binned logarithmically (20 bins/decade) and plotted against the square root transformation of the ordinate (number of events/bin). The distribution was fitted by a sum of exponential probability density functions using the maximum-likelihood method. With this type of representation, peaks in the histogram correspond to the time constant of the exponential (Sigworth and Sine, 1987). A correction was made for the rise time of the filter (Colquhoun and Sigworth, 1983) and all bins were used for fitting. The number of statistically significant components was determined by the method of maximum likelihood ratios (Horn and Lange, 1983). Data were not corrected for missed events. Channel open probability (P_o) was estimated as NP_o, the product of the open probability times the number of channels. NP_o was calculated using ReadEvents v1.37 (Scott Eliasof, Portland, OR), as the sum of (dwell time × level number) divided by the total time. N was estimated as the number of simultaneously open channels at a $P_o > 0.5$. Finally, P_o was obtained by dividing NP_o by N. Where applicable, values were expressed as mean ± SD, and P values were derived from unpaired two-tailed Student's t-tests. Results were considered significantly different at $P < 0.01$. Oocyte expression and recordings of cloned hSK1 channels were performed as described previously for rSK2 (Hirschberg et al., 1998).

We observed a variable degree of channel "run-down" in the absence of any treatment. In the presence of a fixed concentration of Ca^{2+}, ~30% of patches exhibited SK channels whose activity was lost within the first minute of recording. Of the remaining patches, ~70% exhibited SK channels whose activity was lost within the first 10 min of recording. Loss of activity occurred during high and low P_o behavior and was abrupt, complete, and irreversible; i.e., no channel openings were observed over minutes even in increased [Ca^{2+}] or after removal and reapplication of Ca^{2+}. Generally, there was no change in gating leading up to the loss of activity. The loss of SK channel activity in excised inside-out patches may suggest that channel activity requires cytoplasmic factors. All reagents were obtained from Sigma, except α- and β-dendrotoxin (Alomone, Israel), $CaCl_2$ (Fluka, NY), and HEPES (Calbiochem, CA).

RESULTS

Ca^{2+}- and voltage-dependence of hippocampal SK channels

In ~90 of 600 patches, application of Ca^{2+} to the inner face of inside-out membrane patches caused activation of a small-amplitude channel. Increasing the concentration of Ca^{2+} from 0.1 to 1 μM caused a progressive increase in channel P_o (Fig. 1 *A*). A plot of P_o as a function of Ca^{2+} concentration was described by the Hill equation, with an EC_{50} of 0.56 μM and a Hill coefficient (n_H) of 4.6 (Fig. 1 *B*). This observed Ca^{2+}-dependence of channel P_o agrees well with the macroscopic Ca^{2+}-dependence of both hSK1 and rSK2 (EC_{50} values 0.7 and 0.63 μM and n_H values of 3.9 and 4.8, respectively; Köhler et al., 1996).

The voltage-dependence of channel P_o was determined in the presence of a fixed Ca^{2+} concentration. Fig. 2 *A* shows traces from a patch recorded at three membrane potentials containing a single SK channel activated by 1 μM Ca^{2+}. Channel gating was not obviously dependent on membrane potential, with P_o values being similar over a membrane potential range of −100 to +60 mV (Fig. 2 *B*). Measurement of the single channel amplitude at each voltage gave rise to the current-voltage relationship shown (Fig. 2 *B, inset*), yielding a slope conductance of 9.8 pS. Determination of slope conductance in six patches gave a mean value of 10.1 ± 0.5 pS. The Ca^{2+} sensitivity, voltage-independence, and single channel conductance of this channel were not significantly different from those obtained from both hSK1 and rSK2 clones (see Köhler et al., 1996 and Hirschberg et al., 1998), identifying it as an SK channel.

Kinetic properties of hippocampal SK channels

SK channel gating is voltage-independent

The decay of the slow AHP in hippocampal pyramidal neurons is insensitive to membrane potential (Lancaster and Adams, 1986). This may arise from the open-state kinetics of the underlying SK channel being insensitive to voltage, as occurs with cloned rSK2 channels (Hirschberg et al., 1998). In the presence of a fixed concentration of Ca^{2+}, the P_o of hippocampal SK channels was voltage-independent (Fig. 2 *B*). Fig. 3 *A* shows open duration histograms at −60 mV and +60 mV constructed from a single channel patch bathed in 1 μM Ca^{2+}. Both open duration histograms were best fit by the sum of two exponentials with similar time constants (Fig. 3 *A*). The time constant of each exponential component is shown as a function of membrane potential in Fig. 3, *B* and *C*, with each symbol reflecting data from one patch. Data from hippocampal neurons are shown as closed symbols, and data from cloned hSK1 channels expressed in *Xenopus* oocytes are shown for comparison (*open symbols*). The long and short open-time constants seen with hippocampal SK channels were indistinguishable from those obtained with hSK1, and both open-time constants were independent of voltage. In addition, voltage did not signif-

