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Low affinity block of native and cloned hyperpolarization-activated I_h channels by Ba^{2+} ions

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Abstract

Ba^{2+} is commonly used to discriminate two classes of ion currents. The classical inward-rectifying K^+ current, I_{Kir}, is blocked by low millimolar concentrations of Ba^{2+}, whereas the hyperpolarization-activated cation current, I_h, is assumed not to be sensitive to Ba^{2+}. Here we investigated the effects of Ba^{2+} on I_h currents recorded from rat hippocampal CA1 pyramidal neurons, and on cloned I_h channels composed of either HCN1 or HCN2 subunits transiently expressed in Human Embryonic Kidney (HEK) 293 cells. The results show that low millimolar concentrations of Ba^{2+} reduce the maximal I_h conductance (IC_{50}~3–5 mM) in both CA1 pyramidal neurons and in HEK 293 cells without specificity for HCN1 or HCN2 subunits. In addition, Ba^{2+} decreases the rate of activation and increases the rate of deactivation of I_h currents. Neither the half-maximal voltage of activation, V_h, nor the reversal potential of the I_h channels were affected by Ba^{2+}. The combined results suggest that Ba^{2+}, at concentrations commonly used to block I_{Kir} currents, also reduces the conductance of I_h channels without subunit specificity, and affects the kinetics of I_h channel gating.

Keywords: Ba^{2+}; Hyperpolarization-activated cation channels; In situ patch clamp; HEK 293 cells

1. Introduction

Hyperpolarization-activated I_h channels are a subset of voltage-gated ion channels which are expressed in both peripheral and central neurons (Pape, 1996; Robinson and Siegelbaum, 2003). I_h currents have been identified as a component of the anomalous inward rectification (an increase of conductance upon hyperpolarization) observed in many neurons (DiFrancesco, 1981a; Pape, 1996). I_h currents are distinguished from the classical inward-rectifying K^+ currents (I_{Kir}) by the fact that I_h channels are permeable to both Na^+ and K^+, resulting in a reversal potential well above the equilibrium potential of K^+, and I_h currents activate more slowly than I_{Kir} currents (DiFrancesco, 1981b; Pape, 1996; Robinson and Siegelbaum, 2003). Furthermore, it is widely assumed that I_{Kir} currents can be distinguished from I_h currents by their sensitivity to Ba^{2+} ions (Robinson and Siegelbaum, 2003). However, there are a number of studies which show that low millimolar concentrations of Ba^{2+} can reduce I_h currents recorded from a variety of preparations (Takahashi, 1990; Kamondi and Reiner, 1991; Bayliss et al., 1994; Wollmuth, 1995). In addition, we observed in a previous study that Ba^{2+} blocks I_h currents recorded from cell-attached patches in rat hippocampal CA1 pyramidal neurons (van Welie et al., 2002).

A family of four subunits, HCN1–4, has been cloned which underlies the molecular diversity of I_h channels (Ludwig et al., 1998; Santoro et al., 1998; Monteggia et al., 2000). When expressed in heterologous expression systems, each subunit gives rise to a hyperpolarization-activated inward current, albeit with distinct functional properties. It has been shown that the HCN subunits are differentially distributed among neurons, giving rise to a functional heterogeneity (Moosmang et al., 1999, 2001; Santoro et al., 2000). In hippocampus, HCN1 and HCN2 are predom-

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inantly expressed, whereas HCN3 and HCN4 are present at very low levels, if present at all (Moosmang et al., 1999; Monteggia et al., 2000; Santoro et al., 2000; Bender et al., 2001). In this study, we examined the effect of Ba^{2+} on I_h currents in rat hippocampal CA1 pyramidal neurons, and tested whether the block by Ba^{2+} is subunit-specific by expressing HCN subunits in Human Embryonic Kidney (HEK) 293 cells.

2. Materials and methods

2.1. Slice preparation

Hippocampal slices were prepared as described previously (van Welie et al., 2004). Briefly, male Wistar rats (14–28 days old) were decapitated and parasagittal slices (250 μm) of the hippocampus were cut on a vibroslicer (725M, Campden Instruments, Loughborough, UK). Slices were allowed to recover for 1 h at 31 °C in artificial cerebrospinal fluid containing (in mM): 120 NaCl, 3.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, and 25 glucose, continuously bubbled with 95% O_2 and 5% CO_2 (pH 7.4). Slices were kept at room temperature until use.

