GPD1L links redox state to cardiac excitability by PKC-dependent phosphorylation of the sodium channel SCN5A

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[1]*Department of Medicine, University of Wisconsin-Madison, Madison, Wisconsin and* [2]*Departments of Medicine, Pediatrics, and Molecular Pharmacology and Experimental Therapeutics/Divisions of Cardiovascular Diseases and Pediatric Cardiology and the WindlandSmith Rice Comprehensive Sudden Cardiac Death Program, Mayo Clinic, Rochester, Minnesota*

Valdivia CR, Ueda K, Ackerman MJ, Makielski JC. GPD1L links redox state to cardiac excitability by PKC-dependent phosphorylation of the sodium channel SCN5A. *Am J Physiol Heart Circ Physiol* 297: H1446–H1452, 2009. First published August 7, 2009; doi:10.1152/ajpheart.00513.2009.—The *SCN5A*-encoded cardiac sodium channel underlies excitability in the heart, and dysfunction of sodium current (I_{Na}) can cause fatal ventricular arrhythmia in maladies such as long QT syndrome, Brugada syndrome (BrS), and sudden infant death syndrome (SIDS). The gene *GPD1L* encodes the glycerol phosphate dehydrogenase 1-like protein with homology to glycerol phosphate dehydrogenase (GPD1), but the function for this enzyme is unknown. Mutations in *GPD1L* have been associated with BrS and SIDS and decrease I_{Na} through an unknown mechanism. Using a heterologous expression system, we show that GPD1L associated with SCN5A and that the BrS- and SIDS-related mutations in *GPD1L* caused a loss of enzymatic function resulting in glycerol-3-phosphate PKC-dependent phosphorylation of SCN5A at serine 1503 (S1503) through a GPD1L-dependent pathway. The direct phosphorylation of S1503 markedly decreased I_{Na}. These results show a function for GPD1L in cell physiology and a mechanism linking mutations in *GPD1L* to sudden cardiac arrest. Because the enzymatic step catalyzed by GPD1L depends upon nicotinamide adenine dinucleotide, this GPD1L pathway links the metabolic state of the cell to I_{Na} and excitability and may be important more generally in cardiac ischemia and heart failure.

cardiac arrhythmia; sodium current; ion channel; cell metabolism; glycerol 3-phosphate dehydrogenase 1-like; protein kinase C; cardiac sodium channel α-subunit

THE *SCN5A* GENE ENCODES THE cardiac sodium channel α-subunit (SCN5A; also called Nav1.5) that carries the sodium current (I_{Na}) that underlies excitation and conduction of the cardiac impulse. Loss-of-function and gain-of-function mutations in *SCN5A* or other genes that encode sodium channel interacting proteins (ChIPs) cause potentially lethal inheritable arrhythmia syndromes or cardiac channelopathies including long QT syndrome (LQTS) (10, 19, 23, 24), Brugada syndrome (BrS) (3, 9, 25), and sudden infant death syndrome (SIDS) (1, 4). The gene *GPD1L* on chromosome 3p22.3 encodes the protein glycerol 3-phosphate dehydrogenase 1-like (GPD1L); the letters stand for glycerol 3-phosphate dehydrogenase 1-like because it shares 84% homology with glycerol 3-phosphate dehydrogenase 1 (GPD1) (13). *GPD1L* was first noted as part of mammalian gene sequence collection program (18) in 2002, but the function or importance of GPD1L, if any, was unknown. Mutations in *GPD1L* were linked to BrS in a large family (26),

and in 2007 the mutation (GPD1L-A280V) (9) and the novel SIDS-associated mutation (GPD1L-E83K) (22) were both shown to decrease cardiac I_{Na} amplitude. This decrease could account for their arrhythomogenesis, but the mechanism by which GPD1L mutations decreased I_{Na} was unknown.

The related gene *GPD1* encodes a NAD-dependent cytosolic enzyme that is an important link between the glycolytic pathway and triglyceride synthesis. GPD1 catalyzes the reversible conversion of glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate (DHAP). GPD1 has direct and indirect interactions with genes and proteins involved in the insulin pathway and cellular metabolism, and GPD1 dysfunction has been linked to obesity, diabetes, and other diseases (14). We hypothesized that like its namesake GPD1, GPD1L catalyzes the reaction of G3P to DHAP. A loss of function of the enzymatic activity of GPD1L would be expected to increase levels of G3P as has been observed in a mouse GPD1-knockout model (2). By a GPD1L-dependent SCN5A phosphorylation pathway (Fig. 2*B*), decreased GPD1L activity would then increase the substrate G3P and feed the PKC-mediated phosphorylation of SCN5A at serine residue 1503 (S1503) where such phosphorylation is known to decrease I_{Na} (11). To investigate this hypothesis, we performed experiments with wild-type (WT) and mutant SCN5A and GPD1L constructs coexpressed in heterologous cell system [human embryonic kidney (HEK)293 cells].

