Blockade of SK-Type Ca$^{2+}$-Activated K$^+$ Channels Uncovers a Ca$^{2+}$-Dependent Slow Afterdepolarization in Nigral Dopamine Neurons

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Ping, Han Xian and Paul D. Shepard. Blockade of SK-type Ca$^{2+}$-activated K$^+$ channels uncovers a Ca$^{2+}$-dependent slow afterdepolarization in nigral dopamine neurons. J. Neurophysiol. 81: 977–984, 1999. Sharp electrode current-clamp recording techniques were used to characterize the response of nigral dopamine (DA)-containing neurons in rat brain slices to injected current pulses applied in the presence of TTX (2 μM) and under conditions in which apamin-sensitive Ca$^{2+}$-activated K$^+$ channels were blocked. Addition of apamin (100–300 nM) to perfusion solutions containing TTX blocked the pacemaker oscillation in membrane voltage evoked by depolarizing current pulses and revealed an afterdepolarization (ADP) that appeared as a shoulder on the falling phase of the voltage response. ADP were preceded by a ramp-shaped slow depolarization and followed by an apamin-insensitive hyperpolarizing afterpotential (HAP). Although ADPs were observed in all apamin-treated cells, the duration of the response varied considerably between individual neurons and was strongly potentiated by the addition of TEA (2–3 mM). In the presence of TTX, TEA, and apamin, optimal stimulus parameters (0.1 nA, 200-ms duration at −55 to −68 mV) evoked ADP ranging from 80 to 1,020 ms in duration (355.3 ± 56.5 ms, n = 16). Both the ramp-shaped slow depolarization and the ensuing ADP were markedly voltage dependent but appeared to be mediated by separate conductance mechanisms. Thus, although bath application of nifedipine (10–30 μM) or low Ca$^{2+}$, high Mg$^{2+}$ Ringer blocked the ADP without affecting the ramp potential, equimolar substitution of Co$^{2+}$ for Ca$^{2+}$ blocked both components of the voltage response. Nominal Ca$^{2+}$ Ringer containing Co$^{2+}$ also blocked the HAP evoked between −55 and −68 mV. We conclude that the ADP elicited in DA neurons after blockade of apamin-sensitive Ca$^{2+}$-activated K$^+$ channels is mediated by a voltage-dependent, L-type Ca$^{2+}$ channel and represents a transient form of the regenerative plateau oscillation in membrane potential previously shown to underlie apamin-induced bursting activity. These data provide further support for the notion that modulation of apamin-sensitive Ca$^{2+}$-activated K$^+$ channels in DA neurons exerts a permissive effect on the conductances that are involved in the expression of phasic activity.

INTRODUCTION

Mesencephalic dopamine (DA)-containing neurons in vivo exhibit a variety of activity patterns ranging from a tonic single spike to a multiple spike bursting discharge (Buney et al. 1973; Grace and Bunney 1984a,b; Shepard and German 1988; Wilson et al. 1977). During the past decade, converging lines of evidence suggested that alterations in DA neuronal firing pattern are of considerable physiological importance (reviewed by Overton and Clark 1997). Increases in the incidence and intensity of bursting activity are observed in a variety of species in response to auditory and visual stimuli (Freeman et al. 1985; Horvitz et al. 1997; Mirenowicz and Schultz 1996). In the primate, transient increases in bursting activity are linked to novel appetitive stimuli whose rewarding properties are unexpected, suggesting that changes in the temporal organization of DA neuronal spike trains may encode errors in reward prediction (Schultz et al. 1997). Functionally, bursting activity is associated with enhanced DA release in terminal field areas (Chergui et al. 1994; Garris et al. 1994; Gonon 1988; Gonon and Buda 1985; Manley et al. 1992; Nissbrandt et al. 1994) and a regionally selective increase in c-fos expression (Chergui et al. 1996). It was also recently shown that DA released during bursts of action potentials exerts a delayed excitatory effect on striatal neurons via activation of extrasynaptic D1 receptors (Gonon 1997). Thus, by transiently increasing release, bursting activity could trigger a change in the nature of DA neurotransmission from a conventional synaptic to a paracrine modality (Fuxe and Agnati 1991). Taken together, these data indicate that changes in the temporal organization of DA neuronal spike trains represent a mechanism through which these neurons alter their influence on target cells in the forebrain.

