Muscarinic Inhibition of Persistent Na\(^+\) Current in Rat Neocortical Pyramidal Neurons

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Mittmann, Thomas and Christian Alzheimer. Muscarinic inhibition of persistent Na\(^+\) current in rat neocortical pyramidal neurons. J. Neurophysiol. 79: 1579–1582, 1998. Muscarinic modulation of persistent Na\(^+\) current (\(I_{\text{NaP}}\)) was studied using whole cell recordings from acutely isolated pyramidal cells of rat neocortex. After suppression of Ca\(^{2+}\) and K\(^+\) currents, \(I_{\text{NaP}}\) was evoked by slow depolarizing voltage ramps or by long depolarizing voltage steps. The cholinergic agonist, carbachol, produced an atropine-sensitive decrease of \(I_{\text{NaP}}\) at all potentials. When applied at a saturating concentration (20 \(\mu M\)), carbachol reduced peak \(I_{\text{NaP}}\) by 38% on average. Carbachol did not alter the voltage dependence of \(I_{\text{NaP}}\) activation nor did it interfere with the slow inactivation of \(I_{\text{NaP}}\). Our data indicate that \(I_{\text{NaP}}\) can be targeted by the rich cholinergic innervation of the neocortex. Because \(I_{\text{NaP}}\) is activated in the subthreshold voltage range, cholinergic inhibition of this current would be particularly suited to modulate the electrical behavior of neocortical pyramidal cells below and near firing threshold.

INTRODUCTION

Activation of muscarinic receptors exerts a wide spectrum of actions in neocortical neurons, including modulation of K\(^+\) currents, Ca\(^{2+}\) currents, and a nonselective cation current (Haj-Dahmane and Andrade 1996; McCormick 1993). In the present study, we investigated whether muscarinic receptor stimulation also would affect the persistent Na\(^+\) current (\(I_{\text{NaP}}\)) of neocortical neurons, a functionally important ion conductance in the subthreshold voltage range (Crill 1996). It is firmly established that Na\(^+\) currents can be modulated by protein kinase A- and C-mediated phosphorylation of the principal (\(\alpha\)) subunit of Na\(^+\) channel proteins (Catterall 1992). Because muscarinic receptors use protein kinase C (PKC)-mediated phosphorylation as one of their signal transduction pathways (Durieux 1996), Na\(^+\) channel gating represents a putative target of cholinergic modulation in rat neocortex. With whole cell recordings from acutely isolated neocortical pyramidal cells, we report here that activation of muscarinic receptors decreases persistent Na\(^+\) current, without changing its voltage-dependent properties or its slow inactivation kinetics.

METHODS

Details of the slicing and dissociation procedures have been described elsewhere (Alzheimer et al. 1993a). Briefly, 400-\(\mu m\)-thick coronal slices were taken from sensorimotor cortex of ether-anesthetized rats 13–19 days old. Before mechanical trituration, small pieces of slice tissue were incubated for 90 min at 29\(^\circ\)C in N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered oxygenated saline solution containing 19 U/ml papain. After suppression of Ca\(^{2+}\) and K\(^+\) currents, \(I_{\text{NaP}}\) was evoked by slow depolarizing voltage ramps or by long depolarizing voltage steps. The cholinergic agonist, carbachol, produced an atropine-sensitive decrease of \(I_{\text{NaP}}\) at all potentials. When applied at a saturating concentration (20 \(\mu M\)), carbachol reduced peak \(I_{\text{NaP}}\) by 38% on average. Carbachol did not alter the voltage dependence of \(I_{\text{NaP}}\) activation nor did it interfere with the slow inactivation of \(I_{\text{NaP}}\). Our data indicate that \(I_{\text{NaP}}\) can be targeted by the rich cholinergic innervation of the neocortex. Because \(I_{\text{NaP}}\) is activated in the subthreshold voltage range, cholinergic inhibition of this current would be particularly suited to modulate the electrical behavior of neocortical pyramidal cells below and near firing threshold.

RESULTS

To isolate voltage-dependent Na\(^+\) currents, Ca\(^{2+}\) and K\(^+\) currents were blocked by external Cd\(^{2+}\) and internal Cs\(^+\), respectively. When applied under these conditions, long depolarizing voltage steps or slow depolarizing voltage ramps evoked \(I_{\text{NaP}}\) of typical voltage dependence and amplitude (cf. Alzheimer et al. 1993b). The effect of the cholinergic agonist carbachol (20–100 \(\mu M\)) was tested in 25 visually identified pyramidal-shaped neurons. The nicotinic receptor antagonist, mecamylamine (10 \(\mu M\)) was added routinely to the bathing solutions to block nicotinic responses to carbachol. In the first set of experiments, slow depolarizing voltage ramps (−70–0 mV) were applied at 15-s intervals, and peak \(I_{\text{NaP}}\) amplitudes were determined before, during, and after superfusion of carbachol (Fig. 1, A and B). In 5 of 25 cells, carbachol did not affect \(I_{\text{NaP}}\). In the remaining cells (80%), carbachol consistently reduced \(I_{\text{NaP}}\). At 20 \(\mu M\), carbachol decreased peak \(I_{\text{NaP}}\) to 62.4 ± 2.6% (\(n = 17\)) of
Fig. 2. Cholinergic inhibition of $I_{\text{NaP}}$ is mediated by muscarinic receptors. Atropine (1 μM) abolished reduction of $I_{\text{NaP}}$ by carbachol (20 μM).

