Biophysical Characterization and Functional Consequences of a Slowly Inactivating Potassium Current in Neostriatal Neurons

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Gabel, Lisa A. and Eric S. Nisenbaum. Biophysical characterization and functional consequences of a slowly inactivating potassium current in neostriatal neurons. J. Neurophysiol. 79: 1989–2002, 1998. Neostriatal spiny projection neurons can display a pronounced delay in their transition to action potential discharge that is mediated by a slowly developing ramp depolarization. The possible contribution of a slowly inactivating A-type K⁺ current (I_{h}) to this delayed excitation was investigated by studying the biophysical and functional properties of I_{h}, using whole cell voltage- and current-clamp recording from acutely isolated neostriatal neurons. Isolation of I_{h} from other voltage-gated, calcium-independent K⁺ currents was achieved through selective blockade of I_{h} with low concentrations (10 μM) of the benzazepine derivative, 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (APB; SKF82958) and subsequent current subtraction. Examination of the voltage dependence of activation showed that I_{h} began to flow at approximately −60 mV in response to depolarization. The voltage dependence of inactivation revealed that 50% of I_{h} channels were available at the normal resting potential (−80 mV) of these cells, but that only 20% of the channels were available at membrane potentials corresponding to spike threshold (about −40 mV). At these depolarized membrane potentials, the rate of activation was moderately rapid (τ = 60 ms), whereas the rate of inactivation was slow (τ = 1.5 s). The time course of removal of inactivation of I_{h} at −80 mV also was relatively slow (τ = 1.0 s). The subthreshold availability of I_{h} combined with its rapid activation and slow inactivation rates suggested that this current should be capable of dampening the onset of prolonged depolarizing responses, but over time its efficacy should diminish, slowly permitting the membrane to depolarize toward spike threshold. Voltage recording experiments confirmed this hypothesis by demonstrating that application of APB at a concentration (10 μM) that selectively blocks I_{h} substantially decreased the latency to discharge and increased the frequency of firing of neostriatal neurons. The properties of I_{h} suggest that it should play a critical role in placing the voltage limits on the recurring episodes of subthreshold depolarization which are characteristic of spiny neurons recorded in vivo. However, the voltage dependence and recovery kinetics of inactivation of I_{h}, predict that its effectiveness will vary exponentially with the level and duration of hyperpolarization which precedes depolarizing episodes. Thus long periods of hyperpolarization should increase the availability of I_{h} and dampen succeeding depolarizations; whereas brief epochs of hyperpolarization should not sufficiently remove inactivation of I_{h}, thereby reducing its ability to limit subsequent depolarizing responses.

INTRODUCTION

A distinctive electrophysiological property of spiny projection neurons within the neostriatum is their ability to attenuate the amplitude of voltage transients evoked by subthreshold depolarizing current pulses (Bargas et al. 1989; Calabresi et al. 1987b; Galarraga et al. 1994; Jiang and North 1991; Kawaguchi et al. 1989; Kita et al. 1985a,b; Nisenbaum and Wilson 1995; Nisenbaum et al. 1994). This attenuation is manifested as a slowing of the rate of depolarization giving rise to a subthreshold ramp potential that can last for hundreds of milliseconds. When the depolarizing input is maintained, the ramp potential eventually will culminate in action potential discharge with the latency to first spike being prolonged (Bargas et al. 1989; Nisenbaum et al. 1994). Recently, we have studied the voltage dependence, kinetics, and pharmacological properties of this delayed excitation (Nisenbaum et al. 1994). Results showed that the slope of the ramp potential was dependent on the level of depolarization with the ramp becoming evident at membrane potentials near −65 mV. The magnitude of the delayed excitation also was largely dependent on the membrane potential from which it was evoked. More specifically, prior depolarization decreased the slope of the ramp potential and the latency to spike discharge, whereas a conditioning hyperpolarization produced the opposite effect. In addition, the kinetics of recovery from inactivation of the delayed response showed that relatively long periods (2–3 s) of hyperpolarization were required for the ramp depolarization to be developed fully. Pharmacologically, the delayed excitation was reduced by extracellular application of low concentrations (≤100 μM) of 4-aminopyridine (4-AP), implicating a potassium (K⁺) current(s) in this response (Bargas et al. 1989; Kita et al. 1985b; Nisenbaum et al. 1994).

A similar 4-AP–sensitive delayed excitation has been described in other cell types, including lateral geniculate nucleus (LGN) relay neurons (McCormick 1991), hippocampal CA1 pyramidal cells (Storm 1988), and prefrontal cortical neurons (Hammond and Crepel 1992). Several lines of evidence from these studies indicate that the delayed excitation depends on a slowly inactivating outward K⁺ current, termed I_{D} or I_{h}. These currents become available at subthreshold membrane potentials and have relatively rapid kinetics of activation and slow kinetics of inactivation. These properties of slowly inactivating K⁺ currents suggest that they should be capable of limiting the initial response to subthreshold depolarizing input but over time should slowly permit the membrane potential to depolarize toward spike threshold. In addition, both the delayed excitation and the slowly inactivating currents can be blocked by low micromolar concentrations of 4-AP, further supporting a role for these currents in the mediating this response (McCormick 1991; Storm 1988).

Voltage-clamp recording experiments from neostriatal spiny neurons have shown that these cells possess at least...
three types of calcium-independent, depolarization-activated K⁺ currents. These include 4-AP–sensitive fast (\(I_{\text{f}}\)) and slowly (\(I_{\text{s}}\)) inactivating A currents and a 4-AP–resistant, persistent K⁺ current (\(I_{\text{p}}\)) (Nisenbaum et al. 1996; Surmeier et al. 1988, 1991). The hypothesis that, similar to other cell types, \(I_{\text{f}}\) underlies the delayed excitation in neostriatal neurons has not been tested because it has not been possible to isolate this current from \(I_{\text{s}}\) and \(I_{\text{p}}\). However, we recently have shown that \(I_{\text{f}}\) can be selectively inhibited by low micromolar concentrations of the dopamine D₁ receptor agonist, 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (APB) (Nisenbaum et al. 1998). Moreover, the APB-induced reduction in \(I_{\text{f}}\) is not mediated by stimulation of D₁ receptors but rather by blocking the channels giving rise to this current. Therefore \(I_{\text{f}}\) can now be isolated from \(I_{\text{s}}\) and \(I_{\text{p}}\) by taking advantage of its sensitivity to APB, and a biophysical characterization of this current can be performed.

On the basis of our previous analysis of the delayed excitation in neostriatal spiny neurons (Nisenbaum et al. 1994), we predict that \(I_{\text{f}}\) should be endowed with several properties, including a relatively hyperpolarized voltage dependence of activation and inactivation, rapid kinetics of activation, slow kinetics of inactivation, and slow kinetics of recovery from inactivation. In addition, similar to the effects of 4-AP, depression of \(I_{\text{f}}\) by APB should reduce the delayed excitatory response of these neurons. These hypotheses were tested in the present experiments using both voltage- and current-clamp recording from acutely isolated neostriatal neurons.

