Muscarnine Reduces Calcium-Dependent Electrical Activity in Substantia Nigra Dopaminergic Neurons

REES E. SCROGGS,1 CARLA G. CARDENAS,1 JOSEPH A. WHITTAKER,2 AND STEPHEN T. KITAI 1
1Department of Anatomy and Neurobiology, Health Science Center, University of Tennessee, Memphis, Tennessee 38163; and 2Morehouse School of Medicine, Neuroscience Institute, Atlanta, Georgia 30310

Received 7 May 2001; accepted in final form 31 July 2001

Scroggs, Reese S., Carla G. Cardenas, Joseph A. Whittaker, and Stephen T. Kitai. Muscarine reduces calcium-dependent electrical activity in substantia nigra dopaminergic neurons. J Neurophysiol 86: 2966–2972, 2001. The effect of muscarine on Ca2+ dependent electrical activity was studied in dopamine (DA) neurons located in the substantia nigra pars compacta (SNc) in brain slices from young rats, using sharp electrodes. In most DA neurons tested, muscarine (50 μM) reduced the amplitude of spontaneous oscillatory potentials and evoked Ca2+ dependent potentials recorded in the presence of TTX. Muscarine also reduced the amplitude of the slow afterhyperpolarization (sAHP) following action potentials in most DA neurons. These data suggest that muscarine reduces Ca2+ entry in SNc DA neurons. The reduction of the amplitude of the sAHP by muscarine in DA neurons may facilitate bursting initiated by glutamatergic input by increasing the frequency at which DA neurons can fire. The reduction of the sAHP via activation of muscarinic receptors in vivo may provide a mechanism whereby cholinergic inputs to DA neurons from the tegmental peduncular pontine nucleus could modulate dopamine release at dopaminergic targets in the brain.

INTRODUCTION

Basal ganglia (BG) function is highly dependent on dopaminergic (DA) activity originating in the substantia nigra pars compacta (SNc). SNc DA neurons in vivo exhibit various patterns of firing behavior including a slow irregular firing rate interspersed with bursts of action potentials (Bunney et al. 1973; Grace and Bunney 1984a,b; Pucak and Grace 1994). It is possible that the different firing patterns exhibited by DA neurons encode information, as various changes in their firing have been associated with different behaviors and movements in awake rats (Freeman et al. 1985; Horvitz et al. 1997; Miller et al. 1981; Schultz 1998) as well as changes in dopamine release at DA neuron synaptic targets (Garris and Wightman 1994; Garris et al. 1994; Gonon and Buda 1985; Manely et al. 1992; Nissbrandt et al. 1994; Pucak and Grace 1994). However, the mechanisms controlling DA neuronal firing behavior are poorly understood.

One likely participant in the regulation of DA neuron firing behavior is cholinergic inputs to the SNc from the pedunculopontine tegmental nucleus (PPN) (Clarke et al. 1987; Futami et al. 1995; Takakusaki et al. 1996, 1997; Woolfe and Butcher 1986). Acetylcholine activates both nicotinic and muscarinic receptors on DA neurons, which depolarizes them and increases their firing rate (Calabresi et al. 1989; Lacey et al. 1990; Pidoplichko et al. 1997; Sorenson et al. 1999). The M1 receptor-mediated component of the depolarization and firing rate increase appears to involve a simultaneous increase in a nonselective cation current and blockade of an outward K+ current (Lacey et al. 1990). More recent work has shown that the depolarization produced by M1 receptor activation in DA neurons is preceded by a brief hyperpolarization. This response, which rapidly desensitizes, appears to involve M1 receptor-mediated Ca2+ release from intracellular Ca2+ stores, which activates an apamin-sensitive Ca2+-dependent K+ current (Fiorillo and Williams 2000).

Calcium entry also appears to be an important factor in the regulation of SNc DA neuronal firing behavior; participating in the slow pacemaker-like depolarization that leads to action potential firing as well as activation of Ca2+-dependent AHPs that follow action potentials (Fujimura and Matsuda 1989; Grace and Bunney 1984a,b; Grace and Onn 1989; Harris et al. 1989; Kang and Kitai 1993; Mercuri et al. 1994; Nedergaard 1993; Ping and Shepard 1996, 1999; Shepard and Bunney 1991; Yung et al. 1991). In addition bursting activity of DA neurons may be influenced by intracellular Ca2+ levels (Grace and Bunney 1984b; Overton and Clark 1997) as well as cholinergic inputs (Gronier and Rasmussen 1998). In several other types of neurons, including rat striatal medium spiny neurons, cortical pyramidal neurons, and sympathetic ganglion neurons, activation of M1 receptors has been shown to reduce voltage-dependent Ca2+ currents (Bernheim et al. 1991; Howe and Surmeier 1995; Stewart et al. 1999). However, whether muscarinic receptor activation reduces Ca2+ entry in SNc DA neurons has not been studied.

