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Muscarinic Receptors Involved in the Subthreshold Cholinergic Actions of Neostriatal Spiny Neurons

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ABSTRACT Administration of the peptide MT-1 (48 nM), a selective agonist of muscarinic M_1-type receptors, mimicked the subthreshold actions of muscarine (1 μM) on neostriatal neurons, i.e., it produced a reduction in subthreshold inward rectification leading to an enhancement in input resistance (R_N) and evoked discharge. In all recorded cells, MT-1 effects remained in the presence of the specific peptidergic antagonist of the M_4-type receptor, MT-3 (10 nM), but were blocked by the specific M_1-type receptor antagonist MT-7 (5 nM). These results suggest that most muscarinic facilitatory actions in the subthreshold voltage range occur through M_1-type receptors. However, in a fraction of cells (40%) muscarine produced an excitability enhancement not blocked by MT-7. This additional facilitatory action, not present when using MT-1, was blocked by MT-3, suggesting it was mediated by M_4-type receptor activation. This facilitation could not be blocked by Cs^+, TTX, or Cd^{2+}, but only by a reduction in extracellular sodium. This result is the first evidence that M_4-type receptor activation enhances a cationic inward current in a fraction of neostriatal projection neurons. **Synapse 46:215–223, 2002.**

INTRODUCTION

It has been demonstrated by both current- and voltage-clamp studies that muscarinic receptor agonists modulate the Cs^+-sensitive inward rectification of neostriatal projection neurons (Dodt and Misgeld, 1986; Galarraga et al., 1999a; Hsu et al., 1996). Inward rectification dominates the subthreshold voltage behavior of spiny neurons (Galarraga et al., 1994; Nisenbaum and Wilson, 1995; Mermelstein et al., 1998; Reyes et al., 1998), and thus condition their activation properties. Voltage-clamp analysis has imputed subthreshold rectification to Kir2-type channels (Mermelstein et al., 1998). Current-clamp experiments disclosed that activation of muscarinic receptors causes a decrease in inward rectification, which in turn produces an enhancement in neuronal input resistance (R_N) and a facilitation of evoked discharge (Galarraga et al., 1999a,b; Calabresi et al., 2000). These actions can be elicited by endogenous acetylcholine (Galarraga et al., 1999a). Blockade of rectification by Cs^+ occludes most muscarinic actions (Galarraga et al., 1999a; Hsu et al., 1996). However, it has not been conclusively demonstrated which muscarinic receptors are involved in this action (Calabresi et al., 2000), since only nonselective muscarinic ligands have been tested (Galarraga et al., 1999a).

Another question is if inward rectification explains all muscarinic actions in the subthreshold range. Muscarinic receptor agonists may enhance R_N at more positive membrane potentials (i.e., between −60 mV and −45 mV) (Shen and North, 1992; Galarraga et al., 1999a,b), where inward-rectifying channels are not likely to be activated. This effect may also help to facilitate evoked discharge and it is not sensitive to Cs^+, Cd^{2+}, TTX, or Co^{2+} (Galarraga et al., 1999a). Hence, it cannot be attributed to the inward-rectifying conductance.

Therefore, the present current-clamp analysis made use of peptides that selectively target muscarinic M_1- and M_4-type receptors (Bradley, 2000; Jerusalinsky et al., 1995; Karlsson et al., 2000; Liang et al., 1996;

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Purkerson and Potter, 1998) to discover: 1) the muscarinic receptors that modulate subthreshold voltage behavior in spiny neurons, 2) the percentage of cells that exhibit these subthreshold voltage behaviors, and 3) their ionic nature. A preliminary report of this study has been presented in abstract form (Figueroa et al., 1999).

MATERIALS AND METHODS

The present experiments were performed on rat dorsal neostriatal slices maintained in vitro as previously reported (Galarraga et al., 1999a). Briefly, Wistar rats (100–200 g) were deeply anesthetized with ether and perfused transcardially with 50 ml of an iced-cold (4°C) saline solution containing (in mM): 125 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 10 D-glucose, 0.0002 thiourea, and 0.0002 L-ascorbic acid (saturated with 95% O_2 and 5% CO_2; 300 mOsm/L, pH = 7.4). The brain was rapidly removed and placed into this solution before slicing. Sagittal slices (350 μm thick) of the neostriatum were obtained with a vibratome and incubated 60 min at 25°C before recording in the same saline. The slices were recorded in a submerged chamber and superfused with the same saline (except that choline-Cl or N-methyl-glucamine, NMG, substituted NaCl when using low Na^+ saline) at 2 ml/min (34–36°C). Intracellular recordings were performed with microelectrodes filled with 3 M K-acetate (DC resistance ~80–120 MΩ). Records were obtained with an active bridge electrometer (Neuro Data; Cygnus Technology, Delaware Water Gap, PA), digitized, and saved on VHS tapes (40 kHz) to be analyzed off-line with a PC-clone computer. The preferred stimulus for the present study was a current ramp injected intracellularly (0.5–1 nA/s; 1 mV/ms) (Galarraga et al., 1994; 1999a,b; Pacheco-Cano et al., 1996). In current-clamp conditions, responses to ramp stimuli allows one to easily test the actions of a transmitter in the subthreshold voltage range (from ca. −100 to ca. −45 mV) and at the same time evaluate the influence of any subthreshold change on evoked discharge (e.g., Pacheco-Cano et al., 1996; Pineda et al., 1995). A change in the slope of the current-voltage relationship (I–V plot) built with ramp responses can be interpreted as an input resistance (R_N) change induced by the transmitter (Galarraga et al., 1994, 1999b; Pacheco-Cano et al., 1996). The I–V slope was defined quantitatively as the derivative (dV/dI) of the I–V plot at resting membrane potential (Galarraga et al., 1994). In the present study, stimulus intensity was regulated so that only a few spikes were evoked in the control condition. To take into account the variability between the samples experiments were paired, so that measurements in the presence or absence of drugs were compared in the same neuron and in the same sample with a nonparametric test (Wilcoxon's T). Although not shown for the sake of figure clarity, responses described were reversible with the exception of MT-1 toxin. Means ± SEM, medians, and ranges of R_N changes are reported. N-methyl D-glucamine, cesium chloride (Cs^+), cadmium chloride (Cd^{2+}), and tetrodotoxin (TTX) were obtained from Sigma (St. Louis, MO). Muscarinic ligands were: muscarine (RBI, Natick, MA), the muscarinic toxins MT-1 and MT-3 from *Dendroaspis angusticeps* (Alomone Lab, Jerusalem, Israel) and MT-7 (Peptides International, Louisville, KY). All reagents were added from stock solutions to the bath saline.