**METHODS**

**Acute isolation of neostriatal neurons**

Neurons from adult (28–42 days old) rats were acutely isolated from the neostriatum using previously described procedures (Nisenbaum et al. 1996). Male, Sprague-Dawley rats were anesthetized deeply with methoxyflurane and decapitated. Their brains were removed rapidly from the skull and immersed in a cold (\(-2^\circ\)C) NaHCO₃-buffered saline solution, which contained (in mM) 126.0 NaCl, 2.0 KCl, 2.0 CaCl₂, 2.0 MgCl₂, 26.0 NaHCO₃, 1.25 NaH₂PO₄, 1.0 pyruvic acid, and 10.0 glucose; pH = 7.4, osmolarity = 300 ± 5 mOsm/l. The brains were blocked, and 400 μm thick coronal sections were cut through the rostrocaudal extent of the neostriatum using a Vibroslice (Campden Instruments, London, UK). Slices then were incubated at room temperature (\(20^\circ\)–\(22^\circ\)C) for 0.5–6.0 h in a holding chamber containing the continuously oxygenated (95% O₂-5% CO₂) NaHCO₃-buffered saline solution. After the incubation period, slices were transferred to a glass petri dish containing a low Ca²⁺, N-[2-hydroxyethyl]-piperazine-N’-[2-ethanesulfonic acid] (HEPES)-buffered saline solution that contained (in mM) 140.0 NaHCO₃, 6.0 CH₃SO₃H, Na isethionate, 2.0 KCl, 4.0 MgCl₂, 0.1 CaCl₂, 23.0 glucose, and 15.0 HEPES; pH = 7.4, osmolarity = 300 ± 5 mOsm/l and placed under a dissecting microscope. The dorsal neostriata from each hemisphere was dissected from the surrounding white matter and cortex. The neostriata were placed into a holding chamber containing protease Type XIV (1 mg/ml, Sigma Chemical, St. Louis, MO) dissolved in a HEPES-buffered Hank’s balanced salt solution (HBSS 6136; Sigma Chemical) maintained at 37°C and oxygenated (100% O₂). After 30–45 min of incubation in the enzyme solution, the neostriata were rinsed three times with the low Ca²⁺, HEPES-buffered saline solution and triturated using two fire-polished Pasteur pipettes having tips of decreasing diameter. Before whole cell recording, the cell suspension was placed into a 35-mm transparent plastic petri dish that was mounted onto the stage of an inverted microscope (Nikon, Tokyo, Japan). All voltage- and current-clamp recordings were performed at 22°C.

**Whole cell recordings**

The whole cell variant of the patch-clamp technique (Hamill et al. 1981) was used for recording from acutely isolated neostriatal neurons. Electrodes were pulled from borosilicate capillary tubing (Corning 7052, WPI, Sarasota, FL) using a multistage puller (Sutter Instruments, Novato CA). The electrodes were fire-polished with a microforge before use. The internal electrode filling solution contained (in mM) 140.0 NaCl, 1.0 KCl, 4.0 CaCl₂, 1.0 MgCl₂, 0.4 CdCl₂, 1.0 HEPES, 10.0 glucose, and 0.001 tetrodotoxin (TTX); pH was adjusted to 7.4 with NaOH to 1.0 M; osmolarity was adjusted to 300 ± 5 mOsm/l. TTX and CdCl₂ were added to block voltage-dependent Na⁺ and Ca²⁺ channels, respectively. Application of drugs was accomplished using a five-barrel pipette array made from small diameter (~500 μm) glass capillary tubing. Solutions were contained in 10-ml syringes and positioned ~12 in above the recording chamber. Gravity-induced flow of each solution from the syringe to the corresponding barrel was controlled by electronic valves. The pipette array was positioned 100–200 μm from the cell before seal formation. The isolated cells were bathed continuously in HBSS, which facilitated washing of drug solutions. The solutions from the drug array were rapidly changed (<20 ms) by altering the array position with a DC motorized actuator (Warner Instruments, Hamden, CT).

Upon placing the recording electrode in the bath, offset potentials were corrected and electrode resistances ranged between 2 and 7 MΩ. To reduce the effect of changes in junction potentials associated with alterations in ionic conditions, the bath ground was a 3 M KCl agar bridge to a Ag/AgCl ground well. A small amount of constant positive pressure (2–3 cm H₂O) was applied to the electrode as it was advanced through the bath. Once the electrode tip made contact with the cell membrane, negative pressure was applied to the back of the electrode to form a high-resistance seal between the electrode tip and the cell membrane and subsequently to achieve the whole cell configuration. After achieving the whole cell configuration, series resistance was compensated (60–80%) and monitored periodically. Because the whole cell K⁺ currents recorded typically were ~<2 nA, errors in voltage resulting from inadequate compensation would not have exceeded a few millivolts. Voltage-clamp recordings were conducted using an Axon Instruments 200A amplifier (Axon Instruments, Foster City, CA). Currents were digitized and monitored with pClamp software (Axon Instruments) running on a PC pentium clone computer.

For some experiments, current-clamp recording of isolated neurons was performed. In these experiments, the whole cell configuration was achieved in voltage-clamp mode using the procedures described above. The I-CLAMP NORMAL mode of the Axopatch 200A amplifier was used during voltage recordings to minimize errors in the response times. At the end of each recording, any pipette offset potentials were measured and used for determining the membrane potential recorded during the experiment.

**Pharmacological agents**

APB (SKF82958) was obtained from Research Biochemicals International (Natick, MA). A stock solution of 2 mM APB dis-
solved in a 0.1% ascorbic acid solution was apportioned into 50-μl aliquots and stored a −30°C until the day of recording. Solutions containing APB were kept covered to protect from exposure to light during the experiments. BAPTA, GTP, leupeptin, Na₂ATP, and TTX were purchased from Calbiochem (La Jolla, CA). All other chemicals were obtained from Sigma Chemical.

RESULTS

The results of the present study were collected from recordings of 77 neurons. Acutely isolated neurons selected for recording were all ~10 μm in diameter and had an average whole cell capacitance of 7.2 ± 0.4 pF (mean ± SD; range = 1.4–11.8 pF). Although the morphological identity of these neurons was not determined, several anatomic studies have shown that the spiny projection neurons of the neostriatum (~95%) (Chang et al. 1982; DiFiglia et al. 1976; Kemp and Powell 1971). In addition, previous studies have shown that acutely isolated spiny neurons that were identified after an injection of retrogradely transported fluorescent beads into the substantia nigra have whole cell capacitance values within the range reported here (Surmeier et al. 1992a, 1995). Therefore the population of unidentified neurons recorded here was assumed to be composed primarily of spiny projection cells.

