Reconstitution of Expressed K_{Ca} Channels from *Xenopus* Oocytes to Lipid Bilayers

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ABSTRACT Reconstitution of large conductance calcium-activated potassium (K_{Ca}) channels from native cell membranes into planar lipid bilayers provides a powerful method to study single channel properties, including ion conduction, pharmacology, and gating. Recently, K_{Ca} channels derived from the *Drosophila Slowpoke* (*Slo*) gene have been cloned and heterologously expressed in *Xenopus* oocytes. In this report, we describe the reconstitution of cloned and expressed *Slo* K_{Ca} channels from *Xenopus* oocyte membranes into lipid bilayers. The reconstituted channels demonstrate functional properties characteristic of native K_{Ca} channels. They possess a mean unitary conductance of ≈260 pS in symmetrical potassium (250 mM), and they are voltage- and calcium-sensitive. At 50 μM Ca^{2+}, their half-activation potential was near −20 mV; and their affinity for calcium is in the micromolar range. Reconstituted *Slo* K_{Ca} channels were insensitive to external charybdotoxin (40–500 nM) and sensitive to micromolar concentrations of external tetraethylammonium (K_D = 158 μM, at 0 mV) and internal Ba^{2+} (K_D = 76 μM, at 40 mV). In addition, they were blocked by internally applied "ball" inactivating peptide (K_D = 480 μM, at 40 mV). These results demonstrate that cloned K_{Ca} channels expressed in *Xenopus* oocytes can be readily incorporated into lipid bilayers where detailed mechanistic studies can be performed under controlled internal and external experimental conditions.

INTRODUCTION

Large conductance calcium-activated K channels are widely distributed among many cell types (Latorre et al., 1989; McManus, 1991). Their voltage sensitivity, calcium dependence, and regulation by neurotransmitters and hormones suggest that they are important modulators of cellular processes such as contraction of smooth muscle (Suarez-Kurtz et al., 1991; Brayden and Nelson, 1992; Toro, 1994, in press; Anwer et al., 1993). Because of their large unitary conductance and the ability to reconstitute K_{Ca} channels from native membranes into lipid bilayers, these channels have served as models for ion permeation, gating mechanisms, and channel pharmacology (Vergara et al., 1984; MacKinnon and Miller, 1988; Miller, 1988). Reconstitution into lipid bilayers allows the study of single channels with simultaneous control of internal and external solutions, and possibly with a better control of the microenvironment of the channel than in excised membrane patches (Ertel, 1990), because cytoplasmic remnants are more likely to be absent in reconstituted channels. This accessibility to the internal and external side of the channel protein has proven valuable in modulatory studies (Toro et al., 1990; Reinhart et al., 1991; Chung et al., 1991; Scornik et al., 1993). Recently, a family of cloned K_{Ca} channels derived from the *Slowpoke* locus of *Drosophila melanogaster* (*Slo*) (Atkinson et al., 1991; Adelman et al., 1992;) and from its mammalian homolog *mSlo* (Butler et al., 1993) have been functionally expressed in *Xenopus* oocytes, providing a mechanism to study the molecular anatomy of these channels in relation to their function. Thus, we have isolated membranes from oocytes expressing *Slo* K_{Ca} channels and have transferred the cloned channels into lipid bilayers.

Received for publication 26 August 1993 and in final form 10 January 1994.
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0006-3495/94/04/1022/06 $2.00

MATERIALS AND METHODS

Oocyte expression

The cDNA encoding the *Slo* K_{Ca} channel A1/C2/E1/G3/I0 (Adelman et al., 1992) was transcribed using T7 RNA polymerase in the presence of 1 mM NTP, 0.6 mM GpppG (Pharmacia, Piscataway, NJ), and 0.5 units/μl of Inhibit-ACE RNase inhibitor (5 Prime → 3 Prime, Boulder, CO). Oocytes were injected with 20–30 ng of cRNA dissolved in sterile water and kept at 18°C for 7–10 days.