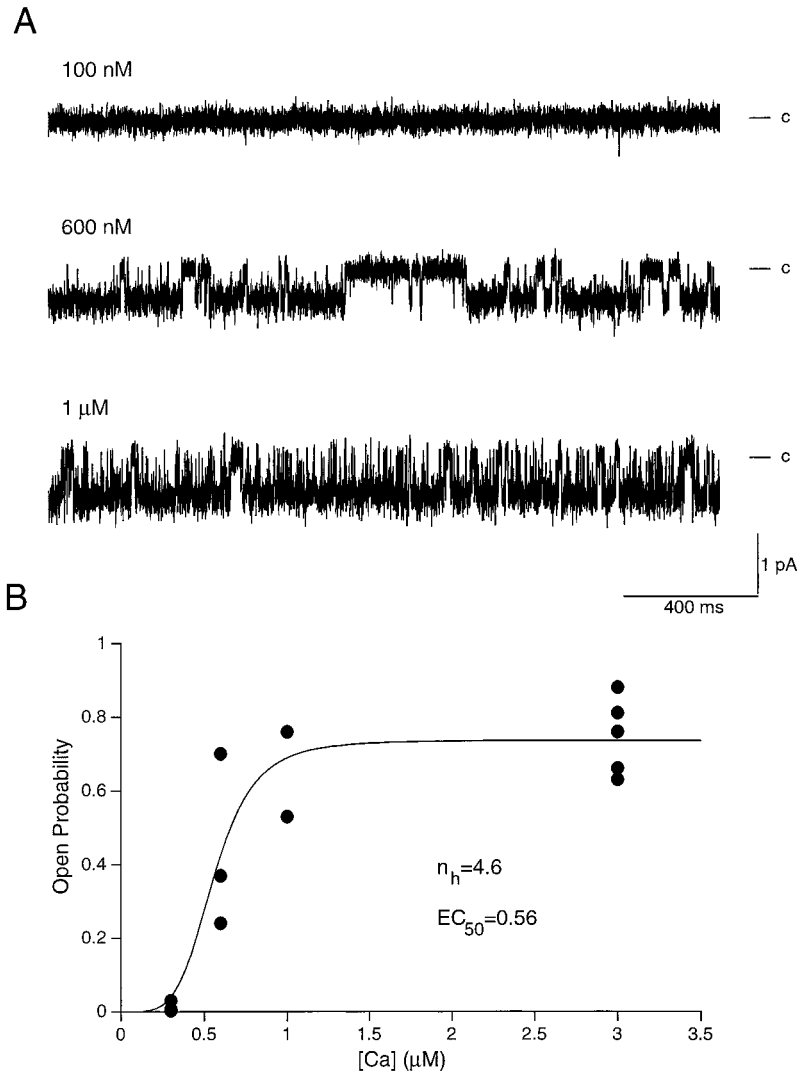

FIGURE 1 Ca^{2+}-dependence of hippocampal SK channel activity. (*A*) Current traces from an inside-out patch excised from a hippocampal CA1 neuron and held at −60 mV. As the Ca^{2+} concentration bathing the patch was raised from 0.1 to 1 μM, SK channel activity increased. Channel openings are shown as downward deflections. (*B*) Open probabilities calculated from the patch shown in *A* are plotted as a function of the Ca^{2+} concentration bathing the patch. Least-squares fitting of the data to the Hill equation yielded NP_{omax} 0.74, EC_{50} 0.56 μM, and n_H 4.6, where NP_{omax} is the open probability in saturating Ca^{2+} concentrations.

icantly affect the relative contribution of each exponent to the open duration histogram (Fig. 3 *D*).

Two gating behaviors distinguished by open- and closed-time kinetics

rSK2 channels have been shown to exhibit and switch between two dominant modes of gating, low and high P_o behavior (Hirschberg et al., 1998). The open-time distribution of rSK2 channels exhibiting either behavior was best fit by the sum of two exponentials, with a larger fraction of short-duration events occurring during low P_o (Hirschberg et al., 1998). However, the main factor determining the observance of low P_o was the presence and weighting of a very slow closed-time component (Hirschberg et al., 1998). During high P_o activity this closed time was absent, being replaced by a closed time an order of magnitude faster (Hirschberg et al., 1998).

rSK2 channels were found to switch P_o behaviors most frequently in Ca^{2+} concentrations close to the EC_{50} (Hirschberg et al., 1998). In 8 of 10 patches exposed to 0.6 μM Ca^{2+} and three of three patches exposed to 1 μM Ca^{2+}, hippocampal SK channels were observed to spontaneously switch P_o behaviors during the time of recording. This is illustrated in Fig. 4. During the first 2 min of recording in the presence of 1 μM Ca^{2+}, a patch containing a single SK channel exhibited relatively high P_o (Fig. 4, *A* and *B*). Analysis of open and closed times showed that the majority of openings were of long duration (τ = 6.3 and 1.2 ms), with closed times being best described by the sum of two exponentials with time constants of 0.94 and 5.7 ms (Fig. 4 *C*). After ~114 s of recording, SK channel activity switched to an extremely low P_o behavior (Fig. 4, *A* and *B*). Observed openings were rare and of short duration, being described by a single exponential of time constant 0.64 ms (Fig. 4 *D, left*). In contrast to high P_o behavior, the closed-time distribution was best fit by the sum of three exponentials (τ = 1.7, 5.6, and 208 ms) (Fig. 4 *D, right*). As with cloned rSK2 channels, the short and intermediate closed times were similar to those seen during high P_o behavior with the