Thus in the present study, we examined the effects of muscarine on various types of Ca2+-dependent electrical activity in SNc DA neurons. We found that muscarine reduced the amplitude of the Ca2+-dependent spontaneous oscillatory potentials (SOPS) in SNc DA neurons recorded from in the presence of TTX. In addition, we found that muscarine reduced the amplitude and rate of rise of Ca2+-dependent potentials evoked with depolarizing current pulses and reduced the amplitude of the slow afterhyperpolarization (sAHP) in many SNc DA neurons. These experiments suggest that muscarine reduces Ca2+ entry into SNc DA neurons through voltage-dependent Ca2+ pathways.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
channels. We hypothesize that an associated reduction of Ca2+-dependent K+ current(s) may facilitate bursting in response to large glutamatergic excitatory postsynaptic potentials (EPSPs) by raising the upper limit of the rate at which SNc DA neurons can fire.

METHODS

Brain slices containing the SNc were made from young Sprague-Dawley rats (Harlan) 13–19 days of age. Rats were anesthetized with medetomidine and decapitated, and whole brains were removed and immediately placed in ice-cold artificial cerebral spinal fluid (ACSF) for 3–5 min to cool. The ACSF contained (in mM) 124 NaCl, 3.5 KCl, 1.2 KH2PO4, 26 NaHCO3, 1 MgSO4, 2.4 CaCl2, and 10 glucose and was continuously bubbled with 95% O2-5% CO2, pH 7.3. After cooling, the brains were trimmed to blocks containing the substantia nigra, glued to the stage of a vibratome, immersed in ice-cold ACSF, and 300 μm parasaggital or coronal sections containing the substantia nigra were cut. The slices were maintained for between 1 and 6 h in ACSF at room temperature before recording. For recording, the slices were transferred to a 1-ml bath and continuously superfused with ACSF preheated to 34°C. The SNc was visualized with an Olympus BX50WI microscope fitted with Nomarski optics, and DA neurons were impaled with sharp electrodes containing 2 M KCl and 2% neurobiotin (resistance, 45–120 MΩ).

Dopamine neurons were initially recognized by their characteristic electrical properties (Fig. 1, A–C): >2-s duration action potential (at threshold), large AHP, prominent time-dependent rectification, and delayed repolarization following cessation of a strong hyperpolarizing current pulse (Grace and Onn 1989; Harris et al. 1989; Yung et al. 1991). Following recordings, the slices were fixed in 4% paraformaldehyde for later immunohistochemical analysis of the recorded neurons regarding expression of tyrosine hydroxylase and anatomical location. To determine if the recorded neurons expressed tyrosine hydroxylase (TH), slices were preincubated in Texas Red-Avidin (1:500) (Vector) in 0.1 M phosphate-buffered saline (PBS) and 0.5% Triton X-100 for 2 h at 23°C and then overnight at 4°C with mouse monoclonal anti-TH (1:2000) (Diasorin) in PBS 3% normal horse serum and 0.5% Triton-X. The slices were then incubated for 3 h at 23°C in FITC-labeled horse anti-mouse IgG (1:150; Vector) with 2.5% 1,4-diazabicyclo-[2,2,2,]-octane and 50% glycerol in PBS. The neurobiotin-labeled neurons were analyzed for double-labeling with TH antibodies using a confocal laser scanning microscope. Forty-six of 51 neurons included in the study were confirmed to express tyrosine hydroxylase. For the five remaining neurons, the immunohistochemical data were inconclusive, and they were assumed to be dopaminergic based on their expression of the preceding characteristic DA neuron electrical properties.