MATERIALS AND METHODS

Reagents and antibodies. Cell culture media were Opti-MEM, RPMI 1640, and fetal calf serum from Invitrogen (Carlsbad, CA) and FuGENE6 from Roche Molecular Biochemicals (Basel, Switzerland). Staurosporine, 1-oleoyl-2-acetylglycerol (OAG), thimerosal, and propranolol were from Sigma-Aldrich (St. Louis, MO). Rabbit FITC-conjugated anti-FLAG antibody, rabbit anti-SCN5A antibody, and goat horseradish peroxidase-conjugated anti-rabbit IgG (H+L) antibody were from Immunology Consultants Laboratory (Newberg, OR), Upstate (Charlottesville, VA), and Bio-Rad laboratories (Hercules, CA), respectively.

Plasmids construction. The cDNA of *GPD1L* (GenBank Accession No. BC028726) was subcloned into pIRES2EGFP (Clontech, Palo Alto, CA) to generate pGPD1L-IRES2EGFP. E83K and A280V mutants in *GPD1L* were incorporated into WT clones by the site-directed mutagenesis. The cDNAs of *SCN5A* (GenBank Accession No. AB158469), and β$_1$-subunit gene, *SCN1B* (GenBank Accession No. BC112922) were subcloned into pcDNA3, and the FLAG peptide of DYKDDDDK was incorporated at R45 in *SCN1B* and confirmed by sequencing. S1503A in *SCN5A* was incorporated by the site-directed mutagenesis. For glutathione *S*-transferase (GST)-fusion GPD1L constructs, the coding region of *GPD1L* was subcloned pGEX-5X-2 (Amersham Biosciences, Piscataway, NJ). All clones were sequenced and confirmed.

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Cell culture and transfection. Cells were maintained in MEM supplemented with 10% heat-inactivated fetal calf serum. For transfections, cells were grown in 60-mm dishes and then transfected with 1.5 μg DNA of SCN5A and 0.3 μg DNA of GPD1L-IRES-GFP per dish using FuGENE6.

Electrophysiological study. Macroscopic voltage-gated sodium current was recorded using the whole-cell method of the patch clamp technique in the HEK293 cells 48 h after transfection as described previously (12) at room temperature with cells continuously perfused with solution containing (in mM) 140 NaCl, 4 KCl, 1.8 CaCl$_2$, 0.75 MgCl$_2$, and 5 HEPES (pH 7.4 set with NaOH). The pipette (intracellular) solution contained (in mM) 120 CsF, 20 CsCl$_2$, 5 EGTA, and 5 HEPES (pH 7.4 set with CsOH). Except where indicated otherwise, drugs and substrates were added to the culture medium and incubated for 3–5 h and then washed before the electrophysiological experiment. I_{Na} density was measured at −20 mV from holding potential −140 mV and normalized to cell capacitance.

Flowcytometry. The transfected HEK293 cells were harvested by incubation with 0.5 mM EDTA-PBS for 10 min at 37°C and washed with RPMI 1640 supplemented with 1 mM EDTA (pH 7.4), 3%
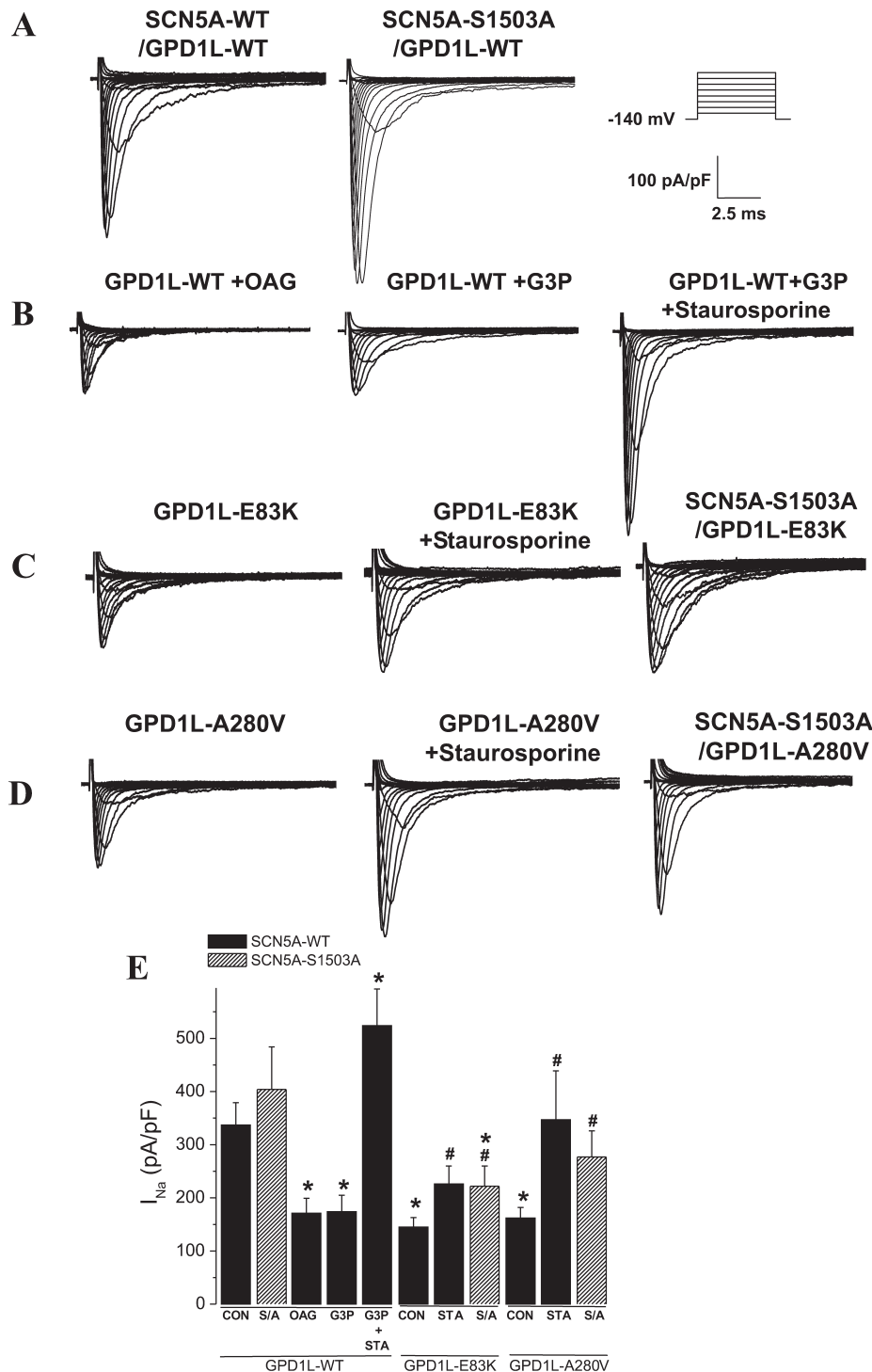