RESULTS

A linear depolarizing current ramp typically evokes a nonlinear voltage behavior in the subthreshold range (Fig. 1A,D; stimulus ramp is on top in this and other figures). The nonlinearity of the voltage trajectory towards firing is mainly due to inward rectification (Galarraga et al., 1994; Nisenbaum and Wilson, 1995). The voltage trajectory can be used to build current–voltage relations (I–V plots) (Fig. 1C). Notice that apparent R_N (I–V plot slope) keeps increasing during constant depolarization (Fig. 1C). This inward rectification can also be observed after steady-state I–V plots built with step stimuli (not shown, but see Galarraga et al., 1994; Nisenbaum and Wilson, 1995; Reyes et al., 1998). Voltage-clamp and single-cell polymerase chain reaction (scRT-PCR) analyses demonstrated that one cause for this voltage behavior is the presence of an inward rectifying current of the Kir2 type expressed by neostriatal spiny neurons (Mermelstein et al., 1998).

The addition of muscarinic receptor agonists such as muscarine (1 μM) (Fig. 1B) produced an apparent increase in R_N in the subthreshold range (Fig. 1C, arrows) (Dodt and Misgeld, 1986; Pineda et al., 1995; Galarraga et al., 1999a,b; Calabresi et al., 2000). This is accompanied by a decrease in the inward-rectifying current (Hsu et al., 1996). In the present work, 18 neurons were analyzed quantitatively before and during muscarine administration (see Materials and Methods). Muscarine had effects in every cell tested (100%): R_N increased from (mean ± SEM) 44 ± 1.8 MΩ, median = 42 MΩ, range = 31–61 MΩ in the controls, to 58 ± 3.2 MΩ, median = 56 MΩ, range = 39–80 MΩ in the presence of 1 μM muscarine ($P < 0.001$; Wilcoxon's T-test). Therefore, a significant increase in R_N of about 33% (with medians) was detected after muscarine.

The peptidic muscarinic receptor agonist, MT-1 (48 nM), which is selective but not specific for muscarinic M_1-type receptors, was tested to see if it mimicked the effects of 1 μM muscarine on R_N (Fig. 1D–F). The MT-1 peptide also had effects in every cell tested: R_N was enhanced from 45 ± 12 MΩ, median = 42 MΩ, range = 17–78 in the controls to 54 ± 12 MΩ, median = 48, range = 24–88 in the presence of the MT-1 peptide ($n = 6$, $P < 0.03$, Wilcoxon's T-test). This effect was significant and about half of the effect produced by muscarine (Cf. Fig. 1C,F). However, the concentration of MT-1 employed was not saturating, since higher concentrations might affect other receptor types.