Isolation of membranes

Initial attempts to reconstitute ion channels from membranes of noninjected oocytes were performed with Dr. R. Miledi from University of California at Irvine. Membrane isolation was performed according to Colman (1984) with some modifications. The entire procedure was performed at 4°C. Oocytes (30–40) were rinsed using a solution containing (in mM): 400 KCl, 5 PIPES, pH 6.8 (*high K buffer*) supplemented with 100 μM phenylmethylsulfonylfluoride, 1 μM pepstatin, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μM p-aminobenzamidine, and 300 mM sucrose. Oocytes were transferred to a 1-ml ground glass tissue grinder (Kontes Duall) and homogenized manually with the same solution (≈10 μl/oocyte) for about 5 min. The homogenate (~200 μl) was layered onto a discontinuous sucrose gradient (0.75 ml of each 50 and 20%, w/v in *high K buffer* plus protease inhibitors), and centrifuged at 30,000 × *g* for 30 min in a TLS 55 rotor. The top lipid layer was eliminated, and the 20%:50% interface (visible band) was collected and diluted about 3 times with *high K buffer*. Membranes were pelleted at 50,000 × *g* for 30 min in a TLA 100.3 rotor, and resuspended in a final volume of ≈8–10 μl with a micropipet using buffer A containing (in mM): 300 sucrose, 100 KCl, 5 MOPS, pH 6.8. Membranes were frozen in liquid N_2 and stored at −70°C. By using this method, proper membrane fusion and reasonably stable recordings (>1 h) were obtained. An important step in this procedure was to dilute the membrane-enriched sucrose interface with *high K buffer*, otherwise reconstitution of channel activity was poor,

and bilayers were unstable. This step may enhance the separation of fatty acids and other components that may destabilize vesicles from the membrane preparation.

Reconstitution into lipid bilayers

Membrane vesicles from oocytes expressing *Slo* K_{Ca} channels were incorporated into lipid bilayers composed of phosphatidylethanolamine: phosphatidylcholine:phosphatidylserine in a ratio of 5:3:2 at 25 mg/ml in n-decane, as previously described (Toro et al., 1991). In most cases multiple channels were fused; thus, to obtain single channel incorporation, the membrane fraction was diluted 2–5 times in *buffer A*. Unless otherwise stated, channel recordings were performed in symmetrical conditions using (mM): 245 KMethanesulfonate, 5 KCl (or 250 KCl), 10 MOPS-K, 0.55 K_2EGTA, 0.6 $CaCl_2$, pCa 4.3, pH 7.4, or 250 KCl, 5 MOPSK, 0.1 $CaCl_2$, pH 7.4. Potassium methanesulfonate was used to avoid endogenous chloride conductances (Young et al., 1984; Mallinowska and Cuppoletti, 1992). Reconstituted membranes from noninjected oocytes (2 preparations) or injected with cRNA from Shaker H4-Δ6–46-T449Y K channels (fast and slow inactivation removed; López-Barneo et al., 1993) (2 preparations) did not induce maxi K_{Ca} channel-like activity. A rapid perfusion system was implemented that allowed complete exchange of the experimental solution in ≈15–30 s. In this way, problems of diffusion due to unstirred layers were avoided, and reversibility experiments were feasible. The vesicles were applied with a glass rod to the preformed bilayer from the *cis* chamber (the voltage-controlled side), whereas the *trans* chamber was referred to ground. Application of the sample to the preformed bilayers was readily effective in allowing channel incorporation and required very small volumes (≤1 μl of membrane preparation/experimental session), maximizing the use of the micropreparation. The internal side of the channels was determined by their Ca^{2+}- and voltage-sensitivity. Channels were incorporated with a random orientation; thus, the sign of the recordings was assigned referring to their orientation and is the convention used for cells (positive depolarizing pulses favor a higher activity). Data before acquisition were typically filtered at 500 Hz or 1 kHz and acquired at 400 or 200 μs/point, respectively. Single channel analysis was performed using TRANSIT (A. M. J. VanDongen, Duke University, Chapel Hill, NC). Open probability was obtained from the ratio between the open time and the total time. Channel amplitude was obtained from total point histograms. Kinetic analysis was performed in bilayers with only one active channel. Solutions with different calcium concentrations were obtained by adding different amounts of EGTA to the experimental solution. Free calcium concentrations were calculated according to Fabiato (1988). Unless otherwise stated, mean values ± SD are given.

RESULTS AND DISCUSSION

Slowpoke K_{Ca} channel reconstitution

Membranes isolated from oocytes expressing K_{Ca} channels were applied to the preformed bilayers. Subsequent voltage pulses usually elicited stable channel activity. Fig. 1 *A* shows an example of channel activity after reconstitution in symmetrical 245 mM KMes, 5 mM KCl (see Methods) at 40 mV and 50 μM free Ca^{2+}. At this potential channel open probability was high (P_O = 0.98), and channel openings were interrupted by abundant brief closings (≈1 ms) and sporadic silent periods (≈200 ms); in this case, the mean open time was 71 ms, and the mean closed time was 1.4 ms. Fig. 1 *B* illustrates the channel amplitude at different potentials in symmetrical K (250 mM). Channel amplitude was a linear function of voltage; the reversal potential was 0 mV. The fitting of similar data from eleven experiments gave a mean conductance of 262 ± 7 pS. The higher conductance obtained here with respect to previous measurements in the

