that cannot deactivate themselves, such as G protein βγ subunits. It has been argued that the effects of βγ on cardiac K^+ channels (*20*) and phospholipase C-β occur at high, physiologically irrelevant concentrations when compared with the effects of GTP-γ-S–G_α. Half-maximal stimulation of phospholipase C-β by GTP-γ-S–$G_{q\alpha}$ occurs at concentrations of approximately 300 pM. It is estimated that phospholipase C-β increases the k_{cat} for hydrolysis of GTP by $G_{q\alpha}$ approximately 100-fold to a value greater than 80 min^{-1} (*18*). These values (on the assumption that $k_1 = 10^9$ M^{-1} min^{-1}) yield a $K_{app,50}$ greater than 80 nM, which is similar to the concentration of βγ required to activate phospholipase C-β. Thus, the necessary concentrations of GTP-G_α and βγ may be comparable. (iv) High values of $K_{app,50}$ (compared to the low values of K_d obtained with GTP-γ-S–G_α) are consistent with the high concentrations of $G_{i\alpha}$, and in particular $G_{o\alpha}$, found in many cells. We suggest that the affinities of activated $G_{o\alpha}$ for its effectors are low, that these effectors are GAPs, or that both of these conditions exist.

REFERENCES AND NOTES

1. A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987); H. R. Bourne, D. A. Sanders, F. McCormick, *Nature* **348**, 125 (1990); M. I. Simon, M. P. Strathmann, N. Gautam, *Science* **252**, 802 (1991).
2. T. Katada, G. M. Bokoch, J. K. Northup, M. Ui, A. G. Gilman, *J. Biol. Chem.* **259**, 3568 (1984); T. Katada, G. M. Bokoch, M. D. Smigel, M. Ui, A. G. Gilman, *ibid.*, p. 3586.
3. W.-J. Tang, J. Krupinski, A. G. Gilman, *J. Biol. Chem.* **266**, 8595 (1991).
4. R. Taussig, L. M. Quarmby, A. G. Gilman, *ibid.* **268**, 9 (1993).
5. W.-J. Tang and A. G. Gilman, *Science* **254**, 1500 (1991).
6. B. Gao and A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10178 (1991).
7. A. Yatani, J. Codina, A. M. Brown, L. Birnbaumer, *Science* **235**, 207 (1987).
8. M. E. Linder, D. A. Ewald, R. J. Miller, A. G. Gilman, *J. Biol. Chem.* **265**, 8243 (1990).
9. S. M. Mumby, R. O. Heuckeroth, J. I. Gordon, A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 728 (1990); M. E. Linder *et al.*, *ibid.* **90**, 3675 (1993).
10. Y. H. Wong, B. R. Conklin, H. R. Bourne, *Science* **255**, 339 (1992); Y. H. Wong *et al.*, *Nature* **351**, 63 (1991).
11. M. E. Linder *et al.*, *J. Biol. Chem.* **266**, 4654 (1991).
12. R. Taussig and A. G. Gilman, unpublished data.
13. C. Gallego, S. K. Gupta, S. Winitz, B. J. Eisfelder, and G. L. Johnson [*Proc. Natl. Acad. Sci. U.S.A.* **89**, 9695 (1992)] observed that the myristoylation of $G_{i\alpha}$ was necessary for downstream signaling in transfected cells.
14. G. E. Breitwieser and G. Szabo, *J. Gen. Physiol.* **91**, 469 (1988).
15. K. H. Jakobs, K. Aktories, G. Schultz, *Arch. Pharmacol.* **310**, 113 (1979).
16. P. C. Sternweis and I.-H. Pang, *Trends Neurosci.* **13**, 122 (1990).
17. K. H. Muntz, A. G. Gilman, S. M. Mumby, *Circulation* **86**, 764 (1992).
18. G. Berstein *et al.*, *Cell* **70**, 411 (1992).
19. D. C. May, E. M. Ross, A. G. Gilman, M. D. Smigel, *J. Biol. Chem.* **260**, 15829 (1985); M. Freissmuth and A. G. Gilman, *ibid.* **264**, 21907 (1989).
20. L. Birnbaumer, *Trends Pharmacol. Sci.* **8**, 209 (1987).
21. To construct the recombinant baculovirus encoding type V adenylyl cyclase, the appropriate complementary DNA (cDNA) was excised from plasmid cDNA 1-113-72 [Y. Ishikawa *et al.*, *J. Biol. Chem.* **267**, 13553 (1992)] by digestion with Sma I and Xba I and was ligated into pVL1393. The resulting DNA was cotransfected into Sf9 cells with linearized AcRP23-LacZ viral DNA. The recombinant virus was isolated and plaque purified, and infected cells were screened for adenylyl cyclase activity.
22. M. D. Smigel, *ibid.* **261**, 1976 (1986).
23. M. P. Graziano, M. Freissmuth, A. G. Gilman, *ibid.* **264**, 409 (1989).
24. P. C. Sternweis and J. D. Robishaw, *ibid.* **259**, 13806 (1984).
25. E. M. Ross, M. E. Maguire, T. W. Sturgill, R. L. Biltonen, A. G. Gilman, *ibid.* **252**, 5761 (1977).

