

# Cholinergic Suppression of KCNQ Channel Currents Enhances Excitability of Striatal Medium Spiny Neurons

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In response to glutamatergic synaptic drive, striatal medium spiny neurons *in vivo* transition to a depolarized “up state” near spike threshold. In the up state, medium spiny neurons either depolarize enough to spike or remain below spike threshold and are silent before returning to the hyperpolarized “down state.” Previous work has suggested that subthreshold K<sup>+</sup> channel currents were responsible for this dichotomous behavior, but the channels giving rise to the current and the factors determining its engagement have been a mystery. To move toward resolution of these questions, perforated-patch recordings from medium spiny neurons in tissue slices were performed. K<sup>+</sup> channels with pharmacological and kinetic features of KCNQ channels potentially regulated spiking at up-state potentials. Single-cell reverse transcriptase-PCR confirmed the expression of KCNQ2, KCNQ3, and KCNQ5 mRNAs in medium spiny neurons. KCNQ channel currents in these cells were potentially reduced by M<sub>1</sub> muscarinic receptors, because the effects of carbachol were blocked by M<sub>1</sub> receptor antagonists and lost in neurons lacking M<sub>1</sub> receptors. Reversal of the modulation was blocked by a phosphoinositol 4-kinase inhibitor, indicating a requirement for phosphatidylinositol 4,5-bisphosphate resynthesis for recovery. Inhibition of protein kinase C reduced the efficacy of the muscarinic modulation. Finally, acceleration of cholinergic interneuron spiking with 4-aminopyridine mimicked the effects of exogenous agonist application. Together, these results show that KCNQ channels are potent regulators of the excitability of medium spiny neurons at up-state potentials, and they are modulated by intrastriatal cholinergic interneurons, providing a mechanistic explanation for variability in spiking during up states seen *in vivo*.

**Key words:** linopirdine; XE991; M<sub>1</sub> knock-out; cholinergic interneuron; PIP<sub>2</sub>; PKC

## Introduction

Striatal medium spiny neurons undergo shifts in their membrane potentials in response to coordinated glutamatergic synaptic input, moving from a hyperpolarized “down state” to a depolarized “up state” (Wilson and Kawaguchi, 1996; Wilson, 2004). In the up state, medium spiny neurons either depolarize sufficiently to generate spikes or remain below spike threshold and are silent (Wilson and Kawaguchi, 1996; Wickens and Wilson, 1998; Tseng et al., 2001; Wilson, 2004). Understanding the mechanisms underlying this dichotomous behavior is of obvious importance to models of striatal information processing, because a silent up state will be indistinguishable from a maintained down state to targets in the globus pallidus and substantia nigra.

Previous studies of the up state have focused on voltage-dependent K<sup>+</sup> channels (Wilson and Kawaguchi, 1996). Blockade of K<sup>+</sup> channels has profound effects on up-state potentials. Several voltage-gated K<sup>+</sup> channels might contribute to this de-

pendence. Kv4 channels activate in this voltage range, but they inactivate rapidly, making them poor regulators of sustained depolarizing inputs (Tkatch et al., 2000). Kv1.2 channels are also active in this range (Nisenbaum et al., 1994; Shen et al., 2004). However, these channels inactivate as well, albeit more slowly than Kv4 channels. This characteristic makes it unlikely that they are central players in the phenomenon observed *in vivo*, in which the up state can reside at subthreshold potentials for seconds.

Another K<sup>+</sup> channel that is known to regulate subthreshold membrane potential and excitability in several central and peripheral neurons is the KCNQ (Kv7) channel (Brown and Adams, 1980; Marrion, 1997; Wang et al., 1998; Jentsch, 2000; Shapiro et al., 2000). These channels open at subthreshold membrane potentials and do not inactivate. As with other members of the Kv class, these channels are multimeric (Jentsch, 2000; Hadley et al., 2003). Four known subunits contribute to KCNQ channels found in the brain (KCNQ2–5) (Jentsch, 2000). *In situ* hybridization (Saganich et al., 2001) and immunocytochemical (Cooper et al., 2001) studies have shown that KCNQ subunits are expressed in the striatum. However, it is unclear from these studies whether they are expressed by medium spiny neurons, because the most prominent labeling appears to be of large interneurons.

Another feature of KCNQ channels that makes them attractive candidates for controlling up-state silencing is their susceptibility to neuromodulation. They were originally called “M-

Received April 8, 2005; revised July 1, 2005; accepted July 1, 2005.

This work was supported by National Institutes of Health Grants NS 34696 (D.J.S.) and NS 26920 (N.M.N.). We thank Dr. Savio Chan for his participation in the initial phase of the experiments and Dr. Tatiana Tkatch, Sasha Ulrich, and Yu Chen for their excellent technical support.

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DOI:10.1523/JNEUROSCI.1381-05.2005

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channels” because of their suppression by muscarinic receptor signaling (Brown and Adams, 1980; Adams and Brown, 1982; Jones, 1985). One of the most prominent modulators of medium spiny neurons is acetylcholine (Bolam et al., 1984; Kawaguchi, 1993). All medium spiny neurons express high levels of the  $M_1$  muscarinic receptor, the receptor known to modulate KCNQ channels in other cell types (Hersch et al., 1994; Yan et al., 2001). Could cholinergic interneurons toggle medium spiny neurons between spiking and silent up states by regulating KCNQ channel opening? The data presented below are consistent with this hypothesis, showing that medium spiny neurons express functional KCNQ channels that regulate spiking at up-state potentials and that these channels are potently modulated by  $M_1$  receptor activation.

## Materials and Methods

**Slice preparation.** All experiments were conducted in accord with the guidelines approved by the Northwestern University Animal Care and Use Committee. Standard techniques were used for the preparation of slices for recording (Shen et al., 2004). Briefly, Sprague Dawley rats of either sex, 16–23 d of age, and  $M_1$  muscarinic receptor knock-out mice (Hamilton et al., 1997) were anesthetized deeply with ketamine–xylazine and perfused transcardially with 5–10 ml of ice-cold artificial CSF (ACSF) comprising the following (in mM): 125 NaCl, 2.5 KCl, 1.25  $\text{NaH}_2\text{PO}_4$ , 2.0  $\text{CaCl}_2$ , 1.0  $\text{MgCl}_2$ , 25  $\text{NaHCO}_3$ , and 14 glucose, bubbled continuously with carbogen (95%  $\text{O}_2$  and 5%  $\text{CO}_2$ ). The brain was quickly removed, blocked in either coronal or parasagittal plane, glued to the stage of a VT1000S slicer (Leica, Nussloch, Germany), and immersed in the ice-cold ACSF. Sections through the striatum were cut at a thickness of 275–300 nm and then transferred to a holding chamber, where they were completely submerged in ACSF and maintained at 35°C for 30–40 min. Slices were then kept in the holding chamber at the room temperature (22°–23°C) for another 20 min before recording.

**Electrophysiological recordings.** Individual slices were transferred to a recording chamber and were perfused continuously (2–3 ml/min) with carbogenated ACSF for the duration of the experiment. A 40× water immersion objective (Olympus, Melville, NY) was used to examine the slice with standard infrared differential interference contrast video microscopy. Experiments were performed at room temperature unless otherwise specified.

Patch pipettes were pulled from thick-walled borosilicate glass (outer diameter, 1.5 mm) on a Sutter P-97 puller (Sutter Instruments, Novato, CA) and fire polished before recording. Pipette resistance was typically 3–4 M $\Omega$  when filled with recording solution. The internal pipette solution contained the following (in mM): 126  $\text{KMeSO}_4$ , 14 KCl, 3  $\text{MgCl}_2$ , 0.5  $\text{CaCl}_2$ , 5 EGTA, 10 HEPES, pH adjusted to 7.25 with NaOH and osmolarity adjusted to 275–280 mOsm/L. Electrical access was achieved through the perforated-patch method using amphotericin B (Rae et al., 1991). The perforated-patch method was used to preserve the intracellular integrity of the neurons, thus maintaining functional KCNQ current and muscarinic responses that would be lost after cell dialysis in these neurons during whole-cell recording. For perforated-patch experiments, a stock solution of amphotericin B (60 mg/ml) in dimethyl sulfoxide was prepared and diluted in the recording solution immediately before use yielding a final concentration of 180  $\mu\text{g/ml}$ . Capacitance current was monitored continuously by applying a 5–10 mV pulse from a holding potential of –70 mV. Series resistance (20–35 M $\Omega$  in voltage-clamp recordings and 40–50 M $\Omega$  in current-clamp recordings) was monitored periodically during experiments. To minimize voltage error in voltage-clamp recordings, series resistance was typically compensated by 60–70%. Given small amplitude of the KCNQ currents, this should have produced an error of ~1–2 mV. If series resistance changed >15% during recording, the data were discarded. The membrane potentials were corrected routinely for a –7 mV liquid junction potential (Neher, 1992).