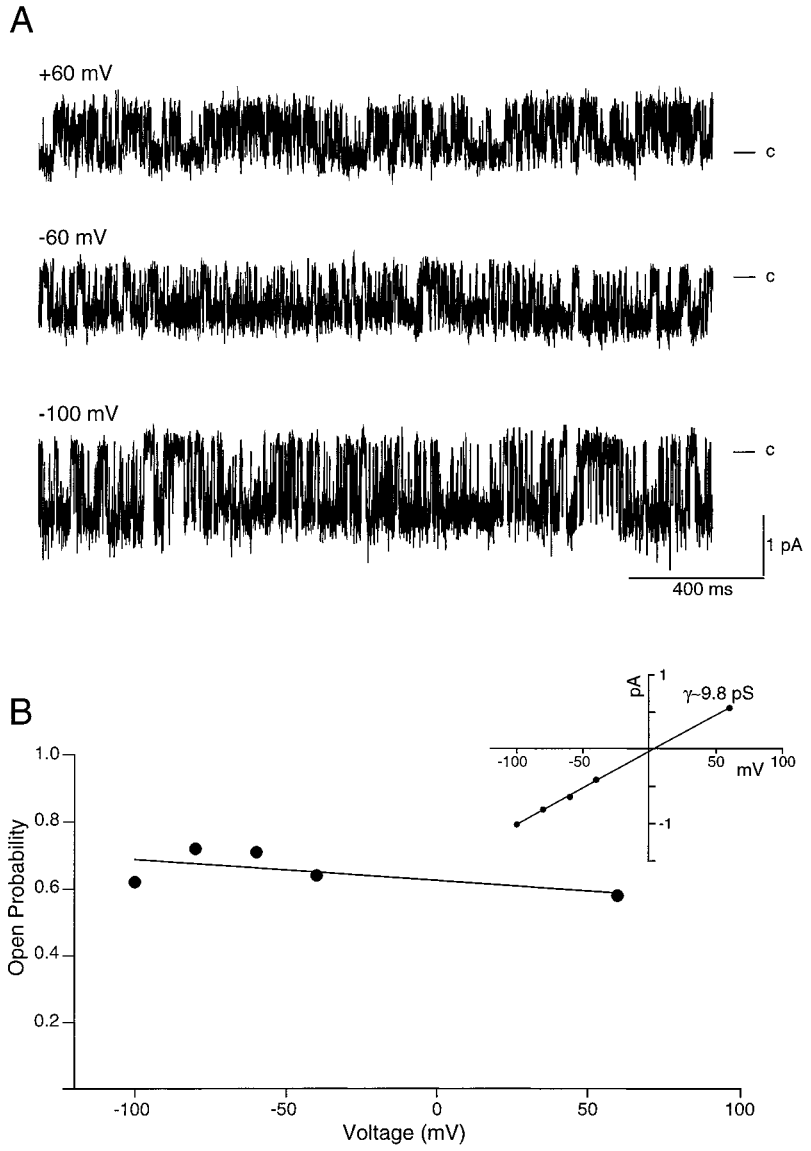

FIGURE 2 Hippocampal SK channel activity as a function of membrane potential. (*A*) In the presence of 1 μM Ca^{2+}, SK channel activity is shown at the illustrated membrane potentials. Openings are upward at +60 mV and downward at negative potentials. As is apparent, SK channel activity was not markedly voltage-sensitive. (*B*) SK channel open probability is plotted as a function of membrane potential for the patch shown in *A*. The solid line is fit by linear regression analysis, showing little voltage-dependence to SK channel P_o. *Inset:* single channel amplitudes obtained by the fitting of a single Gaussian function to amplitude histograms constructed from the patch shown in *A* are plotted as a function of membrane potential. Linear regression analysis yielded a slope conductance of 9.8 pS.

appearance of an additional very long closed time. After ~60 s of low P_o behavior, SK channel activity abruptly switched back to high P_o behavior (Fig. 4, *A* and *B*). In three patches bathed in 1 μM Ca^{2+}, average P_o values were 0.67 ± 0.13 for the high P_o behavior and 0.03 ± 0.02 for the low P_o behavior. Therefore, in the presence of a fixed concentration of Ca^{2+}, hippocampal SK channels can spontaneously and rapidly switch between two P_o behaviors, a property observed with cloned rSK2 channels (Hirschberg et al., 1998).