(+)-Muscarine and tetrodotoxin (Sigma) were diluted to their appropriate concentrations in ACSF and delivered to the slice via the bath. In some experiments, a local perfusion system was employed which decreased the time for drug onset and washout. For this local perfusion system, the drugs were dissolved in Tyrode’s solution and delivered from the end of a glass capillary tube placed in the bath near the recording site. The Tyrode’s solution contained (in mM) 140 NaCl, 4 KCl, 2 MgCl2, 2 CaCl2, 10 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH and was heated to the same temperature as the bath. In these experiments, control responses were assessed in the presence of drug-free Tyrode’s solution.

Data acquisition was performed using an Axoclamp 1B amplifier and a Digidata A/D D/A converter controlled by P-Clamp (Axon Instruments). All data were acquired at a frequency of 100–150 Hz. Data regarding spontaneous action potential firing and spontaneous oscillatory potentials (SOPs) were acquired in the gap-free mode. Data regarding evoked action potentials, Ca2+-dependent potentials, and input resistance were obtained using the episodic stimulation mode. Data analysis was accomplished using the Clampfit and Fetch-chan analysis programs included with pClamp.

The frequency of SOPs of DA neurons were estimated from a representative 10-s segment for each experimental condition by constructing power spectrum graphs in Clampfit 8.0 (Axon Instruments). The average amplitude of SOPs was estimated from representative 10- to 30-s segments for each experimental condition, using the pick peak program in Origin (Microcal Software). ACSII files constructed in Clampfit were imported into Origin and plotted so that the positive going or negative going peaks could be selected. The voltages of the positive peaks were subtracted from adjacent negative peaks and the resultant amplitudes averaged.

Changes in the amplitude of the sAHPs following action potentials were assessed relative to a point 6 ms after the peak of the action potential. This 6-ms reference point corresponded approximately to the transition between the fast AHP (fAHP) and sAHP, which was apparent in some DA neurons but not others (Fig. 1). The sAHP amplitude was measured between 30 and 50 ms from the reference point at a position where the greatest change was produced by muscarine relative to control sweeps.

Data are expressed as the means ± SE. Statistical analyses were performed on paired data using the paired t-test (Systat, SPSS). Probabilities <0.05 were considered significant.

RESULTS

Effects of muscarine on spontaneous oscillatory potentials

To assess the possibility that muscarinic receptor activation reduces Ca2+ entry in DA neurons, we examined the effect of muscarine on SOPs. SOPs are spontaneous fluctuations in membrane potential that can be observed in DA neurons in the presence of TTX to block Na+ currents (see METHODS for measurement protocols). In seven DA neurons tested, muscarine (50 μM) initially reduced SOP amplitude to 4.4 ± 1.4 mV from a control amplitude of 8.7 ± 1.5 mV (P < 0.05, paired t-test; Fig. 2A, a and b). This initial reduction in amplitude lasted ~10–30 s, and was then followed by a gradual resurgence of SOP amplitude, which peaked after ~3 min at an
FIG. 2. Effects of muscarine on spontaneous oscillatory potentials and evoked Ca\(^{2+}\)-dependent potentials in dopamine neurons. A: amplitude and frequency of spontaneous oscillations observed in the presence of 500 nM TTX (a), 2 min after initiation of superfusion with 50 \(\mu\)M muscarine (b), 3.7 min after initiation of superfusion with muscarine (c), and after washout of muscarine with TTX (d). B: voltage deflections induced by a 50-pA hyperpolarizing current pulse and a subsequent 200-pA depolarizing current pulse in the same dopamine neuron depicted in A in the presence of 500 nM TTX, under control conditions (control), and after superfusion of the neuron with 50 \(\mu\)M muscarine (Musc). The spans of the voltage transients designated by numbers bracketed inside arrows define (under control conditions) the amplitude of the evoked Ca\(^{2+}\)-dependent potential (1), the oscillation (3), and the area where the slope was estimated (2). The holding current was −84 pA for (a) and −125 pA for (b).

Average of 7.4 ± 1.5 mV (Fig. 2A, c). The resurgence of SOP amplitude was associated with a significant increase in SOP frequency to 2.3 ± 0.3 Hz compared with an average control frequency of 1.3 ± 0.2 Hz (P < 0.05, paired t-test). After washing muscarine out of the bath for an average of ~15 min, SOP amplitude and frequency returned to near control values (Fig. 2A, d).

