Kv1.2-Containing $K^+$ Channels Regulate Subthreshold Excitability of Striatal Medium Spiny Neurons

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Shen, Weixing, Salvador Hernandez-Lopez, Tatiana Tkatch, Joshua E. Held, and D. James Surmeier. Kv1.2-containing $K^+$ channels regulate subthreshold excitability of striatal medium spiny neurons. J Neurophysiol 91: 1337–1349, 2004. First published September 17, 2003; 10.1152/jn.00414.2003. A slowly inactivating, low-threshold $K^+$ current has been implicated in the regulation of state transitions and repetitive activity in striatal medium spiny neurons. However, the molecular identity of the channels underlying this current and their biophysical properties remain to be clearly determined. Because previous work had suggested this current arose from Kv1 family channels, high-affinity toxins for this family were tested for their ability to block whole cell $K^+$ currents activated by depolarization of acutely isolated neurons. α-Dendrotoxin, which blocks channels containing Kv1.1, Kv1.2, or Kv1.6 subunits, decreased currents evoked by depolarization. Three other Kv1 family toxins that lack a high affinity for Kv1.2 subunits, r-agitoxin-2, dendrotoxin-K, and r-margatoxin, failed to significantly reduce currents, implicating channels with Kv1.2 subunits. RT-PCR results confirmed the expression of Kv1.2 mRNA in identified medium spiny neurons. Currents attributable to Kv1.2 channels activated rapidly, inactivated slowly, and recovered from inactivation slowly. In the subthreshold range (ca. −60 mV), these currents accounted for as much as 50% of the depolarization-activated $K^+$ current. Moreover, their rapid activation and relatively slow deactivation suggested that they contribute to spike afterpotentials regulating repetitive discharge. This inference was confirmed in current-clamp recordings from medium spiny neurons in the slice preparation where Kv1.2 blockade reduced first-spike latency and increased discharge frequency evoked from hyperpolarized membrane potentials resembling the "down-state" found in vivo. These studies establish a clear functional role for somato-dendritic Kv1.2 channels in the regulation of state transitions and repetitive discharge in striatal medium spiny neurons.

INTRODUCTION

In response to temporally coherent glutamatergic synaptic input, striatal medium spiny neurons move from a hyperpolarized "down-state" to a depolarized "up-state" that is near spike threshold (Stern et al. 1997; Wilson and Groves 1981; Wilson and Kawaguchi 1996). Previous studies of these state transitions have highlighted the importance of intrinsic $K^+$ channel currents in determining membrane potential range visited during these states (Wilson and Kawaguchi 1996). In the down-state, Kir2 family channels dominate the conductance profile of the medium spiny neuron (Mermelstein et al. 1998; Nisenbaum and Wilson 1995). When the membrane potential moves away from the $K^+$ equilibrium potential in response to synaptic input, these channels close as Mg$^{2+}$ and polyamines are swept into the pore (Ficker et al. 1994; Ishihara et al. 1989). Near −60 mV, another family of $K^+$ channels activate and begin to brake the synaptically driven depolarization. One class of channel that activates quickly in this voltage range is composed of Kv4 family subunits (Tkatch et al. 2000). Early in development, these channels are the predominant depolarization-activated $K^+$ channels in medium spiny neurons (Akins et al. 1990; Surmeier et al. 1988). However, these channels inactivate relatively rapidly, making them poor regulators of sustained depolarizing inputs of the sort seen in adult neurons. This role is played by another $K^+$ channel that emerges later in development. This channel opens in a subthreshold voltage range but unlike Kv4 channels inactivates slowly. Studies in vitro have suggested that a channel with these properties helps set the membrane potential and discharge rate in the up-state (Nisenbaum et al. 1994). In many respects, this current resembles what has been referred to as the D-type current (Storm 1988) or the slowly inactivating A-type current (Albert and Nerbonne 1995; Locke and Nerbonne 1997; Surmeier et al. 1991). In several types of central neuron, this current slows the subthreshold ramp potential induced by depolarizing current injection and slows repetitive spike rate (Bekkers and Delaney 2001; Foehring and Surmeier 1993; McCormick 1991; Storm 1988, 1990).

The molecular identity (or identities) of this slowly inactivating $K^+$ channel in medium spiny neurons has yet to be determined. However, the biophysical and pharmacological data gathered to date point to a Kv1 family channel. In addition to its activation at subthreshold membrane potentials and slow inactivation, two properties are telling. First, the slowly inactivating current is sensitive to 4-aminopyridine (4-AP) at micromolar concentrations and to α-dendrotoxin (α-DTX) (Nisenbaum et al. 1994, 1996). Second, recovery from inactivation is slow, having a recovery time constant greater than a second at −90 mV (Surmeier et al. 1991). Both features are similar to those of Kv1.1, Kv1.2, and Kv1.6 channels studied in heterologous expression systems (Coetzee et al. 1999). Several other lines of evidence that are consistent with this inference as members of the Kv1 family are increasingly being seen as key determinants of somatodendritic excitability in neurons. In peripheral sensory neurons, these channels help determine somatic spike threshold and spike frequency in response to depolarizing stimuli (Glazebrook et al. 2002). Similarly, in neurons of the medial trapezoid body, heteromers of Kv1.1, Kv1.2, and Kv1.6 subunits are important regulators of repetitive spiking (Dodson et al. 2002). Cortical pyramidal neurons also express an α-DTX-sensitive current with profound effects on action potential firing and spike duration in vitro (Storm et al. 2000; Raman et al. 2002). In this study the authors have identified the Kv1.2-containing $K^+$ current as the subthreshold activating channel that contributes to the regulation of the membrane potential and repetitive activity in striatal medium spiny neurons.
on repetitive discharge near rheobase (Bekkers and Delaney 2001). The experiments described here were undertaken to pinpoint the molecular identity of the slowly inactivating $K^+$ channel in striatal medium spiny neurons, to quantitatively characterize its gating properties, and to determine its impact on spiking. Our results show that although Kv1.1, Kv1.2, and Kv1.6 mRNA are expressed in identified medium spiny neurons, the pharmacological profile of the slowly inactivating current in the somatic and proximal dendritic membrane is that of a channel containing Kv1.2 subunits. The biophysical features of the channel currents are consistent with this identification. Moreover, in the subthreshold voltage range, this Kv1.2 current in the somatic and proximal dendritic membrane is that of a channel containing Kv1.2 subunits. The biophysical features of the channel currents are consistent with this identification. Moreover, in the subthreshold voltage range, this Kv1.2 current constitutes nearly half of the total somatic $K^+$ current, leading to a major role in regulating spike threshold and repetitive discharge.