**Data analysis.** Data acquisition used Clampex 8.2 software in combination with a MultiClamp 700A amplifier (Molecular Devices, Union

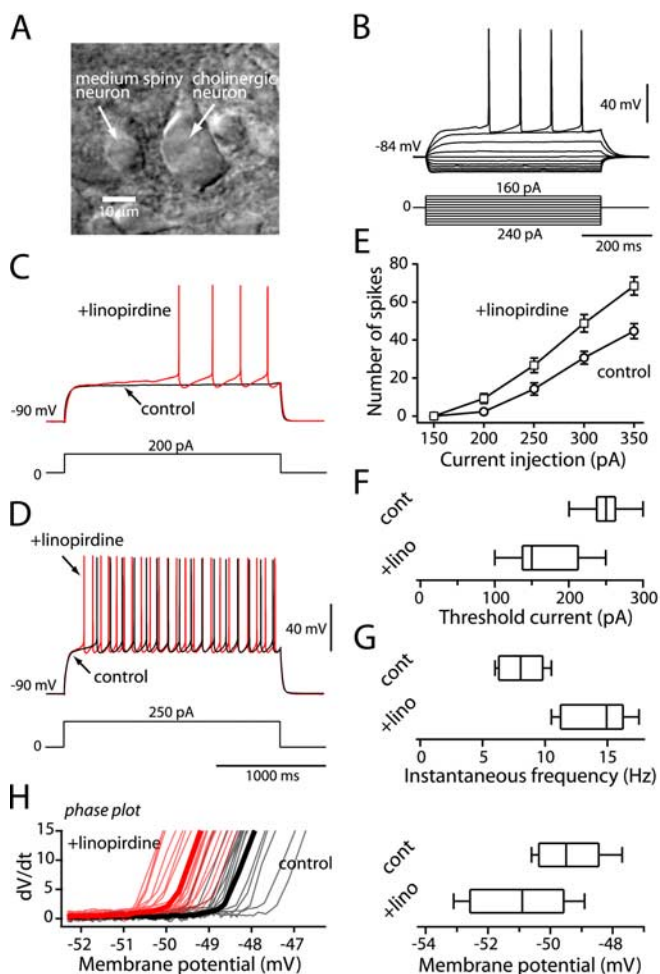
City, CA). All data were analyzed with Igor Pro 5 (WaveMetrics, Lake Oswego, OR) and Clampfit 8.2. Statistical analyses were performed with SigmaStat 3.0 (SPSS, Chicago, IL). Central tendency of numerical data were estimated by computing means  $\pm$  SEM for samples  $\geq 10$  or median and range for smaller samples. Box plots were used for graphic presentation of the data with the small sample size. Differences between small samples were analyzed with nonparametric tests.

**Single-cell reverse transcriptase-PCR analysis.** Dissociated, individual medium spiny neurons were patched in the cell-attached mode and then aspirated into an electrode. Electrodes contained 1–2  $\mu\text{l}$  of diethyl pyrocarbonate (DEPC)-treated water and 1.5 U/ml SUPERase-In (Ambion, Austin, TX). The glass used for making electrodes was heated to 200°C for 2–3 h before use. Sterile gloves were worn at all times during the procedure to minimize RNase contamination. After aspiration of the neuron, the electrode tip was broken in a 0.6 ml presiliconized tube (Midwest Scientific, Valley Park, MO) containing 1.9  $\mu\text{l}$  of DEPC-treated water, 0.7  $\mu\text{l}$  of SUPERase-In (20 U/ml), 0.7  $\mu\text{l}$  of oligo-dT (50 mM), 0.7  $\mu\text{l}$  of BSA (143 mg/ml), 1.0 ml of dNTPs (10 mM), and the contents were ejected. The mixture was heated to 65°C for 5 min to denature the nucleic acids and then placed on ice for at least 1 min. Single-strand cDNA was synthesized from the cellular mRNA by adding 1.2  $\mu\text{l}$  of SuperScript II reverse transcriptase (RT) (200 U/ml), 1  $\mu\text{l}$  of 10× RT buffer, 2  $\mu\text{l}$  of  $\text{MgCl}_2$  (25 mM), 1  $\mu\text{l}$  of DTT (0.1 M), and 0.5 ml of RNase Out (40 U/ml) and then incubating the mixture at 42°C for 90 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  $\mu\text{l}$  of RNase H (2 U/ml) and incubating at 37°C for 20 min. All reagents except SUPERase-In 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 6.7; Molecular Biology Insights, Cascade, CO). Primers for substance P (SP) and enkephalin (ENK) have been described previously (Surmeier et al., 1996). KCNQ2 (Kv7.2) mRNA (GenBank accession number AF087453) was detected with a pair of primers, 5'-GCAACAGAGCCAGTACCGAGTTC (position 2335) and 5'-CACATTGACCTGAGAGCGA (position 2811). The predicted PCR product length was 496 bp. KCNQ3 (Kv7.3) mRNA (GenBank accession number NM031597) was detected with a pair of primers, 5'-ACCAGGTGCCCATCGAC (position 2529) and 5'-GCCAGTGA CCCCTTCTAAGTG (position 3043). The predicted PCR product length was 535 bp. KCNQ4 (Kv7.4) mRNA (GenBank accession number AF249748) was detected with a pair of primers, 5'-GCTTCCGGGCTCTCTAAGACT (position 159) and 5'-TGGACCCTTATCGCCCTTC (position 484). The predicted PCR product length was 344 bp. KCNQ5 (Kv7.5) mRNA (GenBank accession number AF263836) was detected with a pair of primers, 5'-GTTGCTCCAAGGAAAGTGTC (position 2192) and 5'-ACCGTGACCTCCAGTCCTTAGA (position 2545). The predicted PCR product length was 376 bp. A two-round protocol was used for more efficient amplification of single-cell cDNA. This was done by implementing 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and elongation at 72°C for 70 s, followed by elongation for 7 min at 72°C.

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 verify that genomic DNA did not contribute to the PCR products, neurons were aspirated and processed in the normal manner, except that the reverse transcriptase was omitted. Contamination from extraneous sources was checked by replacing the cellular template with buffer solution. Both controls were consistently negative in these experiments.

**Chemicals and drugs.** Reagents were obtained as follow: linopirdine and 10,10-bis(4-pyridinylmethyl)-9(10H)anthracenone dihydrochloride (XE991; Sigma, St. Louis, MO, or Tocris Cookson, Ellisville, MO); (RS)-3,5-dihydroxyphenylglycine (DHPG), 7-(hydroxy-imino) cyclopropa[b]chromen-1 $\alpha$ -carboxylate ethyl ester (CPCCOEt), and 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Tocris Cookson); muscarinic toxin 7



**Figure 1.** Blockade of KCNQ channels increased evoked activity. **A**, Photomicrograph of a medium spiny neuron and a large neuron resembling a cholinergic interneuron. **B**, Typical membrane responses to somatic current injection (shown below). Note the strong inward rectification and delayed firing. **C**, The response of a medium spiny neuron to a near rheobase current step (200 pA, 2 s) before and after application of linopirdine (10  $\mu$ M). Linopirdine increased the rate of rise of the membrane potential toward spike threshold (red line). **D**, The response to a suprathreshold current step (250 pA, 2 s) before and after application of linopirdine. Linopirdine increased firing frequency (red line). **E**, Average number of spikes evoked to the values of the current injected. Linopirdine reduced the amount of current required to generate a given number of spikes. Error bars represent SEM. Box plots illustrate the difference in rheobase current required for action potential firing (**F**) and the difference in instantaneous frequency change to a current step (250 pA) (**G**). **H**, The phase plane analysis in **D** shows that spike threshold is lowered in linopirdine (red lines). A box plot summary showing reduction in spike threshold in linopirdine is displayed. cont, Control; lino, linopirdine.