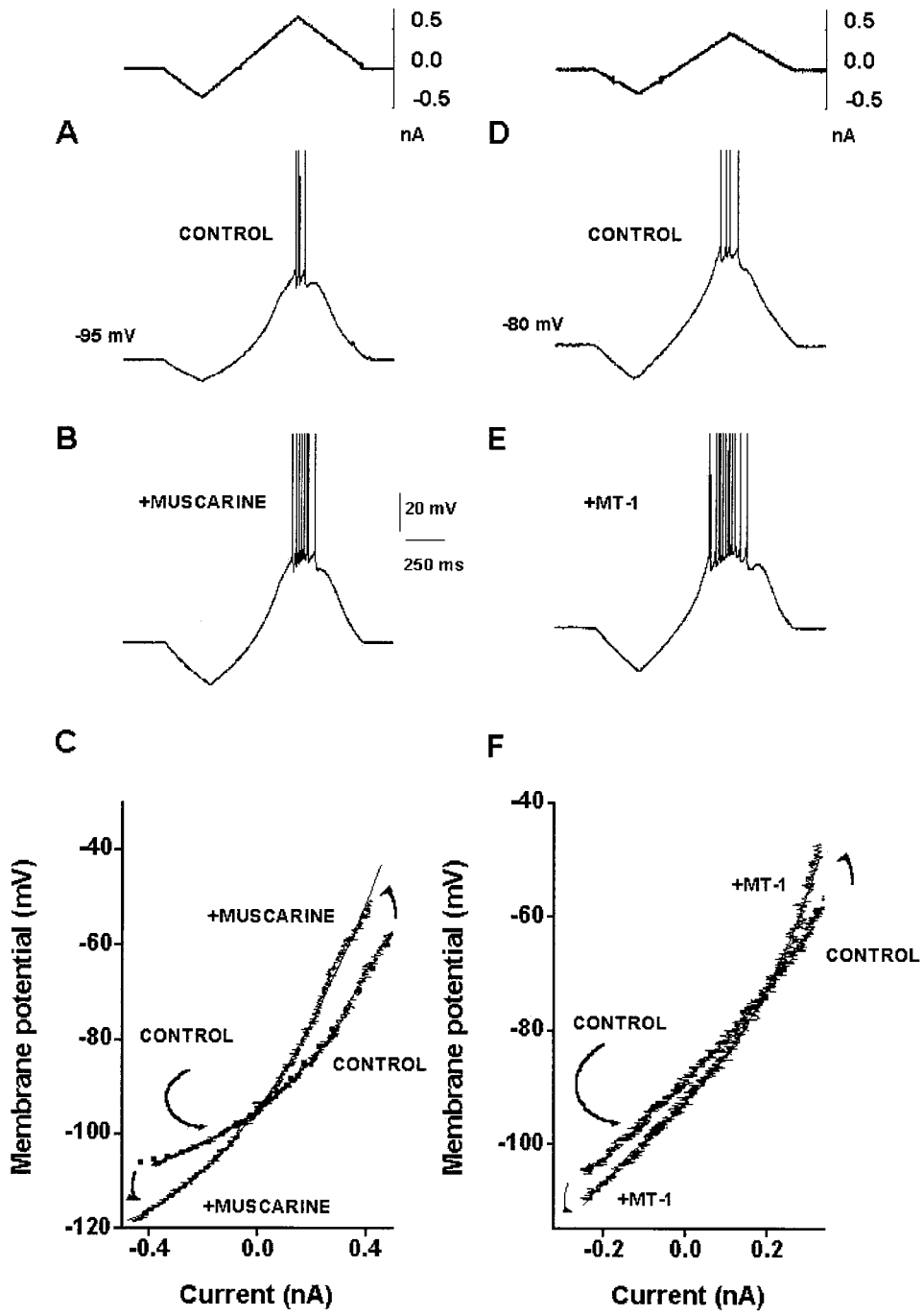


Fig. 1. Cholinergic muscarinic receptor agonists change subthreshold membrane properties of neostriatal spiny projection neurons. **A:** Firing is evoked with a linear current ramp (top). The subthreshold voltage response to the current ramp is not linear. **B:** The cholinergic muscarinic receptor agonist muscarine (1 μM) enhances firing. **C:** When ascending voltage trajectories toward firing, in **A** and **B**, are used to build current–voltage relationships (I–V plots), it is seen that muscarine increases the slope of the I–V plot, that is, it increases input resistance (R_N), and thus favors the depolarization towards firing. **D–F:** A similar result was obtained when testing the MT-1 muscarinic peptide (48 nM).

These results suggest that, at doses that mainly bind M_1-type receptors, the MT-1 peptide could mimic the effects of muscarine on the subthreshold voltage behavior of spiny neurons. This suggested that M_1-type receptor activity modulates the subthreshold voltage behavior of spiny neurons. To confirm the role of M_1-type receptors a specific antagonist, the MT-7 peptide, was tested. MT-7 (5 nM) blocked the actions of both muscarine (1 μM) ($n = 2$) and the MT-1 peptide (48 nM) ($n = 2$). Figure 2A–C illustrates a representative experiment using MT-1. The experiments with muscarine yielded similar results (not shown). R_N did not change during the course of the experiment (at least 1 h recording) when MT-7 accompanied MT-1. Thus, the results confirmed that M_1-type receptor activity modulates subthreshold membrane behavior. This conclusion has been suggested previously (e.g., Galarraga et al., 1999a) using less selective antagonists (Caulfield and Birdsall, 1998), but this is the first report on the physiological actions of the muscarinic peptides on the subthreshold behavior of spiny cells.