Effects of muscarine on evoked Ca\(^{2+}\)-dependent potentials

We extended our study by testing the effects of muscarine on Ca\(^{2+}\)-dependent potentials evoked by depolarizing current pulses in the presence of 500 nM TTX (Fig. 2B). The amplitude of the evoked Ca\(^{2+}\)-dependent potentials was defined as the voltage change from a point immediately before the onset of the depolarizing current pulse to the peak voltage measured during the depolarizing current pulse (Fig. 2B, 1). In 15 of 18 SNc DA neurons tested, 50 \(\mu\)M muscarine reversibly reduced the amplitude of these Ca\(^{2+}\)-dependent potentials by an average of 2.4 ± 0.3 mV. This effect was statistically significant (P < 0.05, paired t-test, n = 18) when data from all 18 neurons tested were included in the analysis. The decrease in the amplitude of the evoked Ca\(^{2+}\)-dependent potential by muscarine was associated with a significant decrease in upward slope of the potential. The slope of the evoked Ca\(^{2+}\)-dependent potentials was measured over the linear portion of their initial rising phase following the initial electronic response (Fig. 2B, 2). It decreased from a control value of 0.153 ± 0.012 to 0.117 ± 0.011 mV/ms in the presence of muscarine (P < 0.05, paired t-test, n = 15). Also there was a significant 3.2 ± 0.7 mV decrease in the amplitude of the overall membrane potential oscillation that occurred during the depolarizing current pulse (P < 0.05, paired t-test, n = 15). The amplitude of the oscillation was measured from the first peak of the evoked Ca\(^{2+}\)-dependent potential to the subsequent trough (Fig. 2B, 3).

In five DA neurons, muscarine was tested on both evoked Ca\(^{2+}\)-dependent potentials and SOPs, and there was a good match regarding the effects of muscarine on SOP amplitude versus evoked Ca\(^{2+}\)-dependent potential amplitude. Figure 2 illustrates an example where muscarine decreased SOP amplitude (Fig. 2A) and evoked Ca\(^{2+}\)-dependent potential amplitude (Fig. 2B) in the same neuron. In four DA neurons, where 50 \(\mu\)M muscarine produced an average 62 ± 6% decrease in SOP amplitude (5.7 ± 1.4 mV), a subsequent application of muscarine also markedly reduced the amplitude of the evoked Ca\(^{2+}\)-dependent potential (2.1 ± 0.3 mV). On the other hand, in the other DA neuron, 50 \(\mu\)M muscarine decreased SOP amplitude by only 13%, and a subsequent application of 50 \(\mu\)M muscarine did not noticeably reduce the amplitude of the evoked Ca\(^{2+}\)-dependent potential.

FIG. 3. Occlusion by Mn\(^{2+}\) and time course of the muscarine induced decrease in evoked Ca\(^{2+}\)-dependent potentials in DA neurons. A: voltage deflections in response to a −0.025-nA hyperpolarizing current pulse followed by a 0.15-nA depolarizing current pulse, under control conditions (a) and after 2 min of superfusion with 50 \(\mu\)M muscarine (b). The holding current was −112 pA for a and −160 pA for b. B: voltage deflections recorded from the same neuron as depicted in A under control conditions (a), after superfusion of the neuron with 1 mM Mn\(^{2+}\) (b), and after superfusion of the neuron with 1 mM Mn\(^{2+}\) + 50 \(\mu\)M muscarine for 2 min (c). The current injection protocol was the same as in A. The holding current was −168 pA for a, −181 pA for b, and −203 pA for c. C: voltage transients recorded from another dopamine neuron under control conditions in the presence of 500 nM TTX (a), 36 s after initiation of superfusion with 50 \(\mu\)M muscarine (b), and 2 min after initiation of superfusion with muscarine (c). For this experiment, a local perfusion system was used (see METHODS for details) that reduced the time required for muscarine to reach the neuron under study. The holding current was −106 pA for a and b and −131 pA for c. D: flow chart depicting the interaction over time between the increase in input resistance and the reduction in the amplitude of the evoked Ca\(^{2+}\)-dependent potential produced by muscarine.
As illustrated in Fig. 3A, muscarine also produced a significant decrease (3.0 \pm 0.4 mV) of the hyperpolarization that followed cessation of the current pulse to evoke the Ca\(^{2+}\)-dependent potentials (\(P < 0.05\), paired \(t\)-test, \(n = 11\)). The amplitude of this hyperpolarization was defined as the voltage change from a point immediately before cessation of the current pulse to a point where peak negativity was observed under control conditions (Fig. 3A). The hyperpolarizations, resembled AHPs that followed action potentials evoked by depolarizing current pulses in the absence of TTX (Fig. 1A). They were more negative than the resting membrane potential of the DA neurons and were reduced by blockade of Ca\(^{2+}\) channels with 1–2 mM Mn\(^{2+}\) (Fig. 3, A and B). These characteristics suggested involvement of active Ca\(^{2+}\)-dependent \(K^+\) conductances evoked during the depolarization.