Fig. 1. Glycerol 3-phosphate dehydrogenase 1-like (GPD1L) mutations decreased sodium current (I_{Na}) density mediated by PKC phosphorylation at serine 1503 (S1503). *A–D*: representative traces for families of macroscopic I_{Na} measured from human embryonic kidney (HEK293) cells co-expressing wild-type (WT) and mutant constructs of cardiac sodium channel α-subunit (SCN5A) and GPD1L as indicated. The cells were depolarized for 24 ms to different potentials in increments of 10 mV from a holding potential of −140 mV (see protocol *inset*) in control conditions and after 3–5 h incubation with 1-oleoyl-2-acetylglycerol (OAG; 20 μM), glycerol-3-phosphate (G3P; 10 μM), and G3P plus staurosporine (Sta; 1 μM) before patch-clamp recordings. *E*: summary data for I_{Na} density measurements at −20 mV from a holding potential of −140 mV for SCN5A with GPD1L-WT and mutants (n = 5–32). S/A, SCN5A-S1503A *P < 0.05 vs. GPDlL-WT control (Con) and vs. GPD1L-WT/SCN5A-S1503A; #P < 0.05 vs. GPD1L-mutant/SCN5A-WT in Con.
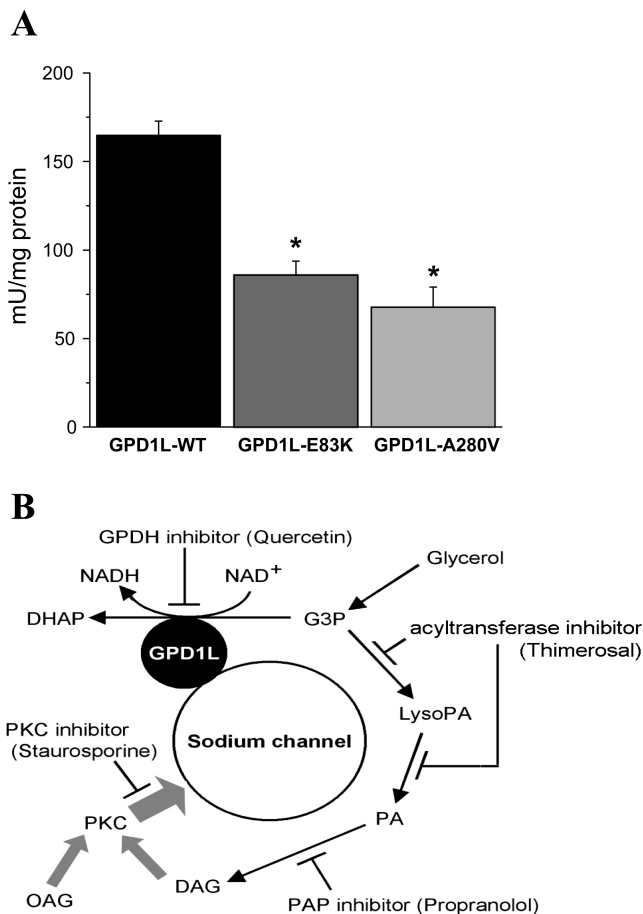
Fig. 2. Enzymatic activity of GPD1L decreased by the GPD1L missense mutations E83K and A280V. *A*: G3P oxidation was measured spectrophotometrically at 37°C from the WT and glutathione *S*-transferase (GST)-labeled GPD1L proteins cleaved by factor Xa to be separated from GST-tag (MATERIALS AND METHODS; $n = 6$ in each group). *B*: diagram of proposed GPD1L-PKC pathway with substrates and pharmacological blockers (indicated by T-shaped lines) that affect SCN5A-GPD1L association via PKC activation. DAG, diacylglycerol; PA, phosphatidic acid; LysoPA, lsophoshpatidic acid; DHAP, dihydroxyacetone phosphate. *$P < 0.05$ vs. GPD1L WT.