Delayed excitation is present in acutely isolated neostriatal neurons

The acutely isolated cell preparation was chosen for whole cell voltage-clamp recording because the dendritic arbor of these cells is dramatically truncated, permitting complete voltage control of the cell membrane and thus an accurate quantitative description of $I_{\text{A}}$. However, a critical assumption in these experiments was that $I_{\text{A}}$ and other currents giving rise to the delayed excitation in neostriatal neurons still would be present in this preparation. To test this hypothesis, whole cell current-clamp recordings from acutely isolated neurons initially were performed. Similar to intracellular recordings from spiny neurons in vivo or in neostriatal slice preparations, subthreshold intracellular depolarizing current pulses (500-ms duration) consistently evoked a slowly depolarizing ramp potential in isolated neostriatal neurons ($n = 26$; Fig. 1A). When threshold depolarizing current pulses were delivered, the ramp depolarization culminated in an action potential discharge at the end of the response (Fig. 1B). Identical current pulses reduced the latency to spike discharge and increased the frequency of firing when the resting membrane potential was more depolarized (Fig. 1C). These results indicate that the currents underlying the ramp depolarization and prolonged latency to discharge are present in the somata of spiny cells and that recruitment of dendritic currents is not required to elicit the delayed excitatory response.

APB selectively blocks $I_{\text{A}}$

An accurate description of the biophysical properties of $I_{\text{A}}$ required that this current be isolated from the other polarization-activated K⁺ currents. Previous studies have isolated slowly inactivating K⁺ currents by taking advantage of their preferential sensitivity to low micromolar concentra-

![Figure 1](https://example.com/figure1.png)

**FIG. 1.** Delayed excitation is present in acutely isolated neostriatal neurons. A: voltage deflection produced by a subthreshold intracellular depolarizing current pulse (0.01 nA, 500-ms duration) from relatively hyperpolarized membrane potentials displayed a pronounced ramp potential. B and C: injection of the same amplitude current pulse when the membrane was depolarized evoked action potential discharges with decreasing latencies.
of the APB-sensitive current with $I_{D}$.

Comparison of the APB-insensitive current from the total $K_p$ currents, $I_{Af}$, and $I_{KRP}$, could be evoked by stepping the membrane potential to $-40 \text{ mV}$ for 5 s and then delivering a brief (25 ms) hyperpolarizing voltage step before a test step to $+35 \text{ mV}$ (Fig. 2A). Subtraction of these rapidly recovering currents from the total current evoked by the test step alone isolated $I_{As}$ (Fig. 2B). In the same neuron, application of 10 $\mu$M APB reduced the total $K^+$ current evoked by the test step alone, leaving the APB-insensitive current (Fig. 2C). Subsequent subtraction of the APB-insensitive current from the total $K^+$ current isolated the APB-sensitive current (Fig. 2D). Comparison of the APB-sensitive current with $I_{As}$ showed that their time courses were nearly identical (Fig. 2E). These results confirm that $I_{As}$ can be isolated on the basis of its preferential sensitivity to APB, and in subsequent experiments, $I_{As}$ was defined as the 10 $\mu$M APB-sensitive current.

Indicates that the ramp depolarization emerged from a competition between depolarizing inward Na$^+$ and Ca$^{2+}$ currents and polarizing outward K$^+$ currents (Bargus et al. 1989; Kita et al. 1985a; Nisenbaum et al. 1994). If $I_{As}$ contributes to the outward K$^+$ flux, then this current must flow at subthreshold membrane potentials. This hypothesis was tested by examining the voltage dependence of steady-state activation of $I_{As}$. The whole cell $K^+$ current was evoked by 500-ms depolarizing voltage steps from $-70$ to $+35 \text{ mV}$ in 15-mV increments (holding potential $= -90 \text{ mV}$) during control conditions (Fig. 3A). With the same protocol, $K^+$ currents were elicited in the presence of 10 $\mu$M APB (Fig. 3B). Subsequent subtraction of the APB-insensitive currents from the control currents yielded $I_{As}$ (Fig. 3C). This same subtraction procedure was used to isolate $I_{As}$ in all of the following figures. Having isolated $I_{As}$, the voltage dependence of activation was assessed by first converting the current amplitudes to chord conductances and then normalizing each conductance by the maximum conductance evoked by the voltage step to $+35 \text{ mV}$. For all cells tested ($n = 15$), the normalized conductances at each membrane potential were calculated and a plot of the average normalized conductance as a function of membrane potential was constructed (Fig. 3D). The plotted points were fit with a Boltzmann function of the form

$$g/g_{max} = 1/(1 + \exp([V_m - V_0]/V_1))$$

where $g$ is conductance, $g_{max}$ is the maximum conductance.
Approximately one-half of $I_{As}$ channels are available at the resting membrane potential

Previous studies have shown that the magnitude of the delayed excitation is directly related to the degree of membrane polarization (Nisenbaum et al. 1994). More specifically, the slope of the ramp potential and the latency to spike discharge increase with membrane hyperpolarization to potentials near rest and decrease with depolarization to potentials near spike threshold. One contributing factor to the voltage dependence of the delayed excitatory response could be that there are fewer $I_{As}$ channels available at more depolarized membrane potentials. This possibility was tested by measuring the voltage dependence of steady-state inactivation of $I_{As}$. The membrane potential was stepped from $-80 \text{ mV}$ to potentials between $-110$ and $-10 \text{ mV}$ for $5 \text{ s}$ before delivering a test step to $+35 \text{ mV}$ for $250 \text{ ms}$. The largest currents were elicited following the most hyperpolarized conditioning potentials (Fig. 4A). For all cells ($n = 7$), the current amplitudes were normalized relative to the maximum amplitude and the average normalized current was plotted as a function of the conditioning membrane potential (Fig. 4B). The plotted points were fit with a Boltzmann function of the form

$$I/I_{\text{max}} = 1/(1 + \exp[(V_m - V_h)/V_c])$$

where $I$ is the current evoked by the test step to $0 \text{ mV}$ from each conditioning potential, $I_{\text{max}}$ is the current evoked by the test step following the conditioning step to $-110 \text{ mV}$, $V_m$ is the membrane potential, $V_h$ is the half-inactivation voltage, and $V_c$ is the slope factor. Inspection of the inactivation curve shows that $I_{As}$ was slightly inactivated at $-100 \text{ mV}$ and $\sim 90\%$ inactivated at $-50 \text{ mV}$. The average $V_h$ and $V_c$ values of $I_{As}$ were $-78.8 \pm 5.1 \text{ mV}$ and $10.4 \pm 2.2 \text{ mV}$, respectively. These data indicate that $\sim 50\%$ of $I_{As}$ channels are available at $-80 \text{ mV}$, which is near the resting potential of spiny neurons recorded in vivo and in neonatal slice preparations using similar extracellular concentrations of $K^+$ (Nisenbaum and Wilson 1995; Wilson and Kawaguchi 1996). By contrast, fewer than $10\%$ of $I_{As}$ channels are available at membrane potentials near spike threshold (about $-40 \text{ mV}$). The relatively large percentage of $I_{As}$ channels inactivated at the resting potential coupled with the moderately steep slope factor indicates that sustained depolarizations from rest will significantly reduce the availability of this current. This reduction in $I_{As}$ should consequently contribute to the decreased slope of the ramp potential and latency to discharge when evoked from more depolarized membrane potentials (Nisenbaum et al. 1994).
Activation and deactivation kinetics of \( I_{As} \) are voltage dependent