Ca^{2+}-dependence of SK channel P_o behavior

It has been reported that rSK2 channels spent more time in high P_o activity as the Ca^{2+} concentration was increased (Hirschberg et al., 1998). A similar relationship was observed with hippocampal SK channels. In the presence of 0.3 μM Ca^{2+}, SK channel activity was of very low P_o throughout, with only brief sojourns to an intermediate value (Fig. 5*A*i). Open- and closed-time analysis revealed that the majority of openings were of short duration (τ = 0.8 and 6.5 ms) and the closed-time distribution was best described by the sum of three exponentials (τ = 1.1, 5.1, and 711 ms) (Fig. 5 *A*ii). Increasing the concentration of Ca^{2+} bathing the patch to 3 μM evoked predominantly high P_o activity (Fig. 5 *B*i). The majority of openings were of long duration (τ = 12.6 ms) with a minor component of short-duration openings (τ = 0.9 ms). Most closures were described by exponentials with short and intermediate time constants (τ = 0.68 and 3.3 ms), with a few events of longer time constant (τ = 150 ms) corresponding to the very brief period of low P_o behavior shown in Fig. 5 *B*i (*asterisk*; Fig. 5 *B*ii). Therefore, high and low P_o behaviors can be observed over a range of Ca^{2+} concentrations, and raising the Ca^{2+} concentration promotes high P_o SK channel gating.

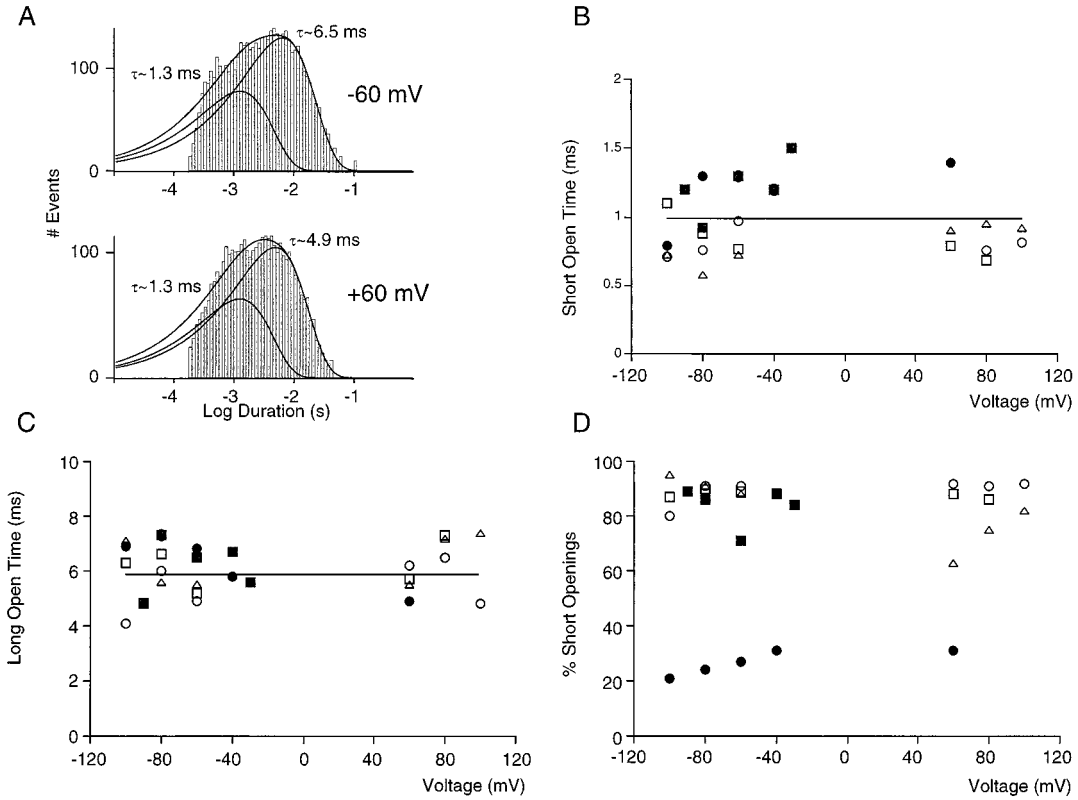

FIGURE 3 Voltage-independence of SK channel gating. (*A*) Open-time histograms from a patch exposed to 1 μM Ca^{2+} and held either at −60 mV (*top*) or at +60 mV (*bottom*). Histograms were fit to the sum of two exponentials with illustrated time constants. (*B*) Time constants of the short open-time component are shown as a function of membrane potential for two patches excised from hippocampal CA1 neurons, one bathed in 1 μM Ca^{2+} (*closed circles*) and the other in 0.6 μM Ca^{2+} (*closed squares*). For comparison, open symbols represent data from three patches excised from *Xenopus* oocytes expressing recombinant hSK1 channels and bathed in 0.2 μM Ca^{2+}. The lines in panels *B* and *C* were drawn for display purposes only. (*C*) Time constants representing the long open-time component for the same patches as in *B*. (*D*) Data represent the percentage of openings contributing to the short open-time constant for the patches also used in *B* and *C*. For each patch the percentage of short openings was essentially voltage-independent. The absolute values differ from patch to patch due to differences in Ca^{2+} concentration and P_o behavior (see text).