analog IPTG and purified by MagenGST particles. The purified proteins were cleaved by factor Xa (Novagen). G3P oxidation activity of the GPD1L WT and mutants were measured spectrophotometrically at 25°C by following the disappearance of the oxidation of G3P and formation of NADH at 340 nm (28). The assay mixture contained 1 mM EDTA and 1 mM 2-mercaptoethanol, 5 mM NAD + and 5 mM G3P, buffered by 50 mM Tris·HCl (for pH 6.5–7.4).

Statistics. Summary data are presented as means ± SE and statistical significance for the difference between mean values in GPD1L-WT versus GPD1L mutants or control versus treatment and tested by the Student's *t*-test.
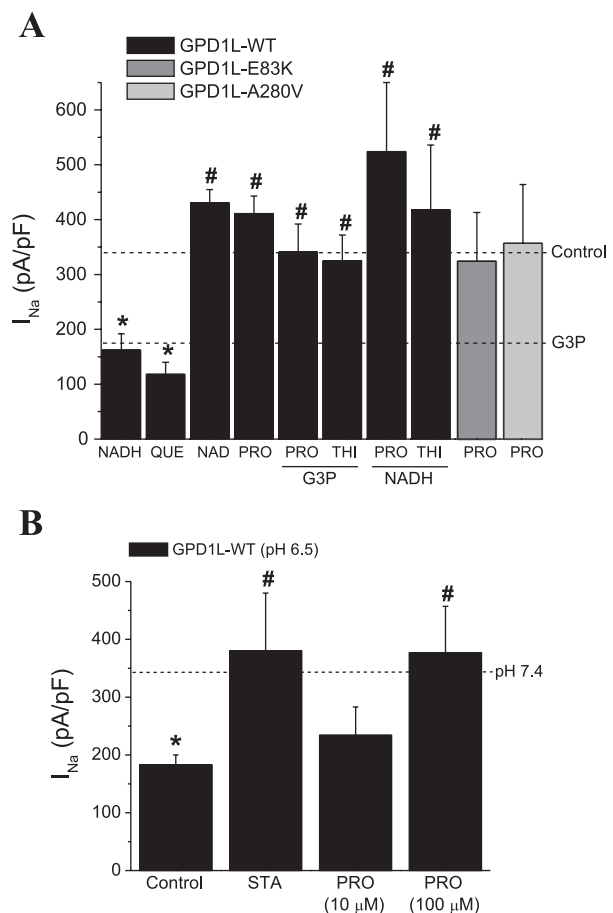

Fig. 3. GPD1L-PKC stimulation through the G3P pathway decreased I_{Na} density for SCN5A with GPD1L-WT. *A*: summary data for I_{Na} density at -20 mV from a holding potential of -140 mV for SCN5A with GPD1L-WT after treatment with various pathway substrates and inhibitors: 10 μM G3P, 300 μM NADH, 10 μM OAG, 100 μM quecertin (Que), 300 μM NAD, 10 μM propranolol (Pro), 1 μM staurosporine (Sta), and 10 μM thimerosol (Thi). For columns 5–8, the treatment was with 2 compounds as indicated by the labels with a column, with G3P present or NADH present. For GPD1L-E83K (dark gray) and GPD1L-A280V (light gray), recordings were performed after incubation with 100 μM Pro added 3–5 h before patch-clamp recording. The dotted lines represent the I_{Na} density in GPD1L-WT for control conditions and in G3P from Fig. 1*E* ($n = 5$–23). *$P < 0.05$ vs. control; #$P < 0.05$ vs. G3P, Que, or NADH alone. *B*: acidosis decreased I_{Na} density through a PKC-dependent mechanism. HEK293 cells were transfected with SCN5A-WT and GPD1L-WT and incubated overnight at pH of 7.4 or pH 6.5 before transfer and recording of I_{Na} in control (normal pH) bath solution. The dotted line represents the value for SCN5A-WT + GPD1L-WT at pH 7.4 from Fig. 1*E*. The I_{Na}-attenuating effects of acidic pH with GPD1L-WT were prevented by both Sta and Pro ($n = 6$–12). *$P < 0.05$ vs. control at pH 7.4; #$P < 0.05$ vs. control at pH 6.5.