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mSlo, a Complex Mouse Gene Encoding "Maxi" Calcium-Activated Potassium Channels

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Complementary DNAs (cDNAs) from *mSlo*, a gene encoding calcium-activated potassium channels, were isolated from mouse brain and skeletal muscle, sequenced, and expressed in *Xenopus* oocytes. The *mSlo*-encoded channel resembled "maxi" or BK (high conductance) channel types; single channel conductance was 272 picosiemens with symmetrical potassium concentrations. Whole cell and single channel currents were blocked by charybdotoxin, iberiotoxin, and tetraethylammonium ion. A large number of variant *mSlo* cDNAs were isolated, indicating that several diverse mammalian BK channel types are produced by a single gene.

Calcium-dependent K^+ channels [K(Ca) channels] are associated with a broad spectrum of cell physiology, including bursting in neurons (*1*), secretion in endocrine and exocrine cells (*2*), contraction in muscle cells (*3*), activation of T cells (*4*), and the regulation of myogenic tone in arterial smooth muscle (*5*). Although all K(Ca) channels are dependent on Ca^{2+} for their activation, some are synergistically activated by voltage and Ca^{2+}, whereas others are insensitive to voltage (*6*). K(Ca) channels from different cells vary widely in their conductance, regulation, and sensitivity to Ca^{2+} and voltage. This diversity may reflect the existence of a multigene family encoding homologous channel proteins or a mechanism such as alternative RNA splicing that produces variant channels from a single gene. Both mechanisms generate the diversity of purely voltage-dependent K^+ channels (*7*). We show here that in the mouse, many variant K(Ca) channel peptides are produced by a single gene.

The *Drosophila slo* gene (*8–10*) encodes K(Ca) channels present in both neurons (*11*) and muscle (*8, 12*). To isolate a mammalian homolog of *slo*, we first isolated a *Drosophila slo* cDNA. Polymerase chain reaction primers based on published sequence (*9*) were used to amplify *slo* gene fragments directly from genomic DNA. Two ~200–base pair (bp) fragments representing either end of hydrophobic segments S1 to S6 (Fig. 1A) were generated and used as hybridization probes to isolate a *slo* cDNA containing a large portion of the coding region. A 1300-bp fragment spanning the S1 to S6 region was then used as a probe for hybridization to a mouse brain cDNA library (Clontech, Palo Alto, California) under low stringency conditions (*13*). Clone mbrl was found to contain a single open reading frame encoding a peptide homologous to the *Drosophila* slo protein from amino acid 96 to 448 (Fig. 1C). The mbrl clone was then used as a hybridization probe for the isolation of *mSlo* cDNAs from both mouse brain and skeletal muscle cDNA libraries.

The deduced translation product of *mSlo* (mSlo) displays extensive sequence conservation with the *Drosophila* slo protein (Fig. 1). Similar to slo, as well as the extended family of voltage-dependent K^+ channels (*7*), mSlo contains six hydrophobic segments (S1 to S6; Fig. 1) that are presumed to span the membrane and surround the pore

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(Fig. 1, B and C). Of potential importance, the mSlo and slo peptides share four additional conserved hydrophobic segments not found in purely voltage-dependent channels (S7 to S10; Fig. 1, B and C). Indeed, S10 and an adjacent region are the most conserved segments of the protein. Segments S7 to S10 may be membrane-spanning segments or intramembrane structures that enter and exit the membrane on a single face. Alternatively, these segments may constitute the hydrophobic interior of protein structure on either side of the membrane.