Ca^{2+}-dependence of SK channel open and closed times

A Ca^{2+}-dependence of the long closed time is apparent from histograms shown in Figs. 3–5. In contrast, the time constants describing channel openings were similar in different Ca^{2+} concentrations, and only their relative contribution varied. Hippocampal SK channel kinetics were determined over a range of 0.3 to 3 μM Ca^{2+}, and data from cloned hSK1 channels are shown as the open symbols for comparison (Fig. 6). The open-time constant of hippocampal SK channels did not change significantly over this range of Ca^{2+}, and was similar to that seen with hSK1 (Fig. 6, *A* and *B*). The Ca^{2+}-dependence of channel P_o was in part due to Ca^{2+}-dependent changes in the relative fractions of long and short openings. Fig. 6 *C* shows that the percentage of openings described by the short time constant exponent decreased monotonically as the Ca^{2+} concentration was increased.

The time constants reflecting short and intermediate closures were not dependent on Ca^{2+} concentration (Fig. 6 *D*), while the magnitude of the time constant describing long-duration closures was obviously sensitive to the concentration of Ca^{2+}. The time constant describing long closures progressively declined as the concentration of Ca^{2+} was increased (Fig. 6 *E*). As the magnitude of this time constant decreased, the number of described closings also declined, with a concomitant increase in the number of short-duration closures (Fig. 6 *F*). These data are very similar to the Ca^{2+}-dependence of open and closed states observed for rSK2 channels (Hirschberg et al., 1998). Therefore, the increase in SK channel P_o observed on increasing the concentration of Ca^{2+} is attributed to a decrease in the number and lifetime of long closures and an increase in the number of long-duration openings.

DISCUSSION

The conductance, Ca^{2+} sensitivity, and voltage-independent properties of SK channels in hippocampal CA1 pyramidal neurons were very similar to those observed for cloned apamin-sensitive rSK2 channels (Hirschberg et al., 1998). However, hippocampal SK channels are insensitive to the bee venom toxin, apamin (Lancaster and Adams, 1986). We have initiated a study of the properties of the apamin-insensitive cloned hSK1 channel. We have observed a sin-

