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Original article

Expression of the sialyltransferase, ST3Gal4, impacts cardiac voltage-gated sodium channel activity, refractory period and ventricular conduction

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ABSTRACT

The sequential glycosylation process typically ends with sialic acid residues added through trans-Golgi sialyltransferase activity. Individuals afflicted with congenital disorders of glycosylation often have reduced glycoprotein sialylation and present with multi-system symptoms including hypotonia, seizures, arrhythmia and cardiomyopathy. Cardiac voltage-gated Na^+ channel (Na_v) activity can be influenced by sialic acids likely contributing to an external surface potential causing channels to gate at less depolarized voltages. Here, a possible pathophysiological role for reduced sialylation is investigated by questioning the impact of gene deletion of the uniformly expressed beta-galactoside alpha-2,3-sialyltransferase 4 (ST3Gal4) on cardiac Na_v activity, cellular refractory period and ventricular conduction. Whole-cell patch-clamp experiments showed that ventricular Na_v from ST3Gal4 deficient mice ($ST3Gal4^{-/-}$) gated at more depolarized potentials, inactivated more slowly and recovered from fast inactivation more rapidly than WT controls. Current-clamp recordings indicated a 20% increase in time to action potential peak and a 30 ms decrease in $ST3Gal4^{-/-}$ myocyte refractory period, concurrent with increased Na_v recovery rate. Na_v expression, distribution and maximal Na^+ current levels were unaffected by ST3Gal4 expression, indicating that reduced sialylation does not impact Na_v surface expression and distribution. However, enzymatic desialylation suggested that $ST3Gal4^{-/-}$ ventricular Na_v are less sialylated. Consistent with the shortened myocyte refractory period, epicardial conduction experiments using optical mapping techniques demonstrated a 27% reduction in minimum ventricular refractory period and increased susceptibility to arrhythmias in $ST3Gal4^{-/-}$ ventricles. Thus, deletion of a single sialyltransferase significantly impacts ventricular myocyte electrical signaling. These studies offer insight into diseases of glycosylation that are often associated with pathological changes in excitability and highlight the importance of glycosylation in cardiac physiology.

1. Introduction

In the heart electrical signaling is responsible for its rhythmicity and is necessary to initiate muscle contraction. The net electrical activity in a cardiac myocyte during a contraction cycle is observed as the action potential (AP), which describes a change in membrane potential as a function of time. In ventricular cardiac myocytes, voltage-gated sodium channel (Na_v) activity is responsible for AP initiation and contributes to conduction. Functional changes in the primary cardiac Na_v isoform, $Na_v1.5$ [1], as evidenced by those characterized in various congenital and/or acquired etiologies, can lead to severe cardiac pathologies [2,3].

Na_v are large transmembrane proteins that can be extensively post-translationally modified through processes that include glycosylation [4]. Protein glycosylation is a sequential process that involves the activities of hundreds of glycosylation-related gene (glycogene) products [5]. Glycans account for up to 35% of the mature Na_v mass, and can contribute to Na_v activity in isoform and cell-specific manners (for review see [6]). Typically, an electrostatic mechanism was assigned, with the negatively charged terminal residues, sialic acids, added to N- and O-glycans, contributing to the extracellular surface potential and thereby causing channels to gate following smaller depolarizations [6]. Developmentally regulated Na_v sialylation was shown previously to affect dorsal root ganglion and cardiomyocyte Na_v function, suggesting a dynamic process [5,7,8].

Growing evidence suggests that reduced glycosylation contributes significantly to human disease. There are a number of pathological states resulting in reduced glycoprotein glycosylation, including a

Abbreviations: ST3Gal4, beta-galactoside alpha-2,3-sialyltransferase 4; Na_v, voltage-gated Na^+ channel; $ST3Gal4^{-/-}$, mouse strain homozygous for a ST3Gal4 null transgene; AP, action potential; Glycogene, gene encoding a protein involved in glycosylation; SAs, sialic acids; CDG, congenital disorders of glycosylation; WT, littermate control homozygous for the ST3Gal4 gene; LVA, left ventricular apex; ICS, intracellular recording solution; I_{Na}, voltage-gated Na^+ current; BCA, bicinchoninic acid; MW, apparent molecular weight; kD, kilodalton; RH237, N-(4-sulfobutyl)-4-(6-(4-(dibutylamino)phenyl) hexatrienyl)pyridinium: voltage sensitive dye; CMOS, Complementary metal-oxide-semiconductor; G–V, conductance–voltage; V_a, voltage of half-activation; SSI, voltage-dependent steady-state inactivation; V_i, voltage of half-inactivation; τ_{inat}, time constant of fast inactivation; τ_{rec}, time constant of recovery from fast inactivation; AU, arbitrary units; *T. cruzi*, *Trypanasoma cruzi*.

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large family of congenital disorders of glycosylation (CDG) that contain approximately 40 different types [9]. CDG result in a relatively modest but variable (among subtypes) reduction in glycoprotein glycosylation that leads to dramatic multi-system effects with a high infant mortality rate [10,11]. CDG are characterized by the glycogene that is mutated and while most mutations occur with genes that encode enzymes that act proximally in the glycosylation pathway to sialyltransferases (enzymes that attach sialic acids to N- and O-glycans), reduced sialylation is common to most disease types. In fact the preliminary diagnostic test for all CDG is based on the number of SA-linked glycan structures attached to the serum protein transferrin [12]. Those who suffer from CDG often present with severe cardiac and neuromuscular deficits [10,13,14]. The mechanisms by which modest reductions in glycosylation lead to the observed symptoms that include hypotonia, developmental delay, seizures, and dilated or hypertrophic cardiomyopathies with related arrhythmias, are not yet understood. The screening for cardiac dysfunction in all CDG patients, and for CDG in all young patients suffering from cardiomyopathy of unknown etiology, is now recommended because of the prevalence of cardiac involvement among CDG patients [10,15–17]. In addition to CDG, there are other potentially glycosylation-associated pathologies that are acquired or result from diseases that impact cardiac function including Chagas disease, which afflicts 16–18 million people in the Americas alone [18–21].