The mechanisms responsible for generation of bursting activity in DA neurons are incompletely understood but are likely to involve both extrinsic and intrinsic components. Activation of afferent projections are undoubtedly involved in mediating the transient increases in bursting activity evoked by sensory stimuli. Unpatterned synaptic input may also contribute to the spontaneous firing patterns exhibited by these cells because DA neurons recorded in coronal brain slices, a preparation devoid of medium- and long-length afferents, exhibit a homogenous pacemaker-like firing pattern that is not observed in the intact animal (Grace and Onn 1989; Kita et al. 1986; Sanghera et al. 1984; Shepard and Bunney 1988). On the other hand, the ability of DA neurons to exhibit spontaneous spiking in the absence of synaptic input indicates that intrinsic mechanisms also contribute to the firing properties exhibited by these cells. Although DA neurons possess a variety of voltage- and ligand-gated conductances (Silva et al. 1990), their respective contributions to the electrophysiological properties exhibited by these neurons are for the most part incompletely understood. One notable exception is the small conductance Ca$^{2+}$-activated K$^+$ channel (gK$_{Ca^{2+}}$), which is potently antagonized by the
neurotoxin apamin (Hugues et al. 1982; Köhler et al. 1996; Sah 1996). Blockade of gKCa2+-inhibits the postsynaptic afterhyperpolarization (AHP) and changes the firing pattern of DA cells in brain slices from a pacemaker-like discharge to an irregular single spike or a multiple spike bursting pattern (Gu et al. 1992; Nedergaard et al. 1993; Shepard and Bunney 1988, 1991). Apamin-induced bursting activity appears to result from a modification in the cell’s autogenous pacemaker mechanisms. Thus, in contrast to the sinusoidal oscillation in membrane voltage characteristic of pacemaking DA neurons (Fujiura and Matsuda 1989; Harris et al. 1989; Kang and Kitai 1993a; Yung et al. 1991), bursting activity, elicited after blockade of gKCa2-, is driven by a plateau-like oscillation in membrane potential (Ping and Shepard 1996). Although spontaneous plateau oscillations are not observed among nonbursting DA neurons, they can be evoked by intracellular injection of hyperpolarizing bias currents. These data suggest that all DA neurons possess the capacity to generate plateau potentials and imply that conditions favoring their development (i.e., inhibition of gKCa2-) may be associated with a change in the electrophysiological properties of the neuron, as was demonstrated for other cells exhibiting bistable membrane characteristics (Hsiao et al. 1998; Russo and Honnsgaard 1996). To test this hypothesis, current-clamp recording techniques were used to characterize the response of DA neurons to injected current pulses applied after blockade of fast Na+ channels and gKCa2-. The results show that apamin-induced blockade of gKCa2- results in the development of an afterdepolarization (ADP) that appears to be mediated by the same conductance mechanisms underlying bursting plateau potentials.

METHODS

Brain slices were prepared from male Sprague-Dawley rats (Charles River, Raleigh, NC) as previously described (Shepard and Bunney 1991; Wu et al. 1994). Tissue harvesting procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23) and policies of the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine. Rats weighing between 120 and 170 g were anesthetized with chloral hydrate (400 mg/kg ip) and decapitated. The brain was rapidly removed and placed in an ice-cold Ringer solution (pH 7.4) consisting of (in mM) 125 NaCl, 4.0 KCl, 1.25 NaH2PO4, 1.2 MgSO4, 26 NaHCO3, 2.5 CaCl2, and 11 glucose. The ventral midbrain was dissected from the surrounding tissue and placed on the stage of a manual tissue chopper for slicing. Two coronal slices (300–400 μm thick) containing the substantia nigra (SNc) were immediately transferred to an interface recording chamber and perfused with normal Ringer at a rate of 2–3 ml/min. Bath temperature was maintained between 35 and 36°C. Tissue slices were incubated for ≥1 h before the start of the recording studies.

