The Journal of Physiological Sciences (2019) 69:335–343
https://doi.org/10.1007/s12576-018-0650-4

ORIGINAL PAPER

Asparagine-linked glycosylation modifies voltage-dependent gating properties of $Ca_V3.1$-T-type Ca^{2+} channel

Yangong Liu[1,2] · Pu Wang[1,2] · Fangfang Ma[1,2] · Mingqi Zheng[1] · Gang Liu[1] · Shinichiro Kume[2] · Tatsuki Kurokawa[2] · Katsushige Ono[2]

Received: 16 August 2018 / Accepted: 5 December 2018 / Published online: 1 January 2019

Abstract

T-type channels are low-voltage-activated channels that play a role in the cardiovascular system particularly for pacemaker activity. Glycosylation is one of the most prevalent post-translational modifications in protein. Among various glycosylation types, the most common one is asparagine-linked (*N*-linked) glycosylation. The aim of this study was to elucidate the roles of *N*-linked glycosylation for the gating properties of the $Ca_V3.1$-T-type Ca^{2+} channel. *N*-linked glycosylation synthesis inhibitor tunicamycin causes a reduction of $Ca_V3.1$-T-type Ca^{2+} channel current ($Ca_V3.1$-$I_{Ca.T}$) when applied for 12 h or longer. Tunicamycin (24 h) significantly shifted the activation curve to the depolarization potentials, whereas the steady-state inactivation curve was unaffected. Use-dependent inactivation of $Ca_V3.1$-$I_{Ca.T}$ was accelerated, and recovery from inactivation was prolonged by tunicamycin (24 h). $Ca_V3.1$-$I_{Ca.T}$ was insensitive to a glycosidase PNGase F when the channels were expressed on the plasma membrane. These findings suggest that *N*-glycosylation contributes not only to the cell surface expression of the $Ca_V3.1$-T-type Ca^{2+} channel but to the regulation of the gating properties of the channel when the channel proteins were processed during the folding and trafficking steps in the cell.

Keywords Glycosylation · T-type Ca^{2+} channel · $Ca_V3.1$ · α1G channel · Tunicamycin

Introduction

T-type calcium channels, known as low-voltage-activated calcium channel or transient-type calcium channel, are predominantly expressed in the atria and nodal cells in the heart. In particular, T-type Ca^{2+} channels contribute to the generation of action potentials in nodal cells at the phase of slow diastolic depolarization. Genetic analysis demonstrated that two types of T-type Ca^{2+} channel isoforms, $Ca_V3.1$ and $Ca_V3.2$, are widely expressed in the heart [1–3]. Studies of genetically modified mice with targeted inactivation of the T-type Ca^{2+} channel suggest that $Ca_V3.1$-T-type Ca^{2+} channel underlies the functional T-type Ca^{2+} channel in the heart for the generation of pacemaker rhythm [1, 2, 4] particularly with adult animals [1]. Regarding the automaticity in the pacemaker cells, failure to generate the nodal electrical impulse underlies congenital or acquired bradycardia and bradycardia-associated conditions, such as atrial fibrillation [5]. Sick sinus syndrome is the term used to describe the inability of the sinus node to generate heart rhythms that meet the physiologic needs of an individual. Sinus node dysfunction occurs as a result of disorders in automaticity, conduction, or both. Although several causes of the acquired sick sinus dysfunction including idiopathic degenerative disease have been postulated, molecular mechanisms for the pacemaker dysfunction are mostly unknown.

Ion channels are subject to complex regulation by a wide range of cytosolic modulators [5, 6]. Ion channels are also subject to multiple post-transcription regulatory mechanisms, including glycosylation, formation of disulfide bonds, enzyme digestion, phosphorylation, and ubiquitination [7–9]. Asparagine (Asn, *N*)-linked glycosylation (*N*-glycosylation) has been investigated to affect the structural folding, membrane targeting, expression level, stability, and voltage-dependent properties of many ion channels including the T-type Ca^{2+} channels. For instance, reduction

✉ Katsushige Ono
ono@oita-u.ac.jp

1 Department of Cardiology, The First Hospital of Hebei Medical University, 89 Donggang Road, Shijiazhuang, Hebei Province 050031, People's Republic of China

2 Department of Pathophysiology, Oita University School of Medicine, Yufu, Oita 879-5593, Japan