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Cardiac Na^+ Current Regulation by Pyridine Nucleotides

Man Liu, PhD[1], **Shamarendra Sanyal, MD, PhD**[2], **Ge Gao, MD, PhD**[1], **Iman S. Gurung, MD, PhD**[3], **Xiaodong Zhu, PhD**[4], **Georgia Gaconnet, MS**[1], **Laurie J. Kerchner, BS**[4], **Lijuan L. Shang, PhD**[1], **Christopher L-H. Huang, MD PhD**[3], **Andrew Grace, MD, PhD**[3], **Barry London, MD, PhD**[4], and **Samuel C. Dudley Jr., MD, PhD**[1]

[1]Division in Cardiology, University of Illinois at Chicago and the Jesse Brown VAMC, Chicago, IL, USA

[2]Department of Medicine, Pulmonary Division, Duke University

[3]Department of Biochemistry, University of Cambridge, UK

[4]Cardiovascular Institute, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Rationale—Mutations in glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) protein reduce cardiac Na^+ current (I_{Na}) and cause Brugada Syndrome (BrS). GPD1-L has >80% amino acid homology with glycerol-3-phosphate dehydrogenase, which is involved in nicotinamide adenine dinucleotide (NAD)-dependent energy metabolism.

Objective—Therefore, we tested whether NAD(H) could regulate human cardiac sodium channels ($Na_v1.5$).

Methods and Results—HEK293 cells stably expressing $Na_v1.5$ and rat neonatal cardiomyocytes were used. The influence of NADH/NAD^+ on arrhythmic risk was evaluated in wild-type or $SCN5A^{+/-}$ mouse heart. A280V GPD1-L caused a 2.48 ± 0.17-fold increase in intracellular NADH level (P<0.001). NADH application or co-transfection with A280V GPD1-L resulted in decreased I_{Na} (0.48 ± 0.09 or 0.19 ±0.04 of control group, respectively; P<0.01), which was reversed by NAD^+, chelerythrine, or superoxide dismutase (SOD). NAD^+ antagonism of the Na^+ channel downregulation by A280V GPD1-L or NADH was prevented by a protein kinase A (PKA) inhibitor, $PKAI_{6–22}$. The effects of NADH and NAD^+ were mimicked by a phorbol ester and forskolin, respectively. Increasing intracellular NADH was associated with an increased risk of ventricular tachycardia (VT) in wild-type mouse hearts. Extracellular application of NAD^+ to $SCN5A^{+/-}$ mouse hearts ameliorated the risk of VT.

Conclusions—Our results show that $Na_v1.5$ is regulated by pyridine nucleotides, suggesting a link between metabolism and I_{Na}. This effect required protein kinase C (PKC) activation and was mediated by oxidative stress. NAD^+ could prevent this effect by activating PKA. Mutations of GPD1-L may downregulate $Na_v1.5$ by altering the oxidized to reduced NAD(H) balance.

Keywords

Arrhythmias; Electrophysiology; Ion channels; Sudden death

Address correspondence to: Dr. Samuel C. Dudley, Jr., Section of Cardiology, University of Illinois at Chicago/Jesse, Brown VA Medical Center, 840 S. Wood Street, MC715, Chicago, IL 60612, Phone: (312) 413-8870, FAX: (312) 413-2948.

Disclosures

Dr. Dudley has a patent pending related to this work, Oxidized nicotinamide adenine dinucleotide (NAD^+) treatment for arrhythmia.

Introduction

Brugada syndrome (BrS) is characterized by an electrocardiographic pattern of ST-segment elevation in the right precordial leads and an increased risk of sudden cardiac death.[1] BrS has been associated with cardiac sodium channels (SCN5A or $Na_v1.5$) mutations causing decreased sodium current (I_{Na}).[2–6] We have reported that the A280V mutation in the glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) causes BrS by reducing I_{Na}.[7] Other mutations in GPD1-L have also been linked to reduced I_{Na} and sudden infant death syndrome (SIDS).[8]

GPD1-L is highly expressed in heart tissue and has a significant degree of homology (81% at the amino acid level) with glycerol-3-phosphate dehydrogenase (GPD).[7] The GPD family of genes is involved in nicotinamide adenine dinucleotides (NAD)-dependent energy metabolism. Therefore, we investigated whether the cardiac $Na_v1.5$ channel might be modulated by alterations in NAD(H) and whether these changes might explain the effects of GPD1-L mutations on I_{Na}.

While regulation by pyridine nucleotides would be a novel finding, $Na_v1.5$ is known to be upregulated by protein kinase A (PKA) and downregulated by protein kinase C (PKC) activation.[9–11] This regulation most likely involves channel phosphorylation. In the case of protein kinase A (PKA) regulation, there is evidence of changes in channel trafficking.[12, 13] Pyridine nucleotides have a number of reported effects that could regulate activity of these kinases. For example, 1 mmol/L NADH enhances superoxide production in smooth muscle by ~2-fold,[14] and superoxide production can both be caused by and result from PKC activation.[15] Extracellular NAD^+ results in PKA activation in human granulocytes human granulocytes and osteoblastic cells.[16, 17] Therefore, it is conceivable that pyridine nucleotides would regulate $Na_v1.5$ through one or more of these established kinase pathways.

Materials and Methods

Cell Culture

Full descriptions of the methods are available in the supplemental material. As previously described,[18] we maintained a human embryonic kidney (HEK) cell line stably expressing the human cardiac $Na_v1.5$ channel (SCN5A cells). Expression of $Na_v1.5$ was linked to green fluorescent protein (GFP) expression by an internal ribosomal entry site (SCN5A-IRES-GFP). Cardiomyocytes were isolated from neonatal rat hearts by collagenase treatment (Worthington Biochemical Corporation, Lakewook, NJ). For whole-cell patch clamping experiments to study GPD1-L effects on $Na_v1.5$, SCN5A cells or cardiomyocytes were transient transfected with wild type (WT) or A280V GPD1-L and an expression vector containing red fluorescent protein (RFP) as described previously.[8] In these experiments, cells expressing both GFP and RFP were studied.

Intracellular NADH and NAD^+ Levels

Intracellular NADH and NAD^+ levels ($[NADH]_i$ and $[NAD^+]_i$) were detected by using the EnzyChromTM NAD^+/NADH Assay Kit (BioAssay Systems, Hayward, CA) in SCN5A cells with or without infection of an adeno-associated virus containing WT or A280V GPD1-L as described previously.[7] NAD^+ (500 μmol/L) was applied extracellularly ($[NAD^+]_o$) for 18 h to WT or A280V GPD1-L groups to detect whether it affected the intracellular NADH level. The intensity difference of the reduced product color, measured at 565 nm at time zero and 15 min later is proportionate to the NAD^+/NADH and is not affected by NADP(H).