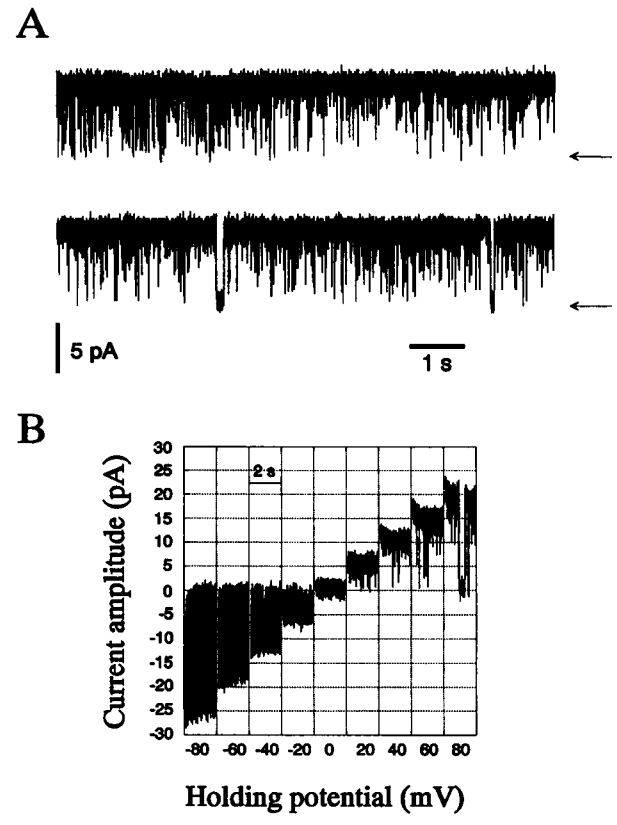

FIGURE 1 *Slowpoke* channel reconstitution. (*A*) Potassium channel activity recorded at 40 mV in symmetrical 245 mM KMethanesulfonate, 5 mM KCl. Free $[Ca^{2+}]$ = 50 μM. In this and following figures, arrows indicate the closed state of the channel. (*B*) Current-voltage relationship. Single channel activity recorded in symmetric potassium conditions (250 mM KCl) during a continuous stimulation of 2-s step pulse at the indicated voltages. The current amplitude was fitted as a linear function of the applied voltage giving a conductance for this channel of 265 pS.

oocyte (Adelman et al., 1992) may be due to a higher K concentration (250 vs. 120 mM) and the use of charged phospholipids in the bilayer experiments (Moczydlowski et al., 1985). The figure also illustrates how channel gating changes with potential. For example, at depolarized potentials channels opened for relatively long periods interrupted by brief closures similar to the ones described for Fig. 1. In addition, at higher voltages (e.g., 80 mV) occasional longer closed times (>400 ms), probably due to Ca^{2+} blockade (Vergara and Latorre, 1983) were also observed. On the other hand, at hyperpolarizing potentials channel openings and closings were short-lived (e.g., at −40 mV, τ_o = 2.9 ms; τ_c = 1.3 ms) (see also Fig. 2 *A*). This type of channel activity was only observed when membranes from oocytes injected with *Slo* K_{Ca} channel cRNA were reconstituted, but not with membranes from uninjected oocytes or oocytes injected with other cRNAs (n = 4 preparations, see Methods).

Voltage and calcium dependence

Large conductance ("maxi") K_{Ca} channels are characteristically sensitive to both voltage and calcium (Latorre et al.,