The mSlo protein (Fig. 1C, mbr8) is 1196 amino acids long, approximately twice the length of voltage-dependent K^+ channels (*7*). The additional length is at the COOH-terminus, which includes S7 to S10. The mSlo and slo peptides show >60% amino acid identity over two regions spanning a total of 997 amino acids (Fig. 1). Conservation of the first region, S1 to S8, abruptly terminates at a precisely conserved RNA splice junction (splice junction A; Fig. 1, A and C). The adjacent nonconserved region had been predicted to encode a calcium binding loop (EF-hand) of the type present in calmodulin (9). However, the EF-hand motif is not present here or elsewhere in mSlo. The second

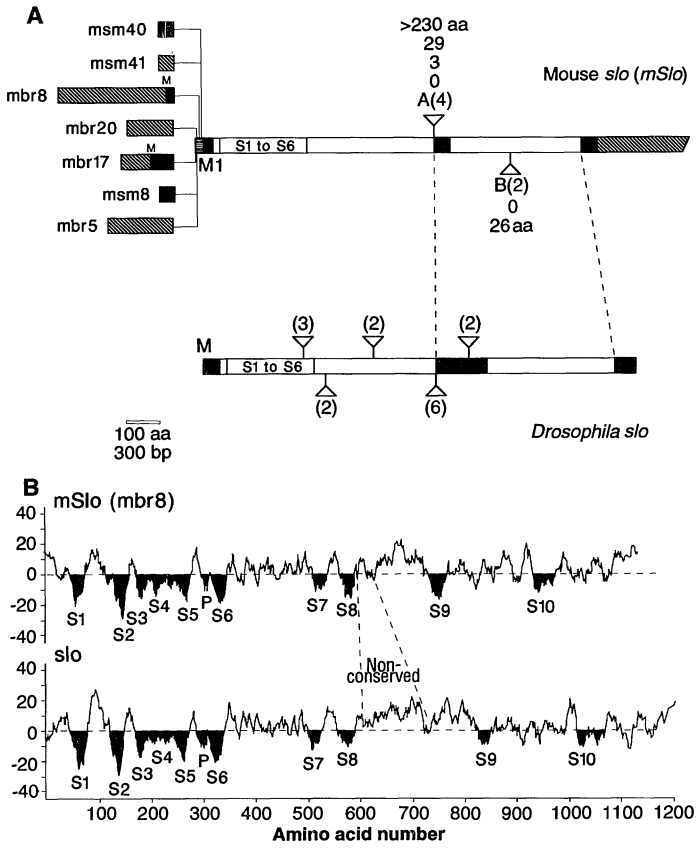

Fig. 1. (**A**) Organization of *mSlo* cDNAs showing alternative splice sites A and B and variant 5′ ends. The number of alternative exons at A and B is indicated in parentheses, and the numbers above and below indicate the size of each. Abbreviations: M1, initiator Met common to several variant cDNAs that have a closed upstream reading frame; M, possible initiator Met; mbr, mouse brain origin; msm, mouse skeletal muscle. Open boxes, conserved (>60% amino acid identity) region; filled boxes, nonconserved translated region; cross-hatching, untranslated region; horizontal hatching, polyserine. (**B**) Hydrophilicity profiles of mouse and *Drosophila* slo proteins. The profiles were computed with a window size of 19 amino acids (*23*). Negative (downward) index values indicate hydrophobic groups, and positive values indicate hydrophilic groups. Ten conserved hydrophobic segments (S1 to S10) are indicated as filled areas. Splice junction A is at the right boundary of S8. P, putative pore region. (**C**) Deduced amino acid sequence of mSlo (upper sequence) and alignment with *Drosophila* slo (*24*). Identical residues are shaded. Proposed membrane-spanning regions are underlined and designated S1 to S6. Additional hydrophobic regions with uncertain topology are underlined and designated S7 to S10. M1 is the initiator Met for mbr5. "PORE" indicates the putative pore region. Box at circled "A" indicates a three-residue alternative exon at splice junction A. Circled "B" indicates an additional site of alternative splicing. Both expressed cDNAs, mbr8 and mbr5, were incomplete at their 3′ termini; expression constructs were made with the mbr3 COOH-terminus. Nucleotide sequence of cDNAs reported in this paper have been deposited in GenBank under the accession number L16912. Single-letter abbreviations for amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

conserved region, encompassing S9 to S10, terminates ~110 residues before the COOH-terminus of mSlo.