Effects of Treatments on I_{Na}

Na^+ currents were measured using the whole-cell patch clamp technique in voltage-clamp mode at room temperature. Pipettes (1–2 MΩ) were filled with a pipette solution containing (in mmol/L): CsCl 80, cesium aspartate 80, EGTA 11, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10, and Na_2ATP 5 (adjusted to pH 7.4 with CsOH). The bath solution consisted of (in mmol/L): NaCl 130, CsCl 5, $CaCl_2$ 2, $MgCl_2$ 1.2, HEPES 10 and Glucose 5 (adjusted to pH 7.4 with CsOH). A stepped voltage protocol from −100 to +60 mV from a holding potential of −100 mV was applied to establish the presence of voltage-gated $Na_v1.5$ channels. Peak currents obtained during steps to −20 or −30 mV were used for comparison in determining the relative reduction in I_{Na}.

SCN5A RNA Abundance

Total RNA (from approximately 2×10^6 number of cells/well) was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA). Quantitative SYBR real time polymerase chain reaction (RT-PCR) was carried out as described using primer pair HE27F and HSCN5AE28A/R.[19] β-actin was used as a reference in all cases. The experiment was carried out in triplicate.

Na^+ Channel Localization

Na^+ channel localization was performed by two methods, fluorescent microscopy and biotin labeling of sarcolemmal $Na_v1.5$. For imaging, HEK293 cells were transfected with SCN5A-GFP cDNA, in which the cDNA sequence encoding GFP was fused to the C-terminus of SCN5A.[18] Cells were incubated with lactate:pyruvate (10 mmol/L:1 mmol/L) to raise intracellular NADH[20–22] or treated with PMA (30 nmol/L) for 10 min and then fixed for fluorescent imaging with a Zeiss LSM510 META confocal microscope (Carl Zeiss, Jena, Germany). To determine the proportion of SCN5A expression at the sarcolemma, total cell fluorescence and that of the membrane region were compared. For biotin labeling, SCN5A cells were incubated as above for 2–10 min. Biotinylation of cell surface proteins was performed with the Pinpoint Cell Surface Protein Isolation Kit (Pierce, Rockford, IL) as previously described.[7] For detection of SCN5A, the primary antibody (rabbit anti-SCN5A, Alomone Labs, Jerusalem, Israel) was diluted 1:100. Alkaline phosphatase-conjugated goat anti-rabbit IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA) was diluted 1:7500.

The Effects of NAD(H) Alterations on Ventricular Arrhythmia

Hearts from wild-type mice were isolated and Langendorff-perfused (3 mL/min at 37° C) with control Krebs-Henseleit buffer (in mmol/L: 119 NaCl, 25 $NaHCO_3$, 4 KCl, 1.2 KH_2PO_4, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose and 2 sodium pyruvate, pH 7.4). Lactate:pyruvate (10:1) Krebs-Henseleit buffer was similarly composed but of 110 mmol/L NaCl, 10 mmol/L Na^+-lactate and 1 mmol/L Na^+-pyruvate, pH 7.4. Monophasic action potentials (MAPs) were recorded on the left ventricular epicardial surface. A custom made, paired-platinum electrode was placed on the right ventricle to deliver 2 ms (0.5–2 mV, twice the threshold) stimuli (GRASS S48 stimulator) to pace the heart at different intervals. The standard pacing protocol consisted of 8 Hz stimuli (basic cycle length, BCL 125 ms) and was delivered for ~20 min to measure MAPs. This was followed by programmed electrical stimulation (PES) consisting of trains of eight paced beats at 125 ms BCL (S_1) followed by an extra stimulus (S_2). Intervals between S_1 and S_2 were gradually decreased by 1 ms each sweep until S_2 overlaid on the 8^{th} S_1. PES was repeated three times each before and after drug application in each heart. Average APD_{90} (action potential duration) was calculated by averaging 240 MAPs recorded at three different locations on each heart (80 MAPs at each location).

Hearts from mice genetically modified to ablate one allele of SCN5A[23] were isolated and Langendorff-perfused (3 ml/min at 37° C). This was followed by assessing arrhythmia using PES and MAP measurements in control condition. Hearts were then perfused with $[NAD^+]_o$ containing Krebs-Henseleit buffer for 20 min, and measurements were repeated.

Statistical Analysis

Data are shown as the mean ± SEM. Determinations of statistical significance were performed with ANOVAs with Bonferroni testing for comparisons of multiple means. A value of $P<0.05$ was considered statistically significant.

Results

A280V GPD1-L Increased $[NADH]_i$

We measured $[NADH]_i$ and $[NAD^+]_i$ in SCN5A cells after infection of WT or A280V GPD1-L. Fig. 1 shows that control and WT GPD1-L groups had similar amounts of $[NADH]_i$ as compared with untreated cells. On the other hand, $[NADH]_i$ was increased 2.48 ± 0.17-fold in A280V GPD1-L group ($P<0.001$). Both WT and A280V GPD1-L groups reduced $[NAD^+]_i$ significantly when compared to untreated SCN5A cells (data not shown). Because of subsequent findings that $[NAD^+]_o$ could prevent the changes in I_{Na} mediated by A280V GPD1-L or NADH, we measured the effect of $[NAD^+]_o$ on $[NADH]_i$. Incubation of 500 μmol/L $[NAD^+]_o$ with the WT GPD1-L group did not alter $[NADH]_i$ compared with control and WT GPD1-L groups. Nevertheless, incubation with $[NAD^+]_o$ prevented the increase of $[NADH]_i$ by A280V GPD1-L.

Increasing $[NADH]_i$ Reduced I_{Na}

Because A280V GPD1-L altered $[NADH]_i$ levels, we tested whether increased $[NADH]_i$ contributed to a reduction in Na^+ current. Fig. 2A shows examples of I_{Na} traces obtained with SCN5A cells, which are reduced by 100 μmol/L $[NADH]_i$. Fig. 2B shows the dose dependence of NADH effect on I_{Na}. Significantly reduced I_{Na} was observed with 20–1000 μmol/L $[NADH]_i$, within the biologically relevant range.[24, 25] The effect reached a maximum at 100 μmol/L $[NADH]_i$ with a peak current at −30 mV of 0.54 ± 0.04 of control ($P<0.01$). The time course for the NADH effect on I_{Na} was rapid, suggesting a post-translational effect. Within 2–4 min, the NADH effect of decreasing I_{Na} became stable and durable, lasted for more than 15 min. The peak current-voltage relationships of control SCN5A group and three doses of $[NADH]_i$ are shown in Fig. 2C. NADH only slightly affected channel gating parameters in a manner likely too small to explain the reduction in current (Fig. 2D and online data). Furthermore, macroscopic inactivation was unaffected by altering $[NADH]_i$.