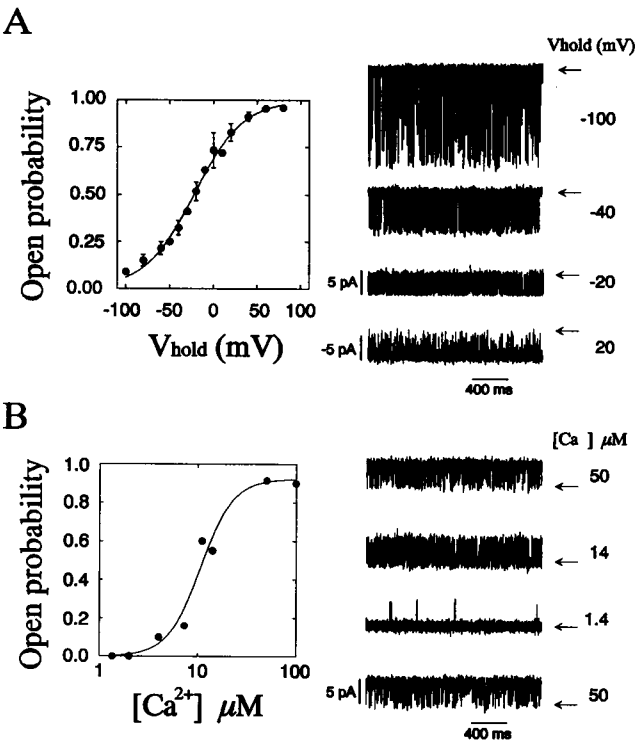

FIGURE 2 Voltage and Ca^{2+} dependence of single Slowpoke K_{Ca} channels. (*A*) Open probability versus membrane potential plot and examples of channel activity. Single channel experiments were analyzed (1–12 bilayers). Experimental points (mean ± SEM) were fitted to a Boltzmann distribution (*continuous line*) of the form: $P_0 = 1/(1 + \exp((V_{1/2} - V)/k))$; where $V_{1/2}$ = half-activation potential, and $k = RT/zF$, (slope factor). *R*, *T*, and *F* have their usual thermodynamic meanings, and *z* is the effective valence. Fitted values were: $V_{1/2} = -22 \pm 1$ mV, and $z = 0.9 \pm 0.05$. Similar values were obtained by fitting the voltage dependence of each channel separately ($V_{1/2} = -22 \pm 15$ mV; $z = 0.97 \pm 0.21$; $n = 12$) (see text). Corresponding single channel traces on the left illustrate how channel P_0 diminishes with hyperpolarizing voltages. Current polarity was inverted in the last trace (20 mV) for illustrating purposes. (*B*) Relationship between internal free Ca^{2+} and open probability and examples of channel activity. Experimental points (●) were obtained in the same channel by perfusing the *trans* chamber (internal) with solutions containing different free Ca^{2+} concentrations (see Methods). The points were fitted to a Hill function: $P_0 = P_{0-max}/(1 + (K_{1/2}/[Ca^{2+}])^N)$ where $(K_{1/2})^N$ is the dissociation constant, $K_{1/2}$ is the half activation concentration, and *N* is the Hill coefficient. $K_{1/2}$ was 11 μM, and *N* was 2.6. Traces on the left illustrate the change in channel activity during a sequential change in internal free Ca^{2+}. Holding potential was 20 mV.

1989; McManus, 1991). These properties are intimately associated, suggesting that calcium ions responsible for channel opening bind to a region within the membrane electric field (Latorre et al., 1983). Fig. 2 shows that reconstituted, cloned K_{Ca} channels display these two defining properties (Adelman et al., 1992). As discussed above, channel activity changes with variations in voltage; this voltage dependence can be quantitatively described by fitting the experimental points to a Boltzmann distribution. Analysis of 12 reconstituted K_{Ca} channels, at $[Ca^{2+}]_i = 50$ μM, gave half activation potentials ($V_{1/2}$) ranging from ≈−60 to −3 mV, and a calculated effective valence (*z*) of near 1 (range 0.7–1.4). Mean values were −22 ± 15 mV and 0.97 ± 0.21, respectively (Fig. 2 *A*). This variation in $V_{1/2}$ may result from heterogeneous channel affinities for Ca^{2+}. With respect to the weaker voltage dependency ($z \approx 1$), this property seems to be a distinct characteristic of *Drosophila Slo* K_{Ca} channels because the majority of channels of this class from mammalian tissues, including the recently cloned *mSlo* K_{Ca} channel, have equivalent charges of around 2 (Latorre et al., 1989; Butler et al., 1993). It is interesting to note that the putative voltage sensor (S4 region) of *Slo* and *mSlo* K_{Ca} channels possesses less charged amino acid residues than other K channels with larger voltage dependence (Adelman et al. 1992; Jan and Jan, 1992; Butler et al., 1993).

Fig. 2 *B* illustrates a Ca^{2+} activation curve at 20 mV obtained by perfusing solutions of defined $[Ca^{2+}]_i$. The records at right show channel activity at different $[Ca^{2+}]_i$ and the recovery of channel activity after return to the initial $[Ca^{2+}]_i$ (compare top and bottom traces). The concentration necessary to half-activate this channel ($K_{1/2}$) was 11 μM, and the Hill coefficient (*N*) was 2.6. Ca^{2+} dependence of K_{Ca} channels is known to vary not only among tissues (Latorre et al., 1989; McManus, 1991), but within the same cell type. In reconstitution and patch experiments, variations from channel to channel in the same preparation can be up to fourfold (Moczydlowski and Latorre, 1983; McManus and Magleby, 1991); this property has been ascribed to molecular diversity among K_{Ca} channels (Reinhart et al., 1989; Toro et al., 1991). However, cloned K_{Ca} channels, homogeneous at the molecular level, seem to also display this property as reflected in the range of P_0 at a constant voltage and internal Ca^{2+} concentration. For example, at −20 mV and with 50 μM internal Ca^{2+}, P_0 values ranged from 0.28 to 0.78 ($n = 12$). These differences are clearly illustrated in Fig. 4 *B*, which shows two K_{Ca} channels simultaneously incorporated with low and high P_0. These variations in channel activity may explain the apparent differences between the bilayer experiments described here and those in excised membrane patches (Adelman et al., 1992). In our experiments it is possible that such variations are due to different posttranslational modifications such as phosphorylation. Alternatively, this may reflect either changes due to the isolation procedure or the activity of channel proteins from intracellular membranes as well as the plasmalemma membranes, which may vary in the extent of posttranslational modifications.

