Muscarinic Receptors Differentially Modulate the Persistent Potassium Current in Striatal Spiny Projection Neurons

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Gabel, Lisa A. and Eric S. Nisenbaum. Muscarinic receptors differentially modulate the persistent potassium current in striatal spiny projection neurons. J. Neurophysiol. 81: 1418–1423, 1999. Cholinergic regulation of striatal spiny projection neuron activity is predominantly mediated through muscarinic receptor modulation of several subclasses of ion channels. Because of its critical role in governing the recurring episodes of hyperpolarization and depolarization characteristic of spiny neurons in vivo, the 4-aminopyridine-insensitive, persistent potassium (K\(_{\text{Kr}}\) ) current, I\(_{\text{Krp}}\) , would be a strategic target for modulation. The present results show that I\(_{\text{Krp}}\) can be either suppressed or enhanced by muscarinic receptor stimulation. Biophysical analysis demonstrated that the depression of I\(_{\text{Krp}}\) was associated with a hyperpolarizing shift in the voltage dependence of inactivation and a reduction in maximal conductance. By contrast, the enhancement of I\(_{\text{Krp}}\) was linked to hyperpolarizing shifts in both activation and inactivation voltage dependencies. Viewed in the context of the natural activity of spiny neurons, muscarinic depression of I\(_{\text{Krp}}\) should uniformly increase excitability in both hyperpolarized and depolarized states. In the hyperpolarized state, the reduction in maximal conductance should bolster the efficacy of impending excitatory input. Likewise, in the depolarized state, the decreased availability of I\(_{\text{Krp}}\) produced by the shift in inactivation should enhance ongoing synaptic input. The alterations associated with enhancement of I\(_{\text{Krp}}\) are predicted to have a more dynamic influence on spiny cell excitability. In the hyperpolarized state, the negative shift in activation should increase the flow of I\(_{\text{Krp}}\) and attenuate subsequent excitatory synaptic input; whereas once the cell has traversed into the depolarized state, the negative shift in inactivation should reduce the availability of this current and diminish its influence on the existing excitatory barrage.

INTRODUCTION

Clinical evidence indicates that dysfunction of the cholinergic system within the striatum plays a critical role in the manifestation of a variety of neurological disorders such as Parkinson’s disease (Barbeau 1962). The striatum has one of the highest concentrations of acetylcholine (ACh) in the mammalian brain (Phelps et al. 1985), and this rich innervation arises from a small population of ACh-containing interneurons (Bolam et al. 1984). One of the primary targets of the cholinergic interneurons is the spiny neurons that give rise to the major striatal projection pathways (Bolam et al. 1984; Wilson et al. 1990). Given the relationship between the activity of spiny neurons and several aspects of motor function (Hikosaka 1994), some of the behavioral consequences of normal and abnormal ACh release in striatum are likely to be mediated by changes in the activity of these spiny cells.

The natural activity of spiny neurons recorded in vivo is characterized by recurring episodes of membrane hyperpolarization (approximately −85 mV) followed by subthreshold depolarization (approximately −45 mV) from which spike discharges can arise (Wilson and Groves 1981). Although the shifts between hyperpolarized and depolarized states require excitatory input from cortico- and thalamostriatal afferents (Wilson et al. 1983), recent evidence indicates that depolarization-activated potassium (K\(^+\) ) currents play a central role in regulating these transitions (Wilson and Kawaguchi 1996). In particular, K\(^+\) currents have been shown to shape the transitions to the depolarized state, as well as govern the voltage limits on this state (Wilson and Kawaguchi 1996). Three types of K\(^+\) currents have been identified including, 4-aminopyridine-sensitive (4-AP), fast (I\(_{\text{A}}\) ), and slowly (I\(_{\text{AK}}\) ) inactivating A-type currents and a 4-AP-insensitive, persistent current (I\(_{\text{Krp}}\) ) (Gabel and Nisenbaum 1998; Nisenbaum et al. 1996; Surmeier et al. 1991). Of these K\(^+\) currents, I\(_{\text{Krp}}\) is expected to contribute significantly to limiting the depolarizing periods due to its availability at subthreshold membrane potentials and slow kinetics of inactivation (Nisenbaum and Wilson 1995; Nisenbaum et al. 1996).