METHODS

Acute dissociation procedure and slice preparation

Striata were dissected from young adult (3–4 wk) Sprague-Dawley rats, and medium spiny neurons were acutely dissociated using previously described procedure (Surmeier et al. 1992). The rats were anesthetized with isoflurane and decapitated. The brains were quickly removed and immersed in an ice-cold, oxygenated sucrose solution comprising (in mM) 250 sucrose, 4 glucose, 2.5 KCl, 1 Na$_2$HPO$_4$, 4 MgSO$_4$, 15 HEPES, 1 kynurenic acid, 0.1 L-arginine, and 0.005 glutathione (pH 7.4, 300 mOsm/l). After incubation, the dorsal striata from each hemisphere were dissected and transferred to oxygenated HEPES-buffered Hank’s balanced salt solution (HBSS). The holding solution was bubbled with carbogen (5% CO$_2$ and 95% O$_2$) and supplemented with (in mM) 1 kynurenic acid, 0.1 L-nitro-arginine, and 0.005 glutathione (pH 7.4, 300 mOsm/l). After enzyme digestion, tissue was rinsed three times in a dissociation solution, and the neurons were dissociated by triturating using three progressively smaller fire-polished Pasteur pipettes. The dissociation solution was oxygenated and comprised (in mM) 140 sodium isethionate, 2 KCl, 4 MgCl$_2$, 23 glucose, 15 HEPES, 1 kynurenic acid, 0.1 L-nitro-arginine, and 0.005 glutathione (pH 7.4, 300 mOsm/l). The cell suspension was then placed into a 35-mm plastic petri dish (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 1 MgCl$_2$, 1 CaCl$_2$, 23 glucose, and 15 HEPES (pH 7.4, 300 mOsm/l). All recordings were conducted at 22–24°C.

For slice preparation, 250 µM coronal striatal slices were made in an artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 1 MgCl$_2$, 1 CaCl$_2$, 1.25 NaH$_2$PO$_4$, 24 NaHCO$_3$, and 10 glucose (pH 7.4 with 5% CO$_2$, 300 mOsm/l). The slices were then allowed to recover in ACSF for 1 h before recording.

Electrophysiology

Whole cell voltage and current recordings were performed using standard techniques (Baranauskas et al. 1999; Hamill et al. 1981). Electrodes were pulled from 7052 glass on a Sutter P-97 puller and fire-polished before use. For recordings from acutely dissociated neurons, the internal pipette solution contained (in mM) 120 KMeSO$_4$, 2 MgCl$_2$, 5 EGTA, 10 HEPES, 2 Na$_2$ATP, 0.2 Na$_2$GTP, 12 phosphocreatine, and 0.1 leupeptin; pH was adjusted to 7.2 with KOH and osmolality to 270–280 mOsm/l. The external recording solution had the following (in mM): 140 NaCl or sodium isethionate, 3 or 0.5 KCl, 2 CaCl$_2$, 2 MgCl$_2$, 10 glucose, and 10 HEPES; pH was adjusted to 7.2 with NaOH and osmolality to 300 mOsm/l. TTX (1 µM) and 0.1 mM CdCl$_2$ were added to block voltage-dependent Na$^+$ and Ca$^{2+}$ channels, respectively. Solutions were applied with a gravity-driven sewer pipe system. An array of application capillaries was positioned a few hundred micrometers from the cell under study. Solution changes were made by altering the position of the array with a PMC200-P2 motion controller and an 850B high-speed actuator (Newport, Irvine, CA). Solution change was complete within 1 s. For the current-clamp study in tissue slices, the internal pipette solution contained (in mM) 135 KMeSO$_4$, 5 KCl, 0.5 CaCl$_2$, 5 EGTA, 5 HEPES, 2 MgATP, and 0.5 Na$_2$GTP, pH 7.2 and 280–290 mOsm/l. Agents dissolved in bath solution were perfused with a rate of 2–3 ml/min.

Whole cell recordings were obtained with an Axopatch 200 or a Multiclamp 700A amplifier and monitored with a PC running Clampex 8 (Axon Instruments, Union City, CA). Electrode resistance in the bath was typically 1.5–2.5 MΩ for recording of acutely isolated neurons and 3–4 MΩ for slice recording. After achieving the whole cell configuration, series resistance (7–13 MΩ in dissociated cells and 10–20 MΩ in slices) was compensated (70–90%) and periodically monitored. A sample of 38 dissociated medium spiny neurons showed a mean whole cell capacitance of 7.6 ± 0.2 (SE) pF.

Data were analyzed with Igor Pro (Version 4, WaveMetrics, Lake Oswego, OR), Clampfit 8 (Axon Instruments, Union City, CA) or Mini Analysis (Synaptosoft, Decatur, GA). Spike shape parameters were measured using the Mini Analysis routines. Specifically, spike threshold was determined by locating the membrane voltage at which the second derivative of the spike waveform exceeded three times its SD in a period before the spike onset. Spike amplitude was defined as the difference between the peak and the baseline of action potential. Spike width was measured at half the spike amplitude. The afterhyperpolarization was defined as the difference between the spike baseline and the minimum membrane voltage after the action potential peak. For samples >10, central tendency was estimated by computing a mean (±SE); for smaller samples, medians were computed. Differences between samples were analyzed with a Student’s-t-test (large samples) or a paired Wilcoxon signed-rank test (small samples).

Computer simulation

Kv1.2 currents were modeled using NEURON (Hines and Carnevale 1997). Channel gating was assumed to conform to a Hodgkin-Huxley-like formalism: $I = G_{\text{max}}m^2(t,V)(\alpha(t,V)h(t,V) + (1 - a)(V - E_k))$, where $I$ is current, $G_{\text{max}}$ is the maximum conductance, $V$ is the transmembrane voltage, $E_k$ is the $K^+$ equilibrium potential, $m$ is the gating particle to control activation, and $h$ is the gating particle to control inactivation and $a$ is the fraction of the current that inactivates. Both $m$ and $h$ were functions of time and transmembrane voltage that satisfied the partial differential equations

$\frac{dm}{dt} = \left( [m(V), V] - m(t,V) \right) \tau_m(V)$ and

$\frac{dh}{dt} = \left( [h(V), V] - h(t,V) \right) \tau_h(V)$

where

$m(V), V = 1/(1 + \exp(V - V_{m}))/V_{m}$

$\tau_m(V) = \tau_m + C_m \exp\left(-[(V - V_{m})/V_{m}]\right)$

$h(V), V = 1/[1 + \exp(-(V - V_h))/V_{h}]$

$\tau_h(V) = C_h/[\exp(V - V_{h})/V_{h}] + \beta \exp(-(V - V_{h})/V_{h})$
This formalism was coded in a NEURON mod file and then virtual channels were distributed in the membrane of a spherical neuron (diameter = 20 μm). To estimate the parameters of the governing activation and inactivation processes, three types of experiment were performed. First, the rising phase of α-DTX-sensitive currents evoked by voltage steps in acutely isolated neurons were fit with the model. This was done using Igor Pro. These gave reasonable estimates of \( t_{\text{m}} \) at depolarized membrane voltages (≥−30 mV) where the current was readily resolvable. The deactivation time constant at −60 mV was extracted from mono-exponential fits to α-DTX-sensitive tail currents. Next, the recovery kinetics of the α-DTX-sensitive currents at −90 mV were estimated using voltage ramps. Third, voltage ramps of varying speed were used to generate an estimate of inactivation voltage dependence and kinetics. α-DTX-sensitive ramp currents were generated in dissociated neurons with biphasic (“up and down”) ramps of three different speeds. The parameters of the ramp protocol initially were chosen to span a range of kinetics thought to be relevant to Kv1.2 channels at room temperature. These ramps were then tested in a NEURON simulation for their ability to generate data that allowed NEURON’S parameter estimation utility (Multirun Fitter) to reliably converge to accurate estimates of the model’s starting values even when it was given initial estimates that deviated significantly from these values. Based on these simulations, voltage-clamp protocols were designed for use with real neurons as described in the following text.