Pharmacological profile

The pharmacology of potassium channels has been exploited to map their structure and to understand basic features of their function. K_{Ca} channels in particular are known to be sensitive to micromolar concentrations of external tetraethylammonium (TEA), internal Ba^{2+} (Latorre et al., 1989), and internal "ball" inactivating peptide (BP) (Foster et al., 1992; Toro et al., 1992). A majority of K_{Ca} channels are also sensitive to nanomolar concentrations of external charybdotoxin (Latorre et al., 1989), although in brain a charybdotoxin-insensitive K_{Ca} channel has been reported (Reinhart et al., 1991). The results shown below demonstrate that *Slo* K_{Ca}

channels share pharmacological characteristics with their mammalian counterparts.

Fig. 3 *A* shows that reconstituted *Slo* K_{Ca} channels are blocked by TEA with a typical fast-blocking mechanism (Yellen, 1984; Benham et al., 1985; Villarroel et al., 1988) that gives a decrease in channel amplitude. The K_D at 0 mV was 158 ± 8 μM (n = 5), and the Hill coefficient was 1.0. This K_D value agrees with previous ensemble current analyses (Adelman et al., 1992) and suggests that the TEA receptor is well conserved, because similar values have been obtained in K_{Ca} channels from many different sources (Latorre et al., 1989).

Fig. 3 *B* illustrates that the "ball" peptide also inhibits channel activity with a characteristic "flickery" type of block, which is sufficiently slow (medium block) to permit the detection of the whole transitions from the open to the blocked state (Yellen, 1984). At 40 mV, the estimated K_D value was 480 ± 88 μM, and the Hill coefficient was near 1 (0.72 ± 0.09, n = 2). Compared to other K channels, *Slo* K_{Ca} channels have ≈fivefold lower affinity for BP than K_{Ca} channels from coronary smooth muscle (Toro et al., 1992), ≈20-fold lower affinity than *Shaker* B K channels (Zagotta et al., 1990), and ≈50-fold lower affinity than K_{Ca} channels expressed in rat brain (Foster et al., 1992). These reduced affinities suggest that, although there is a consensus receptor site for BP in K channels, their structural properties vary. Indeed, the BP receptor has been localized to the intracellular loop between the fourth and fifth transmembrane segments of cloned voltage-dependent potassium channels (Isacoff et al., 1991). Accordingly, the sequence alignment of *Shaker* B K and *Slo* K_{Ca} channels in this region (Scheme 1) reveals that amino acid residues important in the BP binding site have been conserved (T388, S392, and L396; residues in boxes), but E395, a residue critical for the BP receptor is not present in *Slo* K_{Ca} channel (shaded box). This difference in primary structure may contribute to the reduced affinity of *Slo* K_{Ca} channels compared to the affinity of Shaker B K channels (Zagotta et al., 1990); the negative charge might favor electrostatic interaction between the positively charged domain of BP and its receptor in the channel protein.

Shaker B (382):	L	Q	I	L	G	R	T	L	K	A	S	M	R	E	T				
Slo K_{Ca} (239):	L	N	V	L		K	T	S		S	S	I	R		T				

Scheme 1

Fig. 4 *A* illustrates the internal block by Ba^{2+} in *Slo* K_{Ca} channels that had similar characteristics to the Ba^{2+} blockade in mammalian K_{Ca} channels (Vergara and Latorre, 1983). The K_D was 76 ± 6 μM, which is similar to the K_D found in rabbit smooth muscle K_{Ca} channels (Benham et al., 1985) and larger than the reported value for skeletal muscle channels (Latorre et al., 1989); also, the Hill coefficient was near 1 (0.7 ± 0.05, n = 3). This difference in affinity indicates that Ba^{2+} blockade (similar to BP), although conserved in evolution, may result from different amino acid composition

