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The role of protein kinase C in the metabolic regulation of the cardiac Na⁺ channel

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Abstract

Background—NADH increases in cardiomyopathy, activates protein kinase C (PKC), upregulates mitochondrial reactive oxygen species (mitoROS), and downregulates the cardiac Na⁺ channel (Na_v1.5).

Objective—The objective was to determine how NADH signals downregulation of Na_v1.5.

Methods—Isolated mouse cardiomyocytes were used for patch clamp recording and to monitor mitoROS with MitoSOXTM Red. HEK293 cells were used for transient transfections. HEK293 cells stably expressing human Na_v1.5 were utilized for single channel recording, whole-cell patch clamp recording, activity measurements of phospholipase C and D (PLD), channel protein purification, and co-immunoprecipitation with PKC isoforms. HL-1 cells were used for mitochondria isolation.

Results—NADH enhanced PLD activity (1.6±0.1-fold, P<0.01) and activated PKCδ. Activated PKCδ translocated to mitochondria and upregulated mitoROS (2.8±0.3-fold, P<0.01) by enhancing the activities of mitochondrial complexes I, II and IV (1.1- to 1.5-fold, P<0.01). PKCδ also interacted with Na_v1.5 to downregulate Na⁺ current (I_{Na}). Reduction in I_{Na} by activated PKCδ was prevented by antioxidants and by mutating the known PKC phosphorylation site

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S1503. At the single channel level, the mechanism of current reduction by PKC and recovery by PKA was a change in single channel conductance.

Conclusion—NADH activated PKC δ by enhancing PLD activity. PKC δ modulated both mitoROS and Na_v1.5. PKC δ elevated mitoROS via enhancing the mitochondrial oxidative phosphorylation complex activities. PKC δ -mediated channel phosphorylation and mitoROS were both required to downregulate Na_v1.5 and altered single channel conductance.

Keywords

PKC δ ; mitochondria; arrhythmia; NADH; channel phosphorylation; metabolism; cardiomyopathy

Introduction

Human cardiomyopathy is associated with activated protein kinase C (PKC)^{1–4} and decreased cardiac Na⁺ current (I_{Na}).^{5,6} Altered cardiac Na⁺ channel (Na_v1.5) function has been implicated in the increased risk of sudden death in heart failure.^{5–7} PKC is a family of serine/threonine-specific protein kinases, composing three subgroups with at least ten isoforms.⁸

Activated PKC triggers many signaling pathways, and different PKC isoforms impact myocardial function distinctively.⁹ For example, transgenic mice with higher PKC α activity show decreased cardiac contractility, ventricular dilation, and secondary hypertrophy,^{10–12} while transgenic mice with inducible cardiac expression of a dominant negative PKC α mutant showed partial protection from cardiac decompensation after myocardial infarction injury.¹³ PKC δ and PKC ϵ play opposing roles in cardiac ischemia and reperfusion.¹⁴ PKC δ causes increased damage from ischemic insults,¹⁵ while PKC ϵ plays a role in cardioprotection.^{16,17}

Previously, we have found that elevated NADH activates PKC, causing mitochondrial reactive oxygen species (mitoROS) overproduction and I_{Na} reduction,¹⁸ both of which can be ameliorated by NAD⁺ through PKA activation.^{6,18,19} Na_v1.5 S1503 has been reported as a PKC phosphorylation site.^{20–23} Our studies show that the changes of I_{Na} induced by NADH, PKC, and mitoROS are rapid (detectable in 5 minutes)^{18,19} and, therefore, are most likely to be a result of changes in channel properties rather than the number of channels in the membrane.

In this work, we described data in support of a potential signaling cascade whereby NADH activates PKC, PKC induces mitoROS overproduction, and PKC affects the cardiac sodium channel directly by phosphorylation and indirectly by modification of mitoROS generation.

Materials and Methods

For detailed methods, please see Supplementary Materials. Animal care was provided in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Experimental Animals, and all protocols were approved by the Lifespan Institutional Animal Care and Use Committee.