Cholinergic modulation of outward K\(^+\) currents would be expected to provide an additional layer of regulation of spiny cell activity. In situ hybridization studies indicate that much of the postsynaptic effect of ACh on spiny neurons will be transduced by stimulation of muscarinic receptors (Bernard et al. 1992; Hersch et al. 1994). Indeed, activation of muscarinic receptors has been shown to produce a voltage-dependent modulation I\(_{\text{AF}}\) in cultured striatal neurons (Akins et al. 1990). Because the whole cell K\(^+\) current in cultured neurons is dominated by I\(_{\text{AF}}\) modulation of other currents was not readily testable. Given the critical role of I\(_{\text{Krp}}\) in governing the subthreshold responses of spiny neurons, the possibility that this current may be a target of muscarinic modulation was investigated in acutely isolated cells from adult tissue.

METHODS

Neurons from young, adult (28–42 days old) male, Sprague-Dawley rats were acutely isolated from the striatum using previously described procedures (Nisenbaum et al. 1996). Briefly, animals were deeply anesthetized with methoxyflurane and perfused intracardially with a cold (−2°C) NaHCO\(_3\)-buffered saline solution (in mM): 126 NaCl, 2 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 26 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), 1 pyruvic acid, and 10 glucose; pH 7.4; osmolarity, 300 ± 5 mOsm/l. After the perfusion, their brains were removed rapidly from the skull, and 400-μm-thick coronal sections were cut through the rostrocaudal extent of the striatum. Slices then were incubated at room temperature (−22°C) for 0.5–6.0 h in a continuously oxygenated...
(95% O₂-5% CO₂) NaHCO₃-buffered saline solution. After the incubation period, the dorsal striata were placed into a HEPES-buffered Hank’s balanced salt solution (HBSS) containing protease Type XIV (1 mg/ml; Sigma Chemical, St. Louis, MO) maintained at 37°C and oxygenated (100% O₂). After 30–45 min of incubation in HBSS, the striata were triturated using fire-polished Pasteur pipettes, and the cell suspension was placed into a plastic Petri dish mounted onto the stage of an inverted microscope. Whole cell voltage-clamp recordings were performed at 22°C using glass micropipettes containing (in mM) 72 KF, 2 MgCl₂, 40 HEPES, 3 bis-(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA), 12 phosphocreatine, 2 Na₂ATP, 0.2 GTP, and 0.1 leupeptin; pH 7.2; osmolarity, 275 mOsm/liter. The extracellular solution contained (in mM) 140 NaHOCH₂CH₂SO₃, 1 KCl, 5 CaCl₂, 1 MgCl₂, 0.4 CaCl₂, 10 HEPES, 10 glucose, and 0.001 tetrodotoxin; pH 7.4; osmolarity, 300 ± 5 mOsm/liter. For a subset of experiments, striatal neurons were retrogradely labeled following bilateral injections of rhodamine latex microspheres (400 μl each hemisphere) into the substantia nigra 2–5 days before electrophysiological recording (Surmeier et al. 1992).

RESULTS

Carbachol can enhance or depress Iₖrp in spiny neurons

Consistent with previous reports (Nisenbaum et al. 1996), Iₖrp could be isolated from the other calcium-independent, depolarization-activated K⁺ currents in the presence of 10 mM 4-AP. In initial experiments, Iₖrp was evoked by 500-ms depolarizing voltage steps from −80 to +35 mV. Carbachol (2 μM) either reduced or enhanced Iₖrp. C and D: histograms of the average (± SD) carbachol-induced decrease or increase in peak Iₖrp: E: plot of peak Iₖrp as a function of time and extracellular solution. Application of atropine (2 μM) alone did not affect Iₖrp nor did coapplication of carbachol (2 μM). However, carbachol alone produced a reversible enhancement in the current. F: atropine also blocked the carbachol-induced depression of Iₖrp.
from spiny cells. To verify this assumption we conducted additional recordings from identified spiny projection neurons that were retrogradely labeled following injections of rhodamine microspheres into the substantia nigra. The effects of carbachol on $I_{Krp}$ recorded from these identified striatonigral spiny cells showed that the agonist produced either a depression (41%, 9 of 22 cells), an enhancement (45%, 10 of 22 cells), or no change in (14%, 3 of 22 cells) in $I_{Krp}$. The muscarinic receptor specificity of the carbachol-induced enhancement and depression of $I_{Krp}$ was tested using the specific muscarinic antagonist, atropine. $I_{Krp}$ was evoked by voltage steps from $-80$ to $+20$ mV delivered every 7 s, and the peak amplitude of $I_{Krp}$ was measured as a function of time and extracellular solution. Application of atropine (2 μM) alone had no effect on $I_{Krp}$ nor did subsequent addition of carbachol (2 μM; Fig. 1E). However, the carbachol-induced enhancement of $I_{Krp}$ was evident following removal of atropine. The peak amplitude of $I_{Krp}$ returned to control levels during the wash period. Atropine also blocked the carbachol-induced depression of $I_{Krp}$ (Fig. 1F). The potential contribution of nicotinic receptors to these responses was tested using the antagonist, mecamylamine (10–20 μM). Neither the carbachol-induced enhancement nor depression of $I_{Krp}$ was affected by nicotinic receptor blockade. Collectively, these results demonstrate that the modulatory effects of carbachol on $I_{Krp}$ depend on stimulation of muscarinic receptors.