FCS, and 0.02% azide (staining medium). The FITC-conjugated anti-FLAG antibody (Sigma-Aldrich) incubations were performed in staining medium at 4°C and then washed by PBS supplemented with 1 mM EDTA (pH 7.4) and 1% FCS. The stained cells were examined for surface expression with FACSCalibur (BD Biosciences, San Jose, CA).

GST-pulldown assay. Rosetta gami 2 bacteria (Novagen, Madison, WI) expressing GST-labeled GPD1L induced by isopropyl-β-D-thiogalactopyranoside (IPTG) were sonicated in PBS with 1% Triton X-100, purified and fixed on MagneGST particles (Promega, Madison, WI) per manufacturer instructions. Purity and concentration of fusion proteins were determined by SDS-PAGE followed by Coomasie Blue staining. GST-bait was incubated in protein-binding buffer overnight at 4°C with cell lysates obtained from stably expressing SCN5A cells that were precleared for 1 h at 4°C with MagneGST particles without GPD1L. Samples were washed four times in binding buffer. Bound proteins were liberated by boiling in Laemmli buffer with 50 mM DTT and subjected to SDS-PAGE and immunoblotted with anti-SCN5A antibody on 7.5% SDS-polyacrylamide gels (Bio-Rad).

G3P oxidation activity assay. WT and mutated GPD1L with GST label were obtained from bacterial expression induced by lactose

RESULTS

Stimulation of the GPD1L-WT PKC pathway reduces I_{Na} density. GPD1L-WT was coexpressed with SCN5A-WT, or with SCN5A-S1503A, a channel with an engineered mutation at serine 1503 to an alanine at a consensus PKC phosphorylation site (11). The I_{Na} density from whole-cell voltage clamp showed a small but insignificant increase with SCN5A-S1503A compared with SCN5A-WT (Fig. 1, *A* and *E*). Activation of the GPD1L-PKC pathway using the direct PKC agonist OAG and the substrate G3P both significantly de-

creased I_{Na} density, and this decrease was abrogated by the PKC blocker staurosporine (Fig. 1, *B* and *E*). As reported previously (15), OAG shifted inactivation in the negative direction (by 7 mV; data not shown), which would tend to further reduce I_{Na} from holding potentials more positive than those used in this study. The SIDS mutation GPD1L-E83K (22) (Fig. 1, *C* and *E*) and the BrS mutation GPD1L-A280V (9) (Fig. 1, *D* and *E*) significantly decreased I_{Na} density compared with GPD1L-WT, and the pathway blocker staurosporine abrogated this decrease. In addition, the decreased I_{Na} density
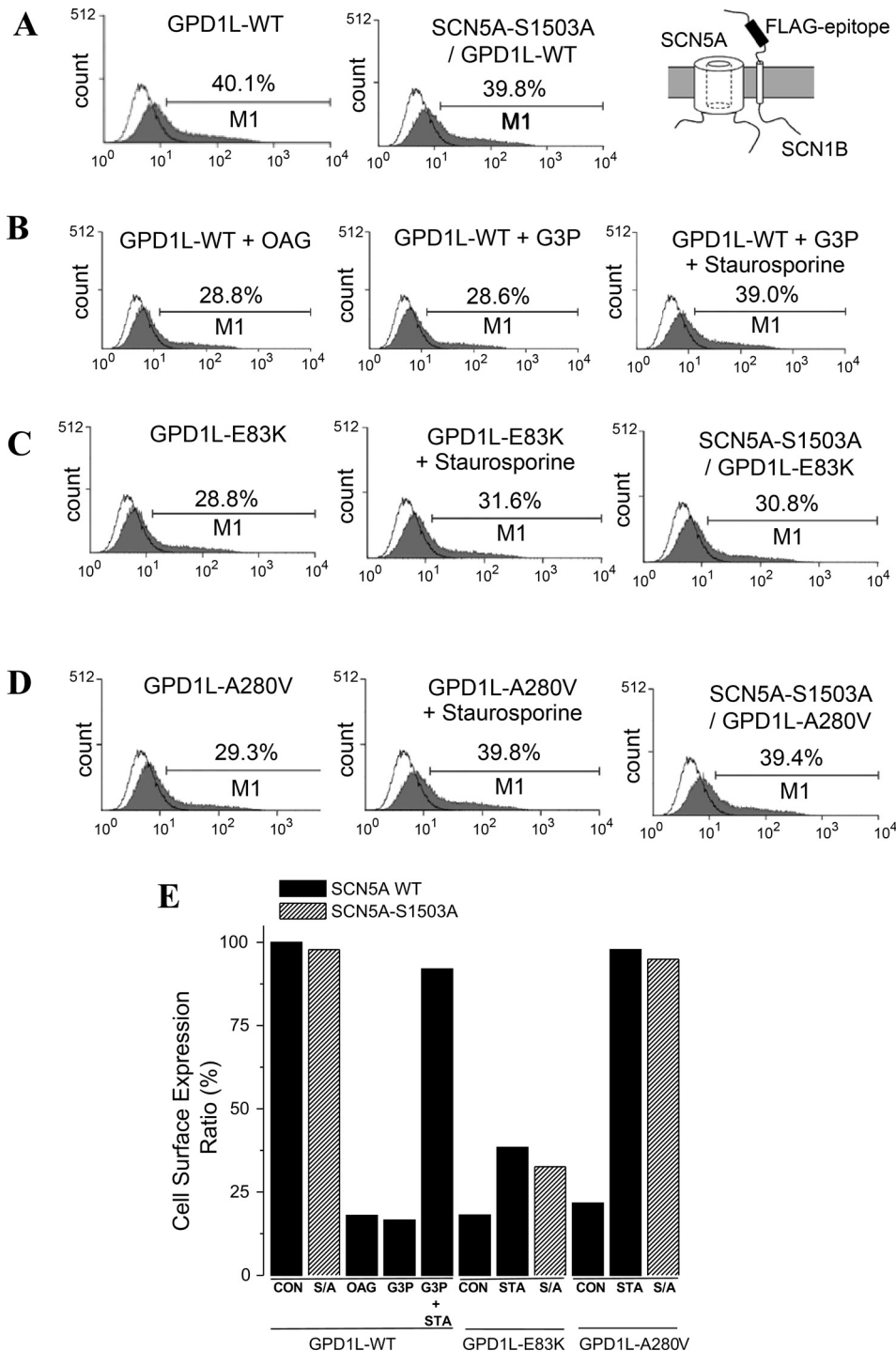

