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Contribution of slowly inactivating potassium current to delayed firing of action potentials in NG108-15 neuronal cells: Experimental and theoretical studies

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Abstract

The properties of slowly inactivating delayed-rectifier K^+ current ($I_{K_{dr}}$) were investigated in NG108-15 neuronal cells differentiated with long-term exposure to dibutyryl cyclic AMP. Slowly inactivating $I_{K_{dr}}$ could be elicited by prolonged depolarizations from −50 to +50 mV. These outward K^+ currents were found to decay at potentials above −20 mV, and the decay became faster with greater depolarization. Cell exposure to aconitine resulted in the reduction of $I_{K_{dr}}$ amplitude along with an accelerated decay of current inactivation. Under current-clamp recordings, a delay in the initiation of action potentials (APs) in response to prolonged current stimuli was observed in these cells. Application of aconitine shortened the AP initiation in combination with an increase in both width of spike discharge and firing frequency. The computer model, in which state-dependent inactivation of $I_{K_{dr}}$ was incorporated, was also implemented to predict the firing behavior present in NG108-15 cells. As the inactivation rate constant of $I_{K_{dr}}$ was elevated, the firing frequency was progressively increased along with a shortening of the latency for AP appearance. Our theoretical work and the experimental results led us to propose a pivotal role of slowly inactivating $I_{K_{dr}}$ in delayed firing of APs in NG108-15 cells. The results also suggest that aconitine modulation of $I_{K_{dr}}$ gating is an important molecular mechanism through which it can contribute to neuronal firing.

Keywords: Delayed-rectifier K^+ current; Slow inactivation; Action potential; Aconitine; Computer simulations

1. Introduction

Delayed firing or facilitation of action potentials (APs) was thought to be property present in a variety of neurons. The slowly inactivating K^+ currents in neurons or neuroendocrine cells have been suggested to exert a role in producing different firing patterns of APs (Rudy and McBain, 2001; Golomb et al., 2007). Slow inactivation of these currents can be elicited by prolonged depolarizations of hundreds of milliseconds or seconds or by long trains of brief repetitive depolarizations (Rudy and McBain, 2001; Fernandez et al., 2003). The membrane potential to which a cell goes to is determined by a fine dynamic balance between available Na^+ and K^+ conductances. Therefore, the role of this type of K^+ current is thought to produce a progressive decrease in K^+ conductance, thus leading to a shorter inter-spike interval or delayed facilitation of APs in time-coding neurons (Marom, 1998; Klemic et al., 2001; Rudy and McBain, 2001).

The slowly inactivating K^+ channels also exhibit a range of state dependencies of inactivation. C-type inactivation of *Shaker*-related channels was thought to be coupled to channel opening and exhibit little or no voltage dependence

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(Marom, 1998). Upon depolarization, the K^+ channel opens quickly, but inactivates slowly, which results in large K^+ currents that decay slowly (Rasmusson et al., 1998; Klemic et al., 2001). A subfamily (i.e., Kv3 or KCNC) of K^+ channels have very fast activation and deactivation kinetics associated with a high threshold for activation (Marom, 1998; Rudy and McBain, 2001). Kv3 channels assemble from subunits that in mammals are coded by four genes, named KCN1-4 (Kv3.1–3.4 subunits) (Rasmusson et al., 1998; Kurata and Fedida, 2006).

NG108-15 cell has been used as a neuron model in electrophysiology and pharmacology researches (Brown and Higashida, 1988). It is a hybrid cell line derived from the fusion of two separate cell lines, mouse neuroblastoma (N18TG-2, a subclone of mouse C1300 neuroblastoma cells) and rat glioma (C6BV-1, a subclone of rat C6 glioma) (Brown and Higashida, 1988). This cell line was previously reported to express Kv3.1a mRNAs and to exhibit the activity of the slowly inactivating delayed rectifier K^+ (K_{dr}) channels (Yokoyama et al., 1993; Wu et al., 2001; Lo et al., 2003). K_{dr} channels from the Kv3.1–Kv3.2 types, which are the major determinant of $I_{K_{dr}}$ in NG108-15 cells, are responsible for spike repolarization and after-hyperpolarization of these cells (Yokoyama et al., 1993; Wu et al., 2001). This cell line was thought to be a suitable model for investigating the mechanisms of neuronal development and differentiation (Yokoyama et al., 1993; Kawaguchi et al., 2007).

