Phospholemman Phosphorylation Mediates the Protein Kinase C–Dependent Effects on Na\(^+\)/K\(^+\) Pump Function in Cardiac Myocytes

Fei Han, Julie Bossuyt, Sanda Despa, Amy L. Tucker, Donald M. Bers

Abstract—Because phospholemman (PLM) regulates the Na\(^+\)/K\(^+\) pump (NKA) and is a major cardiac phosphorylation target for both protein kinase A (at Ser68) and protein kinase C (PKC) (at both Ser63 and Ser68), we evaluated whether PLM mediates the PKC-dependent regulation of NKA function and protein kinase A/PKC crosstalk in ventricular myocytes. PKC was activated by PDBu (300 nmol/L), and we measured NKA-mediated [Na\(^+\)] decline (fluorescence measurements) and current (\(I_{\text{pump}}\)) (voltage clamp). In wild-type mouse myocytes, PDBu increased PLM phosphorylation at Ser63 and Ser68, \(I_{\text{pump}}\) (both at 10 and 100 nmol/L Na\(^+\) in the pipette solution) and maximal NKA-mediated Na\(^+\) extrusion rate (\(V_{\text{max}}\)) from 7.9±1.1 to 12.7±1.9 mmol/L\(^{-1}\) per minute without altering NKA affinity for internal Na\(^+\) (\(K_{\text{0.5}}\)). In PLM knockout mice, PDBu had no effect on either \(V_{\text{max}}\) or \(K_{\text{0.5}}\). After pretreatment with isoproterenol (ISO) (1 \(\mu\)mol/L), PDBu still increased the NKA \(V_{\text{max}}\) and PLM phosphorylation at Ser63 and Ser68. Conversely, after pretreatment with ISO, PDBu further increased the Na\(^+\) affinity of NKA and phosphorylation at Ser68, as it did alone without PDBu. The final NKA activity was independent of the application sequence. The NKA activity in PLM knockout myocytes, after normalizing the protein level, was similar to that after PDBu and ISO treatment. We conclude that (1) PLM mediates the PKC-dependent activation of NKA function in cardiac myocytes, (2) PDBu and ISO effects are additive in the mouse (affecting mainly \(V_{\text{max}}\) and \(K_{\text{0.5}}\), respectively), and (3) PDBu and ISO combine to activate NKA in wild-type to the level found in the PLM knockout mouse. 

Key Words: Na\(^+\) pump ■ phospholemman ■ PKA ■ PKC

The autonomic nervous system is an important modulator of heart function. The sympathetic transmitters, epinephrine and norepinephrine, bind to and stimulate adrenergic receptors in the membrane of cardiac myocytes. Stimulation of \(\beta\)-adrenergic receptors (\(\beta\)-ARs) activates protein kinase A (PKA) via adenylyl cyclase–mediated increase in cAMP concentration. The signaling pathway initiated by stimulation of \(\alpha\)-ARs involves activation of coupled G proteins (G\(\alpha\)), which in turn cause the activation of phospholipase C (PLC). This leads to the formation of diacylglycerol and activation of protein kinase C (PKC).

