Behavioral/Systems/Cognitive

Control of Spontaneous Firing Patterns by the Selective Coupling of Calcium Currents to Calcium-Activated Potassium Currents in Striatal Cholinergic Interneurons

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The spontaneous firing patterns of striatal cholinergic interneurons are sculpted by potassium currents that give rise to prominent afterhyperpolarizations (AHPs). Large-conductance calcium-activated potassium (BK) channel currents contribute to action potential (AP) repolarization; small-conductance calcium-activated potassium channel currents generate an apamin-sensitive medium AHP (mAHP) after each AP; and bursts of APs generate long-lasting slow AHPs (sAHPs) attributable to apamin-insensitive currents. Because all these currents are calcium dependent, we conducted voltage- and current-clamp whole-cell recordings while pharmacologically manipulating calcium channels of the plasma membrane and intracellular stores to determine what sources of calcium activate the currents underlying AP repolarization and the AHPs. The $Ca_v2.2$ (N-type) blocker ω-conotoxin GVIA (1 μM) was the only blocker that significantly reduced the mAHP, and it induced a transition to rhythmic bursting in one-third of the cells tested. Ca_v1 (L-type) blockers (10 μM dihydropyridines) were the only ones that significantly reduced the sAHP. When applied to cells induced to burst with apamin, dihydropyridines reduced the sAHPs and abolished bursting. Depletion of intracellular stores with 10 mM caffeine also significantly reduced the sAHP current and reversibly regularized firing. Application of 1 μM ω-conotoxin MVIIC (a $Ca_v2.1/2.2$ blocker) broadened APs but had a negligible effect on APs in cells in which BK channels were already blocked by submillimolar tetraethylammonium chloride, indicating that $Ca_v2.1$ (Q-type) channels provide the calcium to activate BK channels that repolarize the AP. Thus, calcium currents are selectively coupled to the calcium-dependent potassium currents underlying the AHPs, thereby creating mechanisms for control of the spontaneous firing patterns of these neurons.

Key words: **tonically active neurons; high-voltage-activated calcium currents; basal ganglia; ongoing activity; potassium currents; afterhyperpolarization; calcium-induced calcium release; intracellular stores; ryanodine; xestospongin C**

Introduction

The cholinergic interneurons of the striatum exhibit a variety of spontaneous firing patterns *in vivo* (Wilson et al., 1990; Reynolds and Wickens, 2004) and *in vitro* (Bennett and Wilson, 1999). In slices, these include two periodic patterns: single spiking and rhythmic bursting. These patterns differ in the outward currents that give rise to hyperpolarizations. In the single-spiking pattern, action potentials trigger the activation of an apamin-sensitive calcium-dependent potassium [small-conductance calcium-activated potassium (SK) channel] current that produces medium-duration afterhyperpolarizations (mAHPs), lasting ~200 ms (Bennett et al., 2000). Rhythmic bursting is promoted by a slow apamin-insensitive calcium-dependent potassium current that gives rise to slow afterhyperpolarizations (sAHPs) that last several seconds and that follow bursts or prolonged subthreshold depolarizations (Wilson and Goldberg, 2005). The dominance of a pattern in any given neuron depends on the relative strengths of its underlying conductances, including these two calcium-dependent potassium conductances. For example, the bursting pattern cannot be expressed if the current underlying the mAHP is strong enough to prevent repetitive firing. Conversely, application of 100 nM apamin causes all cholinergic interneurons to burst (Bennett et al., 2000).

The properties of calcium-dependent potassium conductances present in the cholinergic interneurons have been studied widely in various central neurons. In pyramidal cells, repolarization of action potentials, similar to cholinergic interneurons (Bennett et al., 2000), results from a potassium current through large-conductance calcium-activated potassium (BK) channels (Lancaster and Adams, 1986; Lancaster and Nicoll, 1987); mAHPs are generated by SK currents (Schwindt et al., 1988); and the calcium-dependent component of the sAHP that follows long trains of action potentials (Hotson and Prince, 1980; Gustafsson and Wigstrom, 1983; Schwindt et al., 1988) results from a potassium current that has yet to be identified (Sah and Faber, 2002; Vogalis et al., 2003). These calcium-dependent potassium currents rely on calcium entry through high-voltage-activated calcium channels (Viana et al., 1993; Sah, 1995; Williams et al., 1997; Pineda et al., 1998; Cloues and Sather, 2003). Several such cal-

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cium channels have been shown to contribute to barium currents recorded from dissociated cholinergic interneurons (Yan and Surmeier, 1996). One possible function for a diversity of calcium currents is the selective coupling of these channels to calcium-dependent potassium channels (Viana et al., 1993; Sah, 1995; Williams et al., 1997; Marrion and Tavalin, 1998; Pineda et al., 1998; Shah and Haylett, 2000; Vilchis et al., 2000; Cloues and Sather, 2003). Because the calcium currents in cholinergic interneurons are targets of neuromodulation (Yan and Surmeier, 1996; Yan et al., 1997; Song et al., 2000; Pisani et al., 2002), finding such a selective coupling would provide a possible mechanism by which the firing patterns and the cholinergic output of these neurons are controlled by neuromodulators. In this work, we combine voltage- and current-clamp recording in the whole-cell configuration with pharmacological manipulation of calcium channels (both of the plasma membrane and of intracellular stores) to study their coupling to the calcium-dependent potassium currents in cholinergic interneurons. The spontaneously firing cholinergic interneurons provide a unique opportunity to observe the effect of this coupling on their ongoing firing patterns.

Materials and Methods

Slice preparation. Sprague Dawley rats of either sex, aged 14–23 d, were anesthetized deeply with ketamine–xylazine and perfused through the heart with 10–30 ml of ice-cold modified artificial CSF (ACSF), which had been bubbled with 95% O_2 and 5% CO_2 and contained the following (in mM): 2.5 KCl, 26 $NaHCO_3$, 1.25 Na_2HPO_4, 0.5 $CaCl_2$, 10 $MgSO_4$, 230 sucrose, and 10 glucose. The brain was removed rapidly, blocked in the sagittal plane, glued to the stage of a VT1000S vibratome (Leica, Nussloch, Germany), and immersed in ice-cold modified ACSF. Three-hundred-micrometer-thick slices containing the neostriatum were cut and then transferred to a holding chamber where they were submerged in ACSF, which was bubbled continuously with 95% O_2 and 5% CO_2 and contained the following (in mM): 2.5 KCl, 126 NaCl, 26 $NaHCO_3$, 1.25 Na_2HPO_4, 2 $CaCl_2$, 2 $MgSO_4$, and 10 glucose. In some experiments, we used a nominally calcium-free ACSF that contained the following (in mM): 2.5 KCl, 123 NaCl, 26 $NaHCO_3$, 1.25 Na_2HPO_4, 1 EGTA, 3 $MgSO_4$, and 10 glucose. Slices were held at room temperature in this chamber for at least 1 h before recording.