Fig. 4. Flowcytometric analysis of cell surface expression of the sodium channel complex. HEK293 cells transiently transfected with SCN5A, GPD1L, and FLAG-SCN1B were harvested (see MATERIALS AND METHODS), and cell surface staining was performed by FITC-conjugated anti-FLAG M2 antibody, without permeabilization. Gray areas reflect signals from cells expressing SCN5A, GPD1L, and FLAG-SCN1B constructs, and the black line indicates signals from SCN5A, GPD1L, and SCN1B without FLAG as negative control. In this method, the count of cells with surface expression is reflected in the long tail rather than the peak. Marker 1 (M1) was established at a point containing 5% of negative control. The numbers above M1 represent the percentage of positive events falling within the M1 region and reflect cell surface expression. *A* and *B*: cell surface expression of the SCN5A. GPD1L complex was measured from HEK cells coexpressing SCN5A, SCN1B, and GPD1L-WT under control conditions (*A*) and after 3–5 h incubation with OAG (20 µM), or G3P (10 µM) and G3P plus staurosporine (1 µM) 1 µM stausporin (*B*), or with GPD1L-E83K or GPD1L-A280V (*C* and *D*). GPD1L-E83K and -A280V decreased cell surface expression, and this effect was reversed by staurosporine and by expression of SCN5A with a mutated PKC phosphorylation site (SCN5A-S1503A; *C* and *D*). *E*: summary data for I_{Na} density measurements shown in *A–D*. The data represent the percent ratio with the minimal (26.4%) surface expression obtained by testing SCN5A-G1743R, an established trafficking defective mutation (Ref. 20) (data not shown) and maximal (40.1%) surface expression seen in control conditions.

with the GPD1L mutations was not observed when coexpressed with the phosphorylation-deficient mutant channel SCN5A-S1503A (Fig. 1, *C–E*). Together these data support the hypothesis that GPD1L plays a role in regulating I_{Na} by a GPD1L-PKC phosphorylation pathway depicted in Fig. 2*B*.

GPD1L enzymatic activity decreased by mutations. A G3P oxidation assay with GPD1L-WT and mutant GPD1L showed that both E83K and A280V exhibited significantly less enzymatic activity than GPD1L-WT (Fig. 2*A*). These results support the hypothesis that these mutations affect I_{Na} by a direct decrease in enzymatic activity, although other conformational effects of the mutations on the complex such as chaperoning functions cannot be excluded.

