Cellular/Molecular

Slick (Slo2.1), a Rapidly-Gating Sodium-Activated Potassium Channel Inhibited by ATP

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Neuronal stressors such as hypoxia and firing of action potentials at very high frequencies cause intracellular Na^+ to rise and ATP to be consumed faster than it can be regenerated. We report the cloning of a gene encoding a K^+ channel, *Slick*, and demonstrate that functionally it is a hybrid between two classes of K^+ channels, Na^+-activated (K_{Na}) and ATP-sensitive (K_{ATP}) K^+ channels. The Slick channel is activated by intracellular Na^+ and Cl^- and is inhibited by intracellular ATP. Slick is widely expressed in the CNS and is detected in heart. We identify a consensus ATP binding site near the C terminus of the channel that is required for ATP and its nonhydrolyzable analogs to reduce open probability. The convergence of Na^+, Cl^-, and ATP sensitivity in one channel may endow Slick with the ability to integrate multiple indicators of the metabolic state of a cell and to adjust electrical activity appropriately.

Key words: **channel; chloride; hippocampus; metabolism; potassium; sodium**

Introduction

The wide variety of electrical patterns in the brain can be attributed in large part to the diversity of K^+ channels and their different properties. In excitable cells, K^+ channels shape action potentials, set resting membrane potentials, regulate repetitive firing, and modulate the release of neurotransmitters and hormones (Levitan and Kaczmarek, 2002). Some K^+ channels are modulated by intracellular metabolites. For example, K_{ATP} channels may hyperpolarize cells during periods of high metabolic activity or limited energy availability. Some other types of K^+ channels are regulated directly by changes in intracellular ion concentrations. These properties allow currents to be regulated by the recent history of activity of an excitable cell, as reflected in the accumulation of intracellular ions. Well studied examples include BK channels, which are activated by both intracellular Ca^{2+} ($[Ca^{2+}]_i$) and voltage and participate in rapid repolarization of action potentials (Lancaster and Nicoll, 1987; Lancaster et al., 1991) and SK channels, which are activated solely by $[Ca^{2+}]_i$ and thus progressively hyperpolarize cells as $[Ca^{2+}]_i$ accumulates during a train of action potentials (Lancaster and Nicoll, 1987).

By contrast, less is known electrophysiologically or at the molecular level about another class of ion-sensitive K^+ channels, those activated by elevation of cytoplasmic Na^+. This family, collectively known as K_{Na} channels, was first identified in guinea pig cardiomyocytes (Kameyama et al., 1984). Subsequently, similar channels were reported in a variety of neurons (Bader et al., 1985; Dryer et al., 1989; Schwindt et al., 1989; Dryer, 1991; Egan et al., 1992a; Haimann et al., 1992; Dale, 1993; Safronov and Vogel, 1996; Bischoff et al., 1998). Like Ca^{2+}-activated BK and SK channels, the properties of K_{Na} channels appear to be diverse. The reported unitary conductances of these channels range from 105 to 200 pS, and half-maximal activation by Na^+ occurs between 7 and 80 mM, depending on cell type and recording conditions (Dryer, 1994). Moreover, the physiological roles of these channels appear to be distinct in different cell types. For example, in quail trigeminal ganglion neurons and in dorsal root ganglion neurons, it has been suggested that K_{Na} channels regulate the resting membrane potential (Haimann et al., 1992; Bischoff et al., 1998). In other neurons, K_{Na} channels have been implicated in an apamin-insensitive, Na^+-dependent slow afterhyperpolarization (AHP) that follows a burst of action potentials (Dryer, 1994). In ferret perigeniculate neurons, such a Na^+-dependent slow AHP is an important component of spindle wave activity (Kim and McCormick, 1998). It has also been proposed that K_{Na} channels may be activated by a single action potential and may therefore play a role in determining the duration of action potentials (Bertrand et al., 1989), although is unclear whether the amount of Na^+ influx through a TTX-sensitive Na^+ channel during a single action potential is normally sufficient to activate K_{Na} channels (Dryer, 1991, 1994). Activation may depend on the relative rates of influx, diffusion, and extrusion of Na^+, the proximity of K_{Na} channels to the source of Na^+, and the particular geometry of the space occupied by K_{Na} channels in a given cell type (Dryer, 1991, 1994). Resolution of these questions requires molecular identification of K_{Na} channels.

It has recently been shown that K^+ channels encoded by the *Slack* (Slo2.2) gene are gated by Na^+ and that the distribution of Slack in the brain coincides with neuronal types reported to possess K_{Na} channels (Joiner et al., 1998; Bhattacharjee et al., 2002; Yuan et al., 2003). We now report the cloning and expression of a second Na^+-activated K^+ channel gene, *Slick* (Slo2.1), which is selectively expressed in the nervous system and heart. With a

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similar single-channel conductance to Slack, Slick also rectifies outwardly and is activated by intracellular Na^+. In marked contrast to Slack, however, Slick activation occurs very rapidly with step changes in voltage, whereas Slack activation increases with time after a step depolarization. In addition, the activity of Slick channels is substantially more sensitive to changes in intracellular Cl^-, and Slick open probability is significant even in the absence of Na^+. Moreover, Slick contains a regulatory nucleotide-binding site that is responsible for ATP-dependent inhibition of channel activity. Thus, the properties of the Slick channel indicate that its activation by intense neuronal activity or by hypoxia may hyperpolarize specific neurons in the brain and the heart, where the channel is expressed, thereby limiting excitability and energy consumption to maintain cell viability.

Materials and Methods

Molecular biology. The Basic Local Alignment Sequence Tool (BLAST) algorithm (Altschul et al., 1990) was used to screen the human genome in GenBank for sequences that, when translated, are homologous to the amino acid sequence of the rat *Slack* gene. Other than the human ortholog of Slack, the highest match was identified as a long stretch of DNA on chromosome 1. Portions of the homologous sequence were aligned to Slack, and primers were designed for amplifying cDNA using the PCR. Using a rat cDNA library as template, a specific product was amplified, subcloned using the TA cloning kit (Invitrogen, Carlsbad, CA), and sequenced (W. M. Keck Biotechnology Resource Center, Yale University, New Haven, CT). Using this sequence, one specific primer was designed to amplify the missing 5′ end of the cDNA in conjunction with a second, nonspecific primer derived from library vector sequence. Similarly, using sequence from the original PCR product, another specific primer was designed to amplify the missing 3′ end of the cDNA in combination with nonspecific library vector-derived primer. Amplified products were subcloned and sequenced as before. Specific primers were then designed against the beginning of the 5′ product and against the end of the 3′ product in opposite orientations and subsequently used to amplify a single 3515 bp product from the cDNA library using Pfu Turbo (Stratagene, La Jolla, CA). After subcloning this product using the TA Cloning Kit (Invitrogen) and sequencing it in both directions (W.M. Keck Biotechnology Resource Center), it was found to match the overlapping original three PCR products and to contain in-frame stop codons before the first methionine of a long open reading frame and before the end of the novel DNA sequence. Finally, the rat Slick cDNA was subcloned into the *Not*I and *Xba*I sites of the expression plasmid pTRACER (Invitrogen) for expression in Chinese hamster ovary (CHO) cells.

Full-length human Slick cDNA was obtained by first comparing the open reading frame of rat Slick with genomic sequence of the Slick ortholog on human chromosome 1. Primers were designed against regions of significant mismatch 5′ and 3′ to the regions of homology on human chromosome 1, and these were used to clone full-length human Slick from first strand human cDNA (Stratagene, La Jolla, CA) using Pfu Turbo. The PCR product was subsequently subcloned using the TA Cloning Kit (Invitrogen) and sequenced in both directions (W.M. Keck Biotechnology Resource Center).

Site-directed mutagenesis was performed using sense and antisense primers designed against the region of channel cDNA in pTRACER that was targeted for change, that is, regions of Slick (residues 869–877) or Slack (residues 923–931) homologous to the bowl region of Slo or the consensus sequence for ATP binding to Slick (GXXXXGKT, residues 1032–1039). In each primer pair, one or more base mismatches were included to alter the final sequence that was obtained by PCR of a given cDNA by Pfu Turbo. PCR products were digested with *Dpn*I to eliminate nonamplified cDNA, and the remaining products were used to transform *Escherichia coli*. Miniprep DNA was then sequenced to verify that the designated changes had been made. In the end, four mutants were made: Slick bowl replaced the Slack bowl, Slack bowl replaced the Slick bowl, all six charged residues in the bowl of Slack were converted to glutamines, and glycine at position 1032 of Slick was converted to a serine (G1032S).

