Properties of Slo1 K\(^+\) channels with and without the gating ring

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High-conductance Ca\(^{2+}\)- and voltage-activated K\(^+\) (Slo1 or BK) channels (KCNN4) play key roles in many physiological processes. The structure of the Slo1 channel has two functional domains, a core consisting of four voltage sensors controlling an ion-conducting pore, and a larger tail that forms an intracellular gating ring thought to confer Ca\(^{2+}\) and Mg\(^{2+}\) sensitivity as well as sensitivity to a host of other intracellular factors. Although the modular structure of the Slo1 channel is known, the functional properties of the core and the allosteric interactions between core and tail are poorly understood because it has not been possible to study the core in the absence of the gating ring. To address these questions, we developed constructs that allow functional cores of Slo1 channels to be expressed by replacing the 827-amino acid gating ring with short tails of either 74 or 11 amino acids. Recorded currents from these constructs reveals that the gating ring is not required for either expression or gating of the core. Voltage activation is retained after the gating ring is replaced, but all Ca\(^{2+}\)- and Mg\(^{2+}\)-dependent gating is lost. Replacing the gating ring also right-shifts the conductance-voltage relation, decreases mean open-channel and burst duration by about sixfold, and reduces apparent mean single-channel conductance by about 30%. These results show that the gating ring is not required for voltage activation but is required for Ca\(^{2+}\) and Mg\(^{2+}\) activation. They also suggest possible actions of the unliganded (passive) gating ring or added short tails on the core.

Significance

High-conductance Slo1 (BK) K\(^+\) channels are synergistically activated by Ca\(^{2+}\), voltage, Mg\(^{2+}\), and additional factors to modulate membrane excitability in many key physiological processes. Slo1 channels are of modular design with allosteric interactions between the core transmembrane modules and a large cytoplasmic gating ring module, providing a model system to study allosteric principles in channel gating and protein function. To examine the allosteric interactions, we developed constructs that replace the large gating ring module with short peptides and then characterized the altered properties of the gating. Our studies, which provide insight into functional and allosteric interactions between the core and the gating ring, may be useful in understanding the disease processes associated with Slo1-channel dysfunction.


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which has seven transmembrane segments containing a voltage
sensor and a K⁺-selective pore (labeled “core”) and a larger
cytoplasmic region containing two tandem regulators of K⁺
conductance (RCK1 and RCK2), each with a Ca²⁺ sensor (la-
beled “tail”). Two impediments to the functional expression of
truncated core constructs are their entrapment within the ER
and their failure to form tetramers (24). We addressed the
first of these problems by creating a construct (Slo1C-KvT) which
contained a 74-amino acid C-terminal region (labeled Kv1.4 tail
in Fig. 1) from the Kv1.4 voltage-sensitive K⁺ channel which
contains a conserved motif (Fig. 1, highlighted in blue) reported
to facilitate the efficiency of channel expression and export to the
plasma membrane (25). This added region is less than 10% the
size of the normal Slo1-WT tail domain (Fig. 1). The second
problem was addressed by including the last 16 residues of the
core which extend into the cytoplasm from the base of S6 (Fig. 1,
highlighted in yellow). This region was reported to be important
to the formation of channel tetramers (24, 26).

Amazingly, this channel construct expressed currents, and the
magnitudes of the expressed currents for both whole-cell re-
cording and inside-out patches were as large as those achieved by
the expression of WT channels (Fig. 2A and B; Slo1C-KvT). Curiously,
however, large, slowly decaying tail currents were present in these current records (arrows) which were not seen in
Slo1-WT channels. These tail currents reversed at the K⁺ equi-
librium potential, indicating that the currents are the product of
a K⁺-selective channel. To examine whether these tail currents
might be a property resulting from a motif present in the added
74-residue Kv1.4 tail; Slo1C-Kv-minT is a Slo1 core with a Kv1.4 11-residue mini tail; Slo1C-KvTNAFQ is a Slo1 core with a 74-residue Kv1.4 tail with NAFQ substituted for KKFR in the tail; Slo1C-KvT R207E is Slo1C-KvT with R207E in S4 in the core.