The Na\(^+\)/K\(^+\) pump (NKA) is the main pathway for Na\(^+\) extrusion from the cells and therefore plays an essential role in the regulation of intracellular Na\(^+\) concentration ([Na\(^+\)]), which, via the Na\(^+\)/Ca\(^{2+}\) exchanger, is essential in controlling intracellular Ca\(^{2+}\) and contractility in the heart. There is evidence that sympathetic transmitters can adjust the activity of the NKA to the functional demands of the heart (reviewed previously\(^{1,2}\)). We have shown recently\(^{3}\) that \(\beta\)-AR stimulation enhances the NKA activity in mouse ventricular myocytes and the effect is mediated primarily by phosphorylation of phospholemman (PLM), a small transmembrane protein. PLM belongs to a family of proteins (FXYD gene family) that bind to and regulate the NKA in various tissues.\(^{4-7}\) PLM is the only FXYD protein present in cardiac myocytes, where it is a major substrate for both PKA- (at site Ser68) and PKC-dependent (at sites Ser63 and Ser68) phosphorylation.\(^{8,9}\) \(\alpha\)-AR stimulation and PKC have been shown to activate the NKA in myocytes from rat and guinea pig,\(^{10-13}\) but the mechanism involved is unclear. Thus, the first aim of this report was to investigate whether PKC-dependent regulation of NKA function is also mediated by PLM. We combined measurements of NKA-mediated [Na\(^+\)] decline and NKA current (\(I_{\text{pump}}\)) to determine the effect of PKC activation by PDBu (300 nmol/L) on NKA function in ventricular myocytes from mice in which the PLM gene was targeted\(^9\) (PLM knockout [PLM-KO]) and wild-type (WT) littermates.

Both PKA and PKC are activated during sympathetic stimulation of the heart. However, a potential crosstalk between PKA- and PKC-dependent regulations of the NKA has not been studied widely. One study found that in guinea pig ventricular myocytes the effects of PKA and PKC...
stimulation on $I_{\text{pump}}$ were additive and the basal activity of each kinase was involved in the modulation of the pump.\textsuperscript{10} It is however not known how the signaling pathways for the 2 kinases interact in regulating the NKA. Because PLM modulates the activity of the NKA and can be phosphorylated by both PKA and PKC at Ser68 and by PKC alone at Ser63, it may integrate the PKA- and PKC-dependent effects on the NKA function. Therefore, the second aim of this report was to test whether the sequential effects of PKA and PKC on the NKA are additive, synergistic, or antagonistic and whether crosstalk. We determined the effect of successive application of isoproterenol (ISO) (PKA activation) and ISO plus PDBu (PKC activation with PKA still fully activated by ISO) and vice versa on the NKA function and PLM phosphorylation at Ser63 and Ser68 in mouse ventricular myocytes.

### Materials and Methods

#### Generation of PLM-KO mice

PLM-KO mice were generated as previously described,\textsuperscript{14} except that they are now congenic on a pure C57B/6 background. Heterozygous breeding pairs were used to generate PLM-KO and WT littermates. Mice of 3 to 4 months of age were used, before PLM-KO mice developed hypertrophy.\textsuperscript{14} All animal protocols were approved by the animal care and use committees at Loyola University Chicago and University of Virginia.

#### Myocyte Isolation

Mouse ventricular myocytes were isolated as previously described.\textsuperscript{15} Briefly, hearts were excised quickly after mice were anesthetized (inhalation of 5% isoflurane) and mounted on a gravity-driven Langendorff perfusion apparatus. Hearts were perfused for 8 minutes (inhalation of 5% isoflurane) and mounted on a gravity-driven Langendorff perfusion apparatus. Hearts were perfused for 8 minutes at 37°C with nominally Ca-free solution with 24 mmol/L NaHCO\textsubscript{3} bubbled with 95% O\textsubscript{2}:5% CO\textsubscript{2} (pH 7.4). Perfusion was then switched to DMEM solution containing 1.27 mg/mL collagenase (type B, Sigma). When the heart became flaccid (~6 to 8 minutes), ventricular tissue was removed, dispersed, and filtered, and myocytes suspensions were rinsed several times.

#### Intracellular [Na\textsuperscript{+}] Measurements in Intact Myocytes

Isolated myocytes were plated on laminin-coated coverslips and incubated with 10 μmol/L sodium-binding benzofuran isophthalate/acetoxymethyl ester (SBFI-AM) in the presence of Pluronic F-127 (0.05% wt/vol) for 90 minutes at room temperature. After washing out the external dye, SBFI-AM was allowed to further desensitize for 20 minutes. The normal Tyrode’s solution contained (in mmol/L): 140 NaCl, 4 KCl, 1 MgCl\textsubscript{2}, 1 CaCl\textsubscript{2}, 10 HEPES, and 10 glucose (pH 7.4). Perfusion was then switched to DMEM solution containing 1.27 mg/mL collagenase (type B, Sigma). When the heart became flaccid (~6 to 8 minutes), ventricular tissue was removed, dispersed, and filtered, and myocytes suspensions were rinsed several times.