With rat neonatal cardiomyocytes, we observed similar effects of $[NADH]_i$ on reducing I_{Na} (Fig. 2E). With 500 μmol/L $[NADH]_i$, I_{Na} was decreased to 0.48 ± 0.08 of the control myocyte group ($P<0.001$). Transfection of A280V GPD1-L to myocytes decreased I_{Na} to 0.19 ± 0.04 of control group ($P<0.001$), consistent with our previous observation in SCN5A cells with co-transfection of A280V GPD1-L.[7] Myocytes appeared somewhat less sensitive to pyridine nucleotides than were SCN5A cells, since 20 μmol/L of $[NADH]_i$ was enough to alter I_{Na} significantly in the model cell type.

Quantitative real-time PCR was undertaken to evaluate the possibility of alterations in mRNA transcription or stability. We did not observe any reductions in SCN5A mRNA abundances when SCN5A cells were transfected with WT or A280V GPD1-L or treated with extracellular pyridine nucleotides. The mRNA abundances were 98.0 ± 3.3%, 104.2 ± 3.5%, 102.2 ± 2.5%, 96.9 ± 2.1% and 96.2 ± 2.0% for the control, WT GPD1-L, A280V GPD1-L, NADH and

NAD^+ groups, respectively (P>0.05), consistent with a post-transcriptional regulation of I_{Na} by A280V GPD1-L or NADH.

Antagonism of the NADH Effect

Since NADH is in a redox couple with NAD^+, we tested whether NAD^+ could reverse the NADH effect on I_{Na}. SCN5A cells were incubated with different doses of $[NAD^+]_o$ (50–1000 μmol/L) for ~18 h prior to the patch clamp recording. With 100 μmol/L $[NADH]_i$ added in the pipette solution, the expected reduction of I_{Na} was blocked in a dose dependent manner by $[NAD^+]_o$ (Fig. 3A). Internal NAD^+ had a similar effect but at lower doses (data not shown).

Since PKC has been reported to acutely downregulate I_{Na}[10] and NADH is a substrate for the NADH oxidase,[26] we tested whether PKC and oxidative stress were involved in NADH regulating $Na_v1.5$. Chelerythrine inhibits the PKC catalytic domain[27] and PKC translocation to the membrane.[28] As shown in Fig. 3B, both chelerythrine (5 μmol/L) and superoxide dismutase (SOD; 3 μmol/L) blocked the decrease in I_{Na} seen with $[NADH]_i$. Neither agent affected I_{Na} when applied in the absence of $[NADH]_i$ (0.77 ± 0.10 and 1.02 ± 0.03 of control group, respectively, P>0.05). The I_{Na} reduction seen with $[NADH]_i$ could be recapitulated by a PKC activator, PMA. As shown in Fig. 3C, PMA (30 nmol/L) caused a decrease of I_{Na} (0.47 ± 0.05 of control group, P<0.01), which was prevented by 5 μmol/L SOD (0.75 ± 0.01 of control group, P<0.05).

Activation of PKA increases cardiac Na^+ current acutely.[11, 29, 30] Therefore, we investigated whether PKA was involved in the signaling pathway by which NAD^+ antagonized the downregulation of I_{Na} by NADH. As before, $[NAD^+]_o$ (500 μmol/L) was applied to SCN5A cells or transfected cells (WT and A280V GPD1-L) for an ~18 h incubation. $PKAI_{6–22}$ (100 nmol/L), a specific inhibitor for PKA[31], prevented the $[NAD^+]_o$ antagonism of $[NADH]_i$ on reducing I_{Na} (Fig. 3D). $PKAI_{6–22}$ alone at the same dose had no effect on I_{Na} (1.05 ± 0.15 of the A280V GDP1-L and 0.99 ± 0.32 of the SCN5A groups, respectively, P>0.05). Channel gating was relatively unaffected between experimental conditions (online table II). As expected if the NAD^+ effect was mediated by PKA, 1 μmol/L forskolin, a PKA activator, blocked the NADH effect on reducing I_{Na} (Fig. 3D), while it showed no influence on I_{Na} when applied alone (0.88 ± 0.05 of the control group, P>0.05). Application of $[NAD^+]_o$, $[NAD^+]_o$ + $PKAI_{6–22}$, chelerythrine, and SOD had the comparable effects on the reduction in I_{Na} mediated by A280V GPD1-L (Fig. 3E). In summary, these experiments suggest that PKC activation and superoxide are involved in NADH downregulation of $Na_v1.5$, mutant GPD1-L most likely has its effect through a similar mechanism, and oxidative stress is a downstream of PKC activation.

Surface Expression of $Na_v1.5$ is Unchanged by Treatments Reducing I_{Na}

Expression of $Na_v1.5$ near the surface membrane was monitored by confocal microscopy and biotinylation. Both methods showed that $Na_v1.5$ membrane expression was not affected by incubation with PMA or lactate:pyruvate (Fig. 4), conditions known to increase intracellular NADH level[20–22] and reduce I_{Na} (0.54±0.04 of control, P<0.01).

The Effects of NAD(H) Alterations on Ventricular Arrhythmia

If increased $[NADH]_i$ reduces I_{Na}, then elevated $[NADH]_i$ might be associated with increased arrhythmic risk. Since NADH is impermeable to the sarcolemma, external lactate:pyruvate (10:1) was used to increase $[NADH]_i$. The average action potential duration was not significantly changed following 20 min perfusion with lactate:pyruvate versus control (38 ± 2 vs. 39 ± 3 ms, respectively). PES failed to induce VT in hearts perfused with control Krebs-Henseleit buffer (Fig. 5A). When PES was repeated following 20 min perfusion with lactate:pyruvate buffer, VT was induced in 2 out of 5 hearts (Fig. 5B). The arrhythmogenic effect was reversed when control Krebs-Henseleit buffer was re-introduced (Fig. 5C).

We tested the biological significance of our findings about NAD^+ amelioration of reduced I_{Na} by using a gene-targeted mouse in which one allele of the cardiac $Na_v1.5$ channel has been ablated ($SCN5A^{+/-}$).[23] This model shows decreased I_{Na} and is characterized by a high proclivity towards inducible ventricular tachycardia. MAPs were recorded from six $SCN5A^{+/-}$ mice and two WT mouse hearts. Each MAP consists of a rapid upstroke and smooth repolarization phase giving a triangular shape action potential that was similar to MAPs observed previously in murine heart.[32, 33] The morphology of MAPs was similar to transmembrane action potentials recorded by the patch-clamp technique (Fig. 5D–E). Perfusion for 20 min with 100 μmol/L $[NAD^+]_o$ produced no significant change in MAP duration or morphology (Fig. 5F). Mean values of APD_{90} from six $SCN5A^{+/-}$ hearts after $[NAD^+]_o$ perfusion were close to the control value (29.9 ± 1.7 ms in control versus 27.9 ± 1.9 ms in 100 μmol/L $[NAD^+]_o$, $p = 0.46$) as shown in Fig. 5G.