Manipulation of the GPD1L-PKC pathway. The proposed pathway (Fig. 2*B*) makes a number of predictions regarding the effects of various substrates and blockers on I_{Na} density, which we tested (Fig. 2*A*). As predicted, NADH and the glycerol phosphate dehydrogenase (GPDH) inhibitor quercetin both significantly decreased I_{Na} density compared with the control GPD1-L WT. NAD^+ did not significantly increase I_{Na} density above control levels (Fig. 3*A*), suggesting the relatively low basal state of phosphorylation of SCN5A under our study conditions, also consistent with the nonsignificant increase with SCN5A-S1503A compared with SCN5A-WT (Fig. 1, *A* and *E*). The NADH effect to decrease I_{Na} was nearly completely abrogated by the GPD1L-dependent SCN5A phosphorylation pathway blockers the phosphatidic acid phosphatase inhibitor propranolol and the acelytransferase inhibitor thimerosal (Fig. 3*A*), supporting the hypothesis that this pathway is a predominant mechanism for NADH modulation of I_{Na}. As with the mutations, propanolol abrogated the effects of GPD1L-E83K and GPD1L-A280V mutants. These data (Fig. 3*A*) were obtained after 3–5 h incubation with drugs and reagents, and the reagents were removed before testing for I_{Na}. In more acute experiments, inclusion of 10 μM G3P in the pipette solution significantly reduced peak I_{Na} to 69 ± 79 pA/pF ($n = 4$) compared with peak I_{Na} of 379 ± 68 pA/pF ($n = 6$), and thimerosol significantly increased peak I_{Na} to 309 ± 58 pA/pF ($n = 5$) from 248 ± 53 pA/pF ($n = 6$) in cells coexpressing SCN5A and GPD1L-WT ($P < 0.05$). Together, these results support the hypothesis that GPD1L-E83K and GPD1L-A280V decrease I_{Na} by the pathway indicated in Fig. 2*B*, where loss of enzymatic function and accumulation of G3P leads to increased PKC-dependent phosphorylation of SCN5A.

Acidosis is another important component of metabolic state, and the enzymatic activity of GPD1L was reduced under more acidic conditions (data not shown). I_{Na} density was also significantly reduced after incubation at pH 6.5 compared with pH 7.4 (Fig. 3*B*), and this reduction was abrogated by the PKC inhibitor staurosporine and by propranalol. Protons have long been known to affect I_{Na} by both pore-blocking and surface charge effects (7, 8), but the voltage clamp data were obtained after transfer to control pH, suggesting additional important longer term pH effects on I_{Na} may be mediated through G3P metabolism and the GPD1L-dependent SCN5A phosphorylation pathway.

GPD1L mutants decrease SCN5A cell surface expression and I_{Na} through a GPD1L-SCN5A phosphorylation pathway. Previously, the mutations GPD1L-A280V (9) and GPD1L-E83K (22) were shown to decrease I_{Na} and A280V was noted

to decrease cell surface expression of SCN5A by quantitative immunostaining and confocal microscopy (9). We used flow-cytometry and I_{Na} measurements to confirm that A280V as well as E83K decreased I_{Na} and SCN5A cell surface expression compared with GPD1L-WT (Fig. 4, *A–E*). When the E83K and A280V mutations were expressed with the channel lacking the PKC-dependent phosphorylation site at S1503 (SCN5A-S1503A), cell surface expression and the I_{Na} density were partially restored compared with when they were expressed with SCN5A-WT (Fig. 4, *C–E*). The decrease in cell surface expression and I_{Na} density caused by the GPD1L mutants was reversed (partially for E83K, completely for A280V) by the PKC inhibitor staurosporine or by the S1503A SCN5A mutant lacking the PKC consensus site (Fig. 4, *C–E*), further supporting the hypothesis that these GPD1L mutations decrease I_{Na} through PKC phosphorylation, but the less than complete reversal by PKC block for E83K suggests other mechanisms may contribute to the effect of this mutation on I_{Na}. Gating properties of SCN5A such as activation and inactivation were not affected by the GPD1L mutations (data not shown) in agreement with previous reports (9, 22) suggesting the major effect is a reduction in the cell surface expression of Nav1.5.

SCN5A and GPD1L associate. GPD1L was used as bait to pull-down SCN5A in a GST-fusion protein assay to show that GPD1L was associated with SCN5A. Neither the SIDS-associated (E83K) nor the BrS-associated (A280V) GPD1L mutation disrupted the association between GPD1L and SCN5A since the GST-GPD1L fusion protein containing these mutations also pulled down SCN5A (Fig. 5). This suggests that the hypothesized loss of GPD1L function is not caused by disruption of the complex and is consistent with our data that the mutations interfere directly with the enzymatic activity (Fig. 2*A*).
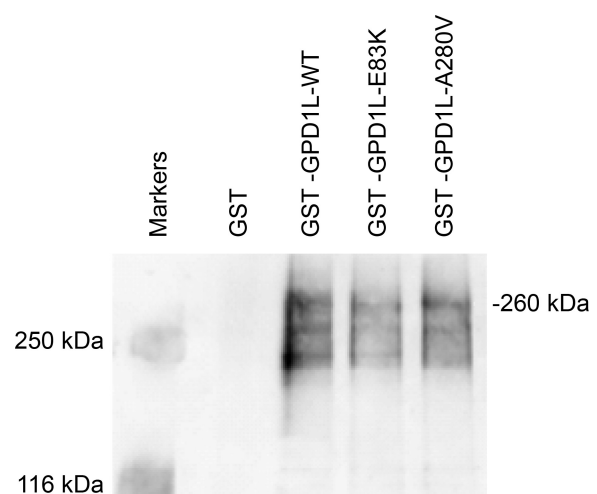

Fig. 5. GPD1L associated with SCN5A and GPD1L mutations do not disrupt this association. HEK cells stably expressing SCN5A were transfected with GST constructs of GPD1L-WT, E83K, or A280V and lysed 48 h after transfection. Cell lysates were examined for protein interaction using the pull-down approach. GST-GPD1L-bound proteins were analyzed by SDS-PAGE and immunoblotted with anti-cardiac sodium channel antibody.