Fig. 1. Slo1 channel constructs used in this study. The Slo1 channel constructs used in this study are based on the mouse mbr5 cDNA (12) and the mouse
Shaker family Kv1.4 channel (25). The “Slo1 core and tail” refers to the first 342 and the last 827 amino acid residues. The “Kv1.4 tail” refers to the last 74
amino acid residues of Kv1.4. The different channel constructs are designated as follows: Slo1-WT is Slo1 full-length WT; Slo1C-KvT is a Slo1 core with a 74-
residue Kv1.4 tail; Slo1C-Kv-minT is a Slo1 core with a Kv1.4 11-residue mini tail; Slo1C-KvTNAFQ is a Slo1 core with a 74-residue Kv1.4 tail with NAFQ
substituted for KKFR in the tail; Slo1C-KvT R207E is Slo1C-KvT with R207E in S4 in the core.

Fig. 2. Slo1 constructs without gating rings express large currents in Xenopus oocytes. (A) Whole-cell current recordings from oocytes injected with cRNAs of
the indicated constructs (see Fig. 1 and legend). Currents were not observed when a stop codon was placed immediately after the tetramerization domain at
position 342 (Slo1C-Stop). With a two-electrode voltage clamp, oocytes were held at −70 mV, and 20-ms step pulses were applied from −70 mV to 250 mV in
10-mV increments followed by a step to 0 mV to see outward tail currents in an ND96 bath solution. Not all traces are shown. (B) Currents recorded from
inside-out macropatches from oocytes injected with the constructs in A. Asymmetric K⁺ with 10 mM K⁺ in pipette and 140 mM K⁺ on the inside of membrane
was used. A 50-ms prepulse to −100 mV was followed by 20-ms step pulses from −100 to 240 mV in 20-mV increments followed by a step to 0 mV for 10 ms.
Arrows indicate prominent tail currents observed for Slo1C-KvT. (C) The G–V curve for Slo1-WT is left-shifted compared with Slo1C-KvT and Slo1C-Kv-minT.
Data were obtained from inside-out macropatch recordings in symmetrical 140 mM K⁺.

macroscopic currents also were expressed by this Slo1C-Kv-minT construct, but the expressed currents lacked the prominent tail currents seen when the full-length Kv1.4 terminal sequence was present (Fig. 2 A and B, Slo1C-Kv-minT). We also observed that during voltage steps Slo1C-KvT currents typically had a greater time-dependent decrease in amplitude than Slo1C-Kv-minT currents.

One possible interpretation of these results is that the added 74-residue Kv1.4 tail blocks the channel and then is expelled upon repolarization, thus producing the prominent tail currents seen in Fig. 2 A and B (arrows), perhaps similar to peptide blocking of Shaker channels (27). To test the involvement of the Kv1.4 tail, we neutralized three positively charged residues grouped in the Kv1.4 tail (Fig. 1, highlighted in magenta). Expression of this construct (Fig. 2 A and B, Slo1C-KvTNAFQ) eliminated the prominent tail currents in Slo1C-KvT channels and produced currents virtually indistinguishable from the Slo1C-Kv-minT construct.

The Recorded Currents Are Not from Endogenous Channels. Our ability to change the properties of the expressed currents by modifying the primary structure of the Kv1.4 sequences added to the C terminus of the Slo1 core provided evidence that the observed currents were from channels encoded by the cRNA injected into Xenopus oocytes. As an additional control, we created a cDNA identical to the Slo1C constructs except that all the Kv1.4 sequence was omitted and a Stop codon was added immediately following the 16-residue tetramerization domain (Fig. 1, highlighted in yellow). Injection of cRNA from this construct into Xenopus oocytes failed to produce any detectable currents (Fig. 2 A and B, Slo1C-Stop). In a further test to verify that the recorded currents resulted from the expression of the constructs without gating rings, we altered the voltage sensor of the Slo1C-KvT channel (R207E) and observed that the conductance-voltage (G-V) curve shifted to the left and decreased its slope (Fig. 3), consistent with results reported for the same mutation in Slo1-WT (28, 29). Having demonstrated that we can express functional Slo1 channels after replacing their gating ring (Fig. 1, highlighted in yellow). Injection of cRNA from this construct into Xenopus oocytes failed to produce any detectable currents (Fig. 2 A and B, Slo1C-Stop). In a further test to verify that the recorded currents resulted from the expression of the constructs without gating rings, we altered the voltage sensor of the Slo1C-KvT channel (R207E) and observed that the conductance-voltage (G-V) curve shifted to the left and decreased its slope (Fig. 3), consistent with results reported for the same mutation in Slo1-WT (28, 29). Having demonstrated that we can express functional Slo1 channels after replacing their gating ring with short 11- and 74-residue tails, we next examined the properties of these constructs.