The α-DTX-sensitive currents generated in these experiments were then used by NEURON’S Multirun Fitter to generate parameter estimates for the inactivation process; the activation process was constrained by the parameter estimates derived from the step data. The model was able to reasonably simulate the response to the ramp currents. For activation, the parameter estimates derived from this curve fitting procedure were: \( V_{\text{th}} = −27 \text{ mV} \); \( V_{\text{nc}} = −16 \text{ mV} \); \( \tau_{\text{m}} = 3.4 \text{ ms} \); \( C_{\text{m}} = 89.2 \text{ } \Omega \text{cm} \); \( V_{\text{tm}} = −34.3 \text{ mV} \); \( V_{\text{rcm}} = 30.1 \text{ mV} \). For the inactivation process, the parameters were: \( V_{\text{hi}} = −33.5 \text{ mV} \); \( V_{\text{hc}} = 21.5 \text{ mV} \); \( C_{\text{h}} = 548.7 \text{ } \Omega \text{cm} \); \( V_{\text{t1}} = −0.96 \text{ mV} \); \( V_{\text{r1}} = 29.01 \text{ mV} \); \( V_{\text{t2}} = −0.96 \text{ mV} \); \( V_{\text{r2}} = 100 \text{ mV} \). To estimate Kv1.2 currents flowing during repetitive spike activity, whole cell recordings from medium spiny neurons in a slice preparation were ported to NEURON and used to drive a simulated voltage-clamp electrode attached to a spherical neuron containing modeled Kv1.2 channels. To correct for the difference in temperature, \( V_{\text{m}}(V_{\text{th}}) \) and \( V_{\text{m}}(V_{\text{i}}) \) functions were corrected using a Q10 of 3 (Hille 2001).

Single-cell RT-PCR analysis

Isolated individual neurons were patched in the cell-attached mode and lifted into a stream of the physiological saline. Neurons were then aspirated into the electrode. Electrodes contained 1−2 μl of diethylpyrocarbonate (DEPC)-treated water. The glass used for making electrodes was heated to 180°C for 2−3 h. Sterile gloves were worn at all times during the procedure to minimize RNase contamination. After aspiration of the neuron, the electrode was removed from the holder, the tip was broken in a 0.5-ml Eppendorf tube containing 3.6 μl of DEPC-treated water, 0.7 μl of RNasin (28,000 U/ml), and 0.7 μl of oligo-dT or random hexamer (0.5 mg/ml), and the contents were ejected. The mixture was heated to 70°C to denature the nucleic acids and then placed on ice for >1 min. Single-strand cDNA was synthesized from the cellular mRNA by adding SuperScript II RT (1 μl; 200 U/ml), 10× PCR buffer (1 μl), MgCl2 (2 μl; 25 mM), DTT (2 μl; 0.1 M), and mixed dNTPs (1 μl; 10 mM) and then incubating the mixture at 42°C for 120 min. The reaction was terminated by heating the mixture to 70°C for 15 min. The RNA strand in the RNA-DNA hybrid was then removed by adding 0.5 μl RNase H (2 U/ml) and incubating at 37°C for 20 min. All reactions contained RNasin (Promega, Madison, WI) were obtained from Invitrogen (Carlsbad, CA).

The single-cell cDNA generated from the reverse transcription step was subjected to conventional PCR using a programmable thermal cycler (P-200, MJ Research, Watertown, MA). PCR primers were developed from GenBank sequences with commercially available OLIGO software (Version 6.6, National Biosciences, Plymouth, MN). Primers for substance P (SP), enkephalin (ENK), Kv1.1, and Kv1.2 have been described previously from this laboratory (Song et al. 1998; Surmeier et al. 1996). Kv1.3 mRNA (GenBank Accession No. X16001) was detected with a pair of primers 5’-GCT GCC AGC ACC TCT CCT C (position 1368) and 5’-AAC CAG GCA CTT GTT CTA GTA GA (position 1740), which give a PCR product of 396 base pairs (bp). Kv1.2 mRNA (GenBank Accession No. X17621) was detected with a pair of primers 5’-ACC TGA AGG CCA CGG ACA ATG (position 1896) and 5’-ACC CAG GCA TGA GAA CTC AGT CTT (position 2322), which give a PCR product of 450 bp. Because of the apparent low abundance of mRNA for Kv1.2, two-round PCR using nested primers was designed. For the first-round outer primers, the upper primer was 5’-GTT GTC CAA AGA TCC GTG CCT C (position 1863), and the lower primer was 5’-GCT GCA TTG TGC CTT TCT GAG A (position 2304). The inner primers were 5’-CAA CTG TAC TCT GGT TAA CAC AAA (position 2002) and 5’-CTC CAT GGG TGA CTC TCA TCT (position 2276), yielding a predicted PCR product of 296 bp. “Touch-down” protocols were implemented for more efficient amplification of single-cell cDNA. This was done by modifying a standard 45-cycle PCR protocol in the following way: first, 35 cycles were run at the optimal annealing temperature for each primer set, then the annealing temperature was decreased by 1 for two cycles—five times—for a total of 45 cycles.

For an assessment of developmental change in Kv1.2 mRNA, serial dilutions of striatal cDNA from different ages were made to determine the detection threshold for Kv1.2 and β-actin. The detection threshold for Kv1.2 cDNA in each sample was then normalized to that for β-actin. The Kv1.2 expression level is presented as a percentage of β-actin.

PCR procedures were performed using procedures designed to minimize the chance of cross-contamination (Cimino et al. 1990). Negative controls for contamination from extraneous and genomic DNA were run for every batch of neurons. To ensure that genomic DNA did not contribute to the PCR products, neurons were aspirated and processed in the normal manner except that the reverse transcription was omitted. Contamination from extraneous sources was checked by replacing the cellular template with water. Both controls were consistently negative in these experiments.

