Ionic Basis of ON and OFF Persistent Activity in Layer III Lateral Entorhinal Cortical Principal Neurons

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Tahvildari B, Alonso AA, Bourque CW. Ionic basis of ON and OFF persistent activity in layer III lateral entorhinal cortical principal neurons. J Neurophysiol 99: 2006–2011, 2008. First published February 6, 2008; doi:10.1152/jn.00911.2007. Principal neurons in layer III of the rat lateral entorhinal cortex (LEC) generate a self-sustained plateau potential and persistent spiking following the application of a brief suprathreshold excitatory stimulus delivered in the presence of the muscarinic receptor agonist carbachol. This persistent activity can be terminated by application of a second excitatory stimulus, and these cells can be repeatedly toggled between ON and OFF states by consecutive excitatory stimuli. However, the ionic mechanisms that underlie the production of ON and OFF states in layer III LEC neurons are unknown but seem to involve activity-dependent conductances, since they can be initiated by trains of action potentials evoked by either depolarizing current pulses applied to the cell or by repetitive spiking induced by activation of excitatory synaptic inputs. In this study, we obtained intracellular recordings from rat layer III LEC neurons in vitro, and a series of pharmacological and ionic substitution experiments were performed to identify mechanisms involved in the induction and termination of persistent spiking. Our data indicate that initiation of the ON state depends on spike-evoked calcium influx and subsequent activation of calcium-activated nonselective cationic current. Moreover, we show that termination of persistent firing in response to an excitatory stimulus can be blocked by tetraethylammonium or ibeberiotoxin, suggesting that the activation of calcium-activated potassium current mediated by large conductance calcium-activated K+ (i.e., BK) channels is required to induce the OFF state.

INTRODUCTION

Previous studies in humans and animals have indicated that neurons of the lateral entorhinal cortex (LEC) may generate a persistent increase in electrical activity following the presentation of a sensory stimulus (Stern et al. 2001; Suzuki et al. 1997; Young et al. 1997). In monkeys, for example, an increase in neural activity is observed during the delayed match-to-sample task, in which a subject is required to recognize that a second (i.e., test) stimulus is identical to a “sample” sensory stimulus after a brief delay (Suzuki et al. 1997). Neurophysiological (reviewed in Goldman-Rakic 1995) and modeling studies (Fransen et al. 2002, 2006) have suggested that this sustained activity may be important for maintaining information related to the sample sensory stimulus during the delay phase of the task. Interestingly, deafferentation of the cholinergic input to the entorhinal cortex significantly impairs the performance of short-term memory tasks (McGaughy et al. 2005; Turchi et al. 2005). Moreover, functional MRI studies have shown that activation of the medial temporal lobe during such tasks is reduced by systemic administration of scopolamine, a muscarinic cholinergic antagonist (Schon et al. 2005). These data suggest that generation of poststimulus sustained spiking in entorhinal cortex neurons may require cholinergic activation.

We have recently shown that principal neurons in layer III of the rat LEC can generate poststimulation self-sustained persistent activity in presence of a cholinergic agonist (Tahvildari et al. 2007). This persistent increase in electrical activity is expressed in the form of an afterdischarge that is driven by a depolarizing plateau potential following a suprathreshold excitatory stimulus. Interestingly, we showed that poststimulation persistent firing in these cells could also be terminated by application of a second excitatory stimulus, providing a cellular mechanism by which the activity of layer III LEC principal neurons can be toggled ON and OFF in response to an identical stimulus. Toggling between ON and OFF states could also be induced by a brief (4 s) repetitive activation (~20 Hz) of excitatory synapses on electrical stimulation of the perirhinal cortex, suggesting that toggled ON-OFF firing could be promoted by this pathway in situ (Tahvildari et al. 2007).

Previous studies in other cortical neurons have indicated that activation of a calcium-dependent nonselective cation (CAN) current can mediate the onset and maintenance of plateau potentials and spiking induced by depolarizing pulses applied in the presence of cholinergic agonists (Andrade 1991; Constanti and Bagetta 1991; Schwindt et al. 1988). Whether CAN channels are involved in genesis of plateau potentials and persistent spiking in layer III LEC principal neurons remains unknown. Therefore in this study, we performed pharmacological and ionic substitution experiments to identify ionic mechanisms contributing to the induction and termination of muscarinic receptor-dependent persistent firing in layer III LEC principal neurons.