#### Na\textsuperscript{+} Efflux Through the NKA

NKA flux was determined as the rate of pump-mediated [Na\textsuperscript{+}], decline.\textsuperscript{16} Myocytes were Na\textsuperscript{+} loaded by inhibiting the NKA in a K-free solution containing (in mmol/L): 145 NaCl, 2 EGTA, 10 HEPES, and 10 glucose (pH 7.4). [Na\textsuperscript{+}], decline was measured on pump reactivation in solution containing (in mmol/L): 140 tetraethylammonium chloride, 4 KCl, 1 MgCl\textsubscript{2}, 2 EGTA, 10 HEPES, and 10 glucose (pH 7.4). Because cell volume does not change with this solution (described in Silverman et al\textsuperscript{20}) were used to detect phosphorylation at site Ser63 and Ser68 respectively (CP-63 and CP-68 antibody). Blots were developed using enhanced chemiluminescence (Pierce supersignal west dura substrate). Signals were recorded with an UVP EpiChemi II darkroom imaging system and quantitated with ImageJ software (NIH). Signals were normalized to control sample signals on the same gel and equal protein loading was ensured by reprobing with GAPDH.

#### Statistical Analysis

Data are expressed as mean±SEM. Statistical discriminations were performed with Student’s t test (paired when appropriate) with P<0.05 considered significant (*).

### Results

#### Effect of PDBu on NKA-Mediated Na\textsuperscript{+} Efflux in Myocytes From WT and PLM-KO Mice

NKA flux was determined as the rate of pump-mediated [Na\textsuperscript{+}], decline as previously described.\textsuperscript{3,16} Myocytes were Na\textsuperscript{+} loaded by inhibiting the NKA in a K-free solution (Figure 1A). Then the pump was reactivated with K (4 mmol/L, in the absence of external Na\textsuperscript{+}), and [Na\textsuperscript{+}],...
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1.9 mmol/L. PDBu did not significantly change either Vmax (9.0 ± 0.7 mmol/L, 8 cells, 4 hearts) on NKA activation with 4 mmol/L external K in the continuous presence of PDBu. Because PDBu enhances Na+ influx (see below), the initial [Na+] on NKA activation was often different under control and PDBu conditions. However, this does not affect the I$_{\text{pump}}$ versus [Na+] plot (Figure 3C and 3F) because the plots for 2 consecutive control runs with different initial [Na+] were superimposable (not shown).

Figure 3A through 3C shows a representative experiment in myocytes from WT mouse. The plot of I$_{\text{pump}}$ versus [Na+] (Figure 3C) emphasizes that I$_{\text{pump}}$ was significantly higher in the presence of PDBu for a given [Na+], (0.33 ± 0.04 versus 0.45 ± 0.07 pA/pF at [Na+] = 12.3 ± 0.5 mmol/L, 8 cells, 4 hearts; Figure 3C, inset). In contrast, PDBu had no effect on the [Na+] dependence of I$_{\text{pump}}$ in myocytes from PLM-KO mouse (Figure 3D through 3F; 0.35 ± 0.05 versus 0.36 ± 0.05 pA/pF at [Na+] = 12.4 ± 0.6 mmol/L, 9 cells, 4 hearts, Figure 3F, inset). Thus, I$_{\text{pump}}$ data are consistent with a PLM-dependent stimulation of NKA function by PDBu. This further confirms that PDBu stimulation of NKA is mediated primarily by PLM.