(MT 7; Peptides International, Louisville, KY); and calphostin C, thapsigargin, and wortmannin (Calbiochem, La Jolla, CA). Tetraethylammonium (TEA), 4-aminopyridine (4-AP), mecamylamine, and all other chemicals were obtained from Sigma. When TEA was applied at concentrations  $\geq 1$  mM, the osmolality was adjusted by reducing the concentration of NaCl in ACSF.

## Results

### Blockade of KCNQ channels increases excitability at up-state potentials

Perforated-patch recordings were obtained from medium-sized neurons (somal diameter, 10–15  $\mu$ m). These neurons were distinguished readily from large cholinergic interneurons by their size, as illustrated in Figure 1A. To distinguish medium spiny neurons from other interneurons (Kawaguchi, 1993), electrophysiological criteria were used (Fig. 1B). Recordings were made

only from neurons having (1) a relatively hyperpolarized resting potential ( $-88.4 \pm 1.1$  mV;  $n = 27$ ); (2) strong inward rectification; and (3) a slow voltage ramp to near rheobase current injection. They were classified as medium spiny neurons (Kerr and Plenz, 2002; Shen et al., 2004; Wilson, 2004).

As a first step toward testing the hypothesis that KCNQ channels modulated the subthreshold excitability of medium spiny neurons, the effect of linopirdine, a selective KCNQ channel blocker, on the response to 2 s current steps was examined (Fig. 1C,D, bottom). Linopirdine (10  $\mu$ M) had no effect on the resting membrane potential ( $-89.5 \pm 1.0$  mV in control;  $-88.5 \pm 0.8$  mV in linopirdine;  $n = 6$ ;  $p > 0.05$ ; Wilcoxon signed rank test). However, near rheobase, linopirdine accelerated the rate of rise of the characteristic slow voltage ramp (Fig. 1C). The median rheobase current was reduced by nearly 100 pA by linopirdine ( $n = 6$ ;  $p < 0.05$ ; Wilcoxon) (Fig. 1F). With larger current steps in which repetitive spiking was evoked, linopirdine increased the number of spikes evoked (Fig. 1D), shifting the frequency–current relationship to the left (Fig. 1E). Using a 250 pA step, the median discharge frequency increased from  $\sim 8$  to  $\sim 15$  Hz in the presence of linopirdine ( $p < 0.05$ ; Wilcoxon) (Fig. 1G). Phase plane analysis of spiking revealed that linopirdine lowered spike threshold, defined by the discontinuity in the relationship between  $dV/dt$  and  $V$  (Fig. 1H) without significantly affecting the maximum  $dV/dt$ . The median change in spike threshold was 1.4 mV (median,  $-49.5$  mV in control,  $-50.9$  mV in linopirdine;  $p < 0.05$ ; Wilcoxon) (Fig. 1H). XE991, another selective KCNQ channel blocker, had similar effects on the excitability of medium spiny neurons ( $n = 2$ ; data not shown).

In whole-cell recordings (as opposed to perforated-patch recordings), linopirdine-sensitive currents were lost in the first few minutes (data not shown). As a consequence, all of our subsequent experiments used perforated-patch recording.

### Medium spiny neurons express a slow, noninactivating KCNQ channel currents

Somatic point clamp experiments were performed to generate a biophysical description of the gating properties of the putative KCNQ channel currents. In an attempt to isolate the KCNQ channel currents from other voltage-gated  $K^+$  currents, the membrane potential was held at a relatively depolarized potential ( $V_H$ ,  $-30$  mV) to activate KCNQ channels (Brown and Adams, 1980; Adams and Brown, 1982) and to inactivate many of the other  $K^+$  channels that activate in this membrane potential region (Nisenbaum et al., 1996; Tkatch et al., 2000; Shen et al., 2004). The membrane potential was then stepped down to more hyperpolarized potentials for 4 s to deactivate the KCNQ channels (Fig. 2A, top). The inward tail currents decayed biexponentially (Fig. 2A, D). Bath application of XE991 (20  $\mu$ M) reduced the holding current and blocked the deactivating current (Fig. 2A). The calculation of difference currents by subtracting the XE991-resistant currents from control currents yielded records expected of KCNQ channels (Fig. 2A, bottom). Stepping back to  $-30$  mV led to the reactivation of the KCNQ channels and the generation of an outward current.

To measure the activation threshold and activation time constant of the KCNQ current, standard activation protocol was applied by stepping to depolarized potentials from a holding potential of  $-90$  mV (Fig. 2B). Again, subtraction of XE991-resistant currents from control currents yielded KCNQ difference currents. The voltage dependence of activation and deactivation was assessed by converting the current amplitudes to chord conductances (Fig. 2C). Data points were then fitted

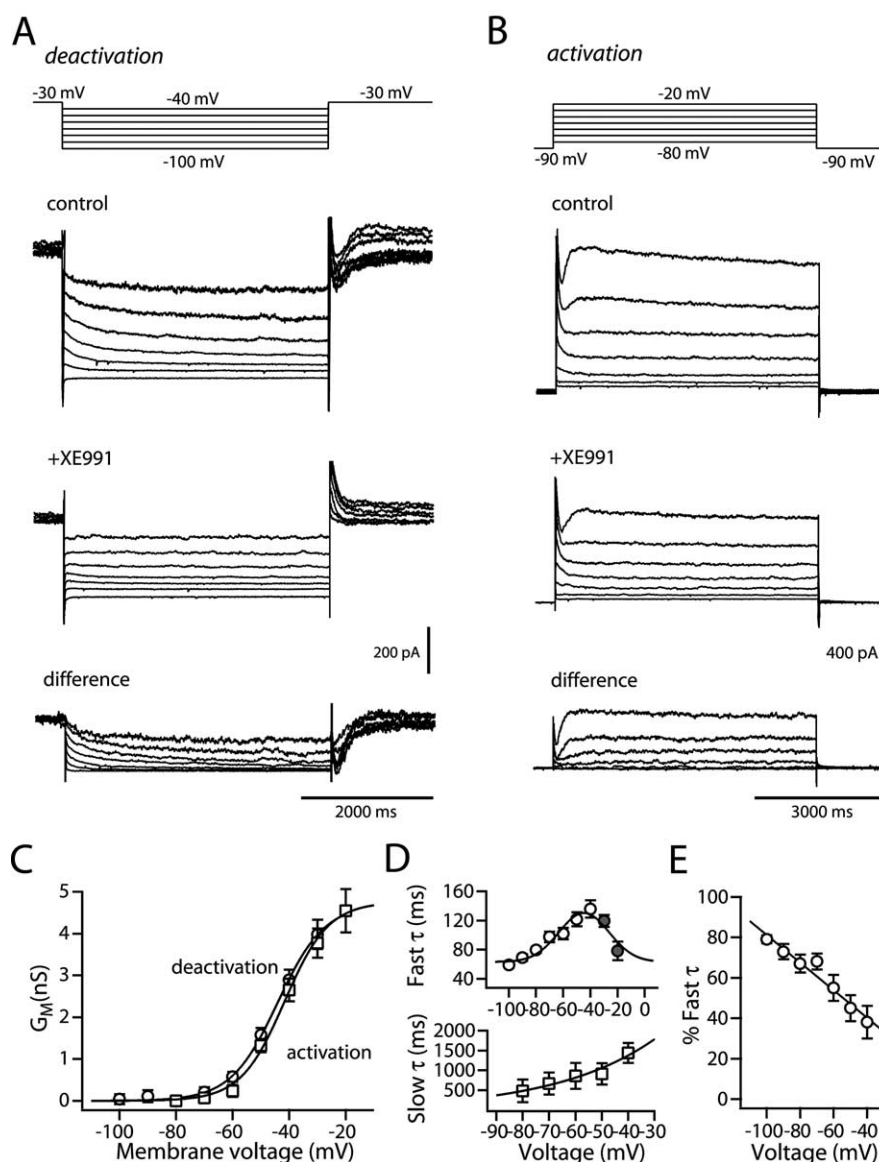
with a first-order Boltzmann function of the following form:  $G_M = G_{\max}/(1 + \exp((V - V_{1/2})/k))$ , where  $V_{1/2}$  is the half-deactivation or activation voltage, and  $k$  is the slope factor. KCNQ channel gating measured using the deactivation or the activation protocol yielded the same results: average half-deactivation voltage was  $-43.3 \pm 1.0$  mV ( $n = 7$ ), and the slope factor was  $-8.1 \pm 0.4$  mV; the average half-activation voltage was  $-41.6 \pm 0.8$  mV ( $n = 7$ ), and the slope factor was  $-7.9 \pm 0.6$  mV ( $V_{1/2}$ ,  $p > 0.05$ , Mann–Whitney rank-sum test;  $k$ ,  $p > 0.05$ , Mann–Whitney).