Visualized whole-cell recording. Individual slices were transferred to the recording chamber and were perfused continuously with oxygenated ACSF at 32–35°C. A 40× water-immersion objective (Axioskop; Zeiss, Oberkochen, Germany) was used to examine the slice using standard infrared differential interference contrast video microscopy (Stuart et al., 1993). The cholinergic interneurons were identified by their large (>20 μm) somata. In many cases, two or three thick primary dendrites could be seen emanating from soma (Kawaguchi, 1992). Somatic recordings were made using patch pipettes prepared from thin-wall borosilicate glass (outer diameter, 1.5 mm; inner diameter, 1.17 mm; Warner Instruments, Hamden, CT) on a P-87 Flaming/Brown electrode puller (Sutter Instruments, Novato, CA). Pipettes were filled with a solution containing the following (in mM): 130.5 K-$MeSO_4$, 10 KCl, 7.5 NaCl, 0.01 phosphocreatine, 10 HEPES, 0.2 EGTA, 0.2 Na_2GTP, and 2 $Mg_{1.5}ATP$. The pH and osmolarity of the intracellular solution were 7.3 (with KOH) and 275–300 mOsm/kg, respectively. Biocytin (5% w/v) was added to the internal solution. The resistance of the filled pipettes ranged from 4 to 8 MΩ, and the junction potential was 7 mV (voltage traces are corrected for the junction potential). Recordings in the whole-cell configuration were made using an Axopatch 200B amplifier (Molecular Devices, Foster City, CA) in the voltage-clamp and fast current-clamp modes. Signals were digitized at 5–100 kHz and logged onto a personal computer with the pClamp 8.0.1 software (Molecular Devices).

Drugs. Drugs were bath applied. Ca_v1 (L-type) channels were blocked with 10 μM of either nifedipine or nimodipine (because both dihydropyridines gave similar results, we pooled their data). Nifedipine was prepared in a stock solution of 10 mM in 1 ml of 95% ethyl alcohol and diluted 1:1000, and 20 mM nimodipine was prepared in stock solution in 1 ml of 100% ethyl alcohol and diluted 1:2000. Both drugs were protected from light during use and storage. $Ca_v2.2$ (N-type) channels were blocked with 1 μM ω-conotoxin GVIA (GVIA) (Boland et al., 1994). At the population level, $Ca_v2.1$ (P/Q-type) currents were considered blocked when the current measured was affected by the broad-band $Ca_v2.1/2.2$ channel blocker ω-conotoxin MVIIC (MVIIC) (Randall and Tsien, 1995; McDonough et al., 1996) but not by GVIA. ω-Agatoxin (AgTx) IVA or TK were used as an alternative $Ca_v2.1$ blocker on a small number of cells. Agatoxins were used at two concentrations: 40 nM to block P-type and 200 nM to also block the Q-type channels (Randall and Tsien, 1995). $Ca_v2.3$ (R-type mediated by the α-1E subunit) currents were blocked with 100 nM SNX-482 (Newcomb et al., 1998). A low dose of Ni^{2+} (50 μM) was used as an additional less-specific blocker of R-type currents (Randall and Tsien, 1995). All measurements of the effect of these calcium currents blockers were included in the group statistics if the drugs had at least 5 min to wash in, except in the case of the dihydropyridines, which were given 9 min to wash in. SK channels were blocked with apamin. BK channels were blocked with low doses (≤1 mM) of tetraethylammonium (TEA) chloride. Tetrodotoxin (TTX; 1 μM) was added in some experiments to block action potentials. Ba^{2+} (100 μM) was used to block KIR channels in one experiment. Intracellular stores were depleted nonspecifically with 10 mM caffeine. Additionally, xestospongin C (XeC; 1 μM) was used to selectively block the inositol 1,4,5-trisphosphate (IP_3)-mediated calcium release from stores (Gafni et al., 1997). XeC was prepared in a 1 mM stock solution in 225 μl of dimethyl sulfoxide and diluted 1:1000. Ryanodine (15 μM) was used to selectively deplete ryanodine-sensitive stores. Cytochrome *c* (0.01% w/v in final bathing solution) was added as a carrier whenever ryanodine and agatoxins were used (Bargas et al., 1994). In some experiments, a 2 mM concentration of the fast calcium chelator BAPTA was added to the pipette solution.

Histochemical processing, data analysis, and statistics. At the end of recording, slices were fixed by immersion into 4% paraformaldehyde in 0.15 M phosphate buffer and refrigerated for a period of 7–12 d. Consequently, the filled cells were stained using the avidin–biotin complex (ABC) nickel-enhanced 3,3′-diaminobenzidine tetrahydrochloride reaction (Horikawa and Armstrong, 1988). Histochemical processing demonstrated the ramification of these dendrites into higher-order dendritic processes. Often, the intricate axonal arborization of the neuron was also visible (Bennett and Wilson, 1999).

Data were analyzed using custom software and Mathematica 5 (Wolfram Research, Champaign, IL). All voltage-clamp traces depicted in the figures are an average of five consecutively recorded current traces. All measurements of tail currents were taken from these averaged traces. These measurements were very sensitive to changes in series resistance. Therefore, we only used cells in which this resistance was stable (and <30 MΩ) throughout the experiment. Widths of action potentials were defined as the duration from (1) the first data point at which the derivative of the voltage trace exceeded a threshold of 10 mV/ms to (2) the return of the voltage trace to the voltage at which this threshold occurred.

Two-tailed paired *t* tests were used to test for the effect of drugs on currents measured in voltage clamp. Tests based on ranks (Siegel and Castellan, 1988) were used to test for the effect of drugs on action potential widths. The threshold for statistical significance was set to a *p* value of 0.05. All drugs and reagents were acquired from Sigma (St. Louis, MO) except for the XeC, which was acquired from Cayman Chemical (Ann Arbor, MI); the SNX-482, which was acquired from Alomone Labs (Jerusalem, Israel); and the ABC kits, which were acquired from Vector Laboratories (Burlingame, CA).

Results

Firing patterns and afterhyperpolarizations of the striatal cholinergic interneurons

Cholinergic interneurons of the striatum discharge spontaneously *in vitro* displaying a variety of firing patterns. Most neurons discharged tonically in a single spiking mode, whereas others discharge in rhythmic bursts interrupted by large hyperpolariza-