Effect of PDBu on I$_{\text{pump}}$ in Myocytes From WT and PLM-KO Mice

We determined the effect of PKC stimulation on I$_{\text{pump}}$ by simultaneously monitoring I$_{\text{pump}}$ and [Na+], under voltage-clamp conditions at a range of relevant [Na+]. For these experiments, we used relatively small patch pipettes (initial resistance was 3 to 5 MΩ, resulting in a series resistance ≥10 MΩ) and larger cells in an attempt to allow the transsarcolemmal Na+ fluxes, rather than the pipette solution to mainly control [Na+]. This way, we could measure I$_{\text{pump}}$ over a range of [Na+], (Figure 3). After reaching the whole-cell configuration (allowing the fluorescent indicator to diffuse into the cell), NKA was inhibited for 5 to 10 minutes in an external K-free solution. This resulted in Na+-loading of the myocyte. Then, the pump was abruptly re-activated by restoring external K (4 mmol/L) while measuring membrane current (I$_{\text{pump}}$, Figure 3A and 3D) and [Na+], (Figure 3B and 3E). Pump reactivation resulted in a rapid activation of outward I$_{\text{pump}}$ followed by a decay (Figure 3A and 3D), which accompanied the decline in [Na+], driven by pump function (Figure 3B and 3E). Then, the myocyte was Na+ loaded again in the presence of 300 mmol/L PDBu, followed by measurement of I$_{\text{pump}}$ and [Na+] on NKA activation with 4 mmol/L external K in the continuous presence of PDBu. Because PDBu enhances Na+ influx (see below), the initial [Na+] on NKA activation was often different under control and PDBu conditions. However, this does not affect the I$_{\text{pump}}$ versus [Na+] plot (Figure 3C and 3F) because the plots for 2 consecutive control runs with different initial [Na+], were superimposable (not shown).

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Immunoblots with phosphorylation-specific antibodies (Figure 2B) show a time-dependent increase in PLM phosphorylation at Ser68 and Ser63 in myocytes exposed to PDBu for up to 20 minutes. No PLM was detected in PLM-KO mouse, as indicated by our previous results.3

Effect of PDBu on I$_{\text{pump}}$ in Myocytes From WT and PLM-KO Mice

We determined the effect of PKC stimulation on I$_{\text{pump}}$ by simultaneously monitoring I$_{\text{pump}}$ and [Na+], under voltage-clamp conditions at a range of relevant [Na+],. For these experiments, we used relatively small patch pipettes (initial resistance was 3 to 5 MΩ, resulting in a series resistance ≥10 MΩ) and larger cells in an attempt to allow the transsarcolemmal Na+ fluxes, rather than the pipette solution to mainly control [Na+]. This way, we could measure I$_{\text{pump}}$ over a range of [Na+], (Figure 3). After reaching the whole-cell configuration (allowing the fluorescent indicator to diffuse into the cell), NKA was inhibited for 5 to 10 minutes in an external K-free solution. This resulted in Na+-loading of the myocyte. Then, the pump was abruptly re-activated by restoring external K (4 mmol/L) while measuring membrane current (I$_{\text{pump}}$, Figure 3A and 3D) and [Na+], (Figure 3B and 3E). Pump reactivation resulted in a rapid activation of outward I$_{\text{pump}}$ followed by a decay (Figure 3A and 3D), which accompanied the decline in [Na+], driven by pump function (Figure 3B and 3E). Then, the myocyte was Na+ loaded again in the presence of 300 mmol/L PDBu, followed by measurement of I$_{\text{pump}}$ and [Na+] on NKA activation with 4 mmol/L external K in the continuous presence of PDBu. Because PDBu enhances Na+ influx (see below), the initial [Na+] on NKA activation was often different under control and PDBu conditions. However, this does not affect the I$_{\text{pump}}$ versus [Na+] plot (Figure 3C and 3F) because the plots for 2 consecutive control runs with different initial [Na+], were superimposable (not shown).