2.2. Cell culture

Human Embryonic Kidney 293 (HEK 293) cells were maintained in minimum essential medium (MEM) supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were plated on 12 mm cover slips, and were transiently transfected 2 days later with expression vectors for mHCN1, mHCN2, or hHCN2 using the calcium phosphate precipitation method. Results obtained with mHCN2 and hHCN2 did not differ significantly (data not shown). To detect transfected cells, a vector encoding Enhanced Green Fluorescent Protein was cotransfected at a ratio of 1:5. Medium was refreshed every 2–3 days, and cells were used for experiments 1–3 days after transfection.

2.3. Electrophysiology

Whole-cell voltage clamp recordings were made from CA1 pyramidal neurons in hippocampal slices, which were visualized using infrared differential interference contrast microscopy on a Zeiss FS2 microscope with a

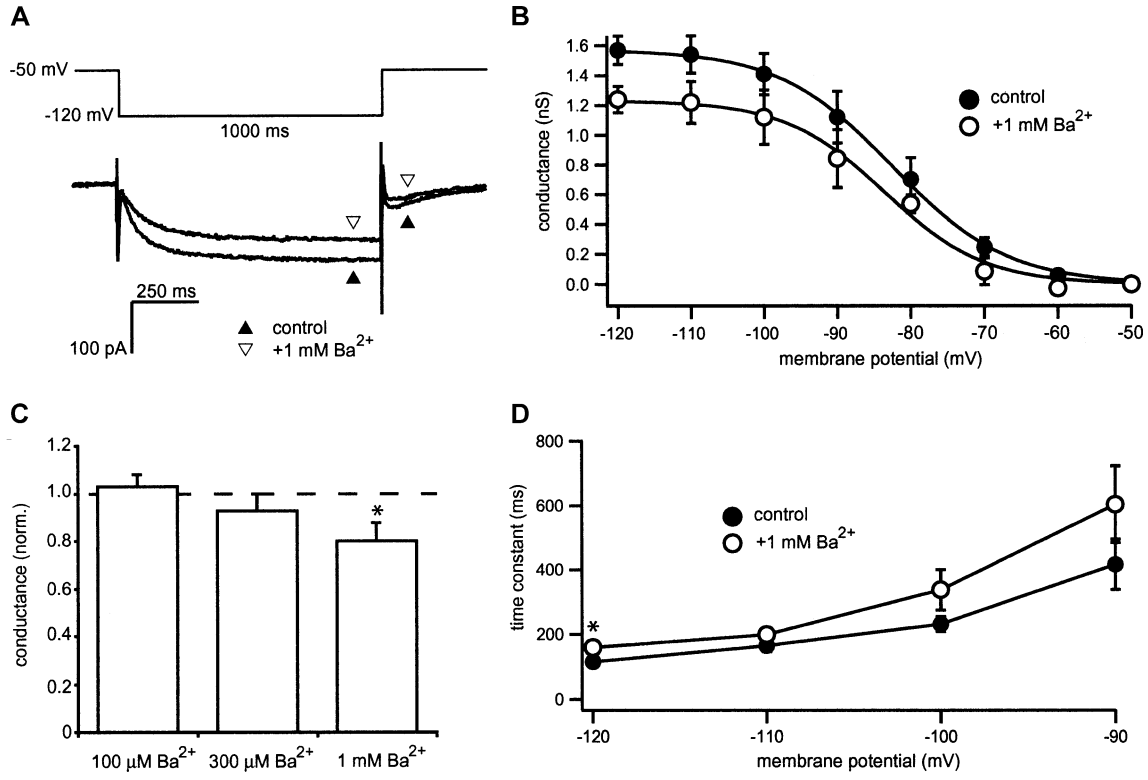

Fig. 1. Ba^{2+} ions reduce the maximal conductance and reduce the rate of activation of I_h currents in hippocampal CA1 pyramidal neurons. (A) I_h currents recorded from a whole-cell voltage clamped pyramidal neuron in the absence and presence of 1 mM Ba^{2+}. (B) Conductance of I_h versus membrane potential in the presence and absence of 1 mM Ba^{2+}. The maximal conductance in the presence of 1 mM Ba^{2+} is decreased, but the V_h and V_c are unchanged. Solid curves represent the fit to the Boltzmann equation (see Materials and methods). Data points represent the mean±S.E.M of 6–10 cells. (C) Concentration-dependence of the block of maximal conductance of I_h by Ba^{2+}. Bars represent the mean±S.E.M. of 6 cells. Asterisk indicates $P<0.05$. (D) Time constant of activation of the I_h currents is increased by Ba^{2+}. Asterisk indicates significant difference from control. Data points represent the mean±S.E.M. of 4–6 cells. Absence of error bars indicates that the S.E.M. is smaller than the symbol size.