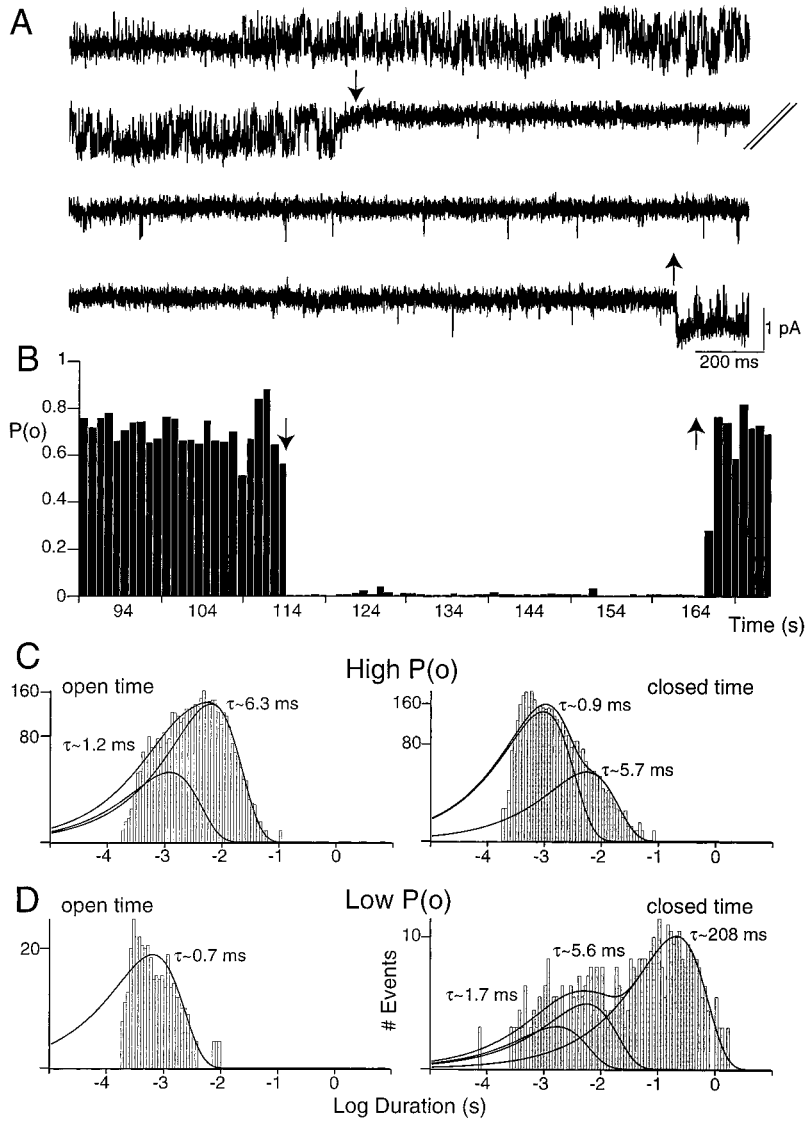

FIGURE 4 Switches between two gating behaviors. (*A*) Current traces from a patch containing a single SK channel bathed in 1 μM Ca^{2+}, at a holding potential of −60 mV. The parallel lines mark a break in the display during low P_o activity; 114 s after patch excision, 25 s after the start of the recording (*downward arrow*), channel activity spontaneously and abruptly decreased to a very low open probability. The rare openings were all of short duration. Channel activity abruptly increased back to its previous level 46 s later (*upward arrow*). (*B*) Stability plot for the patch in *A* showing the open probability during 1-s intervals as a function of time after patch excision. The arrows indicate the abrupt switch between high P_o activity and low P_o behavior (see *A*). (*C*) Open- (*left*) and closed-time (*right*) histograms assembled from the two periods of high open probability for the recording in *A*. The open-time histogram was fit by two exponentials with time constants 6.3 and 1.2 ms. The closed-time histogram was fit with two exponentials, with most closures being of very short duration. (*D*) Open- and closed-time histograms representing the period of low open probability shown in *A* and *B*. The open-time histogram was fit by a single exponential with a time constant of 0.7 ms, and the closed-time histogram was fit by the sum of three exponentials with time constants 1.7 ms, 5.6 ms, and 208 ms.

gle channel conductance of 9.2 ± 0.3 pS ($N = 3$) in isotonic potassium, a value not statistically different from that observed with hippocampal SK channels ($P > 0.01$; Köhler et al., 1996). hSK1 channels were half-activated by 0.7 ± 0.06 μM Ca^{2+}, with an n_H value of 3.9 ± 0.45 (Köhler et al., 1996), values very similar to hippocampal SK channels (see text). Combining data from both P_o behaviors, mean open-time constants for hippocampal SK channels were τ_1 = 1.1 ± 0.3 ms and τ_2 = 7.7 ± 2.9 ms ($N = 11$), while hSK1 open times were τ_1 = 1.0 ± 0.4 ms and τ_2 = 6.5 ± 2.3 ms ($N = 10$). These comparisons suggest that hippocampal SK channels may be the native correlate for the SK1 gene product. Support for this proposal comes from RT-pcr experiments on RNA isolated from acutely dissociated rat CA1 neurons. Primers were used to sequences within the core of the molecule that were conserved between rSK1 and hSK1, but divergent in rSK2. Control experiments showed that these primers were indeed subtype-specific (data not shown). These experiments show that an abundance of SK1 mRNA is present in hippocampal CA1 neurons, with only a minor presence of SK2 transcript (J. P. Adelman, unpublished observation). However, it should be noted that our comparison is between the SK channel native to rat hippocampal neurons and the heterologously expressed human SK1 channel. The biophysical properties of rSK1 are not known.

The slow rise of the slow AHP in hippocampal neurons has been proposed to arise from the diffusion of Ca^{2+} from its point of entry to the SK channel (Lancaster and Adams, 1986). The main support of this proposal is that the slow AHP can be eliminated by intracellular EGTA, a relatively slow Ca^{2+} chelator (Lancaster and Nicoll, 1987). The observed sensitivity of hippocampal SK channels to Ca^{2+} (EC_{50} 0.56 μM, see Fig. 1) demands that a substantial rise in intracellular Ca^{2+} concentration must underlie generation of the slow AHP. This argument is supported by the finding that the P_o of the SK channel at the peak of the slow AHP is 04−0.6 (Sah and Issacson, 1995; Valiante et al., 1997),