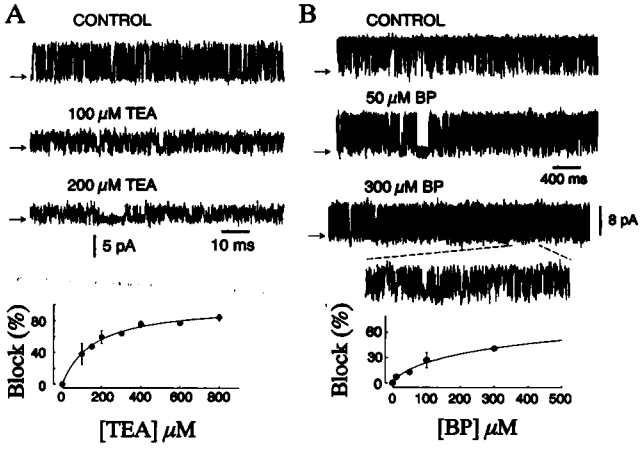

FIGURE 3 Slowpoke K_{Ca} channel sensitivity to external TEA and internal inactivating "ball" peptide (BP). (*A*) Sensitivity to external TEA at 0 mV. Single channel traces illustrate the change in channel amplitude at different TEA concentrations (250/50 KCl, 50 μM free Ca^{2+}). The dose-response curve was obtained from five different experiments. The points were fitted using the following equation: %Block = $100/(1 + (K_{1/2}/[TEA])^N)$. $(K_{1/2})^N$ and N were defined in Fig. 2. %Block at each [TEA] was $(1-i_{TEA}/i_0) * 100$, where i is the unitary current before (i_0) and after TEA (i_{TEA}). The Hill coefficient obtained from the fit (*continuous line*) was 1.0, and the dissociation constant (K_D) was 158 μM. (*B*) Sensitivity to BP at 40 mV in symmetrical 250 KCl and 100 μM $CaCl_2$. BP blocked *Slo* K_{Ca} channel activity in a dose-dependent manner. Note that the main effect was the production of fast transitions from the open to the blocked state (≈0.7 ms). In this example, mean open time was reduced from 12 ms in the control to 1.5 ms after 300 μM BP, whereas mean closed times remained practically the same (1.8 vs. 1.5 ms). Number of transitions: control = 6885; BP = 29866. Dotted lines show a section of the trace at a different time scale. The dose-response curve was fitted using the same function as in *A*, but using %Block = $(1-P_{0-BP}/P_{0-control}) * 100$. N was 0.7, and the estimated value for K_D was 480 μM.

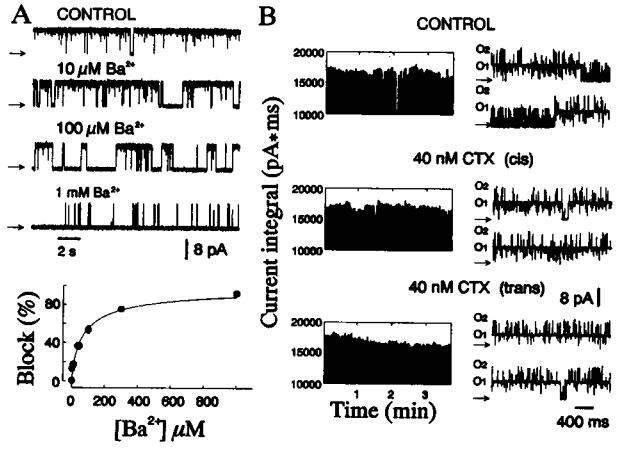

FIGURE 4 Sensitivity to internal Ba^{2+} and charybdotoxin (CTX). (*A*) Perfusion of micromolar concentrations of internal Ba^{2+} promoted the appearance of slow closings whose frequency increased by increasing $[Ba^{2+}]$. The dose-response curve at 40 mV gave an N value of near 1 and a K_D of 76 μM. Data were fitted as in Fig. 3 *B*. Symmetrical 250 mM KCl, 100 μM Ca^{2+}. Sampling rate 1 ms/point; filter 500 Hz. (*B*) Current integral versus time plots obtained from two active channels at 0 mV in 250/50 mM KCl (*cis/trans*). CTX (40 nM) did not modify channel activity when applied in either side of the channels. Right panels show corresponding channel activities; arrows indicate the closed state, and O1 and O_2 the first and the second open level of the channels, respectively.

and/or spatial array conferring the binding site various affinities in distant channels of this family. In this context, in DRK1 K channel, the amino acid V374, which would correspond to V302 of *Slo* K_{Ca} channel (Adelman et al., 1992), has been identified as a critical pore residue for Ba^{2+} blockade (Taglialatela et al., 1993); however, the K_D for internal Ba^{2+} blockade in DRK1 is smaller (13 μM) than in *Slo* K_{Ca} channel (76 μM), suggesting that other residues are involved in the binding of Ba^{2+} ion to its site.

