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ORIGINAL PAPER

Membrane Potential Dependent Duration of Action Potentials in Cultured Rat Hippocampal Neurons

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Abstract (1) Fluctuations of the membrane potential states are essential for the brain functions from the response of individual neurons to the cognitive function of the brain. It has been reported in slice preparations that the action potential duration is dependent on the membrane potential states. (2) In order to examine whether dependence of action potential duration on the membrane potential could happen in isolated individual neurons that have no network connections, we studied the membrane potential dependence of the action potential duration by artificially setting the membrane potentials to different states in individual cultured rat hippocampal neurons using patch-clamp technique. (3) We showed that the action potential of individual neurons generated from depolarized membrane potentials had broader durations than those generated from hyperpolarized membrane potentials. (4) Furthermore, the membrane potential dependence of the action potential duration was significantly reduced in the presence of voltage-gated K^+ channel blockers, TEA, and 4-AP, suggesting involvement of both delayed rectifier I_K and transient I_A current in the membrane potential dependence of the action potential duration. (5) These results indicated that the dependence of action potential duration on the membrane potential states could be an intrinsic property of individual neurons.

Keywords Action potential · Action potential duration · Voltage-gated K^+ channel · Membrane potential · Neuron

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Abbreviations

4-AP	4-Aminopyridine
AP	Action potential
APD	Action potential duration
I_A	Transient outward K^+ current
I_K	Persistent delay rectifier K^+ current
MP	Membrane potential
TEA	Tetraethylammonium

Introduction

The cognitive ability of both humans and animals depends on the brain states. The brain states are believed to be ultimately attributed to the fluctuations of the membrane potential (MP) states of individual neurons (Fujisawa et al. 2006). It has been found that many kinds of neurons fluctuate between two sustained levels of MP states both under anesthesia and during quiet wakefulness. These states are referred to as the down-state and the up-state, which modify the responses of neurons to sensory stimuli (Wilson and Kawaguchi 1996; Anderson et al. 2000; Petersen et al. 2003). In the down-state, neurons are relatively hyperpolarized (−75 to −85 mV), while in the up-state neurons sit at a MP close to the threshold for a spike generation (between −65 and −50 mV). Many kinds of neurons have been shown to have such a bistability of their MP states (O'Donnell and Grace 1995; Wilson and Kawaguchi 1996; Anderson et al. 2000; Heyward et al. 2001; Sachdev et al. 2004). While the up-states are predominantly a network phenomenon, or at least crucially depend on synaptic input, these forms of bistability also exist at the single neuron level (Loewenstein et al. 2005). These states influence the neural processing of sensory inputs; that is to say, the intensity and propagation of neuronal activity vary depending on whether neurons reside in the up- or down-state (Anderson et al. 2000; Petersen et al. 2003; Sachdev et al. 2004).

In slice preparations, it has been reported that the action potential duration is dependent on the membrane potential states, from which the action potentials are generated (Shu et al. 2006). However, it is not clear whether this is a network phenomenon or an intrinsic property of single neurons. If it originates from single neurons, what kinds of ionic currents are involved in and how the MP state regulates the properties of the action potential (AP) by modifying these ionic currents. In the present study, therefore, we studied the relationship of the action potential duration (APD) and the MP states of single hippocampal pyramidal neurons by artificially setting the MP to different states. We demonstrate that the MP states affect the APD through regulation of the voltage-dependent transient outward current (I_A) and the late outward rectifying current (I_K) in cultured hippocampal neurons.