Intracellular recordings were made with sharp electrodes prepared from borosilicate tubing (1.0 mm OD, WPI, Sarasota, FL) with a horizontal electrode puller (Sutter Instruments, Novato, CA) and filled with 3 M KCl (DC resistance: 40–80 MΩ). Electrodes were positioned within the zona compacta of the substantia nigra (SNc) with the aid of a dissecting microscope and advanced vertically with a piezoelectric microdrive (Burleigh Instruments, Fishers, NY). Immediately after impalement, cells were temporarily hyperpolarized to facilitate sealing of the recording electrode. Electrode potentials were amplified with an Axoclamp 1A amplifier (Axon Instruments, Foster City, CA). Timed current pulses were generated with a digital stimulator (NeuroData, New York, NY) and applied to the cell through the balanced bridge circuit of the amplifier. Neurons were identified as dopaminergic on the basis of their well-characterized active and passive electrical properties, including voltage-dependent, slow depolarization preceding spike initiation (Grace and Onn 1989), apamin-sensitive, postsynaptic AHP (Shepard and Bunney 1991), and pronounced time-dependent anomalous rectification (Mercuri et al. 1995).

Membrane voltage and current monitor outputs were observed continuously with a digital oscilloscope. Analog data were digitized in real-time with a pulse code modulator (NeuroData, New York, NY) and stored on videotape. Recorded data were analyzed with the pCLAMP data acquisition package (Axon Instruments, Foster City, CA). Input resistance was estimated by measuring the change in membrane voltage produced by rectangular current pulses of sufficient duration (>250 ms) to fully charge membrane capacitance. Cells were hyperpolarized slightly (−65 to −68 mV) to suprathreshold spiking during the measurements.

The following drugs were added to normal Ringer solution in the concentrations indicated: TTX (2 μM), tetrodymehematicium chloride (TEA; 2–3 mM), apamin (100–300 nM), nifedipine (10–30 μM; RBI, Natick, MA), and ω-conotoxin GVIA (1.7–5 μM; Alomone, Jerusalem, Israel). Low Ca2+-, high Mg2+- Ringer was prepared by reducing the Ca2+ concentration from 2.5 to 0.3 mM and increasing the Mg2+ concentration from 1.2 to 3.4 mM (Kang and Kitai 1993a). Co2+-containing Ringer was prepared by equimolar substitution of CoCl2 for CaCl2, Cesium chloride (5–10 mM) and nickel chloride (100 μM) were added directly to normal Ringer. All drugs were obtained from Sigma (St. Louis, MO) unless otherwise indicated. Compounds were dissolved in Ringer solution and applied directly to the chamber with a four-channel perfusion system (Adams and List, Westbury, NY). All data are expressed as means ± SE.

RESULTS

Stable intracellular recordings were obtained from a total of 101 neurons within the SNc. All cells included in this study exhibited electrophysiological characteristics previously ascribed to neurochemically identified DA neurons (Grace and Onn 1989; Yung et al. 1991) and are therefore referred to as such. Mean values for membrane resistance (154 ± 7 MΩ), spike threshold (−38.4 ± 0.5 mV), duration (1.75 ± 0.04 ms), and amplitude, measured from the peak of the spike to the peak of the AHP (82.5 ± 1.3 mV), were obtained from a representative sample of 30 DA neurons. Eighty-four percent (85/101) of the neurons recorded exhibited spontaneous action potentials after recovery from impalement, whereas the remaining 16 cells were silent at rest (−42.5 ± 1.2 mV).

Intracellular injection of depolarizing current pulses (200- to 800-ms duration) applied from membrane potentials between −60 and −68 mV elicited repetitive spiking in all cells tested (n = 17). Individual action potentials were preceded by a ramp-shaped slow depolarization and followed by a postspike AHP (Fig. 1A). Addition of TTX (2 μM) blocked current evoked spiking (Fig. 1B) and occasionally led to the appearance of an oscillation in membrane potential that was potentiated by addition of TEA (2–3 mM; n = 25, Fig. 1C). Addition of apamin (100–300 nM) to Ringer containing TTX and TEA blocked the stimulus-evoked oscillation in membrane potential and led to the development of a complex voltage response comprised of a ramp-shaped depolarization followed by an afterdepolarization (ADP) that appeared as a shoulder on the falling phase of the voltage response (Fig. 1D). ADPs were followed by an apamin-insensitive hyperpolarization, henceforth referred to as the hyperpolarizing artifact potential (HAP) to distinguish it from the AHP after individual spikes. Both the ramp potential and the ADP were observed in all cells tested.
The voltage-dependent nature of the ADP is further illustrated in Fig. 3. In these experiments, the amplitude of the stimulus current was adjusted to provide a comparable net depolarization regardless of the initial membrane potential. Intracellular injection of increasingly negative bias currents reduced and eventually eliminated the ADP without significantly affecting the ramp potential (Fig. 3A, top panel). Decreases in the duration of the ADP were accompanied by the progressive development of a HAP, which persisted at membrane potentials approximating $E_K$ ($-90$ mV). Both the amplitude and the slope of the falling phase of the HAP increased as the membrane was progressively hyperpolarized. Bath application of Cs$^+$, in concentrations capable of blocking the fast hyperpolarization-activated cation current in DA neurons (5–10 mM; Fig. 3B), attenuated the HAP ($n = 4$; Fig. 3A). These effects were fully reversed after washout with control Ringer.