Results

NADH induced PKC activation via enhancing PLD activity

Previously, we have shown that NADH affects sodium channels through activation of PKC in minutes.¹⁸ Conventional and novel PKCs require DAG for activation. Therefore, we studied whether NADH could elevate DAG levels. DAG can be formed from hydrolysis of phosphatidylinositol 4,5-bisphosphate by PLC or from hydrolysis of phospholipids by PLD. As shown in Figure 1A, NADH elevation (induced by PL buffer) enhanced PLD activity to 1.6 ± 0.1 -fold ($P < 0.01$ vs. untreated cells) but not PLC activity (0.93 ± 0.02 -fold, $P = \text{NS}$ vs. untreated cells). A PLD inhibitor ($\text{IC}_{50} = 25 \text{ nM}^{27,28}$), FIPI ($0.5 \mu\text{mol/L}$) completely restored I_{Na} decreased by NADH (control: $-310 \pm 19 \text{ pA/pF}$; the NADH group: $-134 \pm 21 \text{ pA/pF}$, $43 \pm 7\%$ of control, $P < 0.05$ vs. control; the NADH+FIPI group: $-309 \pm 25 \text{ pA/pF}$, $100 \pm 10\%$ of control, $P = \text{NS}$ vs. control), as shown in Figure 1B. This confirmed that PLD was downstream of NADH. FIPI alone did not affect I_{Na} ($-282 \pm 15 \text{ pA/pF}$, $P = \text{NS}$ vs. control).

PKC δ modulated mitoROS and downregulated $\text{Na}_v1.5$ predominately

In order to identify which PKC isoform was responsible for mitoROS elevation and I_{Na} reduction, we tested specific inhibitors for three PKC isoforms (all at $2 \mu\text{mol/L}$ in bath solution). Figure 2A shows the ratios of the peak I_{Na} (white bars) and mitoROS levels (black bars) under various experimental conditions normalized to their respective levels in untreated mouse cardiomyocytes. NADH treatment resulted in a significant decrease of I_{Na} and an increase of mitoROS (0.5 ± 0.1 - and 2.8 ± 0.4 -fold of untreated control group, respectively, $P < 0.01$ vs. control myocytes). Coapplication of $\delta\text{V1-1}$ (a specific PKC δ inhibitor¹⁵) completely restored the peak I_{Na} and mitoROS to control levels (0.9 ± 0.1 - and 1.2 ± 0.1 -fold of control, respectively). Partial recovery of I_{Na} was observed with coapplication of $\alpha\text{V5-3}$ (a specific PKC α inhibitor²⁹; 0.7 ± 0.1 -fold of control, $P < 0.05$ vs. control or the NADH group), although $\alpha\text{V5-3}$ showed no effects on NADH-induced mitoROS overproduction (1.9 ± 0.3 -fold of control, $P = \text{NS}$ vs. the NADH group, $P < 0.01$ vs. control). This suggested that PKC α participated in I_{Na} downregulation but not mitoROS stimulation. Coapplication of $\epsilon\text{V1-2}$ (a specific PKC ϵ inhibitor¹⁶) exerted no influence on either decreased I_{Na} or increased mitoROS level (0.6 ± 0.1 - and 2.5 ± 0.6 -fold of control, respectively, $P < 0.01$ vs. control and $P = \text{NS}$ vs. the NADH group). Figure 2B shows representative fluorescent confocal images of mitoROS levels with NADH application and PKC isoform inhibition. These results suggest that NADH activated predominately PKC δ to evoke mitoROS excess.

To confirm the role of PKC δ in the regulation of I_{Na} , we exposed WT and PKC $\delta^{-/-}$ cardiomyocytes to NADH. Cardiomyocytes isolated from PKC $\delta^{-/-}$ mice showed similar I_{Na} density ($-37.2 \pm 3.1 \text{ pA/pF}$) compared with WT mice ($-39.8 \pm 3.9 \text{ pA/pF}$). NADH application ($500 \mu\text{mol/L}$) to WT cardiomyocytes led to significant I_{Na} reduction ($-17.8 \pm 2.1 \text{ pA/pF}$, $P < 0.05$ vs. WT). On the other hand, NADH did not alter I_{Na} in PKC $\delta^{-/-}$ cardiomyocytes ($-41.7 \pm 4.2 \text{ pA/pF}$, $P = \text{NS}$ vs. PKC $\delta^{-/-}$; Figure 2C). Increasing mitoROS production with a complex III blocker antimycin A ($20 \mu\text{mol/L}$) led to significant reduction of I_{Na} on PKC $\delta^{-/-}$ cardiomyocytes ($-16.8 \pm 2.0 \text{ pA/pF}$, $P < 0.01$ vs. PKC $\delta^{-/-}$) that was identical to the reduction

seen in WT myocytes. Coapplication of antimycin A and α V5-3 (2 μ mol/L) partially restored I_{Na} to -29.5 ± 1.4 pA/pF ($P < 0.05$ vs. PKC $\delta^{-/-}$ or PKC $\delta^{-/-}$ + antimycin A). This partial inhibition of Na_v1.5 downregulation by mitoROS in the presence of a PKC α inhibitor implied that PKC α was activated by mitoROS and played a role on Na_v1.5 downregulation.