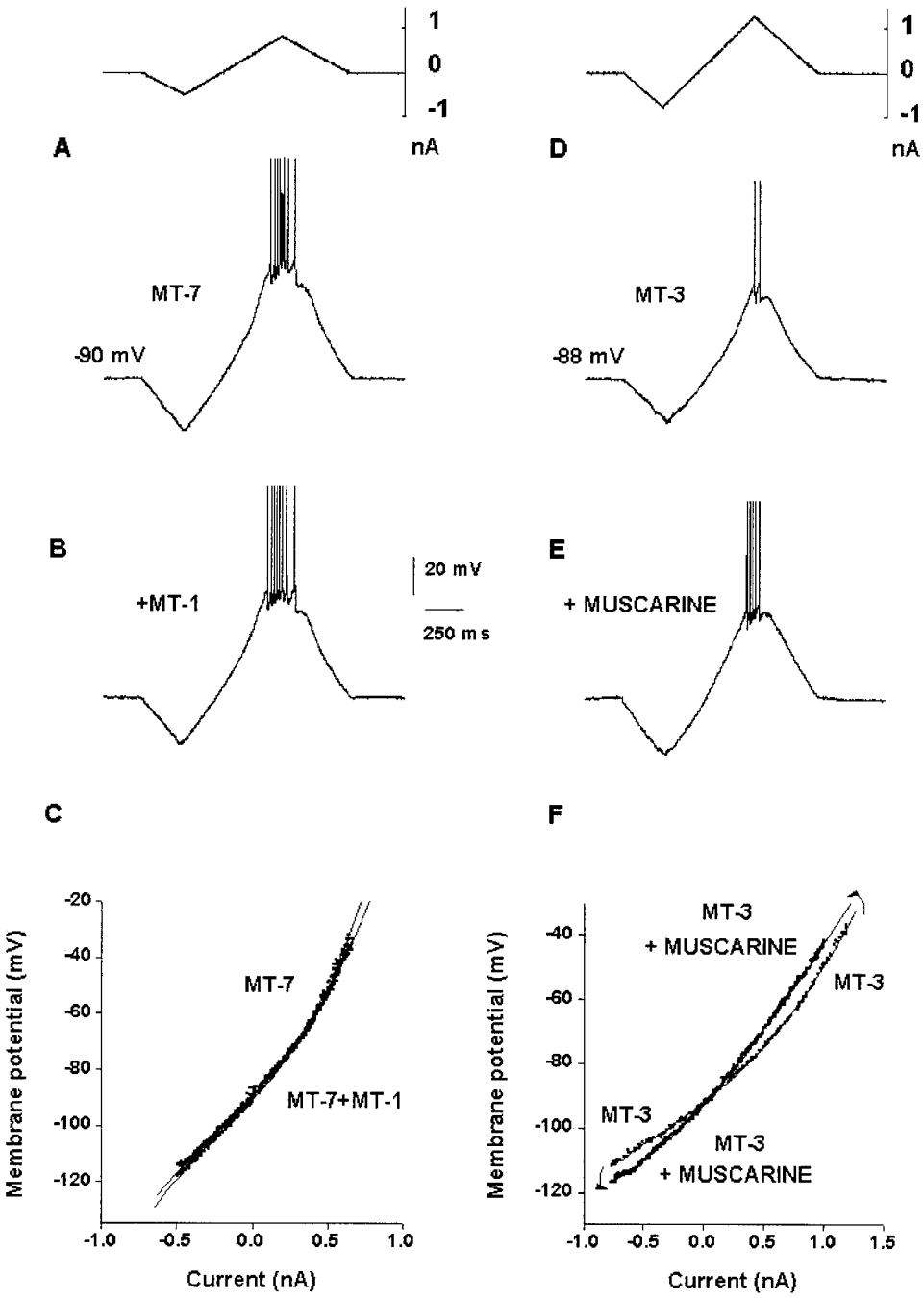


Fig. 2. Subthreshold facilitatory actions of muscarinic agonists are blocked by MT-7 but are not blocked by MT-3. **A:** Firing is evoked with a current ramp (top), as in Figure 1. The MT-7 (5 nM) peptide did not change subthreshold voltage trajectory or evoked discharge. **B:** However, MT-7 blocked the action of the agonist peptide MT-1 (48 nM), which now is unable to enhance the evoked discharge. **C:** I–V plots built from ascending records in **A** and **B** show no change when MT-1 (48 nM) was administered with MT-7 (5 nM). The same result can be obtained with muscarine (not shown). **D:** The MT-3 peptide (10 nM) did not change the subthreshold response to a current ramp. **E:** Addition of muscarine (1 μM) had the usual effect of enhancing the evoked discharge in the presence of MT-3. **F:** I–V plots show that the MT-3 peptide could not block the usual change in slope (R_N) as a result of muscarine. The same result can be obtained with MT-1 (not shown).

To further support the above conclusion, we tested whether the specific muscarinic M_4-type receptor antagonist, the MT-3 peptide, could block the actions of muscarine or the MT-1 peptide. Figure 2D–F illustrates a representative experiment using muscarine as the muscarinic receptor agonist. The MT-3 peptide (5–10 nM) was unable to block the R_N enhancement produced by muscarine (1 μM) (n = 11). The MT-3 peptide was also unable to block the action of the MT-1 peptide (n = 2) (not shown). The fact that a specific M_4-type receptor antagonist did not block most actions of muscarine, or the MT-1 peptide, whereas a specific M_1-type receptor antagonist easily blocked these effects allows a conclusive statement about the role of M_1-type muscarinic receptors in the modulation of the subthreshold behavior of spiny cells. These results also show that muscarinic peptides acted as expected on neostriatal projection cells.

Nevertheless, in a sample of neurons the MT-3 peptide could block a part of the facilitatory response produced by muscarine. This additional facilitatory response was not present during MT-1 action and was only present after muscarine in a fraction of cells (7/18 or 40%). One case is illustrated in Figure 3A,B. Mus-