Materials and Methods

Cell Culture

Hippocampal neurons were acutely dissociated according to a previous method (Brewer et al. 1993; Chen et al. 2005) with slight modification. All animal protocols were approved by the animal research ethical committee (Institute of Biophysics, CAS). Briefly, the hippocampi were dissected from neonatal Sprague–Dawley rats, and neurons were dissociated by

incubation (7 min, 37°C) in Trypsin–EDTA (GIBCO) and triturated in DMEM (Life Technologies) supplemented with 10% bovine serum (Hyclone, Logan, Utah). The resulting hippocampal cells were plated at a density of 2×10^5 cells/cm^2 onto poly-L-lysine (Sigma, St. Louis, MO, USA) coated glass coverslips. The coverslips were then incubated at 37°C in a humidified atmosphere of 95% O_2 and 5% CO_2. The medium was replaced 7 h later with Neurobasal^{TM}-A Medium, B-27 (GIBCO) and 0.5 mM glutamine without antibiotic solution. After 48 h the medium was changed to Neurobasal and B27.

Electrophysiological Recording

Current-clamp and voltage-clamp recordings were performed on the hippocampal pyramidal neurons between 6 and 10 days in vitro by using the EPC-10 patch-clamp amplifier (HEKA, Germany). Pipette and membrane capacitances were compensated automatically with the amplifier. Currents were corrected offline for a linear leak current measured at −90 mV. A program package Pulse + Pulsefit (HEKA, Germany) was used for data acquisition and analysis. HANKS' Balanced salts solution (HBSS, Sigma) was taken as extracellular solution (in mM): 1.3 $CaCl_2$, 0.8 $MgSO_4$, 5.4 KCl, 0.4 KH_2PO_4, 136.9 NaCl, 0.3 Na_2PO_4, 10 D-glucose, and 4.2 $NaHCO_3$ (pH 7.3–7.5). The intracellular solution contained (in mM): 155 KCl, 2 NaCl, 0.1 $CaCl_2$, 1 EGTA, 2 MgATP, and 10 HEPES at pH 7.4. All experiments were performed at room temperature (22–25°C). For current clamp, neurons were held at given MPs at a range of −95 to −45 mV by injection of steady currents range from −180 to −20 pA. APs were evoked by passing 5 ms depolarizing pulses of increasing magnitude (−50 to 250 pA in 50 pA steps) through the patch electrode. The APDs were measured at one-third the peak amplitude of AP. K^+ currents were evoked by depolarizing voltage steps from indicated holding potentials to +70 mV in a step of 20 mV increment with duration of 200 ms. For activation curves, the experimental data were fitted with a modified Boltzmann equation: $G/G_{\max} = P_v/\{1 + \exp[-(V - V_{1/2})/k]\}$,where $G_{\max}$ is the maximum conductance (usually achieved at −120 mV) among all the hold potentials, P_v is the maximum open probability of the channel at a given holding potential, V is the membrane potential, $V_{1/2}$ is the voltage at half-maximal activation, and k is the slope factor. All the data are mean ± SE for at least three experiments. Significance among multiple groups was determined by one-way analysis of variance (ANOVA).

Results

Effect of the MP States on the APD in Individual Cultured Neurons

In order to understand the effect of the MP states on the neuronal excitability, we studied the properties of the AP generated in single cultured pyramidal neurons at different MP states, which were artificially set by injections of different currents under current clamp. The APD was dependent on the MP, from which the AP was generated. For example, in an experiment shown in Fig. 1a, the AP generated from a MP of −82 mV had a duration (measured at one-third of the peak amplitude) of 0.5 ms. In contrast, the APD increased to 0.9, 1.3, and 2.0 ms when the APs were generated from MPs of −76, −68, −58 mV, respectively. The falling phase of AP had a time constant of 0.6 ms at MP of −82 mV. In contrast, the time constant increased to 1.2, 1.9, and 4.6 ms when the MP is shifted to more depolarized levels of −76, −68, and −58 mV. In order to clearly show the statistic result, the MP values from −95 to −45 mV were divided into