Both deactivation and activation kinetics were best fit with two exponentials over a range of potentials (Fig. 2*D,E*). The time constants in medium spiny neurons were close to those found in rat sympathetic neurons and those reported for heterologously expressed KCNQ2/KCNQ3 heteromeric channels (Hadley et al., 2000; Shapiro et al., 2000; Pan et al., 2001). These data were pooled and then fit with a two-state kinetic model plotted as a solid line in Figure 2*D*. Deactivation accelerated with increasing hyperpolarization; both  $\tau_{\text{fast}}$  and  $\tau_{\text{slow}}$  shortened, with  $\tau_{\text{fast}}$  falling  $e$ -fold for a 52.6 mV hyperpolarization (Fig. 2*D*).

### KCNQ channels of medium spiny neurons are comprised of KCNQ2/3 subunits

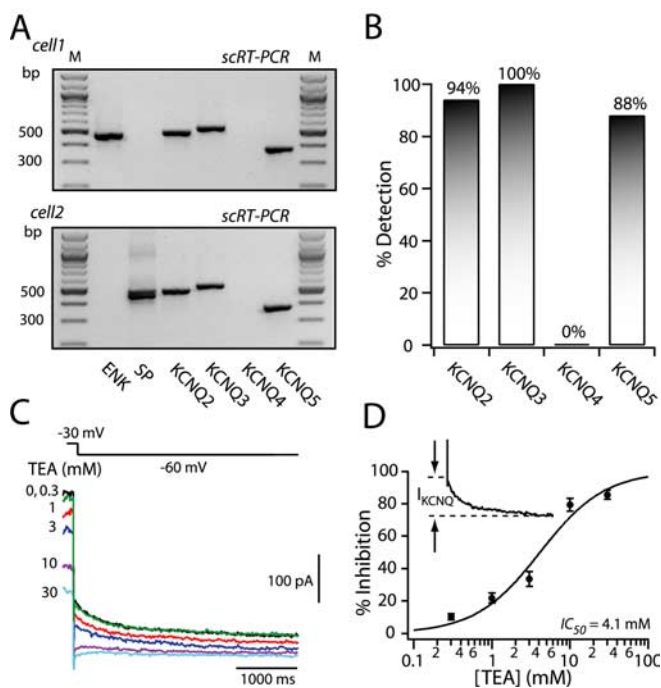
The biophysical and pharmacological data presented thus far suggest that medium spiny neurons express KCNQ channels. Previous studies have shown KCNQ transcripts are present in the striatum (Saganich et al., 2001), an observation confirmed by our tissue level RT-PCR analysis (data not shown). However, localization of KCNQ transcripts to medium spiny neurons is less clear. To examine directly cellular localization of KCNQ mRNAs, single-cell RT-PCR (scRT-PCR) profiling was performed on 16 medium spiny neurons identified by their expression of ENK or SP mRNA. Neurons were profiled for their expression of KCNQ2, KCNQ3, KCNQ4, and KCNQ5 mRNAs. KCNQ2, KCNQ3, and KCNQ5 mRNAs were consistently detected in both SP- and ENK-expressing medium spiny neurons (Fig. 3*A,B*). KCNQ4 mRNA was not detected in any cell ( $n = 16$ ). There was no correlation between SP or ENK detection and the detection of KCNQ mRNAs.

Although KCNQ subunits are capable of forming homomeric channels in heterologous expression systems, in native systems, KCNQ3 subunits are thought to form heteromeric channels with KCNQ2, 4, or 5 subunits (Jentsch, 2000). For example, KCNQ channels in sympathetic neurons are thought to be heteromers of KCNQ2 and KCNQ3 subunits (Wang et al., 1998; Shapiro et al.,



**Figure 2.** Striatal medium spiny neurons express a slow, noninactivating  $K^+$  channel current. **A**, Representative deactivation currents recorded from a medium spiny neuron before and after application of XE991. The cell was held at  $-30$  mV to generate sustained outward currents. The channels were then deactivated by 4 s hyperpolarizing steps in increments of  $-10$  mV (shown above). Subtraction of current traces obtained in the presence of XE991 ( $20 \mu\text{M}$ ) from in control gives rise to the difference currents in the bottom panel of **A** is shown. **B**, Representative activation currents recorded from another medium spiny neuron before and after application of XE991. The cell was held at  $-90$  mV and then activated by 4 s depolarizing steps in increments of  $-10$  mV (shown above). Subtraction of current traces obtained in the presence of XE991 ( $20 \mu\text{M}$ ) from control yielded the difference currents in the bottom panel of **B**. **C**, Conductance–voltage relationship.  $G_M$  was determined from the amplitude of the current relaxations during the hyperpolarizing and depolarizing steps. Data points were fit with the following equation:  $G_M = G_{\max}/(1 + \exp((V - V_{1/2})/k))$ , where  $V_{1/2} = -43.3$  mV,  $k = -8.1$  mV for deactivation and  $V_{1/2} = -41.6$  mV,  $k = -7.9$  mV for activation. **D**, Plots of the voltage dependence of fast and slow time constants. Data points were fit with a Boltzmann equation. **E**, The percentage of the current that was fast, plotted as a function of voltage. Data were fit to a simple linear equation. Error bars represent SEM.

2000; Selyanko et al., 2001). Immunoprecipitation studies have shown that in many regions of the brain, KCNQ3 subunits associate with either KCNQ2 (Cooper et al., 2000; Yus-Najera et al., 2003; Devaux et al., 2004) or KCNQ5 (Yus-Najera et al., 2003) subunits. KCNQ2 and KCNQ5 subunits do not appear to associate (Yus-Najera et al., 2003). One way of distinguishing KCNQ channels with differing subunit composition is to determine their sensitivity to TEA (Hadley et al., 2000; Hadley et al., 2003). KCNQ2 homomeric channels have a very high affinity for TEA,