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Crosstalk Between PKC- and PKA-Induced NKA Stimulation: Role of PLM

We previously showed\(^3\) that β-AR stimulation with ISO (1 μmol/L) activates NKA in WT mouse myocytes mainly by increasing its affinity for internal Na\(^+\) and has no effect in PLM-KO mouse. To investigate a possible crosstalk between PKC- and PKA-dependent regulations of the NKA, we determined the effect of PDBu on \(-d[\text{Na}^+]_i/dt\) in myocytes that were pretreated with ISO (Figure 5A). PDBu still increased the \(V_{\text{max}}\) of NKA-mediated Na\(^+\) extrusion to the same level as in the absence of ISO (\(V_{\text{max}}\) increased from 7.6±0.5 mmol/L\(^{-1}\) per minute with ISO alone to 13.6±0.9 mmol/L\(^{-1}\) per minute with ISO plus PDBu) without a significant change in \(K_{\text{i,Na}}\) (12.0±0.9 versus 10.6±1.1 mmol/L).

Figure 5B shows the converse experiment where after PDBu exposure, ISO further stimulated the NKA function by significantly enhancing the affinity for Na\(^+\) (\(K_{\text{i,Na}}\) decreased from 17.4±1.4 to 11.2±1.2 mmol/L) without further changes in \(V_{\text{max}}\) (11.3±0.6 versus 12.3±0.8 mmol/L\(^{-1}\) per minute). Notably, application of ISO and PDBu resulted in a similar level of NKA stimulation regardless of the application sequence.

The different target of NKA modulation by PKA (\(K_{\text{i,Na}}\)) and PKC (\(V_{\text{max}}\)) activation may be attributable to PLM phosphorylation at different sites: PKA phosphorylates PLM at Ser68, whereas PKC phosphorylates at both Ser68 and Ser63. Figure 5C shows immunoblots with phospho-specific PLM antibodies. ISO increased phosphorylation at Ser68 (2-fold), but not Ser63, whereas PDBu enhanced phosphorylation at both sites (nearly 4-fold at Ser68 and 2-fold at Ser63).

PDBu application after ISO (10 minutes each) increased the Ser63 and Ser68 phosphorylation substantially (Figure 5C). When ISO was applied after PDBu there was further increase in phosphorylation at Ser68. Thus, prior activation of PKA or PKC did not prevent further PLM phosphorylation and stimulation of the NKA by the other kinase. Moreover, dual exposure to PDBu and ISO either simultaneously (for 10 or 20 minutes) or sequentially in either order (for 10 minutes each) raised PLM phosphorylation at both sites to similar apparent maxima (Figure 5C). Functionally, this resulted in a similar level of NKA stimulation.

Effect of PDBu on Resting [Na\(^+\)], in Myocytes From WT and PLM-KO Mice

We previously showed that PKA-dependent stimulation of NKA in WT myocytes caused a decline in resting [Na\(^+\)], but no effect in PLM-KO myocytes.\(^3\) Figure 6 shows the effect of PDBu on [Na\(^+\)], in WT and PLM-KO myocytes. In WT, PDBu did not significantly alter resting [Na\(^+\)], (Figure 6B; 9 cells from 3 hearts). However, in PLM-KO myocytes PDBu increased [Na\(^+\)], by 2.4±0.6 mmol/L (Figure 6B; 13 cells from 3 hearts). Because PKC activation can stimulate the Na\(^+/\)H\(^+\) exchange,\(^{21,22}\) PDBu may enhance Na\(^+\) influx, independent of PLM or NKA. This may explain why PDBu increased resting [Na\(^+\)], in PLM-KO myocytes, despite unaltered NKA function (Figure 2A).

