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Protein arginine methyl transferases-3 and -5 increase cell surface expression of cardiac sodium channel

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ABSTRACT

The α-subunit of the cardiac voltage-gated sodium channel ($Na_V1.5$) plays a central role in cardiomyocyte excitability. We have recently reported that $Na_V1.5$ is post-translationally modified by arginine methylation. Here, we aimed to identify the enzymes that methylate $Na_V1.5$, and to describe the role of arginine methylation on $Na_V1.5$ function. Our results show that protein arginine methyl transferase (PRMT)-3 and -5 methylate $Na_V1.5$ in vitro, interact with $Na_V1.5$ in human embryonic kidney (HEK) cells, and increase $Na_V1.5$ current density by enhancing $Na_V1.5$ cell surface expression. Our observations are the first evidence of regulation of a voltage-gated ion channel, including calcium, potassium, sodium and TRP channels, by arginine methylation.

Structured digital abstract:
PRMT5 physically interacts with **Nav1.5** by fluorescent resonance energy transfer (View interaction)
PRMT3 physically interacts with **Nav1.5** by fluorescent resonance energy transfer (View interaction)
Nav1.5 physically interacts with **PRMT3** by anti tag coimmunoprecipitation (View interaction)
PRMT1 physically interacts with **Nav1.5** by fluorescent resonance energy transfer (View interaction)
Nav1.5 physically interacts with **PRMT1** by anti tag coimmunoprecipitation (View interaction)

1. Introduction

Cardiac ion channels are essential for the generation of cardiomyocyte action potentials (AP). The cardiac voltage-gated sodium channel is responsible for the sodium inward current that initiates the AP, and consists of an α-subunit ($Na_V1.5$), which is necessary and sufficient for the generation of sodium currents, and accessory proteins [1]. The fine regulation of $Na_V1.5$ current is vital for proper cardiac function, and single mutations in $Na_V1.5$ can lead to cardiac arrhythmia conditions [2].

$Na_V1.5$ is predicted to be composed of four homologous domains, DI to DIV, joined by interdomain linkers. Interdomain linkers, N and C termini of the protein are cytosolic, and are accessible to post-translational modifications such as phosphorylation [3], and ubiquitination [4] which modulate $Na_V1.5$ function. Noteworthy, we have recently reported initial (40% sequence coverage) mass spectrometry evidence that identified arginine methylation (ArgMe) as a novel post-translational modification of $Na_V1.5$ [5]. ArgMe was observed in Arg residues within the interdomain linker between DI and DII of $Na_V1.5$ ($L_{I–II}$).

Abbreviations: AP, action potential; ArgMe, arginine methylation; bmp, beats per minute; CFP or YFP, cyan or yellow fluorescent protein; co-IP, co-immunoprecipitation; FRET, Förster resonance energy transfer; HEK, human embryonic kidney; IP, immunoprecipitation; $L_{I–II}$, linker between domains DI and DII of $Na_V1.5$; $Na_V1.5$, cardiac isoform of the voltage-gated sodium channel α subunit; PRMT, protein arginine methyltransferase; SAM, *S*-(5′-adenosyl)-L-methionine

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ArgMe is catalysed by protein arginine methyltransferases (PRMT), which transfer methyl groups from the cofactor *S*-adenosyl-L-methionine (SAM) to the protein, leading to mono- or dimethylated arginines [6]. In the latter case, methylation can be asymmetric or symmetric [7]. To date, 9 PRMTs have been identified in the human genome [8], namely PRMT1 to -9. PRMT1, -2, -3, -4, -6 and -8 are Type I PRMT that produce monomethyl and asymmetric dimethyl arginines [9]. PRMT5 is a Type II PRMT that produces monomethyl and symmetric dimethyl arginines [10], while PRMT7 leads to monomethyl arginines only [11]. PRMT9 has not been biochemically characterised to date. The addition of methyl groups modifies the shape of the arginine residue, turning it more bulky and hydrophobic, and may block a potential hydrogen bond donor.

Evidence indicating that $Na_V1.5$ is modified by ArgMe has opened new questions in the sodium channel field. In this context, we set ourselves to identify the PRMTs that mediate $Na_V1.5$ ArgMe, and to describe their effect on $Na_V1.5$ function.

2. Materials and methods

2.1. Plasmids

A pcDNA3 plasmid that encodes $Na_V1.5$ as a FLAG fusion, which has previously been described [12–14], was used in electrophysiological, co-IP, and biotin pull-down experiments. For Förster resonance energy transfer (FRET) measurements, a $Na_V1.5$-CFP construct, which localises to the endoplasmic reticulum in HEK cells, was used [15].

PRMTs for in vitro methylation assays were cloned into pGEX or Myc vectors. GST-PRMT1, -2, -3, -4, -6, and -7, as well as Myc-PRMT5, have been described [11,16]. To perform methylation studies in cells, FRET, co-IP, electrophysiological, and biotin pull-down experiments, PRMT-YFP fusion proteins were produced. PRMT1, -2, -3, -5, -6 and -7 were cloned from human cardiac cDNA (the study was approved by the institutional ethical committee of the Dr. Josep Trueta Hospital, Girona, Spain) into pcDNA3.1 (Invitrogen) as C-terminal YFP fusion proteins. PRMT4 could not be amplified from cardiac cDNA and a plasmid template was used.