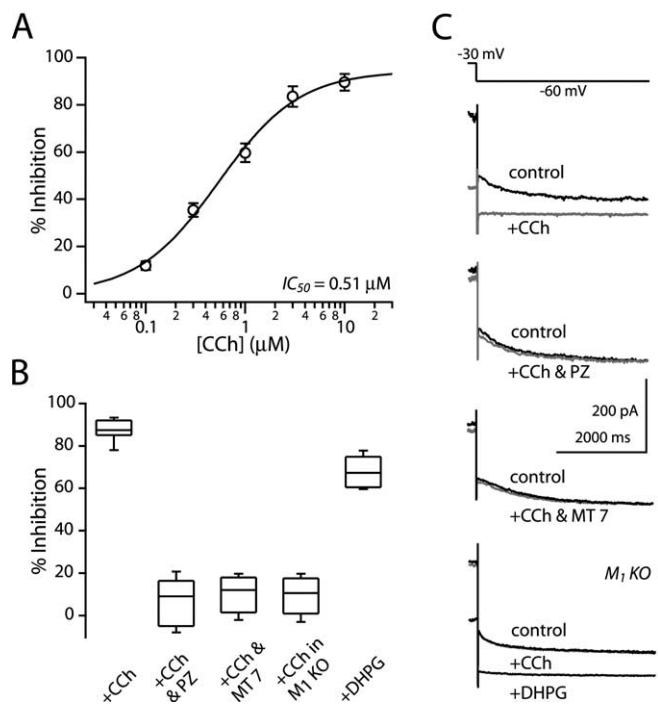


**Figure 3.** Medium spiny neurons express functional KCNQ2/KCNQ3 channel subunits. *A*, scRT-PCR from an ENK-expressing neuron (top) and an SP-expressing neuron (bottom). M, Marker. *B*, In a sample of medium spiny neurons ( $n = 16$ ), 94% had detectable levels of KCNQ2 mRNA, 100% had detectable levels of KCNQ3 mRNA, and 88% had KCNQ5 mRNA, with none of the cells having detectable levels of KCNQ4 mRNA. *C*, KCNQ currents recorded by stepping membrane voltage from  $-30$  to  $-60$  mV (shown above) for 4 s in the absence and presence of increasing concentrations of TEA (0.3–30 mM; different-colored lines). *D*, The amplitude of the KCNQ channel current was measured as the difference between the instantaneous current at the onset of hyperpolarization and the steady-state current at the end of voltage command, as shown in the inset. The percentage of inhibition was plotted as a function of TEA concentration. The data were fit with a logistic function:  $IC_{50} = 4.1$  mM and slope factor = 1.07. Error bars represent SEM.

whereas the affinity of KCNQ2/KCNQ3 heteromeric channels is  $\sim 10$ -fold lower because KCNQ3 channels are insensitive to TEA. KCNQ3/KCNQ5 heteromeric channels are blocked poorly by TEA (Schroeder et al., 2000). In medium spiny neurons, KCNQ channel tail currents evoked by stepping to  $-60$  mV from a holding potential of  $-30$  mV had an intermediate sensitivity to TEA (Fig. 3*C,D*). The dose–response relationship could be well fit by a logistic equation of the following form:  $Y = ((Y_0 - Y_\infty)/(1 + (C \times IC_{50}^{-1})^B)) + Y_\infty$ , where  $Y_0$  is the amplitude in the absence of TEA,  $Y_\infty$  is the maximal response to TEA,  $B$  is the slope factor, and  $C$  is the concentration of TEA. The  $IC_{50}$  was  $4.1 \pm 0.5$  mM, and the slope factor was  $0.95 \pm 0.09$  ( $n = 7$ ). This value is close to that reported for KCNQ2/KCNQ3 heteromeric channels (Wang et al., 1998; Hadley et al., 2000, 2003; Shah et al., 2002).

#### M<sub>1</sub> muscarinic receptor mediates suppression of KCNQ channel current in medium spiny neurons

KCNQ channel opening is reduced by activation of M<sub>1</sub>-class receptors (Brown and Adams, 1980; Marrion, 1997; Wang et al., 1998; Shapiro et al., 2000; Robbins, 2001). Both striatonigral and striatopallidal medium spiny neurons express M<sub>1</sub>-class receptors (Yan et al., 2001). As expected, application of the cholinergic agonist carbamylcholine chloride (carbachol) in the presence of the nicotinic receptor antagonist mecamylamine (10  $\mu$ M) reduced KCNQ channel currents (Fig. 4*A*). The carbachol dose–response relationship could be fitted by a logistic equation with



**Figure 4.** M<sub>1</sub> muscarinic receptor mediates the modulation of KCNQ channel currents. *A*, Dose–response relationship of the carbachol modulation of KCNQ channel current. The fitting parameters were  $IC_{50} = 0.51$   $\mu$ M and slope factor = 1.07. Error bars represent SEM. *B*, The box plot summary illustrates the M<sub>1</sub> receptor-mediated modulation. Carbachol (10  $\mu$ M) blocked 86% of the KCNQ current ( $n = 6$ ). The blockade was reversed in the presence of pirenzepine (100 nM;  $n = 4$ ) and MT 7 (100 nM;  $n = 5$ ) and was lost in M<sub>1</sub> muscarinic receptor knock-out mice (M<sub>1</sub> KO;  $n = 5$ ). The KCNQ current recorded in M<sub>1</sub> KO mice was reduced by the group 1 metabotropic glutamate agonist DHPG (50  $\mu$ M;  $n = 5$ ). *C*, Typical KCNQ current traces and their responsiveness to M<sub>1</sub> agonist and antagonists and DHPG. CCh, Carbachol; PZ, pirenzepine.

$IC_{50}$  of  $0.52 \pm 0.14$   $\mu$ M and slope factor of  $1.06 \pm 0.12$  ( $n = 5$ ). In agreement with the attribution of the modulation to M<sub>1</sub>-class receptors, pirenzepine (100 nM) blocked the effects of carbachol (10  $\mu$ M;  $n = 4$ ) (Fig. 4*B,C*).

There are three M<sub>1</sub>-class receptors (M<sub>1</sub>, M<sub>3</sub>, M<sub>5</sub>) that are expressed by medium spiny neurons, with the M<sub>1</sub> receptor being the predominant subtype (Hersch et al., 1994; Yan et al., 2001; Zhang et al., 2002). All three receptors are coupled to phospholipase C $\beta$  (PLC $\beta$ ) through G<sub>q</sub>-proteins, and all three have been implicated in the modulation of KCNQ currents (Robbins et al., 1991; Guo and Schofield, 2003). In sympathetic ganglion neurons, M<sub>1</sub> receptors have been firmly established as mediating the cholinergic modulation of KCNQ channels (Hamilton et al., 1997; Robbins, 2001). However, in central neurons, the situation is less clear. Several recent studies showed that muscarinic modulation of KCNQ channel currents is not altered in neurons from M<sub>1</sub> receptor knock-out mice (Rouse et al., 2000; Fisahn et al., 2002) or by an M<sub>1</sub>-specific toxin (Rouse et al., 2000).

To determine whether M<sub>1</sub> muscarinic receptors mediate the effects of carbachol on KCNQ channels in striatal medium spiny neurons, two approaches were taken. First, MT 7, a highly selective, irreversible antagonist of the M<sub>1</sub> receptor, was used (Max et al., 1993). Bath perfusion of the toxin (100 nM) for 40–50 min completely abolished the effects of carbachol on KCNQ channel currents (Fig. 4*B,C*). Second, medium spiny neurons from M<sub>1</sub> receptor knock-out mice were examined for their responsiveness to carbachol. Consistent with the result from the toxin treatment, KCNQ channel currents in medium spiny neurons from M<sub>1</sub> knock-out mice ( $n = 5$ ) were completely insensitive to carbachol

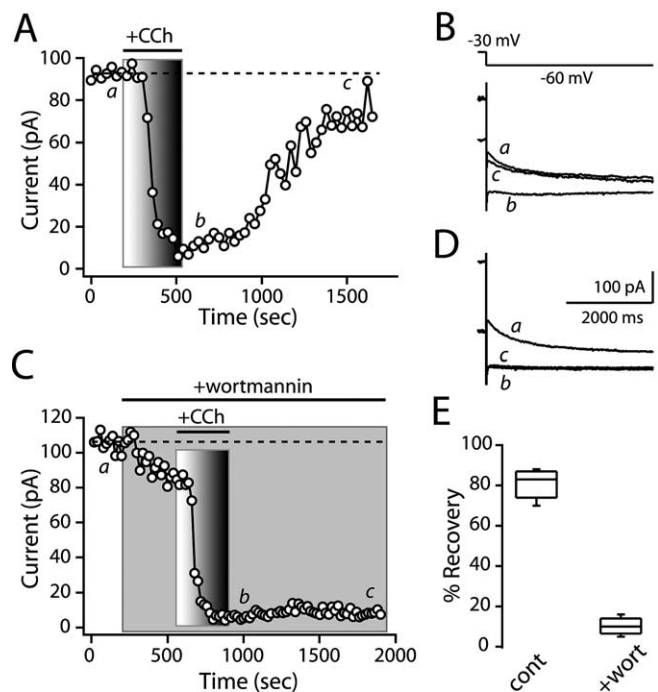
( $n = 6$ ) (Fig. 4*B,C*). The failure of carbachol to alter currents was not caused by a defect in the KCNQ channel itself or PLC $\beta$  signaling, because the group 1 metabotropic glutamate receptor agonist DHPG (50  $\mu\text{M}$ ) potently reduced KCNQ channel currents in neurons from the  $M_1$  receptor knock-out ( $n = 5$ ) (Fig. 4*B,C*), as it did in wild-type neurons ( $n = 3$ ; data not shown). The absence of an effect of carbachol was not species dependent either because carbachol potently reduced KCNQ channel currents in wild-type mice (median reduction, 81%;  $n = 5$ ; data not shown). These results argue that muscarinic receptor mediating the reduction in KCNQ channel currents in medium spiny neurons is the  $M_1$  receptor.

