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Protein kinase D regulates the human cardiac L-type voltage-gated calcium channel through serine 1884

Yusuke Aita [a], Nagomi Kurebayashi [b], Shigehisa Hirose [a], Andrés D. Maturana [c,d,*]

[a] Department of Biological Sciences, Tokyo Institute of Technology, Yokohama 226-8501, Japan
[b] Department of Cellular and Molecular Pharmacology, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan
[c] Global Edge Institute, Tokyo Institute of Technology, Tokyo 152-8550, Japan
[d] Department of Bioengineering, Nagaoka University of Technology, Niigata 940-2188, Japan

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ABSTRACT

Protein kinase D (PKD) regulates the activity of the L-type calcium channel in rat ventricular cardiomyocytes. However, the functional target residues of PKD on the L-type calcium channel remain to be identified. Our aim was to identify the functional phosphorylation sites of PKD on the human L-type calcium channel. The pore subunit of the human CaV1.2 (hCaV1.2) was stably expressed in HEK293 cells. Both the expression of a dominant-negative mutant of PKD and the mutation of serine 1884 but not serine 1930, putative targets of PKD, strongly reduced L-type calcium currents and single channel activity without affecting the channel's expression at the plasma membrane. Our results suggest that serine 1884 is essential for the regulation of hCaV1.2 by PKD.

1. Introduction

L-type voltage-gated calcium channels are the major gate for the entry of calcium into the cytosol of many cell types such as neurons, myocytes and endocrine cells [1]. These channels play an essential role in the excitation-contraction coupling of muscle contraction, hormone secretion, transcription and neuronal activity [1]. In the heart, the calcium entering through the L-type voltage-gated calcium channels initiates the cardiac contraction. Cardiac L-type calcium channels are also involved in cardiac arrhythmia and heart failure [2]. In response to stress and neuro-humoral stimulation, the activity of L-type calcium channels is acutely modified by post-translational modification, mainly phosphorylation [3]. The regulation of the cardiac L-type calcium channel by various protein kinases, particularly Protein Kinase A (PKA) and Protein Kinase C (PKC), has been extensively studied [4].

Protein kinase D is a serine/threonine kinase playing an important role in the heart [5]. We have shown that the cardiac L-type calcium channel is regulated by PKD upon α-adrenergic stimulation in ventricular cardiomyocytes of newborn rats [6]. The regulation of L-type calcium channel by PKD was subsequently confirmed to occur in adult rat cardiomyocytes [7].

The mechanisms of this regulation and whether the human L-type calcium channel is regulated by PKD have not yet been studied. Analysis of the amino-acid sequence of the pore subunit of the L-type calcium channel, CaV1.2, showed two serine residues possess an amino-acid sequence specific for PKD. Both residues and their neighboring sequence are fully conserved in various species [8]. In the present study, we aimed at testing the functional role of these two serine residues, potential targets of PKD, in the regulation of the human cardiac L-type calcium channel activity.

2. Materials and methods

For this study, L-type calcium currents were measured by patch-clamp in the whole-cell and cell-attached configuration from HEK293 cells stably expressing the human CaV1.2. Expression of hCaV1.2 and mutants was verified by western blot (WB) and cell surface biotinylation. Details of Section 2 are found in Supplementary data.

3. Results

3.1. Protein kinase D regulates the basal activity of the human CaV1.2

We have previously shown that in ventricular cardiomyocytes of newborn rats, protein kinase D (PKD) regulates the activity of the L-type calcium channels upon α-adrenergic stimulation [6].

* Corresponding author.
E-mail address: maturana@vos.nagaokaut.ac.jp (A.D. Maturana).