The *mSlo* transcripts are alternatively spliced. One splice site (A; Fig. 1, A and C) is conserved in *Drosophila slo* (*9*, *10*) and is a major point of variation in both species. Four alternative exons have been identified in *mSlo* cDNAs. In contrast to *Drosophila slo* transcripts, *mSlo* transcripts appear to show extensive 5′ sequence variation. We isolated nine variant *mSlo* cDNAs with 5′ ends that diverged within a region encoding polyserine upstream from residue 22 (Fig. 1C). Of ten randomly selected cDNAs (six from brain, four from muscle), nine had unique 5′ sequences; one muscle cDNA shared sequence homology with a brain cDNA. Northern (RNA) blot analysis of polyadenylate-selected whole brain RNA revealed an *mSlo* transcript larger than 12 kb and a smeared signal consistent with heterogeneous transcripts of smaller sizes (*14*).

When we injected *mSlo* cRNA transcribed from the mouse brain–derived cDNA mbr5 (Fig. 1C) into *Xenopus* oocytes, we observed currents in both the single electrode patch clamp and in two-electrode whole cell configurations. Patch-clamp experiments revealed a large conductance, K^+-selective channel characteristic of BK-type K(Ca) channels (6). The mSlo channels were absolutely dependent on Ca^{2+} at physiological voltages; single channel currents were not observed below +70 mV in Ca^{2+}-free saline. Inside-out patches were pulled from oocytes 2 to 5 days after injection with cRNA. Single channel amplitudes varied linearly with voltage in symmetrical potassium concentrations ($[K^+]$) (156 mM) with a slope conductance of 272 pS and a reversal potential of −1.6 mV (Fig. 2A). Such a large conductance is typical of that displayed by K(Ca) channels of the BK type, (~260 pS) (6) and is more than twice that reported for the *Drosophila* slo channel (*10*). This difference is intriguing because mSlo and slo differ by only a few residues in the putative pore region (Fig. 1C). When recording solutions approximated physiological conditions (2 mM K^+, 140 mM Na^+ external; 156 mM K^+ internal), single channel amplitudes conformed to the Goldman-Hodgkin-Katz current equation, which predicts the observed outward rectification (*15*). The best fit of the current equation to data predicts a reversal potential of −92 mV, with a permeability ratio of Na^+ to K^+ equal to 0.02. Data acquired from voltage ramps of patches containing single channels were consistent with current-voltage relations constructed from voltage jumps (Fig. 2A).