The analysis of the voltage dependence of activation and inactivation of \( I_{As} \) indicated that \(~50\%\) of these channels are available at the resting membrane potential and that this current can be recruited by subthreshold depolarizations. The availability of this current supports the hypothesis that it contributes to the subthreshold ramp potential in spiny neurons. However, for \( I_{As} \) to limit the initial response to depolarizing input also requires that its kinetics of activation be relatively rapid at subthreshold membrane potentials. The kinetics of activation of \( I_{As} \) were assessed by delivering 150-ms voltage steps (15-mV increments) from \(-70\) to \(+35\) mV (holding potential, \(-90\) mV; \(n = 16\)). Examination of the current traces showed that \( I_{As} \) had a delayed onset that decreased with larger depolarizations (Fig. 5A). Because of

![Figure 4](image1)

**Fig. 4.** Approximately 50% of \( I_{As} \) channels are available at the resting potential of neostriatal neurons. A: voltage dependence of steady-state inactivation of \( I_{As} \) was evaluated by stepping the membrane potential to values between \(-110\) and \(-10\) mV (10-mV increments) for 5 s before delivering a test step to 0 mV. Responses of \( I_{As} \) to the test step are shown with the largest current evoked after the most hyperpolarizing conditioning step \((-110\) mV) and the smallest current elicited after the most depolarizing conditioning step \((-10\) mV). B: relationship between the average normalized peak current and conditioning membrane potential are plotted. A Boltzmann fit of the points shows that approximately one-half of the channels are available at membrane potentials corresponding to the resting potential in vivo. Note that the \( V_i \) and \( V_c \) values for \( I_{As} \) were derived from the Boltzmann fit of the average normalized conductance values presented in the plot. These values differ slightly from average \( V_i \) and \( V_c \) values presented in the text.

![Figure 5](image2)

**Fig. 5.** Activation and deactivation kinetics of \( I_{As} \) are voltage dependent. A: \( I_{As} \) was evoked by 150-ms voltage steps from \(-70\) to \(+35\) mV (15-mV increments, holding potential, \(-90\) mV). Currents exhibited a delayed onset, with the delay becoming shorter with stronger depolarizations. Second-order kinetics were used to fit activation of the \( I_{As} \). Fitted curves for each trace are superimposed as solid lines. B: kinetics of deactivation of \( I_{As} \) were studied by stepping the membrane potential from \(-90\) to \(+35\) mV for 100 ms before delivery of 150-ms test steps from \(-25\) to \(-115\) mV (15-mV increments). Tail currents elicited upon deactivation were fit using single exponential functions, and the fitted curves are superimposed as solid lines. C: average time constants of activation and deactivation derived from curve fits like those presented in A and B are plotted. Examination of the entire activation/deactivation profile illustrates the voltage dependence of these processes.
this delay, the currents were best fit using a single second-order exponential function

\[ I_{\text{max}} = I_{\text{max}} \left(1 - \exp \left(\frac{t - \tau}{\tau} \right) \right)^2 \]

where \( I_{\text{max}} \) is the maximum amplitude of each current, \( t \) is time after the onset of the voltage step, and \( \tau \) is the time constant of activation of each current. In Fig. 5A, the exponential fits of the currents are plotted as overlapping lines. The time constants of activation were dependent on the test by the fact that the time constants of activation (73.4 ± 11.9 ms) and deactivation (76.1 ± 13.5 ms) were similar for test steps to −25 mV. The entire activation/deactivation time constant profile had an inverted-U shape with values increasing as a function of step potential to −25 mV and decreasing at depolarized membrane potentials (Fig. 5C). The average time constants of inactivation varied as a function of step potential, becoming faster at more hyperpolarized membrane potentials.

**Fig. 6.** \( I_{\text{max}} \) has slow kinetics of inactivation. A: \( I_{\text{max}} \) was evoked by 8-s depolarizing voltage steps from −70 to +35 mV (15-mV increments, holding potential, −90 mV). Decay of \( I_{\text{max}} \) was well fit by single exponential functions, which are superimposed over the traces as solid lines. Values adjacent to each curve fit reflect the time constants of inactivation for those currents at the membrane potential indicated. B: relationship between the average time constants of inactivation and membrane potential for \( I_{\text{max}} \) are plotted. C: for most neurons, the amplitudes of the currents evoked by steps to membrane potentials greater than or equal to −55 mV were relatively small, making it difficult to accurately measure inactivation kinetics. To obtain a more accurate measure of inactivation kinetics at these potentials, currents were elicited by stepping the membrane potential from −90 mV to the desired potential (e.g., −55 mV) for 1 ms and then stepping to +35 mV for 20 ms before delivering a hyperpolarizing step to −55 mV. Successive steps from −55 to +35 mV then were delivered at intervals ranging from 10 ms to 8.3 s. Amplitude of the currents evoked in this manner decreased as a function of time after the initial voltage step to −55 mV. D: when the peak current values were plotted as a function of time after the initial voltage step, the points could be fit with a single exponential function. Responses evoked from −55 mV decayed with an average time constant of ~600 ms. E: with the fitted parameters from the Boltzmann functions describing the voltage dependencies of activation and inactivation of \( I_{\text{max}} \), normalized activation (\( m_+ \)) and inactivation (\( h_+ \)) curves were generated. Comparison of the activation curve for the nonactivating component of \( I_{\text{max}} \) (dashed line) with the \( m_+ \) curve shows that their voltage dependencies are very similar. Minimal overlap in the \( m_+ \) and \( h_+ \) curves suggests that their is very little window current associated with \( I_{\text{max}} \). F: normalized window conductance was calculated by multiplying the \( m_+ \) and \( h_+ \) curves and the graph shows that at approximately −55 mV, the maximal conductance was only ~0.3% of the total conductance for \( I_{\text{max}} \).
activation for subthreshold membrane potentials ranged from 23.7 ± 9.2 ms at −85 mV to 59.6 ± 10.5 ms at −40 mV. These kinetics of activation of \( I_{\text{Na}} \) at subthreshold voltages are relatively rapid and would be expected to be sufficiently fast to attenuate the initial component of a depolarizing voltage transient.