Voltage Sensitivity is Retained and the Voltage for Half Activation is Right-Shifted for Slo1C-KvT and Slo1C-Kv-minT Channels. The retention of voltage-dependent gating in Slo1 channels without gating rings might be expected because the voltage sensor (S1–S4) is contained in the core of the channel (Fig. 1). Indeed, as shown in Figs. 2 C and 3, the manipulation of the voltage-dependent gating of the Slo1C-KvT and Slo1C-Kv-minT channels through mutation helped establish that the observed currents were the products of the expressed constructs. The voltage sensitivity of Slo1C-KvT and Slo1C-Kv-minT channels was found to be similar to that of Slo1-WT channels (comparable slopes), but with the voltage for half activation (V1/2) right-shifted 27.8 ± 5.8 mV for Slo1C-KvT and 49.2 ± 3.0 mV for Slo1C-Kv-minT relative to Slo1-WT (Fig. 2C). These significant right-shifts (P < 0.001, n ≥ 5) indicate that replacing the unliganded Slo1 gating ring with the KvT or Kv-minT sequences allosterically alters the voltage range of activation. The change in activation to more positive voltages could arise from a possible lack of pull on S6 through the RCK1–S6 linkers because of the absence of the gating ring (see ref. 21) or from the short tails inhibiting open probability (Po) in some manner. In either case, these observations indicate that direct allosteric input from the gating ring to the core is not required for voltage-dependent channel activation.

The Gating Ring is Required for Ca2+ and Mg2+ Sensitivity of Slo1 Channels. Structure–function studies have suggested that Ca2+ and Mg2+ activation of Slo1 channels works through the gating ring (13–16, 21, 30, 31). We now test this suggestion directly by examining Ca2+ and Mg2+ sensitivity in Slo1 channels in which the gating ring has been replaced by the KvT or Kv-minT sequences using three different experimental approaches. In all cases, no significant sensitivity to Ca2+ or Mg2+ was observed. Single-channel recordings showed that exposing inside-out patches to 100 μM Ca2+ or 10 mM Mg2+ greatly increased Po in Slo1-WT channels by 530 ± 110- or 53 ± 12-fold, respectively, compared with negligible effects on SloC-Kv-minT channels (Fig. 4 A and B, Fig. S1, and Table S1). When voltage ramps were applied to inside-out patches expressing Slo1-WT, Slo1C-KvT, or Slo1C-Kv-minT channels, channel activity increased as the membrane potential was made more positive for all three channel types (Fig. 4C Top). Application of 200 μM Ca2+ or 10 mM Mg2+ greatly increased channel activity for Slo1-WT channels but had no apparent effect on Slo1C-KvT or Slo1C-Kv-minT channels (Fig. 4C; note calibration bars). A similar result was observed when G–V relationships were obtained in the absence and presence of intracellular Ca2+ and Mg2+; the V1/2 for Slo1-WT channels was left-shifted toward more negative potentials by 228 ± 5.5 mV

Fig. 3. Verification that the Slo1C-KvT construct without a gating ring is expressed and functional. The R207E mutation in S4 in the voltage sensor of Slo1-KvT left-shifted the voltage-dependent activation of Slo1C-KvT as expected from previous Slo1-WT experiments (22, 23), indicating that the isolated core of Slo1 channels without gating rings is being expressed. (A) Sequence of S4 (Upper) in the voltage sensor of Slo1 and with the R207E mutation (Lower). (B) Currents from inside-out macropatches from oocytes injected with Slo1C-KvT and Slo1C-KvT-R207E. G–V plots (n = 5) are shown on the right. The voltage protocol was —80 mV for 20 ms followed by a 40-ms voltage step of —80 to +295 mV (in 25-mV increments), followed by steps to 0 mV for 20 ms to measure tail currents. Asymmetric K+ with 10 K+ in pipette and 150 K+ at intracellular side was used. (C) Whole-cell currents recorded from Slo1C-KvT and Slo1C-KvT-R207E channels. Oocytes were held at —70 mV and 20-ms step pulses applied from —90 mV to +240 mV with a step back to 0 mV. G–V plots are shown on the right. Solutions are as in Fig. 2 for whole-cell recording.
with 200 μM Ca\(^{2+}\) and by 51.2 ± 1.9 mV with 10 mM Mg\(^{2+}\) (Fig. 5A). In contrast, the \(V_{1/2}\) of Slo1C-Kv-minT was not shifted by Ca\(^{2+}\) or Mg\(^{2+}\) (Fig. 5B), and normalized I–V curves for Slo1C-KvT channels were not shifted when exposed to 200 μM Ca\(^{2+}\) (Fig. S2). Hence, the gating ring is required for Ca\(^{2+}\) and Mg\(^{2+}\) sensitivity of Slo1 channels.

