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Peripheral N- and C-terminal domains determine deactivation kinetics of HCN channels

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Abstract

Among four subtypes of mammalian HCN channels, HCN1 has the fastest activation and deactivation kinetics while HCN4 shows the slowest. We previously showed that the activation kinetics are determined mainly by S1, S1–S2, and the S6-cyclic nucleotide binding domain. However, the effects of those regions on the deactivation kinetics were relatively small. Therefore, we investigated the structural basis for deactivation kinetics. Substitution of the core region (from S3 to S6) between HCN1 and HCN4 did not affect deactivation kinetics. This suggests that the peripheral regions (outside of S3 to S6) determine subtype-specific deactivation kinetics. Furthermore, we examined whether peripheral regions determined the deactivation kinetics across species by introducing the core region of DMIH (*Drosophila* homologue) into both HCN1 and HCN4. The DMIH core with HCN1 activated and deactivated more than threefold faster than that with HCN4. Taken together, the peripheral domains are diversified to create distinct kinetics.

Keywords: Hyperpolarization; HCN channels; HCN1; HCN4; Chimeras; Deactivation kinetics; Activation kinetics

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels contribute to many physiological functions such as control of pacemaker activity, resting potential, and membrane excitability [1]. There are four HCN subtypes in mammals, HCN1–4 [2]. HCN channels possess the same subunit architecture as voltage-gated potassium channels, with six transmembrane domains and a pore region. The cyclic nucleotide binding domain (CNBD) resides within the intracellular C-terminal domain. The CNBD is a common structure to HCN channels, cyclic nucleotide-gated channels, and the human ether go–go (HERG) K^+ channel [3,4]. Among the four HCN subtypes, HCN1 shows the fastest activation and deactivation kinetics in response to voltage commands, and the lowest sensitivity to intracellular cAMP [3]. In contrast, HCN4 shows the slowest activation and deactivation kinetics, and is more sensitive to cAMP [5–7]. The different kinetics and cyclic nucleotide sensitivities of HCN channels are thought to be important for their distinct physiological functions [8,9]. The C-terminal region, especially the S6–CNBD loop and CNBD, determines the distinct subtype sensitivities to cyclic nucleotides [10–12].

Using chimeras, we previously found that S1 and the S1–S2 loop, and the S6–CNBD loop are mainly responsible for the different activation kinetics between HCN1 and HCN4 [13]. However, those regions did not markedly affect deactivation kinetics. Using chimeras between HCN2 and HCN4, it was recently reported that the S1–S2 region plays a crucial role in deactivation kinetics [14]. To examine whether the interaction between the core (S3–S6) and the periphery (outside of S3–S6) was conserved in evolution, we constructed chimeras among HCN1, HCN4, and DMIH (*Drosophila* homologue of HCN channels) and found that the peripheral regions of HCN channels (from the N-terminus to S3 and from S6 to CNBD) contain the determinants for subtype-specific deactivation kinetics.

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Materials and methods

Molecular biology. Deletion mutants and chimeras were constructed as previously described [13]. Briefly, 1△C lacks 289 amino acid residues at the C-terminus in mouse HCN1 (Accession No. AJ225123). 4△N△C lacks 214 amino acid residues at the N-terminus and 422 amino acid residues at the C-terminus in rabbit HCN4 (Accession No. AB022927). The central core region (from S3 to S6) of DMIH-$A_1C_1B_1$ (Accession No. AY928069) [15] was cloned from *Drosophila melanogaster* cDNA (a generous gift from Dr. T. Usui, Kyoto University) by PCR. Chimeras were created by overlap PCR using PfuTurbo (Stratagene, La Jolla, CA, USA). All PCR products were verified by sequencing (BigDye Terminator Cycle Sequencing, Applied Biosystems, Inc., Foster City, CA).

Functional expression and electrophysiology. All channel subunits and green fluorescent protein (GFP) S65A cDNA were subcloned into independent mammalian expression vectors pCI (Promega, Madison, WI) and the mixture of vectors was transfected into COS-7 cells (RIKEN, Wako, Japan) using LipofectAMINE (Invitrogen, Carlsbad, CA). Currents were recorded from transfected COS-7 cells at 35.0 ± 0.5 °C with the whole-cell patch recording technique using an EPC-8 amplifier (List, Darmstadt, Germany), and the whole-cell current and the tail current were fitted to a single exponential function except for the initial lag as previously described [13]. The intracellular (pipette) solution contained (mM): 135 KCl, 5 EGTA, 5 NaCl, 10 Hepes, and 5 KOH (pH 7.4). The extracellular (bath) solution contained (mM): 155 NaCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 17 glucose, 10 Hepes, and 5 KOH (pH 7.4). Data are given as means ± SEM (number of experiments). Statistical differences were determined using Student's unpaired t test; p values <0.05 were considered significant.