Northern blot analysis and immunohistochemistry. To determine the regional expression of the rat Slick cDNA, a 627 bp fragment was first excised by restriction digestion with *Hind*III and *Xba*I. This fragment was labeled by random priming using a Prime-It II kit (Stratagene) and ^{32}P-dCTP to a specific activity of $\sim 10^9$ dpm/μg. The ^{32}P-labeled fragment was then used to probe a commercial multiple tissue rat Northern blot (BD Biosciences; Clontech, Palo Alto, CA).

To determine where Slick protein is expressed in the brain, the synthetic peptide CXKDVKDPGHHRSIHRN representing 15 amino acids within the C-terminal region of rat Slick was synthesized (W.M. Keck Biotechnology Resource Center). The X represents aminocaproic acid, which acted as a spacer molecule. The N-terminal cysteine was added to allow conjugation to the keyhole limpet hemocyanin peptide and to permit coupling to a column for affinity purification. Generation of chicken polyclonal anti-Slick IgY was performed by Aves Labs, Inc. (Tigard, OR). Antibodies were affinity-purified, and immunohistochemistry was performed as previously described (Bhattacharjee et al., 2002).

Electrophysiology and analysis of channel activity. CHO cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal bovine serum, hypoxanthine—thymidine, and penicillin–streptomycin (all from Invitrogen, Gaithersburg, MD). Cells were plated on plastic 35 mm dishes at a confluence of 20% and then transfected with 1 μg of Slick/pTRACER (or Slack/pTRACER) cDNA and 5 μl of Lipofectamine (Invitrogen) in IMDM as previously described. Recordings of green fluorescent cells were performed 1–2 d after transfection. Electrodes had a resistance of 2–3 MΩ for whole-cell recordings and 8–10 MΩ for excised patch recordings. For whole-cell recordings, the bath solution contained (in mM): 140 NaCl, 1 $CaCl_2$, 5 KCl, 29 glucose, and 25 HEPES, pH 7.4. The pipette solution contained (in mM): 130 KCl, 5 EGTA, and 10 HEPES, pH 7.3, and when Cl^- was varied, gluconate was used for ionic replacement. For excised patch recordings, cells were initially bathed in a solution containing (in mM): 130 KCl, 5 Na-gluconate, 10 HEPES, 5 EGTA, and 29 glucose, pH 7.3. When Na^+ was varied, *N*-methylglucamine was used for cationic replacement. Pipette solution contained 127 K-gluconate, 3 KCl, 5 Na-gluconate, 10 HEPES, 5 EGTA, and 29 glucose, pH 7.3. Data were acquired on-line at 5–20 kHz and filtered at 1–2 kHz. For single-channel analysis, pClamp 9.0 (Axon Instruments, Union City, CA) was used. Data derived from these and other experiments is expressed as mean ± SE. Statistically significant differences were assessed using a paired *t* test.

Dose–response relations were obtained only up to physiologically maximal ion concentrations of Na^+ and Cl^- (<130 mM). We found that increasing the ionic strength by raising salt concentrations to 300 mM reduced channel activity significantly. Dose–response curves for Na^+ and Cl^- were fit with the equation: $I = I_{min} + I_{max}/[1 + (C_{50}/C)^n]$, where I is the measured current density, I_{min} is the minimal current density, I_{max} is the peak current density, C is the concentration of agonist used, C_{50} is the concentration of agonist required to achieve half-maximal activation (i.e., EC_{50}), and n is the degree of cooperativity.

Accession numbers. The rat and human Slick sequences have been submitted to GenBank. They were assigned the accession numbers AY359443 and AY359444, respectively. The accession numbers for rat Slack and nSlo2 are AF089730 and AF232770, respectively.

Results

Cloning of the Slick channel

Using the TBlastN algorithm at the National Center for Biotechnology website and the entire *Slack* amino acid sequence, we determined that a gene closely related to *Slack* is located on human chromosome 1. To determine if the novel homolog of *Slack* indeed encodes a functional K^+ channel, PCR was performed on a rat cDNA library using primers designed against specific sequences of DNA in the middle of the predicted open reading frame and other primers designed against library phage arms. Using this RACE-like PCR strategy, putative 5′ and 3′ untranslated regions (UTRs) were identified that extended beyond the apparent start and stop codons of the open reading frame. Based

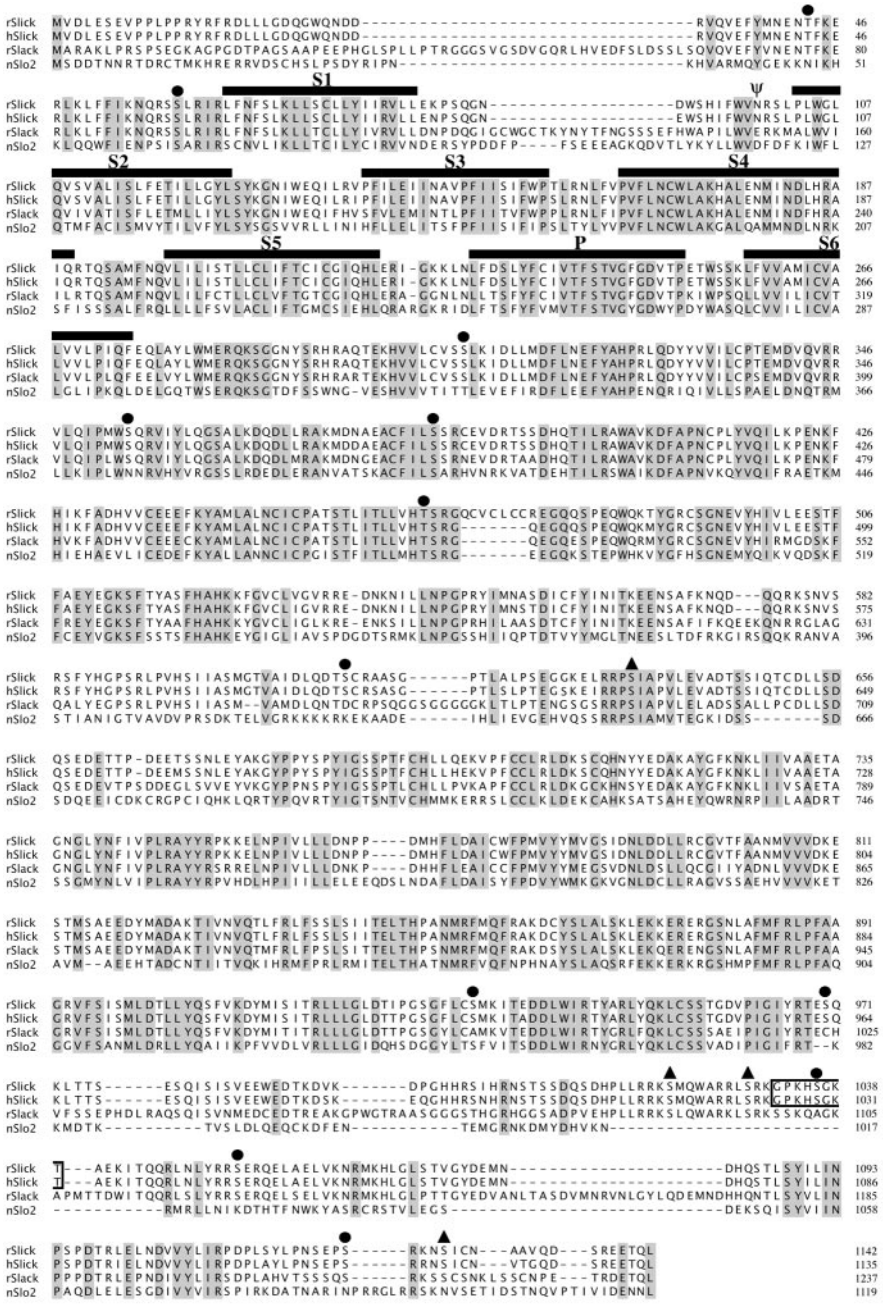


Figure 1. Amino acid sequence alignment of rat Slick (rSlick), human Slick (hSlick), rat Slack (rSlack) and the Slack ortholog from *C. elegans*, nSlo-2. Sequences were aligned using the web-based program ClustalW from the European Bioinformatics Institute. Residues in gray represent identical amino acids in all four aligned sequences. Gaps are represented by dashed lines. Consensus site for ATP binding to rSlick and hSlick is GXXXXGKT and is boxed. All other designations refer specifically to the rSlick sequence. Transmembrane domains are marked by bars and are designated S1-S6 or P (for pore). Consensus sites are indicated for phosphorylation by protein kinase C (circles) and by cAMP-dependent protein kinase (triangles) and for N-linked glycosylation (ψ).