five groups: −95 to −86 (−88.6 ± 0.9 mV), −85 to −76 (−79.7 ± 0.6 mV), −75 to −66 (−70.7 ± 0.7 mV), −65 to −56 (−60.8 ± 0.6 mV), and −55 to −46 (−52.0 ± 0.5 mV). A summary of this MP-dependent APD was shown in Fig. 1b. In all the neurons tested, the APD ?tul?> of the AP triggered from relatively depolarized states, was longer than that from relatively hyperpolarized states (Fig. 1b). The mean APD was increased from 0.61 ± 0.04 ms at −88.6 ± 0.9 mV to 0.98 ± 0.05 ms at −79.7 ± 0.6 mV, 1.29 ± 0.06 ms at −70.7 ± 0.7 mV, 1.88 ± 0.07 ms at −60.8 ± 0.6 mV, and 2.39 ± 0.11 ms at −52.0 ± 0.5 mV (Fig. 1b). In contrast to significant difference ($P < 0.001$) of APD between different groups, the AP overshoots were not significantly different (Fig. 1b, c).

Holding MP-Dependent Changes of Gating of the Outward K^+ Currents

The above results indicated that main changes in the APDs occurred through changes in falling phase, suggesting involvement of the voltage-dependent K^+ currents in the AP repolarization. No significant difference from the whole cell current was observed under high (10 mM) or low (nominal free) extracellular Ca^{2+} concentration (data not shown), suggesting that the

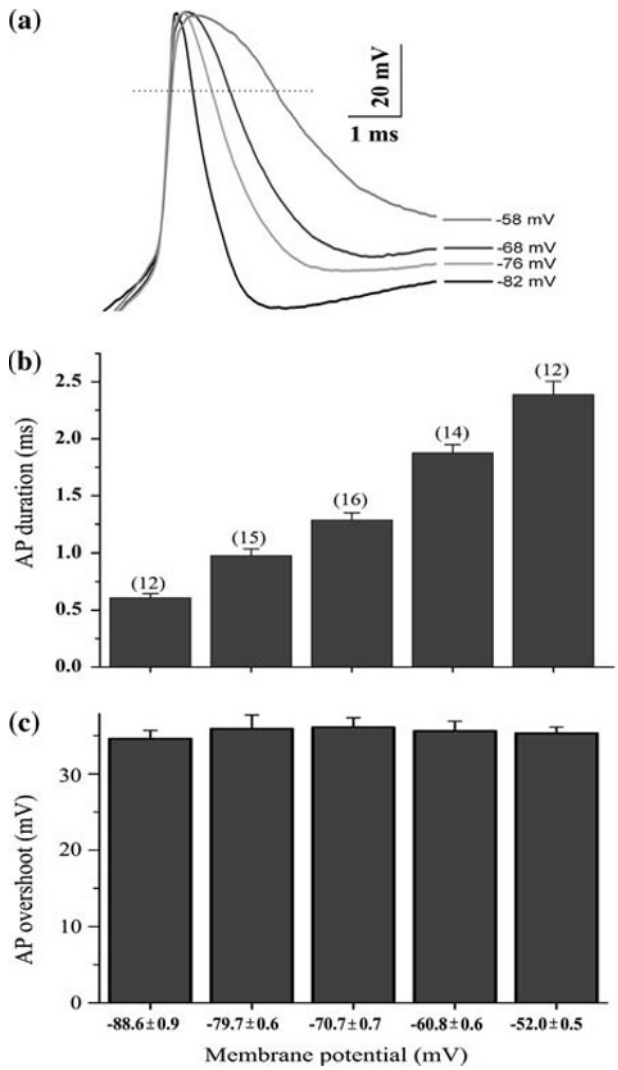

Fig. 1 Effect of the MP states on the APDs in cultured rat hippocampal neurons. (**a**) AP traces generated from different MPs, the values of which are indicated in the *right*. Action potentials were aligned at the time of maximal upstroke to allow comparison of time course. (**b**) Plot of the action potential duration measured at one-third of AP amplitude (*dotted line*) versus different MP states. (**c**) AP overshoot from different membrane potentials. Times of each experiment are indicated in the *parenthesis* in (**b**)

component of Ca^{2+} activated currents could be neglected. Therefore, the outward voltage-dependent potassium currents were split into two major components: a fast transient A-type, 4-aminopyridine sensitive I_A and a delayed rectifier, TEA sensitive I_K current as previously suggested (Klee et al. 1995). In order to find factors that determine the MP dependence of APD, we applied 5 mM TEA and 3 mM 4-AP to dissect the outward K^+ current into I_A and I_K, respectively. The current traces for I_K and I_A generated from different holding MPs (Fig. 2a, b) indicated that the magnitude of both I_K and I_A current was strongly dependent on the holding MP states. For I_K current, the current was only 0.9 nA at +70 mV when it was generated from a holding MP of −40 mV. This value increased to 2.5 and 3.2 nA when the holding MPs were changed to −80 and −120 mV. For I_A current, there was almost no I_A current when the