**Inactivation kinetics of \( I_{\text{Na}} \) are slow**

Perhaps the most obvious attribute of the delayed excitation is the long latency to spike discharge. Depending on the amplitude of injected current, this delay can easily last for hundreds of milliseconds (Nisenbaum et al. 1994). For \( I_{\text{Na}} \) to contribute to the prolonged latency to spike discharge, this current must be endowed with relatively slow kinetics of inactivation. The time constants of inactivation of \( I_{\text{Na}} \) (\( n = 14 \)) were assessed by delivering 8-s voltage steps from −70 to +35 mV in 15-mV increments (holding potential, −90 mV) and fitting the decay of the evoked currents with a single exponential function of the form

\[
I = A(\exp(-t/\tau)) + c
\]

where \( A \) is the amplitude of the inactivating component of the current, \( t \) is time, \( \tau \) is the time constant of inactivation, and \( c \) is a constant term representing the noninactivating component of the current. It is important to note that for all currents, a component of \( I_{\text{Na}} \) was noninactivating even after 8-s depolarizations, requiring the added constant term in the exponential equation (see further text). The small amplitude of the currents elicited by voltage steps to potentials more hyperpolarized than −40 mV precluded an accurate measurement of their inactivation kinetics (Fig. 6A). Therefore, only the responses to more depolarized potentials were evaluated in this manner. The time constants of inactivation varied as a function of test step potential. The currents evoked by the most depolarized test steps (+5 to +35 mV) inactivated with time constants of ~2 s. The longest time constants of inactivation were associated with voltage steps to −10 mV (2.9 ± 1.6 s) and −25 mV (2.9 ± 1.7 s). At membrane potentials corresponding to spike threshold (−40 mV), the time constant of inactivation was 1.5 ± 0.6 s (Fig. 6B).

The kinetics of inactivation at more hyperpolarized membrane potentials were evaluated by stepping from −90 to −55 mV for 1 ms and then delivering successive 20-ms steps from −55 to +35 mV at intervals ranging from 10 ms to 8.3 s (\( n = 6 \)). The currents evoked by the test steps +35 mV are shown in Fig. 6C. The largest current was elicited after the 1-ms step to −55 mV; the smallest current was evoked after 8.3 s at −55 mV. The amplitude of the currents decreased as a function of time after the initial voltage step to −55 mV. The peak amplitudes of the currents evoked by the successive steps to +35 mV were plotted against the time after the initial test stimulus onset, and the points were fit with a single exponential function (Fig. 6D). With this method, the average time constant of inactivation at −55 mV was 0.6 ± 0.2 s.

As described earlier, a noninactivating component of the current was evident even after 8-s depolarizations. This noninactivating component could arise from several different mechanisms, including a window current predicted by the steady-state voltage dependencies of activation and inactivation, a noninactivating component of \( I_{\text{Kr}} \), and/or contamination from the persistent K⁺ current, \( I_{\text{Kp}} \), as a result of partial blockade of this current by APB. To distinguish between the first two possibilities, the predicted window current was assessed by examining the degree of overlap of the normalized activation (\( m_\infty \)) and inactivation (\( h_\infty \)) curves for \( I_{\text{Na}} \). The \( m_\infty \) and \( h_\infty \) curves for voltages ranging from −115 to +35 mV were generated using the fitted parameters from the Boltzmann functions describing the voltage dependencies of activation and inactivation of \( I_{\text{Na}} \) (Fig. 6E). Inspection of the curves shows that they overlap minimally, suggesting that the predicted window current should be small. Indeed, calculation of the window conductance by multiplying the \( m_\infty \) and \( h_\infty \) curves that at approximately −55 mV, the maximal conductance was only ~0.3% of the total conductance for \( I_{\text{Na}} \) (Fig. 6F). In addition, if the noninactivating component arises from a window conductance, then their voltage-dependent properties should be the same. Conversely, if the noninactivating component represents a true sustained portion of \( I_{\text{Na}} \), then its voltage dependence should be similar to the \( m_\infty \) curve. To test these hypotheses, the activation curve for the nonactivating component was cal-

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**FIG. 7.** Kinetics of recovery from inactivation of \( I_{\text{Na}} \) are relatively slow. A: recovery kinetics were assessed by stepping the membrane potential from −80 to −40 mV for 4 s, then delivering a conditioning step to −80 mV for periods of time ranging from 1 ms to 10 s before delivering a 150-ms test step to 0 mV. Largest currents were evoked after the longest conditioning steps. B: for each cell tested, the current amplitudes were normalized relative to the amplitude after a 10-s conditioning step, and the average normalized currents were plotted as a function of conditioning step duration. Time constant of recovery from inactivation of these averaged normalized values was 1.0 s. Note this value differs slightly from average time constant of recovery from inactivation presented in the text.
culated by plotting the normalized conductance values of the constant term, \(c\), derived from the exponential fits of the decay phase of each current by membrane potential, and fitting these points with a Boltzmann function. Comparison of the activation curve for the noninactivating component of \(I_{\text{As}}\) (dashed line) with the \(m_a\) curve shows that their voltage dependencies are very similar (Fig. 6E). More importantly, the voltage dependence of the noninactivating component does not parallel that of the window conductance (Fig. 6F).

The last possibility that the noninactivating component reflects a contribution from \(I_{\text{Krp}}\) also seems unlikely because the voltage dependence of activation of the noninactivating component \((V_h = -2.0 \text{ mV})\) is \(\sim 10 \text{ mV}\) more depolarized than that for \(I_{\text{Krp}}\) \((V_h = -13.0 \text{ mV})\) (Nisenbaum et al. 1996). In addition, we previously have shown that \(I_{\text{Krp}}\) can be isolated in the presence of 10 mM 4-AP (Nisenbaum et al. 1996) and that this current is not affected by APB (Nisenbaum et al. 1998). Collectively, these data indicate that the noninactivating component of \(I_{\text{As}}\) reflects a sustained portion of this current.