### Phosphatidylinositol 4,5-bisphosphate is required for KCNQ channel gating

The signaling cascade mediating muscarinic receptor modulation of KCNQ channels is controversial (Marrion, 1997; Brown and Yu, 2000; Ikeda and Kammermeier, 2002; Shapiro, 2004). Recent studies have suggested that PLC-dependent depletion of a membrane lipid, phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), results in decreased KCNQ channel opening with membrane depolarization (Suh and Hille, 2002, 2005; Ford et al., 2003; Zhang et al., 2003; Suh et al., 2004; Winks et al., 2005). One of the strongest pieces of evidence for the hypothesis that PIP<sub>2</sub> is an allosteric regulator of KCNQ channel gating is that inhibition of the enzyme that maintains membrane PIP<sub>2</sub> levels [phosphatidylinositol 4 kinase (PI4K)] dramatically slows recovery from G-protein-coupled receptor modulation (Suh and Hille, 2002; Zhang et al., 2003; Winks et al., 2005). To determine whether the  $M_1$  receptor modulation of KCNQ channels in medium spiny neurons had the same PIP<sub>2</sub> dependence, the PI4K inhibitor wortmannin was applied after establishment of the KCNQ modulation. Normally, KCNQ channel currents recovered nearly completely after carbachol application (recovery to  $81\% \pm 3.2\%$  of control amplitudes;  $n = 5$ ) (Fig. 5*A,B*). However, application of wortmannin (50  $\mu\text{M}$ ) significantly reduced recovery of the KCNQ channel currents after termination of carbachol exposure (recovery to  $10\% \pm 1.8\%$  of control amplitudes;  $n = 5$ ;  $p < 0.05$ ; Mann–Whitney) (Fig. 5*C,D*).

The products of PLC $\beta$  metabolism of membrane PIP<sub>2</sub> are diacylglycerol (DAG) and inositol trisphosphate (IP<sub>3</sub>). DAG is a potent activator of protein kinase C (PKC) isoforms. Attempts to determine whether PKC affects KCNQ channels have yielded seemingly contradictory results. Namely, KCNQ channels appear to be responsive to PKC activators but resistant to PKC inhibitors (Marrion, 1997; Brown and Yu, 2000). Recent work reconciles these discrepant results by showing that inhibitors interacting with the PKC DAG binding site attenuate KCNQ channel modulation, but those that interact with the PKC catalytic domain are less effective because this site may be shielded by scaffolding proteins (Hoshi et al., 2003). In agreement with this view, calphostin C (1  $\mu\text{M}$ ), a PKC inhibitor acting on the DAG binding site (Kobayashi et al., 1989), reduced the effect of low concentrations of carbachol on KCNQ channel currents (Fig. 6*A,B*). However, higher concentrations of carbachol were equally effective in the presence of calphostin C. More complete analysis of this result revealed that calphostin C reduced the efficacy of carbachol in modulating KCNQ channels, approximately tripling the IC<sub>50</sub> (Fig. 6*C,D*). These results suggest that PKC activation acts cooperatively with PIP<sub>2</sub> depletion to reduce KCNQ channel opening.

The other leg of the PLC $\beta$  signaling cascade involves IP<sub>3</sub> liberation and release of Ca<sup>2+</sup> from intracellular stores. Several studies have implicated intracellular Ca<sup>2+</sup> in KCNQ regulation

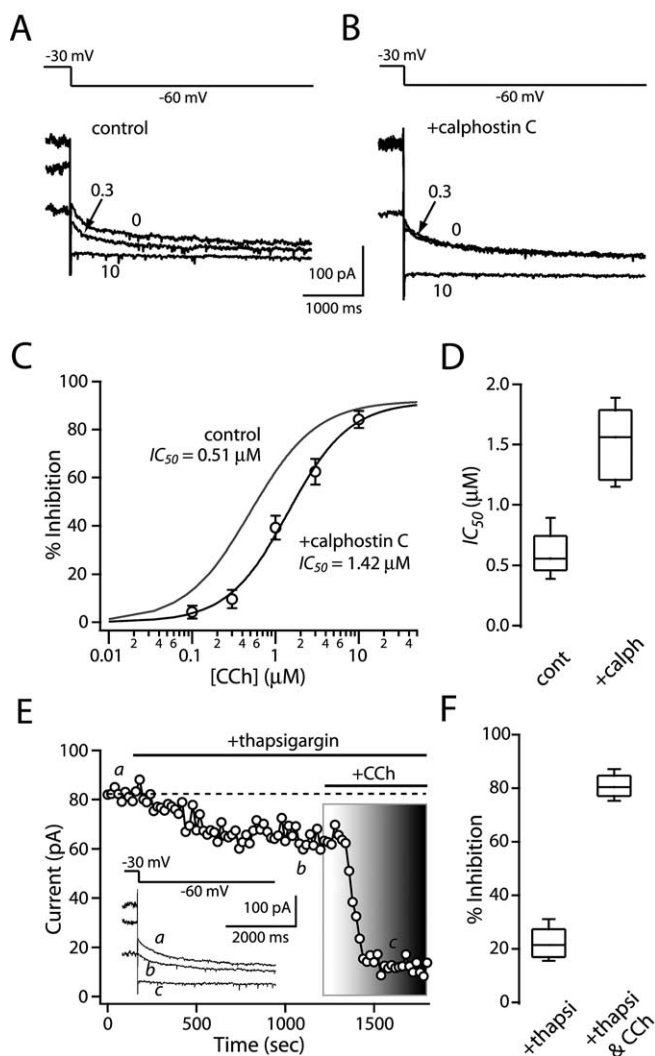


**Figure 5.** Recovery from  $M_1$  muscarinic inhibition requires PIP<sub>2</sub> synthesis. **A, C**, Plots of inhibition and recovery of KCNQ currents elicited by steps from a holding potential of  $-30$  mV. **A**, Carbachol (3  $\mu\text{M}$ ) modulation and recovery in the standard external ACSF solution. **B**, Representative current traces from **A**. **C**, Application of wortmannin (50  $\mu\text{M}$ ) prevented reversal of the CCh (3  $\mu\text{M}$ ) modulation. **D**, Current traces used to construct **C**. **E**, Box plot summary of the percentage of recovery from muscarinic inhibition (control, 81%,  $n = 5$ ; wortmannin, 10.2%,  $n = 5$ ;  $p < 0.05$ ; Mann–Whitney). CCh, Carbachol; cont, control; wort, wortmannin.

(Delmas et al., 2002; Wen and Levitan, 2002; Gamper and Shapiro, 2003). The open probability of KCNQ channel appears to be enhanced by modest elevations in cytosolic Ca<sup>2+</sup> concentration (50–150 nM) and inhibited by greater elevations (>200 nM) (Marrion et al., 1991; Yu et al., 1994; Selyanko and Brown, 1996; Gamper and Shapiro, 2003). To determine whether intracellular Ca<sup>2+</sup> release was a necessary component of the signaling cascade mediating  $M_1$  receptor reductions in KCNQ channel currents, these stores were depleted with Ca<sup>2+</sup>-ATPase inhibitor thapsigargin (2  $\mu\text{M}$ ) before application of carbachol. Bath application of thapsigargin alone reduced KCNQ tail currents by  $\sim 20\%$  ( $n = 5$ ) (Fig. 6*E,F*). Although intracellular Ca<sup>2+</sup> levels were not directly measured, inhibition of the Ca<sup>2+</sup>-ATPase has been shown to elevate these levels significantly (Cruzblanca et al., 1998; del Rio et al., 1999), consistent with the proposition that high free-Ca<sup>2+</sup> levels reduce KCNQ opening. However, subsequent application of carbachol still resulted in a profound reduction in KCNQ channel currents (Fig. 6*E,F*), arguing that intracellular Ca<sup>2+</sup> release was not necessary for the  $M_1$  receptor effects.

