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Phospholemman-Mediated Activation of Na/K-ATPase Limits $[Na]_i$ and Inotropic State During β-Adrenergic Stimulation in Mouse Ventricular Myocytes

Sanda Despa, PhD, **Amy L. Tucker, MD**, and **Donald M. Bers, PhD**
From the Department of Physiology, Loyola University Chicago, Maywood, III (S.D., D.M.B.), and Cardiovascular Division, University of Virginia, Charlottesville (A.L.T.).

Abstract

Background—Cardiac Na/K-ATPase (NKA) regulates intracellular Na ($[Na]_i$), which in turn affects intracellular Ca and thus contractility via Na/Ca exchange. Recent evidence shows that phosphorylation of the NKA-associated small transmembrane protein phospholemman (PLM) mediates β-adrenergic-induced NKA stimulation.

Methods and Results—Here, we tested whether PLM phosphorylation during β-adrenergic activation limits the rise in $[Na]_i$, Ca transient amplitude, and triggered arrhythmias in mouse ventricular myocytes. In myocytes from wild-type (WT) mice, $[Na]_i$ increased on field stimulation at 2 Hz from 11.1±1.8 mmol/L to a plateau of 15.2±1.5 mmol/L. Isoproterenol induced a decrease in $[Na]_i$ to 12.0±1.2 mmol/L. In PLM knockout (PLM-KO) mice in which β-adrenergic stimulation does not activate NKA, $[Na]_i$ also increased at 2 Hz (from 10.4±1.2 to 17.0±1.5 mmol/L) but was unaltered by isoproterenol. The PLM-mediated decrease in $[Na]_i$ in WT mice could limit the isoproterenol-induced inotropic state. Indeed, the isoproterenol-induced increase in the amplitude of Ca transients was significantly smaller in the WT mice (5.2±0.4- versus 7.1±0.5-fold in PLM-KO mice). This also was the case for the sarcoplasmic reticulum Ca content, which increased by 1.27 ±0.09-fold in WT mice versus 1.53±0.09-fold in PLM-KO mice. The higher sarcoplasmic reticulum Ca content in PLM-KO versus WT mice was associated with an increased propensity for spontaneous Ca transients and contractions in PLM-KO mice.

Conclusions—These data suggest that PLM phosphorylation and NKA stimulation are an integral part of the sympathetic fight-or-flight response, tempering the rise in $[Na]_i$ and cellular Ca loading and perhaps limiting Ca overload–induced arrhythmias.

Keywords

arrhythmia; calcium; sodium

In the heart, intracellular Na concentration ($[Na]_i$) affects excitation-contraction coupling by modulating the intracellular pH and Ca through Na/H exchange and Na/Ca exchange (NCX), respectively. $[Na]_i$ is determined by a fine balance between Na influx and efflux. There are many Na entry pathways, including NCX, Na channels, and Na/H exchange, whereas the Na/

Correspondence to Donald M. Bers, PhD, Department of Pharmacology, University of California at Davis, Genome Building 3511, Davis, CA 95616-8636. dmbers@ucdavis.edu.

Disclosures
None.

K-ATPase (NKA) is the main route for Na extrusion and therefore is essential in $[Na]_i$ regulation.

A family of proteins, FXYD (FXYD-1 to FXYD-10, named for a conserved Pro-Phe-X-Tyr-Asp motif1), has emerged recently as tissue-specific regulators of NKA. Members of the FXYD family, including phospholemman (PLM) and the NKA γ subunit, are small single-membrane-spanning proteins. PLM is the only FXYD protein that is highly expressed in the heart and is unique within the family in that it has multiple phosphorylation sites at its cytosolic carboxy-terminus. In the heart, PLM phosphorylation by the cAMP-dependent protein kinase A (PKA) during β-adrenergic receptor (β-AR) stimulation is quantitatively comparable to that of troponin I and phospholamban.2,3 PLM has been shown to associate with and modulate NKA activity.4–9