**Kinetics of recovery from inactivation of \(I_{\text{As}}\) are slow**

Our previous analysis of the delayed excitation in spiny neurons revealed that the prolonged latency to spike discharge evoked by a depolarizing current pulse was highly dependent on the preceding duration of hyperpolarization. That is, a delayed spike discharge was evident only after the membrane potential had been hyperpolarized for several seconds before delivery of the test current pulse (Nisenbaum et al. 1994). To the extent that \(I_{\text{As}}\) contributes to this delay, these data suggest that the availability of this current should increase with longer periods of hyperpolarization. This hypothesis was tested by measuring the kinetics of recovery from inactivation of \(I_{\text{As}}\). The voltage protocol involved stepping the membrane potential from \(-80\) to \(-40 \text{ mV}\) for 4 s and subsequently delivering a conditioning step to \(-80 \text{ mV}\) for periods of time ranging from 1 ms to 10 s before delivery of a test step to 0 mV. The currents evoked by the test step are shown in Fig. 7A. The largest currents were evoked after the longest conditioning step durations. For all cells \((n = 10)\) tested, the current amplitudes were normalized relative to the amplitude after a 10-s conditioning step, and the average normalized currents were plotted as a function of conditioning pulse duration. The average time constant of recovery from inactivation of \(I_{\text{As}}\) was \(1.2 \pm 0.4 \text{ s}\) (Fig. 7B). The range of recovery kinetics was \(0.9-2.2 \text{ s}\). The relatively slow recovery kinetics of \(I_{\text{As}}\) are consistent with a contribution of this current to the delayed excitation after prolonged periods of hyperpolarization.

**APB-induced decrease in delayed excitation is voltage dependent**

The selectivity of 10 \(\mu\text{M}\) APB for \(I_{\text{As}}\) permitted a direct examination of the contribution of this current to the delayed...
excitation in spiny neurons. During control conditions, a threshold depolarizing current pulse delivered to a cell resting at a relatively hyperpolarized membrane potential evoked a single spike discharge at the end of the response (Fig. 8A). The ramp potential underlying the prolonged latency to discharge also was evident. Subsequent application of APB (10 μM) decreased the latency to discharge from $346.0 \pm 148.0$ ms during control conditions to $157.8 \pm 65.9$ ms [$t(6) = 3.1, P < 0.05$] in the presence of the drug. The frequency of firing and the duration of action potential discharge also were increased after blockade of $I_{Na}$ (Fig. 8B). These results support the hypothesis that $I_{Na}$ contributes to the ramp depolarization and delayed spike discharge in spiny neurons.

The results of the biophysical analysis of $I_{Na}$ showed that this current had a relatively hyperpolarized voltage dependence of inactivation. In particular, ~50% of $I_{Na}$ channels were inactivated at $-80$ mV and >90% of these channels were unavailable at $-40$ mV. One predicted consequence of this property of $I_{Na}$ is that sustained depolarization should inactivate these channels and thus diminish the latency to discharge and increase the frequency of discharge in response to a test pulse. This hypothesis was confirmed by recording from cells at several membrane potentials and showing that the latency to discharge decreased and the frequency of firing increased as a function of membrane depolarization (Fig. 8, C–E). An additional implication of the voltage dependence of inactivation of $I_{Na}$ is that blockade of these channels should have little effect on the latency and frequency of discharge when evoked from a depolarized membrane potential due to the large degree of inactivation of this current. To test this prediction, a suprathreshold stimulus was delivered to a neuron at approximately $-60$ mV during control conditions and elicited a high frequency of discharge having a short delay to onset (Fig. 9A). In contrast to effects observed at more hyperpolarized membrane potentials, subsequent application of APB (10 μM) had little effect on the latency or frequency of the response (Fig. 9B).

**DISCUSSION**

$I_{Na}$ underlies the delayed excitatory response of spiny neurons

The delayed transition to discharge of neostriatal spiny neurons is an electrophysiological property that distinguishes these cells from other neurons in the neostriatum (Kawaguchi 1992, 1993). A similar delayed excitatory response was described first in hippocampal pyramidal CA1 neurons and was shown to depend on recruitment of a slowly inactivating $K^+$ conductance (Storm 1988). Voltage-clamp recordings from neostriatal neurons also have identified a slowly inactivating A-type $K^+$ current ($I_{Na}$), raising the possibility that, similar to CA1 neurons, this current is responsible for the delayed excitation in neostriatal cells (Surmeier et al. 1991). In the present experiments, the hypothesis that $I_{Na}$ contributes to the delay in transition to discharge of neostriatal neurons was investigated. Toward this end, a biophysical analysis of $I_{Na}$ initially was performed to determine if the voltage dependence and kinetics of this current were consistent with that of the delayed excitatory response (Nisenbaum et al. 1994). However, a prerequisite for such an analysis was a method for isolating $I_{Na}$ from the other voltage-gated $K^+$ currents in neostriatal neurons. Our recent observation that the dopamine D1 receptor agonist, APB, selectively blocks $I_{Na}$ provided a pharmacological tool by which $I_{Na}$ could be isolated (Nisenbaum et al. 1998). By taking advantage of its preferential sensitivity to APB, $I_{Na}$ could be separated from $I_{AI}$ and $I_{Ksp}$ through current subtraction, and a biophysical characterization of this current then could be conducted. In addition, the selectivity of APB for $I_{Na}$ permitted a direct assessment of the contribution of this current to the transition to discharge of neostriatal neurons in current-clamp experiments.

The results from the biophysical experiments strongly support a role for $I_{Na}$ in the delayed excitation of neostriatal neurons. First, the availability of $I_{Na}$ at subthreshold membrane potentials is consistent with the subthreshold nature of the ramp depolarization (Bargas et al. 1989; Kawaguchi et al. 1989; Kita et al. 1985a,b; Nisenbaum et al. 1994). Second, the voltage dependence of inactivation of $I_{Na}$ is similar to that of the delayed spike discharge of spiny neurons. More specifically, a conditioning depolarization to membrane potentials near $-60$ mV inactivated ~80% of $I_{Na}$ channels and produced a corresponding decrease in the latency to spike discharge. In contrast a conditioning hyperpolarization to approximately $-100$ mV deinactivated nearly 100% of $I_{Na}$ channels and produced a concomitant increase in the latency to discharge. Third, the kinetics of activation of $I_{Na}$...
are relatively rapid at subthreshold membrane potentials, having time constants of activation ranging from 20.2 to 59.6 ms. Assuming a temperature coefficient of 2–3 (Huguenard and Prince 1991), the time constant of \( I_{\Delta h} \) activation should be \( \sim 15–25 \) ms at \(-60 \) mV, which is consistent with the predicted ability of this current to limit the initial response to depolarizing input. Fourth, the slow time course of inactivation of \( I_{\Delta h} \) at subthreshold membrane potentials is consistent with the shallow trajectory of the ramp potential. For example, the time constant of inactivation of \( I_{\Delta h} \) at \(-40 \) mV (approximately spike threshold) was 1.5 s and should be sufficiently long to permit this current to dampen threshold depolarizing responses and account for the delay to spike discharge, which can last for hundreds of milliseconds (Bargas et al. 1989; Kawaguchi et al. 1989; Nisenbaum et al. 1994). Finally, the moderately slow kinetics of recovery from inactivation of \( I_{\Delta h} \) are similar to the slow recovery kinetics of the delayed excitation. Previous results have shown that full development of the ramp potential and delayed spike discharge requires prior hyperpolarization of the membrane for 1–2 s (Nisenbaum et al. 1994; Surmeier et al. 1992b). The time constant of recovery from inactivation of \( I_{\Delta h} \) at \(-80 \) mV was 1.2 s, which is consistent with this requirement. Collectively, the similarities between the voltage dependence and kinetics of \( I_{\Delta h} \) and the delayed excitation support the hypothesis that this current makes a significant contribution to the transition to firing of neostriatal spiny neurons.