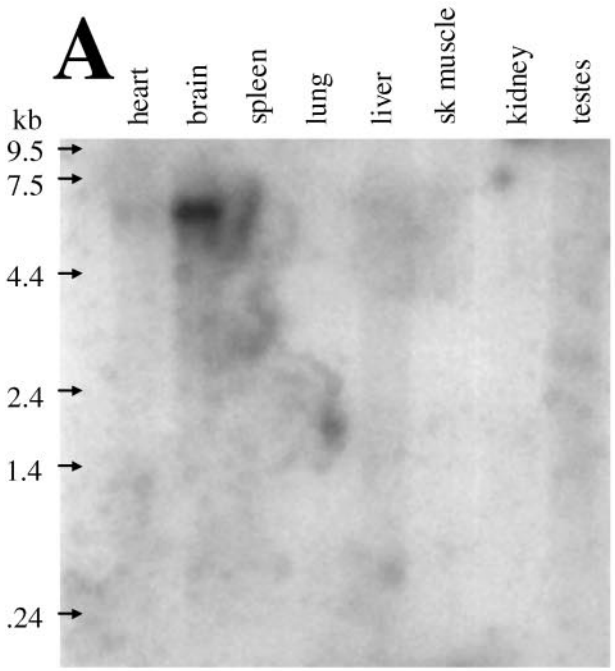
on the sequences of the UTRs, new primers were synthesized, and a full-length clone encoding a protein of 1142 amino acids was amplified by PCR. The putative start codon was preceded by stop codons in frame, indicating that the entire 5′ end of the open reading frame was present in the PCR product. The open reading frame of this new gene encodes a protein sharing 74% sequence identity to rat Slack (Fig. 1). Because of its resemblance to *Slack*, we named this sister channel "*Slick*" (Sequence like an intermediate conductance K channel). After determination of the open reading frame of rat *Slick*, the human *Slick* gene was isolated by designing primers based on the putative untranslated regions encoded by the human genomic sequence. Rat and human *Slick* are ~98% identical at the level of amino acid sequence (Fig. 1). In both species, the Slick polypeptide, like that of Slack, contains six putative membrane-spanning domains, a P-region between predicted transmembrane domains 5 and 6, and an extensive C-terminal region. The C-terminal region is predicted to encode two RCK domains, similar to the *Slo* gene (Jiang et al., 2003). The greatest divergence between Slick and Slack occurs in the N-terminal region before the first predicted transmembrane domain (Fig. 1). Slack has an N terminus that is almost double in size to that of Slick, and unlike Slo, the N termini of both Slick and Slack are predicted to be cytosolic. Curiously, a sequence resembling the N terminus of Slick is also encoded by a putative alternative exon of the *Slack* genomic sequence on human chromosome 9, located ~20 kb upstream from the predicted start site of the human ortholog of the cloned *Slack* gene. It is therefore possible that there exists a Slack isoform that contains a N terminus like that of Slick.

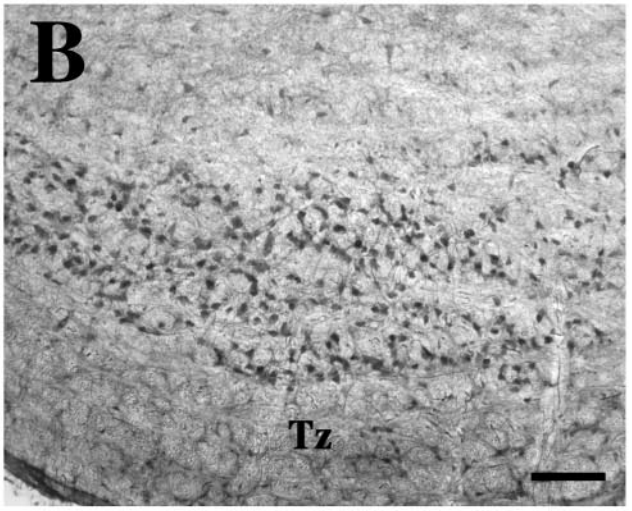
Slick, like Slack, also contains a consensus PDZ-binding motif at the end of its long C-terminal tail. This may be important functionally, because Na^+ channels (Gee et al., 1998) and glutamate receptors (Niethammer et al., 1996), which conduct the K_{Na} activator Na^+, are also known to cluster by binding to PDZ-containing proteins. Unlike Slack, however, Slick contains a consensus ATP binding motif in its C-terminal tail (Fig. 1, boxed residues). Slick also contains multiple consensus sites for phosphorylation by cAMP-dependent protein kinase and protein kinase C. Many of these are found in the vicinity of the putative ATP-binding motif, suggesting that this region may be specialized for regulation of channel activity.

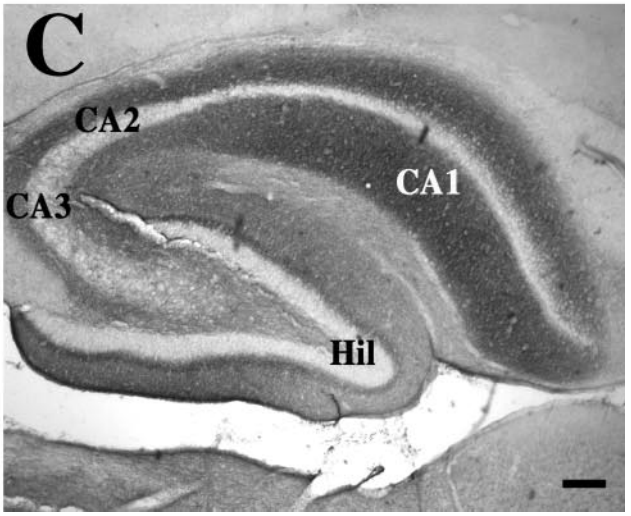
Localization of Slick expression

To date, K_{Na} channels have been recorded primarily in excitable tissues such as heart, brain, and spinal cord, although they have also been detected in *Xenopus* oocytes (Egan et al., 1992b). In a comparison of expression levels in different tissues of the rat by Northern blotting, Slick was found predominantly in brain as a transcript of ~6.9 kb (Fig. 2*A*). We also detected a signal of similar size in rat heart, although it was considerably weaker than in brain.

We used an affinity-purified antibody targeted against the C-terminal region of Slick to examine localization of the channel in rat brain. Slick immunoreactivity is widely distributed in rat







brain and overlaps many regions that have previously also been reported to express the Slack subunit (Bhattacharjee et al., 2002). Examples include the dorsal root ganglion, thalamic neurons, the olfactory bulb, and the medial nucleus of the trapezoid body (MNTB) in the auditory brainstem, which is capable of firing action potentials at high frequencies (Fig. 2*B*). One notable exception to the general pattern of overlap in expression of Slick and Slack is the CA1 region of the hippocampus (Fig. 2*C*). Slack is undetectable by immunoreactivity or *in situ* hybridization in this region (Bhattacharjee et al., 2002). In contrast, strong Slick immunoreactivity was detected in the CA1 region (Fig. 2*C*).

Characteristics of Slick currents

We next characterized the electrophysiological properties of Slick channels cloned from rat brain and compared them to those of Slack. We expressed Slick in both *Xenopus* oocytes ($n = 5$) and in CHO cells (Fig. 3). However, because it has been reported that nearly half of patches from water-injected oocytes have endogenous K_{Na} channels (Egan et al., 1992b), we restricted our studies to CHO cells to avoid any possible contamination from endogenous currents. Currents in *Slick*-transfected cells activated nearly instantaneously with step changes in voltage (Fig. 3*A*). In contrast, as reported previously (Joiner et al., 1998), whole-cell currents of CHO cells transfected with *Slack* cDNA have an apparently instantaneous component followed by a slow time-dependent increase in current, particularly at higher voltages (Fig. 3*B*). Although neither Slick nor Slack possesses the positively charged S4 domain characteristic of voltage-sensitive Na^+, K^+, and Ca^{2+} channels (Fig. 1), both Slick and Slack rectify strongly in the outward direction in physiological saline (Fig. 3*A*,*B*). As a control we recorded from CHO cells transfected with vector alone and, as previously demonstrated (Joiner et al., 1998), observed no appreciable whole-cell currents ($n = 10$).

Partial replacement of external Na^+ with K^+ (5 mM $[K^+]_o$ to 100 mM $[K^+]_o$) shifted the reversal potential of the whole-cell current by 57 ± 3 mV ($n = 5$). Under ideal conditions, a current perfectly selective for potassium ions would be expected to shift by ~75 mV. These results for Slick are similar to what has been reported previously for Slack (Joiner et al., 1998) and indicate that currents carried by these channels are predominantly, although perhaps not exclusively, selective for K^+ ions. For example, the shift in reversal potentials is consistent with what would be expected if the Na^+ permeability were ~ 1/20 of that for K^+. For whole-cell patch experiments we used 130 mM Cl^- in the internal recording solution because an internal solution containing low Cl^- produced a significant reduction in current (Fig. 3*C*,*D*). Slick currents were blocked at high voltages (more than approximately +100 mV) when the pipette solution contained small amounts of intracellular Na^+ ($n = 20$), consistent with "cation-block" which has been reported for native K_{Na} channels (Wang et al., 1991).