We further tested whether PDBu alters Na\(^+\) influx by measuring the rate of Na\(^+\) rise on NKA inhibition (as in...
PDBu enhanced Na\(^+\) influx rate in both WT and PLM-KO myocytes (Figure 6C, upper bars) at 12 mmol/L [Na\(^+\)], resting [Na\(^+\)], in both WT and PLM-KO mice\(^3\). We compared this to the PDBu-induced increase in Na\(^+\) efflux, based on NKA function curves in Figure 2A (also at 12 mmol/L [Na\(^+\)]; Figure 6C, lower bars). For WT myocytes, the PDBu-induced increase in Na\(^+\) influx (2.9 ± 1.1 mmol L\(^{-1}\) min\(^{-1}\)) was nearly counter-balanced by the enhancement of NKA activity (2.5 ± 1.2 mmol L\(^{-1}\) per minute at 12 mmol/L), explaining the unaltered [Na\(^+\)] in WT myocyte on PDBu exposure. In PLM-KO mice, the PDBu-induced increase in Na\(^+\) influx exceeds the negligible effect on NKA function, resulting in increased [Na\(^+\)], (as in Figure 6A). Thus, PKC-dependent NKA activation may compensate for PKC-dependent stimulation of Na\(^+\) influx.

**Discussion**

The present study shows that PKC stimulates the NKA in mouse ventricular myocytes by increasing its V\(_{\text{max}}\) with no significant effect on the affinity for internal Na\(^+\). The absence of NKA stimulation in PLM-KO mouse suggests that PKC-dependent effects on the pump are mediated primarily by PLM, rather than direct NKA phosphorylation. Additionally, PKC and PKA appear to have additive effects on NKA function.

Regulation of the NKA by PKC in Mouse Ventricular Myocytes: Role of PLM

There is some consensus that α-AR or PKC activation enhance the activity of the NKA,\(^10\) but the mechanism responsible is still unknown. PKC can directly phosphorylate the α subunit of the NKA in intact rat cells, but this does not seem to affect either the V\(_{\text{max}}\) or the apparent Na\(^+\) affinity of the pump.\(^23\) An alternative mechanism involves phosphorylation of a NKA regulatory protein. We and others have shown previously that PLM, a member of the FXYD family of proteins that associate with and modulates the NKA in various tissues, coimmunoprecipitates with the α subunit of the NKA.\(^3\)–\(^5\),\(^7\),\(^19\),\(^20\),\(^24\) Moreover, we demonstrated that PLM inhibits the NKA and this inhibition is relieved by PLM phosphorylation on ISO stimulation.\(^3\) β-AR stimulation of the NKA was mediated primarily by PLM phosphorylation, as no effect was observed in the PLM-KO mice.\(^3\) Studies on shark-like PLM (PLMS) indicated that phosphorylation of PLMS by PKC or selective proteolysis of the C terminus increase the NKA activity.\(^25\),\(^26\) Here we show that PKC activation with PDBu stimulates the NKA in intact and voltage-clamped mouse ventricular myocytes and the effect is absent in cells from PLM-KO mice. This suggests that PKC-dependent effects on the NKA are also mediated primarily by PLM.

**Figure 3.** Simultaneous I\(_{\text{pump}}\) and [Na\(^+\)] measurements in myocytes from WT (A through C) and PLM-KO (D through F) mice. A and D, I\(_{\text{pump}}\) on abrupt NKA reactivation after a period of pump blockade with and without 300 nmol/L PDBu; PDBu was applied 3 to 5 minutes before activating the pump and then throughout measuring the NKA function. B and E, Time course of [Na\(^+\)]. C and F, [Na\(^+\)], dependence of I\(_{\text{pump}}\) using simultaneous measurements of both. Data are representative of 8 cells from 4 WT hearts and 9 cells from 4 PLM-KO hearts. Insets in C and F are mean values of I\(_{\text{pump}}\) at 13 mmol/L Na\(^+\) (or closest [Na\(^+\)]) applicable. Dashed vertical line (in C and F) indicates the [Na\(^+\)], at which I\(_{\text{pump}}\) was compared. **P<0.01 (PDBu vs control; paired).
Our data indicate that PKC stimulates the NKA in mouse ventricular myocytes by increasing its maximum Na\(^+\)/H\(^+\) extrusion rate with no effect on the affinity for intracellular Na\(^+\)/H\(^+\). We found a 60% increase in V\(_{\text{max}}\) following PDBu treatment in intact cells (d[Na\(^+\)]/dt experiments). The effect was reduced, but still significant, in ruptured-patch experiments. V\(_{\text{max}}\) increased by 30% when using small pipette (3 to 5 M\(\Omega\), series resistance \(\geq\)10 M\(\Omega\); experiments as in Figure 3) and by only 10% with larger pipettes used to optimize dialysis with high [Na\(^+\)]; (<2 M\(\Omega\), series resistance <5 M\(\Omega\); experiments as in Figure 4). This reduction in the PKC effect may be caused by dialysis of PKC in the voltage-clamp experiments. Indeed, immunoblots showed that PLM phosphorylation by both PDBu and ISO was approximately 50% lower at both Ser68 and Ser63 in myocytes permeabilized with saponin compared with intact myocytes (not shown). The rise in V\(_{\text{max}}\) found here is in agreement with data from rat and guinea pig that show a higher I\(_{\text{pump}}\) at an internal Na\(^+\) that likely saturates the pump.\(^{10-13}\) However, prior data on PKC effects on NKA \(K_{0.5}\) are lacking.