$L_{I–II}$ ($Na_V1.5$ residues 416–711) was cloned into pcDNA3.1 as $L_{I–II}$-FLAG-YFP fusion. Plasmids for expression in *Escherichia coli* were pETM15b (EMBL) in which $L_{I–II}$ or the C-terminal domain ($Na_V1.5$ residues 1773–2016) were cloned as N-terminal His-tagged proteins.

For electrophysiological and FRET assays, HEK cells were transfected using calcium phosphate precipitation. For all other experiments, cells were transfected using GeneCellin (BioCellChallenge).

2.2. Detection of methylated $L_{I–II}$ in cells

HEK cells were transiently transfected with the $L_{I–II}$-FLAG-YFP plasmid (2 μg), and harvested 30 h later. Soluble lysates were incubated with α-GFP antibody (Abcam) coupled to protein A beads (GE Healthcare). Immunoprecipitated proteins were detected with α-FLAG (M2, Sigma), α-ASYM24, or α-SYM11 (Merck).

2.3. In vitro methylation assays

$L_{I–II}$ and the C-terminal domain of $Na_V1.5$ were expressed in *E. coli* as 6×His fusions, and purified using Ni-NTA chromatography. In vitro methylation assays were conducted as described [17]. Recombinant GST-PRMT1, -2, -3, -4, -6 and -7 were expressed and purified from *E. coli*. Myc-tagged human PRMT5 was expressed and purified from HeLa cells. Reactions were done in the presence of 0.5 μCi *S*-adenosyl-L-[methyl-3H]methionine (^{3}H-SAM, GE Healthcare) for 1 h at 30 °C in a final volume of 30 μl.

2.4. FRET between $Na_V1.5$ and PRMTs

HEK cells expressing $Na_V1.5$-CFP (0.5 μg plasmid) together with the corresponding PRMT–YFP (1.5 μg plasmid), or YFP as a negative control (0.5 μg plasmid), were grown for 24–48 h after transfection. For every $Na_V1.5$/PRMT pair, 4 independent transfections were done. In every measuring session, 5–10 negative controls were measured in parallel. Cells were examined by confocal microscopy on a LSM 710 (Zeiss, Jena, Germany) using a C-Apochromat 40×/1.2 NA water-immersion objective lens at room temperature. FRET was calculated using the sensitised emission method. Reported apparent FRET efficiencies ($FRET_{app}$) are the mean ± S.E.M. of individual cell $FRET_{app}$ values for a given $Na_V1.5$-CFP/PRMT–YFP pair.

2.5. Co-IP of PRMT1 and -3, with $Na_V1.5$

HEK cells were transiently transfected with FLAG $Na_V1.5$ (1 μg plasmid), together with GFP (0.5 μg plasmid), or PRMT1-, or -3–YFP (1.5 μg plasmid). Cells were lysed 48 h after transfection, and $Na_V1.5$ was immunoprecipitated ($n = 3$) using FLAG chromatography (Sigma). PRMTs were detected using an α-GFP antibody (Abcam), and $Na_V1.5$ was detected using an α-$Na_V1.5$ antibody (Alomone).

2.6. Electrophysiological recordings

Sodium currents in HEK cells transiently expressing FLAG-$Na_V1.5$ (1.5 μg plasmid) together with the corresponding PRMT–YFP (0.5 μg plasmid), were measured between 25 and 35 h after transfection. The total number of independent transfections was 5 (PRMT3 and -5), 3 (PRMT1), or 2 (PRMT2, -4, and -7). The day after transfection, SAM (0.15 mM, final concentration) was added to the medium, and sodium currents were measured at room temperature. In every session, 3–10 negative controls (i.e. cells co-expressing $Na_V1.5$ and YFP) were measured in parallel.

Currents were obtained by patch clamp whole-cell recording using an Axopatch 200B amplifier (Molecular Devices). Activation data at test potentials of −80 to +50 mV (in 5 or 10 mV steps) were fitted to a Boltzmann equation, of the form $g = g_{max}/(1 + \exp[(V_{1/2} - V_m)/s])$, where g is the conductance, g_{max} the maximum conductance, V_m the membrane potential, $V_{1/2}$ the voltage at which half of the channels are activated, and s the slope factor. Inactivation data at test potentials of −140 to −30 mV (in 5 or 10 mV steps, for 250 ms) were fitted to a Boltzmann equation of the form $I = I_{max}/(1 + \exp[(V_m - V_{1/2})/s])$, where I is the peak current amplitude, I_{max} the maximum peak current amplitude, V_m the membrane potential, $V_{1/2}$ the voltage at which half of the channels are inactivated, and s the slope factor. Kinetics of $Na_V1.5$ inactivation were fitted to a monoexponential function, from which time constant τ (inactivation) between −40 and 30 mV was estimated. $Na_V1.5$ recovery from inactivation data (at recovery times of 250 μs, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 100, 250, 500, 1000 and 1900 ms at −120 mV) were fitted to monoexponential functions to determine recovery time constant τ. All reported errors are S.E.M.

2.7. Biotin pull-down of cell surface proteins

Biotin pull-down of FLAG-$Na_V1.5$ was performed from transiently transfected HEK cells ($n = 2$) and from a previously developed [5] cell line stably expressing FLAG-$Na_V1.5$ ($n = 2$). Cells were transfected with $Na_V1.5$ (1 μg plasmid) together with YFP (0.5 μg plasmid), PRMT1, -3 or -5 (1.5 μg plasmid). After 24 h, cells were treated with reactive biotin (Pierce) for 60 min at 4 °C, and

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