The muscarine-induced decrease in the amplitude and hyperpolarization of Ca\(^{2+}\)-dependent potentials was significantly occluded by preapplication of 1–2 mM Mn\(^{2+}\) to block Ca\(^{2+}\) channels (Fig. 3, A and B). In four neurons studied, 1–2 mM Mn\(^{2+}\) reduced the amplitude and hyperpolarization by 6.1 \pm 1.7 and 6.0 \pm 1.0 mV, respectively. In these same four neurons, 50 \(\mu\)M muscarine applied before the addition of Mn\(^{2+}\) reversibly reduced the amplitude and hyperpolarization by 2.3 \pm 0.5 and 4.0 \pm 0.8 mV, respectively (Fig. 3A, a and b). However, when muscarine was reapplied in the presence of Mn\(^{2+}\), the amplitude and hyperpolarization were changed by only \(-0.1 \pm 0.2\) and \(0.2 \pm 0.4\) mV, respectively (\(P < 0.05\), each, paired \(t\)-test; Fig. 3B, a–c).

In three DA neurons, where a rapid local drug application technique replaced the usual bath application (see METHODS), a transient decrease in the amplitude of evoked Ca\(^{2+}\)-dependent potentials by muscarine was resolved that appeared to be correlated with changes in input resistance (Fig. 3C, see also D). In these three neurons, 50 \(\mu\)M muscarine decreased the amplitude of the evoked Ca\(^{2+}\)-dependent potential by 3.8 \pm 0.3 mV within 40 s after the valves controlling the solution flow were switched from control solution to one containing muscarine. At this time, input resistance was little changed, averaging 138 \pm 19 vs. 136 \pm 19 M\(\Omega\) under control conditions (Fig. 3C, a and b). However, within 2 min, input resistance had increased to 173 \pm 11 M\(\Omega\) (Fig. 3C, c). This increase in input resistance was associated with a return of the amplitude of the evoked Ca\(^{2+}\)-dependent potential toward control levels, reducing the decrease in amplitude of the potentials to 1.7 \pm 1.2 mV (Fig. 3C, c). Overall in 18 experiments on evoked Ca\(^{2+}\)-dependent potentials, muscarine increased input resistance by an average of 20 \pm 5\% from 152 \pm 14 M\(\Omega\) under control conditions to 176 \pm 13 M\(\Omega\). However, in most of the experiments where muscarine was applied by bath application, a transient effect of muscarine on the amplitude evoked Ca\(^{2+}\)-dependent potentials was not apparent, possibly due to the slower change in concentration of muscarine around the neurons.

An interesting side effect of Mn\(^{2+}\) application in the preceding experiments on evoked Ca\(^{2+}\)-dependent potentials was an attenuation of the increase in input resistance usually associated with muscaricne treatment (Fig. 3, A and B). In the four DA neurons subjected to occlusion experiments with Mn\(^{2+}\), muscarine increased input resistance by 37 \pm 7 M\(\Omega\) (25\%) over 2–5 min in the absence of Mn\(^{2+}\) compared with a change of only 2 \pm 3 M\(\Omega\) (\(-1.5\%\)) over a similar time period when muscarine was reapplied in the presence of Mn\(^{2+}\).

**Effects of muscarine on the sAHP following action potentials**

Because the preceding experiments suggested that muscarine reduced Ca\(^{2+}\) entry into most DA neurons, we tested the effects of muscarine on Ca\(^{2+}\)-dependent sAHPs following action potentials in DA neurons. Our initial experiments were performed on DA neurons that were spontaneously firing. In these experiments, 50 \(\mu\)M muscarine decreased in amplitude of the sAHP in 13 of 16 neurons tested. Most of the time it could not be determined whether or not the decreases in sAHP amplitude were secondary to muscarine-induced increases in firing rate and membrane potential depolarization (which also decrease the sAHP). However, in five DA neurons, obvious changes in the sAHP amplitude occurred during the initial onset of the effects of muscarine when firing rate and membrane potential had not changed much. Also in these five neurons, there existed periods of control firing activity which closely matched the firing activity during the initial onset of muscarine regarding rate and membrane polarization (Fig. 4, A and B). In these five DA neurons, 50 \(\mu\)M muscarine produced a 2.1 \pm 0.4-mV reduction of the sAHP that could not be explained by changes in firing rate or membrane potential (Fig. 4, A and B).