Chemicals and drugs

α-Dendrotoxin (α-DTX), r-agitoxin-2 (AgTX), dendrotoxin-K (DTX-K), r-margatoxin (MgTX), and TTX were obtained from Alomone Labs (Jerusalem, Israel). Bovine serum albumin (BSA, 0.1%) was added to α-DTX-, AgTX-, DTX-K-, and MgTX-containing solutions (Martina et al. 1998). To control for any effects of BSA, it was included at equal concentrations in all of external solutions in experiments using these toxins. GTP, leupeptin, and ATP were purchased from Calbiochem (La Jolla, CA). All other chemicals were obtained from Sigma Chemicals (St. Louis, MO).

RESULTS

Pharmacological profile of the slowly inactivating K+ channels implicates Kv1.2 channels

α-DTX has a high affinity for three Kv1 family subunits—Kv1.1, Kv1.2, and Kv1.6 (Coetzee et al. 1999). These subunits can form homomeric or heteromeric ion channels, which retain their sensitivity to α-DTX. To determine if similar channels were expressed in medium spiny neurons, α-DTX and other specific Kv1 subunit blockers were examined for their ability to block depolarization-activated K+ currents. To this end,
voltage-clamp recordings were made from acutely isolated medium spiny neurons (Baranauskas et al. 1999). To isolate K+ currents, voltage-dependent Na+, Ca2+, and Ca2+-dependent K+ channels were blocked by addition of 1 μM TTX and 0.1 mM CdCl2 to the perfusion solution. After a prepulse of 2 s to −90 mV to diminish resting voltage-dependent inactivation, currents were evoked by a depolarizing voltage step to +30 mV for 1 s. These currents decayed bi-exponentially (Fig. 1A). Application of α-DTX at concentrations from 0.4 to 1 μM reduced current amplitude both at the peak of the transient and at latter time points in the current trace, suggesting that a rapidly activating, slowly inactivating current was being blocked (Fig. 1A). This inference was confirmed by examination of difference currents (Fig. 1A, bottom). α-DTX (1 μM) reduced the current measured at the end of the step to +30 mV by 15.1 ± 1.9% (n = 18, P < 0.01, Fig. 1B).

As mentioned in the preceding text, α-DTX blocks three types of Kv1 channel. To provide some insight into the particular subunits forming the channels here, a battery of Kv1 potassium channel toxins were applied. DTX-K only blocks channels containing Kv1.1 subunits (Robertson et al. 1996; Wang et al. 1999b). Application of 100 nM DTX-K failed to reduce K+ currents in medium spiny neurons (peak current = 98.8 ± 1.3% of control in DTX-K, n = 10, P > 0.05), arguing against the involvement of Kv1.1 subunits. Application of r-margatoxin (MgTX; 30 nM), a selective blocker of Kv1.3 channels (Garcia-Calvo et al. 1993), also failed to reduce currents (99.2 ± 1.1% of control in MgTX, n = 16, P > 0.05, Fig. 1B). Finally, application of AgTX (10 nM), a high-affinity blocker of Kv1.1, Kv1.3, and Kv1.6, but not Kv1.2 (Garcia et al. 1994), also failed to reduce K+ current amplitude (peak current = 98.7 ± 1.6% of control, n = 12, P > 0.05). In the presence of TEA (10–20 mM)—concentrations block both Kv1.1 and Kv1.6 channels but have no effect on Kv1.2 subunits (Coetzee et al. 1999), the addition of α-DTX (1 μM) continued to block residual currents (n = 6, data not shown), suggesting that Kv1.2 subunits do not form a heteromultimers with Kv1.1 and/or Kv1.6 subunits. Taken together, these data strongly implicate Kv1 channels containing Kv1.2 subunits, but lacking Kv1.1, Kv1.3, or Kv1.6 subunits, in the generation of depolarization-activated K+ current in medium spiny neurons.

Medium spiny neurons express Kv1.2 mRNA

If the pharmacological analysis is correct, medium spiny neurons should express Kv1.2 mRNA. Previous studies have shown Kv1.2 mRNA is present in the striatum (Scott et al. 1994), an observation confirmed by tissue level RT-PCR analysis (data not shown). To test the hypothesis that medium spiny neurons express Kv1.2 mRNA, single-cell RT-PCR profiling was performed. Initially, 15 medium spiny neurons—identified by their expression of enkephalin (ENK) or substance P (SP) mRNA—were examined using a single round of amplification. Kv1.1, Kv1.2, Kv1.3, and Kv1.6 mRNAs were examined. All four transcripts were readily detected at the tissue level. But, in individual medium spiny neurons the expression pattern was more restricted. Kv1.3 subunit mRNA was not detected in identified medium spiny neurons. On the other hand, the other transcripts were frequently evident (Fig. 2A). Kv1.1 was the most readily detected, being seen in >90% of the neurons profiled. Kv1.2 was less readily detected, being seen in ~40% of the neurons with a single round of amplification.

There are two interpretations of this result. One is that Kv1.2 mRNA is expressed in a subpopulation of medium spiny neurons. The other is that Kv1.2 mRNA abundance was near the detection threshold for the single-cell assay, leading to inconsistent detection. There was no correlation between Kv1.2 detection and the expression of SP or ENK mRNA, a common predictor of medium spiny phenotype (Anderson and Reiner 1991; Gerfen 1992). Moreover, α-DTX reduced K+ currents in all neurons examined (n > 20). To provide a more direct test of the second possibility, the sensitivity of the assay for Kv1.2 mRNA was increased by employing a nested, two-stage priming strategy (Brytting et al. 1991). Using this protocol, Kv1.2 was seen in the majority of the cells (25/28; Fig. 2B). Gels from ENK and SP expressing medium spiny neurons are shown in Fig. 2B. As stated in the preceding text, there was no correlation between SP/ENK expression and the detection of Kv1.2 mRNA.

To provide an additional test of the involvement of Kv1.2 channels, a developmental profile of Kv1.2 expression was generated. The depolarization activated K+ currents in early postnatal medium spiny neurons are dominated by Kv4, rapidly inactivating channels (Surmeier et al. 1988). The slowly inactivating K+ currents emerge in the third and fourth postnatal weeks. If this current is attributable to Kv1.2 channels in whole or part, Kv1.2 mRNA levels should increase in parallel.

FIG. 1. α-Dendrotoxin (α-DTX) blocks a component of depolarization activated K+ currents. A: K+ current traces in control conditions and in the presence of external α-DTX. Currents were evoked by depolarizing test pulses to +30 mV after a prepulse of 2 s to −90 mV. Subtraction of current trace obtained in the presence of α-DTX from in control gives rise to the difference current in A, bottom. B: percentage blockade by α-DTX, r-agitoxin-2 (AgTX), dendrotoxin-K (DTX-K), r-margatoxin (MgTX). *P < 0.01.
Semi-quantitative RT-PCR analysis of Kv1.2 expression revealed a roughly threefold increase in the relative levels of Kv1.2 mRNA abundance between the first and fourth postnatal weeks (Fig. 2C).