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We first aimed at verifying if the cardiac human L-type calcium channel is also regulated by PKD. For this purpose, we established a stable cell line co-expressing the human CaV1.2 (hCaV1.2) pore subunit with the human β2a subunit. The expression of hCaV1.2 was under the control of a tetracycline-inducible promoter. The expression of hCaV1.2 was strongly induced 24–48 h after the addition of 20 μg/ml of doxycycline (Fig. 1A). In these cells, L-type calcium currents could be measured by eliciting 200 ms voltage depolarization steps from −90 to +70 mV from a holding potential of −90 mV in the whole-cell configuration of the patch-clamp technique (Fig. 1B).

Because no specific activator or inhibitor of PKD are commercially available, we used a constitutive active mutant of PKD tagged with a green fluorescent protein (GFP-PKD-CA) and a dominant-negative mutant of PKD (GFP-PKD-DN) [9,10] to investigate the regulation of the human L-type calcium channel by PKD. GFP-PKD-CA expression did not change the amplitude of the L-type calcium current density compared to the control (Fig. 1B). However, in hCaV1.2-HEK293 cells expressing GFP-PKD-DN, L-type calcium currents were significantly reduced between +20 and +40 mV (Fig. 1B). These results suggested the activity of the human cardiac L-type calcium channel might be regulated at the basal level by PKD.

To test this hypothesis, we measured the activity of the L-type calcium channel at the single channel level in the cell-attach configuration (Supplementary data Fig. S1). The single channel activity was observed in both GFP-PKD-DN and GFP-PKD-CA expressing cells, however, the frequency of spontaneous events was much lower when cells expressing the dominant-negative mutant of PKD (Fig. 2A). The open probability in cells expressing GFP-PKD-DN was significantly reduced compare to control cells or to GFP-PKD-CA expressing cells (Fig. 2B). These results corroborate the observation made in the whole-cell experiments and suggest that PKD regulates the activity of the human L-type calcium channel.

3.2. Ser 1884 but not S1930 is important for the PKD-dependent regulation of the human L-type calcium channel

We next aimed at identifying the functional target residues of PKD on the hCaV1.2. The amino-acid sequence of the hCaV1.2 showed two serine residues in the C-terminal domain of hCaV1.2 with an amino-acid context specific for PKD: LXRXXS [8]: Ser 1884 and Ser 1930 (Fig. 3A). To investigate their functional role these residues were mutated to alanine, individually: Ser1884 to Ala1884 (S1884A) and Ser1930 to Ala1930 (S1930A) or both together (S1884A/S1930A). Mutated hCaV.1.2 was also stably expressed in HEK293 cells. WB analysis showed that the expression of all three hCaV1.2 mutants was not modified compare to the wild-type hCaV1.2 at 24 and 48 h after the induced-expression (Fig. 3B). On the same PVDF membrane, the detection of α-tubulin was used as a loading control. In addition, the mutations did not affect the expression of hCaV1.2 at the surface of the plasma membrane as shown by the cell surface biotinylation experiment. No difference in surface expression of the serine-mutated hCaV1.2 was observed compared to the wild-type hCaV1.2 (Figs. 3C and