Using isolated myocytes from PLM-KO versus wild-type (WT) mice, we have recently shown that PLM inhibits NKA by reducing its affinity for intracellular Na.[10] In WT mice, activation of β-AR with 1 μmol/L isoproterenol resulted in phosphorylation of PLM at Ser-68 and stimulated NKA by inducing a leftward shift in the Na activation curve. With isoproterenol, the apparent $K_{1/2}$ for internal Na in myocytes from WT mice became comparable to that found in PLM-KO mice. Moreover, isoproterenol had no significant effect on the Na/K pump function in myocytes from PLM-KO mice. These data indicate that unphosphorylated PLM inhibits NKA, mostly by reducing its affinity for internal Na, whereas PLM phosphorylation enhances NKA activity by relieving this inhibition, a mechanism similar to the way phospholamban modulates sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), a close relative of NKA.

The aim of this study was to investigate whether the activation of NKA, mediated by PLM phosphorylation, plays an integral role in the adrenergic fight-or-flight response. Stimulation of the β-ARs results in larger Ca transients and thus increased inotropy.11 The larger Ca transients drive greater Ca extrusion, and thus greater Na influx, via NCX at each beat. β-AR stimulation also accelerates the heart rate; thus, there is more frequent Na current via Na channels (which also may be larger as a result of phosphorylation by PKA12) and more frequent Ca transient–driven Na entry via NCX. Combined, the inotropic and chronotropic effects of β-AR stimulation may lead to a large increase in Na influx into cardiac myocytes and thus an increase in $[Na]_i$. The rise in $[Na]_i$ may add another component to the inotropic effect of β-AR stimulation13,14 by favoring more Ca influx and limiting Ca efflux through NCX (mechanistically analogous to cardiac glycoside–induced inotropy). Excessive cellular Na and Ca loading also can lead to spontaneous sarcoplasmic reticulum (SR) Ca release and arrhythmias.[11] PLM phosphorylation and the subsequent enhancement of NKA activity may limit the $[Na]_i$ increase and thus limit the inotropic state and arrhythmogenesis. These are the 2 working hypotheses that we test here: that isoproterenol will induce a greater rise in $[Na]_i$ in myocytes from PLM knockout (PLM-KO) mice (in which it does not stimulate NKA) compared with WT mice and that, because of this, isoproterenol will have a greater inotropic effect in PLM-KO than in WT mice.

Methods

Generation of PLM-KO Mice and Myocyte Isolation

PLM-KO mice were generated as previously described[15] except that they are now congenic on a pure C57B/6 background. Heterozygous breeding pairs were used to generate PLM-KO and WT littermates. All animal protocols were approved by the Animal Care and Use Committee at the University of Virginia or by the Loyola University Chicago Animal Welfare Committee.

Isolation of mouse ventricular myocytes was done as previously described.[16] Briefly, PLM-KO mice and age-matched WT littermates (3 to 4 months of age) were anesthetized in a gas chamber with 3% to 5% isoflurane (100% O_2). Hearts were excised quickly and mounted on a gravity-driven Langendorff perfusion apparatus. Hearts were digested by perfusion with 0.8 mg/mL collagenase (type B, Boehringer Mannheim, Indianapolis, Ind). When the heart became flaccid (7 to 12 minutes), ventricular tissue was removed, dispersed, and filtered, and myocytes suspensions were rinsed several times.

Intracellular [Na] and [Ca] Measurements in Intact Myocytes

Isolated myocytes were plated on laminin-coated coverslips and loaded with SBFI-AM (10 μmol/L for 90 to 120 minutes) as previously described[17] for $[Na]_i$ measurements or Fluo-3AM (10 μmol/L for 40 minutes) for $[Ca]_i$ measurements. Both indicators (from Molecular Probes, Eugene, Ore) were allowed to de-esterify for 20 minutes in normal Tyrode's solution containing (mmol/L) 140 NaCl, 4 KCl, 1 $MgCl_2$, 10 glucose, 5 HEPES, and 1 $CaCl_2$ (pH 7.4). SBFI was excited alternately at 340 and 380 nm (F_{340} and F_{380}) with an Optoscan monochromator (Cairn Research, Faversham, UK), and fluorescence was collected at 535±20 nm. The SBFI fluorescence ratio (F_{340}/F_{380}) was calculated after background subtraction and converted to $[Na]_i$ by calibration at the end of each experiment in the presence of 10 μmol/L gramicidin and 100 μmol/L strophanthidin[17] (Sigma, St Louis, Mo). Fluo-3 was excited at 480 nm, and fluorescence was measured at 535±20 nm. All measurements were at room temperature.