To begin to investigate whether the reduced sialylation associated with these diseases contributes to the often observed aberrant electrical signaling, we utilized a mouse model in which a uniformly expressed cardiac glycogene responsible for glycoprotein sialylation, the sialyltransferase beta-galactoside alpha-2,3-sialyltransferase 4 (ST3Gal4) was deleted [5,22]. We investigated whether and how the resulting reduced glycoprotein sialylation that occurs in the ST3Gal4$^{-/-}$ heart affects Na_v activity and electrical signaling in the adult ventricle. The data indicate that gene deletion of a single sialyltransferase, ST3Gal4, affects Na_v sialylation and gating as well as myocyte and ventricular refractory periods in the adult mouse heart, and leads to markedly increased susceptibility to ventricular arrhythmias. Therefore, we describe a role for aberrant sialylation in cardiac Na_v gating and ventricular electrical signaling and identify it as a potential substrate for disease-related arrhythmias.

2. Materials and methods

An expanded methods section is available online as a supplement.

2.1. Disclosure

All chemicals, reagents, enzymes, and animal tissue were stored, used, and disposed of following guidelines outlined in product literature, and established by the National Institutes of Health and approved through the University of South Florida (USF) Institutional Animal Care and Use Committee.

2.2. Generation of the ST3Gal4$^{-/-}$ transgenic strain

The ST3Gal4 enzyme was previously reported to be an important mediator in hemostasis, and leukocyte arrest [23–26]. Creation and characterization of the ST3Gal4$^{-/-}$ mouse was performed by others previously [23] and the strain was generously provided by Dr. Jamey Marth. Animals were genotyped using standard PCR methods.

2.3. Cardiac myocyte isolation

Adult (12–14 weeks old) male mice homozygous for the normal ST3Gal4 gene (WT) and for the ST3Gal4 null-transgene (ST3Gal4$^{-/-}$) were anesthetized using isoflurane (5%) then euthanized by cervical dislocation. Hearts were rapidly excised from the body and cannulated through the aorta. Using a modified Langendorff apparatus, the heart was perfused and digested following a protocol described by others [27]. Then, the bottom 2 mm of the left ventricular apex (LVA) was cut and dispersed in a high K^+ solution for approximately 10 min to obtain individual myocytes. Myocytes were maintained at room temperature in the high K^+ solution until use 1–4 h following isolation.

2.4. Electrophysiology

All electrophysiological recordings were performed at 22 ± 1 °C. Patch pipettes were fabricated using a horizontal pipette puller (Sutter P97) and filled with an intracellular recording solution (ICS). Immediately prior to seal formation, the solid/liquid junction offset was zeroed manually using the pipette offset on an Axopatch 200B amplifier with β set to 0.1. Following seal formation of at least one gigaohm, the whole cell-configuration of the patch-clamp method was obtained. Series resistance was compensated as necessary to a minimum of 80%. Analog signals were low-pass filtered at 5 kHz then digitized at a rate of 50 kHz using a Digidata 1440A analog/digital interface connected to a personal computer. Voltage and current clamp protocols were written and executed using Clampex 10.2 and the ICS was allowed to dialyze for 5 min following cell rupture.

2.5. Voltage and current clamp protocols

Standard pulse protocols were used to measure whole-cell current–voltage, conductance–voltage, steady state inactivation, the time constants of fast inactivation and recovery from fast inactivation of voltage-sensitive I_{Na} as described by us previously, and in more detail in the online supplement [5,8]. APs were recorded without EGTA in the ICS in order to more closely mimic normal myocyte electrical activity and to ascertain how any potential differences in ST3Gal4$^{-/-}$ APs may contribute to cardiac conduction. Following dialysis of the ICS and immediately prior to executing the current-clamp protocols, the membrane potential was measured using the amplifier. Cells were injected with increasing positive current pulses (4 ms) to determine threshold. Cells were then stimulated with a suprathreshold (125% of threshold) current for 4 ms followed by a second pulse 5 ms later and then each additional pulse came with a delta of 8 ms. The pulse number that elicited an overshooting AP that preceded at least 2 more similar APs was used to estimate the refractory period. To measure single AP waveforms, cells were stimulated with five 125% suprathreshold pulses (as described above) at a frequency of 1 Hz. The replicate AP waveforms were averaged and parameters were measured and calculated using Clampfit and Excel software packages.

2.6. Membrane protein enrichment

The lower 2/3 of 12–14 week old male excised hearts consisting of both the right and left ventricles was separated and snap frozen in liquid nitrogen. Sarcolemmal protein was isolated using a protocol described by others [28]. Protein concentrations were determined using a BCA assay (Pierce 23237) and the protein solutions were stored at −80 °C until use.

2.7. Western blotting and immunodetection

Western blotting for densitometry analysis was performed using protocols adapted from Invitrogen's Novex system. Briefly, 20 μg of sarcolemmal enriched protein was denatured and separated electrophoretically through an 8% Bis–Tris resolving gel using a 3-N-morpholinopropanesulfonic acid (MOPS)-SDS buffer at 200 V. Protein was transferred to nitrocellulose membranes (GE Healthcare RPN3032D) using a tank transfer system (Bio-Rad 170-3930). Na_v visualization was performed using Millipore's SNAP ID system following the manufacturer's recommendations. A pan-Na_v antibody [8,29] was used as the primary antibody and a goat anti-rabbit horseradish-peroxidase