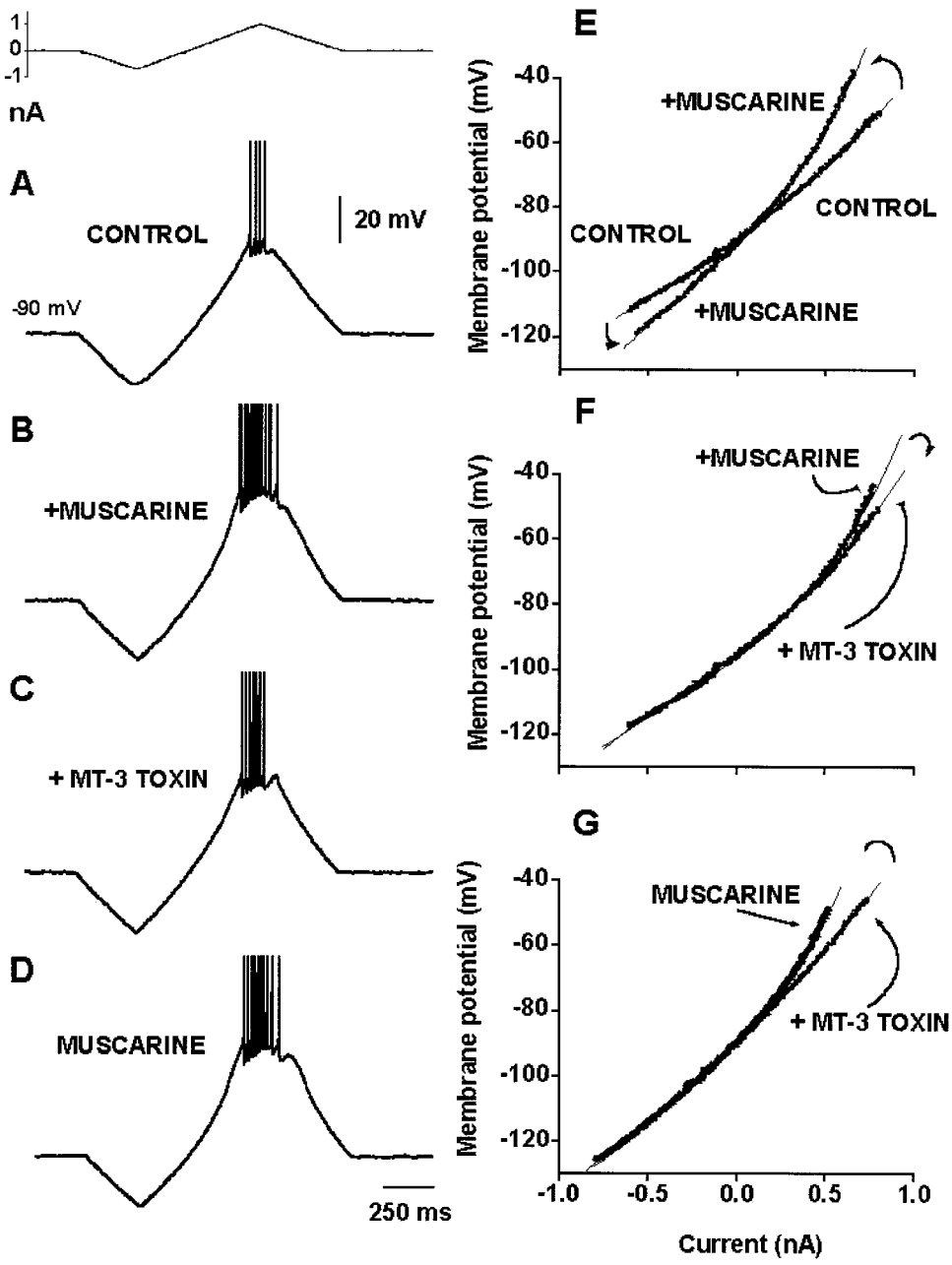
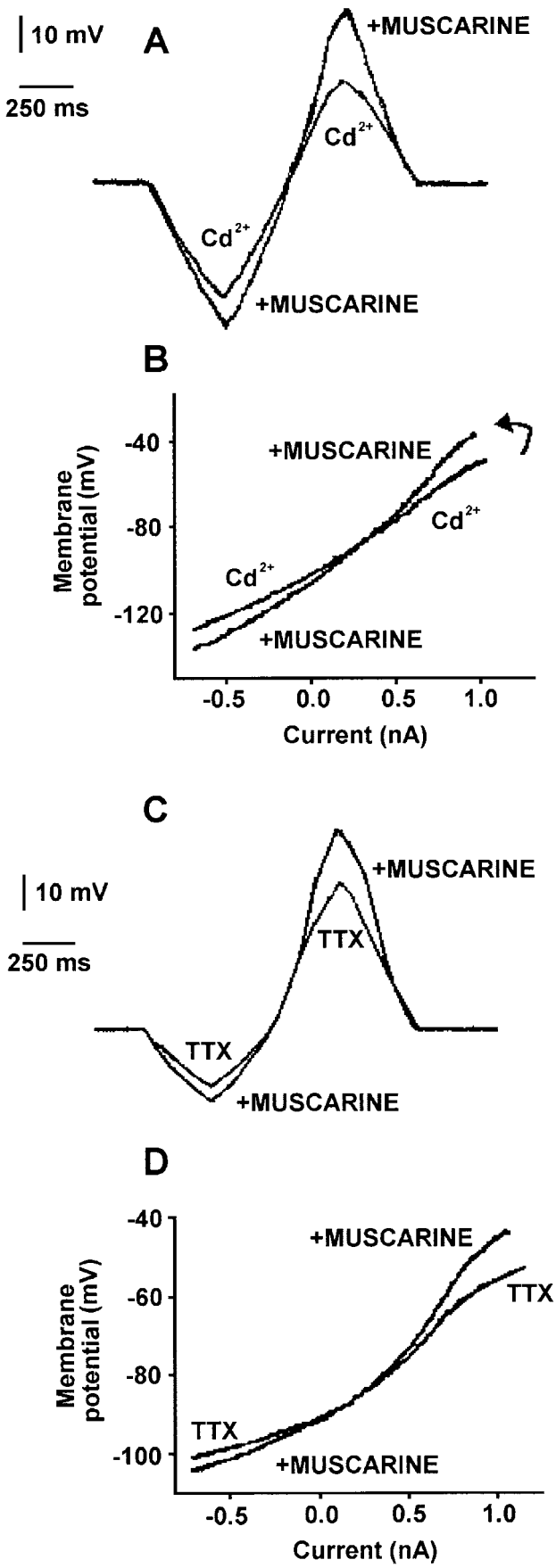


Fig. 3. The MT-3 peptide had some blocking action in some cells. **A:** Voltage response to a current ramp (top). **B:** Addition of muscarine (1 μM) produced a robust increase in the evoked discharge. **E:** Corresponding I–V plots (from ascending records in **A** and **B**) show an increase in R_N (I–V slope) after muscarine. **C:** In this cell, the MT-3 peptide had some blocking effect on the evoked discharge facilitated by muscarine. **F:** The superimposition of I–V plots taken from records **B** and **C** shows that a reduction in slope took place after MT-3. This change in slope only occurs at the most depolarized subthreshold potentials. Inward rectification in most subthreshold ranges did not change. **D:** The effect of the MT-3 peptide could be reversed after washing the toxin. **G:** Corresponding I–V plots (from ascending records **C** and **D**) show that the change in I–V slope was reversed after the toxin was washed.

carine (1 μM) produced an enhancement of R_N and evoked discharge (see corresponding I–V plots in Fig. 3E). As expected, most subthreshold actions were not reversed by MT-3, since corresponding I–V plots in Figure 3F (muscarine vs. muscarine plus MT-3) superimposed almost completely. However, Figure 3C also shows that the MT-3 peptide could reverse a part of these effects. At potentials just below firing threshold, a change in I–V slope could be detected (Fig. 3F). This was accompanied by a corresponding decrease in evoked discharge (Fig. 3C). When the MT-3 peptide was removed from the superfusion, the full I–V relation and evoked discharge obtained with muscarine alone could be recovered (Fig. 3D,G). Thus, the MT-3 peptide did not block most effects of muscarine at subthreshold membrane potentials, especially the most negative, but partially reversed the effects of muscarine at the most positive subthreshold potentials in 40% of the neurons. This suggests the participation of an ion conductance, different from the inward rectifier, in the subthreshold response.