Statistical Analysis

Data are expressed as mean±SEM. Statistical comparisons between groups were performed with Student's *t* test (paired when appropriate) with values of $P<0.05$ considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Effect of Isoproterenol on $[Na]_i$ in Myocytes From WT and PLM-KO Mice

Na influx into cardiac myocytes is greatly enhanced during sympathetic activation of the heart because of the combined inotropic and chronotropic effects of physiological β-AR activation. This is expected to lead to an increase in $[Na]_i$. Stimulation of NKA via PLM phosphorylation[10] may limit this rise in $[Na]_i$. To investigate this, we measured the effect of 1 μmol/L isoproterenol on $[Na]_i$ in contracting myocytes from WT and PLM-KO mice using SBFI (Figure 1). The SBFI ratio was converted to $[Na]_i$ on the basis of a 3-point calibration that was done at the end of each experiment (Figure 1A). We first measured $[Na]_i$ at rest, and then myocytes were paced at 2 Hz (Figure 1B), a rudimentary simulation of the pure chronotropic effect of β-AR stimulation in vivo. As expected, $[Na]_i$ increased on pacing in myocytes from both WT and PLM-KO mice. After 7 to 8 minutes, when $[Na]_i$ reached a new steady state, we applied isoproterenol. With isoproterenol, $[Na]_i$ decreased markedly in the WT mice, in which isoproterenol enhances the NKA activity to extrude more Na,[10] and did not change significantly in the PLM-KO mice, in which NKA is not affected by isoproterenol[10] (Figure 1B). Mean data from these experiments (Figure 1C) show that resting $[Na]_i$ is similar in myocytes from PLM-KO and WT mice (10.4±1.2 versus 11.1±1.8 mmol/L; $P>0.05$, unpaired *t* test), as we have previously reported.[10] $[Na]_i$ increased with pacing by 6.6 ±0.6 mmol/L (to a new level of 17.0± 1.5 mmol/L) and 4.1±0.8 mmol/L (to 15.2±1.5 mmol/L) in myocytes from PLM-KO and WT mice, respectively. With isoproterenol, $[Na]_i$ decreased significantly to 12.0±1.2 mmol/L ($P=0.004$, paired *t* test) in WT mice and remained high (17.3 ±1.8 mmol/L; change not significant) in PLM-KO mice. Thus, the PLM-dependent

enhancement of NKA activity limits $[Na]_i$ during β-AR activation in mouse ventricular myocytes.

Effect of Isoproterenol on Ca Transients in Myocytes From WT and PLM-KO Mice

Myocytes loaded with the Ca indicator Fluo-3 were paced at 2.0 Hz under control conditions for 7 to 8 minutes (Figure 2A). Pacing was then stopped for 5 seconds, followed by application of 10 mmol/L caffeine to empty the SR of Ca and to assess its Ca content. Pacing was restarted, and when Ca transients recovered to the precaffeine level, isoproterenol was applied for another 7 to 8 minutes. Caffeine was applied again at the end to determine the SR Ca load in the presence of isoproterenol.

Figure 2A (top) shows a typical record from a myocyte from a PLM-KO mouse. The amplitude of Ca transients increased rapidly on application of isoproterenol and reached a stable plateau in ≈3 minutes. The effect of isoproterenol was somewhat different in myocytes from WT mice (Figure 2A, bottom); in 12 of 20 cells, the Ca transient amplitude started to increase on application of isoproterenol, reached a maximum in ≈2 minutes, and then decreased to a new steady state. Individual Ca transients under control conditions, at 2 minutes after isoproterenol application, and at steady state in the presence of isoproterenol are shown in Figure 2B. We did not measure $[Na]_i$ and Ca transients simultaneously in the same cell; however, even in a comparison of Na and Ca in different cells, it is apparent that there is good temporal correlation between the isoproterenol-induced decrease in $[Na]_i$ and the decline in the Ca transient amplitude after the maximum. This is shown clearly in the mean data for WT myocytes (Figure 3A). $[Na]_i$ starts decreasing ≈2 minutes after the addition of isoproterenol and reaches a plateau ≈3 mmol/L below the preisoproterenol level. The amplitude of Ca transients increases rapidly and reaches a brief plateau. Then, as $[Na]_i$ declines, Ca transients also start to decline. In contrast, $[Na]_i$ remains practically constant, and Ca transient amplitude does not show any secondary decrease in myocytes from PLM-KO mice (Figure 3B).