**Mean Channel Open Time and Burst Duration Are Greatly Reduced for Slo1C-Kv-minT Channels.** The single-channel kinetics of Slo1C-Kv-minT channels differed markedly from those of the Slo1-WT channels. Mean open-interval duration and mean burst duration were decreased significantly, by 5.5- and 6.3-fold, respectively, for Slo1C-Kv-minT channels compared with Slo1-WT channels (\(P < 0.02\), \(n \geq 3\)) (Fig. 6 and Table S2). These marked changes in single-channel kinetics show that replacing the unliganded gating ring in Slo1 channels with the Kv-minT sequence has profound effects on channel gating. Whether these properties represent the true baseline properties of the core in isolation from allosteric input from the gating ring or whether the Kv-minT peptide is a contributing factor remains to be determined.

**The Apparent Mean Single-Channel Conductance Is Reduced for Slo1C-Kv-minT Channels.** The high conductance of Slo1-WT channels compared with other K\(^{+}\)-selective channels is one of the defining properties of Slo1-WT channels (10). Unexpectedly, we found that replacing the gating ring with the KV-minT construct decreased single-channel current amplitudes (Figs. 4A and 6), suggesting an apparent decreased conductance. When measurements of currents were restricted to openings of sufficient duration so that their amplitudes should not be attenuated by the low-pass filtering, replacing the gating ring decreased apparent mean single-channel conductance by ~30%, from 307 ± 7 pS for Slo1-WT channels to 213 ± 6 pS for Slo1C-Kv-minT channels (\(P < 0.001\), \(n = 3\) patches, in each case with mean conductance for each patch determined...
for data typically collected from +80 to +140 mV). The ring of negative charge (E321 and E324) at the entrance to the inner cavity that doubles the outward conductance of Slo1-WT channels (32, 33) is retained in the Slo1C-Kv-minT channels, so the reduced conductance does not involve a reduction in the ring of negative charge.

The Gating Ring Is Not Required for Block by External Iberiotoxin and Tetraethylammonium. Because the single-channel conductance was unexpectedly reduced upon replacement of the gating ring with the Kv-minT construct, the possibility arises that other properties of the core pore-gate domain might be altered also. To explore this possibility, we first tested the effect of the highly specific Slo1 channel blocker iberiotoxin (34) on Slo1C-KvT channels. The application of 60 nM iberiotoxin to the external membrane surface of outside-out macropatches (+180 mV) reduced currents 78 ± 3% (n = 5), which was not significantly different from the 82 ± 3% (n = 7, P = 0.46) reduction for Slo1-WT channels (Fig. S3A). We next tested the effect of external application of the generic K+ channel blocker tetraethylammonium (TEA), because Slo1 channels are known to be highly sensitive to blocking by external TEA (35, 36). We found that 2 mM TEA (+70 mV) reduced Slo1C-KvT whole-cell currents by 81 ± 2%, which was not significantly different from the 85 ± 4% reduction seen for Slo1-WT currents (n = 6, P = 0.43) (Fig. S3B). Both blocking agents also were tested on Slo1C-Kv-minT with similar effects. Hence, as might be expected, the gating ring is not required for external blocking by iberiotoxin and TEA.