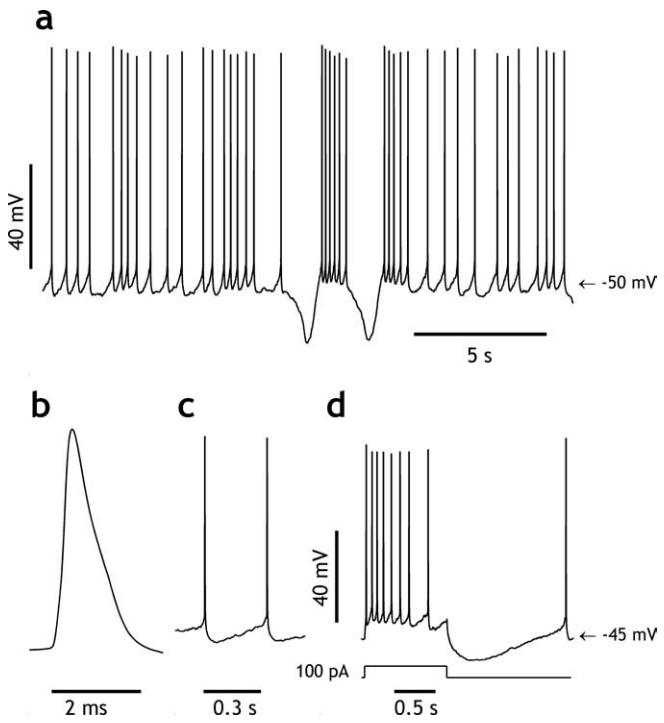

Figure 1. Firing properties of striatal cholinergic interneurons *in vitro*. ***a***, A 20 s trace depicting a spontaneous firing pattern of a cholinergic interneurons *in vitro*, which includes transitions from tonic to burst discharge. The bursts are followed by prominent hyperpolarizations. ***b***, Action-potential waveform of a cholinergic interneuron. ***c***, During spontaneous discharge, each action potential is followed by a medium AHP. ***d***, A somatic current injection elicits a slow AHP after its termination.

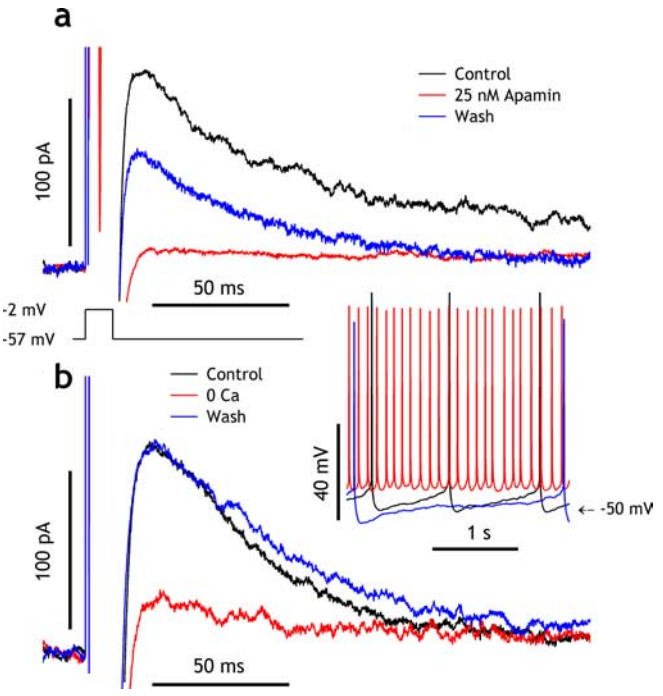

Figure 2. A calcium-dependent apamin-sensitive outward current contributes to the medium AHPs. ***a***, A brief 10 ms voltage step to −2 mV from a holding potential of −57 mV elicits an outward tail current (black trace), which is abolished by bath application of 25 nM apamin (red trace), an effect that is partially reversed after washout (Wash; blue trace). ***b***, This current (black trace) is abolished in a calcium-free bathing solution (red trace) but then fully recovers after washing in normal ACSF (Wash; blue trace). Inset, In current clamp, the reduction of this current in a calcium-free bath corresponds to a reduction in the medium AHPs and leads to an increased firing rate (red trace) relative to control (black trace). This effect is reversible after the wash-in of normal ACSF (blue trace). Thus, the outward current generated by this short pulse will be called the mAHP current. The current traces are averages of five trials.

tions (Bennett and Wilson, 1999). Occasionally, a neuron transiently shifted between these patterns as shown in Figure 1*a*. This figure also demonstrates that the firing pattern of the cholinergic interneuron is sculpted by the differences between hyperpolarizations that follow single action potentials and those that follow bursts.

The action potential of the cholinergic neurons is broad (Fig. 1*b*), with a mean width of 2.87 ± 0.11 ms (mean ± SEM; $n = 10$ cells), as measured from the time of threshold crossing until the return to the threshold potential on the falling phase, for action potentials that were at least 80 mV from threshold to peak. BK calcium-activated potassium channels contribute to the repolarization process (Bennett et al., 2000). Each action potential is followed by a prominent mAHP (Fig. 1*c*) that is apamin sensitive, indicating that it is generated by calcium-activated potassium current through SK channels (Bennett et al., 2000). After termination of a long somatic current injection, the neuron displays a long-lasting sAHP (Fig. 1*d*). Often, the cell exhibits a powerful and stereotyped hyperpolarization after a long current injection (such as the ones occurring spontaneously in Fig. 1*a*), which is deeper and faster in onset than the sAHP depicted in Figure 1*d*. The differences in size of these hyperpolarizations are primarily determined by the degree to which they are amplified by a regenerative outward current through KIR channels and not by the size of the sAHP current itself (Wilson, 2005)

The potassium current underlying the mAHP is coupled to $Ca_v2.2$ calcium currents

Because both the mAHP and the sAHP are generated by calcium-dependent potassium currents (Bennett et al., 2000; Wilson and Goldberg, 2005), we used voltage-clamp protocols to study each of these currents and its coupling to calcium currents. To evoke the outward current underlying the mAHP, we used a 10 ms pulse to −2 mV from a holding potential of −57 mV (values corrected for junction potential). This pulse elicits an unclamped action current that is followed by a 100–200 ms long outward tail current. This tail current is reversibly abolished in 25 nM apamin (Fig. 2*a*), indicating that it flows through SK channels. As expected for the calcium-activated SK channels, this outward current is reversibly abolished in a calcium-free bathing solution (Fig. 2*b*). In current clamp, the calcium-free bath reversibly reduced the mAHPs that follow each action potential during single-spiking discharge, thereby dramatically increasing the spontaneous firing rate (Fig. 2*b*, inset) of the neuron. Hence, we will refer to the current evoked by the 10 ms pulse as the mAHP current.

In several cell types, BK and SK potassium channels are coupled selectively to the various calcium channels (Viana et al., 1993; Sah, 1995; Williams et al., 1997; Marrion and Tavalin, 1998; Pineda et al., 1998; Cloues and Sather, 2003). Therefore, we sought to identify which calcium channels were responsible for the influx needed to activate the mAHP current. To the extent that the cholinergic interneuron exhibits such selectivity, we wished to study how this selectivity could serve as a mechanism to modulate the mAHP. Because the cholinergic interneuron is spontaneously active *in vitro*, it provides an opportunity to study the direct effect of this selectivity on the ongoing firing patterns. To this end, we used pharmacological means to dissect the contribution of each calcium current to the mAHP current and studied the outcome of these treatments on the spontaneous and driven activity of these cells in current clamp.