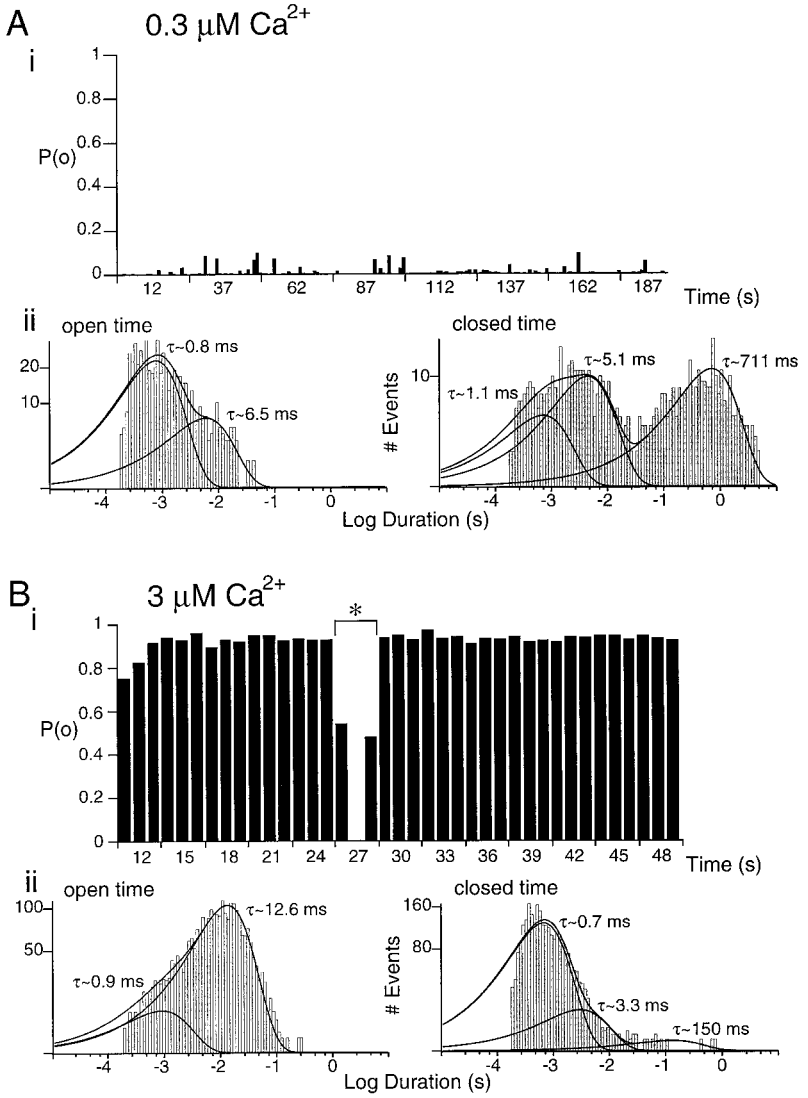

FIGURE 5 Ca^{2+}-dependence of SK channel gating. (*A*i) Open probability during 1-s intervals as a function of time during recording for a patch held at −60 mV and bathed in 0.3 μM Ca^{2+}. At this concentration of Ca^{2+}, SK channel activity was of low P_o behavior throughout. (*A*ii) Open- (*left*) and closed-time (*right*) histograms obtained from the recording illustrated in *A*i. The open-time histogram was fit with the sum of two exponentials, with most openings being of short duration (τ = 0.8 ms and 6.5 ms). The closed-time histogram was fit to the sum of three exponentials with time constants 1.1 ms, 5.1 ms, and 711 ms. The low P_o activity was characterized by the presence of the long closed-time component in the histogram. (*B*i) Open probability during 1-s intervals for the same patch as in *A* (holding potential −60 mV), after the concentration of Ca^{2+} was increased to 3 μM Ca^{2+}. A very brief switch to low open probability gating was seen ~26 s into the recording (*asterisk*). (*B*ii) Open- and closed-time histograms obtained from channel activity promoted by 3 μM Ca^{2+}. The open-time histogram was fit with two exponentials with time constants 0.9 and 12.6 ms. In contrast to low P_o activity, the closed-time histogram was fit to the sum of three exponentials, but with most of the closures being of short duration (τ = 0.68 ms and 3.3 ms). The presence of the small number of events contributing to the slowest component (τ = 150 ms) likely arises from the short period of low P_o activity (see above).

values that can only be observed with an intracellular Ca^{2+} concentration of 0.6–1 μM (see Fig. 1). However, bulk increases of intracellular Ca^{2+} only up to 0.1 μM have been measured (Knöpfel et al., 1990).

The slow AHP in hippocampal neurons is blocked by nimodipine, implying that L-type channels provide Ca^{2+} for SK channel activation (Rascol et al., 1991; Moyer et al., 1992; Tanabe et al., 1998). The subcellular location of SK channels in hippocampal neurons is not known. It has been proposed that they may be somatic (Lancaster et al., 1991) or located in the proximal dendritic tree (Sah and Bekkers, 1996). SK channels recorded in this study were somatic, as is the distribution of L-type Ca^{2+} channels in these neurons (Hell et al., 1993). This distribution, within a soma of ~10 μm in diameter, appears inconsistent with the slow rise of the slow AHP. It has been suggested that the characteristic time course of the slow AHP results from SK channels activating slowly in response to an increase in cytosolic Ca^{2+} (Sah and Clements, 1999). This was suggested because it was assumed that SK channels are located in the proximal apical dendrite (Sah and Bekkers, 1996) and the kinetics of the intracellular Ca^{2+} transient observed in this region are too rapid (Sah and Clements, 1999). However, this proposal assumes that SK channels are not somatic in their distribution, contradicting evidence from single channel recording (Lancaster et al., 1991; this study). In addition, to permit a model to be constructed reproducing the time course of the slow AHP, it was assumed that SK channels are activated by Ca^{2+} with an EC_{50} of 150 nM (Sah and Clements, 1999). This assumption is not supported by measurements of both cloned (Köhler et al., 1996; Hirschberg et al., 1998) and native (Lang and Ritchie, 1987; Lancaster et al., 1991; Grissmer et al., 1992; Park, 1994; Selyanko et al., 1998; this study) SK channels. Finally, this suggestion is not in agreement with the rapid activation of cloned SK channels by intracellular Ca^{2+} (Xia et al., 1998) and the finding in this study that the closed-time kinetics of hippocampal SK channels are consistent with them responding rapidly to a rise of intracellular Ca^{2+} (see Figs. 4–6).