The Gating Ring Is Not Required for β1 Subunits to Slow the Activation of Slo1 Channels. The β subunits are integral membrane proteins with two transmembrane segments that interact with Slo1-WT α subunits and alter various channel properties such as kinetics (37). Although some of the various β subunits may interact with both the core and tail of Slo1-WT channels, the β1 subunit may interact only with the core, slowing the rate of activation of the current (38, 39). Consistent with the previous results, we observed that coexpression of β1 with Slo1-WT produced currents that activated more slowly than those of Slo1-WT α subunits alone (Fig. S3C). In a similar manner, coexpression of the β1 subunit with the Slo1C-KvT and Slo1C-Kv-minT constructs also produced currents that activated significantly more slowly than in the absence of the β1 subunits (Fig. S3C). In addition, the slow current decrease observed after activation was absent for all three constructs (Slo1-WT, Slo1C-KvT, and Slo1C-Kv-minT) when coexpressed with β1 subunits (Fig. S3C). Thus, the gating ring is not required for β1 subunits to interact functionally with Slo1 channels.

Discussion

The Core Can Be Expressed Without the Gating Ring. The fact that Slo1 channels are conserved in invertebrates as well as vertebrates implies that core and tail may have become so interdependent that the core no longer can function without the tail. Our laboratories and others had tried to express the core without the gating ring but with success. A detailed analysis of truncated Slo1 constructs which failed to express currents indicated difficulties in protein processing, tetramerization, and export from the ER to the plasma membrane (24). We achieved robust expression of Slo1 cores without gating rings by preserving the tetramerization domain of Slo1 (Fig. 1) and either replacing the S277-residue tail that forms the gating ring with the last 74 residues of the Kv1.4 C terminus or attaching a much shorter 11-residue C terminus that included the five-residue motif from Kv1.4 for processing and surface expression (25) and also the last three residues of the C terminus of Kv1.4 (Fig. 1).

Functions of the Core and Gating Ring. Our experiments directly show the major functions of the core and gating ring of Slo1 channels. Voltage-dependent gating (Figs. 2–5 and S2), blocking by iberiotoxin, TEA, and Mg2+ (Fig. 4A and Fig. S3A and B), and slowed activation with β1 subunits (Fig. S3C) did not require the gating ring. Hence, these are properties of the core. In contrast, Ca2+ and Mg2+ activation was not observed in the absence of the gating ring (Figs. 4 and 5, Figs. S1 and S2, and Table S1). Hence, Ca2+ and Mg2+ activation are conferred by the gating ring through allosteric interactions with the core. These observations confirm previous prognostications (31). It follows from these findings that allosteric interactions of the gating ring with the core are not required for channel opening or closing or for voltage-dependent gating. Hence, the core of Slo1 with attached short tails is sufficient to form a voltage-gated channel. Our observations of no significant Ca2+ and Mg2+ activation in the absence of the gating ring indicates that no functional Ca2+ and Mg2+ activation sites are restricted to the core, as is consistent with previous studies showing that mutating sites on the gating ring can eliminate all Ca2+ and Mg2+ activation (13, 14, 40).

In addition we found that replacing the gating ring with the short tails (i) inhibited activation by right-shifting the V1/2 to more positive voltages (Fig. 2C); (ii) greatly decreased mean open-interval duration and burst duration (Fig. 6C and Table S2); and (iii) decreased the apparent mean single-channel conductance (Fig. 6A and B). If these alterations in gating and conductance reflect the baseline properties of the core in the absence of the gating ring, then the right-shift in activation and decreased burst duration could arise if the unliganded gating ring normally applies a passive tension to S6 through the RCK1–S6 linkers to facilitate activation. Consistent with this possibility, decreasing passive tension in the linkers of Slo1-WT by lengthening the RCK1–S6 linkers also right-shifts the activation curve and decreases burst duration (21). The apparent decreased conductance may reflect gating to subconductance levels in the absence of the gating ring. However, at this time we cannot rule out the possibility that the tetramerization domains and appended tails are determinants of these properties. Peptides are known to alter gating of Slo1-WT channels (27). Removal of the gating ring in Slo1-WT channels immediately after the S6 helix without appending tails could resolve this issue, but attempts to express such constructs have not been successful so far (24).