NADH induced PKC δ translocation to mitochondria

As shown in Figure 3A, NADH increased levels of PKC δ , phospho-PKC δ , and the ratio of phospho-PKC δ to PKC δ in the mitochondrial fraction, compared with untreated group ($P < 0.05$). NADH did not alter PKC α translocalization to mitochondria. Figure 3B shows that there was significant colocalization of Na_v1.5 and PKC δ . On the other hand, no colocalization was detected for PKC α and Na_v1.5.

PKC δ modulated mitoROS by enhancing the mitochondrial electron transport

In order to understand how PKC δ modulated mitoROS level, we monitored the activities of the mitochondrial ETC complexes with HL-1 cells. As shown in Figure 4, the activities of complex I, II and IV were all significantly enhanced by PKC activation (1.1 ± 0.0 -, 1.4 ± 0.1 -, and 1.5 ± 0.1 -fold of control, respectively, $P < 0.001$ vs. control).

PKC-dependent phosphorylation of Na_v1.5 at S1503

To investigate the direct effect of PKC on Na_v1.5, we obtained site-directed mutants of the known PKC phosphorylation site, S1503.^{20–23} As shown in Figure 5A, NADH (100 μ mol/L), PMA (30 nmol/L), and antimycin A (20 μ mol/L) induced significant reduction of peak I_{Na} in WT Na_v1.5. NADH and PMA failed to induce significant I_{Na} reductions ($81 \pm 6\%$ and $87 \pm 11\%$ of untreated S1503A, $P = 0.06$ and $P = 0.82$ vs. S1503A, respectively), indicating that this site was important for PKC downregulation of Na_v1.5. The possibility of slight reductions with NADH and PMA cannot be completely ruled out, however. In this case, it is possible that another site on the channel has a minor role in the signaling cascade. Nevertheless, antimycin A failed to reduce the current from S1503A ($107 \pm 18\%$ of untreated S1503A, $P = \text{NS}$) as well, indicating that mitoROS alone without channel phosphorylation at S1503 by PKC was not sufficient to downregulate Na_v1.5. Unexpectedly, the peak I_{Na} of the phosphomimetic mutant S1503D (Figure 5B) was not significantly reduced compared to the WT channel. Application of NADH, PMA, or antimycin A successfully decreased I_{Na} of S1503D ($52 \pm 4\%$, $45 \pm 7\%$, and $67 \pm 6\%$ of untreated S1503D, respectively, $P < 0.05$ vs. S1503D), indicating that an elevation of mitoROS in addition to phosphorylation of the 1503 site was required to decrease I_{Na} . Consistent with the idea that both mitoROS and site specific phosphorylation are necessary to decrease I_{Na} , mitoTEMPO and SOD could block the PMA effect on I_{Na} reduction ($87 \pm 13\%$ and $75 \pm 9\%$ of the untreated group, respectively, $P = \text{NS}$ vs the untreated group, $P < 0.01$ vs PMA group), as shown in Figure 5C. Since PMA is expected to activate mitoROS and PKC simultaneously, the ability of mitoROS scavengers to prevent the downregulation of current suggests that, without concomitant mitoROS overproduction, PKC-mediated channel phosphorylation alone is not sufficient to downregulate I_{Na} .

PKC activation reduced Na_v1.5 single channel conductance

Previously, we have shown that NADH/NAD⁺ regulation of I_{Na} was independent of Na_v1.5 membrane protein expression.^{6,19} Figure 6A exhibits representative traces of the single channel currents at -80 mV with no treatment (control) or with NADH (100 μmol/L), PMA (30 nmol/L), NADH+NAD⁺ (100+500 μmol/L), or NADH+forskolin (100+1 μmol/L). The single channel currents were -1.09±0.12, -0.55±0.04, -0.49±0.01, -1.01±0.07, -1.11±0.05 pA for these groups, respectively. The single channel conductance of Na_v1.5 was significantly decreased by NADH and PMA (5.5±1.4 and 6.3±0.9 pS, respectively, P<0.01 vs. 12.0±1.8 pS of untreated cells, n = 4–12 cells; Figure 6B). Coapplication of NAD⁺ or forskolin with NADH restored the conductance back to the control level (13.9±1.6 and 15.1±1.5 pS, respectively, P=NS vs. the untreated cells, P<0.01 vs. NADH group, n = 4–7 cells). The reduction in channel conductance (46–53% of reduction compared with the untreated group) was comparable to that in whole-cell macroscopic currents and consistent with the unaltered Na_v1.5 protein membrane expression reported previously.^{6,19} The reduction in single channel current explained why the maximum effect of NADH or PKC did not completely eliminate I_{Na}.¹⁸