To examine the biophysical properties of Slick, inside-out patches were excised from transfected CHO cells and perfused with a solution containing 5 mM Na^+/130 mM Cl^-. Slick channels have a chord conductance of 141 ± 3 pS measured at +80 mV from a holding potential of 0 mV in 130 mM symmetrical K^+

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Figure 2. Tissue distribution of Slick. *A*, Northern blot analysis of rat Slick transcripts. A single 6.9 kb band was detected in both brain and heart. *B*, Slick immunoreactivity in the MNTB and the hippocampus (*C*). Strong immunolabeling was found in the CA1 section. The antibody was designed against the C-terminal region of Slick and immunohistochemistry was performed using an avidin— biotin– diaminobenzidine reaction system. Scale bars: *B*, 133 μm; *C*, 200 μm.

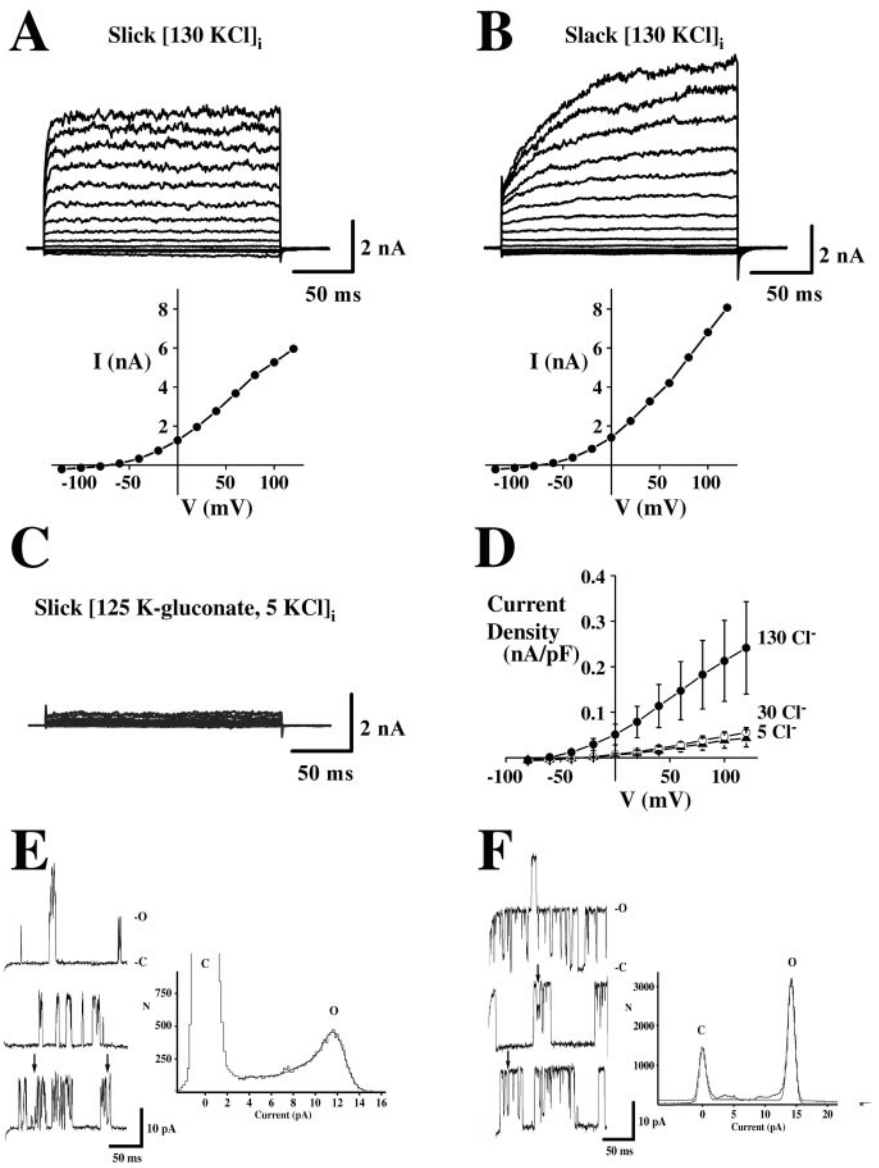


Figure 3. Expression of Slick and Slack in CHO cells. Whole-cell recordings and associated current–voltage relationships of representative cells transfected with Slick (*A*) and Slack (*B*). Recordings were made in bath solutions containing (in mM): 5 NaCl, 140 KCl, 29 glucose, 1 $CaCl_2$, and 25 HEPES, pH 7.4. The voltage protocol consisted of 200 msec steps from −120 to +120 mV in 20 mV increments from a holding potential of −70 mV. Pipette solution contained (in mM): 130 KCl, 5 EGTA, and 10 HEPES, pH 7.2. *C*, Representative Slick-transfected cell under identical recording conditions as in *B* except that [Cl^-] was reduced to 5 mM in the pipette solution by replacement with gluconate. *D*, Cl^- sensitivity of Slick. Average current–density versus voltage of cells recorded in 130 mM Cl^- ($n = 5$), 30 mM Cl^- ($n = 5$), and 3 mM Cl^- ($n = 4$). *E*, Single-channel properties of Slick and Slack (*F*) channels. Excised inside-out patches of transfected CHO cells were recorded in a symmetrical solution containing (in mM): 130 KCl, 5 Na-gluconate, 5 EGTA, 10 HEPES, and 29 glucose. All-points histograms for channel recordings are shown in insets. Subconductances for both Slack and Slick were often observed. Recordings were conducted at +80 mV. The "C" and "O" labels represent data from the closed and main open state, respectively. Arrows indicate subconductance states.

identical (Fig. 4*A*,*B*) but differ significantly from that of large conductance (BK) Ca^{2+}-activated K^+ channels produced by the Slo channel subunit. Whereas 1 mM tetraethylammonium (TEA) had little effect on either Slick or Slack ($n = 10$), 20 mM TEA inhibited both channels by >60% (Fig. 4*A*). In contrast, 100 nM iberiotoxin and 100 nM charybdotoxin had little effect on Slick or Slack (Fig. 4*A*,*B*) at concentrations sufficient to block nearly all Slo current (Gribkoff et al., 1997). Likewise, apamin, an inhibitor of small conductance (SK) Ca^{2+}-activated K^+ channels, also had no effect on Slick or Slack at a concentration of 100 nM, which far exceeds the K_d of the toxin for SK channels. In contrast, quinidine, a cardiac antiarrhythmic drug, inhibited Slick and Slack currents by >90% at a concentration of 1 mM (Fig. 4*A*,*B*). The effects of TEA and quinidine were both reversible.

Ba^{2+}, another general K^+ channel blocker, also inhibited Slick and Slack currents. Curiously, this inhibition appeared to be voltage-dependent (Fig. 4*C*), because greatest inhibition was seen at high voltages and was associated with a decay in current after a step depolarization (Fig. 4*C*, inset). It is possible that this time-dependent component resulted from trace amounts of Ba^{2+} entering the cell and blocking the pore from the intracellular side because the effect did not reverse after wash-out.