To assess the inducibility of ventricular arrhythmia, PES was applied as described in the methods. PES induced multiple episodes of ventricular tachycardia (VT) of varying durations (0.5–48 s) in 6 out of 6 $SCN5A^{+/-}$ hearts (Fig. 5H) but not in WT hearts (n=2). This is consistent with previous observation that a decrease in sodium conductance in mouse ventricle by disrupting SCN5A causes ventricular tachycardia.[23] After 20 min of perfusion with 100 μmol/L $[NAD^+]_o$, PES failed to induce VT in 5 out of 6 hearts, suggesting an antiarrhythmic property of $[NAD^+]_o$ in $SCN5A^{+/-}$ hearts (Fig. 5I). The effect of $[NAD^+]_o$ was reversible. Multiple episodes of PES-induced VTs were observed in 4 out of 6 hearts when $[NAD^+]_o$ was removed.

Discussion

Our data demonstrate that the cardiac sodium channel current can be modulated by pyridine nucleotides. Elevated intracellular NADH resulted in a rapid decrease in I_{Na} in both HEK cells and cardiomyocytes that was large enough to be clinically significant[34] and of a magnitude seen in BrS.[35] The immediacy of the NADH effect on reducing I_{Na} observed and the lack of change in mRNA abundances under various experimental conditions suggested that the effect of NADH was post-transcriptional. Membrane expression of SCN5A showed no changes, suggesting that decreased I_{Na} may be the result of changes in channel gating instead of a decrease of available channels in the membrane. The reduction in current could be prevented by SOD or a PKC inhibitor. PKCα-mediated phosphorylation of the channel has been previously reported to result in immediate downregulation of $Na_v1.5$ channels.[36] SOD prevented the downregulation of current in the presence of a PKC activator, suggesting that superoxide was downstream of PKC activation.

NADH is in a redox couple with NAD^+. Internally or externally applied NAD^+ antagonized the downregulation of current seen with a rise of internal NADH. Nevertheless, the NAD^+ effect did not seem to occur by the same signaling mechanism as did NADH and could be recapitulated by a PKA activator or prevented by a PKA inhibitor. This is consistent with the known effect of NAD^+ to activate PKA in human granulocytes[16] and osteoblastic cells[17] and of PKA activation to increase sodium channel current.[11, 36] A general scheme for pyridine regulation of the sodium channel is presented in Fig. 6.

Changes in the Na^+ current induced by pyridine nucleotides are consistent with alterations observed in other channels. Tipparaju et al.[37, 38] have reported that NAD(P)H to $NAD(P)^+$ ratio regulates K^+ currents, although the regulation mostly affects gating rather than peak current. Some transient receptor potential currents are increased by NAD^+ in a manner similar to that seen in our experiments.[39] A non-selective cation channel conductance is also increased by NAD^+.[40] The NADH/NAD^+ ratio affects Ca^{2+} flux in red blood cells.[41] Zima et al.[42] show that cytosolic NADH inhibited cardiac sarcoplasmic reticulum Ca^{2+} release channels, while NAD^+ had activating effects on this channel. Analogously to our results with the Na^+ channel,

NADH has been reported to decrease Ca^{2+}-activated K^+ channel currents, while NAD^+ increases the current.[43] Although our buffering conditions were considerably stronger, these effects of NAD(H) on local Ca^{2+} homeostasis may help explain some of the changes seen in I_{Na}, since recent data shows $Na_v1.5$ is regulated acutely by Ca^{2+}.[44, 45]

The findings provide a possible explanation for the mechanism by which GPD1-L mutations result in BrS[7] and SIDS.[8] GPD1-L has a high degree of homology with GPD, a protein that mediates cytoplasmic reduction of dihydroacetone to glycerol using NADH as the electron donor.[46] If GDP1-L were to serve a similar function, the expectation would be that mutations could result in decreased function and an increase in $[NADH]_i$. This appears to be the case, since transfection of cells with mutant A280V GPD1-L resulted in an increased $[NADH]_i$ level. Other observations suggesting that this GPD1-L mutant may be working through modulation of pyridine nucleotide levels include: 1) elevations of $[NADH]_i$ reduced Na^+ current to a comparable extent as A280V GPD1-L did; 2) WT GPD1-L had no effect on $[NADH]_i$ level or I_{Na}; and 3) the effect of both A280V GPD1-L and increased $[NADH]_i$ on $Na_v1.5$ could be reversed by NAD^+, PKC inhibitor, or SOD. On the other hand, while a strong case may be made for alterations of the NAD(H) levels mediating the effect of mutant GPD1-L to cause BrS, this assertion needs to be confirmed with other mutations known to cause the disease.

The physiological relevance of pyridine nucleotide regulation of $Na_v1.5$ is suggested by the experiments in whole hearts showing manipulations in NAD(H) alter arrhythmic risk. Furthermore, our results suggest that NAD^+ may be a treatment strategy for GPD1-L-mediated BrS or any arrhythmic state associated with reduced I_{Na}, if the acute results are sustained over time. The finding that the balance of oxidized and reduced pyridine nucleotides regulates the Na^+ current suggests that the metabolic state of myocytes may influence Na^+ channel. NADH is known to oscillate with mitochondrial injury, as occurs in ischemic myocardial injury, and mitochondrial injury is associated with increased $[NADH]_i$ and reactive oxygen species levels.[47, 48] Given the acute nature of effects on Na^+ channels, both of these changes could contribute to reduced I_{Na} and arrhythmic risk known to exist with ischemia. Moreover, heart failure is associated with increased oxidative stress, less $[NAD^+]_i$, and increased $[NADH]_i$.[49–51] The increased $[NADH]_i$ levels may contribute to the reduced I_{Na} in this condition.[19, 52, 53]

In summary, A280V GPD1-L can induce elevation of $[NADH]_i$, which can downregulate I_{Na} acutely through a PKC activation and increased superoxide. This can be antagonized by PKA activation mediated by NAD^+, or by application of a PKC inhibitor or SOD. Our results identify a heretofore unknown regulation of cardiac Na^+ channels that may help explain the link between metabolism and arrhythmic risk[19, 54] and may suggest that NAD^+ could lessen arrhythmic risk resulting from reduced Na^+ current.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Non-standard Abbreviations and Acronyms

APD_{90}, action potential duration at 90% repolarization
BCL, basic cycle length
BrS, Brugada syndrome
GFP, green fluorescent protein
GPD, glycerol-3-phosphate dehydrogenase
GPD1-L, glycerol-3-phosphate dehydrogenase 1-like
HEK, human embryonic kidney
IRES, internal ribosomal entry site