The mean amplitude of Ca transients under control conditions was slightly (not significantly) larger in WT than in PLM-KO mice (F/F_0=0.98±0.08 versus 0.83±0.07; Figure 4A); the same was true for the SR Ca content (F/F_0=4.8±0.2 versus 4.4±0.2; Figure 4C). The fractional SR Ca release (twitch/caffeine-induced Ca transient amplitude) was similar (0.20±0.01 in WT versus 0.18±0.01 in PLM-KO mice). The time constant of $[Ca]_i$ decline of twitch Ca transients also was similar between the WT and PLM-KO mice (0.33±0.02 versus 0.30±0.02 seconds).

With isoproterenol, the amplitude of steady-state Ca transients was significantly larger in myocytes from PLM-KO versus WT mice (F/F_0=5.5±0.3 versus 4.7±0.3; Figure 4A). Thus, Ca transients in isoproterenol were 5.2±0.4 times larger than control in WT mice and increased by more (7.1±0.5-fold) in PLM-KO mice (Figure 4B). The greater increase in Ca transients in PLM-KO compared with WT mice was due mainly to a larger increase in the SR Ca content (1.53±0.09- versus 1.27±0.09-fold in PLM-KO versus WT mice; Figure 4D). There was no difference in the steady-state fractional SR Ca release in the presence of isoproterenol between myocytes from PLM-KO and WT mice (0.77±0.03 versus 0.74±0.03). The time constant of $[Ca]_i$ decline of twitch Ca transients also was similar between PLM-KO and WT mice (90±3 versus 97±3 ms), although the peak Ca is higher in PLM-KO mice and the Ca decline becomes faster with increased amplitude of Ca transient.18 However, the time constant versus peak [Ca] relationship tends to plateau at large [Ca] (≥1.5 μmol/L),18 and such large systolic Ca is expected with isoproterenol, which may explain why Ca transients are not significantly faster in the PLM-KO versus WT mice during isoproterenol stimulation. The time constant for $[Ca]_i$ decline during caffeine-induced Ca transients (indicative of NCX activity) decreased significantly in the presence of isoproterenol in WT myocytes (2.4±0.2 seconds in control conditions versus 2.0±0.1 seconds with isoproterenol).β-AR stimulation does not directly stimulate the Na/Ca exchanger in cardiac myocytes.19,20 Thus, the faster decay of the caffeine-

induced Ca transient in isoproterenol-treated WT myocytes is due to the lower level of $[Na]_i$ (compared with WT myocytes without isoproterenol; Figures 1C and 3A), which enhances the ability of the Na/Ca exchanger to extrude Ca.

Interestingly, if we compare Ca transients at the time of the peak in myocytes from WT mice, ie, 2 minutes after application of isoproterenol (dashed line in Figure 2A), the amplitude is similar in myocytes from WT and PLM-KO mice (Figure 4A). This further strengthens the idea that the decline in Ca transient amplitude that follows the initial increase in WT mice is due to the decline in $[Na]_i$ and the effects that this has on the Na/Ca exchanger and SR Ca content.