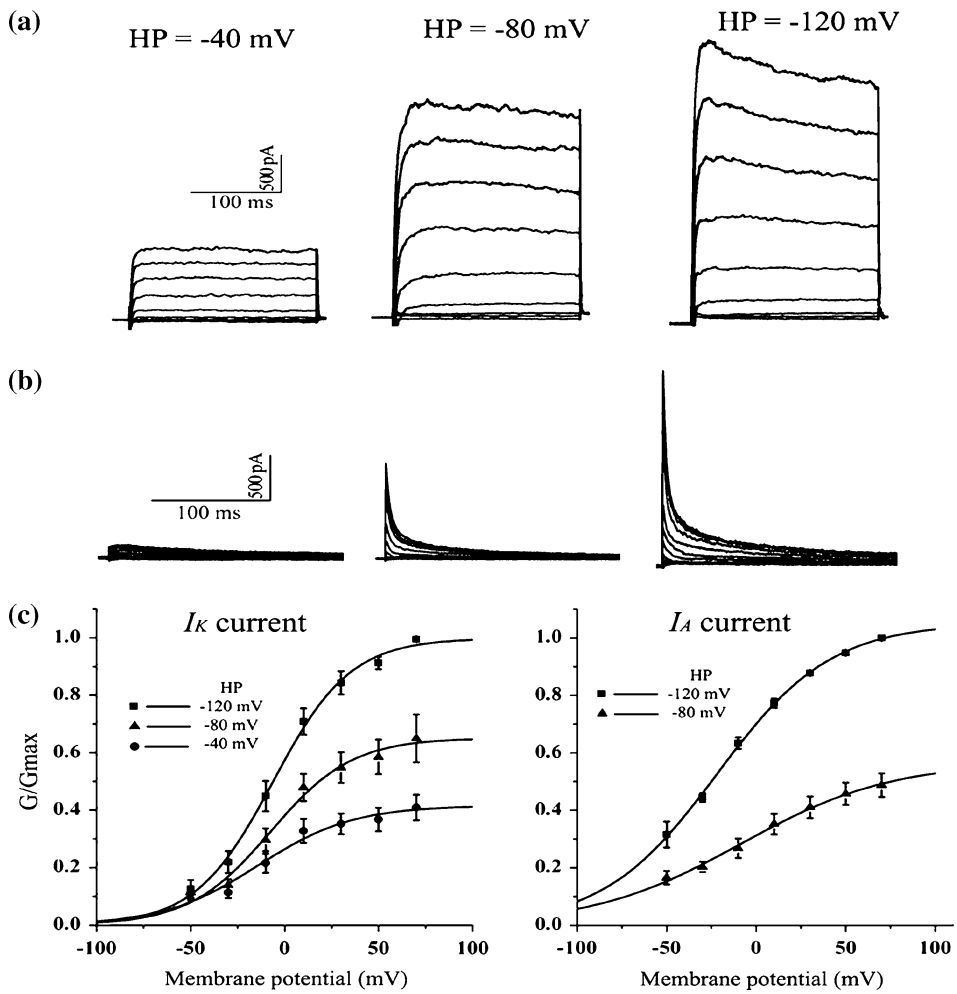

Fig. 2 Effect of MP states on outward K^+ currents. Current traces for I_K (**a**) and I_A (**b**) are shown after holding membrane potentials (HP) for 100 ms at −40 mV, −80 mV, and −120 mV. (**c**) Activation curves for I_K and I_A that were elicited at different MP states (n = 3–15). The normalized conductance (G/G_{max}) is plotted against the membrane potential and fitted with the Boltzmann equation. The values of $V_{1/2}$ and k for I_K were −14.0 mV and 25.3 at HP of −40 mV, −7.5 mV and 21.5 at HP of −80 mV, −6.2 mV and 20.6 at HP −120 mV. Almost no I_A was observed at −40 mV due to inactivation. The values of $V_{1/2}$ and k for I_A were −8.4 mV and 41.9 at HP of −80 mV, −22.0 mV and 31.8 at HP of −120 mV

holding MP was at −40 mV. However, the I_A current increased to 1.1 and 2.1 nA when the holding MP changed to −80 and −120 mV. Furthermore, the corresponding activation curves at different MP states (Fig. 2c) indicated that the activation of I_A current depends on holding potentials more than that of the I_K current.