Fig. 4 *B* illustrates that charybdotoxin (40 nM) was unable to block K_{Ca} channels when added either to the internal or external side of the channel. Similarly, higher external concentrations of the toxin 100–500 nM did not block channel activity ($n = 3$). These data demonstrate at the single channel level that *Slo* K_{Ca} channels do not possess a high affinity charybdotoxin binding site, and confirm previous studies on macroscopic currents (Adelman et al., 1992). Thus, *Slo* K_{Ca} channels share the peculiarity of being charybdotoxin-insensitive with type-2 K_{Ca} channels from brain (Reinhart et al., 1991).

In conclusion, these results demonstrate that K_{Ca} channels expressed in *Xenopus* oocytes can be readily studied after being incorporated into lipid bilayers, that they share common characteristics with their mammalian counterparts, and that similar binding sites for TEA, Ba^{2+}, and "ball" inactivating peptide have been conserved through evolution.

This work was supported by grants HL37044 (E.S.), HL47382 (L.T.), NS28504 (J.P.A.) from the National Institutes of Health. The authors would like to thank Drs. E. Stefani for helpful discussion, A. M. J. VanDongen for TRANSIT, D. Malinowska, J. Cupolleti for discussion on the isolation method, and R. Miledi for pioneering experiments on reconstitution of oocyte's membranes.

REFERENCES

Adelman, J. P., K. Z. Shen, M. P. Kavanaugh, R. A. Warren, Y. N. Wu, A. Lagrutta, C. T. Bond, and R. A. North. 1992. Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron.* 9:209–216.

Anwer, K., C. Oberti, G. J. Perez, N. Perez-Reyes, J. K. McDougall, M. Monga, B. M. Sanborn, E. Stefani, and L. Toro. 1993. Calcium-activated K^+ channels as modulators of human myometrial contractile activity. *Am. J. Physiol.* 265:C976–C985.

Atkinson, N. S., G. A. Robertson, and B. Ganetzky. 1991. A component of calcium-activated potassium channels encoded by the *Drosophila slo* locus. *Science.* 253:551–555.

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 Arch.* 403:120–127.

Brayden, J. E., and M. T. Nelson. 1992. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science.* 256:532–535.

Butler, A., S. Tsunoda, D. P. McCobb, A. Wei, and L. Salkoff. 1993. *mSlo*, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science.* 261:221–224.

Chung, S., P. H. Reinhart, B. L. Martin, D. Brautigan, and I. B. Levitan. 1991. Protein kinase activity closely associated with a reconstituted calcium-activated potassium channel. *Science.* 253:560–562.

Colman, A. 1984. Translation of eukaryotic messenger RNA in *Xenopus* oocytes. *In* Transcription and Translation. B. D. Hames and S. J. Higgins, editors. IRL Press, Oxford, England. 271–302.

Ertel, E. A. 1990. Excised patches of plasma membrane from vertebrate rod outer segments retain a functional phototransduction enzymatic cascade. *Proc. Natl. Acad. Sci. USA.* 87:4226–4230.

Fabiato, A. 1988. Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods Enzymol.* 157:378–417.

Foster, C. D., S. Chung, W. N. Zagotta, R. W. Aldrich, and I. B. Levitan. 1992. A peptide derived from the *Shaker* B K^+ channel produces short and long blocks of reconstituted Ca^{2+}-dependent K^+ channels. *Neuron.* 9:229–236.

Isacoff, E. Y., Y. N. Jan, and L. Y. Jan. 1991. Putative receptor for the cytoplasmic inactivation gate in the *Shaker* K^+ channel. *Nature* 353: 86–90.

Jan, L. Y., and Y. N. Jan. 1992. Structural elements involved in specific K^+ channel functions. *Ann. Rev. Physiol.* 54:537–555.

Latorre, R., A. Oberhauser, P. Labarca, and O. Alvarez. 1989. Varieties of calcium-activated potassium channels. *Ann. Rev. Physiol.* 51: 385–399.

Latorre, R., C. Vergara, and E. Moczydlowski. 1983. Properties of a Ca^{2+}-activated K^+ channel in a reconstituted system. *Cell Calcium.* 4:343–357.

López-Barneo, J., T. Hoshi, S. H. Heinemann, and R. W. Aldrich. 1993. Effects of external cations and mutations in the pore region on C-type inactivation of *Shaker* potassium channels. *Receptors and Channels.* 1:61–71.

MacKinnon, R., and C. Miller. 1988. Mechanism of charybdotoxin block of the high-conductance, Ca^{2+}-activated K^+ channel. *J. Gen. Physiol.* 91:335–349.

Mallinowska, D. H., and J. Cuppoletti. 1992. Expression of gastric Cl^- channels in *Xenopus* laevis oocytes. *Biophys. J.* 61:10*a*. (Abstr.)

McManus, O. B. 1991. Calcium-activated potassium channels: regulation by calcium. *J. Bioenerg. Biomembr.* 23:537–560.