The gating ring has been removed with trypsin digestion from Methanobacterium thermoautotrophicum (MthK), a prokaryotic Ca2+-dependent K+ channel (41). Like Slo1, MthK has an intracellular gating ring formed by four pairs of RCK1 and RCK2 domains, but with differences in gating ring structure (23, 42, 43).
The mean open times and conductance of MthK were little changed by removing its gating ring (41), in contrast to the marked decreases we observed for Slo1 when the gating ring was replaced with short peptide tails. These contrasting results might be related to the fact that, unlike Slo1 channels, the MthKc core includes only a pore-gate domain without voltage-sensor domains (43). Thus, MthK is without potential allosteric input from contact between the gating ring and voltage sensor as in Slo1 channels (42). However, we cannot rule out the possibility that the differences might arise from the short peptide tails used to replace the gating ring in Slo1.

Implications for Allosteric Gating.
Allosteric models for the gating of Slo1 channels typically assume relatively independent action of Ca\(^{2+}\) and voltage to activate the channel (31, 44, 45). Consistent with these models, the channel can be activated without calcium at very high positive membrane potentials (46) or in the presence of high Ca\(^{2+}\) over the range of physiological voltages (7–9, 45). The experimental data and models for gating indicate that the core and gating ring interact allosterically, so that Ca\(^{2+}\) binding can move the voltage sensors and, reciprocally, that voltage-sensor movement can change the calcium-binding affinity (31, 40, 45). The allosteric interactions and transduction pathways between gating ring and core involved in these processes still are not well understood. A number of questions regarding these complex interactions now may be pursued with our constructs, because the allosteric interactions might be easier to understand if the properties of the core are defined without the pushes and pulls normally provided by the gating ring.

Materials and Methods

Standard molecular biology methods (12), Xenopus oocyte expression systems (12, 13, 47), and electrophysiological techniques (12, 13, 21, 48) were used. The indicated concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) were applied to the intracellular membrane surface. Experiments were at room temperature. Error estimates are SEM. See SI Materials and Methods for more details.

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Various PCR and subcloning strategies such as genomic PCR amplification of the C terminal of Kv1.4, making annealed linker oligos containing the Kv1.4 minimal tail sequence (Slo1C-Kv-minT), addition of restriction sites and mutated nucleotides via PCR oligos, site-directed mutagenesis using Stratagene’s Quick Change Mutagenesis Kit, and overlap extension PCR were used to make the constructs in Fig. 1.

Xenopus Oocytes and ND96 Medium for Incubation and Whole-Cell Recording. Defolliculated Xenopus oocytes were injected with 0.5–150 ng of cRNA using a Nanoject II (Drummond Scientific). Injected oocytes were incubated at 18 °C in ND96 complete medium, consisting of ND96 medium plus 2.5 mM sodium pyruvate and penicillin-streptomycin 1 mL/100 mL. The ND96 medium consisted of (in mM) 96 NaCl, 2 KCl, 1.8 CaCl₂, 5 MgCl₂ 5, and Hepes 5 adjusted to pH 7.5. Currents were recorded 2–5 d after injection.

Electrophysiology and Additional Solutions. Two-microelectrode voltage-clamp (whole-cell) recordings from Xenopus oocytes were obtained in ND96 medium with 1 mM 4,4′-disothiocyanatosilbene-2,2′-disulphonic acid disodium salt hydrate (DIDS) to block the endogenous chloride conductances. The currents were obtained with an Oocyte Clamp OC-725C amplifier (Warner Instruments) pulsed with a Sutter Instrument Co. P-87 pipette puller and filled with 3 M KCl.

Patch-clamp recordings were acquired with an Axopatch 200B patch clamp (Molecular Devices), digitized at 100 kHz (macroscopic currents) or at 200 kHz (single channel), and were low-pass filtered at 2 kHz for macropatch recording and at 10 kHz for single-channel analysis, unless otherwise indicated. The data were analyzed using pClamp 9 (Molecular Devices), SigmaPlot (Jandel Scientific), Origin (Microcal Software), and custom software for the burst analysis (1).

Solutions for macropatch recordings shown in Figs. 2 B and C, 3C, 4C, and 5 and Figs. S2 and S3 A and C contained in the pipette were (in mM) 140 KMethasulfonate, 1 MgCl₂, and 10 Hepes (for symmetrical K⁺) or 140 NaMethasulfonate, 10 KMethasulfonate, 1 MgCl₂, and 10 Hepes (for asymmetrical K⁺). Both pipette solutions were adjusted to pH 7.2 using KOH. The bath (intracellular solution) contained (in mM) 140 KMethasulfonate, 10 Hepes, and 1 EGTA, with CaCl₂ and MgCl₂ added as needed to obtain the desired free concentrations, adjusted to pH 7.2 with KOH.

