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ORIGINAL ARTICLE

Disruption of asparagine-linked glycosylation to rescue and alter gating of the $Na_V1.5$-Na^+ channel

Pu Wang[1,2] · Xiufang Zhu[1,2] · Mengyan Wei[1,2] · Yangong Liu[1,2] · Kenshi Yoshimura[2] · Mingqi Zheng[1] · Gang Liu[1] · Shinichiro Kume[2] · Tatsuki Kurokawa[2] · Katsushige Ono[2]

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Abstract

SCN5A gene encodes the voltage-gated sodium channel $Na_V1.5$ which is composed of a pore-forming α subunit of the channel. Asparagine (N)-linked glycosylation is one of the common post-translational modifications in proteins. The aim of this study was to investigate impact of N-linked glycosylation disruption on the Na^+ channel, and the mechanism by which glycosylation regulates the current density and gating properties of the Na^+ channel. The $Na_V1.5$-Na^+ channel isoform (α submit) derived from human was stably expressed in human embryonic kidney (HEK)-293 cells (Nav1.5-HEK cell). We applied the whole-cell patch-clamp technique to study the impact of N-linked glycosylation disruption in Nav1.5-HEK cell. Inhibition of the N-glycosylation with tunicamycin caused a significant increase of $Na_V1.5$ channel current (I_{Na}) when applied for 24 h. Tunicamycin shifted the steady-state inactivation curve to the hyperpolarization direction, whereas the activation curve was unaffected. Recovery from inactivation was prolonged, while the fast phase (τ_{fast}) and the slow phase (τ_{slow}) of the current decay was unaffected by tunicamycin. I_{Na} was unaffected by tunicamycin in the present of a proteasome inhibitor MG132 [N-[(phenylmethoxy)carbonyl]-L-leucy-N-[(1S)-1-formyl-3-methylbutyl]-L-leucinamide], while it was significantly increased by tunicamycin in the presence of a lysosome inhibitor butyl methacrylate (BMA). These findings suggest that N-glycosylation disruption rescues the $Na_V1.5$ channel possibly through the alteration of ubiquitin–proteasome activity, and changes gating properties of the $Na_V1.5$ channel by modulating glycan milieu of the channel protein.

Keywords Glycosylation · $Na_V1.5$ channel · Tunicamycin · MG132 · Ubiquitin–proteasome pathway

Abbreviations

N-linked	Asparagine-linked
HEK cells	Human embryonic kidney
MG132	N-[(phenylmethoxy)carbonyl]-L- leucy-N-[(1S)-1-formyl-3-methylbutyl]- L-leucinamide
BMA	Butyl methacrylate
DMEM	Dulbecco's modified Eagle's medium
I_{Na}	Voltage-gated sodium channel current
LQT3	Long QT syndrome type 3
TEA-Cl	Tetraethylammonium chloride
TEA-OH	Tetraethylammonium hydroxide
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
G	Conductance
I-V curve	Current–voltage curve
ER	Endoplasmic reticulum
FOXO1	Forkhead box protein O1

Introduction

In cardiovascular system many ion channels regulate transmembrane currents that make much contribution to excitation in cardiomyocytes [1–7]. Among them the voltage-gated Na^+ channel current (I_{Na}) plays a critical and significant role for the fast upstroke of the action potentials and is essential for proper conduction of the electrical impulse. Voltage-gated sodium channels are composed of a pore-forming α subunit and auxiliary β subunits. SCN5A gene encodes

Pu Wang, Xiufang Zhu and Mengyan Wei contributed equally to this work.

✉ 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