McManus, O. B., and K. L. Magleby. 1991. Accounting for the Ca^{2+}-dependent kinetics of single large-conductance Ca^{2+}-activated K^+ channels in rat skeletal muscle. *J. Physiol.* 443:739–777.

Miller, C. 1988. Competition for block of a Ca^{2+}-activated K^+ channel by charybdotoxin and tetraethylammonium. *Neuron.* 1:1003–1006.

Moczydlowski, E., O. Alvarez, C. Vergara, and R. Latorre. 1985. Effect of phospholipid surface charge on the conductance and gating of a Ca^{2+}-activated K^+ channel in planar lipid bilayers. *J. Membr. Biol.* 83:273–282.

Moczydlowski, E., and R. Latorre. 1983. Gating kinetics of Ca^{2+}-activated K^+ channels from rat muscle incorporated into planar lipid bilayers. Evidence for two voltage-dependent Ca^{2+} binding reactions. *J. Gen. Physiol.* 82:511–542.

Ottolia, M., E. Stefani, L. Toro, and R. Latorre. 1994. Mapping the "ball" peptide (BP) receptor of K_{Ca} channels. *Biophys. J.* 66:108*a* (Abstr.).

Reinhart, P. H., S. Chung, and I. B. Levitan. 1989. A family of calcium-dependent potassium channels from rat brain. *Neuron.* 2:1031–1041.

Reinhart, P. H., S. Chung, B. L. Martin, D. L. Brautigan, and I. B. Levitan. 1991. Modulation of calcium-activated potassium channels from rat brain by protein kinase A and phosphatase 2A. *J. Neurosci.* 11: 1627–1635.

Scornik, F. S., J. Codina, L. Birnbaumer, and L. Toro. 1993. Modulation of coronary smooth muscle K_{Ca} channels by $G_{\alpha s}$ independent of phosphorylation by protein kinase A. *Am. J. Physiol.* 265:H1460–H1465.

Suarez-Kurtz, G., M. L. Garcia, and G. J. Kaczorowski. 1991. Effects of charybdotoxin and iberiotoxin on the spontaneous motility and tonus of different guinea pig smooth muscle tissue. *J. Pharmacol. Exp. Ther.* 259: 439–443.

Taglialatela, M., J. A. Drewe, and A. M. Brown. 1993. Barium blockade of a clonal potassium channel and its regulation by a critical pore residue. *Mol. Pharmacol.* 44:180–190.

Toro, L. 1994. Calcium-activated K^+ channels from coronary smooth muscle. Mechanisms of modulation and role in cardiac function. *In* Physiology and Pathophysiology of the Heart. N. Sperelakis, editor. Kluwer Academic Publ., Norwell, MA. In press.

Toro, L., J. Ramos Franco, and E. Stefani. 1990. GTP-dependent regulation of myometrial K_{Ca} channels incorporated into lipid bilayers. *J. Gen. Physiol.* 96:373–394.

Toro, L., E. Stefani, and R. Latorre. 1992. Internal blockade of a Ca^{2+}-activated K^+ channel by *Shaker B* inactivating "ball" peptide. *Neuron.* 9:237–245.

Toro, L., L. Vaca, and E. Stefani. 1991. Calcium-activated potassium channels from coronary smooth muscle reconstituted in lipid bilayers. *Am. J. Physiol.* 260:H1779–H1789.

Vergara, C., and R. Latorre. 1983. Kinetics of Ca^{2+}-activated K^+ channels from rabbit muscle incorporated into planar bilayers. Evidence for a Ca^{2+} and Ba^{2+} blockade. *J. Gen. Physiol.* 82:543–568.

Vergara, C., E. Moczydlowski, and R. Latorre. 1984. Conduction, blockade and gating in a Ca^{2+}-activated K^+ channel incorporated into planar lipid bilayers. *Biophys. J.* 45:73–76.

Villarroel, A., O. Alvarez, A. Oberhauser, and R. Latorre. 1988. Probing a Ca^{2+}-activated K^+ channel with quaternary ammonium ions. *Pflügers Arch.* 413:118–126.

Yellen, G. 1984. Ionic permeation and blockade in Ca-activated K-channels of bovine chromaffin cells. *J. Gen. Physiol.* 84:157–186.

Young, G. P. H., J. D.-E. Young, A. K. Deshpande, M. Goldsteon, S. S. Koide, and Z. A. Cohn. 1984. A Ca^{2+}-activated channel from *Xenopus* laevis oocyte membranes reconstituted into planar bilayers. *Proc. Natl. Acad. Sci. USA.* 81:5155–5159.

Zagotta, W. N., T. Hoshi, and R. W. Aldrich. 1990. Restoration of inactivation in mutants of *Shaker* potassium channels by a peptide derived from ShB. *Science.* 250:568–571.