### Activation of striatal cholinergic interneurons suppresses the KCNQ current

The data presented thus far show that KCNQ channel currents in medium spiny neurons are reduced by  $M_1$  muscarinic receptor activation. Activation of these receptors is dependent on acetylcholine release by large aspiny interneurons in the striatum. These interneurons are autonomously active but modulate their spiking rate in response to synaptic input (Bennett and Wilson, 1998). To determine whether modest enhancement of interneuron acetylcholine release would recapitulate the modulation seen with exogenous agonist application, 4-AP (100  $\mu\text{M}$ ) was bath



**Figure 6.** PKC activation increases  $M_1$  receptor efficacy. **A, B**, Representative current traces in the absence (control) and presence of the PKC inhibitor calphostin C ( $1 \mu\text{M}$ ) during application of carbachol at the indicated concentrations (micromolar). **C**, Dose–response relationship in control (gray line from Fig. 4A) and in calphostin C. PKC inhibition shifts the relationship to the right, without changing the maximum modulation. Error bars represent SEM. **D**, The box plot summary illustrates that PKC inhibitors increases  $IC_{50}$  of carbachol inhibition ( $n = 5$ ;  $p < 0.05$ ; Mann–Whitney). **E**, Bath application of thapsigargin ( $2 \mu\text{M}$ ) reduced ( $\sim 20\%$ ) KCNQ currents (inset) but did not disrupt the CCh ( $3 \mu\text{M}$ )-mediated modulation (80%). **F**, Box plot summary from a sample of five neurons. CCh, Carbachol; cont, control; calph, calphostin C; thapsi, thapsigargin.

applied. At this concentration, 4-AP modestly accelerates interneuron discharge rates (Fig. 7B,E). Perforated-patch recording from a medium spiny neuron in the vicinity of an interneuron showing elevated discharge rates revealed a robust 4-AP associated reduction in KCNQ channel currents (Fig. 7C,D). KCNQ currents were reduced by 4-AP over 40% on average (Fig. 7C,D). These recordings were made in the presence of ionotropic glutamate and GABA receptor antagonists [ $20 \mu\text{M}$  CNQX,  $20\text{--}40 \mu\text{M}$  D-AP-5, and  $10 \mu\text{M}$  bicuculline methiodide or  $10 \mu\text{M}$  SR95531 [6-imino-3-(4-methoxyphenyl)-1(6H)-pyridazinebutanoic acid] as well as group 1 metabotropic glutamate receptor antagonists ( $100 \mu\text{M}$  CPCCOEt and  $10 \mu\text{M}$  MPEP), eliminating potentially confounding changes in these transmitters. To verify that the modulation was mediated by acetylcholine release, scopolamine ( $10 \mu\text{M}$ ) was coapplied with 4-AP. As expected, the KCNQ chan-

nel currents in medium spiny neurons were unchanged by this mixture, despite the fact that interneuron discharge rate increased as a consequence of autoreceptor blockade (Fig. 7C,D).

## Discussion

Our studies show that striatal medium spiny neurons express KCNQ  $K^+$  channels that shape the response to depolarizing currents. These widely expressed but little studied channels activate at subthreshold membrane potentials and do not inactivate, making them suitable mediators of variation in up-state potential and spiking observed *in vivo*. What is more, these channels are modulated potently by acetylcholine released by striatal interneurons. This modulation appears to be mediated primarily by membrane depletion of  $PIP_2$  subsequent to activation of an  $M_1$  muscarinic receptor pathway coupled to  $PLC\beta$  and PKC. This modulatory pathway was engaged not only by exogenous agonist application but also by modest acceleration of the autonomous spiking of cholinergic interneurons themselves, demonstrating the functional relevance of the modulation. If cholinergic tone is not uniform in the striatum, then this linkage provides a viable explanation for the variability in up-state spiking seen within the medium spiny population and within individual neurons *in vivo*. It also establishes a novel mechanism by which event driven modulation in the activity of cholinergic interneurons can shape the spiking of medium spiny neurons in response to cortical and thalamic glutamatergic signals.

### Striatal projection neurons express KCNQ channel mRNA and channels

Neuronal KCNQ channels are multimeric transmembrane proteins constructed from a family of at least four subunits (KCNQ2–5) (Wang et al., 1998; Jentsch, 2000; Robbins, 2001). These subunits are expressed widely in the brain and in the striatum in particular (Cooper et al., 2001; Saganich et al., 2001). Our tissue-level RT-PCR analysis has extended this work, showing robust expression of KCNQ2, 3, 4, and 5 mRNA. However, previous attempts to localize KCNQ mRNA and protein in the striatum have focused attention on cholinergic interneurons, leaving it uncertain as to whether KCNQ channels were expressed by the principal medium spiny neurons. Electrophysiological studies of these neurons have not yielded evidence of their expression either. However, KCNQ channels are very sensitive to disruption of the intracellular milieu, as might occur with conventional electrophysiological approaches (Simmons and Schneider, 1998). In our hands, whole-cell dialysis of medium spiny neurons led to the loss of KCNQ-like currents in minutes. With perforated patches, however, linopirdine-sensitive KCNQ channel currents were very stable. The biophysical properties of these pharmacologically isolated currents were very similar to those of KCNQ channel currents found in other neurons (Pan et al., 2001; Shah et al., 2002; Passmore et al., 2003).

The identification of the linopirdine-sensitive channels as KCNQ channels was supported by scRT-PCR. Both striatopallidal and striatonigral medium spiny neurons had readily detectable levels of KCNQ2, 3, and 5 mRNA but not KCNQ4 mRNA (Jentsch, 2000). Based on TEA sensitivity, currents were primarily attributable to heteromeric KCNQ2/KCNQ3 channels (Jentsch, 2000; Schroeder et al., 2000; Hadley et al., 2003).

### $M_1$ receptor activation reduces KCNQ $K^+$ channel currents through $PIP_2$ –PKC signaling pathway

As in peripheral sympathetic neurons (Marrion, 1997), KCNQ channel currents in medium spiny neurons were suppressed by

activation of  $M_1$  muscarinic receptors. This conclusion is based on (1) the broad expression of  $M_1$  receptors in medium spiny neurons (Yan et al., 2001) and (2) the loss of the muscarinic modulation after genetic deletion of the  $M_1$  receptor or pharmacological blockade with muscarinic toxin 7. This signaling configuration differs from that in hippocampal pyramidal neurons in which the muscarinic modulation was not altered by genetic deletion of the  $M_1$  receptor or by the  $M_1$  receptor-specific toxin (Rouse et al., 2000; Fisahn et al., 2002).