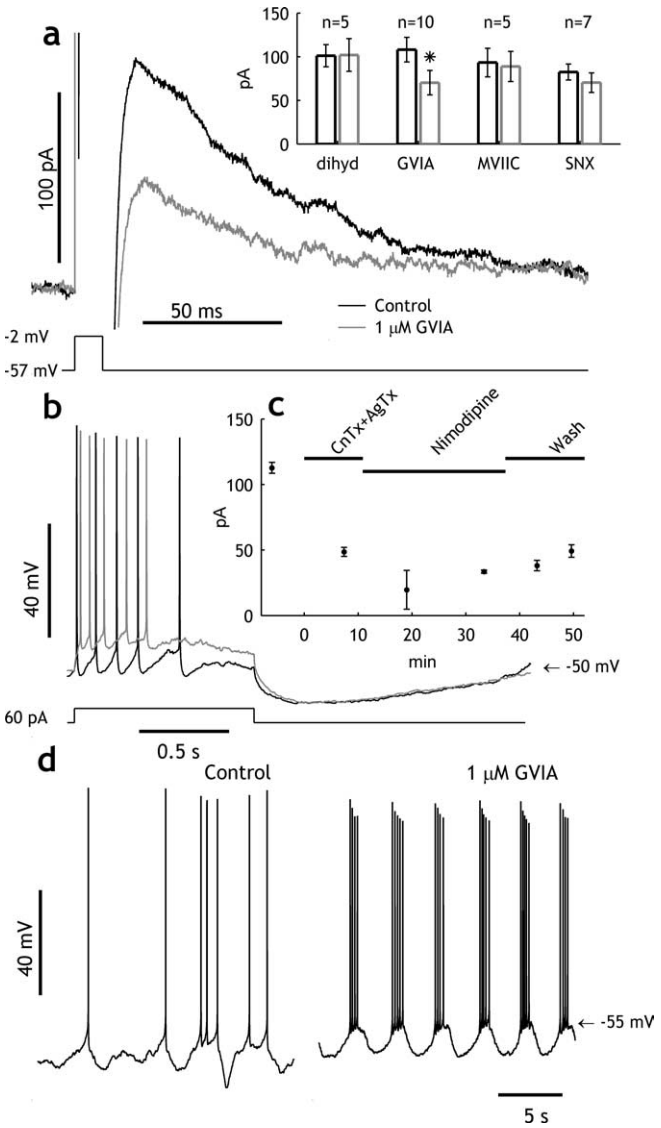

Figure 3. Reduction of the mAHP current by $Ca_v2.2$ channel blockade can induce burst discharge. ***a***, One micromolar GVIA, which blocks $Ca_v2.2$ channels, reduces the mAHP current (gray trace) relative to control (black trace). Inset, The mean ± SEM value across cells of the mAHP current before (black boxes) and after (gray boxes) application of various calcium-current blockers: 10 μM dihydropyridines (dihyd), which block Ca_v1 channels; 1 μM GVIA; 1 μM MVIIC, which blocks $Ca_v2.1/2.2$ channels; and 100 nM SNX-482 (SNX), which blocks $Ca_v2.3$ channels. The current was measured 25 ms after the end of the pulse and averaged over five traces taken in a 20 s interval. Only the GVIA treatment elicited a significant reduction in the value of the mAHP current (*$p < 0.001$; two-tailed paired *t* test; values of *n* indicate the number of cells). ***b***, In current clamp, application of 1 μM GVIA reduced the mAHP after a 60 pA, 1 s somatic current injection (gray trace) relative to control (black trace), without affecting the sAHP that followed the termination of the pulse. ***c***, Mean ± SD (calculated from 5 trials conducted in a 20 s interval) of the mAHP current measured from a cell that was bathed sequentially in (1) a combination of 1 μM GVIA and 100 nM of the $Ca_v2.1$ blocker ω-agatoxin TK (CnTx+AgTx), (2) 10 μM nimodipine, and finally in (3) normal ACSF (Wash). ***d***, Traces of the spontaneous discharge of a neuron in control and after bath application of 1 μM GVIA, which caused it to burst.

When subjecting the cell to calcium channel blockers, only ω-conotoxin GVIA (1 μM; a $Ca_v2.2$ channel blocker) had a significant effect on the mAHP current (Fig. 3*a*). In 10 cells tested, GVIA reduced the current by 8–97% (mean, 35%; $p < 0.001$; two-tailed paired *t* test). In current clamp, the reduction in the mAHP current induced by GVIA generated smaller mAHPs after each action potential. In contrast, this drug had no effect on the sAHP (Fig. 3*b*). Figure 3*c* shows the insensitivity of the mAHP current to blockade of calcium entry through Ca_v1 channels. Application of Ca_v2 blockers dramatically reduced the mAHP current, and it began to recover despite the presence of Ca_v1 blocker in the bathing solution. A similar segregation between the effects of these two channels on mAHPs has been described previously in rat hypoglossal motor neurons (Viana et al., 1993).

The incomplete mean block of the mAHP current by GVIA treatment was attributable in part to variability among neurons. It is possible that the brief voltage pulse used to assess the mAHP current was able to evoke some other outward spike aftercurrent, which could have contaminated the measurement. However, in one of the cells, the block was very nearly complete, and there was a strong correlation between the effectiveness of the mAHP current block seen in voltage clamp and changes in firing pattern seen in current clamp (see below).

Blockade of Cav2.2 channels can generate a transition to burst discharge

Bath application of 100 nM apamin induces a reduction of the mAHP and a rapid transition to burst firing in cholinergic interneurons (Bennett et al., 2000). Apamin application (25 nM) blocked the mAHP current (Fig. 2*a*) and caused most (6 of 10) neurons to switch to burst discharge (data not shown). Because the apamin-sensitive outward current is reduced by GVIA, we expected the latter drug to have similar effects. Of nine neurons in which spontaneous activity was studied in current clamp, all became more irregular after GVIA treatment, and three cells that exhibited >50% block of mAHP current in the voltage-clamp measurement exhibited rhythmic bursting discharge similar to that induced by apamin (Fig. 3*d*).

The sAHP current is activated by the influx of calcium through Ca_v1 channels

To study the current underlying the sAHP, we used an 800 ms pulse from −57 to −2 mV. This pulse evoked a large outward tail that lasted several seconds. The tail current was reversibly blocked in a calcium-free bath (Fig. 4*a*), attaining an average reduction of 81% (Wilson and Goldberg, 2005). At the onset of the tail current evoked by the long voltage pulse, it is contaminated by the mAHP current. Therefore, we measured the magnitude of this sAHP current as the outward current averaged over the interval from 975 to 1025 ms after the end of the pulse (Fig. 4*a*, arrow), a point at which the current is maximal (Wilson and Goldberg, 2005). We then measured the effect of specific calcium channel blockers on this current. In Figure 4*b*, the reversible effect of 10 μM nimodipine on the sAHP current is evident. Of all the blockers we used, only the dihydropyridines (10 μM), which are Ca_v1 channel blockers, had a significant effect on the sAHP current (Fig. 4*b*, inset). The dihydropyridines reduced the sAHP current by 25–52% in all eight cells (mean, 40%; $p < 0.005$; two-tailed paired *t* test). At this dose of dihydropyridines, an ~65% block of L-type channels is obtained in tissue culture in which the drug has complete access to the sites of action (Furukawa et al., 1999; Shen et al., 2000). Figure 4*c* shows an example recorded in current clamp of the reduction in the sAHP (elicited by a 1 s, 100 pA somatic current injection) induced by 10 μM nifedipine. In five other cells, we applied 20 μM nimodipine, which yielded a 52% average block of the sAHP current ($p < 0.05$; two-tailed paired *t* test). In Figure 4*d*, we depict as a function of time the value of the sAHP current measured from a neuron that was subjected sequentially to several calcium channel blockers. In this example, no reduction in the current was observed until 20 μM nifedipine was applied to the bathing solution, at