Carbachol differentially affects the voltage-dependence of $I_{Krp}$

The biophysical mechanisms through which carbachol exerted its effects on $I_{Krp}$ also were investigated. The differential effects of carbachol could have been produced by either shifts in the voltage dependencies of activation, inactivation, and/or changes in maximal conductance. Possible alterations in the voltage dependencies of activation were tested by stepping the membrane potential from $-90$ mV to potentials between $-70$ and $+35$ mV. The normalized conductances were plotted as a function of step potential and fit with a Boltzmann function. The enhancement of $I_{Krp}$ was associated with a hyperpolarizing shift in the voltage dependence of activation. In contrast to the depressive effects of carbachol, the enhancement of $I_{Krp}$ was associated with a hyperpolarizing shift in the voltage dependence of activation (Fig. 2, D–F). The magnitude of the current was considerably larger, particularly at hyperpolarized membrane potentials. The average $V_{h\text{act}}$ values...
shifted toward more hyperpolarized potentials from $6.5 \pm 1.8$ mV during control conditions to $-0.9 \pm 2.0$ mV in the presence of carbachol [$t(6) = 13.4; P < 0.0001, n = 7$]. No change in the slope factor (control $V_C^{\text{act}} = 9.8 \pm 4.4$ mV, $n = 7$) or maximal conductance was observed (control $g_{\text{max}} = 18.6 \pm 4.9$ nS; carbachol $g_{\text{max}} = 18.9 \pm 6.3$ nS, $n = 7$).

Possible shifts in the voltage dependencies of inactivation of $I_{K_{Ir}}$ were assessed by stepping the membrane potential from $-80$ mV to potentials between $-110$ and $-10$ mV for 5 s before delivering a test step to 0 mV for 250 ms (Fig. 3). The current amplitudes were normalized, plotted as a function of the conditioning membrane potential and were fit with a Boltzmann function (Fig. 3, C and F). For cells ($n = 12$) in which carbachol decreased $I_{K_{Ir}}$, the voltage dependence of inactivation of the current was shifted toward hyperpolarized membrane potentials. The average half-inactivation voltage ($V_{h_{\text{inact}}}$) shifted from $-49.0 \pm 4.6$ mV during control conditions to $-59.6 \pm 5.1$ mV in the presence of carbachol [$t(11) = 9.0; P < 0.0001$]. No change in the slope factor ($V_C^{\text{inact}}$) of the current-voltage relationship was observed (control $V_C^{\text{inact}} = 14.0 \pm 0.9$ mV; carbachol $V_C^{\text{inact}} = 14.6 \pm 1.0$ mV). This considerable shift in voltage dependence may have accounted for some of the apparent decrease in maximal conductance given the holding potential of $-90$ mV. A similar hyperpolarizing shift in the voltage dependence of inactivation was associated with the enhancement of this current ($n = 8$). The average $V_{h_{\text{inact}}}$ values were $-37.7 \pm 3.9$ mV and $-45.9 \pm 3.1$ mV for control and carbachol conditions [$t(7) = 5.6; P < 0.001$], respectively. This hyperpolarizing shift in voltage dependence also was accompanied by a change in the steepness of the conductance-voltage relationship [control $V_C^{\text{inact}} = 10.7 \pm 1.0$ mV; carbachol $V_C^{\text{inact}} = 12.7 \pm 1.6$ mV; [$t(7) = 5.7; P < 0.001$]]. An unexpected observation was that the control $V_{h_{\text{inact}}}$ values for cells in which $I_{K_{Ir}}$ was reduced ($-49.0 \pm 4.5$ mV) or enhanced ($-37.7 \pm 3.9$ mV) were significantly different.
ent (t(7) = 5.1; P < 0.005), suggesting that two subpopulations of spiny neurons are present. Indeed, the voltage dependence of inactivation for a given cell could be used to predict its response (i.e., increase or decrease) to muscarinic receptor stimulation. No differences in V_{th} values were found for the two groups of neurons.