Kv1.2 channel currents activate at subthreshold membrane potentials

If slowly inactivating, Kv1.2 channels regulate synaptic integration and repetitive discharge, then these channels should be responsible for a significant portion of the total K⁺ current at subthreshold membrane potentials. This hypothesis was examined first using voltage steps (1 s) from hyperpolarized membrane potentials (−90 mV). The outward currents evoked by steps above −60 mV were reduced by α-DTX (1 μM; Fig. 3A). Subtraction of the α-DTX-sensitive currents revealed them to be activated at subthreshold potentials (ca. −55 mV). The voltage dependence of activation was assessed by converting the current amplitudes to chord conductances and then normalizing (Fig. 3B). Data points were fitted with a second-order Boltzmann function. The α-DTX-sensitive, Kv1.2 chan-

![Image](http://www.jn.org/-/media/jn/issue-files/2004/03/3a/FIG%203%20A.png)

**FIG. 3.** Kv1.2 channel currents activate rapidly and inactivate slowly. A: families of K⁺ currents obtained in the absence and presence of 1 μM α-DTX and the subtraction currents in the lower panel. Currents were evoked during 1-s voltage steps between −75 and +45 mV (15-mV increments) after prepulses of 2 s to −90 mV. The voltage-clamp protocol is illustrated at the top. B: normalized conductance curves were plotted as a function of membrane potentials. The data were fit to a Boltzmann function (see METHODS). The fits were $V_{1/2} = −16.7$ mV and $k = 17.2$ mV for the curve after application of α-DTX: $V_{1/2} = −28.4$ mV and $k = 17.4$ mV for the curve of α-DTX-sensitive current. C: the rising phases of the Kv1.2 currents obtained between 0 and +45 mV were extended for clarity and fit with a modified Hodgkin-Huxley formalism. —, the fitted curves. D: average time constants (tau) of activation derived from curve fits (as in C) are plotted. The deactivation time constant at −60 mV is from Fig. 4B. The function fit to the data are described in METHODS.
nel currents had an average half-activation voltage of $-27.4 \pm 2.5$ mV ($n = 6$) and a relatively shallow slope factor ($16.3 \pm 1.8$ mV). By contrast, the residual currents had a more depolarized half-activation voltage ($-15.3 \pm 2.2$ mV); slope factors were similar to the Kv1.2 currents ($16.8 \pm 1.7$ mV; Fig. 3B). The kinetics of activation were determined by fitting a second-order Hodgkin-Huxley model to the rising phase of the Kv1.2 currents (see METHODS; Fig. 3C). Activation time constants were voltage dependent, decreasing over the potential range where they could be accurately measured from the step currents (Fig. 3D). For example, at $0$ mV, the average activation time constant was $27.8 \pm 2.4$ ms ($n = 6$), whereas at $+45$ mV, it was $2.8 \pm 0.4$ ms. These data were pooled with the deactivation time constant estimate at $-60$ mV to generate a closed form approximation of the relationship between the activation kinetics and voltage. The data were reasonably well fit with commonly used kinetic formula (see METHODS) plotted as a solid line in Fig. 3D.

To provide an alternative way of estimating the activation properties of the Kv1.2 currents, voltage ramps running from $-90$ to $+40$ mV were used. Using intermediate speed ramps ($130$ mV/s) to inactivate Kv4 channel currents, $\alpha$-DTX ($1 \mu$M) produced a marked shift in the current-voltage relation, resembling that seen with steps (Fig. 4A, cf. Fig. 3B). As with the step currents, difference currents were used to estimate the Kv1.2 channel currents (Fig. 4B). Conversion of the currents to conductance estimates allowed fitting of a Boltzmann function (Fig. 4C). These fits were indistinguishable from those generated from the data derived from voltage steps, arguing that channel inactivation was not a significant factor at this ramp speed (at least over much of the voltage range examined; see following text). The average half-activation voltage of the Kv1.2 ramp currents was $-25.3 \pm 2.7$ mV ($n = 7$) and the average slope factor was $13.4 \pm 1.6$ mV. The residual current had a significantly more depolarized voltage dependence, having a half-activation voltage of $-11.2 \pm 2.1$ mV ($n = 7$) and an average slope factor of $11.6 \pm 2.0$ mV. These properties more closely reflect those of Kv2 delayed rectifier currents, which are known to be present in medium spiny neurons (Baranauskas et al. 1999), than the residual from the step data. An added advantage of this type of voltage-clamp protocol is experimental speed. The estimation of current properties is limited only by the kinetics of the $\alpha$-DTX block, making rundown or other changes in channel properties a less significant concern.

For voltage steps to depolarized membrane potentials, the Kv1.2 channel current constituted a small fraction of the total $K^+$ current ($<20\%$). A similar situation is found in cortical pyramidal neurons (Bekkers and Delaney 2001). With this information alone, it is difficult to see how Kv1.2 channels could be of much functional importance. However, these channels may be a more important factor at subthreshold potentials, particularly when the membrane potential is changing slowly. To estimate the relative contribution of Kv1.2 channels across the voltage spectrum, the percentage of the total current evoked by a slow voltage ramp that was attributable to Kv1.2 channels was computed. An average derived from seven neurons is shown in Fig. 4D. Between $-60$ and $-50$ mV, the Kv1.2 current was about half ($54.3 \pm 2.2\%$, $n = 7$) of the total $K^+$ current. This is almost certainly an underestimate, as $1 \mu$M $\alpha$-DTX is unlikely to completely block Kv1.2 channels. At more depolarized potentials, the contribution of Kv1.2 channels diminished, falling to $<20\%$ ($18.6 \pm 1.5\%$, $n = 7$) at $+30$ mV.

In addition to $\alpha$-DTX, Kv1.2 channels are readily blocked by 4-aminopyridine ($4$-AP) at micromolar concentrations (Russell et al. 1994). Application of $100$ $\mu$M $4$-AP reduced currents evoked by the slow voltage ramp by approximately the same amount as did $\alpha$-DTX. At $-20$ mV, $4$-AP reduced the current amplitude by nearly half ($48.3 \pm 2.1\%$, $n = 4$). Holding the membrane at $-60$ mV, to inactivate Kv1.2 channels, reduced the block by $4$-AP to $14\%$ ($\pm 1.8\%$, $n = 4$). The block by

![Figure 4](http://jn.physiology.org/)

**FIG. 4.** Kv1.2 channel currents accounts for half of subthreshold $K^+$ current. A: activation curves obtained by recording membrane current responses to a slowly depolarizing voltage-ramp ($130$ mV/s from $-90$ to $+40$ mV, inset) in the absence and presence of $1 \mu$M $\alpha$-DTX. B: $\alpha$-DTX-sensitive currents obtained by subtraction of the traces shown in A. Inset: tail currents obtained by stepping to $-60$ mV; the deactivation time constant was estimated by fitting the tail currents with a single exponential function with a time constant of 56 ms (—). C: normalized conductance estimates generated from the currents in A and B were plotted as a function of membrane potentials. The data were then fit with a Boltzmann function as described in METHODS. The fits were $\nu = -7.5$ mV and $k = 12.2$ mV for the $\alpha$-DTX-insensitive conductance; $\nu = -21.9$ mV and $k = 15.5$ mV for the $\alpha$-DTX-sensitive conductance. D: percentage of current that was attributable to $\alpha$-DTX-sensitive, Kv1.2 channels over a voltage range of $-60$ and $+40$ mV.
α-DTX was similarly reduced by holding at −60 mV (8.7 ± 1.2%, n = 4).