Recently, L-type Ca^{2+} and SK channels have been observed within the same patch. These experiments have indicated that L and SK channels are colocalized, being sep-

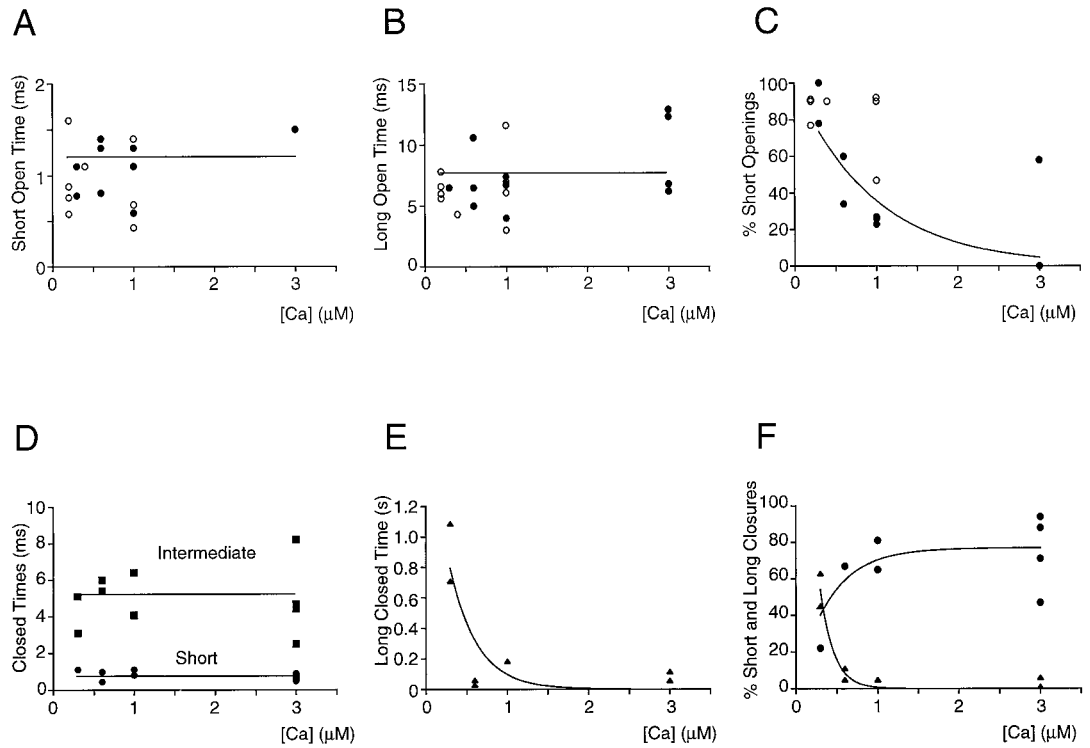

FIGURE 6 Effect of Ca^{2+} concentration on SK channel open- and closed-times. (*A*) Increasing the concentration of Ca^{2+} from 0.3 to 3 μM had no obvious effect on the magnitude of the short open-time constant in patches excised from hippocampal neurons (*closed circles*), and values were similar to those seen in patches from *Xenopus* oocytes expressing recombinant hSK1 channels (*open circles*). Hippocampal patches were held at −60 mV, and oocyte patches at −80 mV. Lines in all panels were drawn for display purposes only and were not fit to the data. (*B*) Increasing the Ca^{2+} concentration had no apparent effect on the magnitude of the long open-time constant (same patches as *A*). (*C*) The increase in SK channel P_o promoted by increasing the Ca^{2+} concentration is partly the result of a loss of short-duration openings. As the concentration of Ca^{2+} was increased from 0.3 to 3 μM, the percentage of short-duration openings decreased monotonically. (*D*) Raising the concentration of Ca^{2+} bathing patches excised from hippocampal CA1 neurons had no effect on the magnitude of either the short (*circles*) or intermediate (*squares*) closed-time components. (*E*) The Ca^{2+}-dependence of SK channel P_o likely results from the reduction in the duration and number of long closures. Plotted in *E* is the magnitude of the long closed-time as a function of Ca^{2+} concentration, showing that the time constant describing these events decreased monotonically as the Ca^{2+} concentration was increased. (*F*) As the concentration of Ca^{2+} was increased, the number of long closures decreased (*triangles*), while the percentage of short-duration closures diametrically increased (*circles*).

arated by only ~100–150 nm (Marrion and Tavalin, 1998). This finding is consistent with requiring ~1 μM Ca^{2+} to be present at the SK channel during the peak of the slow AHP (see above). However, it does not explain the kinetics of activation of the slow AHP. SK channels activate quite rapidly. For example, fast-flow application of 10 μM Ca^{2+} to excised inside-out macropatches found that hSK1 and rSK2 channels activated with time constants of 5.8 and 6.3 ms, respectively (Xia et al., 1998; see also Lancaster and Zucker, 1994). Modeling SK channel gating has predicted that rSK2 channels would activate with a time constant of ~20 ms in the presence of 1 μM Ca^{2+} (Hirschberg et al., 1998). Therefore, the relative proximity would permit a high enough concentration of Ca^{2+} to be present at the SK channel, but the predicted rate of activation does not allow for a slow rise of the slow AHP. It has been proposed that delayed facilitation of L-type Ca^{2+} channels underlies the time course of the slow AHP (Cloues et al., 1997). Delayed facilitation is induced by a train of action potential waveforms and is characterized by prolonged L-type channel activity at membrane potentials negative to −60 mV (Cloues et al., 1997). The time course of delayed facilitation is the same as the slow AHP, with both exhibiting a slow-rising phase and decay (Pedarzani and Storm, 1993; Cloues et al., 1997). Therefore, it is possible that delayed facilitation dictates the time course of SK channel activation, producing the characteristic slow rise and decay of the slow AHP.

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