For Figs. 3B and 4A and B, Fig. S1, and Tables S1 and S2, the 0 Ca⁺ and 0 Mg⁺ bath (intracellular solution) contained (in mM) 150 KCl, 1 EGTA, 1 EDTA, and 5 mM 2-(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid, N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES), with the final solution adjusted to pH 7.0 with KOH. The pipette solution for symmetrical K⁺ recording contained (in mM) 150 KCl, 2 mM MgCl₂, and 5 TES (pH 7.0). The pipette solution for asymmetric K⁺ recording (Fig. 3B) was the same as the 0 Ca⁺ and 0 Mg⁺ solution, except with 140 mM NaCl and 10 mM KCl. Solutions with symmetrical 150 mM KCl were used unless otherwise indicated. Solutions indicated as 0 Ca⁺ solutions had calculated free Ca²⁺ <0.01 μM. To this solution sufficient CaCl₂ or MgCl₂ was added to obtain the calculated free Ca²⁺ and Mg²⁺ levels indicated in the text. Iberiotoxin was from Tocris Bioscience. The other chemicals and reagents were from Sigma-Aldrich.


Fig. S1. Ca²⁺ and Mg²⁺ no longer increased the mean open-interval duration (mean open time) after the gating ring in Slo1-WT channels was replaced with an 11-residue tail to obtain Slo1C-Kv-minT channels (see Fig. 1). Data from Table S1 are plotted for Slo1-WT channels and for Slo1C-Kv-minT channels lacking the gating ring. Ca²⁺ and Mg²⁺ significantly increased mean open time for Slo1-WT channels, (P < 0.05, paired t test, n = 4) but had insignificant effects on mean open time for Slo1C-Kv-minT channels (P > 0.09, n = 3). Symmetrical 150 mM K⁺ was used (see Materials and Methods). Error bars represent SEM.
Fig. S2. Slo1C-KvT channels, which lack a gating ring, are activated by voltage but not by Ca$^{2+}$. Slo1C-KvT channels have the gating ring replaced with a 74-residue tail (Fig. 1). (Left) The macroscopic currents were obtained from inside-out patches in asymmetrical K$^+$ (10 mM K$^+$ in the pipette and 140 mM K$^+$ at the inner membrane surface) with either 0 Ca$^{2+}$ or 200 μM Ca$^{2+}$. Patches were first held at 0 mV. A 50-ms prepulse to −100 mV was applied; then the voltage was stepped from −100 mV to 240 mV in 20-mV intervals, followed by a step back to 0 mV to measure outward tail currents. (Right) Normalized current amplitudes are plotted against voltage in the absence (black) and presence (red) of Ca$^{2+}$. Currents were reduced somewhat in the presence of Ca$^{2+}$, but the reduction was not significant (P values are indicated). Error bars represent SEM.
**Fig. S3.** The gating ring is not required for extracellular block by iberiotoxin (IbTX) or tetraethylammonium (TEA) or for activation by accessory β1 subunits. (A) (Left) Extracellular application of 60 nM IbTX blocks Slo1-WT and Slo1C-KvT currents similarly in outside-out macropatches. Currents were evoked by stepping from −80 to +180 mV in the absence (black) and presence (red) of 60 nM extracellular IbTX. (Right) Normalized I−V plots before (black) and after (red) exposure to IbTX. The blocking effect is virtually the same for Slo1-WT (Upper) and Slo1C-KvT (Lower) (n = 6; P = 0.42). (B) TEA (2 mM) blocks Slo1-WT and Slo1C-KvT currents in a similar manner. (Left) Whole-cell currents evoked at +70 mV before (black) and after (red) 2 mM extracellular TEA. (Right) The blocking effect is the same for Slo1-WT and Slo1C-KvT (n = 6; P = 0.32). (C) The auxiliary β1 subunit modulates Slo1-WT and SloC-KvT currents in a similar manner. Coexpression of β1 with Slo1-WT or with Slo1C-KvT slows activation. Red traces are with β1 subunits; black traces are without β1 subunits. Depolarizing pulses are to +60 mV with a two-microelectrode whole-cell voltage clamp (Upper Left) and to +240 mV in macropatches (Lower Left). The coexpression of β1 significantly slows the activation time constant (τ) for both channel types. The macropatch current rising phase was fitted with a single exponential: Slo1-WT, τ = 1.85 ± 0.28 ms and 7.26 ± 0.56 ms in the presence of β1 (P = 0.0001, n = 4); Slo1C-KvT, τ = 1.12 ± 0.07 ms and 2.94 ± 0.37 ms in the presence of β1 (P = 0.0006, n = 6). The Slo1C-Kv-MinT construct also was found to be modulated by β1, with a significant threefold change of τ, from 0.91 ± 0.09 ms to 2.61 ± 0.32 ms (P = 0.001, n = 5). Thus, coexpression with β1 slows the activation of Slo1 constructs with and without the gating ring by about three- to fourfold, indicating that the gating ring is not required for the β1 subunit to slow the activation kinetics. Error bars represent SEM.
Table S1. Single-channel kinetic properties for Slo1-WT and Slo1C-Kv-minT channels in the absence and presence of Ca\(^{2+}\) and Mg\(^{2+}\)