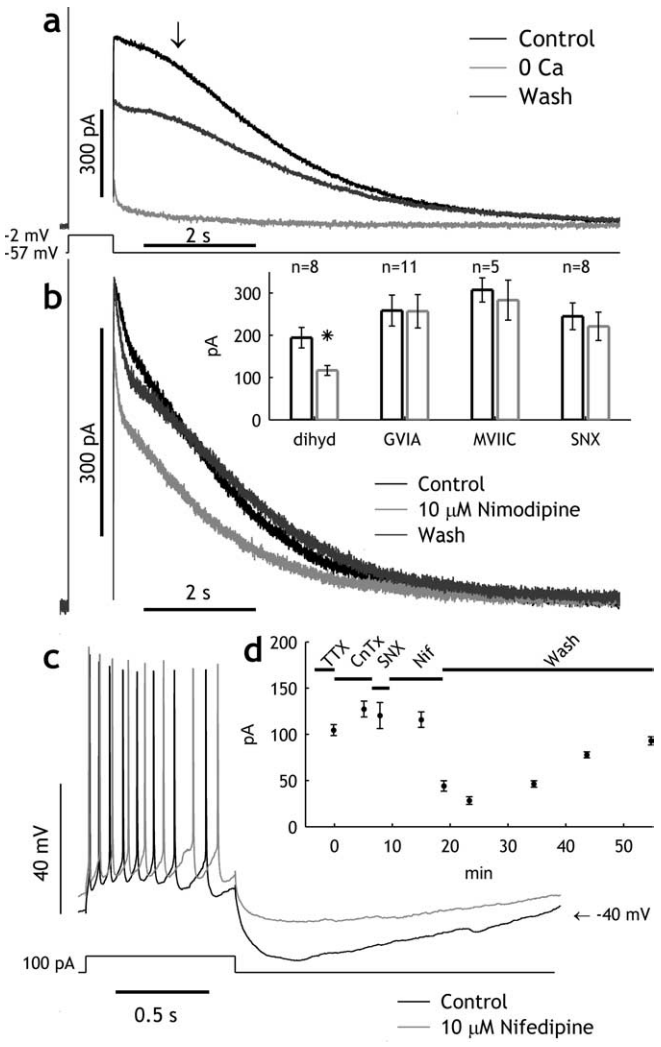

Figure 4. The calcium-dependent slow AHP current is selectively sensitive to dihydropyridine treatment. ***a***, An 800 ms step to −2 mV from a holding potential of −57 mV elicits a large and long-lasting outward current (black trace) that is abolished in the calcium-free bath (light gray trace) and that partially recovers after wash-in (Wash) of normal ACSF (dark gray trace). The current traces are averages of five single trials measure during a 70 s interval. This sAHP current was measured as the temporal mean of the averaged current trace calculated between 975 and 1025 ms past the end of the pulse (arrow) to avoid contamination by the mAHP current. ***b***, The sAHP current (black trace) is reduced by bath application of 10 μM nimodipine (light gray trace). This block was reversible after washout (Wash) of the drug (dark gray trace). Inset, The mean ± SEM value across cells of the sAHP current before and after application of the various calcium-current blockers (the same format as in Fig. 3*a*). Only the dihydropyridine (dihyd) treatment caused a significant reduction in this current (*$p < 0.005$; two-tailed paired *t* test; values of *n* indicate the number of cells). ***c***, In current clamp, application of 10 μM nifedipine reduced the sAHP after a 100 pA, 1 s somatic current injection (gray trace) relative to control (black trace). ***d***, Mean ± SD of the sAHP current measured from a cell that was bathed sequentially in (1) 1 μM TTX, (2) a combination of 2 μM GVIA and 2 μM MVIIC (CnTx), (3) 100 nM SNX, (4) 20 μM nifedipine (Nif), and finally in (5) normal ACSF (Wash). SNX, SNX-482.

which point the sAHP current decreased by 73% and consequently recovered after an extended washout of the drugs.

We used 100 nM SNX-482 to block $Ca_v2.3$ channels. Because this drug does not consistently block all native R-type currents (i.e., resistant to N-, P/Q-, and L-type currents) in various CNS neurons in the rat (Newcomb et al., 1998), we also tested the effect of 50 μM Ni^{2+} on the sAHP current in six neurons. Nickel had no significant effect on the mean current (mean ± SEM, 255 ± 62 pA in control vs 240 ± 43 pA in Ni^{2+}; two-tailed paired *t* test).

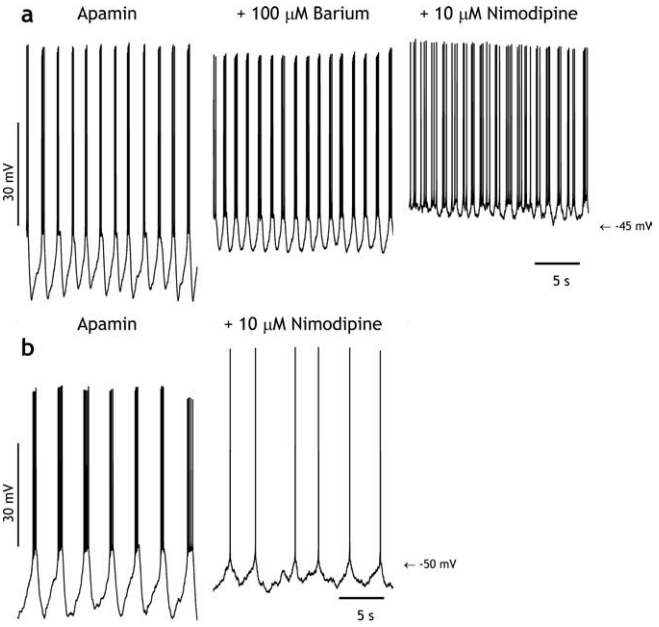

Figure 5. Spontaneous sAHPs during apamin-induced bursting are dihydropyridine sensitive. ***a***, Preincubation of the slice in apamin caused stereotyped burst discharge with prominent regenerative hyperpolarization. Addition of barium (plus 100 nM apamin) blocked the regenerative hyperpolarization but did not suppress the bursting. Subsequent addition of 10 μM nimodipine to the existing mixture dramatically reduced the sAHPs, thereby leading to variable lengths of bursts, each with a variable number of action potentials. ***b***, Application of 10 μM nimodipine alone to a bursting cell preincubated in apamin also reduced the hyperpolarizations and lead to irregular single-spiking discharge.