A role for \( I_{\Delta h} \) in the delayed excitation also was supported by the results of the voltage-recording experiments. The latency to spike discharge was reduced substantially after application of 10 \( \mu \)M APB, which selectively blocks \( I_{\Delta h} \). A similar decrease in spike latency and an increase in frequency of firing was produced by depolarization of the membrane to potentials approaching spike threshold (e.g., \(-60 \) mV) before delivery of the test stimulus. At these membrane potentials, only \( \sim 20\% \) of \( I_{\Delta h} \) channels are available, suggesting that the decreased spike latency and increased frequency of discharge resulted from inactivation of \( I_{\Delta h} \) channels. Moreover, subsequent application of APB at these depolarized membrane potentials had little effect on the delayed excitation as would be expected if \( I_{\Delta h} \) channels already were inactivated. It should be noted that an additional mechanism underlying the APB-induced decrease in the delayed excitation could have come in part from the dopamine \( D_1 \) receptor-mediated effects of this compound on inward \( Na^+ \) and \( Ca^{2+} \) currents. Acutely isolated neostriatal neurons are known to possess \( D_1 \) receptors (Surmeier et al. 1996), and \( D_1 \) receptor stimulation has been shown to reduce both of these inward currents in this preparation (Surmeier et al. 1992a, 1995). However, a reduction in \( Ca^{2+} \) currents most likely did not contribute to the decrease in the delayed excitation because the extracellular solution in these experiments contained the \( Ca^{2+} \) channel blocker, CdCl\(_2\), at a concentration (400 \( \mu \)M) that previously has been shown to block all \( Ca^{2+} \) currents in these neurons (Bargas et al. 1994; Surmeier et al. 1995). A substantial depression of \( Na^+ \) currents also seems unlikely because this effect would be expected to further the delay to spike discharge by increasing the level of depolarization required to reach threshold.

Evidence from several studies indicates that the ramp depolarization and delayed spike discharge of spiny neurons emerges from the competing effects of depolarizing inward \( Na^+ \) and \( Ca^{2+} \) currents and the polarizing outward \( K^+ \) current, \( I_{\Delta h} \). As such, blockade of inward \( Na^+ \) and/or \( Ca^{2+} \) currents diminishes the steepness of the trajectory of the ramp potential (Bargas et al. 1989; Calabresi et al. 1987a; Galarreta et al. 1994; Kita et al. 1985a; Nisenbaum et al. 1994). In addition, selective blockade of \( I_{\Delta h} \) with either low concentrations of 4-AP (Nisenbaum et al. 1994) or APB (present results) decreases the slope of the ramp depolarization and the latency to spike discharge. However, elimination of either the inward currents or \( I_{\Delta h} \) alone does not completely abolish the ramp potential, indicating that none of these currents is solely responsible for the response (Kita et al. 1985a; Nisenbaum et al. 1994). Taken together, these results suggest that subthreshold depolarizing current recruitment inward \( Na^+ \) and \( Ca^{2+} \) currents, which act to further depolarize the membrane, and \( I_{\Delta h} \), which tends to slow the rate of this depolarization. However, differences in their voltage dependencies and kinetics suggest that these currents will predominate at different voltages and times during the response to depolarization. Because \( I_{\Delta h} \) is available at more hyperpolarized membrane potentials compared to either \( Na^+ \) or \( Ca^{2+} \) currents (Bargas et al. 1994; Surmeier et al. 1992a), it would be expected to primarily govern the initial portion of the depolarizing response. As the membrane potential becomes more depolarized, inward \( Na^+ \) and \( Ca^{2+} \) currents will flow more readily and will compete with \( I_{\Delta h} \). As \( I_{\Delta h} \) gradually inactivates, the net ionic flow will shift more toward the inward currents and the membrane potential will slowly depolarize toward spike threshold. Thus the interplay between these inward and outward ionic conductances is postulated to give rise to a slowly developing depolarization, which will proceed until termination of the current pulse or the membrane potential reaches spike threshold (Nisenbaum et al. 1994). Moreover, the presence of the delayed excitation in acutely isolated neostriatal neurons indicates that the ionic channels subserving this response are expressed on the perisomatic membrane of these cells.

The possibility that \( I_{\Delta h} \) and/or \( I_{KRP} \) contribute to the delayed excitation of spiny neurons also should be considered. A role for \( I_{\Delta h} \) in the response seems unlikely because it does not become available until the membrane potential reaches spike threshold and its rapid inactivation rate is not consistent with the slow development of the ramp potential (Surmeier et al. 1988). In contrast to \( I_{\Delta h} \), \( I_{KRP} \) becomes available at subthreshold membrane potentials and has an inactivation rate which is approximately two times slower than \( I_{\Delta h} \), suggesting that it should be capable of contributing to the total outward \( K^+ \) flux during the ramp depolarization. However, the relatively slow activation rate of \( I_{KRP} \) should limit its contribution to extended depolarizing ramp responses (Nisenbaum et al. 1996).

Comparison of \( I_{\Delta h} \) with slowly inactivating \( K^+ \) currents in other neurons

Since the original description of the slowly inactivating \( K^+ \) current, \( I_D \), in hippocampal pyramidal CA3 cells (Storm 1988), similar currents have been identified in other neurons of the central nervous system, including cortical pyramidal
cells (Foehring and Surmeier 1993; Hammond and Crepel 1992; Spain et al. 1991), LGN relay neurons (Budde et al. 1992; McCormick 1991), as well as neostriatal neurons (Surmeier et al. 1991; present results). Comparison of the slowly inactivating K⁺ currents in these various cell types shows that they share similarities in voltage dependence and pharmacology. Examination of the voltage dependence of activation of these currents reveals that they all become available at subthreshold membrane potentials between –60 to –70 mV (Foehring and Surmeier 1993; McCormick 1991; Storm 1988; present results). As described above, the availability of these currents at hyperpolarized membrane potentials enables them to attenuate subthreshold depolarizing inputs. The voltage dependencies of inactivation of the slowly inactivating K⁺ currents also are similar such that each of these currents is half-inactivated at membrane potentials between –75 and –90 mV (Hammond and Crepel 1992; McCormick 1991; Storm 1988; present results). This voltage range corresponds to the resting potentials of these cells, indicating that ~50% of slowly inactivating K⁺ channels normally are inactivated at rest. The slowly inactivating K⁺ currents also share pharmacological properties. For example, all of the these currents are sensitive to low micromolar concentrations of 4-AP (Foehring and Surmeier 1993; Hammond and Crepel 1992; McCormick 1991; Spain et al. 1991; Storm 1988; Surmeier et al. 1991). In contrast, these currents are relatively resistant to blockade by tetraethylammonium (Hammond and Crepel 1992; McCormick 1991; Storm 1988).