Atropine (1 μM) abolished reduction of $I_{\text{NaP}}$ by carbachol (20 μM). The instantaneous surge of transient inward current was followed by a small sustained inward current, $I_{\text{NaP}}$ (cf. Alzheimer et al. 1993b). Application of 20 μM carbachol produced an apparently time-independent decrease of $I_{\text{NaP}}$ that was reversible after drug wash-out. Comparison of the complete $I$-$V$ curves (Fig. 3B) shows that the voltage dependence of $I_{\text{NaP}}$ activation was not altered by muscarinic modulation.

One functionally important feature of $I_{\text{NaP}}$ is the slow inactivation brought about by prolonged depolarization (Fleidervish and Gutnick 1996). To examine whether muscarinic receptor activation would affect this process, activation of $I_{\text{NaP}}$ was tested using depolarizing voltage ramps of variable control. At higher concentrations (50–100 μM), carbachol did not further decrease peak $I_{\text{NaP}}$ amplitude (reduction to 59.6 ± 2.3% of control, n = 5, P = 0.59). The outward-going, nonselective cation current ($I_{\text{cat}}$) appearing at potentials more positive than −30 mV under these experimental conditions (cf. Alzheimer 1994) was not affected by carbachol (100 μM) when studied in isolation (i.e., in the presence of 1 μM tetrodotoxin, n = 4, Fig. 1C). To establish the involvement of muscarinic receptors, responses to carbachol were measured in the absence and presence of the muscarinic antagonist, atropine. Peak $I_{\text{NaP}}$ amplitudes obtained in carbachol solution or in carbachol/atropine solution were normalized to the peak $I_{\text{NaP}}$ amplitude recorded under control conditions.

Figure 2 summarizes the results from five different neurons, where carbachol alone (20 μM) reduced peak $I_{\text{NaP}}$ to 65.8 ± 3.2% of control, whereas the current attained almost its maximum value when carbachol was applied with atropine (1 μM) present in the bathing solution (94.6 ± 3.1% of control, $P = 0.0002$).

To determine the effects of carbachol under steady-state voltage-clamp conditions, membrane potential was depolarized using square voltage commands to various test potentials. Figure 3A shows the typical biphasic current response evoked by a sustained depolarizing voltage step to $-42$ mV. The instantaneous surge of transient inward current was followed by a small sustained inward current, $I_{\text{NaP}}$ (cf. Alzheimer et al. 1993b). Application of 20 μM carbachol produced an apparently time-independent decrease of $I_{\text{NaP}}$ that was reversible after drug wash-out. Comparison of the complete $I$-$V$ curves (Fig. 3B) shows that the voltage dependence of $I_{\text{NaP}}$ activation was not altered by muscarinic modulation.

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BI was independent of ramp speed. In this voltage range, only reduces current amplitude but also slows fast inactivation (Cantrell et al. 1996; Catterall 1992), carbachol did not affect the mechanism(s) responsible for slow inactivation rhythmicity in stellate cells of entorhinal cortex layer II.

DISCUSSION

Our data predict that $I_{NaP}$ of pyramidal cells can be modulated by the dense cholinergic innervation reaching the cerebral cortex. With the cholinergic agonist carbachol in combination with a nicotinic receptor antagonist, we found that stimulation of muscarinic receptors produced about equal reduction of $I_{NaP}$ independent of ramp speed and corresponding $I_{NaP}$ availability. Figure 4B summarizes data from seven such experiments. Each column represents the carbachol-induced reduction in relative $I_{NaP}$ at the ramp speed indicated above. Because the column means were not significantly different, carbachol is unlikely to interfere with the mechanisms underlying slow inactivation of $I_{NaP}$.

How does the (partial) inhibition of a small current like $I_{NaP}$ compare to other actions of acetylcholine in the brain? Despite the well-established and, in part, strong effects of excitatory synaptic input in different neuronal compartments and during carbachol (20 μM) reducing transient Na$^+$ current by ~30% (Cantrell et al. 1996).

Given the almost equal sensitivity of fast and sustained Na$^+$ current to cholinergic input, the ratio of persistent to fast Na$^+$ current should remain largely unaffected by muscarinic modulation. In this respect, the action of carbachol differs from effects of state-dependent Na$^+$ channel blockers such as the anticonvulsant phenytoin, which suppresses $I_{NaP}$ more potently than fast Na$^+$ current (Chao and Alzheimer 1995). Phenytoin and carbachol seem to employ different mechanisms of Na$^+$ current modulation. Because of its higher affinity to inactivated Na$^+$ channels, phenytoin preferentially diminishes the late Na$^+$ channel openings (Segal and Douglas 1997) that are thought to underlie $I_{NaP}$ (Alzheimer et al. 1993a), whereas muscarinic receptor stimulation appears to reduce early and late Na$^+$ channel openings to an approximately equal extent.

REFERENCES


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