Changes in stimulus intensity also had a pronounced and unexpected effect on the temporal characteristics of the ADP. Figure 4A illustrates the response of a representative SNc DA neuron to 400-ms depolarizing current pulses of increasing amplitude applied from $-66$ mV. The duration of the ADP was taken as the interval between termination of the stimulus pulse and the point at which membrane voltage returned to its initial value. Small-amplitude depolarizing current pulses (0.1 nA) evoked ADPs ranging in duration from 81.2 to 1,020.8 ms (355.3 ± 56.5 ms, $n = 16$), whereas higher-intensity stimuli (0.2–0.3 nA) produced a significant decrease in the duration of the response (RMANOVA, $F_{1,30} = 19.6, P < 0.001$; Fig. 4C). Similar effects were observed after constant current pulses (0.2 nA) of increasing duration (Fig. 4B). Thus ADPs evoked in response to a 200-ms stimulus pulse (range: 124.8–868.0 ms; 383.2 ± 57 ms) were significantly longer than those obtained with 400- to 800-ms pulses (RMANOVA, $F_{3,45} = 23.9, P < 0.001$; Fig. 4D).

A Ca$^{2+}$ conductance appeared to be principally responsible for generation of the ADP because perfusion with a modified Ringer solution containing low Ca$^{2+}$ (0.3 mM) and high Mg$^{2+}$ (3.4 mM) blocked it without affecting the initial ramp-shaped depolarization ($n = 2$; Fig. 5A). By contrast, equimolar substitution of Co$^{2+}$ for Ca$^{2+}$ blocked both ramp potential and the ADP as well as the HAP ($n = 5$; Fig. 5B). Bath application of the L-type Ca$^{2+}$ channel blocker nifedipine (10–30 μM; $n = 8$) had no effect on the ramp potential but produced a near-complete blockade of the ADP (Fig. 6A). Notably, however, the drug failed to prevent high-threshold Ca$^{2+}$ spiking evoked by depolarizing current pulses applied from rest (Fig. 6B). Bath application of the T- and N-type Ca$^{2+}$ channel blockers Ni$^{2+}$ (100–300 μM, $n = 2$) and ω-conotoxin GVIA (1.7–5 μM, $n = 2$), respectively, were without effect on either the ADP or the ramp potential (data not shown).

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**FIG. 1.** Electroresponsive properties of substantia nigra (SNc) dopamine (DA)-containing neurons. A: intracellular injection of a depolarizing current pulse (0.2 nA, 400 ms) evokes repetitive single spike activity that is suppressed after bath application of 2 μM TTX (B). Note the dampened oscillation in membrane potential. C: after adding tetraethylammonium chloride (TEA; 3 mM) to the bathing solution, an identical depolarizing current pulse evokes a slow oscillatory potential. Tracings in A–C were obtained from the same cell. D: apamin (APA; 200 nM) completely blocks the stimulus-induced membrane oscillation observed in the presence of TTX and TEA. Under these conditions, the voltage response consists of a slow, ramp-shaped depolarization (R) followed by an afterdepolarization (ADP) and an apamin-insensitive hyperpolarization (HAP). Note that TEA (3 mM) increases input resistance and strongly potentiates the ADP. In this and in all subsequent figures, the dashed line(s) indicate the membrane potential at which the neuron was held by constant current injection.

($n = 65$) but only in the presence of TTX and apamin. TEA, although not required to evoke either component of the voltage response, strongly potentiated the ADP without affecting the ramp potential (Fig. 1D).