METHODS

Preparation of brain slices

All experimental procedures were approved by the McGill University Animal Care Committee and were in compliance with the guidelines of the Canadian Council on Animal Care. Conventional sharp microelectrode intracellular recordings were performed on brain slices obtained from adult Long-Evans rats (male, 150-250 g; Charles River Canada, Saint-Constant, Quebec, Canada). Semicoronal in vitro rat
of a suprathreshold (0.1–0.3 nA, 4 s) depolarizing current pulse, and this activity could be switched on/off by the application of a second depolarizing pulse, as reported previously (Tahvildari et al. 2007). Neurons in which the off state was induced by a depolarizing pulse remained completely silent until another current pulse was delivered to re-establish the on state (≤104 s later).

Previous studies in other cortical neurons have shown that induction and maintenance of muscarinic receptor–dependent postexcitation plateau potentials and afterdischarges depend on influx of extracellular calcium through voltage-gated calcium channels (Andrade 1991; Constanti and Bagetta 1991; Egorov et al. 2002; Schwindt et al. 1988). We therefore examined if calcium influx was required for these features in LEC layer III neurons. Bath application of CdCl₂ (400 μM), a broad spectrum blocker of voltage-gated calcium channels, significantly inhibited the depolarizing plateau potential (10.62 ± 0.94 mV in control vs. −0.4 ± 0.18 mV in Cd²⁺; n = 8; P < 0.001) and the accompanying persistent activity that followed a depolarizing pulse (Fig. 1, A and B). Similarly, the poststimulus depolarizing plateau potential and accompanying persistent activity were eliminated in a Ca²⁺-free solution containing 1 mM EGTA and 4 mM Mg²⁺ (10.33 ± 1.22 mV in control vs. −0.25 ± 0.28 mV in Ca²⁺-free; n = 6; P < 0.001; Fig. 1, C and D). Moreover, in three neurons impaled with microelectrodes containing 200 mM EGTA, the ability to generate poststimulus plateau and persistent spiking was gradually abolished over a period of 20–30 min following the impalement (10.83 ± 0.6 mV immediately after impalement vs. 1.83 ± 0.34 mV 20 min following the impalement; P < 0.01; Fig. 1, E and F). These data suggest that the onset of persistent firing following an excitatory stimulus depends on influx of calcium through voltage-gated channels and on its accumulation inside the neuron.

Previous studies have shown that CAN channels commonly mediate the spike- and burst-evoked plateau potentials and afterdischarges of neurons in different brain areas (Egorov et al. 2002; Ghamari-Langroudi and Bourque 2002; Haj-Dahmane and Andrade 1998). Since the induction of the on state of layer III LEC neurons is calcium dependent, we next examined the possibility that a CAN current contributes to the generation of poststimulus plateau potentials and associated persistent activity. As shown in Fig. 2, bath application of flufenamic acid (FFA, 100 μM), a blocker of the CAN current (Partridge and Valenzuela 2000), significantly reduced the depolarizing plateau potential (11.5 ± 0.92 mV in control vs. 1.25 ± 0.28 mV in FFA; n = 6; P < 0.001) and persistent activity that followed a depolarizing pulse. However, a recent study in lamprey reticulospinal neurons has indicated that FFA may also inhibit calcium channels (Wang et al. 2006), indicating that caution must be used when interpreting the effects of this drug. We therefore examined if FFA could interfere with action potential parameters known to be calcium dependent in layer III EC neurons (Dickson et al. 1997). Bath application of 100 μM FFA had no effect on either action potential duration (Control, 0.98 ± 0.03 ms; FFA, 1.03 ± 0.03 ms; P > 0.15) or the amplitude of the fAHP (Control, −6.0 ± 0.4; FFA: −5.7 ± 0.3 mV; P > 0.3). In contrast, application of CdCl₂ (400 μM) significantly increased the duration of action potentials (Control, 1.00 ± 0.02; Cd²⁺, 1.40 ± 0.03 ms; P < 0.001) and significantly attenuated the amplitude of the fAHP (Control,
The inhibitory effect of FFA on plateau potentials and persistent firing in layer III LEC pyramidal neurons are likely caused by an inhibition of CAN channels rather than to a reduction of calcium influx during spike trains.