<table>
<thead>
<tr>
<th>Property</th>
<th>Slo1-WT</th>
<th>Slo1C-Kv-minT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Ca(^{2+})</td>
<td>100 μM Ca(^{2+})</td>
</tr>
<tr>
<td>nPo</td>
<td>0.0022 ± 0.0008</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>Po normalized to 0 Ca</td>
<td>1.0</td>
<td>530 ± 110</td>
</tr>
<tr>
<td>No. of openings in bursts</td>
<td>1.5 ± 0.2</td>
<td>28 ± 21</td>
</tr>
<tr>
<td>Open-interval duration</td>
<td>0.77 ± 0.14</td>
<td>7.5 ± 2.3</td>
</tr>
<tr>
<td>Intraburst closed-interval duration</td>
<td>0.23 ± 0.03</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>Burst duration</td>
<td>1.2 ± 0.2</td>
<td>*</td>
</tr>
<tr>
<td>Mean duration of gaps between bursts</td>
<td>540 ± 100</td>
<td>*</td>
</tr>
</tbody>
</table>

Data were obtained with single-channel recordings from inside-out patches held at +80 mV. Durations are in milliseconds. Slo1-WT data are from four different patches, each containing a single channel with \( n = 1 \) in nPo, so that the parameters could be determined readily. Slo1C-Kv-minT data are from three different patches, each containing an unknown number of channels. For Slo1C-Kv-minT channels the mean duration of gaps between bursts for single channels would be greater by a factor of \( n \) for the unknown number of channels. For Slo1C-Kv-minT channels the Po was sufficiently low so that openings seldom overlapped; thus the second through fifth parameters could be determined with negligible error. Effective low-pass filtering of 4.47 kHz was used. Data are presented as mean ± SEM. nPo is number of channels in the patch times their average open probability; Po is the average open probability.

*With 100 μM Ca\(^{2+}\) for Slo1-WT channels, the Po was so high that the channel was mostly open, so it was difficult to determine gaps between bursts or burst duration.

Table S2. Mean open-interval duration and burst duration are decreased for Slo1C-Kv-minT channels

<table>
<thead>
<tr>
<th>Property</th>
<th>Slo1-WT</th>
<th>Slo1C-Kv-minT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-interval duration</td>
<td>0.77 ± 0.14</td>
<td>0.14 ± 0.02*</td>
</tr>
<tr>
<td>No. of opening in bursts</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Intraburst closed-interval duration</td>
<td>0.23 ± 0.03</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Burst duration</td>
<td>1.2 ± 0.2</td>
<td>0.19 ± 0.01*</td>
</tr>
</tbody>
</table>

Data reformatted from Table S1 to facilitate comparison. Durations are in milliseconds. Data were obtained using single-channel recording with 0 Ca\(^{2+}\) and 0 Mg\(^{2+}\). Slo1-WT data are from four different patches, each containing a single channel. Slo1C-Kv-minT data are from three different patches, each containing an unknown number of channels. The Po was sufficiently low so that openings seldom overlapped; thus the parameters could be determined. *Open-interval duration and burst duration were significantly decreased (\( P < 0.02 \)) for Slo1C-Kv-minT channels compared with Slo1-WT channels. Effective low-pass filtering of 4.47 kHz was used. Data are presented as means ± SEM.