Changes in membrane potential had a dramatic effect on the response of DA neurons to constant current depolarizing pulses (0.2 nA; 400 ms) applied in the presence of TTX, TEA, and apamin ($n = 14$). At depolarized membrane potentials (positive to $-50$ mV), rectangular current pulses evoked an electrotonic voltage response accompanied by intermittent high-threshold Ca$^{2+}$ spiking (Fig. 2A). Identical stimuli applied closer to the threshold of the slow depolarization leading to spike initiation ($-52$ to $-55$ mV) and produced a passive voltage response followed by a slow relaxation to a prolonged HAP. Both the ramp potential and the ADP were most prominently observed in the voltage region between $-55$ and $-68$ mV. However, the slope of the ramp (Fig. 2A) and the duration of the ADP (Fig. 2B) decreased as the membrane potential approached $-80$ mV. Depolarizing current pulses applied from membrane potentials below $-80$ mV failed to evoke either the ramp component or the ADP, although a HAP continued to be observed (Fig. 2A).
DISCUSSION

In the current series of experiments, we examined the effects of apamin, a potent and selective antagonist of \( g_{KCa} \), on the subthreshold response properties of nigral DA neurons under conditions in which fast Na\(^+\) currents were blocked. In addition to confirming the ability of apamin to suppress the spontaneous pacemaker oscillation in membrane potential, we show that blockade of \( g_{KCa} \) is associated with the development of a slow ADP. ADPs were observed in all cells that were treated with apamin but could not be evoked in TTX and TEA alone, suggesting that blockade of voltage-activated K\(^+\) channels is not sufficient to induce the response. However, apamin-induced ADPs were strongly potentiated by the addition of TEA, which has also been shown to increase the amplitude of the slow, spontaneous oscillation in membrane potential (Nedergaard and Greenfield 1992). These effects are consistent with TEA’s ability to eliminate outward K\(^+\) currents that might otherwise oppose the ADP. It is also possible that by increasing

FIG. 2. Voltage-dependent characteristics of the apamin-induced ADP. A: voltage traces obtained from a representative neuron after TTX (2 \( \mu \)M), TEA (3 mM), and apamin (APA; 200 nM). In these experiments, constant amplitude stimuli (0.2 nA, 400 ms) were applied from membrane potentials ranging from −44 to −88 mV. Note the gradual reduction in the duration of the ADP and in the slope of the initial ramp-shaped depolarization. B: effect of membrane potential on the duration of the ADP. Each curve represents the response of an individual neuron. ADP duration was taken as the interval between termination of the stimulus pulse and the point at which the membrane voltage returned to prestimulus levels.

FIG. 3. Effect of Cs\(^+\) on subthreshold responses to depolarizing and hyperpolarizing current pulses. All records were obtained from the same neuron in the presence of TTX (2 \( \mu \)M), TEA (3 mM), and apamin (APA; 200 nM). A: depolarizing current pulses (400 ms) of varying amplitude were used to produce the same net level of depolarization from different membrane potentials. As the membrane potential was hyperpolarized, the ADP decayed at a faster rate and was eventually supplanted by a HAP. Bath application of Cs\(^+\) (10 mM) reversibly inhibited the HAP without affecting the ADP. Current calibration tracing pertains to control data only. B: bath application of Cs\(^+\) was also associated with a marked increase in input resistance and blockade of the fast component of the anomalous rectification characteristic of SNc DA-containing neurons.
the length constant of the cell TEA facilitated propagation of ADP from a remote location, possibly a dendritic compartment.