Ca_v1 channels regulate burst discharge

The slow decay time course of the sAHP current causes it to accumulate in response to a train of action potentials elicited, for example, during burst discharge. This build-up leads in turn to the termination of the burst (Bennett et al., 2000). Because the dihydropyridines reduce the sAHP current, they should have a dramatic effect on the pattern of burst discharge of the neuron. Figure 5 depicts the effects of nimodipine treatment on the firing pattern of neurons that were induced to burst with apamin (left column) (Bennett et al., 2000). Because KIR channels can amplify the sAHP into a regenerative hyperpolarization (Wilson, 2005), we first applied 100 μM barium to block KIR channels. This reduced the amplitude and duration of the slow hyperpolarization observed between bursts but nevertheless preserved the sAHPs that separate them. Bath application of 10 μM nimodipine reduced the sAHPs that followed each burst, causing the bursts to become more irregular and less stereotyped (Fig. 5*a*). Application of nimodipine alone (without first applying barium) also reduced the spontaneous sAHPs and disrupts the apamin-induced bursting (Fig. 5*b*). We conclude that the sAHP current responsible for slow rhythmic bursting is triggered by calcium influx via Ca_v1 channels.

The sAHP current is activated by calcium release from intracellular stores

A sensitivity of the sAHP current to Ca_v1, but not to Ca_v2, currents has been described in CA3 pyramidal neurons (Tanabe et al., 1998). In these neurons, as well as in others, the sAHP current is activated by calcium release from intracellular stores (Sah and McLachlan, 1991; Berridge, 1998; Pineda et al., 1999). We therefore tested the sensitivity of the sAHP to depletion of intracellular stores. Application of 10 mM caffeine rapidly and reversibly re-

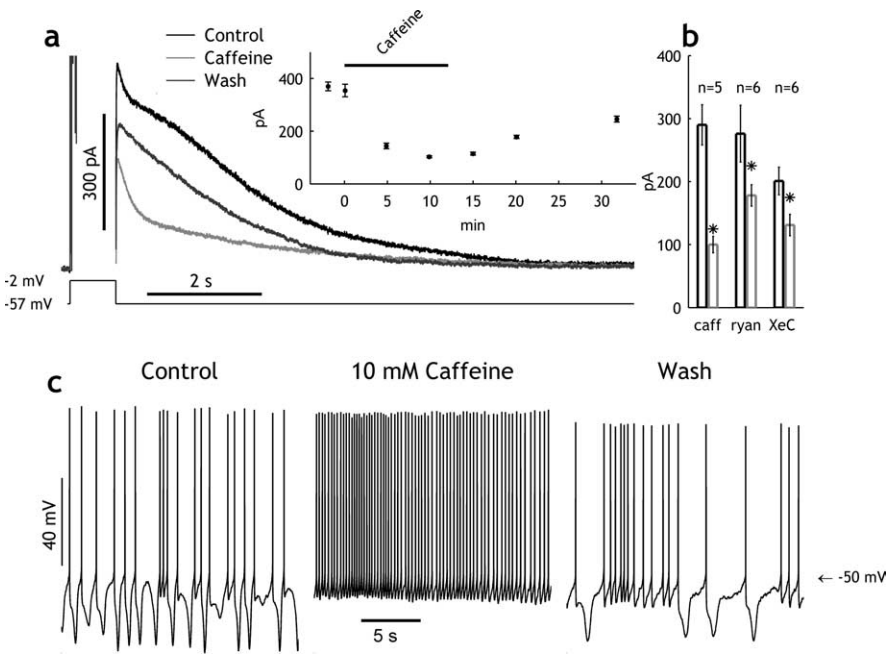

Figure 6. The role of intracellular stores in generating the sAHP current and the spontaneous discharge of cholinergic interneurons. ***a***, The sAHP current, but not the early phase of the tail current, recorded in control (black trace) is reduced by bath application of 10 mM caffeine (light gray trace) and subsequently partially recovers after washing in normal ACSF (Wash; dark gray trace). Each trace is an average of five trials measured during an interval of 70 s. Inset, Time course of the mean ± SD of the sAHP current during caffeine treatment and the subsequent washing in of normal ACSF. ***b***, The mean ± SEM value across cells of the sAHP current before (black boxes) and after (gray boxes) depleting intracellular stores with 10 mM caffeine (caff); 15 μM ryanodine (ryan), which selectively affects ryanodine-sensitive stores; or 1 μM XeC, which selectively blocks IP_3-mediated calcium release from stores. These drugs significantly reduced the sAHP current by 66, 36, and 35%, respectively (*$p < 0.05$; two-tailed paired *t* test; values of *n* indicate the number of cells). ***c***, Caffeine treatment causes a cholinergic interneuron displaying an irregular spontaneous discharge pattern that includes prominent hyperpolarizations to transition to rapid single-spiking discharge. This effect is reversed after washing out (Wash) the drug. Same neuron as that depicted in ***a***.

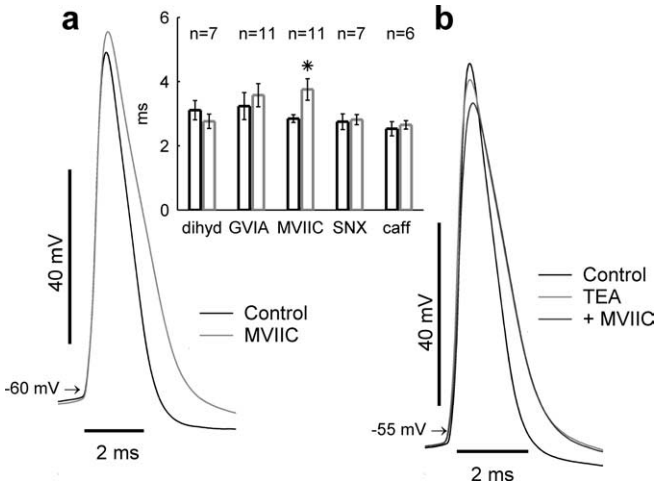

Figure 7. BK channels activated by calcium influx through $Ca_v2.1$ channels contribute to action potential repolarization. ***a***, Action-potential waveform before (black trace) and after (gray trace) bath application of 1 μM MVIIC. Inset, The mean ± SEM across cells of the spike width before and after application of the various calcium-current blockers. Same format as in Figure 3*a*, with the additional treatment with 10 mM caffeine (caff). Only MVIIC generated a significant increase in the width of the action potential (*$p < 0.05$; two-tailed Wilcoxon signed-ranks test; values of *n* indicate the number of cells). dihyd, Dihydropyridine; SNX, SNX-482. ***b***, The effect of MVIIC was occluded by 1 mM TEA, indicating that the sole effect of blocking $Ca_v2.1$ channels is mediated by their contribution to the activation of BK channels. The control action-potential waveform was shifted in the hyperpolarizing direction to correct for a 6.5 mV offset.

duced the sAHP current (Fig. 6*a*). Ten minutes of bathing in caffeine generated a 66% reduction on average in the sAHP current ($n = 5$; two-tailed paired *t* test; $p < 0.01$) (Fig. 6*b*). To determine the contribution of ryanodine-sensitive stores to this activation, we applied 15 μM ryanodine to six cells and found that it significantly reduced the current in all cells by 36% on average (two-tailed paired *t* test; $p < 0.05$) (Fig. 6*b*). Application of 1 μM XeC, which selectively blocks IP_3-sensitive stores (Gafni et al., 1997), reduced the sAHP current by 35% on average ($n = 6$; two-tailed paired *t* test; $p < 0.005$) (Fig. 6*b*). The effect of XeC was unique in that it reduced the long sAHP tail current during the initial 2 s without significantly reducing the later part of tail current relative to control (data not shown).