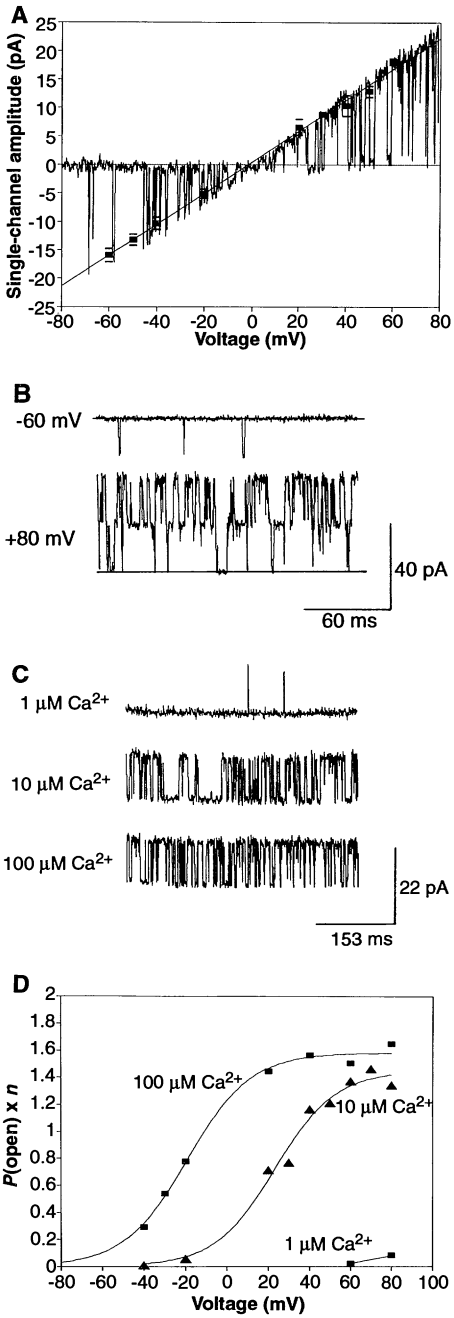

Fig. 2. (**A**) Current-voltage relation of patches in symmetrical $[K^+]$ (100 μM Ca^{2+}) (*25*). Voltage was ramped from −80 mV to +80 mV over 500 ms. Superimposed on the ramp are data points that represent average single-channel amplitudes from two to five patches. Standard deviations are as indicated. A line fit by linear regression to these points predicts a reversal potential of −1.6 mV and a slope conductance of 272 pS. (**B**) mSlo channel activity in an inside-out patch exposed to 100 μM Ca^{2+}. The patch contains two mSlo channels; 200-ms voltage steps were applied from −90 mV to the potentials shown. Note the reversal of current from inward (−60 mV) to outward (+80 mV). Solid lines indicate zero current baseline. The symmetrical $[K^+]$ was 156 mM. (**C**) Representative traces recorded from an inside-out patch containing a single channel exposed to different $[Ca^{2+}]$; the voltage jump is to +40 mV. (**D**) Voltage dependence of open channel probability at a concentration of 1, 10, and 100 μM Ca^{2+}. For each of the data points plotted, ensemble averages of mSlo channel activity were constructed from 25 to 75 voltage jumps of 500-ms duration. Open channel probability × the number of channels in the patch [P(open) × n] for each voltage and Ca^{2+} concentration was determined as the average P(open) × n from 100 to 500 ms. Data shown are from a representative patch that appeared to contain two channels. Boltzmann equations were fit to data points at each Ca^{2+} concentration: at 100 μM Ca^{2+} $V_{1/2}$ = −19.0 mV [maximal P(open) × n = 1.58; k = 15.25 mV, where k is the change in voltage for an e-fold increase in P(open) × n], and at 10 μM Ca^{2+} $V_{1/2}$ = +23.4 mV [maximal P(open) × n = 1.45; k = 14.57 mV]. For all data shown, *Xenopus* oocytes were injected with mbr5 cRNA at least 36 hours before recording; cRNA synthesis was as previously described (*26*).

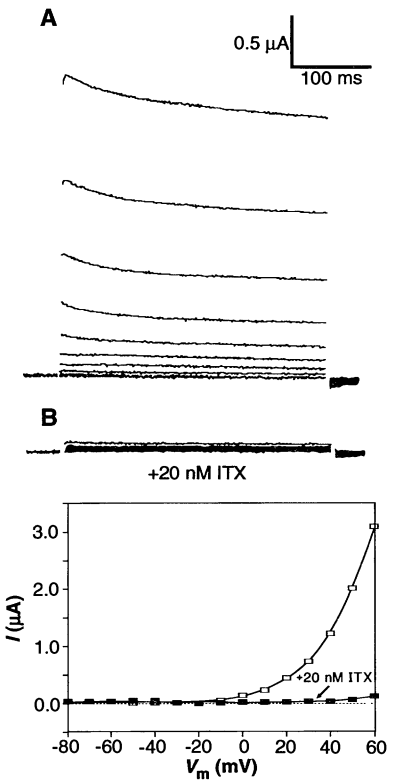

Fig. 3. (**A**) mbr5 whole cell currents measured in normal ND96 saline with two-electrode voltage clamp. Recording conditions were as previously described (*26*). Current records were leak subtracted, and capacitative transients were removed. (**B**) (Traces) Same oocyte as in (A) after addition of 20 nM ITX to the bath. (Inset) Current-voltage relation for (A) and (B).