The stimulus-evoked ADPs appear to be generated by the same mechanism responsible for the prolonged plateau depolarization produced after blockage of gKCa1.1 (Nedergaard et al. 1993; Ping and Shepard 1996). Both phenomena are markedly voltage sensitive and persist in the presence of TTX and are observed within a relatively narrow voltage band. Previous intracellular studies have shown that plateau potentials in DA neurons are mediated by a dihydropyridine-sensitive Ca2+ current (Mercuri et al. 1994; Nedergaard et al. 1993). A Ca2+ conductance also appeared to underlie the ADP because it could be blocked in Ringer containing low Ca2+, high Mg2+, by replacing Ca2+ with Co2+, or after bath application of nifedipine, a selective antagonist of L-type Ca2+ channels (Tsien et al. 1988). By contrast, bath application of high concentrations of ω-conotoxin GVIA had no effect on either the ADP (this study) or the regenerative plateau potential (Nedergaard et al. 1993). Ni2+ (300 μM) was also without effect on the ADP. Although these data suggest that both the ADP and the plateau potential are mediated by an L-type Ca2+ channel, the voltage range over which these potentials are observed is considerably below that typically associated with activation of a high-threshold Ca2+ conductance (Bertolino and Llinás 1992). Moreover, high-threshold Ca2+ spikes evoked by depolarizing current pulses continued to be observed after nifedipine-induced blockade of the ADP. The identification of multiple subtypes of dihydropyridine-sensitive, L-type Ca2+ channels may provide an explanation for these seemingly paradoxical observations (Fisher and Bourque 1996; Williams et al. 1992). As heteroligomeric complexes, L-type Ca2+ channels are comprised of multiple subunits that appear to confer unique kinetic and voltage-dependent properties to the resultant conductance (Miller 1997). Thus recombinant expression of Ca2+ channels consisting of α1D subunits results in a dihydropyridine-sensitive Ca2+ channel that activates at voltages considerably negative to those associated with

FIG. 4. Temporal characteristics of the ADP. All experiments were performed in the presence of TTX (2 μM), TEA (3 mM), and apamin (APA; 200 nM). A: voltage responses produced by intracellular injection of 400-ms depolarizing current pulses of increasing amplitude. Note that increases in stimulus intensity reduced the duration of the ADP. The duration of the ADP was taken as the interval between termination of the stimulus pulse and the point at which membrane voltage crossed the initial membrane potential (horizontal line). B: increases in the duration of constant current (0.2-nA) depolarizing pulses also reduced the time course of the ADP. C and D: curves summarizing the response of 16 cells to the current clamp protocols illustrated in A and B, respectively. Each cell was tested across the entire range of stimulus parameters.
Channels comprised of αtC subunits (Tareilus and Breer 1995; Williams et al. 1992). Low voltage-activated Ca\(^{2+}\) currents were identified in a variety of neurons, including neurosecretory cells of the supraoptic nucleus (Fisher and Bourque 1996), and in certain motoneurons (Hsiao et al. 1998; Russo and Hounsgaard 1996). Cells expressing this conductance exhibit bistable shifts in membrane potential similar to those observed in DA neurons after blockade of gKCa\(^{2+}\). suggesting that these currents may provide the principal depolarizing bias responsible for the generation of the plateau potential associated with some forms of bursting activity (Fisher and Bourque 1996).

Given the marked similarity between the stimulus-evoked ADP and the longer-duration, plateau potential it seems reasonable to conclude that the former simply represents a transient version of the latter. However, unlike the regenerative plateau oscillation, which is exhibited only by those cells in which apamin induces a sustained bursting discharge (Ping and Shepard 1996), ADPs were observed in all cells tested, implying that nonbursting DA neurons retain the capacity to generate bursting oscillations. Moreover, the ability to reliably evoke ADP in DA neurons by intracellular current pulses implies that the plateau properties exhibited by DA neurons could be evoked by afferent synaptic input. The question of whether these properties represent a modification of the mechanisms underlying the slow oscillatory pacemaker potential or a separate process “unmasked” after blockade of gKCa\(^{2+}\) remains to be determined. Evidence favoring a common mechanism of action stems from previous intracellular studies in which nifedipine was reported to block both the slow oscillatory potential and spontaneous pacemaker firing in nigral DA neurons (Mercuri et al. 1994; Nedergaard et al. 1993). By contrast, neither Ni\(^{2+}\) nor ω-conotoxin had any effect on these parameters (Nedergaard et al. 1993). However, it is important to note that other investigators reported that nifedipine in concentrations as high as 100 μM is without effect on pacemaker activity (Fujimura and Matsuda 1989). Similarly, Kang and Kitai (1993b) found that the Ca\(^{2+}\) current underlying the pacemaker oscillation in membrane potential is more sensitive to ω-conotoxin than to nifedipine. Clearly, additional studies will be required to specify the nature of the contribution, if any, made by the Ca\(^{2+}\) current(s) underlying the pacemaker oscillation to the plateau properties exhibited by DA neurons.