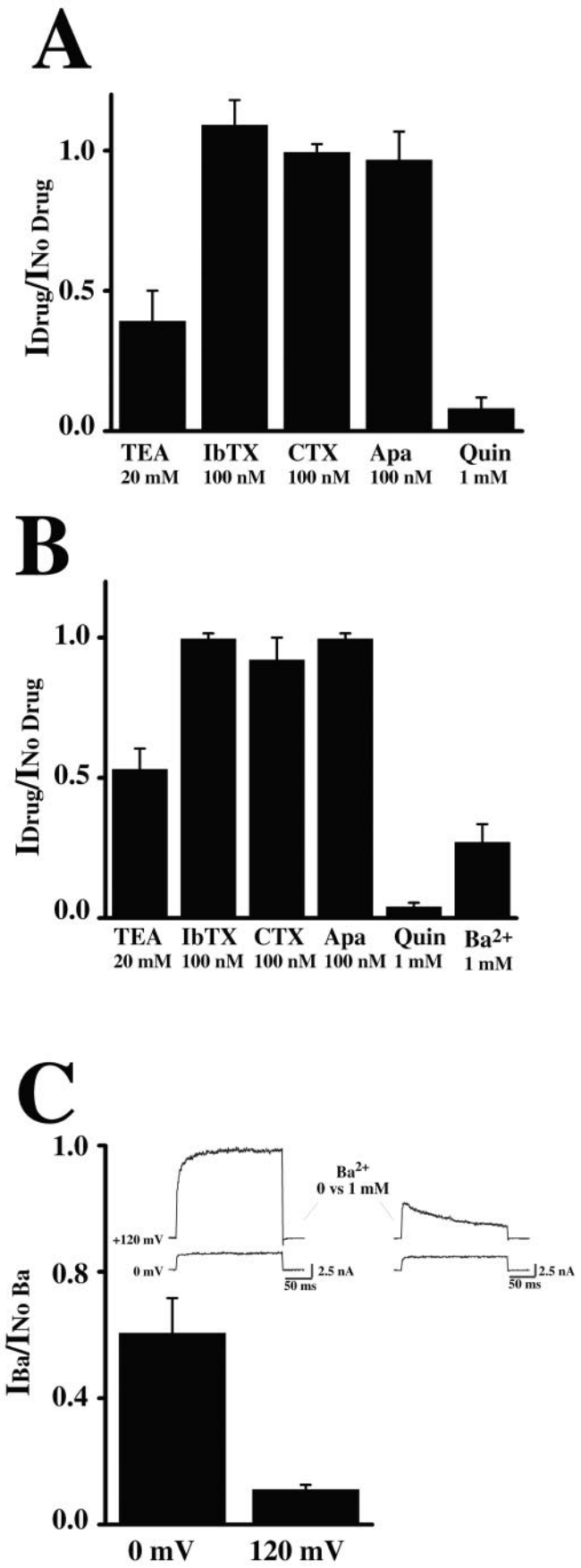
Na^+ dependence of Slick channels

Perfusing the cytoplasmic face of patches expressing either Slick or Slack channels with increasing concentrations of Na^+ resulted in a dose-dependent increase in activity for both types of channels in a reversible manner (Fig. 5). Although channel activity was greatly enhanced, the unitary conductance in the outward direction remained unchanged, as expected for channels that are predominantly K^+-selective. In these experiments NMDG was used for replacement of Na^+. Dependence on Na^+ was also observed when K^+ was substituted for Na^+. Channel activity was always reduced to very low levels when Na^+ was removed from the cytoplasmic face of the channel and replaced by K^+ ($n = 43$).

(Fig. 3*E*). Under the same recording conditions, the unitary conductance for Slack was slightly higher, 183 ± 3 pS (Fig. 3*F*). Slick channels typically exhibited rapid flickering activity (Fig. 3*E*) that contrasted with the longer "box-like" openings of Slack (Fig. 3*F*). Under the conditions of 5 mM Na^+/130 mM Cl^-, Slick channel mean burst duration was 7.24 ± 1.4 msec, whereas that for Slack was 118 ± 26 msec. Subconductances for Slick were detected frequently and ranged from 30 to 60 pS (Fig. 3*E*), consistent with the properties reported for K_{Na} channels in neurons (Dryer, 1994).

The pharmacological profiles of Slick and Slack are nearly

Other than differences in expression levels, the properties of Slick and Slack differed in two significant ways. First, no rate-limiting activation or deactivation kinetics was observable for Slick openings or closures (Fig. 5*B*). In contrast, the slow deactivation of Slack currents at −80 mV mirrored the slow activation kinetics observed in whole-cell recordings in more physiological conditions (Figs. 3*B*, 5*D*). Second, despite their low expression levels, Slick channels were readily detected even in Na^+-free media (Fig. 5*B*,*C*). In contrast, Slack channel activity was negligible



in media devoid of Na^+ (Fig. 5*D,E*), similar to what has been reported for K_{Na} channels in *Xenopus* oocytes (Egan et al., 1992b). As derived from the dose–response curves for activation of each channel by cytoplasmic Na^+, the effective concentrations of Na^+ for 50% activation (EC_{50}) of Slick (89 mM) and Slack (41 mM) were similar and fell within the range reported for other K_{Na} channels in native tissues (Dryer, 1994), supporting the idea that Slick and Slack are true K_{Na} channels. Similarly, like native channels, both channel isoforms were cooperatively gated by Na^+, with Slick and Slack exhibiting Hill coefficients of 1.4 and 2.4, respectively (Fig. 5*C,E*). Because of the inability to rule out effects of osmotic pressure and charge shielding caused by high Na salts, we were unable to test channel activity at Na^+ concentrations exceeding 90 mM. To a first approximation, however, Slick appeared to be somewhat less sensitive to cytoplasmic Na^+ than Slack.

Cl^- dependence of Slick and Slack channels

Although both Slick and Slack are regulated by intracellular Cl^- levels, there is a large difference in their sensitivity to this anion. There is an approximately fivefold increase in Slick channel activity when cytoplasmic Cl^- is raised from 3 to 130 mM in the presence of 5 mM Na^+, whereas Slack channel activity is increased only twofold under the same conditions (Fig. 6*A,B*). This increase is detected both in whole-cell recordings (Fig. 3*D*) and excised patches (Fig. 6*A,B*) This cannot be explained by differences in the Na^+ affinity of each channel isoform because Slick is in fact less sensitive to Na^+ (Fig. 5*C,E*). Indeed, at 50 mM Na^+, Slack channel activities were unaffected by altering concentrations of Cl^-, whereas Cl^- sensitivity for Slick at 50 mM Na^+ was similar to that recorded at 5 mM Na^+ (threefold increase in channel activity from 3 to 130 mM Cl^-).

The *Caenorhabditis elegans* channel nSlo2, which is related to Slick and Slack, has been shown to require both Ca^{2+} and Cl^- for activation (Yuan et al., 2000). It has been proposed that in nSlo2 Cl^- binds to a stretch of positively charged amino acids that has been referred to as a "Cl^--bowl" (Yuan et al., 2000) (Fig. 1, nSlo-2 amino acids 881–892). This name is derived from a chain of negatively charged amino acids at an analogous position in Slo channels that has been referred to as the "Ca^{2+}-bowl" for its hypothesized function of chelating Ca^{2+} ions during BK channel activation (Schreiber and Salkoff, 1997). A comparison of the Slick and Slack sequences reveals that Slick contains a net charge of +2, and Slack contains a net neutral charge over the bowl region (Fig. 1, Slick amino acids 868–879, Slack amino acids 921–932). To test whether the correlation between the positive charge in the bowl region and Cl^- sensitivity could be explained by the bowl participating in binding of Cl^-, we generated three mutants and measured their Cl^- affinities in excised inside-out patches. In the first, the bowl sequence in the Slick channel was replaced by the Slack bowl sequence. In the second mutation, the

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Figure 4. Pharmacology of Slick and Slack. Fractional unblocked whole-cell currents recorded at 0 mV for Slick (*A*) and Slack (*B*) using 20 mM tetraethylammonium (TEA), 100 nM iberiotoxin (IbTX), 100 nM charybdotoxin (CTX), 100 nM apamin (Apa), and 1 mM quinidine (Quin). All recordings were performed in a bath consisting of physiological saline and a pipette solution containing (in mM): 130 KCl, 5 EGTA, and 10 HEPES, pH 7.4. *C*, Voltage-dependent block of Slick by Ba^{2+}. The fraction of unblocked current at the end of a 200 msec voltage step in 1 mM Ba^{2+} was measured at 0 and +120 mV ($n = 3$). Block was greater at the higher voltage. Inset is a recording from a representative cell before and after perfusion of Ba^{2+} at the given voltages. In the presence of Ba^{2+} current decay became stronger at increasingly depolarized potentials. All pharmacological agents were applied extracellularly.