Figure 4 shows that the subthreshold response to cholinergic muscarinic agonists on spiny projection neurons cannot be suppressed with either sodium or calcium channel blockers. Figure 4A,C shows a superimposition of subthreshold voltage trajectories in response to current ramps (not shown) in both control conditions and in the presence of 400 μM Cd^{2+} ($n = 5$)



or 1 μM tetrodotoxin (TTX) ($n = 6$). Figure 4B,D shows the corresponding I–V plots. Notice that the response to 1 μM muscarine persists in both cases. This suggests that the response to muscarine, in the entire subthreshold range, does not depend on either a Na^+ (TTX-sensitive) conductance or on a subthreshold Ca^{2+} conductance. These findings also confirm that muscarinic actions studied are direct, i.e., the receptors activated are on spiny cells (since Cd^{2+} and TTX would suppress most spontaneous, and all evoked, synaptic activity in the preparation).

Figure 5 shows the voltage responses of a cell representative of another set of experiments. In these experiments, both 5 mM Cs^+ and 1 μM TTX were administered. Thus, Figure 5A shows the block of inward rectification by Cs^+ at hyperpolarized potentials. This blockade produced a characteristic voltage trajectory that has been described with both ramp and step stimuli (Galarraga et al., 1994; Reyes et al., 1998). Notice that, under these conditions, addition of muscarine (1 μM) did not affect the hyperpolarizing region of the voltage trajectory (Fig. 5B) (see corresponding I–V plots in Fig. 5D). Thus, Cs^+ occluded most muscarinic responses at the most negative subthreshold voltage range. However, in some neurons the depolarizing region of the voltage response still exhibited an increase in slope due to muscarine (Fig. 5D). In other words, Cs^+ occluded the muscarinic action in the region where the inward-rectifying Cs^+-sensitive conductance activates, but did not affect the muscarinic response at the more depolarized subthreshold region. In this condition we added the MT-3 peptide to the bath saline (Fig. 5C). I–V plots in Figure 5E show that this M_4-type receptor antagonist reversed most of this muscarinic action ($n = 3$). This effect was reversible (not shown). These results confirmed that a part of the facilitatory action of muscarine in some spiny projection neurons may be mediated by M_4-type receptors and further confirmed that they do not depend on a TTX-sensitive conductance.

Since Cs^+, Cd^{2+}, and TTX cannot block the muscarinic response at depolarized subthreshold potentials (Figs. 4, 5) (Galarraga et al., 1999a), we performed a set of experiments in saline with a low concentration of extracellular Na^+. The results in a representative cell

Fig. 4. Muscarinic actions take place in the absence of synaptic activity. **A:** In the presence of Cd^{2+} (200 μM), two subthreshold voltage responses to the same linear ramp (not depicted) were superimposed. One of the traces shows that muscarine kept changing the subthreshold voltage trajectory in the presence of Cd^{2+}. **B:** Corresponding I–V plots show that muscarine increased R_N in the presence of Cd^{2+}. Thus, responses to muscarine were neither dependent on Ca^{2+} currents nor dependent on indirect synaptic activity. **C:** In the presence of tetrodotoxin (TTX 1 μM), two subthreshold voltage responses to the same linear ramp (not depicted) were superimposed. One of the traces show that muscarine kept changing the subthreshold voltage trajectory in the presence of TTX. **D:** Corresponding I–V plots show that muscarine increased R_N in the presence of TTX. Thus, responses to muscarine did not depend on Na^+ currents (TTX-sensitive) or the firing of action potentials.