The mechanisms underlying termination of the ADP have also yet to be established. However, some insights can be obtained from consideration of the voltage- and time-dependent characteristics of the response. Although of nearly constant amplitude, ADPs evoked from −55 to −68 mV were reduced in duration in response to increases in stimulus intensity. Identical results were obtained in recent studies of the plateau generating neurons in the dorsal horn of the spinal cord (Russo and Hounsgaard 1996). It is possible that high-intensity stimuli terminate the ADP by activating an opposing outward current such as an apamin-insensitive gKCa\(^{2+}\). This hypothesis is supported by the observation that the ADP exhibited by DA neurons is followed by a HAP that is blocked in Ca\(^{2+}\)-free Ringer containing Cs\(^{+}\). However, the persistence of the HAP at membrane potentials close to E\(_K\) implies that multiple conductance mechanisms may contribute to the response. Indeed, the ability of Cs\(^{+}\) to attenuate the HAP evoked at membrane potentials below −70 mV suggests that a “sag conductance,” mediated by a hyperpolarization-activated cation current (I\(_h\)), may indirectly contribute to the appearance of HAP-like waveforms at hyperpolarized membrane potentials. For example, depolarizing current pulses of sufficient amplitude to deactivate I\(_h\) would be expected to increase input resistance. As a result, membrane potential would transiently overshoot its original value immediately after termination of the stimulus pulse but depolarize gradually as I\(_h\) reactivated, resulting in the appearance of a HAP-like waveform (Maccarferri et al. 1993; McCormick and Pape 1990; Mosfeldt-Laursen and Rekling 1989; Schwindt et al. 1988; Womble and Moises 1993). Although as an inward current I\(_h\) would not be expected to contribute to the process underlying termination of the ADP, it could alter the time course and amplitude of outward currents that would otherwise act to curtail the potential.

In addition to Ca\(^{2+}\)-sensitive ADP, apamin-treated neurons frequently exhibited a ramp-shaped slow depolarization in response to depolarizing current pulses. The slope of the ramp varied as a function of the membrane potential attained during
the depolarization and was observed over approximately the same voltage region as the ADP. On the basis of these observations, it seems reasonable to conclude that the ramp potential simply reflects slow activation of the dihydropyridine-sensitive Ca$_{2+}$ channel responsible for generating the ADP. Although the ADP was completely blocked in low Ca$_{2+}$, high Mg$_{2+}$ salines or after bath application of nifedipine, these treatments had no effect on the ramp potential. By contrast, Ca$_{2+}$-free Ringer containing Co$_{2+}$ blocked both components of the voltage response. Thus the ramp potential and ADP appear to be mediated by independent conductance mechanisms. Rather than time-dependent activation of an inward current, it is possible that the ramp potential reflects the gradual inactivation of an outward current that opposes the depolarizing effects of the nifedipine-sensitive Ca$_{2+}$ channel. The voltage range over which the ramp potential was observed corresponds to that associated with activation of the transient outward K$^+$ current or $I_A$ (Silva et al. 1990). Although insensitive to 4-AP (Harris 1992), the transient outward current in DA neurons has been shown to be modulated by Co$_{2+}$, which acts to shift both activation and inactivation curves in a depolarizing direction by 30–50 mV (Silva et al. 1990). Modulation of $I_A$ gating could explain why Co$_{2+}$-containing Ringer is effective in blocking both the ramp potential and the transient plateau potential, whereas nifedipine and low Ca$_{2+}$, high Mg$_{2+}$ Ringer affect only the latter component.

In summary, it is becoming increasingly clear that mesencephalic DA-containing neurons are capable of exhibiting nonlinear response properties including regenerative plateau potentials and stimulus-evoked ADP. Although endogenously generated, these characteristics are uncovered only after blockade of outward K$^+$ currents. The ability of apamin, a potent and selective antagonist of gKCa$_{2+}$, to reliably evoke spontaneous bursting and plateau activity in DA neurons suggests that modulation of these channels could exert a permissive effect on the expression of bursting activity in DA neurons. Modulation of Ca$_{2+}$-activated K$^+$ channels by neurotransmitters acting through G-protein–coupled receptors was demonstrated in a wide variety of neurons and represents an important mechanism for the regulation of neuronal excitability (reviewed by Sah 1996). Although direct modulation of gKCa$_{2+}$ in nigral DA neurons has yet to be demonstrated, it is tempting to speculate that a similar mechanism may be involved in the generation of some types of bursting activity exhibited by these neurons, particularly the sustained phasic discharge typically observed in the anesthetized preparation.