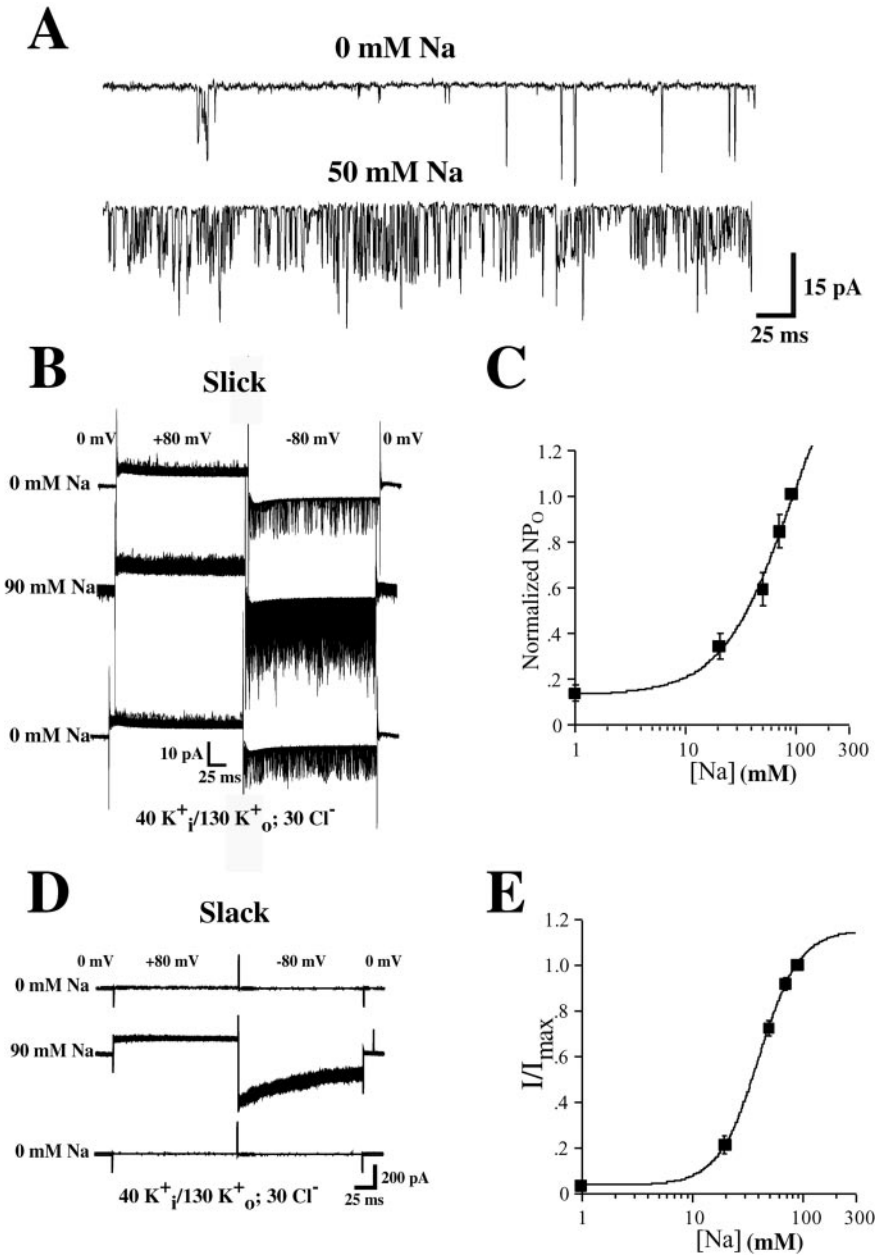

Figure 5. Slick and Slack are activated by intracellular Na^+ in inside-out patches from transfected CHO cells. *A*, Patch recording from a CHO cell transfected with Slick. The cytoplasmic face of the patch was perfused with 0 or 50 mM intracellular Na^+, as indicated. Membrane potential was held at −80 mV. *B*, Representative patch recording of inward and outward Slick channel activity in the presence of either 0 or 90 mM Na^+. Patches were held at 0 mV and stepped to +80 mV for 200 msec and immediately stepped down to −80 mV for another 200 msec. Fifty sweeps were recorded and superimposed onto each other. Basal activity is significant even in the absence of Na^+. Although the unitary conductance appears inwardly rectifying because of the difference in K^+ concentrations across the membrane (external $[K^+] = 130$ mM, internal $[K^+] = 40$ mM), channel openings are favored at positive potentials, consistent with the intrinsic outward rectification of whole-cell currents. *C*, Dose–response relationship of Na^+ for Slick. Patches were perfused with solutions containing concentrations of Na^+ that ranged from 0 to 90 mM, with NMG used as a cationic substitute. $[K^+]$ was kept constant at 40 mM, and $[Cl^-]$ was kept constant at 30 mM. NP_0 values were calculated over 50 sweeps at each dose of Na^+. NP_0 values were then normalized to the NP_0 obtained at 90 mM Na^+. Each point represents the average value from five experiments. Error bars represent SEM. Data was fitted as described in the Materials and Methods section. EC_{50} was determined to be 89 mM with a Hill coefficient of 1.4. *D*, Representative patch of Slack channels perfused with Na^+ as in *B*. Macroscopic currents were easily measured for Slack, and a time-dependent component of the current was always observed. *E*, Dose–response relationship of Na^+ for Slack. Macroscopic currents were measured at each concentration of Na^+ and normalized to the current elicited at 90 mM. Each point represents the average value from five experiments. Error bars represent SEM. EC_{50} was determined to be 41 mM with a Hill coefficient of 2.4. For both Slick and Slack channel recordings, outward currents are smaller than inward currents because of the fact that external $[K^+] >$ internal $[K^+]$.

bowl of the Slack channel was replaced by the Slick sequence. In neither case did the change in positive charge have any effect on Cl^- affinity of the parent channel ($n = 3$ for both mutants). Finally, in the third mutant in which every charged residue of the bowl region of Slack was mutated to the neutral glutamine, Na^+ and Cl^- affinity was unchanged ($n = 6$). These data are not consistent with a role for the bowl region in binding Cl^- and converting this binding energy into channel activation, nor are the data with the Slack bowl neutralization mutant consistent with a role for the bowl region in binding of Na^+.

Slick is an ATP-regulated channel

In contrast to Slack, Slick contains a consensus ATP binding site just C-terminal to the second RCK domain (Fig. 1). To determine whether ATP modulates Slick channels, we measured the current density of *Slick*-transfected cells in the presence of 5 mM MgATP in the recording pipette (Fig. 7*A*). ATP reduced Slick current density by >80% at every voltage. Identical results were obtained using either Na_2ATP or MgATP ($n = 5$), suggesting that the inhibition of Slick by ATP was direct. Additional support for binding of ATP to Slick channels was obtained from recordings in the presence of ATPγS, a slowly hydrolyzable analog of ATP, and AMP-PNP, a nonhydrolyzable analog, which both reduced Slick currents to an extent comparable to that produced by ATP itself (Fig. 7*A*,*B*). The triphosphate group was necessary for the full effect because ADP caused a less marked inhibition than ATP (Fig. 7*A*).

Because the inhibition of K^+ currents by ATP is a defining feature of K_{ATP} channels, we examined the effects of glybenclamide and diazoxide on Slick currents (Fig. 7*C*). Glybenclamide, a classical inhibitor of K_{ATP} channels, had only a very small effect on Slick current. Diazoxide, an activator of K_{ATP} channels had no effect on Slick. Thus, Slick represents a novel ATP-sensitive K^+ channel that is unaffected by typical K_{ATP} agonists or antagonists.

The suppression of Slick channels by ATP was also observed directly in single-channel recordings using the excised inside-out patch configuration. Both MgATP and Na_2ATP reversibly inhibited Slick channel activity (Fig. 8*A*,*B*). Because the open probability of Slick channels decreased equivalently at both +80 and −80 mV (data at −80 mV is not shown), inhibition was most likely caused by ATP and not by Mg^{2+} and Na^+ occluding the pore. Under these conditions blockade of chan-

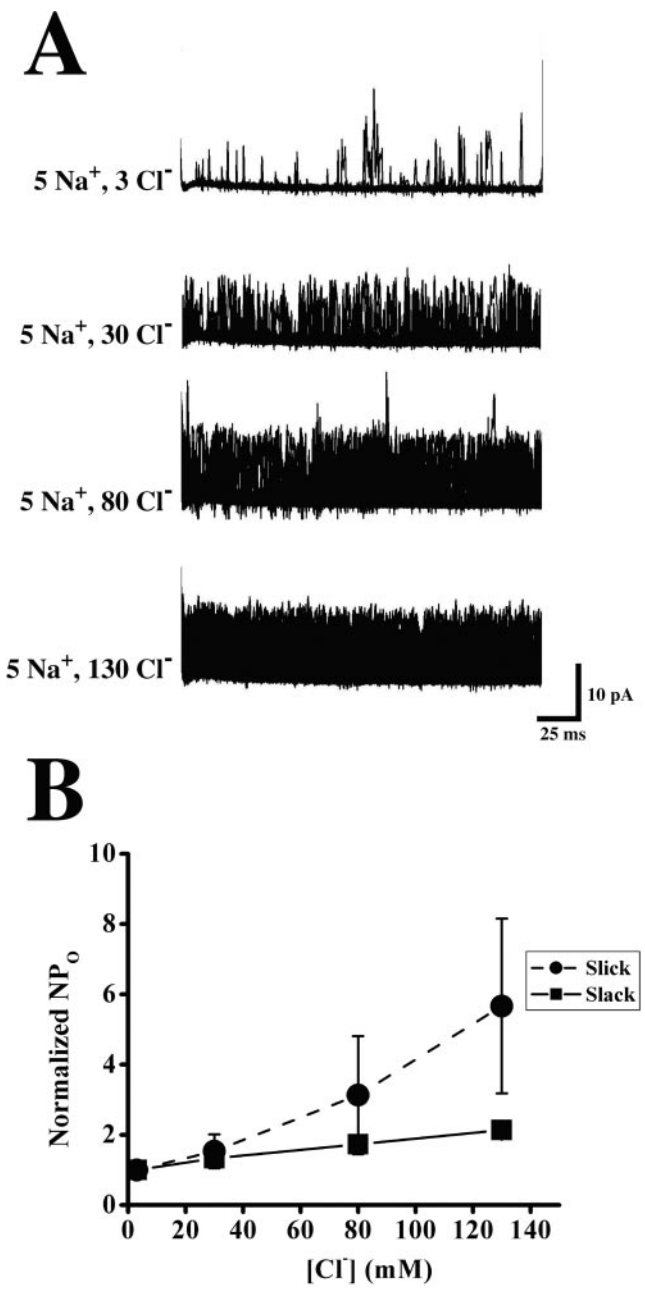

Figure 6. Slick channels are activated by intracellular Cl^-. *A*, Representative excised inside-out patch recording of Slick channels perfused with increasing concentrations of Cl^-. [K^+] was 130 mM in perfusate as well as in the pipette solution. Patches were held at 0 mV and stepped to +80 mV for 200 msec. Gluconate was used as a replacement for Cl^-. *B*, Cl^- dose–response relationship for Slick and Slack. NP_0 values were calculated over 50 sweeps at each dose of Cl^-. NP_0 values were then normalized to the NP_0 obtained at 3 mM [Cl^-] ($n = 3$ for both Slick and Slack). Error bars represent SEM.

nels by cations should be preferentially detected at +80 mV, resulting in inward rectification, as has been previously described (Wang et al., 1991).