Spontaneous Ca Transients in Myocytes From WT and PLM-KO Mice

The elevated SR Ca content, caused by the higher $[Na]_i$, with isoproterenol in myocytes from PLM-KO versus WT mice also appeared to increase the propensity for spontaneous Ca transients and contractions (Figure 5). Figure 5A and 5B shows an example of a spontaneous Ca transient (arrow) occurring between triggered beats (2 Hz) in a myocyte from a PLM-KO mouse. The amplitude of the following triggered Ca transient is reduced, and it takes several beats for Ca transients to return to the level before the spontaneous beat (after going through an overshoot phase; Figure 5B). Such spontaneous Ca transients occurred in 6 of 19 PLM-KO cells and in 3 of 20 WT cells in the presence of isoproterenol. The 3 WT myocytes that had aftercontractions did not show the secondary decline in the amplitude of Ca transients after the initial increase with isoproterenol. No spontaneous Ca transients were observed under control conditions in either PLM-KO or WT mice. We also observed a higher incidence of spontaneous Ca waves or transients during a 5-second pause in the field stimulation (Figure 5C) in myocytes from PLM-KO versus WT mice in the presence of isoproterenol (4 of 16 versus 1 of 15 cells).

Discussion

$[Na]_i$ and Ca Transients During β-AR Stimulation in Myocytes From WT and PLM-KO Mice

We found that β-AR stimulation reduces $[Na]_i$ in isolated, field-stimulated mouse ventricular myocytes. This is most likely due to activation of the Na/K pump via PLM phosphorylation[9,10] because the effect is abolished in myocytes from PLM-KO mice in which β-AR stimulation does not significantly enhance the NKA activity.[10] This decrease in $[Na]_i$ limits the inotropic effect of β-AR stimulation, as indicated by a smaller isoproterenol-induced increase in the amplitude of Ca transients in myocytes from WT versus PLM-KO mice.

Unphosphorylated PLM inhibits NKA, mostly by reducing its affinity for internal Na.[4,10] PLM phosphorylation during β-AR stimulation relieves this inhibition, so the apparent $K_{1/2}$ for internal Na becomes similar to that found in the absence of PLM.[10] In this context, one would have expected a higher $[Na]_i$ in myocytes from WT versus PLM-KO mice under control conditions as a result of NKA inhibition by unphosphorylated PLM in the WT mice. However, this was not the case; resting $[Na]_i$ was similar in WT and PLM-KO mice. This finding might be attributable to reduced NKA expression compensating for the higher apparent Na affinity in PLM-KO mice.[10,15] Myocyte ^{3}H-ouabain binding, myocyte Western blot, and membrane fraction NKA all indicated an ≈20% downregulation of the NKA α subunit in PLM-KO mice.[10] The higher Na affinity and lower NKA expression in PLM-KO mice might offset each other so that at physiological $[Na]_i$ the NKA activity in myocytes from PLM-KO and WT mice is comparable. Our data are in agreement with this in that resting $[Na]_i$ is the same and $[Na]_i$ increases on field stimulation to comparable levels in WT and PLM-KO mice. The difference comes during β-AR stimulation, when NKA is activated to extrude more Na and thus $[Na]_i$ decreases in WT mice.mice. In the PLM-KO mice, however, there is no NKA activation, so $[Na]_i$ stays high.

During sympathetic activation, Ca influx via the larger Ca current must be balanced by the enhanced Ca extrusion via NCX that is driven by larger Ca transients. This should increase Na influx at each beat; therefore, we expected $[Na]_i$ to increase on addition of isoproterenol in field-stimulated myocytes from PLM-KO mice. However, isoproterenol did not have any significant effect on $[Na]_i$. Two factors may account for this result. First, the isoproterenol-induced rise in the integrated Ca influx via L-type Ca channels, and thus the increase in Na influx, may be limited by an increased Ca-dependent inactivation generated by the larger Ca transients. Second, we previously reported that isoproterenol results in a small (not significant) increase in the NKA V_{max} in both WT and PLM-KO mice.[10] This increase might be enough to offset the slightly higher Na influx with isoproterenol. In vivo, however, $[Na]_i$ is likely to increase because of an accelerated heart rate and thus more frequent openings of Na channels and more frequent Ca transient–driven Na entry via NCX.