Results

HCN1 deactivates seven times faster than HCN4

HCN1 and HCN4 were transiently expressed in COS-7 cells and examined by using the whole-cell patch clamp technique [13]. In response to a hyperpolarizing voltage step to −100 mV, HCN1 and HCN4 generated inward currents that were followed by tail currents when the membrane potential was subsequently stepped to potentials between −70 and +20 mV (Fig. 1B). The tail current was well fitted with a single exponential curve, except for the initial lag (Fig. 1E). The HCN1 deactivation time constant was 7.6 ± 1.1 ms ($n = 6$) at 0 mV (Fig. 1D). In contrast, HCN4 deactivated slowly, with a deactivation time constant of 54 ± 6 ms ($n = 7$) at 0 mV (Fig. 1D). Therefore, HCN1 deactivated about seven times faster than HCN4 at 0 mV. Deletion of the C-terminus of HCN1 (1△C) did not change the deactivation kinetics (Fig. 1D) and deletion of both the N- and C-termini of HCN4 (4△N△C) did not significantly affect deactivation

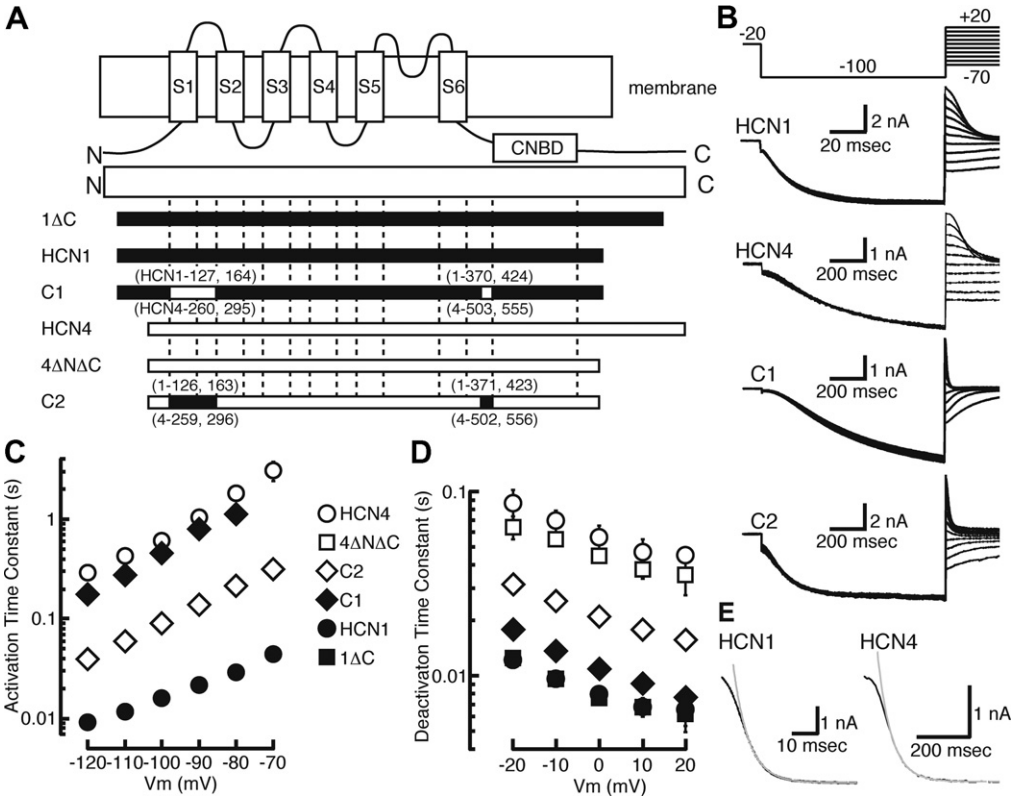

Fig. 1. Activation and deactivation kinetics for chimeras between HCN1 and HCN4 with replacements of S1, S1–S2 loop, and S6–CNBD loop. (A) Schematic representation of mouse HCN1, rabbit HCN4, their deletion mutants, and HCN1–HCN4 chimeras. Black regions represent HCN1 and white regions HCN4 amino acid sequences in this and subsequent figures. The exact points of the crossovers for chimeras are shown in parentheses in this and subsequent figures. (B) Representative current recordings of HCN1, HCN4, C1, and C2. (C) Activation time constants as a function of voltage during hyperpolarizing steps (holding potential = −20 mV) for 1△C ($n = 6$), 4△N△C ($n = 5$), C1 ($n = 6$), and C2 ($n = 6$). (D) Deactivation time constants as a function of voltage during depolarizing steps after the activation by a step to −100 mV for HCN1 ($n = 7$), 1△C ($n = 6$), HCN4 ($n = 6$), 4△N△C ($n = 4$), C1 ($n = 6$), and C2 ($n = 6$). (E) Currents obtained from a 0 mV voltage pulse after activation by a −100 mV hyperpolarizing voltage step. Data were fitted (grey lines) by single exponential functions to obtain the deactivation time constants.