In another series of experiments, we controlled for the effects of muscarine on membrane potential and firing rate by holding DA neurons negative to the membrane potential where they fired spontaneously and evoking action potentials at regular intervals with depolarizing current pulses. Muscarine (50 \(\mu\)M) decreased the sAHP in five of seven DA neurons tested by an average of 2.3 mV \pm 1.2 (Fig. 5A, b). In these comparisons, we instituted further controls by selecting sweeps in the presence and absence of muscarine that had similar holding membrane potentials and latencies between the beginning of the

![FIG. 4. Effects of muscarine on the sAHP in a spontaneously firing dopamine neuron. A: spontaneous action potentials and AHPs recorded before and shortly after the onset of muscarine and after washout of the muscarine for 14 min. B: sequences of 3 action potentials and AHPs taken from the experiment depicted in A before the onset of muscarine (control), shortly after onset of the action of muscarine (muscarine), and after washout of the muscarine (wash). The superscripts (a–c) in B correspond to the same letters in A, indicating where each sequence of 3 action potentials was taken from, sAHP amplitudes (arrows) were measured and defined as described in METHODS.](http://jn.physiology.org/)

**J Neurophysiol • VOL. 86 • DECEMBER 2001 • www.jn.org**
produced a sustained decrease in the sAHP. Where input resistance was not monitored, muscarine produced a significant (Fig. 6), at a later time point when input resistance had increased the initial decrease in the sAHP was reversed to an increase. In two of these neurons, where the experiment continued for several minutes, was little changed (Fig. 6). However, in two of these neurons, where the experiment continued for several minutes, the initial decrease in the sAHP was reversed to an increase at a later time point when input resistance had increased significantly (Fig. 6, A and B). In one additional DA neuron where input resistance was not monitored, muscarine produced a sustained decrease in the sAHP.

**DISCUSSION**

The present study suggests that muscarine reduces Ca\(^{2+}\)-dependent hyperpolarization that followed cessation of the depolarizing current pulse used to evoke the Ca\(^{2+}\)-dependent potentials (the effects of muscarine on evoked Ca\(^{2+}\)-dependent potentials were occluded by blockade of Ca\(^{2+}\) channels with Mn\(^{2+}\)); and muscarine reduced the amplitude of the Ca\(^{2+}\)-dependent sAHP in the majority of SNc DA neurons where valid comparisons could be made. These data can be explained by the hypothesis that muscarine reduces Ca\(^{2+}\) entry into SNc DA neurons. In addition to our data on SNc DA neurons, muscarinic receptor activation has been shown to reduce voltage-gated Ca\(^{2+}\) channel currents in several other neuronal types, including rat striatal medium spiny neurons, cortical pyramidal neurons, and sympathetic ganglion neurons (Howe and Surmeier 1995; Mathie et al. 1992; Stewart et al. 1999).

The above-mentioned reduction by muscarine of the various types of Ca\(^{2+}\)-dependent electrical activity was frequently partly or wholly transient. Application of muscarine resulted in an initial decrease that was followed by a return toward control values. A likely explanation for this pattern is that the reduction of Ca\(^{2+}\) entry into the SNc DA neurons by muscarine occurred more rapidly than the associated increase in input resistance. However, the more gradual increase in input resistance partially or completely obscured the effects of decreased Ca\(^{2+}\) entry on the voltage transients, according to the basic electrical relationship V = IR. This hypothesis is supported by our experiments where input resistance and the amplitude of evoked Ca\(^{2+}\)-dependent potentials were monitored simultaneously in DA neurons and a rapid drug application system was also employed. Under these conditions, we observed that muscarine initially reduced the amplitude of the evoked Ca\(^{2+}\)-dependent potentials at a time point where input resistance was little affected. However, the input resistance gradually increased during continued application of muscarine, which was associated with a resurgence of evoked Ca\(^{2+}\)-dependent potential amplitude back toward control values. Because much of our data regarding the effects of muscarine on evoked Ca\(^{2+}\)-dependent potentials and sAHPs were contaminated by varying degrees of increased input resistance, it is possible that the