Figure 6*a* demonstrates that whereas 10 min of caffeine treatment has a large effect on the sAHP current, the initial fast component of the long tail current, which like the mAHP current is apamin-sensitive (Wilson and Goldberg, 2005), is still quite prominent. Additionally, in four of seven cells treated with caffeine and two of five cells treated with ryanodine, the mAHP current, measured with the 10 ms voltage pulse, increased transiently (for a few minutes) but then returned to control values while these drugs washed on (data not shown).

The effect of depleting intracellular stores with caffeine on the spontaneous discharge patterns is demonstrated in Figure 6*c*. Caffeine caused a significant increase in the firing rates of the neurons by inducing three quiescent neurons to begin firing and four spontaneously active neurons to speed up ($p < 0.05$; two-tailed Wilcoxon signed-ranks test). In all cases, the firing became tonic in a single spiking mode. In the example depicted here, the neuron initially fired irregularly displaying large hyperpolarizations. After reducing the sAHP current with caffeine, the cell was no longer able to trigger these hyperpolarization. However, these hyperpolarizations as well as the irregular firing pattern recovered when the caffeine was washed out.

BK channels contributing to action potential repolarization are activated by calcium influx through $Ca_v2.1$ channels

Iberiotoxin or low concentrations of TEA (0.1–1 mM) have been reported to broaden the action potential of the cholinergic interneuron (data not shown), implicating BK calcium-dependent potassium channels as contributing to action potential repolarization (Bennett et al., 2000). Blocking calcium currents may prevent the opening of BK channels leading to an impaired repolarization process, culminating in a broader action potential. Bathing the slice in 1 μM ω-conotoxin MVIIC (a $Ca_v2.1/2.2$ channel blocker) significantly broadened the action potential in 10 of the 11 cells tested ($p < 0.05$; two-tailed Wilcoxon signed-ranks test) (Fig. 7*a*). In three cells, the action potential was broadened by >75%. In contrast, bathing the cell in the other organic calcium channel blockers or in caffeine had no significant effect on

action potential width (Fig. 7*a*, inset). In two cells for which pretreatment with 1 mM TEA broadened the action potential by 54 and 43%, the subsequent addition of 1 μM MVIIC either did not further broaden the action potential (Fig. 7*b*) or broadened it only by an additional 8%, respectively. These results suggest that MVIIC delays action potential repolarization by reducing the calcium influx, through $Ca_v2.1$ channels, that is needed for opening BK channels. At most 10% of calcium currents expressed in cholinergic interneurons are of the P type (Yan and Surmeier, 1996), suggesting that Q-type channels supply the calcium to activate BK channels. In two cells treated with a low dose (40 nM) of AgTx IVA, which should not appreciably affect Q-type currents but should block P-type channels, we found no change in action potential width (data not shown).

BK channels in hippocampal pyramidal cells are found within tens of nanometers of the calcium channels that provide them with the calcium needed for their activation (Marrion and Tavalin, 1998). One way to demonstrate the proximity of these channels to each other is to show the insensitivity of the function of the BK channels to fast calcium chelators (Velumian and Carlen, 1999). We therefore tested whether the addition of 2 mM BAPTA to the internal solution of the electrode would lead to a broadening of the width of the action potentials of the neurons, presumably by buffering the calcium needed to activate BK channels (Lancaster and Nicoll, 1987). The mean width of action potentials, which were at least 80 mV from threshold to peak, measured later than 12 min from the seal rupture, was 2.26 ± 0.11 ms (mean ± SEM; $n = 6$ cells), which was significantly narrower than in control conditions (two-sided Wilcoxon rank–sum test; $p < 0.001$). We conclude that BAPTA was incapable of suppressing repolarization of the action potential caused by the action of BK channels, suggesting that in cholinergic interneurons the BK and $Ca_v2.1$ channels are in close proximity to each other, a distance that has been estimated at ~30 μm in other neuronal types (Gola and Crest, 1993; Naraghi and Neher, 1997; Marrion and Tavalin, 1998; Velumian and Carlen, 1999).

Discussion

Cholinergic interneurons express a variety of calcium channels with similar voltage sensitivities and kinetics (Yan and Surmeier, 1996), raising the question of the function of such a rich repertoire of calcium channels. We have shown that, as in many other neuron types, the calcium-dependent potassium currents that underlie the three AHPs exhibited by cholinergic interneurons are selectively coupled to specific calcium channels. The sAHP current is activated by calcium entry through Ca_v1 (L-type) channels, the mAHP current through SK channels is activated by calcium entry through and $Ca_v2.2$ (N-type) channels, and the contribution of the BK currents to spike repolarization is activated by calcium influx through $Ca_v2.1$ channels (apparently of the Q type). Each of these examples of specific coupling can be found individually in other neurons (Viana et al., 1993; Tanabe et al., 1998), but the rules of preferential coupling of calcium channels to AHPs are, in general, diverse and cell-type specific (Viana et al., 1993; Sah, 1995; Williams et al., 1997; Marrion and Tavalin, 1998; Pineda et al., 1998; Shah and Haylett, 2000; Vilchis et al., 2000; Cloues and Sather, 2003). Studying this selectivity in cholinergic interneurons provides a unique opportunity to probe the context in which it is played out (i.e., in modulating the ongoing firing patterns exhibited by these neurons). We have seen two dramatic examples of this: (1) a reduction in $Ca_v2.2$ currents can suppress SK currents enough to cause the neuron to burst (Fig. 3*d*) and (2) a reduction in Ca_v1 currents suppresses the sAHP current that helps regularize the rhythmic bursting, thereby leading to irregular firing patterns (Fig. 5). Thus, in cholinergic interneurons, the coupling between calcium currents and calcium-dependent mechanisms apparently maps onto the complex variety of oscillatory mechanisms in these neurons, with each of the calcium currents preferentially participating in a different oscillatory mechanism.