In principle, the termination of an on-going plateau potential and persistent spiking following application of a depolarizing pulse delivered during the ON state (i.e., transition to the OFF state) could be mediated by an activity-dependent reduction of the inward CAN current (Magistretti et al. 2004) or by the activation of an activity-dependent outward current. Previous studies have shown that low concentrations of TEA (e.g., 1 mM) can block large conductance calcium-activated K\(^+\) (i.e., BK) and intermediate conductance calcium-activated potassium (i.e., IK) channels (Vogalis and Goyal 1997). We therefore examined the effects of TEA on ON-OFF responses. Under control conditions, application of a 4-s depolarizing pulse 60 s following induction of the ON state caused an immediate reduction in plateau potential amplitude and spiking frequency. This initial effect was followed by a more gradual but complete...
collapse of the plateau potential and cessation of neural firing (Fig. 3A). In each of seven cells, bath application of 1 mM TEA abolished the ability of depolarizing pulses to cause ON-OFF transitions (Fig. 3A). Indeed, whereas persistent firing always stopped within 16 s of the second pulse under control conditions, neurons exposed to TEA continued to fire at a rate equivalent to the firing frequency observed before the second pulse (prepulse firing rate, 7.6 ± 0.6 Hz vs. postpulse firing rate at 32 s, 7.2 ± 0.6 Hz; n = 7; P > 0.05; Fig. 3B). Interestingly, the prepulse firing rate (i.e., basal rate of activity during persistent activity) was not affected by TEA (control, 7.4 ± 0.5 Hz vs. TEA, 7.6 ± 0.6 Hz; P > 0.05).

Since TEA can block both BK and IK channels (Vogalis and Goyal 1997), we also examined the effects of IBTX, a specific antagonist of BK channels. As shown in Fig. 3C, bath application of 100 nM IBTX also prevented depolarizing pulses from inducing transitions from the ON to the OFF state in LEC layer III principal neurons (Fig. 3, C and D; n = 3). Indeed, cells challenged during the ON state with a depolarizing pulse delivered in presence of IBTX failed to show any reduction in firing rate.

**FIG. 3.** OFF–ON transitions are mediated by large conductance calcium-activated potassium (i.e., BK) channels. CCh (10 μM) was present in all conditions, and dashed lines show baseline voltage. A: intracellular recordings of membrane voltage (top traces; initial voltage, −62 mV) and firing frequency (bottom traces; bin width, 500 ms) observed from a layer III LEC pyramidal neuron during current pulses (middle traces) injected under control conditions (left) and in the presence of 1 mM tetraethyl-ammonium chloride (TEA; right). Pulses applied during the ON state fail to suppress persistent firing in the presence of TEA. Note that depolarizing pulses applied during the ON state induce more action potentials than those applied during the OFF state under both conditions. B: plots show the mean ± SE (n = 7) frequency of firing observed at various time points following the application of a depolarizing pulse delivered during the ON state under control conditions and in the presence of TEA. For this analysis, data were systematically obtained from pulses delivered ~60 s following the onset of the ON state, and identical pulses were used for this comparison in the absence and presence of drug. C: recording from another neuron (layout of traces as in A; initial voltage, −63 mV) shows the effects of 100 nM iberiotoxin (IBTX). Note that pulses applied during the ON state fail to suppress persistent firing in the presence of IBTX. D: plots show the mean ± SE (n = 3) frequency of firing observed at various time points following the application of a depolarizing pulse delivered during the ON state under control conditions and in the presence of IBTX. There was no significant difference between the mean frequency of basal persistent firing observed before application of the 2nd pulse in the 2 groups of cells in the absence of drug (TEA cells: 7.4 ± 0.5 Hz, IBTX cells: 9.6 ± 1.9 Hz; P = 0.14) or in the firing rates observed 32 s after the 2nd pulse in cells treated with TEA (7.2 ± 0.6 Hz) or IBTX (10.1 ± 1.5 Hz; P = 0.15).
firing rate (prepulse firing rate, 10.4 ± 1.3 Hz vs. postpulse firing rate at 32 s, 10.1 ± 1.5 Hz; n = 3; P > 0.05). Furthermore, the prepulse firing rate (i.e., basal rate of activity during persistent activity) was not affected by IBTX (control, 9.6 ± 1.9 Hz vs. IBTX, 10.4 ± 1.3 Hz).

Although persistent firing could not be stopped by the application of depolarizing current steps delivered in the presence of either TEA or IBTX, prolonged hyperpolarizing current pulses (>60 s) applied under these conditions could still effectively restore the off state, as found under control conditions (Tahvildari et al. 2007), and persistent firing (i.e., the on state) could be initiated again by application of a depolarizing pulse. Thus TEA and IBTX seem to interfere specifically with ion channels that mediate transitions to the off state.