We also performed single channel measurements on S1503A with and without NADH treatment to confirm our findings in Fig. 5B. The mutant channel S1503A showed a single channel current of 0.94±0.07 pA at -80 mV (n=8), which was not affected by NADH treatment (0.99±0.07 pA, P=NS vs. untreated S1503A or WT channel). These data are consistent with the macroscopic I_{Na} results of S1503A in Fig. 5B showing that NADH did not affect I_{Na} of S1503A. This observation supports the idea that S1503 is important for PKC-dependent NADH downregulation of I_{Na}.

Discussion

Previously, our group has demonstrated that cardiomyopathy is associated with elevated intracellular NADH level that decreases I_{Na} by activating PKC and promoting mitoROS overproduction.^{18,19,30} In this study, we found that NADH appeared to activate PKC by enhancing PLD activity to promote DAG synthesis. Metabolic downregulation of Na_v1.5 by NADH required simultaneous increases in mitoROS and PKC-dependent phosphorylation of the channel. The single channel conductance of Na_v1.5 was reduced by 45–50% by NADH and PKC activation. These actions were mainly mediated by PKCδ. Upon activation, PKCδ redistributed to the mitochondria. The major source of mitoROS has been reported to be complex I, III, and the reverse electron transfer from complex II to I.^{19,31–33} In this work, we found that PKC activation enhanced the activities of complex I, II and IV significantly. We did not test which types of ROS were involved. Since the reduction in I_{Na} was eliminated by the superoxide scavenger mitoTEMPO, this implies that superoxide is required to carry out the reduction in I_{Na}. Nevertheless, it does not rule out other reactive oxygen species participation in the downstream signaling.

PKCα appeared to play a minor role downstream of mitoROS. PKCα was activated by mitoROS and could partially downregulate Na_v1.5. Our results are consistent with a recent study shows that PKCα mediates angiotensin II-induced I_{Na} reduction.³⁴ It is unclear if the PKCα effects on the channel were the result of direct modification of the channel or an

unidentified indirect effect, however. A scheme of NADH signaling cascades regulating $\text{Na}_v1.5$ is shown in Figure 7.

The residue S1503 of human cardiac $\text{Na}_v1.5$ has been identified as a PKC phosphorylation site.^{20,22} It resides in the linker between domain III and IV of the α subunit. This area is highly conserved in subtypes and species and critical for open-state inactivation.²³ Our work demonstrated unaltered I_{Na} levels with both phosphorylation resistant and phosphomimetic S1503 mutations as compared with WT channels, suggesting phosphorylation at this site alone is insufficient to modulate current. The unaltered I_{Na} with S1503A with respect to the WT channel is in agreement with previous observation on PKC modulating brain and cardiac $\text{Na}_v1.5$.^{20–22} The lack of PKC or mitoROS effects on I_{Na} in the S1503A mutant suggests that phosphorylation at S1503 is necessary for channel downregulation even if mitoROS surplus exists. On the other hand, the unaltered I_{Na} with the phosphomimetic mutant S1503D indicated that PKC phosphorylation at this site alone is not sufficient to decrease I_{Na} . The reduction in current required both the presence of mitoROS and phosphorylation at the 1503 site. The finding that PMA failed to induce I_{Na} reduction in the presence of mitoTEMPO illustrated that mitoROS were essential, too, for channel downregulation even if PKC was activated. MitoROS could have its effect by modification of the channel directly or by some unidentified indirect signaling event. In our study, mitoROS-induced I_{Na} reduction could be reversed by ROS scavenger mitoTEMPO or by PKA activation within minutes. Reversible oxidative PTMs normally involve the sulfur-containing amino acids methionine and cysteine. Nevertheless, dithiothreitol, a reducing agent that could block cysteine oxidation, failed to block NADH effects on I_{Na} reduction (data not shown).