**Fig. 5.** Effects of muscarine on the sAHP following evoked action potentials in a dopamine neuron. A: action potentials were evoked by 180-pA (control) or 220-pA (50 µM muscarine and wash) depolarizing current pulses. The sAHP amplitude (arrows) was defined and measured as described in METHODS. The holding current was ~160 pA under control conditions, ~170 pA in the presence of muscarine, and ~120 pA after washout of the muscarine. B: voltage deflections in response to a family of hyperpolarizing current pulses in the same neuron as A under control conditions and in the presence of 50 µM muscarine. C: plot of the peak voltage deflection vs. amplitude of the current pulse taken from the voltage sweeps shown in B. The input resistance estimated from fitting a straight line to the points was 96 MΩ under control conditions and 97 MΩ in the presence of muscarine.

**Fig. 6.** Transient reduction of the sAHP by muscarine in a dopamine neuron. A: action potential (cropped) and subsequent sAHPs evoked by a 50-pA depolarizing current pulse under control conditions (control), 30 s after initiation of superfusion of 50 µM muscarine (Muscl), and 2 min after initiation of superfusion of muscarine. B: voltage transients produced by a 20-pA hyperpolarizing current pulse delivered 450 ms before initiation of the depolarizing current pulse used to evoke the action potentials, under control conditions (control), 30 s after initiation of superfusion of 50 µM muscarine (Muscl), and 2 min after initiation of superfusion of muscarine. Each trace in A and B is the average of 3 sweeps, which each included both the response to hyperpolarizing depolarizing current pulses. A spike alignment procedure (pClamp 8.0) was used to align the action potentials and AHPs before averaging. This procedure prevented showing the responses to hyperpolarizing and depolarizing current pulses in the same sweep as they were originally recorded. In B, input resistance was 133 MΩ under control conditions, 135 MΩ after muscarine for 30 s, and 196 MΩ after muscarine for 2 min.
MUSCARINIC MODULATION OF Ca\(^{2+}\) ENTRY IN DOPAMINE NEURONS

...figures presented represent somewhat of an underestimation of this effect.

While performing experiments on the occlusion of the muscarine-induced decrease in evoked Ca\(^{2+}\)-dependent potentials with Mn\(^{2+}\), we noted that Mn\(^{2+}\) blocked the increase in input resistance usually produced by muscarine in DA neurons. This observation is consistent with an earlier report by Lacey et al. (1990) showing that inhibition of a K\(^+\) current participates in the depolarization and inward current produced by muscarine in DA neurons and that these effects of muscarine are reduced by low extracellular Ca\(^{2+}\) concentration. They hypothesized that low extracellular Ca\(^{2+}\) concentration may inhibit a Ca\(^{2+}\)-dependent signaling pathway mediating the muscarinic depolarization and increase in inward current. As concluded by Lacey et al. (1990), it seems unlikely that inhibition of the Ca\(^{2+}\)-dependent K\(^+\) current directly or indirectly by muscarine underlies the muscarine-induced increase in input resistance. If this was the case, then one would expect that blockade of Ca\(^{2+}\) channels with Mn\(^{2+}\) would increase input resistance, which we did not observe.

The potential blockade of Ca\(^{2+}\)-dependent signaling pathways by Mn\(^{2+}\) opens up the possibility that the reduction of Ca\(^{2+}\)-dependent electrical activity by muscarine could be due to an increase in a K\(^+\) current that opposes the depolarization produced by Ca\(^{2+}\) entry. Thus occlusion by Mn\(^{2+}\) of the muscarine-induced reduction of evoked Ca\(^{2+}\)-dependent potentials could be due to interruption of the signaling pathway coupling muscarine receptors to this putative K\(^+\) current rather than blockade of Ca\(^{2+}\) channels. In line with this idea, Fiorillo and Williams (2000) reported that DA neurons are hyperpolarized by activation of muscarinic receptors positively coupled to apamin-sensitive SK channels via a Ca\(^{2+}\)-dependent signaling pathway. However, blockade of this hyperpolarization by Mn\(^{2+}\) is an unlikely explanation for its occlusion of the effects of muscarine on evoked Ca\(^{2+}\)-dependent potentials in our study. The hyperpolarization of DA neurons by muscarinic receptor activation lasts for only ~1 s and is strongly desensitized by continued application of agonist (Fiorillo and Williams 2000). In contrast, we observed that the reduction of evoked Ca\(^{2+}\)-dependent potentials by muscarine usually persisted for minutes throughout time periods when the overall input resistance was unchanged or increased. In addition, other studies suggest that the inhibition of voltage-dependent Ca\(^{2+}\) channels in neurons by muscarine is not dependent on Ca\(^{2+}\) entry into the neuron from the extracellular solution. In these voltage-clamp studies, Ca\(^{2+}\) was replaced in the extracellular solution by Ba\(^{2+}\), which served as the charge carrier through the voltage-dependent Ca\(^{2+}\) channels (Bernheim et al. 1991; Howe and Surmeier 1995; Stewart et al. 1999).