MAPs, monophasic action potentials
NAD^+, nicotinamide adenine dinucleotide
NADH, nicotinamide adenine dinucleotide, reduced form
NADP(H), Nicotinamide adenine dinucleotide phosphate
$Na_v1.5$, cardiac sodium channel
PES, programmed electrical stimulation
PKA, protein kinase A
PKC, protein kinase C
RFP, red fluorescent protein
RT-PCR, real-time polymerase chain reaction
SCN5A, cardiac sodium channel
$SCN5A^{+/-}$, a gene-targeted mouse in which one allele of the cardiac $Na_v1.5$ channel has been ablated
SIDS, sudden infant death syndrome
SOD, superoxide dismutase
WT, wild type
VT, ventricular tachycardia

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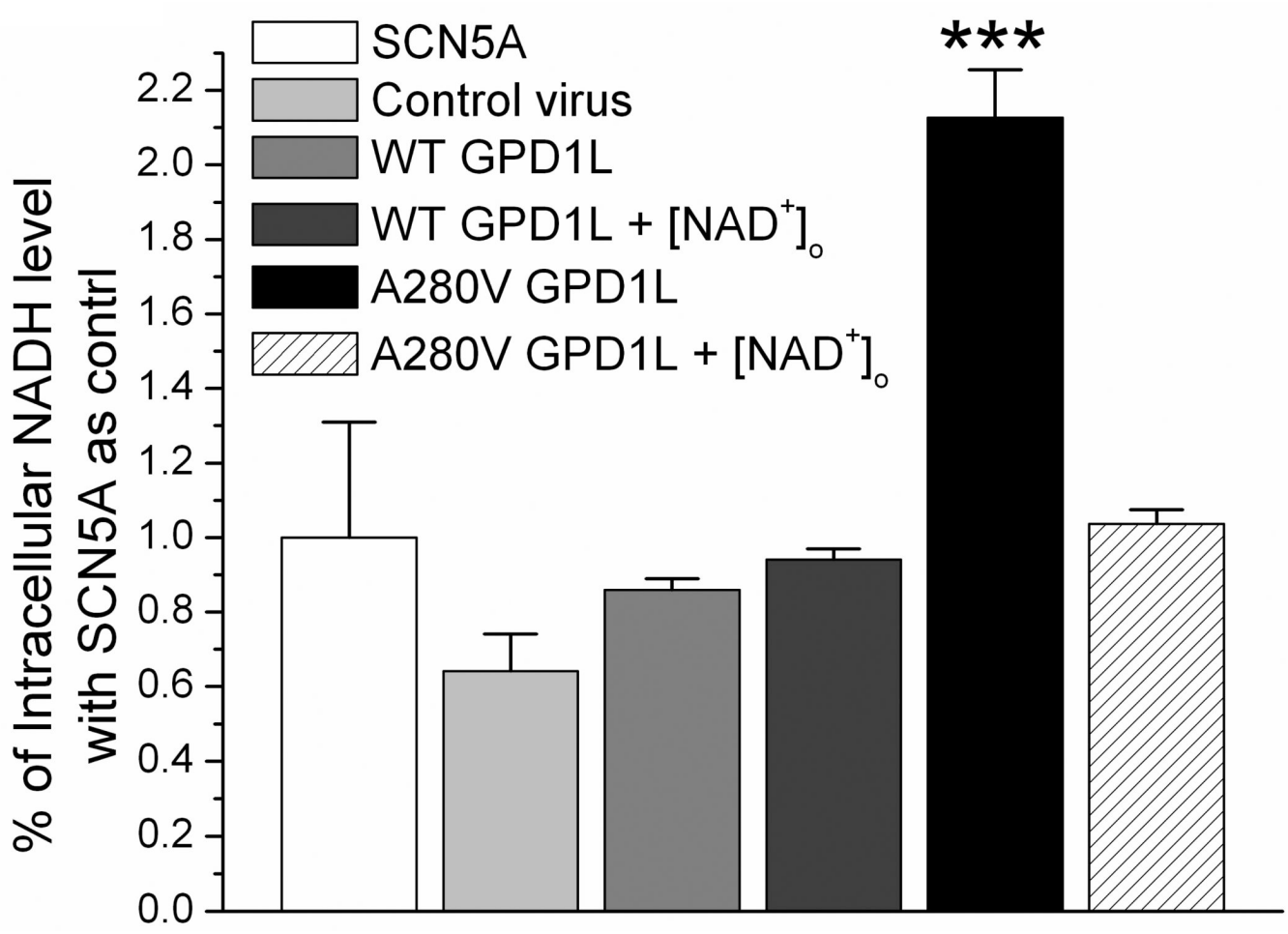

Figure 1.

The intracellular level of NADH is increased by A280V GPD1-L and is reversed by incubation with NAD^+_o. Data from 3–4 samples are normalized to the SCN5A cell group. ***P<0.001 versus all other groups.