Aconitine is a toxic diterpenoid alkaloid occurring in plants of the *Aconitum* genus. It is recognized for their phytomedical effects on the heart, central nervous system, and skeletal muscle (Ameri, 1998). It has been reported that this compound binds with high affinity to the open state of voltage-gated Na^+ channels, thus causing a persistent activation of Na^+ channels (Ameri, 1998; Wang and Wang, 2003; Fu et al., 2006). However, little information is available regarding the actions of aconitine-related alkaloids on voltage-gated K^+ channels.

In this study, we used an experimental and simulation approach to the evaluation of the role of slowly inactivating $I_{K_{dr}}$ in initiation and maintenance of neuronal firing. The results led us to propose that the K^+ conductance sustained by slowly inactivating K^+ channels play a pivotal role in inducing delayed firing of neuronal spikes. We also tested the effects of aconitine, an activator of Na^+ current (Ameri, 1998; Wang and Wang, 2003), on the slowly inactivating $I_{K_{dr}}$ in NG108-15 cells. We have experimentally observed that this compound suppressed $I_{K_{dr}}$ and accelerated current decay.

2. Materials and methods

2.1. Cell preparation and differentiation

The clonal strain NG108-15 cell line, formed by Sendai virus-induced fusion of the mouse neuroblastoma clone N18TG-2 and the rat glioma clone C6 BV-1, was originally obtained from the European Collection of Cell Cultures (ECACC-88112302; Wiltshire, UK). NG108-15 cells were kept in monolayer cultures at a density of $10^6\,ml^{-1}$ in plastic disks containing Dulbecco's modified Eagle's medium supplemented with 100 μM hypoxanthine, 1 μM aminopterin, 16 μM thymidine, and 5% fetal bovine serum as the culture medium, in a humidified incubator equilibrated with 95% air/5% CO_2 at 37 °C (Wu et al., 2001). Media were replenished every 2–3 days with fresh media. The experiments were generally performed after 5 days of subcultivation (60–80%).

To induce neuronal differentiation, culture medium was replaced with medium containing 1 mM dibutyryl cyclic-AMP and cells were cultured in the incubator for 1–7 days. NG108-15 cells could proliferate well in the culture medium; however, they could stop proliferating and induce the growth of neurites in response to dibutyryl cyclic-AMP (Tojima et al., 2003). The numbers of neurites and varicosities were found to be increased in NG108-15 cells treated with 1 mM dibutyryl cyclic AMP.

2.2. Electrophysiological measurements

Before electrophysiological experiments were performed, cells were dissociated with 1% trypsin/EDTA solution and an aliquot of cell suspension was transferred to a recording chamber positioned on the stage of an inverted microscope (DM IL; Leica Microsystems, Wetzlar, Germany). The recording pipettes were fabricated from Kimax-51 glass capillaries (Kimble Glass, Vineland, NJ) using a two-step microelectrode puller (PP-830; Narishige, Tokyo, Japan) and the tips were fire-polished with a microforge (MF-83; Narishige). When filled with pipette solution, their resistance ranged between 3 and 5 MΩ. Ion currents were recorded in whole-cell configuration of the patch-clamp technique as described previously (Wu et al., 2001), with the use of an RK-400 patch-clamp amplifier (Bio-Logic, Claix, France) or an Axopatch-200B amplifier (Molecular Devices, Sunnyvale, CA).

The signals were displayed on an HM-507 oscilloscope (Hameg, East Meadow, NY) and on a Dell 2407WFP-HC LCD monitor (Round Rock, TX). The data were stored online in a Slimnote VX_3 computer (Lemel, Taipei, Taiwan) via a universal serial bus port at 10 kHz through a Digidata-1322A interface (Molecular Devices). This device was controlled by pCLAMP 9.0 software (Molecular Devices). Cell-membrane capacitance of 26–48 pF (35.7 ± 6.4 pF; $n = 27$) was compensated. Series resistance, always in the range of 8–16 MΩ, was electronically compensated. Ion currents were low-pass filtered at 1 or 3 kHz. The signals were stored and analyzed by use of pCLAMP 9.0 (Molecular Devices), Origin 7.5 software (OriginLab, Northampton, MA), or custom-made macros in Microsoft Excel (Redmont, WA) (Wu et al., 2001; Liu et al., 2003). The pCLAMP-generated voltage-step profiles were used to determine the current–voltage (I–V)