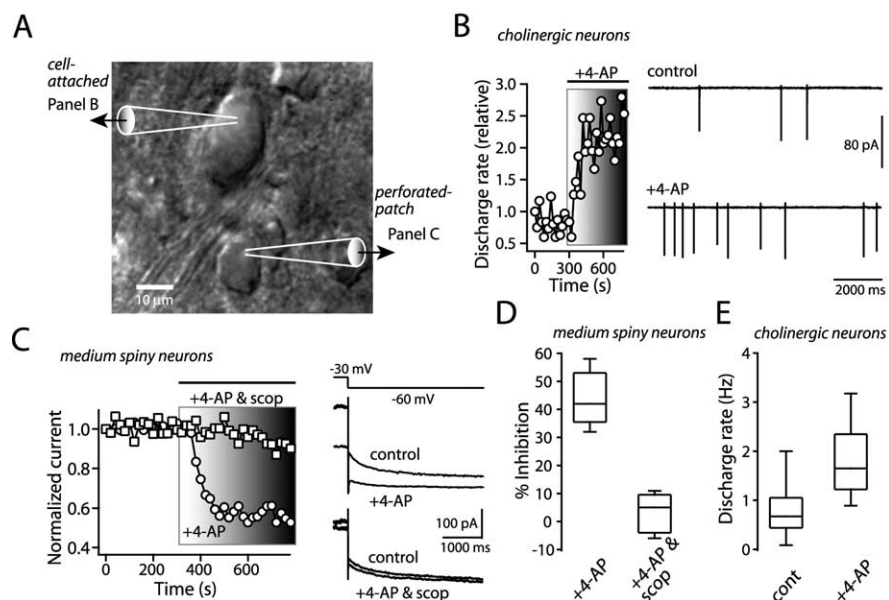
The identity of signaling molecules linking  $M_1$  muscarinic receptors to KCNQ channels has been something of a mystery. Recent work has shown that the recovery of KCNQ channels from muscarinic inhibition was blocked by inhibitors of phosphatidylinositol 4-kinase, suggesting that  $PIP_2$  resynthesis is a necessary step in the recovery process (Suh and Hille, 2002; Ford et al., 2003; Winks et al., 2005). This dependence suggests that  $PIP_2$  binding to the KCNQ subunit is necessary for channel gating (Zhang et al., 2003). Our results show that the  $M_1$  receptor modulation of KCNQ channels in medium spiny neurons has a similar  $PIP_2$  dependence.

In addition to  $PIP_2$  depletion,  $M_1$  receptor activation of PLC leads to the production of DAG and PKC stimulation. Several studies have implicated PKC in the modulation of KCNQ channels (Marrion, 1997; Hoshi et al., 2003). Like recent studies (Hoshi et al., 2003), our results show that PKC activation increases the efficacy of muscarinic agonists without changing the maximal modulation.

### KCNQ channel currents and up states

*In vivo*, medium spiny neurons move between hyperpolarized down states to depolarized up states in response to cortical and thalamic glutamatergic synaptic activity. The up-state event is critical to striatal signaling, because medium spiny neurons lack any autonomous activity. These up-state transitions are of variable duration, sometimes lasting seconds (Wilson and Kawaguchi, 1996; Wickens and Wilson, 1998). Previous work has revealed that  $K^+$  channels are critical determinants of the up-state membrane potential (Wilson and Kawaguchi, 1996). The ability of KCNQ channels to open and not inactivate in the membrane potential range of the up state perfectly suits them for this role. Our work shows that the linopirdine-sensitive KCNQ channels controlled rheobase and spike threshold. Because of their recruitment by sustained depolarization, these same channels dampened spike frequency over a broad range of somatic currents. Although they may subservise other functions when positioned at other cellular locations (Devaux et al., 2004; Martire et al., 2004), the functional role played by KCNQ channels in medium spiny neurons appears to be similar to that found in peripheral and hippocampal neurons (Wang and McKinnon, 1995; Hu et al., 2002).

KCNQ channels in medium spiny neurons do not act alone in shaping the response to excitatory synaptic input. There are at



**Figure 7.** Activation of cholinergic interneurons reduces KCNQ channel current in medium spiny neurons. *A*, Photomicrograph showing the placement of recording electrodes for a cholinergic interneuron and a medium spiny neuron. *B*, Somatic cell-attached recording of autonomous action potential firing in a cholinergic interneuron before and after application of 4-AP ( $100 \mu\text{M}$ ) in the continuous presence of ionotropic glutamate receptor antagonists,  $GABA_A$  receptor antagonist, and group 1 metabotropic glutamate receptor antagonists (see Results). At this concentration of 4-AP, the interneuron discharge rate was increased. Left, Time course plot of the action of 4-AP on the autonomous discharge rate of the cholinergic interneuron shown in the right panel. *C*, KCNQ channel currents in the medium spiny neuron were reduced by the interneuron activation, and this modulation was blocked by scopolamine ( $10 \mu\text{M}$ ). The time course of action of 4-AP and 4-AP plus scopolamine is shown in the left panel. *D*, Box plot summary of the action of 4-AP (4-AP, 56% of control,  $n = 9$ ; 4-AP and scopolamine, 97% of control,  $n = 5$ ;  $p < 0.01$ ; Mann-Whitney). *E*, The box plot illustrates increased discharge rate in 4-AP ( $n = 12$ ; control, 0.8 Hz; 4-AP, 1.8 Hz;  $p < 0.01$ ; Wilcoxon). scop, Scopolamine; cont, control.

least three other  $K^+$  channels that also participate in regulation of the up state. Inwardly rectifying Kir2 channels are major factors in governing the transition from down state to up state and may contribute a modest sustained outward current during the up state itself (Nisenbaum and Wilson, 1995; Wilson, 2004). Rapidly inactivating  $Kv4 K^+$  channels are expressed at modest levels in medium spiny neurons and contribute to the slowing of the membrane potential trajectory to up-state potentials (Nisenbaum and Wilson, 1995; Tkatch et al., 2000). Perhaps the major regulator of this initial phase of the up-state transition is the  $Kv1.2 K^+$  channel (Nisenbaum et al., 1994; Shen et al., 2004). But like  $Kv4$  channels,  $Kv1.2$  (or D-type) channels inactivate, albeit more slowly. As these channels inactivate, KCNQ channels open, providing a sustained hyperpolarizing current to modulate the up-state potential for as long as the up state lasts.

### Cholinergic modulation of KCNQ channels creates a striatal gating mechanism in learning paradigms

Cholinergic interneurons are thought to act as striatal “teachers.” In associative learning paradigms, presentation of primary, and then secondary, reinforcers induces a pause in interneuronal autonomous activity. This dopamine-dependent pause in activity and lowering of striatal cholinergic tone has been hypothesized to coordinate striatal activity in a way that allows execution of motor tasks (Aosaki et al., 1994; Morris et al., 2004; Yamada et al., 2004). How do fluctuations in cholinergic tone modulate the activity of the principal neurons of the striatum, medium spiny neurons? Voltage-dependent  $K^+$  channels regulating state transitions appear to be a major target of cholinergic signaling. Previous studies have suggested that  $M_1$  receptor signaling reduces



the opening of both Kir2 and Kv4 K<sup>+</sup> channels in medium spiny neurons (Akins et al., 1990; Galarraga et al., 1999). Our work complements these studies, showing that KCNQ K<sup>+</sup> channel opening is reduced potently by M<sub>1</sub> receptor activation. The coordinated modulation of these K<sup>+</sup> channels by M<sub>1</sub> receptor activation undoubtedly increases the responsiveness of medium spiny neurons to excitatory cortical and thalamic synaptic inputs. Conversely, pauses in the activity of cholinergic interneurons should transiently reduce the excitability of medium spiny neurons.

The modulation of KCNQ channels is likely to be a particularly important part of this coordinated cholinergic gating of medium spiny neuron responsiveness. In anesthetized animals, transitions to the up state can either result in spiking or silence, because neurons remain several millivolts below spike threshold for as long as seconds (Wilson and Kawaguchi, 1996; Tseng et al., 2001). Our results show that relatively small changes in the discharge rate of cholinergic interneurons can translate into substantial differences in KCNQ availability in nearby medium spiny neurons, providing a potential mechanism by which up-state potential could be modulated. What is less clear is whether differences between nearby medium spiny neurons in up-state potential can be attributed to local variation in cholinergic tone or to differences in intracellular mechanisms, such as regulators of G-protein signaling proteins, which regulate receptor coupling (Dohlmans and Thorner, 1997; Hepler, 1999).

Alterations in cholinergic tone and KCNQ channel are also likely to be important factors in disease states such as Parkinson's disease (PD). In PD, disinhibition of cholinergic interneurons leads to an elevation in striatal cholinergic tone. Our results and those of others shows how elevated cholinergic tone is likely to be translated into a reduction in KCNQ and Kir2/Kv4 channel opening in medium spiny neurons, increasing their excitability. This augmented responsiveness to excitatory inputs could prove to be particularly important to striatopallidal medium spiny neurons in which enhanced cholinergic tone could synergize with diminished D<sub>2</sub> dopamine receptor tone (Albin et al., 1989).

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