**Kv1.2 channels recover slowly from inactivation**

The pharmacological and biophysical data presented thus far are consistent with the attribution of the α-DTX-sensitive current to Kv1.2 channels. Another distinctive feature of these channels (Coetzee et al. 1999; Sprunger et al. 1996) is their slow recovery from inactivation. To determine if the channels in medium spiny neurons had similar properties, channels were inactivated by holding at −40 mV for 4 s and then stepped to −90 mV for varying periods of time before delivering a voltage ramp (Fig. 5A). To isolate the Kv1.2 current, the first conditioning period (25 ms) was used as a control, assuming that there would be little or no recovery in a few milliseconds. Ramp currents were aligned along a voltage axis and the amplitude measured at Ramp currents were aligned along a voltage axis and the average normalized currents were plotted as a function of conditioning step duration in C. Data were well-fit with a single exponential having a time constant of 987 ms.

**Model of Kv1.2 channel gating**

The biophysical data presented thus far are not sufficient to make an adequately constrained model of Kv1.2 channel gating. The principal obstacle to the construction of such a model was the apparent variation in inactivation kinetics estimated from long voltage steps. The problem with these step protocols is that they are very slow, taking tens of minutes to complete over an appropriate voltage range. As a consequence, rundown (or other alterations in channel gating) can contaminate difference currents, leading to random variation in the kinetic estimates. To overcome this obstacle, voltage ramps of varying speed were used to evoke currents. These ramps could be completed in a few seconds (rather than minutes), minimizing the impact of channel instability on parameter estimation. Three ramp speeds were used that preliminary simulation work had shown adequately constrained the inactivation parameters of a modified Hodgkin-Huxley formalism. An example of the α-DTX-sensitive currents evoked by these ramp protocols is shown in Fig. 6A. Note that the peak current amplitude decreased with increasing ramp durations, providing a measure of channel inactivation. Plots of the percent contribution of Kv1.2 channels to the total K⁺ current for the three ramps are shown as an inset. For the intermediate ramp speed, the contribution of Kv1.2 is very similar to what was shown earlier (Fig. 4). However, for faster ramps it is clear that Kv1.2 makes a more substantive contribution to the total current at more depolarized membrane potentials (blue trace). Conversely, for very slow ramps, the contribution of Kv1.2 channels diminishes across a broad range of potentials, reflecting voltage-dependent inactivation of these channels.

With this data in hand, a Hodgkin-Huxley model of the Kv1.2 channel gating was generated using tools in the NEURON simulation program. This was done in two steps. First, the model parameters were constrained by the activation data shown in Fig. 2 and by the inactivation recovery data shown in Fig. 5. Then the Multirun Fitter in NEURON was used to identify inactivation parameters that would allow all three ramp currents to be accurately simulated. The resulting steady-state and kinetic relationships are shown in Fig. 6B (parameter estimates are provided in METHODS). The fits to the ramp currents themselves are shown in Fig. 6D. As mentioned in the preceding text, the inactivation kinetics of α-DTX-sensitive currents evoked by strong depolarizing steps were variable. Three examples are shown in Fig. 6C. The model does a reasonable job of representing the central tendency of these currents using a single inactivation process. The response of the model to standard step activation and inactivation protocols is shown in Fig. 6, E and F. Both sets of data resemble experimentally generated currents in medium spiny neurons as well as currents in heterologous systems expressing Kv1.2 channels (Coetzee et al. 1999; Russell et al. 1994; Sprunger et al. 1996). So, although not perfect, the model captures key features of the Kv1.2 channel currents seen in medium spiny neurons, making it suitable to use as a tool to explore how these channels might contribute to spike generation and repetitive activity.

**Kv1.2 channels regulate first spike latency and repetitive discharge in medium spiny neurons**

From hyperpolarized membrane potentials, rheobase current injection in medium spiny neurons produces a slow voltage ramp to the first spike (Nisenbaum et al. 1994). An example of this phenomenon is shown in Fig. 7A (black line) where the whole cell recording was taken from a medium spiny neuron in a tissue slice preparation. To estimate the contribution of Kv1.2
channels to this behavior, the recording was used as a voltage command in a NEURON simulation of Kv1.2 currents at 33°C ($Q_{10} = 3$). The Kv1.2 channel current evoked by this voltage trajectory is shown as the blue line in Fig. 7A. This simulation shows several interesting features of the current. First, it is significantly activated during the approach to the first spike, suggesting it plays an important role in shaping the voltage trajectory. As shown in the preceding text, in this voltage

**FIG. 6.** Kv1.2 channel kinetics were simulated using a modified Hodgkin-Huxley model. A: $\alpha$-DTX-sensitive Kv1.2 channel ramp currents were estimated by subtraction of control currents evoked by voltage ramps of 3 different speeds from those evoked in the presence of $\alpha$-DTX (1 μM). The percentage contribution of Kv1.2 channels to total K currents for each ramp is illustrated inset. B, left: plots of the activation ($m$) time constant ($\tau_m$), and steady-state ($m_s$) values as a function of membrane potential. Right: corresponding plots inactivation ($h$) time constant ($\tau_h$) and steady-state ($h_s$) values as a function of membrane potential. C: simulated Kv1.2 current (red line) captures inactivation properties of the Kv1.2 channel population as represented by 3 $\alpha$-DTX-sensitive currents evoked by the same membrane potential step. D: $\alpha$-DTX-sensitive Kv1.2 channel ramp currents were well fit with the Kv1.2 channel model (green line). E: simulated currents evoked by progressively more depolarized 1-s voltage steps from a holding potential of −90 mV (protocol is shown at the top). F: simulated currents evoked by a standard inactivation protocol having 5-s prepulses between −110 mV and −10 mV followed by a test step to +20 mV. Note the similarity between the simulated currents and those derived from whole cell recording.
Kv1.2 channels are a major component of the total K⁺ current. Second, the Kv1.2 channels are efficiently activated by the spike and deactivate relatively slowly, leading to substantial activation during the interspike period. This suggests that the fast afterhyperpolarization (fAHP) of the medium spiny neuron is likely to be strongly influenced by Kv1.2 channels. This can be seen more clearly in Fig. 7B, where the current and voltage traces have been scaled up.

To experimentally evaluate the role of Kv1.2 channels, α-DTX was applied to the medium spiny neuron from which the recording in A was obtained. Then, the same current pulse was applied. As shown in Fig. 7C, the first spike latency dramatically shortened, as predicted from the modeling results. Second, the fAHP was reduced and the repetitive discharge rate increased—again as suggested by the Kv1.2 model. Similar results were obtained in every neuron examined (n = 4). Discharge frequency increased on average 340% (±71%, P < 0.05) after blockade of Kv1.2 channels.