Effects of MP States on the APDs in the Presence of Channel Blockers

In order to judge the contribution of I_A and I_K currents to the voltage dependence of the APD, we examined the effects of MP on the APD in the presence of potassium channel blockers. The increase in the APD during depolarization is still evident due to prolongation of APD by externally applied blockers (Fig. 3a–c). However, the relative APD was reduced in the

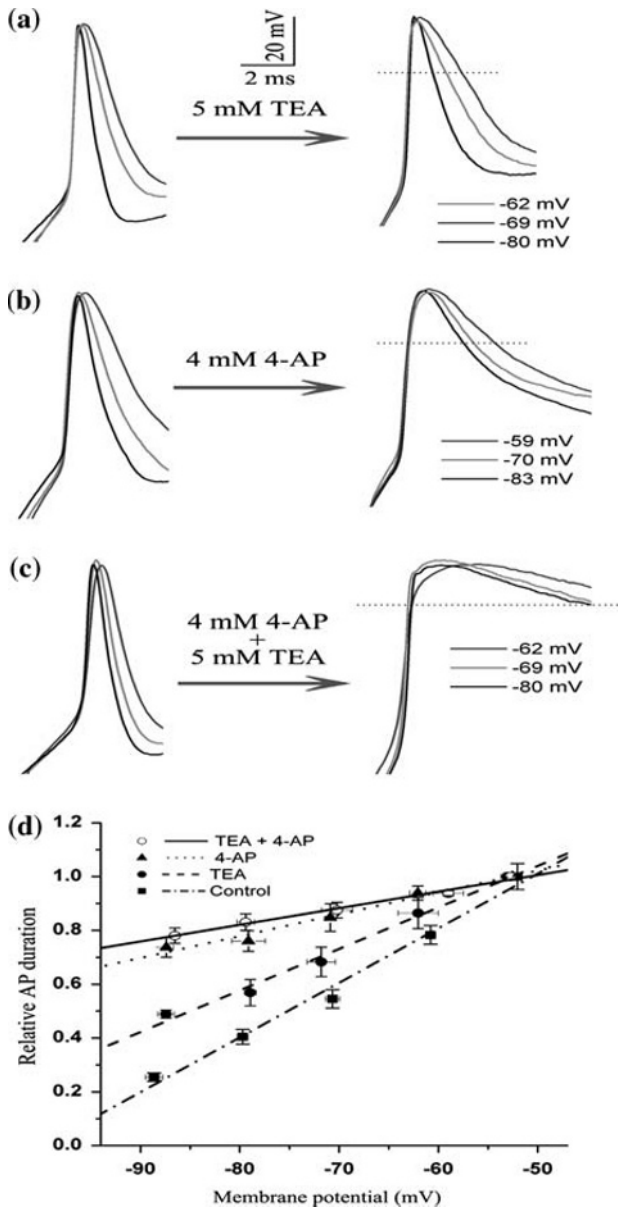

Fig. 3 Effect of the MP states on the APDs in the presence of K^+ channel blockers: TEA (**a**), 4-AP (**b**), both of TEA and 4-AP (**c**). Each experiments in **a**, **b**, and **c** was from the same neuron and representative of 5, 5 and 3 neurons, respectively. Action potentials were aligned at the time of maximal upstroke to allow comparison of time course. (**d**) Summary for relative changes of the MP dependence of the APD in the presence of K^+ channel blockers. Each APD at a given holding membrane potential was normalized to the duration recorded near −50 mV