The mSlo protein is both voltage-dependent at a single Ca^{2+} concentration and sharply Ca^{2+}-dependent. Open channel probability markedly increased when the cytoplasmic face of a single patch was exposed to 10 μM Ca^{2+} and voltage was changed over the range of −60 mV to +80 mV (Fig. 2B). Likewise, the probability of being open at any given voltage was dependent on the internal [Ca^{2+}] (Fig. 2, C and D). Fitting the data with a Boltzmann equation predicts a half-maximal voltage ($V_{1/2}$) of −19 mV in 100 μM Ca^{2+} and +23 mV in 10 μM Ca^{2+}, a shift of 42 mV for a tenfold change in [Ca^{2+}]. The mSlo channels were activated by much lower [Ca^{2+}] than were *Drosophila* slo channels (*10*). For example, ensemble averages showed that mSlo channels exposed to 10 μM Ca^{2+} reached half-maximal conductance at ~+23 mV, whereas *Drosophila* slo channels exposed to the same [Ca^{2+}] were not activated below +50 mV.

It is perhaps because of the greater sensitivity to Ca^{2+} that we were able to record mSlo currents in the two-electrode whole cell configuration, whereas this was not reported for *Drosophila* slo (*10*). Two days after injecting *Xenopus* oocytes with mbr5 cRNA, we observed large outward currents with step depolarizations above 0 mV (Fig. 3A). Whole cell currents showed a rapid onset similar to that seen for the ensemble averages of patch-clamp experiments (*14*). Little or no inactivation was noted with mbr5 currents. As is typical of K(Ca) channels in the BK family, mSlo was sensitive to block by 100 nM charybdotoxin and by the more selective blocker of BK channels, iberiotoxin (ITX) (*16*). Twenty nanomolar ITX virtually eliminated the whole cell current within 10 min (Fig. 3B). BK channels are also reported to be highly sensitive to block by external tetraethylammonium (TEA) (*17*). The TEA concentration producing a 50% block of the mSlo mbr5 channel was 0.14 mM as determined by fitting of the data for several TEA concentrations to a Langmuir function. This high sensitivity to block by TEA is consistent with the presence of an aromatic residue at the mouth of the pore (*18*) at residue 321 (Fig. 1C).

The *mSlo* gene resembles the *Drosophila Shaker* gene, which encodes a voltage-dependent K^+ channel, in that both produce channel peptides with variant NH_2-terminal domains. In *Shaker* peptides, the NH_2-terminal variation produces differences in channel inactivation (*19*). Our preliminary data suggest that the NH_2-terminal variation in mSlo does not affect channel properties (*14*), although we cannot yet conclusively rule this out.

Our results indicate that mSlo channels are members of the BK family of channels as well as of the vast extended family that includes voltage-gated K^+ channels. The mSlo protein is more complex than the voltage-gated K^+ channels, and its homology with the *Drosophila* slo protein is most pronounced in regions outside those shared with the voltage-gated channels. Perhaps over the course of evolution, the Ca^{2+} gating mechanism has been appended to the basic structure of a voltage-gated K^+ channel. BK channels are regulated by a variety of factors, including arachidonic acid (*20*), G proteins (*21*), and angiotensin II (*5*), and may have autocatalytic protein kinase activity (*22*). Thus, the large number of mSlo variants may reflect a need for regulatory diversity as well as a diversity of biophysical properties.