To confirm that the effect of ATP was mediated by the consensus ATP binding site in Slick, we used site-directed mutagenesis to replace a glycine at residue 1032 with a serine to create the mutant G1032S. Application of MgATP to the cytoplasmic face of patches expressing G1032S failed to produce any decrease in channel activity (Fig. 8*C*,*D*). Moreover, in one of four cases, perfusing the Slick mutant channels with MgATP caused a substantial increase in channel activity. This effect waned over time and disappeared entirely during washout of ATP. Such an increase in activity was never seen for wild-type channels (Fig. 8*D*). We hypothesize that in this excised patch addition of MgATP triggered a phosphorylation event through a closely associated kinase, which, in the absence of direct binding of ATP to Slick channels, produced an increase in channel activity.

Discussion

We have identified a gene, *Slick*, that encodes a K^+ channel with very unusual properties. Although Slick is similar to the Slack channel in sequence, single-channel conductance, cooperative activation by intracellular Na^+, and high expression in brain, it differs from Slack in significant ways. In particular, it is expressed in heart, openings of Slick channels can readily be detected even in Na^+-free conditions, it is activated far more robustly than Slack by increases in cytoplasmic Cl^-, and, like a classical K_{ATP} channel, it is inhibited directly by intracellular ATP.

The mechanism by which changes in intracellular Na^+ influence the gating of Slick and Slack is not yet known. It is known that cations can influence gating of potassium channels either by binding within the permeation pathway or by interacting with cytosolic components of the channel. Removal of external potassium reduces voltage-gated K^+ channel currents via the C-type inactivation process (Lopez-Barneo et al., 1993; Yellen, 1998); a corresponding sensitivity to external sodium is also seen in Na^+ channels (Townsend and Horn, 1999). Some permeant ions slow the channel closing in voltage-gated potassium channels (Gomez-Lagunas and Armstrong, 1994). However, these processes would all tend to reduce K^+ current in the presence of intracellular Na^+ rather than increase it as is seen in Slick and Slack channels. Previous work has shown that the Slack channel (Yuan et al., 2003), as well native K_{Na} channels (Dryer, 1994) cannot be activated by substituting Li^+ for Na^+, suggesting that these channels are gated specifically by Na^+.

Both Slick and Slack are also sensitive to Cl^-, although the degree to which channel activity can be influenced by changes in intracellular Cl^- is very much less than that of Na^+. Moreover, we found that the sensitivity of Slick channels was much greater than that of Slack, for which the effects of Cl^- were undetectable at moderate Na^+ concentrations. These results differ somewhat from previous findings for Slack channels (Yuan et al., 2003). It should be noted that those earlier experiments were conducted under conditions of low ionic strength. For ionic and osmotic replacement for Cl^- we used the anion gluconate, instead of dextrose (Yuan et al., 2003), to avoid complications caused by changes in charge shielding. The present experiments reflect the Cl^- sensitivity of K_{Na} channels under more physiological conditions than previously described (Yuan et al., 2003) and demonstrate that Slick is a bona fide Cl^--dependent channel. It is unclear where the ligand-binding sites reside on Slick or Slack, but our results indicate that they do not overlap with the bowl region.

Because Slick is expressed in heart, it may underlie the classic cardiac K_{Na} current, which has been hypothesized to serve a cardioprotective function. By virtue of its low but significant open probability in the absence of Na^+, Slick may also help set the membrane potential in some types of cells such as dorsal root ganglion cells, a cell type in which Slick is found and in which K_{Na} channels are thought to contribute to the resting potential (Bischoff et al., 1998). In addition, robust activation by both intracellular Na^+ and Cl^- makes Slick a likely participant in ion transport processes such as regulation of cell volume that are critical to neuronal viability.

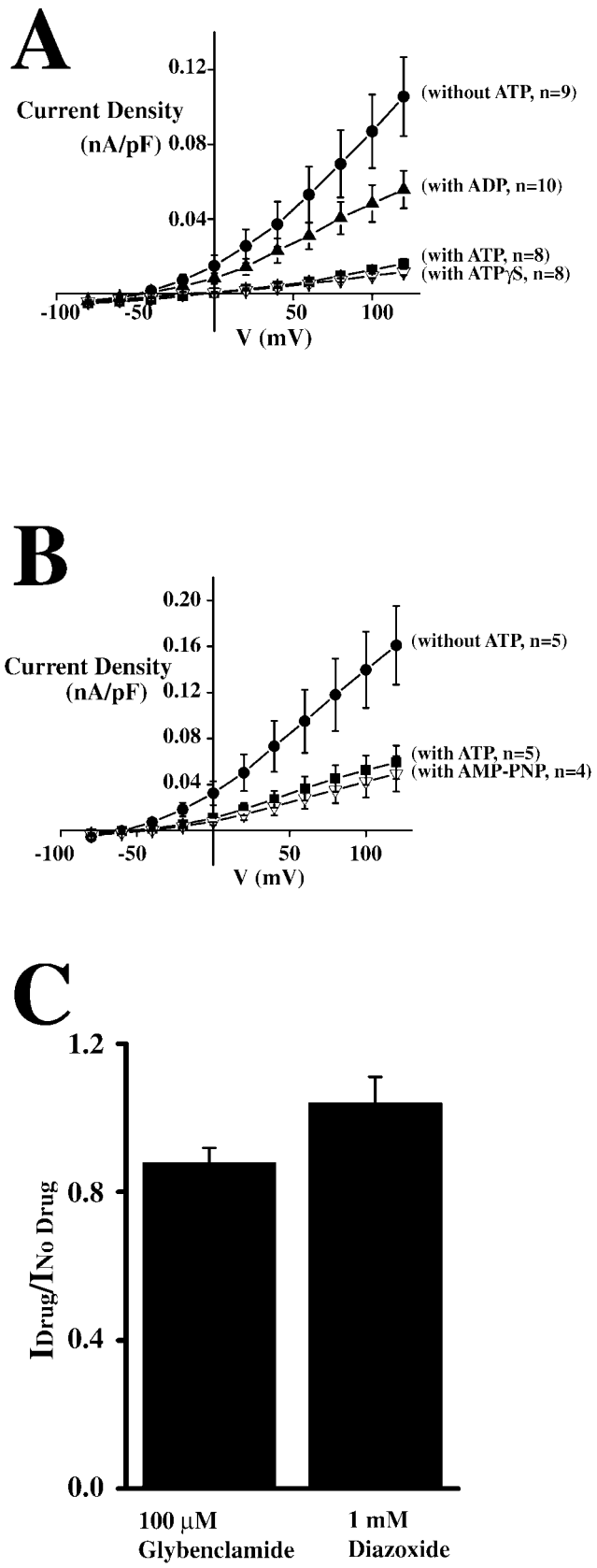

Figure 7. Intracellular ATP reduces Slick whole-cell currents. *A*, Average Slick current densities as a function of voltage recorded from transfected CHO cells with a pipette solution containing (in mM): 130 KCl, 5 EGTA, and 10 HEPES, pH 7.2, and 5 MgATP, MgADP, MgATPγS or no nucleotide. Currents were elicited as 200 msec steps to 0 mV from a holding potential of −70 mV. *B*, Effects of AMP–PNP. The experiment was repeated as in *A*; however, the nonhydrolyzable ATP analog, AMP–PNP, was used in place of nucleotide in some experiments. These experiments represent a different set of transfected cells versus *A*. *C*, Effects of the K_{ATP} channel agonist diazoxide and the K_{ATP} antagonist glybenclamide. Recordings were performed as in Figure 4, *A* and *B*. Glybenclamide (100 μM) and diazoxide (1 mM) were applied to the bath solution.