In the present study, we observed that muscarine reduced the amplitude of the Ca\(^{2+}\)-dependent sAHP following action potentials in DA neurons, as well as a similar Ca\(^{2+}\)-dependent AHP that was observed on cessation of the depolarizing current pulse used to evoke Ca\(^{2+}\)-dependent potentials in the presence of TTX. There are few, if any, examples of neurotransmitters directly coupling to the apamin-sensitive SK channels that generate the sAHP (Vergara et al. 1998). Thus it is plausible that muscarine may reduce the sAHP indirectly via a reduction in Ca\(^{2+}\) entry as seems to be the case regarding the reduction of the sAHP by serotonin in hypoglossal motoneurons (Bayliss et al. 1995).

Our observation that muscarine reduces the sAHP in DA neurons is at odds with the previous study by Lacey et al. (1990) in which this effect of muscarine was not detected. This discrepancy may be due to differences in experimental design. In their study, comparisons of the sAHP recorded during the peak effect of muscarine were made to the sAHP recorded under control conditions. The depolarization produced by muscarine was controlled for in two ways: by depolarizing the DA neurons in the absence of muscarine to the same membrane potentials reached in the presence of muscarine or by hyperpolarizing the DA neurons during the peak effect of muscarine to the control membrane potential (Lacey et al. 1990). However, neither of these techniques could be expected to control for the effects of the muscarine-induced increase in input resistance on the sAHP. Thus during the peak effect of muscarine when comparisons were made to controls, the effects of muscarine-induced reductions in Ca\(^{2+}\) entry on the sAHP could have been masked by increases in input resistance. In the present study, we looked for muscarine-induced decreases of the sAHP at early time points during the onset of drug action, before input resistance had changed considerably, thus circumventing to some degree the problem of overall amplification of voltage transients by increased input resistance.

Cholinergic transmission in the CNS is largely characterized by tonic diffuse release of acetylcholine from varicosities (Descaries et al. 1997), although in the SNc, there is evidence that acetylcholine is also released onto DA neurons at specific synapses (Futami et al. 1995). The data available to date suggest that an increase in cholinergic tone may initiate a sequence of modulatory events. Initially there may be a brief period of inhibition mediated by an increase in conductance through apamin-sensitive SK channels (Fiorillo and Williams 2000). Next there may be brief period where a reduction in Ca\(^{2+}\) entry indirectly reduces SK channel conductance, which is translated into a reduction of the amplitude of the sAHP as seen in this study. During this period, there could be a facilitation of burst firing of DA neurons in response to glutamatergic input from the subthalamus or the PPN due to an increase in the peak rate at which the DA neurons could fire. However, based on the large degree of variability we observed regarding the reduction of the sAHP by muscarine, it is possible that cholinergic facilitation of bursting would be significant for some DA neurons but not others. If the increase in cholinergic tone was prolonged beyond these first two phases, then a general depolarization and overall increase in input resistance could result. This state might not particularly facilitate bursting, as the increased input resistance would tend to counteract the effects of reduced Ca\(^{2+}\) entry on the sAHP. However, one could expect an overall increase in excitability of the DA neurons, making them more likely to fire in response to synaptic inputs. Whether the preceding sequence actually occurs in response to increased cholinergic tone and how this would affect the activity of DA neuronal targets remains to be determined.

The authors thank Dr. Bing Teng for the immunohistochemical identification of the DA neurons included in this study and Dr. Jutta Rohrbacher for collecting some of the preliminary data. This work was supported by National Institute of Neurological Disorders...
and Stroke Grants NS-38963 to J. A. Whittaker and NS-20702 and NS-26473 to S. T. Kitai.

REFERENCES