presence of 4-AP and/or TEA when the APD was normalized to the duration recorded at the most depolarized MP. For example, the relative reductions of a neuron in Fig. 3b of APD for MP of −69 and −82 mV (relative to MP of −59 mV) were 18, 25% in the presence of 4-AP, while the relative reductions of the same neuron in the control condition were 38%, 64%. Similar results could be observed in the presence of TEA and in the presence of both TEA and 4-AP (Fig. 3a, c). A summary of the relative changes of MP dependence of the APD in the presence of K^+ channel blockers is presented in Fig. 3d. In all cases, the relative changes were nearly linear. Thus, the slope of the curve could be used as an index to show the MP dependence of the APD: the larger the slope, the greater the MP dependence of the APD. Figure 3d shows that the slopes of the relative APD enhancements, in the presence of channel blockers, are significantly less than those slopes of the control neurons, suggesting that the voltage dependence of the relative APD enhancement was reduced in the presence of blockers. The relative APD changed 20% per 10 mV in the control condition. In the presence of TEA and 4-AP, this value reduced to 15% and 8%, respectively, (Fig. 3d). Furthermore, the relative APD only changed 6% per 10 mV when both TEA and 4-AP were applied. These results suggest that the 4-AP sensitive I_A current is the major contributor, while the TEA sensitive I_K current only contributes slightly to the voltage dependence of the APD.

Discussion

The MP of the plasma membrane is a key factor in regulating the voltage-gated ion channels, which have important roles in shaping the AP in the excitable cells. Its alteration has been shown to influence the pattern of AP and the transmitter release from the axonal terminals (Shu et al. 2006). The present study suggested that both delayed rectifier I_K and transient I_A current are involved in the MP dependence of the APD. It has been reported that the 4-AP sensitive K^+ currents contribute significantly to the repolarization of the AP (Mitterdorfer and Bean 2002). In agreement with this result, our results indicated a strong effect of 4-AP on the APD. We demonstrated that APs generated at the depolarized MP had a longer duration than those triggered at a hyperpolarized MP. A similar result in slice preparation has been reported to show that the APDs are dependent on the MP levels (Shu et al. 2006).

Neurons process and encode information by generating sequences of APs. Minor modifications of the cell's action potential-generating mechanism can qualitatively change the nature of neuronal encoding (Naundorf et al. 2006). Therefore, the dependence of the APD on the MP states may have important physiological meanings. The MP can be regarded as a "state" of a neuron, evolving continuously over time and space in response to ever changing sensory inputs. A fluctuating MP induced by synaptic inputs and the cooperative interaction of excitatory and inhibitory inputs, are important factors to determine the possible MP states. Thus, the MP states are highly dependent on neuronal activities. APD plays a determinant role in the dynamics of pre-synaptic Ca^{2+} influx (Qian and Saggau 1999), which is important for triggering neurotransmitter release (Hochner et al. 1986). When the MP is altered to an up-state by barrages of input synapses (Mahon et al. 2001), the AP triggered from this relatively depolarized potential will be broader. This spike broadening may contribute to the enhanced transmitter release and a larger post-synaptic change in the follower neuron, as occurs elsewhere (Sabatini and Regehr 1997; Shu et al. 2006). This effect forms a basis in that the excitability of a neuron depends on its recent history of activation.

In conclusion, we found that the membrane potential dependence of the action potential duration could be an intrinsic nature of single neurons and interactions between neurons is not essential. Furthermore, we showed that even though both outward I_K and I_A currents

contributed to the membrane potential dependence of the action potential duration, the I_A current had a greater contribution than the I_K current.

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