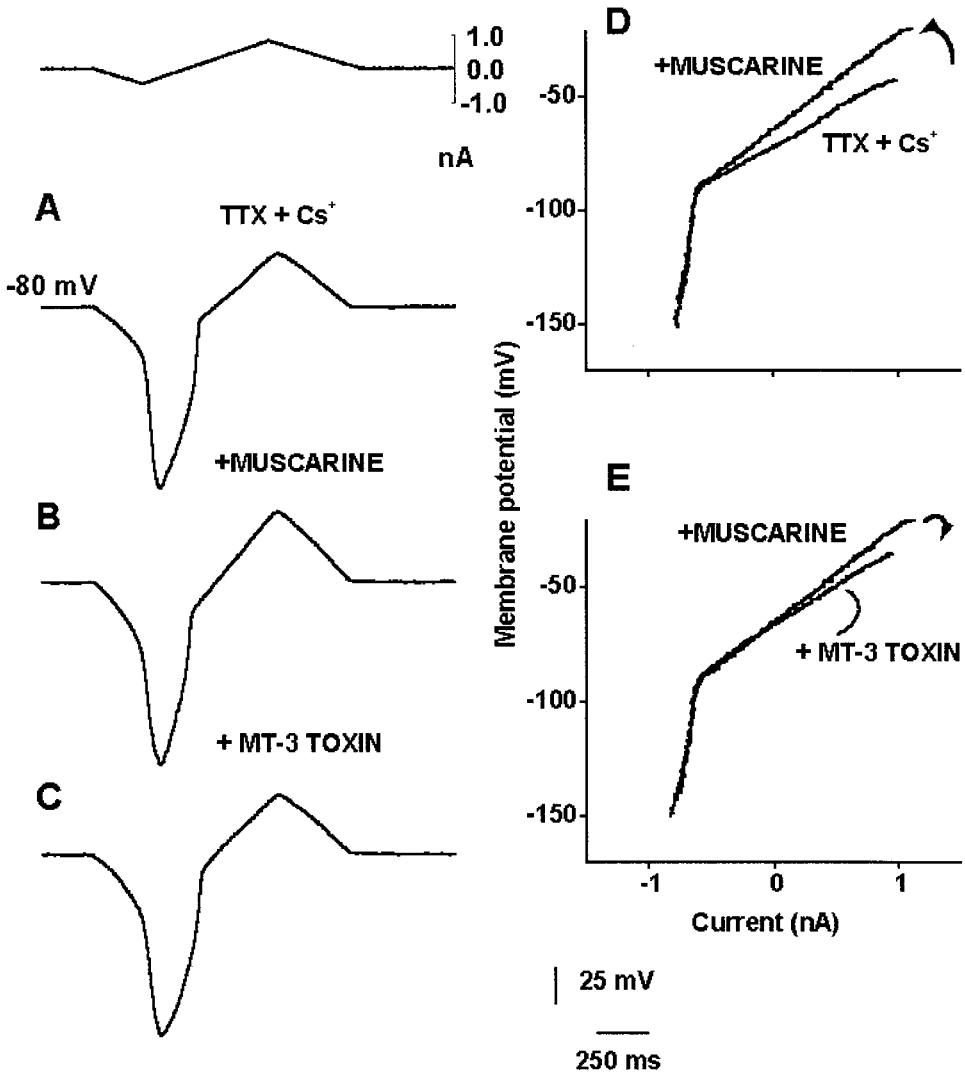


Fig. 5. Subthreshold muscarinic actions after inward rectification block. **A:** Voltage response to a current ramp (top) in the presence of Cs^+ (5 mM) and TTX (1 μM). **B:** Muscarine (1 μM) changed the voltage trajectory of the response at the most depolarized subthreshold potentials. **D:** The effect is evident when comparing the corresponding I–V plots (from ascending records in **A** and **B**). In this cell, muscarine increased I–V slope at the most depolarized voltage range. **C:** Addition of the MT-3 antagonist peptide reversed part of the muscarinic effects on the subthreshold voltage trajectory. **E:** This is evident after superimposing the corresponding I–V plots (from ascending records in **B** and **C**). Note that MT-3 greatly reversed the action of muscarine on I–V slope. It is evident that not all muscarinic effects were occluded by Cs^+, but only those occurring at the most negative subthreshold range.

are depicted in Figure 6: in opposition to Cs^+, a low Na^+-saline occluded muscarinic actions at the most depolarized subthreshold potentials, but muscarinic actions at the most hyperpolarized subthreshold potentials were left intact ($n = 4$). These experiments clearly showed that a part of the muscarinic response, where the action of the M_4-type receptor antagonist may be present, does not depend on the Cs^+-sensitive inward-rectifying conductance.

DISCUSSION

This is the first report on the membrane actions of the muscarinic peptides (Bradley, 2000; Jerusalinsky et al., 1995; Karlsson et al., 2000; Liang et al., 1996; Purkerson and Potter, 1998) on neostriatal projection neurons. The importance of this report relies on the heavy cholinergic modulation of the neostriatal output (Calabresi et al., 2000). This work reports that muscarinic peptides acted as expected according to previous knowledge.

Nonselective antagonists (Caulfield and Birdsall, 1998) had been tested before in these neurons (Galarraga et al., 1999a; Calabresi et al., 2000). The results obtained with the peptidic antagonists supported the general view obtained with the less selective drugs and different techniques, i.e., that the main postsynaptic muscarinic receptor on spiny projection cells is the M_1-type receptor (Alcantara et al., 2001; Consolo et al., 1987; Hersch et al., 1994; Weiner et al., 1990; Yan and Surmeier, 1996). This conclusion was reached based on the fact that a specific M_4-type receptor antagonist (MT-3) did not block most actions of muscarine, or the MT-1 peptide, whereas a specific M_1-type receptor antagonist (MT-7) easily blocked these effects.

The present results confirmed the important subthreshold facilitatory actions of muscarinic agonists in these neurons (Dodt and Misgeld, 1986; Galarraga et al., 1994; Nisenbaum and Wilson, 1995; Mermelstein et al., 1998; Reyes et al., 1998; Calabresi et al., 2000), namely, an increase in apparent R_N with a consequent