Metabolic regulation of I_{Na} by NADH/ NAD^+ appeared to be the result mainly of changes in the single channel conductance. Our study is the first to show a decreased single channel current by PKC activation. It should be noted that we used fenvalerate to resolve single channel currents, which made us unable to investigate whether PKC affects the channel opening time or probability, but there are several reasons we believe that a reduction in single channel current is likely to underlie the change in whole cell current. The reduction in whole cell current is nearly identical to the reduction in single channel current, suggesting a common mechanism. The maximum whole cell current reduction was limited to ~50%, a phenomenon most easily explained by a fixed reduction in single channel current. Channel macroscopic gating parameters and the amount of $\text{Na}_v1.5$ protein in the membrane are unchanged with NADH.^{18,19} While it is possible that fenvalerate may have altered the signaling cascade effect, the drug has been previously shown not to alter Na^+ channels single channel conductance of native or mutated Na^+ channels.^{35–37} A reduction in unitary current is an unusual mechanism. Since it is hard to understand how a modification in the III-IV linker (i.e. S1503) causes this reduction alone, the data suggest that the unknown oxidative modification works in tandem to affect the ion permeation pathway.

We used mouse ventricular myocytes, HEK293 cells, and HL-1 cells for different measurements. When we measured I_{Na} , we used mainly HEK293 cells and mouse ventricular myocytes, and the results were similar in the two cell models. NADH, PMA, and antimycin A decreased Na^+ currents (either macroscopic I_{Na} of Fig. 2 and Fig. 5 or single

channel currents of Fig. 6) to ~50% in both cell types.^{18,19} Therefore, it seems reasonable to conclude that the mechanisms of Na_v1.5 downregulation by NADH, activated PKC, and mitoROS overproduction were similar in these two cell models. To study the translocation of PKC to mitochondria and enzyme activities of the complex I, II, and IV of the mitochondrial ETC, we used the mouse atrial myocyte cell line HL-1 cells because they were stable in culture and yielded a sufficient number of cells and mitochondria to allow for the proteomic analysis. To make sure that these cells were a suitable model, we confirmed a ~50% reduction of I_{Na} with NADH treatment (data not shown). In summary, NADH caused a similar I_{Na} reduction in all three cell types. Therefore, it seems likely that the signaling cascade explored here is shared among all these models.

Conclusions

NADH enhanced PLD activity to activate PKC. PKC, especially PKC δ , regulated cardiac Na_v1.5 in two ways: indirectly through elevated mitoROS production and directly through Na_v1.5 channel phosphorylation at S1503. The combination of these two effects altered Na_v1.5 single channel conductance. Since NADH is increased in cardiac pathologies such as myocardial ischemia^{38,39} and cardiac hypertrophy,⁶ these results may explain the concomitant reduction in I_{Na} seen, and therapies directed with this signaling cascade may have antiarrhythmic effects by raising Na⁺ channel activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Disclosures

Dr. Dudley has filed provisional patents: 1) Modulation of sodium current by nicotinamide adenine dinucleotide and 2) Modulating mitochondrial reactive oxygen species to increase cardiac sodium channel current and mitigate sudden death, related to this work.

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Figure 1.

NADH enhanced PLD activity but not PLC activity. **(A)** The ratios of enzyme activities were obtained by comparison with the control groups of HEK293 cells stably expressing human cardiac $\text{Na}_v1.5$. The NADH group was treated with PL buffer to increase intracellular NADH level. Six samples were measured for each group; * $P < 0.05$ vs. the control group. **(B)** PLD inhibition by FIPI blocked the NADH effect on I_{Na} . The ratios of peak I_{Na} were obtained by comparison with the peak I_{Na} of the untreated control cells at -20 mV. Fifteen to 21 HEK293 cells stably expressing human cardiac $\text{Na}_v1.5$ were measured for each group. * $P < 0.05$ vs. the control group.

Figure 2.