**DISCUSSION**

The present results demonstrate that $I_{Krp}$ can be depressed or enhanced by muscarinic receptor stimulation in striatal spiny neurons and that the biophysical nature of these effects are considerably different. The depression of $I_{Krp}$ is characterized by a hyperpolarizing shift in the voltage dependence of inactivation and a reduction in $s_{\text{max}}$. In contrast, the enhancement of $I_{Krp}$ is associated with hyperpolarizing shifts in both the voltage dependence of activation and inactivation. The functional consequences of these two effects on the responses of spiny neurons to excitatory input in the hyperpolarized and depolarized states should be markedly different. As described above, the membrane potential of spiny neurons resides at potentials near −85 mV in the hyperpolarized state and −45 mV in the depolarized state (Stern 1998; Wilson and Kawaguchi 1996). On the basis of its voltage dependence and kinetic characteristics, $I_{Krp}$ has been postulated to contribute significantly to limiting the level of depolarization associated with the depolarized state (Nisenbaum et al. 1996). As such, muscarinic stimulation in the hyperpolarized state should decrease the subsequent availability of $I_{Krp}$ through a reduction in maximal conductance and thereby enhance the level of depolarization evoked by impending excitatory input once the cell makes the transition into the depolarized state. Likewise, in the depolarized state, the decreased availability of $I_{Krp}$ produced by the hyperpolarizing shift in inactivation voltage dependence should bolster the efficacy of ongoing synaptic input. Thus muscarinic depression of $I_{Krp}$ should uniformly augment spiny cell excitability.

The hyperpolarizing shifts in activation and inactivation voltage dependence associated with the muscarinic receptor–mediated enhancement of $I_{Krp}$ are similar to those previously described for $I_{\text{A}}$ in striatal neurons (Akins et al. 1990). These alterations are predicted to have a more dynamic influence on spiny cell excitability such that in the hyperpolarized state the negative shift in activation should increase the flow of $I_{Krp}$ and attenuate subsequent excitatory synaptic input, thereby maintaining the neuron in this state. In contrast, the cell has traversed into the depolarized state, the negative shift in inactivation of $I_{Krp}$ should reduce the availability of this current, diminishing its influence on the existing excitatory barrage leading to greater depolarization. These hypotheses were tested within the context of the present experiments. Results showed that when $I_{KRP}$ was evoked from negative potentials (e.g., −80 mV) corresponding to the hyperpolarized state, muscarinic stimulation enhanced the current. However, when evoked from potentials corresponding to the depolarized state (e.g., −40 mV), $I_{KRP}$ was reduced (Fig. 3G). Thus these muscarinic effects are postulated to confer a stabilizing effect on the prevailing state (i.e., hyperpolarized or depolarized) of spiny neurons (Akins et al. 1990).

Although the muscarinic receptor subtypes mediating the effects on $I_{Krp}$ were not investigated, of the five receptors that have been cloned, $m_1$ and $m_4$ are preferentially expressed on spiny neurons (Bernard et al. 1992; Hersch et al. 1994). Therefore one explanation for the differential modulation of $I_{Krp}$ is that the nature of the effect depends on selective stimulation of $m_1$ or $m_4$ receptors. However, this hypothesis is difficult to reconcile with the extensive colocalization (~60%) of $m_1$ and $m_4$ receptors on spiny neurons (Bernard et al. 1992). Nonetheless, previous studies in spiny neurons have demonstrated distinct $m_1$ and $m_4$ receptor modulation of calcium (Ca$^{2+}$) currents. The $m_4$ modulation is dependent on intracellular Ca$^{2+}$ concentration [Ca$^{2+}$], and insensitive to pertussis toxin (PTX) and targets L-type Ca$^{2+}$ channels, whereas the $m_1$ modulation is independent of [Ca$^{2+}$] and PTX-sensitive and targets N- and P-type Ca$^{2+}$ channels (Howe and Surmeier 1995). In light of these findings, it is possible that both $m_1$ and $m_4$ modulation of $I_{Krp}$ could be revealed in spiny cells by varying [Ca$^{2+}$] and/or inhibition of PTX-sensitive G-proteins respectively (Howe and Surmeier 1995). Alternatively, the differential modulation of $I_{Krp}$ may indicate that the specific K$^+$ channels subtypes that give rise to $I_{Krp}$ differ between spiny neurons, a possibility supported by the differences in inactivation voltage dependence of the current. Further studies will be required to distinguish between these possibilities.

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