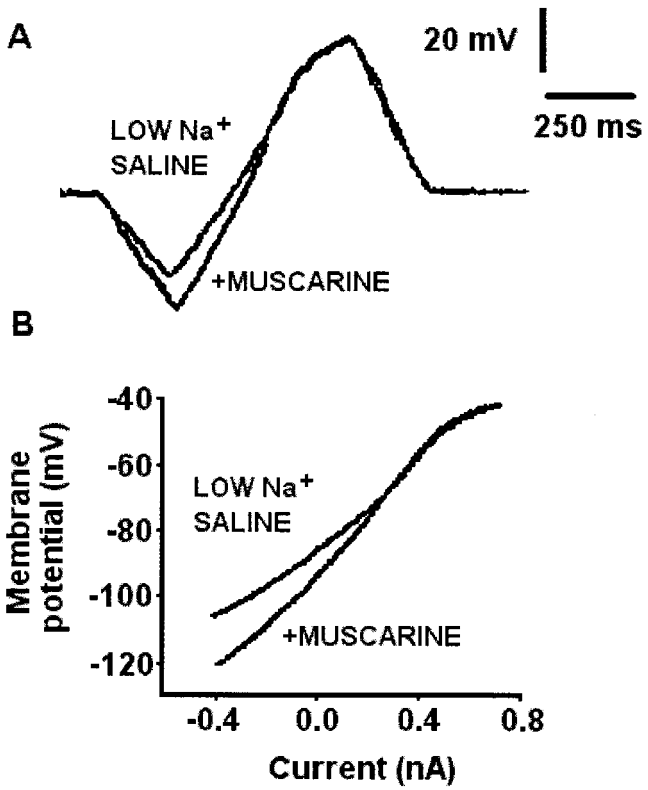


Fig. 6. Low Na^+ saline blocks muscarinic actions at the most depolarized subthreshold potentials. **A:** Two subthreshold voltage responses to a linear current ramp (not depicted), obtained in low Na^+ (25 mM) saline. Na^+ was substituted with choline. Muscarine changed the voltage trajectory of the subthreshold response only at the most hyperpolarized potentials. **B:** Corresponding I–V plots confirm that a change in I–V slope only occurred at the most hyperpolarized potentials. The muscarinic action at more depolarized subthreshold potentials was occluded.

increase in excitability and evoked discharge. Further, they show conclusively that these facilitatory actions are mainly due to M_1-type receptor activation in the great majority of cells. The relevance of this conclusion becomes clear by recalling that endogenous acetylcholine is able to produce these actions (Galarraga et al., 1999a). And since muscarinic receptor activation also decrease outward currents activated by firing in these cells (Pineda et al., 1995; Gabel and Nisenbaum, 1999), the overall postsynaptic result due to muscarinic receptor activation is facilitation. Therefore, the main cellular correlate of the behavioral states produced by increased cholinergic activity in the neostriatum should be a facilitation of neostriatal output (Bickerdike and Abercrombie, 1997).

Other important issues arise from the present experiments: 1) Cs^+ did not occlude all the subthreshold facilitatory actions of muscarine. An additional facilitatory action may be present at less negative, but still subthreshold, membrane potentials. 2) It was confirmed that this additional facilitatory action cannot be blocked by Cs^+, TTX, or Cd^{2+} (Galarraga et al., 1999a) but only by a low Na^+ solution. Therefore, it is not due to the inward rectifier Kir2 channels, but is likely caused by a cationic conductance. 3) Part of this additional facilitation can be induced by activation of M_4-type receptors in some cells (about 40% in the present sample). This last conclusion is supported by the finding that M_4-type receptors are abundant in the neostriatum (Vilaró et al., 1991) but not all projection neurons express large quantities of them (Yan et al., 2001).

In conclusion, the present results provide evidence that the subthreshold response to muscarinic receptor agonists may involve more than a single ion conductance (Shen and North, 1992; Galarraga et al., 1999a) and more than a single receptor type. Further experiments are needed to see if M_1- and M_4-type receptors cooperate to produce this additional facilitation. M_4-type receptors may participate only in some cells. Perhaps both receptor types cooperate to activate the same conductance or a further dissection of ion conductances may be necessary. A difficulty of these studies is that not all cells express large quantities of both receptor types. A synergy between M_1- and M_4-type receptors is expected to produce a strong facilitation and enhanced excitability. It is doubtful if cells expressing M_4-type receptors belong to the direct or the indirect pathway (Yan et al., 2001).

It is intriguing that, postsynaptically, muscarinic activation is facilitatory for the projection neurons (Galarraga et al., 1999a; Pineda et al., 1995; Gabel and Nisenbaum, 1999), whereas presynaptically it is inhibitory, i.e., it decreases the release of both glutamate from cortical afferents (Hernández-Echeagaray et al., 1998; Barral et al., 1999; Calabresi et al., 2000) and GABA from the synaptic contacts that neostriatal interneurons make on spiny neurons (Calabresi et al., 2000; Koos and Tepper, 2002). In the case of cortical afferents the receptors involved appear to be of the M_2- and/or M_3-types (Hernández-Echeagaray et al., 1998) and their affinity seems to be much higher than the postsynaptic receptors (nanomolar vs. micromolar range). That is, at a low level of activity the cholinergic function may be expected to repress desynchronized inputs, thus collaborating with the low level of spontaneous activity seen on the neostriatum. But a strong synchronized input will activate the cholinergic interneuron so that released acetylcholine may reach the concentration necessary to produce output facilitation (Galarraga et al., 1999a). Noticeably, a biphasic modulation has also been found for another important modulator of basal ganglia function: dopamine (Galarraga et al., 1997; Hernandez-Lopez et al., 1997, 2000). In vivo correlated experiments are needed to find out what this means. A feedback system controls the firing of the cholinergic interneuron, since inhibitory M_2-type muscarinic autoreceptors control (Calabresi et al., 1998; Galarraga et al., 1999a; Yan and Surmeier, 1996) the firing of these interneurons and perhaps facilitates the induction of rhythmic firing patterns (Bennett and

Wilson, 1999; Dolezal and Tucek, 1999), whereas nicotinic receptors strongly activate GABAergic interneurons (Koos and Tepper, 2002). Thus, the complexity of muscarinic actions on the network is overwhelming and represents a great experimental and modeling challenge.

Antimuscarinic drugs may ameliorate the symptoms of Parkinson's disease. However, given the weak selectivity of the antagonists used in therapeutics nowadays, it is no surprise that antimuscarinics do not constitute the treatment of choice. However, a sharp division of roles in the excitability and output of the striatal net is now evident, depending on the activation of different muscarinic receptors. Therefore, the advent of a new generation of more selective antimuscarinic drugs may improve the available therapeutic choices in the near future (Eglen et al., 2001).

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