**DISCUSSION**

Recent studies have shown that a variety of neurons in the CNS can operate as toggle switches, where the electrical activity of the cell can be consecutively turned on and off by identical stimuli (Loewenstein et al. 2005; Shu et al. 2003; Tahvildari et al. 2007). This behavior can be expressed through different mechanisms and could play an important role in the short-term encoding or storage of information during signal processing in various parts of the brain. For example, in ferret prefrontal cortical neurons, switching between on and off states can be promoted by changing the proportional balance of excitation and inhibition generated through local recurrent synaptic connections (Shu et al. 2003). In contrast, the on and off states of cerebellar Purkinje cells are each stabilized by intrinsic ionic conductances, and on-off transitions can be induced by stimuli that alternately promote changes in membrane potential toward depolarized and hyperpolarized voltages (Loewenstein et al. 2005). A recent study has shown that principal neurons in layer III of the LEC also display on-off transitions in response to brief excitatory stimuli (Tahvildari et al. 2007), where it may play an important role in the encoding and storage of short-term memory (Goldman-Rakic 1995). This phenomenon was retained even when glutamatergic and GABAergic synaptic transmission was blocked by kynurenic acid and picROTOXIN, suggesting that on-off transitions are caused by intrinsic processes. However, the mechanisms supporting this behavior were not defined.

Our results show that induction of the on state in these neurons is inhibited by blockade of Ca²⁺ influx. Indeed, lowering extracellular Ca²⁺, or blocking voltage-gated calcium channels with Cd²⁺, prevented the emergence of plateau potentials and persistent firing following depolarizing pulses applied during the off state. This process was also abolished by intracellular injection of EGTA. These observations indicate that the on state is triggered by the influx and accumulation of intracellular Ca²⁺ that occurs during the action potentials evoked by a depolarizing stimulus. Our experiments also showed that poststimulus plateau potentials and persistent firing could be abolished by bath application of FFAs, an inhibitor of CAN channels (Partridge and Valenzuela 2000). Taken together, these data indicate that transition from the off to the on state is caused by the depolarizing effect of an inward current that may be mediated by CAN channels that are activated in response to calcium influx during a depolarizing stimulus.

Previous immuno-histochemical and in situ hybridization studies have indicated that BK channels are highly expressed in the outer layers (i.e., layers II and III) of the LEC (Knaus et al. 1996) and throughout the cortex (Wanner et al. 1999). We therefore examined the possible involvement of these channels during transitions from on to off states in layer III LEC pyramidal neurons. We found that blockade of BK channels, either by application of TEA or IBTX, prevented the suppression of plateau potentials and persistent firing that was normally induced by the application of a depolarizing stimulus in control solutions. Thus an activity-dependent activation of BK channels seems to be required to promote transitions from on to off states in these neurons. Taken together our findings suggest that depolarizing pulses promote transitions from the off state to the on state by activating CAN channels and transitions from the on state to the off state by activation of BK channels.

Although both types of channels may be activated to some extent during spike trains delivered during either state, our data suggest that long-lasting activation of BK channels only occurs when a spike train is induced during established persistent activity. Indeed, blockade of BK channels (with IBTX or TEA) did not increase the rate of steady-state firing during the on state (indicating that BK channels are not significantly activated at this time), but it prevented the collapse of the plateau potential (i.e., hyperpolarization) and the inhibition of firing following depolarizing pulses applied during the on state. In principle, this enhanced activation of BK channels during persistent firing could be caused by an increase in Ca²⁺ influx or accumulation under these conditions or the fact that they are stimulated from a more positive membrane potential during an established plateau. Indeed, BK channels are known to be highly sensitive to voltage and intracellular Ca²⁺ concentration (Sah 1996; Vergara et al. 1998). Alternately, BK channels may become more responsive to an equivalent calcium load because of a Ca²⁺- and time-dependent priming effect caused by persistent firing. Further studies are required to investigate these possibilities.

In principle, persistent firing could also increase the relative proportion of BK:CAN channels activated during a depolarizing pulse by promoting the inactivation or desensitization of CAN channels. Indeed, it is conceivable that Ca²⁺ influx and/or accumulation might be greater during an action potential train delivered following a period of persistent firing, and it has been hypothesized that the activity of CAN channels may be reduced, rather than enhanced, by large pulse-induced increases in intracellular Ca²⁺ concentration (Magistretti et al. 2004). However, under conditions where BK channels were blocked (i.e., in the presence of IBTX or TEA), persistent firing could persist indefinitely and could not be terminated by injection of depolarizing pulses. Moreover, in these experiments, spike-mediated Ca²⁺ influx was likely to be enhanced because of the increase in action potential duration caused by TEA (Control, 1.00 ± 0.08 ms; TEA, 1.40 ± 0.04 ms; P < 0.001) and IBTX (Control, 1.10 ± 0.06 ms; IBTX, 1.60 ± 0.05 ms; P < 0.01). Thus a Ca²⁺-dependent inactivation of CAN channels is unlikely to mediate transitions from the on to the off state in LEC layer III pyramidal neurons. Additional studies will be required to examine the possible contribution of other types of ion channels, and the dynamics of CAN and BK channels, during on-off transitions in these cells.
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REFERENCES