DISCUSSION

These results provide a first putative functional role for GPD1L in general cell physiology. The link of GPD1L mutations to sudden death predisposing heritable arrhythmia syndromes such as BrS and SIDS suggests the physiological role of GPD1L may be cardiac specific, but whether GPD1L plays regulatory roles in other excitable tissues remains to be determined. RNA for *GPD1L* was present in the heart (9), but additional information for the tissue distribution of GPD1L is lacking. Even within the heart, whether GPD1L has a regulatory role for proteins other than the cardiac sodium channel is unknown.

We have shown that GPD1L has a direct or closely coupled association with the pore-forming α-subunit of the cardiac sodium channel (SCN5A, Nav1.5). The location of this association is unknown, and it is also possible that the association is indirect and mediated by ChIPs such as syntrophin (6). The association of GPD1L with SCN5A could confer specificity of GPD1L regulation of SCN5A by substrate localization, perhaps analogous to that shown for nitrosylation of SCN5A by the syntrophin complex (19). In that study, mutations in syntrophin were shown to cause SCN5A dysfunction by disrupting the association of important enzymes in the complex (19). Here, however, neither the SIDS-associated (E83K) nor the BrS-associated (A280V) GPD1L mutation disrupted the association between GPD1L and SCN5A since the GST-GPD1L fusion protein containing these mutations also pulled down SCN5A (Fig. 5). Our results suggest that the mutated GPD1L remains associated with SCN5A but has reduced or lost its enzymatic function (Fig. 2*A*).

The results support the idea that GPD1L regulates SCN5A primarily through direct phosphorylation of SCN5A primarily at S1503 by a GPD1L-dependent SCN5A phosphorylation pathway proposed in Fig. 2*B*. The GPD1L mutants, however, also increased phosphorylation of SCN5A-S1503A compared with GPD1L-WT (data not shown), suggesting the possible presence of additional PKC phosphorylation sites in addition to S1503 on SCN5A, perhaps analogous to PKC sites on the domain I-II linker of the brain sodium channel (16). We also cannot exclude the possibility that other proteins that regulate SCN5A may be phosphorylated by the GPD1L mechanism. For example, PKC activates Ras GTPase, Ras GTPase phosphorylation activates ERK, and ERK has been shown to reduce I_{Na} density (27). It is also possible that NAD^+ and NADH may have other direct or indirect effects on SCN5A function, but the complete block of NADH effects on I_{Na} density by pathway inhibitors (Fig. 3*A*) suggests the primary mechanism for NADH effects on I_{Na} is mediated through this GPD1L-dependent SCN5A phosphorylation pathway. In addition, other substrates and inhibitors used in the pathway have many actions in addition to the putative action on the pathway and may have additional effects or exert their action primarily outside the pathway. For example, thimerosol has been used as a thiol oxidant and postulated to have a direct effect on L-type calcium channels (5). Propranalol also has many additional effects, and the concentration used in this study (10 μM) is supratherapeutic so implications for therapy must be considered with caution. A limitation of our study is that the experiments were performed completely in heterologous expression systems and await confirmation in native myocytes. Finally, it is important to note that other pathways, such as ANG II downregulation of I_{Na} by redox-sensitive NF-κβ (17) may also play a role in redox regulation of I_{Na} amplitude.

Despite these limitations, the GPD1L-dependent SCN5A phosphorylation pathway we describe provides a plausible mechanism for the decrease in I_{Na} and the pathogenicity of the BrS2-associated mutation GPD1L-A280V and the SIDS-associated mutation GPD1L-E83K. In addition to suggesting possible therapeutic approaches to loss of SCN5A function, the elucidation of this pathway suggests that the other genes that encode the various proteins responsible for the metabolism of glycerol-3-phosphate may deserve consideration as sudden death-susceptibility candidate genes.

Although the rare heritable arrhythmia syndrome mutations in *GPD1L* are likely to account for a relatively small proportion of sudden cardiac arrests, these results have more general implications for cardiac excitability and arrhythmia. Because metabolism of G3P to DHAP by the enzymatic activity of GPD1L depends upon the ratio of oxidized NAD^+ and reduced NADH (ratio NAD^+-to-NADH; Fig. 2*B*), the GPD1L-dependent SCN5A phosphorylation pathway may be a general regulatory mechanism linking the metabolic state of the cardiocyte to cellular excitability by modulating I_{Na} density. This metabolic pathway may link both acidosis and the oxidation-reduction state of the cardiac cell to I_{Na} and excitability that may play important regulatory roles in arrhythmia vulnerability seen in common diseases such as cardiac ischemia and heart failure.

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