We found previously that NKA activation by ISO in mouse is mainly attributable to an increase in the affinity of the pump for internal Na\(^+\). However, we could not rule out an effect of ISO on V\(_{\text{max}}\), which may or may not be mediated by PLM. Both here and in our previous study,\(^3\) V\(_{\text{max}}\) under control conditions was 10% to 20% higher in myocytes from PLM-KO versus WT mice, but the difference was not significant. However, the NKA expression level is 20% to 25% lower in PLM-KO myocytes.\(^3\) Thus, when normalizing to the protein level, V\(_{\text{max}}\) in PLM-KO mice is \(\approx 50\%\) higher than in WT, which compares well to the rise in V\(_{\text{max}}\) induced by PDBu in intact WT myocytes. Thus, it is likely that PLM inhibits the NKA by reducing both its V\(_{\text{max}}\) and the affinity for intracellular Na\(^+\). Whether the relief of inhibition on PLM phosphorylation occurs through V\(_{\text{max}}\), \(K_{0.5}\) or both may be a
Physiological Context of PKC Activation of NKA
PKC activation in response to α-AR activation stimulates the Na'/H exchanger to extrude protons in exchange for Na⁺. As a result, both intracellular pH and [Na⁺], will rise,21,22 which leads to an increase in the myofilament Ca²⁺ sensitivity and Ca²⁺ transients via Na⁺/Ca²⁺ exchange (NCX), respectively. Action potential duration is typically increased by α-AR activation in parallel to the positive inotropic effect,29 which may be attributable primarily to decreased K currents. The action potential prolongation seen in most myocytes would tend to increase Ca²⁺ influx (by Iₛᵣ and possibly NCX) and also decrease Ca²⁺ efflux via NCX (allowing greater SR Ca²⁺ uptake). This could increase the likelihood of triggered arrhythmias. Enhancement of NKA activity may thus play a role in the sympathetic response of the heart to enhance Na⁺ extrusion, which would offset the higher level of Na⁺ influx (and Ca²⁺ through NCX). Our data in resting [Na⁺], in response to PKC activation fit into this notion that NKA stimulation balances [Na⁺], influx in resting myocytes from WT but not PLM-KO mice (Figure 6). PLM may also directly affect NCX function30,31 and PKC activators regulate NCX,32 which may also alter Na⁺ influx during PDBu application.

In summary, we have shown that (1) PKC-dependent stimulation of the NKA in mouse ventricular myocytes is mediated primarily by PLM phosphorylation and enhancement of Vₘₐₓ, (2) both PKA and PKC are needed to fully activate the NKA, and (3) PKC- and PKA-induced stimulation of the NKA (by enhancing Vₘₐₓ and Na⁺ affinity, respectively) is relatively independent of the other kinase in mouse ventricular myocytes.

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Disclosures
None.

References


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