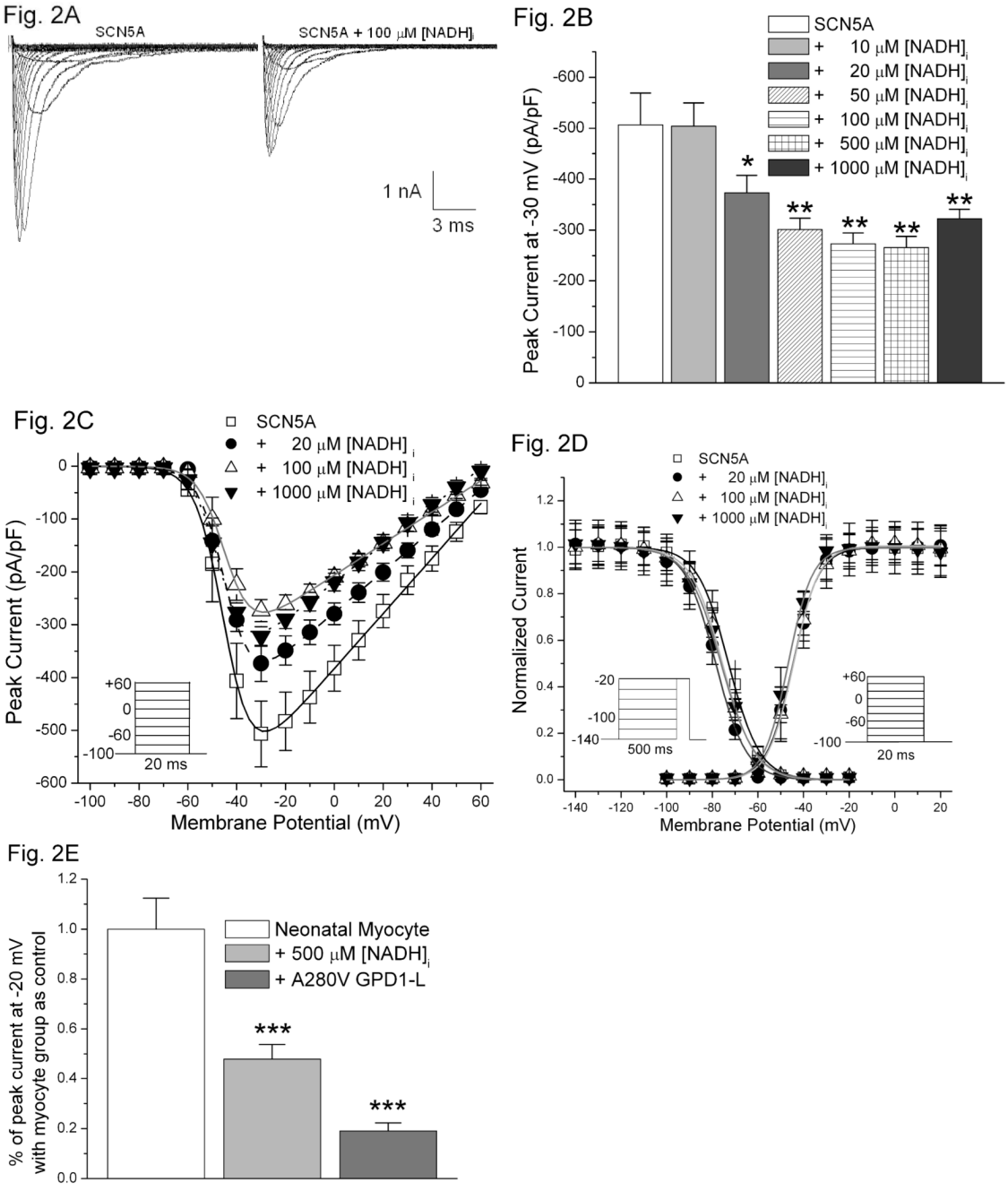

Figure 2.

Intracellular application of NADH reduces cardiac I_{Na}. (A) Representative traces of I_{Na} demonstrate a decrease in current in the presence of $[NADH]_i$ (100 μmol/L). (B) Dose dependence of the $[NADH]_i$ effect on peak I_{Na} averaged from 9–16 samples. *P<0.05 or **P<0.01 versus SCN5A group. There is no significant difference among the NADH groups from 50 to 1000 μmol/L. (C) Peak current-voltage relationships and (D) voltage dependence of steady state activation and inactivation of SCN5A cells (□) or SCN5A cells with $[NADH]_i$ at 20 (●), 100 (Δ), and 1000 (▼) μmol/L. (E) Relative I_{Na} obtained from cardiomyocytes exposed to $[NADH]_i$ (500 μmol/L) or transfection with A280V GPD1-L averaged from 7–13 samples. ***P<0.001 versus cardiomyocytes.

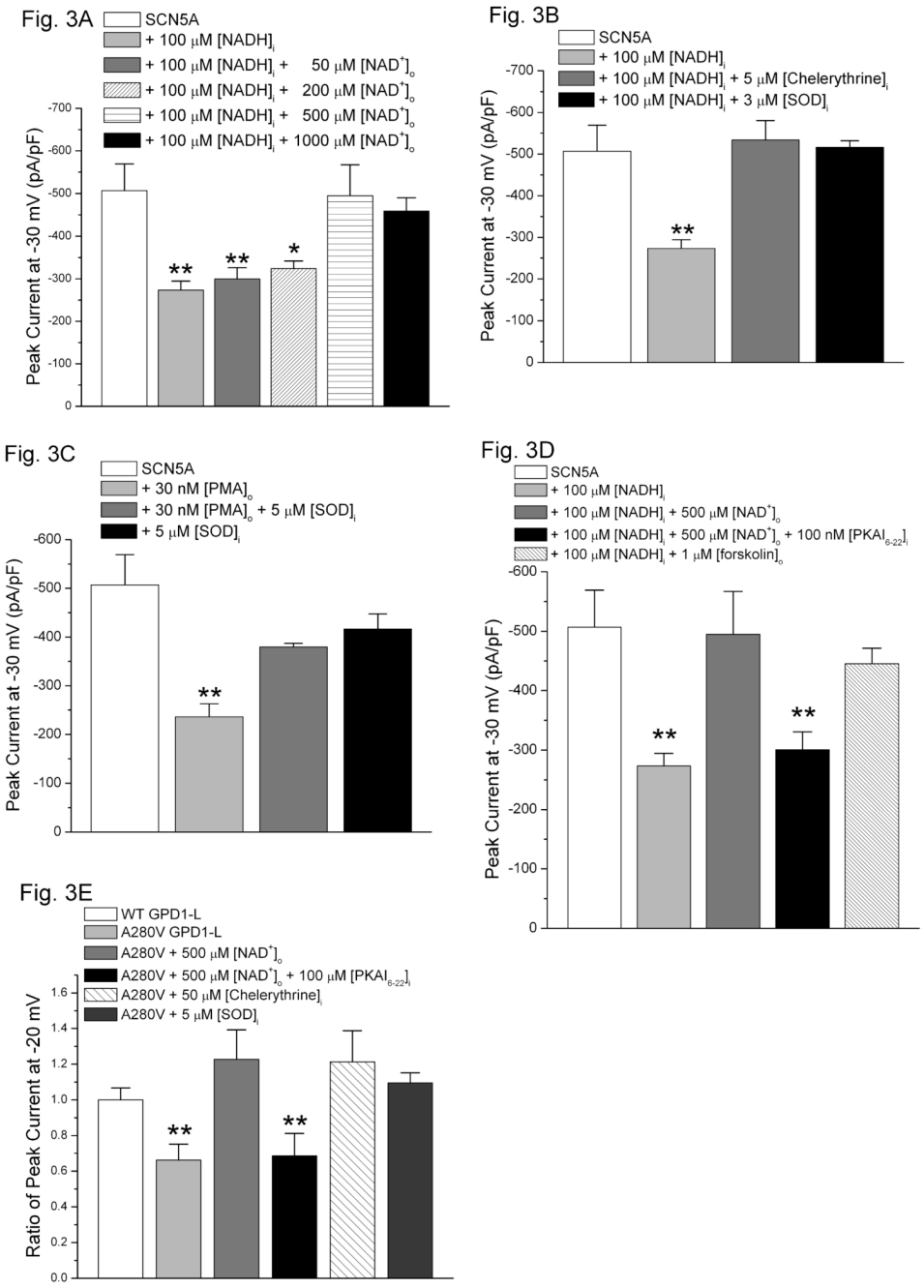

Figure 3.