To characterize the role of Kv1.2 channels on the response to a time varying, suprathreshold current injection, ramp currents were applied to medium spiny neurons recorded in the slice. In the presence of α-DTX, spike threshold—defined by the abrupt increase in dV/dt—decreased by almost 2 mV (1.9 ± 0.11 mV; from −45.07 ± 1.01 mV in control to −47.01 ± 0.99 mV in α-DTX, n = 7, P < 0.02, paired Wilcoxon signed-rank test). As predicted by the slow activation kinetics of Kv1.2 current (Fig. 7B), the fAHP after the first action potential was reduced by 3.45 ± 0.36 mV (−6.78 ± 0.46 mV in control; −3.32 ± 0.20 mV in α-DTX, n = 7, P < 0.02, paired Wilcoxon signed-rank test). There were no alterations in spike amplitude (90.05 ± 2.15 mV in control vs. 92.14 ± 2.15 mV in α-DTX, n = 7, P > 0.05, Wilcoxon signed-rank test) or spike half-width (1.95 ± 0.04 ms in control vs. 1.94 ± 0.04 ms in α-DTX, n = 7, P > 0.05, paired Wilcoxon signed-rank test). In addition, the application of α-DTX (1 μM) consistently shortened first spike latency and increased instantaneous spike frequency (Fig. 7, D–F). The increased discharge frequency was seen throughout the ramp as shown in the plot of frequency as a function of current amplitude (Fig. 7E); α-DTX shifted the relation to the left without significantly altering the slope of the relationship (P > 0.05, Wilcoxon, n = 5). First spike latency was shortened by α-DTX the same amount for a broad range of ramp speeds as expected for the rapid activation kinetics of the Kv1.2 current (Fig. 7F).

The number of spikes evoked during each ramp was increased, shifting the relationship between spike number and ramp slope upward without changing the linearity of the plot or its slope (P > 0.05, Wilcoxon, n = 5, Fig. 7G).

Another important feature of Kv1.2 channels is that prolonged residence at depolarized membrane potentials leads to inactivation. This property has been shown to underlie a form of short-term response plasticity (Turrigiano et al. 1996). In
vivo, medium spiny neurons move between hyperpolarized membrane potentials (ca. −85 mV), where perhaps 30% of Kv1.2 channels should be inactivated, and more depolarized membrane potentials (ca. −55 mV), where −70% of the channels should be inactivated at steady state. A plot modeled Kv1.2 channel availability (h state variable) as a function of time after voltage steps to −60, −50, and −40 mV from a holding potential of −80 mV is shown in Fig. 8A (the simulation was run assuming a temperature of 33°C). The kinetics of change were slow, taking >20 s to stabilize. As predicted by this result, changing the somatic membrane potential to −60 mV in medium spiny neurons in the slice by current injection had an impact on first spike latency and discharge rate that was similar to that of α-DTX (Fig. 8B, cf. Fig. 7C). Although smaller, Kv1.2 current is likely to still to make a contribution to the membrane potential trajectory as judged by currents generated by the model when the current-clamp waveform was used a voltage command (blue line, Fig. 8B). To test this inference, α-DTX (1 μM) was applied to medium spiny neurons held at −60 mV and then the same current step applied. Now, α-DTX did not significantly reduce first spike latency (51 ± 12 ms in control; 34 ± 4 ms in α-DTX, n = 4, P > 0.05, Wilcoxon) but did slightly increase discharge frequency (136 ± 14%, n = 4, P < 0.05, Wilcoxon) and attenuate the fAHP (6.9 ± 0.39 mV in control vs. 3.7 ± 0.47 mV in α-DTX, n = 4, P < 0.05, Wilcoxon) in agreement with the modeling results (Fig. 8C). These results suggest that prolonged residence in the up-state could lead to substantial inactivation of Kv1.2 channels and enhanced excitability. But recordings from medium spiny neurons in anesthetized animals suggest that up-state durations are unlikely to last 10–20 s (Wilson and Kawaguchi 1996). Spiking could make a difference in the extent of inactivation. This hypothesis was tested with the model using a brief (2 ms) depolarization to +20 mV to simulate a spike. The model’s membrane potential was stepped from −80 to −55 mV and spike-like waveforms delivered at varying frequencies. Channel availability was tracked (h state variable) during the course of the simulation. At repetitive frequencies <20 Hz, spiking had virtually no impact on channel availability (Fig. 8D). Above this frequency, spiking had a progressively larger impact, increasing the inactivation of Kv1.2 channels. Simulation runs using 10-, 20-, 50-, and 100-Hz repetitive rates are shown in Fig. 8D. Although the inactivation of Kv1.2 channels increased with high-frequency spiking, the effect was very modest with the total inactivation increasing by just over 10% with 20 s of 100-Hz spiking.

**DISCUSSION**

The pharmacological, molecular, electrophysiological, and computational data presented argue that K+ channels containing Kv1.2 subunits are major contributors to subthreshold K+ currents in striatal medium spiny neurons. Four principal observations support this conclusion. First, K+ currents in medium spiny neurons were sensitive to low micromolar concentrations of α-DTX but not to DTX-K, MgTX, or AgTX. This pharmacological profile clearly points to Kv1.2 channels (Garcia et al. 1994; Harvey 2001; Koch et al. 1997). Second, scRT-PCR profiling of identified medium spiny neurons revealed consistent expression of Kv1.2 mRNA in both ENK- and SP-expressing medium spiny neurons. Previous immunocytochemical studies also have reported Kv1.2 subunit protein in striatal somata (Sheng et al. 1994; Wang et al. 1994). Third, the voltage dependence of the α-DTX-sensitive current in medium spiny neurons were within the range reported for Kv1.2 channel currents in other preparations (Coetzee et al. 1999; Glazebrook et al. 2002; Grissmer et al. 1994; Hopkins et al. 1994), although these estimates vary widely. The activation kinetics for Kv1.2 channels in medium spiny neurons appear to be somewhat slower than those seen in heterologous systems (Coetzee et al. 1999) (12 ms at 0 mV as opposed to 27 ms at 0 mV); however, this difference may be a reflection of co-assembly with other proteins rather than any difference in the pore-forming
subunit. Last, a computational model of the Kv1.2 channel gating derived from voltage-clamp recordings in medium spiny neurons accurately predicted that its blockade should shorten first spike latency, diminish spike afterhyperpolarization and accelerate repetitive discharge.