Perhaps the most unique attribute of Slick is the requirement for ATP to dissociate from a site near the C terminus of the channel (in the presence of elevated intracellular Na^+) to activate an increase in channel activity. The dual requirement for elevated Na^+ and lack of bound ATP suggests that Slick may be selectively activated during conditions such as hypoxia, epileptiform activity, or sustained high-frequency firing of excitable cells. Each of these conditions is associated with elevated intracellular Na^+ and an enhanced demand on mitochondria to maintain ATP levels. For example, during hypoxia, a reduction in the activity of Na,K-ATPase would be expected to compromise normal Na^+ and K^+ gradients, leading to depolarization and a rise in intracellular Na^{2+}. The resulting increase in excitability in either cardiac cells or neurons also leads to enhanced Ca^{2+} influx and reduced extrusion of Ca^{2+} by the Na–Ca exchanger. In the brain such ischemic events can result in cell death within minutes, caused in part by Ca^{2+}-mediated excitotoxicity (Wahlestedt et al., 1993) or failure to regulate cell volume (Pasantes-Morales et al., 2000). It has been suggested that a rise in Cl^- also occurs in ischemia (Inglefield and Schwartz-Bloom, 1998). K^+ channels that open during a hypoxic insult would be expected to protect cells by limiting excitability and maintaining a hyperpolarized membrane potential on which the ion transport processes that regulate cell volume depend. Because Slick is activated by Na^+, Cl^-, and reductions in cellular ATP levels, all of which are thought to result from hypoxic conditions, Slick appears particularly well suited for such a protective role.

It is also possible that Slick plays a role of a "metabolic gatekeeper" in neurons during normal physiological activity, particularly at times of rapid firing and neurotransmitter release, when intracellular Na^+ may rise to very high levels. For example, repetitive stimulation that produces post-tetanic potentiation of neurotransmitter release at the crayfish neuromuscular junction has been shown to elevate presynaptic Na^+ levels up to ~80 mM (Zhong et al., 2001). Indeed, Slick is abundantly expressed in neurons that are capable of firing at very high rates, such as those of the MNTB in the auditory brainstem. As has been suggested for conventional K_{ATP} channels (Liss and Roeper, 2001), Slick may act to adapt the electrical activity of neurons to their rate of ATP consumption. An increased rate of neuronal firing leads to the accumulation of intracellular Na^+ and initial stimulation of the activity of Na,K-ATPase, which is the dominant consumer (40–70%) of neuronal ATP (Erecinska and Dagani, 1990). During hypoxia or sustained firing of action potentials, however, ATP becomes limiting, and intracellular Na^+ cannot be extruded efficiently. Under such conditions, the larger conductance of Slick and the sensitivity of the channel to both Na^+ and ATP would be expected to make its activation even more significant than that of conventional K_{ATP} channels, leading to the coupling of energy consumption to electrical signaling.

Another potential role for ATP-regulated Slick channels in the nervous system is in the generation of spindle activity, a component of slow-wave sleep. Slack (Bhattacharjee et al., 2002) and Slick are expressed in thalamic neurons in which K_{Na}-dependent slow afterhyperpolarizations (sAHPs) are thought to be essential

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for spindle activity (Kim and McCormick, 1998). Sleep is an essential, conserved physiological process that has been postulated to result from the need to restore energy charge in neurons partially depleted of ATP by daily metabolic demands (Bennington and Heller, 1995). An ATP-regulated K_{Na} channel like Slick would be well suited to respond to lowering of cellular energy charge and to provide the Na^+-dependent sAHP of sleep spindles.

In summary, we have identified a K^+ channel, Slick, that functions like a hybrid between K_{Na} and K_{ATP} channels. Thus, the physiological roles of the two mammalian K_{Na} channel subunits, Slick and Slack, may differ. The slow time constant of activation of Slack is well suited to underlie the slow afterhyperpolarization that follows a train of action potentials and the resulting rise in intracellular Na^+ in some neurons. Although both channel types may protect the brain against hypoxic insults, which raise cytoplasmic Na^+ and Cl^- concentrations, Slick may be more specialized for protective functions because of its higher affinity for intracellular Cl^- and its requirement for low intracellular ATP to activate. The functional role of both subunits may be further defined by association with other proteins through their PDZ binding domains. For example, clustering with voltage-dependent Na^+ channels could promote the activation of Na^+-dependent sAHPs. Similarly, clustering with $GABA_A$ and/or glycinergic receptors could potentiate Cl^--mediated inhibition or excitation. The expression of Slick in brain suggests it might be a pharmacological target for regulation of stroke, seizure activity, and sleep.

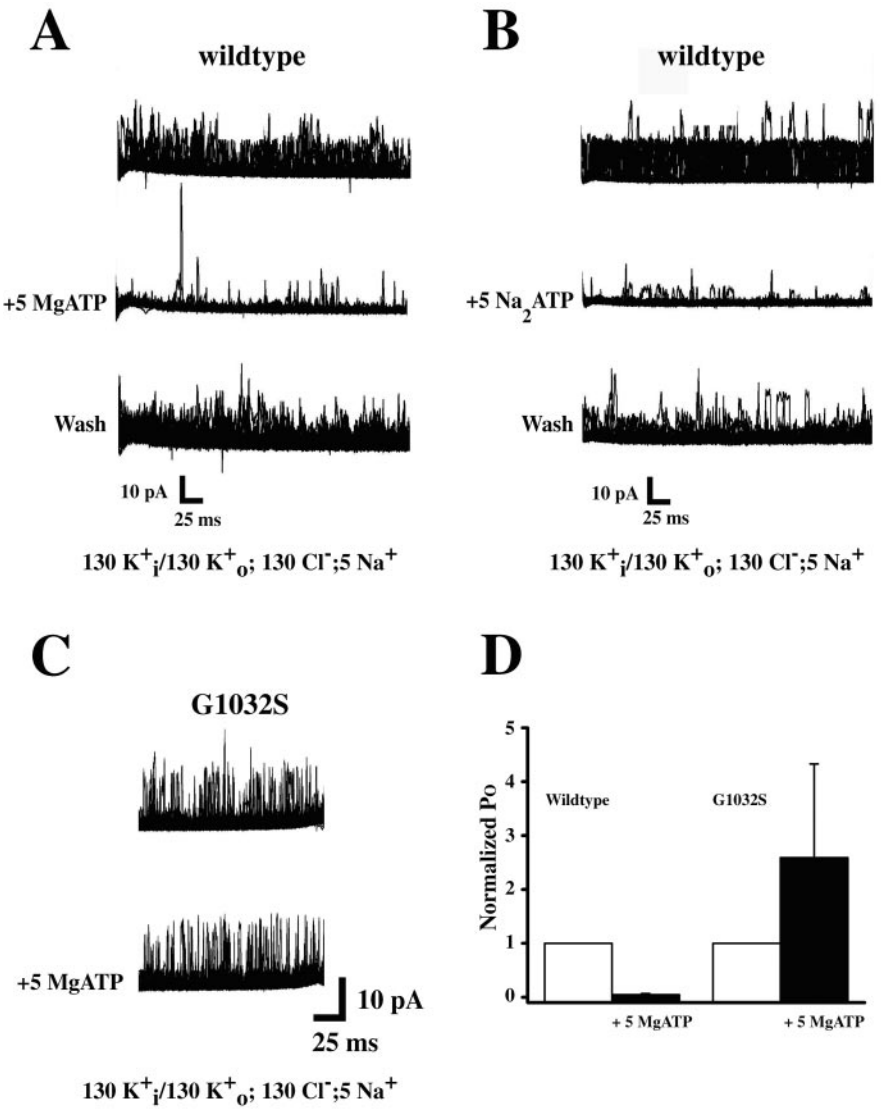

Figure 8. Slick channels are directly inhibited by ATP. Representative excised inside-out patch recordings of Slick channels perfused with a solution containing (in mM): 130 KCl, 5 Na-gluconate, 5 EGTA, 10 HEPES, pH 7.2, and 5 MgATP (*A*) or 5 mM Na_2ATP (*B*). Patches were held at 0 mV and stepped to +80 mV for 200 msec. *C*, Slick channels with mutated consensus ATP binding site (G1032S) perfused with 5 mM MgATP. The activity of the patch remained unchanged after addition of ATP to the bath solution. *D*, Wild-type Slick channels are inhibited by ATP, but G1032S mutants are not. NP_0 values were calculated over 50 sweeps before and after perfusion of patches with ATP. NP_0 values were then normalized to the NP_0 obtained before perfusion of ATP. Average data are from five experiments for wild-type and four experiments for the G1032S mutant. Error bars represent SEM.

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