Downregulation of I_{Na} by $[NADH]_i$ is reversed by incubation with $[NAD^+]_o$, forskolin or intracellular application of chelerythrine or SOD. (A) Dose dependence of $[NAD^+]_o$ prevention of the $[NADH]_i$ effect (100 μmol/L, n=16) on I_{Na} at −30 mV averaged from 8–16 samples. *P<0.05 or **P<0.01 versus SCN5A group. (B) The $[NADH]_i$ effect (100 μmol/L) on reducing I_{Na} is blocked by chelerythrine (5 μmol/L, n=8) and SOD (3 μmol/L, n=10). **P<0.01 versus SCN5A. (C) PMA (30 nmol/L, n=12, extracellularly applied) reproduces the $[NADH]_i$ effect. The PMA-induced reduction in I_{Na} is prevented by SOD (5 μmol/L, n=12). **P<0.01 versus SCN5A. (D) Prevention of the $[NADH]_i$ effect (100 μmol/L, n=16) by $[NAD^+]_o$ (500 μmol/L, n=10) is inhibited by $PKAI_{6–22}$ (100 nmol/L, n=12). Dowregulation of I_{Na} by $[NADH]_i$ is

also reversed by forskolin (1 μmol/L, n=10). (E) Relative I_{Na} at −20 mV obtained with transfection of WT (n=8) or A280V GPD1-L (n=16, **P<0.01 versus WT GPD1-L) with incubation of $[NAD^+]_o$ (500 μmol/L, n=13), with $[NAD^+]_o$ and $PKAI_{6–22}$ (500 μmol/L and 100 nmol/L respectively, n=10, **P<0.01 versus A280V + $[NAD^+]_o$), with SOD (5 μmol/L, n=9), or with chelerythrine (50 μmol/L, n=13). Data are normalized to the WT GPD1-L group.

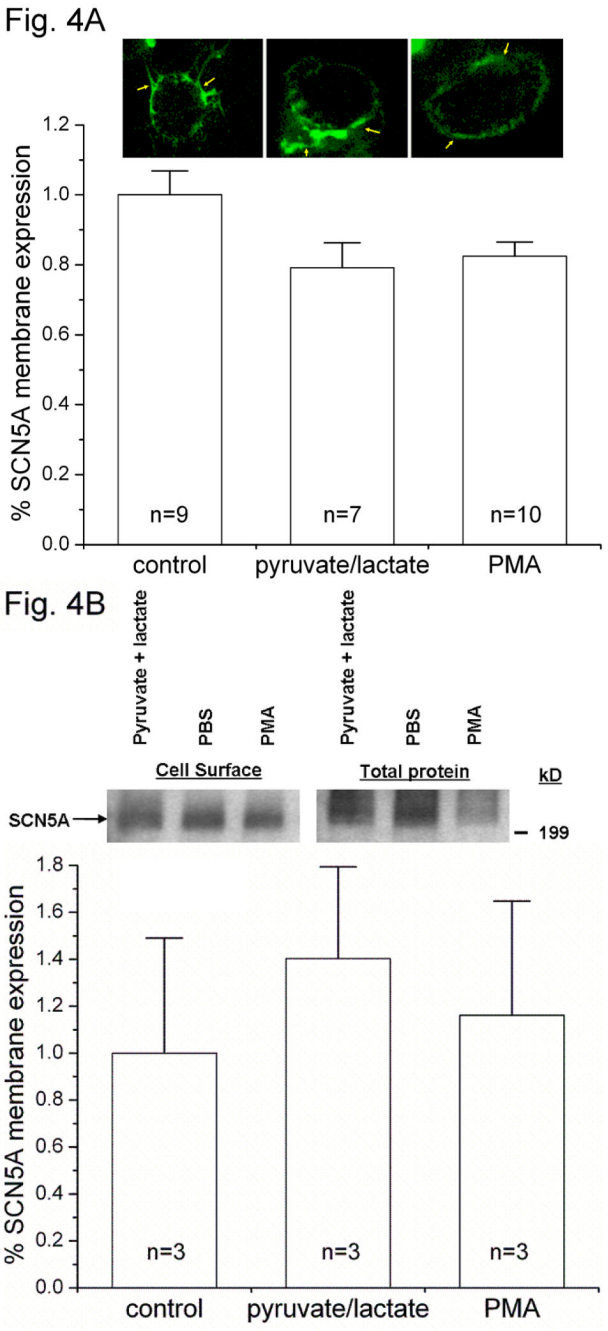

Figure 4.

Cardiac Na^+ channel membrane expression is unaffected by increased $[NADH]_i$ or PKC activation measured with (A) fluorescent imaging and (B) biotinylation. Lactate:pyruvate (10 mmol/L: 1 mmol/L, with PBS group as control) and PMA (30 nmol/L, with DMSO group as control) were used to treat cells to increase $[NADH]_i$ and activate PKC, respectively.