REFERENCES AND NOTES

1. R. Meech, *Annu. Rev. Biophys. Bioeng.* **7**, 1 (1978).
2. O. H. Peterson and Y. Maruyama, *Nature* **307**, 693 (1984); A. Marty, *Trends Neurosci.* **12**, 420 (1989).
3. B. S. Pallotta, K. L. Magleby, J. N. Barrett, *Nature* **293**, 471 (1981).
4. M. Cahalan, K. Chandy, S. Grissmer, *Curr. Top. Membr.* **39**, 357 (1991).
5. L. Toro, M. Amador, E. Stefani, *Am. J. Physiol.* **258**, 912 (1990); L. Toro, L. Vaca, E. Stefani, *ibid.* **260**, 1779 (1991).
6. A. Blatz and K. Magleby, *Trends Neurosci.* **10**, 463 (1987).
7. T. L. Schwarz, B. L. Tempel, D. M. Papazian, Y. N. Jan, L. Y. Jan, *Nature* **331**, 137 (1988); L. Salkoff *et al.*, *Trends Neurosci.* **15**, 161 (1992); A. Wei *et al.*, *Science* **248**, 599 (1990); B. Rudy, C. Kentias, E. Vega-Salenz de Miera, *Mol. Cell. Neurosci.* **2**, 89 (1991).
8. T. Elkins, B. Ganetzky, C.-F. Wu, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8415 (1986).
9. N. S. Atkinson, G. A. Robertson, B. Ganetzky, *Science* **253**, 551 (1991).
10. J. Adelman *et al.*, *Neuron* **9**, 209 (1992).
11. M. Saito and C.-F. Wu, *J. Neurosci.* **11**, 2135 (1991); M. Gho and B. Ganetzky, *J. Exp. Biol.* **170**, 93 (1992).
12. L. Salkoff, *Nature* **302**, 249 (1983), *J. Physiol. (Paris)* **80**, 275 (1985); S. Singh and C.-F. Wu, *Neuron* **2**, 1325 (1989).
13. L. Salkoff *et al.*, *Science* **237**, 744 (1987).
14. A. Butler, S. Tsunoda, D. P. McCobb, A. Wei, L. B. Salkoff, unpublished data.
15. A. Hodgkin and B. Katz, *J. Physiol. (Cambridge)* **108**, 37 (1949).
16. A. Galvez *et al.*, *J. Biol. Chem.* **265**, 11083 (1990).
17. G. Yellen, *J. Gen. Physiol.* **84**, 157 (1984).
18. R. MacKinnon and G. Yellen, *Science* **250**, 276 (1990); R. MacKinnon and L. Heginbotham, *Neuron* **8**, 483 (1992), M. Kavanaugh *et al.*, *ibid.*, p. 493
19. T. Hoshi, W. N. Zagotta, R. W. Aldrich, *Science* **250**, 533 (1990).
20. M. Kirber, R. Ordway, L. Clapp, J. Walsh, Jr., J. Singer, *FEBS Lett.* **297**, 24 (1992).
21. L. Toro, J. Ramos-Franco, E. Stefani, *J. Gen. Physiol.* **96**, 373 (1990); A. Brown and L. Birnbaumer, *Annu. Rev. Physiol.* **52**, 197 (1990)
22. S. Chung, P. H. Reinhart, B. L. Martin, D. Brautigan, I. B. Levitan, *Science* **253**, 560 (1991).
23. J. Kyte and R. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).
24. The cDNAs were bidirectionally sequenced; extensive use was made of synthetic primers. Both M13 single-stranded cloning vectors and Bluescript (Stratagene) cloning vectors were used.
25. External saline for experiments contained the following: 140 mM KCl and 20 mM Hepes (pCa 7) (pH 7.2 with KOH) (with symmetrical [K^+]), and 140 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 10 mM Hepes, and 1 mM $MgCl_2$ (pH 7.0 with HCl) (with asymmetrical [K^+]). Internal solution contained the following: 140 mM KCl, 20 mM Hepes, 5 mM EGTA (pCa 6) (pH 7.0 with KOH) (with 1 μM Ca^{2+}); and 140 mM KCl, 20 mM Hepes, 5 mM HEDTA (pCa 5) (pH 7.0 with KOH) (with 10 μM Ca^{2+}); 140 mM KCl, 20 mM Hepes (pCa 4) (pH 7.0 with KOH) (with 100 μM Ca^{2+}) Free [Ca^{2+}] was calculated with the EGTAETC computer program (written and supplied by E. McCleskey). Single channel currents were recorded with an Axopatch 1B patch-clamp amplifier (Axon Instruments, Foster City, CA). The CCURRENT computer program (courtesy of K. Baker) was used for data acquisition. Analysis of single channel data was performed with VPROC software (written and supplied by C. Lingle). Solutions were perfused onto patches with a large glass pipette carrying three lines of solutions. Flow was gravity-driven through manually controlled solenoid valves. Recordings were done at room temperature. Voltage ramps and ensemble-averaged records were digitized at 507 μs per point; other voltage steps were digitized at 214 μs per point. Data were low-pass filtered at 5 kHz.
26. M. Covarrubias, A. Wei, L. Salkoff, *Neuron* **7**, 763 (1991).

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