The inverse relationship of I_{Na} and mitoROS is mainly mediated by PKC δ . **(A)** Peak I_{Na} and mitoROS levels are altered with treatment of NADH and specific inhibitors of various PKC isoforms. The ratios of peak currents and mitoROS levels were obtained by normalization to untreated control myocytes. The peak I_{Na} were obtained at -20 mV with a holding potential of -100 mV. MitoROS levels were obtained by monitoring MitoSoxTM Red fluorescence intensity. Three to four mice were used for myocyte isolation for each group, and 18–40 cardiomyocytes were measured in each group. * $P < 0.05$ vs. the control group; # $P < 0.05$ vs. the control or NADH group. **(B)** Representative fluorescent confocal images of mouse ventricular myocyte show mitoROS levels monitored with MitoSoxTM Red and MitoTracker Green. Cardiomyocytes were treated with elevated NADH and specific inhibitors of PKC isoforms ($2 \mu\text{mol/L}$). **(C)** Antimycin A but not NADH downregulates peak I_{Na} in cardiomyocytes isolated from PKC $\delta^{-/-}$ mice. The ratios were obtained by comparison with untreated PKC $\delta^{-/-}$ cardiomyocytes. The peak currents were obtained at -20 mV with a holding potential of -100 mV. The treatments were NADH ($500 \mu\text{mol/L}$), antimycin A ($20 \mu\text{mol/L}$), and α V5-3 ($2 \mu\text{mol/L}$). Fifteen to 21 cardiomyocytes isolated from three mice were used in each group. * $P < 0.05$ vs. the untreated PKC $\delta^{-/-}$ group; # $P < 0.05$ vs. the untreated PKC $\delta^{-/-}$ or the antimycin A-treated group.

Figure 3.

NADH induced translocation of PKC δ to mitochondria. **(A)** NADH induced translocation and phosphorylation of PKC δ to the mitochondria. Representative Western blot bands of PKC α , phospho-PKC α , PKC δ , and phospho-PKC δ were obtained from mitochondria isolated from HL-1 cells. Mitochondrial VDAC was used to identify the mitochondrial fraction and to act as a loading control. Three samples were tested in each group. * $P < 0.01$ vs. control. **(B)** PKC δ was co-IPed with membrane Na $_v$ 1.5. Membrane Na $_v$ 1.5 protein was purified from three groups of HEK293 cells stably expressing human cardiac Na $_v$ 1.5: untreated control cells (Ctrl), cells treated with elevated NADH (induced by PL buffer) or cells treated with 30 nmol/L PMA.

Figure 4. PKC activation induced enhanced activities of complex I, II and IV of the mitochondrial electron transport chain. PKC activation was induced by PMA (30 nmol/L) on HL-1 cells. Six samples were measured in each group. *P<0.001 vs. control.

Figure 5.

Phosphorylation at $Na_v1.5$ S1503 and mitoROS overproduction are both essential for $Na_v1.5$ downregulation. **(A)** S1503A, which eliminates the phosphorylation site, blocked the effects of PKC and mitoROS (100 μ mol/L NADH, 30 nmol/L PMA, or 20 μ mol/L antimycin A) on reducing I_{Na} . **(B)** S1503D, a phosphomimetic mutation, required mitoROS induced by NADH, PMA or antimycin A to mediate $Na_v1.5$ downregulation (100 μ mol/L NADH, 30 nmol/L PMA, or 20 μ mol/L antimycin A). **(C)** MitoROS are essential for the PKC-mediated downregulation of $Na_v1.5$. The I_{Na} reduction mediated by PMA (30 nmol/L) was inhibited by co-application of mitoTEMPO or SOD at 5 μ mol/L. WT $Na_v1.5$, mutants S1503A and S1503D channels were transiently transfected in HEK293 cells. The peak currents were obtained at -20 mV with a holding potential of -100 mV. The ratios were obtained by comparison with I_{Na} of WT channel. Fifteen to 28 cells were used in each group. * $P < 0.01$ vs. WT, # $P < 0.01$ vs. S1503D.

Figure 6.

NADH/NAD⁺ and PKC/PKA regulate Na_v1.5 by changing the single channel conductance. (A) Single channel currents traces of human cardiac Na_v1.5 stably expressed in HEK cells were obtained with or without treatment of NADH (100 μmol/L), PMA (30 nmol/L), NADH + NAD (100+500 μmol/L), and NADH+forskolin (100+1 μmol/L), respectively, with a holding potential of -80 mV. Four to twelve cells were used in each group. (B) The linear fitting of current-voltage relationships of the single channel currents give the single channel conductance.

Figure 7.

A scheme for NADH regulation of cardiac $\text{Na}_v1.5$. “+” and “-” indicate activation and inhibition, respectively. DAG, diacylglycerol; ETC, the electron transport chain; I_{Na} , $\text{Na}_v1.5$ channel current; mitoROS, mitochondrial reactive oxygen species; NADH, reduced form of nicotinamide adenine dinucleotide; PKC, protein kinase C; PLD, phospholipase D.