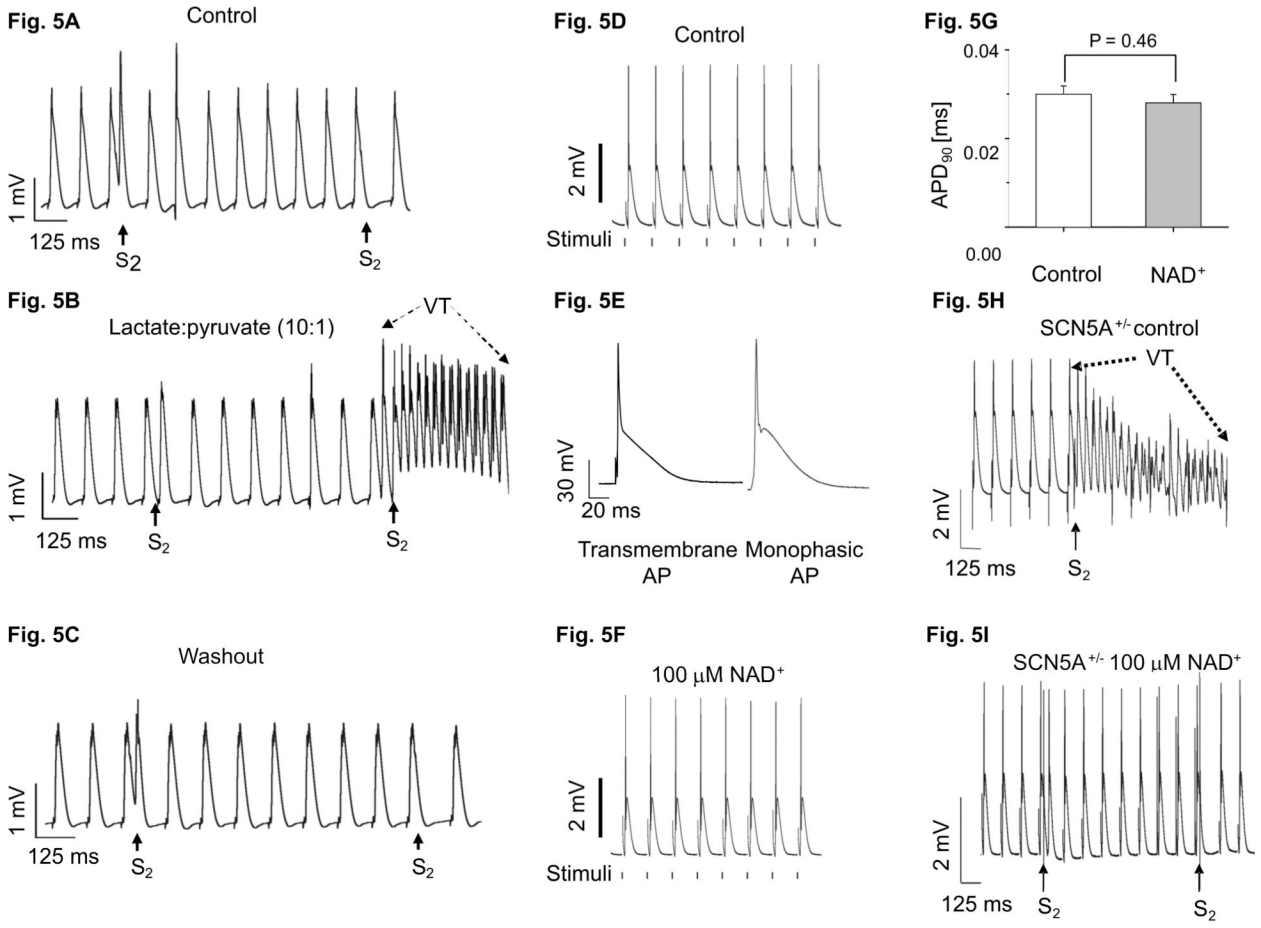

Figure 5.

The effects of altering NAD(H) on arrhythmic risk. Representative monophasic action potential recordings with PES during Krebs-Henseleit buffer perfusion (A), after 20 min perfusion with lactate:pyruvate (10:1) buffer to increase $[NADH]_i$ (B) and re-introduction of Krebs-Henseleit buffer (C). Representative traces of MAPs from left ventricular epicardium of (D) Langendorff-perfused SCN5A$^{+/-}$ heart during standard pacing at BCL of 125 ms in the control condition. Vertical lines below the MAPs represent the times when electrical stimulations were delivered. (E) Action potentials recorded with the patch-clamp technique in single ventricular myocytes and with the MAP electrode on whole heart. (F) MAPs after 20 min of perfusion with 100 μmol/L $[NAD^+]_o$. (G) A histogram of APD_{90} in control condition and with 100 μmol/L $[NAD^+]_o$. (H) Representative MAPs recorded during PES showing PES-induced ventricular tachycardia in SCN5A$^{+/-}$ hearts under control condition. The final six paced beats at 125 BCL (S_1) were followed by an extra stimulus (S_2) delivered at a S_1-S_2 interval of 42 ms. PES induced a polymorphic ventricular tachycardia of frequency, 20–40 Hz, sustained for ≈19 seconds. (I) Representative trace of PES-induced MAP recording in same SCN5A$^{+/-}$ heart after 20 min perfusion with 100 μM $[NAD^+]_o$. S_2 stimuli delivered at a 35 ms S_1-S_2 interval produced a single MAP but failed to induce any arrhythmia.

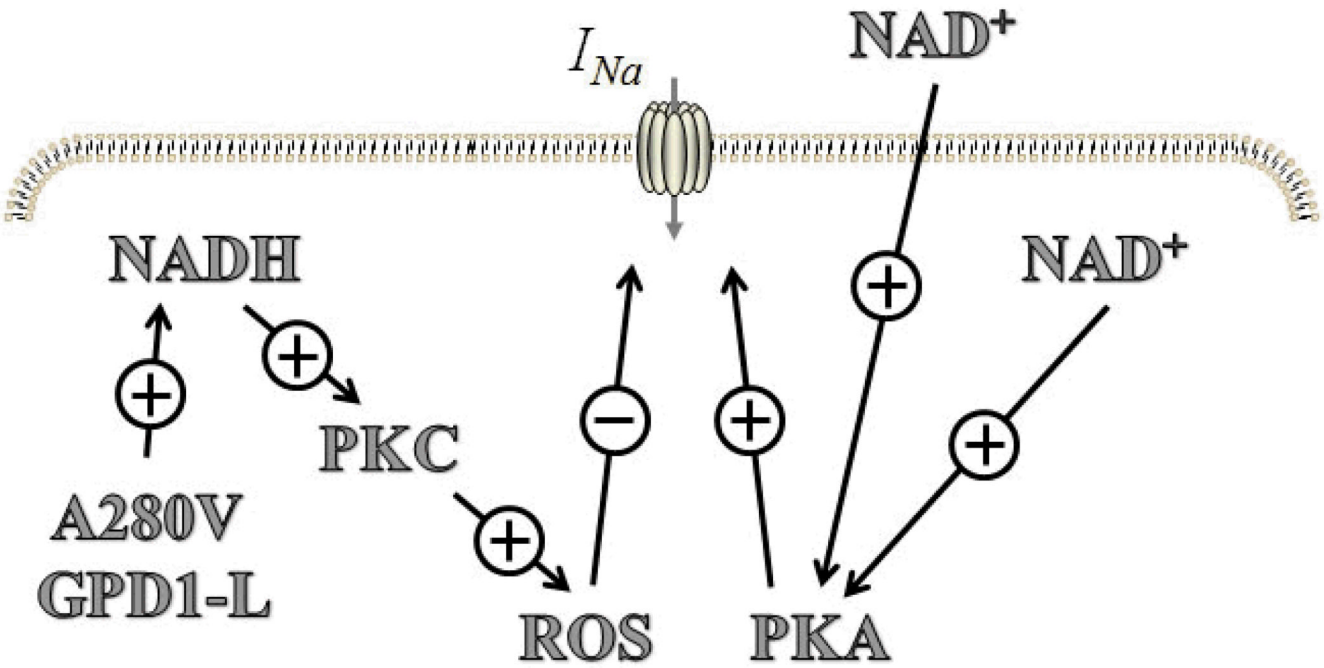

Figure 6.
Possible signaling pathways for the effects of A280V GPD1-L, $[NADH]_i$ and NAD^+ to regulate the $Na_v1.5$.