Control of firing patterns by selective coupling of calcium currents to potassium currents

Cholinergic interneurons exhibit two rhythmic firing patterns when recorded *in vitro*, including: (1) tonic discharge in a single-spiking mode and (2) rhythmic bursting (Bennett and Wilson, 1999). SK channels play an important role in regulating the single-spiking mode. They hyperpolarize the cell after each action potential, thereby activating hyperpolarization-activated cation (HCN) currents, which depolarize the cell enough to engage the activation of persistent sodium current, which can drive the cell to the action potential threshold (Bennett et al., 2000). Because SK currents are activated by calcium influx through $Ca_v2.2$ channels, the latter currents can control the amplitude of the mAHP, the level of depolarization, and the driven firing rate of the cell (Fig. 3*b*).

The sAHP has been studied traditionally in other neurons by eliciting trains of action potentials with somatic current injections (Hotson and Prince, 1980; Gustafsson and Wigstrom, 1983; Schwindt et al., 1988). In the context of the spontaneous activity of the cholinergic interneurons, we have proposed that the sAHP current contributes to burst termination and to the onset of the hyperpolarizations that follow each burst (Wilson and Goldberg, 2005). In the present study, we have shown that blocking Ca_v1 calcium currents reduces the sAHP current, reduces the hyperpolarizations exhibited by the cell, and disrupts apamin-induced bursting (Fig. 5). Similarly, reduction of the sAHP current by depletion of intracellular stores eradicates hyperpolarizations and promotes tonic single-spiking discharge (Fig. 6).

The slow rhythmic bursting pattern of cholinergic interneurons relies primarily on the interaction between the sAHP current and two hyperpolarization-activated currents (Wilson and Goldberg, 2005). During rhythmic bursting, the mAHP current is insufficient to repolarize the cell after each action potential. This leads to a sustained depolarization with a twofold effect: (1) persistent sodium conductance is not sufficiently deactivated, resulting in more action potentials at a higher instantaneous rate, and (2) there is more activation of Ca_v1 calcium currents. These two effects conspire to cause the sAHP current to accumulate, thereby terminating the burst and triggering the ensuing regenerative KIR-induced hyperpolarization (Wilson, 2005). Recovery from the deep hyperpolarization generated by the sAHP and KIR currents depends on the HCN currents. In cells lacking regenerative hyperpolarizations (e.g., after barium treatment, as in Fig. 5*a*), the sAHP current may sufficiently hyperpolarize the cell for HCN currents to be activated thereby initiating the depolarization toward the next burst.

Although similar in voltage sensitivity and kinetics, the result of their specific linkage to different calcium-dependent potassium currents causes Ca_v1 and $Ca_v2.2$ channels to have opponent influences on the spontaneous firing patterns of cholinergic interneurons. Ca_v1 currents promote long hyperpolarizations and rhythmic bursting, whereas $Ca_v2.2$ currents are responsible for mAHPs and sustained rhythmic single spiking. These two firing patterns are mutually exclusive. At any moment, a cholinergic interneuron may fire in only one of these patterns, and the rela-

tive strength of these two currents at any moment is pivotal in determining which oscillatory mechanism will dominate.

We have shown previously that BK currents shape the action potential waveform (Bennett et al., 2000). In the present study, we were interested in revealing the source of calcium that activates these channels. The current-clamp experiment we conducted in which we measured the width of the action potential was sufficient for this purpose. To study the dynamics of BK channels and their contribution to action-potential dynamics relative to other calcium-dependent and calcium-independent potassium channels will require additional voltage-clamp experiments such as those conducted on the SK and sAHP currents. However, these experiments would be more difficult, given the fast time scale of action-potential dynamics.

Selective coupling of calcium currents to potassium currents is a target for neuromodulation

Calcium currents are common targets of neuromodulation (Hille, 1994, 2001). In the striatum, dopamine and acetylcholine are the primary modulators. Ca_v1 in spiny neurons are reduced by the activation of D_2 dopamine receptors (Hernandez-Lopez et al., 2000) and are enhanced by the activation of D_1 dopamine receptors at depolarized potentials (Surmeier et al., 1995; Hernandez-Lopez et al., 1997), leading to an increased mAHP (Hernandez-Lopez et al., 1996). Activation of D_1 receptors also reduce Ca_v2 currents (N and P types) in spiny neurons (Surmeier et al., 1995).

In cholinergic interneurons, activation of muscarinic M_2 receptors reduces Ca_v2 currents (of the N and P type), but Ca_v1 currents are apparently not affected by muscarinic agonists (Yan and Surmeier, 1996). $Ca_v2.2$ currents are also reduced by the activation of D_2 receptors (Yan et al., 1997) and adenosine receptors (Song et al., 2000). Ca_v1 currents are not affected by the D_2 modulation (Yan et al., 1997).

These results suggest that the selective coupling of calcium currents to calcium-activated potassium channels may serve as a mechanism for neuromodulators to influence the firing patterns and spike waveforms of the cholinergic interneurons. Through the selective coupling of $Ca_v2.2$ to SK channels, the release of either dopamine or acetylcholine can presumably reduce the SK current underlying the mAHP by reducing the calcium influx through $Ca_v2.2$ channels. This modulation should promote the irregular and bursty patterns of cholinergic cell firing. Perhaps a different neuromodulator can control the action potential width by modulating $Ca_v2.1$ channels that are linked to BK channels. In spiny neurons, $Ca_v2.1$ are modulated by somatostatin (Vilchis et al., 2002). Because Ca_v1 currents are insensitive to muscarinic agonists and D_2 receptor activation, it is likely that their coupling to the sAHP current is not a mechanism for its modulation. However, in other neurons, the sAHP possesses many alternative mechanisms for direct neuromodulation (Vogalis et al., 2003), which may also be present in the cholinergic interneuron. The experimentally measured effects of neuromodulators on these calcium currents are modest, in the 20–30% range, which in a physiological context may not greatly alter the strength of calcium-activated potassium currents. However, in our experiments, reductions in calcium currents well under 100% produced substantial changes in firing patterns of cholinergic cells, suggesting that such moderate modulations of the currents may be physiologically significant.

Because a reduction in SK currents can lead to bursting, the proposed mechanism by which dopamine release causes a reduction in the SK currents (through the reduction $Ca_v2.2$ currents that are coupled to them) can help illuminate a recent finding from cholinergic interneurons recorded *in vivo* (Reynolds et al., 2004). Reynolds et al. (2004) found that cholinergic interneurons switched to persistent bursting for several minutes after the termination of high-frequency stimulation in the substantia nigra. This stimulation presumably released large amounts of dopamine into the striatum. It is possible that the excessive amount of dopamine, through its action on D_2 receptors, reduced $Ca_v2.2$ currents, which in turn caused a reduction in the SK currents. As we have shown, this can lead to persistent bursting (Fig. 3*d*). In parkinsonism, dopamine depletion leads to an increased release of acetylcholine (Grewaal et al., 1974; DeBoer et al., 1993). Because M_2 receptors also reduce $Ca_v2.2$ currents, a similar mechanism may contribute to the onset of oscillatory discharge in the tonically active neurons of striatum, which are the cholinergic interneurons (Kimura et al., 1984; Wilson et al., 1990; Aosaki et al., 1995), in parkinsonian primates (Raz et al., 1996).

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