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 mechanic 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. Whole cell currents were evoked and recorded using an Axopatch 200 amplifier in conjunction with a TL-1 interface and pClamp 6.0 software (all from Axon Instruments). Current signals were sampled at 2–5 kHz and filtered at 1 kHz (~3 dB). All recordings were performed at room temperature (21–24\(^\circ\)C). Membrane potential was corrected for liquid junction potential. Throughout the experiments, leakage and capacitive currents were determined from hyperpolarizing voltage steps and eliminated using the built-in compensation circuits of the amplifier. Experiments were started when stable I\(_{\text{NaP}}\) responses were obtained, typically 5–8 min after whole cell access was established. During the initial stabilization period, neurons usually displayed a variable degree of I\(_{\text{NaP}}\) rundown. After that period, control recordings (n = 5) showed that I\(_{\text{NaP}}\) remained stable for the time of experimentation (10–15 min). If not stated otherwise, holding potential was ~70 mV. The bath solution contained (in mM) 130 NaCl, 3 KCl, 1.6 CaCl\(_2\), 0.4 CdCl\(_2\), 2 MgCl\(_2\), 25 HEPES/NaHEPES, and 10 D-glucose (pH 7.4). The pipette solution contained (in mM) 110 Cs-gluconate, 3 MgCl\(_2\), 5 ethylene glycol-bis(\(\beta\)-aminoethyl ether)\(-N,N,N’,N’\)-tetraacetic acid, 5 HEPES, 2 Tris-ATP, 0.3 Tris-GTP, 15 phosphocreatine, and 0.1 leupeptin (pH 7.25, adjusted with NaOH). When filled with pipette solution, electrode resistances were 4–6 M\(\Omega\) in the bath and 15–25 M\(\Omega\) in the whole cell configuration before series resistance compensation (~70–75%).

Drugs were bath applied by means of a multiple-inlet system allowing complete bath exchange within ~15 s. Carbachol, atropine, and mecamylamine were all purchased from Sigma (Deisenhofen, Germany). Statistics are presented as means ± SE. Statistical analyses (\(t\)-test and analysis of variance) were done with the use of Graphpad Prism 2.0.

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 \( \mu \)M) abolished reduction of \( I_{\text{NaP}} \) by carbachol (20 \( \mu \)M).

FIG. 3. \( I_{\text{NaP}} \) evoked by square voltage commands. A: reversible inhibition of \( I_{\text{NaP}} \) by 20 \( \mu \)M carbachol. Because of high gain of recording, fast Na\(^+\) current was largely truncated. For illustration, current signals were digitally refiltered at 0.2 kHz. B: \( I-V \) relationship of sustained inward current determined at the end of each voltage step before, during, and after carbachol (20 and 100 \( \mu \)M).

control. At higher concentrations (50–100 \( \mu \)M), carbachol did not further decrease peak \( I_{\text{NaP}} \) amplitude (reduction to 59.6 \( \pm \) 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 \( \mu \)M) when studied in isolation (i.e., in the presence of 1 \( \mu \)M 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 \( \mu \)M) reduced peak \( I_{\text{NaP}} \) to 65.8 \( \pm \) 3.2% of control, whereas the current attained almost its maximum value when carbachol was applied with atropine (1 \( \mu \)M) present in the bathing solution (94.6 \( \pm \) 3.1% of control, \( P = 0.0002 \)).
channel inactivation are separate processes that are regulated independently (Patlak 1991). In view of the small size of $I_{Na}$, the aim of the present study was not to obtain a complete dose-response relationship for cholinergic inhibition of $I_{Na}$ but to establish that $I_{Na}$ is subject to muscarinic modulation. Carbachol hence was applied at a saturating concentration (20 μM) that decreased $I_{Na}$ amplitude by 38% on average. Interestingly, a similar value was reported for muscarinic inhibition of fast Na$^+$ current in hippocampal neurons, with a saturating concentration of carbachol (50 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 $I_{Na}$ more potently than fast Na$^+$ current (Chao and Alzheimer 1995). Thus 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 $I_{Na}$ (Alzheimer et al. 1993a), whereas muscarinic receptor stimulation appears to reduce early and late Na$^+$ channel openings to an approximately equal extent.

How does the (partial) inhibition of a small current like $I_{Na}$ compare to other actions of acetylcholine in the brain? Despite the well-established and, in part, strong effects of muscarinic receptors in the nervous system, given the almost equal sensitivity of fast and sustained Na$^+$ current to cholinergic input, the ratio of persistent to fast Na$^+$ current may be more heavily weighted in the mechanisms underpinning slow inactivation of $I_{Na}$.

**Discussion**

Our data predict that $I_{Na}$ 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 causes an atropine-sensitive decrease of $I_{Na}$ at all potentials without changing the voltage dependence of $I_{Na}$ activation. Unlike muscarinic- or PKC-dependent modulation of fast Na$^+$ current, which not only reduces current amplitude but also slows fast inactivation (Cantrell et al. 1996; Catterall 1992), carbachol did not affect the mechanisms(s) responsible for slow inactivation of $I_{Na}$. This agrees with the notion that fast and slow Na$^+$

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