Thus all four lines of evidence point to channels containing Kv1.2 subunits as playing a major role in subthreshold integration and repetitive activity in medium spiny neurons. A similar finding has recently been reported in cortical pyramidal neurons (Bekkers and Delaney 2001). As in pyramidal neurons, Kv1.2 channels in medium spiny neurons contribute a relatively small proportion of the total K⁺ current (ca. 10–20%) at very depolarized membrane potentials. In spite of this fact, blockade of these channels has a major impact. In part, this is attributable to their strategic location in the spike generating region (Bekkers and Delaney 2001; Dodson et al. 2002). But another factor that governs their functional role is the voltage range in which they open. As shown here, in the voltage range just below spike threshold, Kv1.2 channel currents constitute a much larger fraction of the total K⁺ current—approaching half of the total current. This is particularly true for intermediate speed voltage trajectories in medium spiny neurons where channel inactivation is not prominent. Together these features—strategic location and subthreshold activation—make Kv1.2 channels particularly important contributors to the regulation of excitability.

Do other Kv1 family subunits contribute to α-DTX-sensitive channels in medium spiny neurons? Our scRT-PCR experiments revealed that medium spiny neurons express (in addition to Kv1.2) readily detectable levels of Kv1.1 and Kv1.6 mRNA but not Kv1.3 mRNA. The ease with which these subunits were detected cannot be taken as an indication of mRNA abundance because of uncertainties in the efficiency of the reverse transcription and amplification reactions. Quantitative inferences will require further study. What can be said at this point is that medium spiny neurons express mRNA for all three Kv1 subunits. These subunits are known to form heteromers in vivo (Dodson et al. 2002; Koch et al. 1997; Wang et al. 1999a). However, as heteromers retain their toxin sensitivity (Dodson et al. 2002; Koch et al. 1997) the inability of DTX-K, MgTX or AgTX to block appreciable K⁺ current indicates that Kv1.1 and Kv1.6 subunits do not participate in the somatic/proximal dendritic Kv1 channels in medium spiny neurons. This conclusion is also supported by the ability of α-DTX to block current in the presence of concentrations of TEA that block channels containing Kv1.1 and/or Kv1.6 subunits. Where then do channels containing these subunits reside if not in the somatic membrane? Kv1.1/Kv1.6 (and Kv1.2) subunits are frequently found in axons or axon terminals (Gu et al. 2003; Monaghan et al. 2001; Rasband et al. 1998; Scherer 1999; Shamotienko et al. 1997; Southan and Robertson 2000; Wang et al. 1994), and the terminal fields of medium spiny neurons display immunoreactivity for Kv1.1 protein (Scott et al. 1994). It is also possible that channels containing Kv1.1 or Kv1.6 subunits are found in more distal dendritic regions. However, there is little immunocytochemical support for this proposition and electrophysiological studies suggest that Kv1 family channels are not prominent in these regions in other types of neuron (Golding et al. 1999).

Is D-type current attributable to Kv1 channels?

The biophysical and pharmacological properties of the Kv1 channels in medium spiny neurons strongly resemble those attributed to D-type current in these and other brain neurons (Bekkers and Delaney 2001; Golding et al. 1999; Nisenbaum et al. 1994, 1996; Storm 1988; Surmeier et al. 1991). Given the striking similarities, it is tempting to postulate that all D-type currents are attributable to Kv1 channels. Variations in the α subunit composition of Kv1 channels and inclusion of modifying β subunits (Nakahira et al. 1996; Rhodes et al. 1997) may account for the described variation in biophysical properties of D-type current (Bekkers and Delaney 2001; Foehring and Surmeier 1993; Southan and Robertson 2000; Storm 1988). Variation in subunit composition may not only explain relatively minor differences in the properties of D-type currents but could explain how Kv1 family channels also give rise to delayed rectifiers (Hille 2001).

Kv1.2 channels control spike generation and repetitive activity

Kv1.2 channels in medium spiny neurons effectively play roles ascribed to both D-type channels and delayed rectifiers. That is, the Kv1.2 channel currents effectively delayed first spike latency and contributed to spike afterhyperpolarization and slowing of repetitive discharge. The delay in first spike latency in medium spiny neurons with near rheobase current injection has been associated with a 4-AP-sensitive current previously (Kita et al. 1985; Nisenbaum et al. 1994). Although medium spiny neurons also express Kv4 channels that display a modest affinity for 4-AP (Tkatch et al. 2000), the ability of α-DTX to mimic the effects of 4-AP argues that Kv1.2 channels are largely responsible for these earlier observations. The delay in first spike latency was seen for a broad range of current intensities. These data and those showing a shift in spike threshold with α-DTX application suggest that Kv1.2 channels are key regulators of spike generation in naturally occurring situations.

After spike generation, Kv1.2 channels are strongly activated and then deactivate slowly, allowing them to participate in the afterhyperpolarization, slowing the trajectory to the next spike during sustained depolarization. Kv1.2 blockade accelerated discharge frequency over the entire frequency-intensity plot of medium spiny neurons. The slow deactivation kinetics of Kv1.2 channels are similar to those of Kv2 family channels that are also expressed by medium spiny neurons (Baranauskas et al. 1999), suggesting that both contribute to the voltage trajectory after spikes and the regular spiking pattern. The maintained activation of the Kv1.2 channels during repetitive firing (e.g., Fig. 7) is also dependent on their relatively hyperpolarized activation voltage dependence and slow inactivation kinetics as shown for D-type currents in hippocampal neurons (Storm 1988). The sensitivity of the afterhyperpolarization in medium spiny neurons to holding potential between −90 and −60 mV shown here and previously (Pineda et al. 1992) is consistent with the proposition that Kv1.2 channels contribute to this process and regular firing.

Kv1.2 channels also are likely to be important regulators of up-states in medium spiny neurons (Wilson and Groves 1981; Wilson and Kawaguchi 1996). Previous studies by Wilson and
Kawaguchi (1996) have shown that K⁺ currents are key determinants of the membrane potential achieved during synaptically driven state transitions. Kv1.2 channels undoubtedly figure prominently in this regulation as they contribute nearly half of the total K⁺ current at up-state potentials (ca. −55 mV). The inactivation kinetics of Kv1.2 channels at this potential ensures that their contribution will wane only slowly during sustained up-states, largely unaffected by spiking. Nevertheless, sustained residence in the up-state, as may occur in the awake, behaving state, undoubtedly increases excitability by inactivating Kv1.2 channels. Over long periods (tens of seconds), this process endows medium spiny neurons with a form of activity-dependent, short-term memory (Turrigiano et al. 1998). The available evidence (Dodson et al. 2002; Golding et al. 1999) suggests that this function of Kv1.2 channels is most important in the soma and initial segment where spikes are generated and is not likely to be important in the dendrites receiving synaptic input. This conjecture must be tested, however. Low micromolar concentrations of 4-AP have prominent excitatory effects on glutamate-mediated EPSPs in medium spiny neurons (Bargus et al. 1998). It is possible that in addition to promoting presynaptic depolarization, blockade of Kv1.2 channels in postsynaptic dendritic regions provides a brake on synaptically mediated depolarization—much as Kv4 channels do in hippocampal pyramidal neurons (Yuan et al. 2002).

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