Despite the similarities in voltage dependence and pharmacology, the slowly inactivating K⁺ currents exhibit differences in their kinetics that most likely contribute to the distinct functional properties of these neurons. For example, the time constant of inactivation of Iₐs in neostriatal neurons (1.5 s) at membrane potentials corresponding to spike threshold is faster than that of the slow transient current (3.3 s) (Spain et al. 1991) or Iₐs (3.75 s) (Hammond and Crepel 1992) in cortical neurons, Iₐs (3.2 s) in LGN relay neurons (McCormick 1991), and Iₒ (~5 s) in hippocampal pyramidal cells (Storm 1988). The longer time constants of inactivation for these other cell types probably account for their ability to dampen threshold depolarizing inputs and delay spike discharge for many seconds (e.g., Storm 1988) compared with hundreds of milliseconds for neostriatal neurons (Nisenbaum et al. 1994). The kinetics of recovery from inactivation of Iₐs also differ from slowly inactivating currents in other neurons. The time constant of recovery from inactivation of Iₐs (1.0 s) is intermediate to that for Iₐs in LGN relay neurons (91 ms) (McCormick 1991) and Iₒ in hippocampal CA₁ pyramidal cells (4.7 s) (Storm 1988). In general, slow recovery kinetics combined with slow inactivation rates permit inactivation to accumulate in response to successive depolarizing inputs, thereby enhancing the level of depolarization evoked by later inputs (Nisenbaum et al. 1994; Storm 1988; Surmeier et al. 1992b). The slower recovery kinetics for Iₒ in hippocampal neurons most likely account for the substantially longer periods of time during which hippocampal neurons can integrate depolarizing inputs (Storm 1988). By contrast, the relatively fast recovery kinetics of Iₐs in LGN relay neurons enable it to contribute to repolarization of low-threshold Ca²⁺ spikes which underlie the 1- to 4-Hz rhythmic firing of these cells (McCormick 1991; Steriade et al. 1993).

The comparisons made earlier clearly demonstrate that in addition to their similarities, differences exist in the kinetics of the slowly inactivating K⁺ currents in different cell types. Several types of K⁺ channel subunits have been identified that give rise to slowly inactivating currents, including Kv2.1, Kv3.1, Kv3.2, and Kv3.3 (Drew et al. 1992; Perney et al. 1992; Sheng et al. 1992; Tsaur et al. 1992; Weiser et al. 1994). The kinetic differences in the currents described earlier may likely reflect the differential expression of K⁺ channels subunits in the membranes of these neurons. For example, in situ hybridization studies have shown that high levels of messenger RNA (mRNA) for Kv3.1 and Kv3.2 are expressed in the LGN and CA₁ pyramidal cells of the hippocampus, but low or undetectable levels of these mRNAs are present in spiny neurons of the neostriatum (Perney et al. 1992; Weiser et al. 1994). In addition, high levels of Kv2.1 mRNA are present in CA₁ pyramidal cells but not in neostriatal neurons (Drew et al. 1992). However, neostriatal cells do express mRNA for Kv1.4, Kv1.6, and Kv4.2 subunits (Sheng et al. 1992; Surmeier et al. 1994; Tsaur et al. 1992). An additional contributing factor to the functional differences of slowly inactivating K⁺ currents may arise from the formation of heteromultimeric K⁺ channels (Ruppersberg et al. 1990; Stühmer et al. 1989). Evidence from in vitro expression studies indicates that subunits from the same K⁺ channel subfamily can combine to form heteromultimeric channels (Christie et al. 1990; Ruppersberg et al. 1990; Weiser et al. 1994). Moreover, the assembly of these heteromultimeric channels can give rise to currents that have functional properties intermediate to either channel alone (Ruppersberg et al. 1990; Weiser et al. 1994). Therefore, the differences between slowly inactivating K⁺ currents in different cell types may reflect the differential expression of K⁺ channel subunits and/or the assembly of distinct heteromultimeric channels.

**Role of Iₐs in the spontaneous activity of spiny neurons**

The natural activity of spiny projection neurons recorded intracellularly in vivo is distinguished by recurring episodes of maintained hyperpolarization (about ~80 mV) followed by periods of sustained subthreshold depolarization, which can last for hundreds of milliseconds to seconds (Wilson 1993; Wilson and Groves 1981; Wilson and Kawaguchi 1996). The transitions to the depolarized state depend on powerful excitatory input from cortex and thalamus, and are maintained at a relatively constant potential just below the threshold for spike discharge (Wilson 1993, 1994; Wilson et al. 1983; Wilson and Kawaguchi 1996). Once the cell has entered the depolarized state, action potentials may occur and can have long latencies to the discharge and irregular firing patterns. Recent experiments have demonstrated that the limits placed on the amplitude of the depolarized state depend on K⁺ currents that are recruited by depolarization (Wilson and Kawaguchi 1996). Results from the present experiments would strongly support a role for Iₐs in limiting the subthreshold depolarizations as well as contributing to the latency to discharge and frequency of firing. The availability of Iₐs at approximately ~60 mV coupled with its
relatively rapid rate of activation at subthreshold membrane potentials should enable this current to limit the level of depolarization associated with the onset of the depolarized state. In addition, the slow time course of inactivation of $I_{AS}$ should permit this current to maintain the voltage limits on the depolarized state for moderately long durations. The rapid activation and slow inactivation of $I_{AS}$ suggest that its ability to decrease the likelihood of action potential generation should be greatest as the membrane potential first reaches the depolarized state and should diminish exponentially with time according to its time constants of inactivation. In this manner, $I_{AS}$ is predicted to contribute to the delay to first spike discharge often seen in the depolarized state.

The voltage dependence of inactivation and kinetics of recovery from inactivation suggest that the efficacy of $I_{AS}$ will depend on both the level of polarization associated with the hyperpolarized state as well as the time that the cell previously has spent in this state before receiving an excitatory synaptic barrage. For example, hyperpolarizing episodes lasting for several seconds should substantially remove inactivation of $I_{AS}$, enabling the current to delay spike discharge or even suppress firing entirely. In contrast, brief periods of hyperpolarization should not permit $I_{AS}$ channels to recover from inactivation and would be expected to decrease the efficacy of this current in inhibiting action potential discharge. Thus the voltage history of the neurons should greatly impact on the ability of $I_{AS}$ to shape the subthreshold voltage behavior and discharge characteristics of spiny neurons in response to excitatory synaptic input (Nisenbaum et al. 1994; Wilson 1993).

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REFERENCES