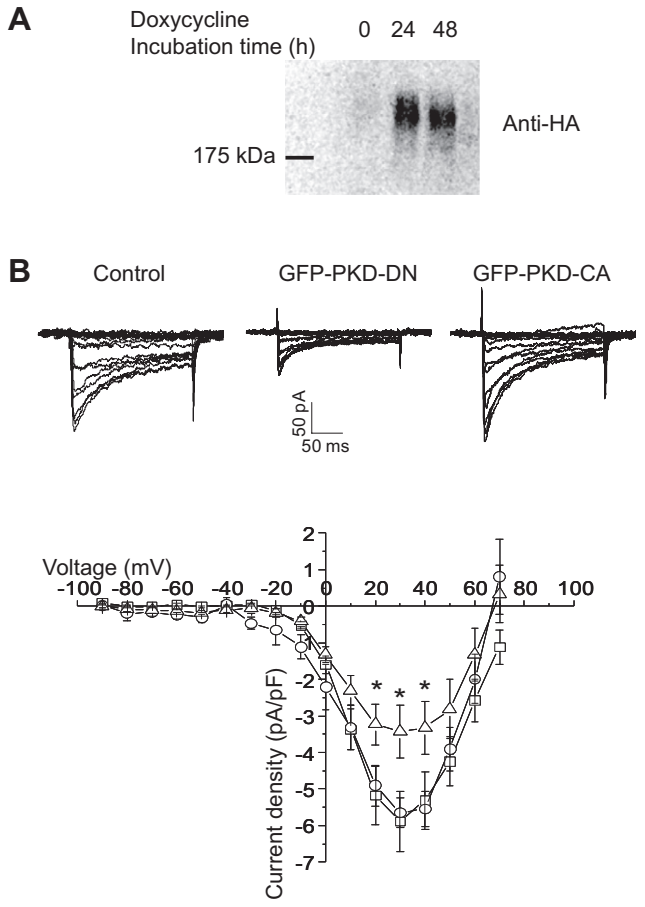

Fig. 1. PKD regulates the activity of human L-type calcium channel. (A) WB showing doxycycline induction of hCaV1.2 at 24 h and 48 h ($n = 3$). (B) Traces of calcium currents recorded at 10 mV voltage steps from a -90 mV holding potential to +70 mV of control cells or cells transfected with GFP-PKD-DN or GFP-PKD-CA. 40 mM Ca^{2+} was used as a charge carrier. The graph is the current density-voltage relationships of control (circles, $n = 12$), GFP-PKD-DN (triangles, $n = 11$) and GFP-PKD-CA (squares, $n = 7$) cells (mean and s.e.m). * is $P < 0.02$.

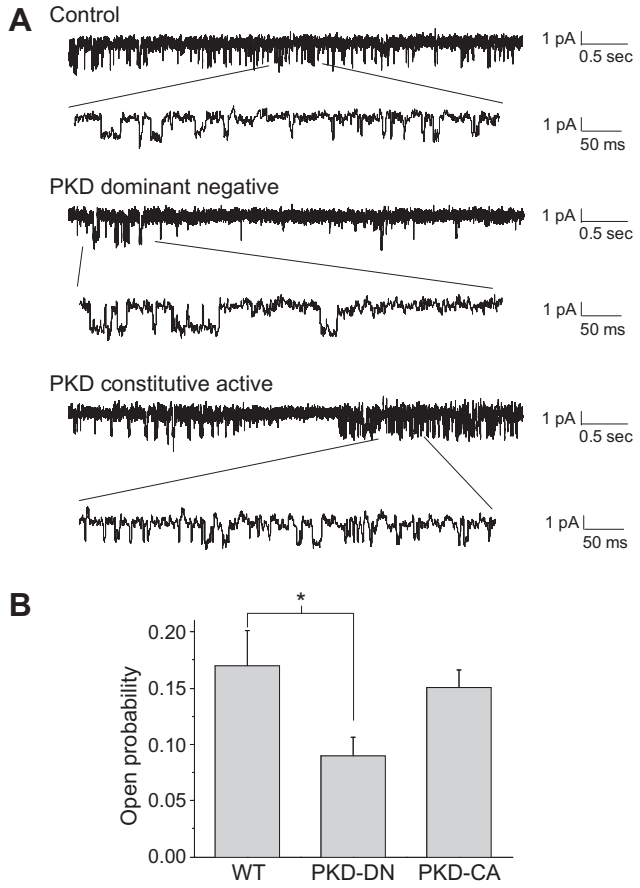

Fig. 2. Single channel activity of hCaV1.2 in cells expressing GFP-PKD-DN or GFP-PKD-CA. (A) Traces of single channel activity recorded in the cell-attached configuration of hCaV12 cells using 100 mM Ba^{2+} as a charge carrier and in presence of 0.2 μM BayK8644 (B) Mean of the open probability of the control ($n = 9$), GFP-PKD-DN ($n = 8$) or GFP-PKD-CA ($n = 6$) expressing cells. * is $P < 0.002$.