In the heart, $[Na]_i$ modulates Ca transients and contractility, mainly via NCX. A decrease in $[Na]_i$ shifts the balance of fluxes on the NCX to favor more Ca efflux, resulting in lower SR Ca content and consequently smaller Ca transients and reduced contractions. Therefore, the isoproterenol-induced decrease in $[Na]_i$ should lead to smaller-amplitude Ca transients than would be observed in the absence of NKA stimulation. Indeed, we found that the steady-state Ca transients in the presence of isoproterenol had significantly lower amplitude in WT than in PLM-KO mice. Thus, the reduction in $[Na]_i$ limits the inotropic effect of β-AR stimulation in mouse ventricular myocytes.

The initial increase in the amplitude of Ca transients with isoproterenol was similar in WT and PLM-KO mice, suggesting that there are no significant differences in the β-AR stimulation of SERCA (via phospholamban phosphorylation), L-type Ca channels, and ryanodine receptors. However, in the majority of WT cells, Ca transients reached a maximum ≈2 minutes after application of isoproterenol and then decreased to a new steady state. This is in agreement with older reports showing that on exposure of Purkinje fibers to catecholamines, the twitch tension rapidly increases to a peak and then decreases, whereas the intracellular Na activity decreases.[21,22] There is good correlation between the time course of $[Na]_i$ and Ca transient amplitude declines. $[Na]_i$ started to decline only after ≈2 minutes following isoproterenol application, shortly preceding the decline in Ca transient amplitudes (Figure 3). The delay in $[Na]_i$ decline versus activation of Ca cycling (see Figure 3) might indicate that β-AR-dependent activation of the Na/K pump lags behind that of Ca cycling. This idea is supported by data indicating a longer time course for the PKA phosphorylation of PLM at Ser68[10] compared with key proteins involved in Ca cycling such as phospholamban[23] and L-type Ca channels.

PLM also may directly affect NCX.[24,25] Zhang et al[26] showed that PLM phosphorylated at Ser68, the PKA site, inhibits cardiac NCX. In the presence of isoproterenol, this would reduce both Na influx and Ca efflux in isolated myocytes from WT mice, resulting in larger Ca transients in WT versus PLM-KO mice, and the NCX inhibition should have slowed $[Ca]_i$ decline during caffeine-induced Ca transient in WT myocytes. These expectations do not fit our experimental data, which show that the amplitude of Ca transients was similar in WT and PLM-KO mice early during isoproterenol application and that isoproterenol accelerated the rate of $[Ca]_i$ decline during caffeine-induced Ca transients in WT myocytes. Thus, we believe that the effect of PLM phosphorylation on NKA activity is the main factor limiting $[Na]_i$ and Ca transients during β-AR stimulation. However, an effect of PLM on NCX may add another level of complexity to the interpretation of the data.

Physiological Role of PLM in the Heart

PLM has long been known as a major target for PKA and protein kinase C phosphorylation in the heart.[2,3] However, its physiological role is poorly understood. In lipid bilayers, PLM forms

ion channels selective for taurine,[27] and recent structural studies support this idea.[28] The selectivity to taurine suggests that PLM might be involved in cell volume regulation.[29]

Recently, it has been established that PLM regulates NKA and its phosphorylation mediates the β-AR effects on NKA.[4,9,10] Here, we show that this process limits the rise in $[Na]_i$ and Ca transient amplitude during β-AR stimulation. Enhancement of NKA activity may thus be an integral part of the sympathetic response of the heart by enhancing Na extrusion to better keep up with the higher level of Na influx (caused by the combined inotropic and chronotropic effects on the heart). The inability of NKA to be activated by β-AR stimulation in the PLM-KO mouse could lead to excessive elevation of $[Na]_i$ during sympathetic activation, which has both the benefits and the risks associated with NKA inhibition by cardiac glycosides (inotropy but enhanced arrhyth-mogenesis) as shown here. Thus, the physiological role of PLM may be to prevent Ca overload and triggered arrhythmias by limiting the rise of $[Na]_i$ during β-AR stimulation.

Conclusions

We have shown that NKA activation via PLM phosphorylation limits $[Na]_i$ and Ca transients and may reduce the propensity for triggered arrhythmias during β-AR stimulation in mouse ventricular myocytes. In this way, β-adrenergic activation of PLM is an integral part of the overall fight-or-flight response in the heart.

CLINICAL PERSPECTIVE

During activation of the sympathetic nervous system, cardiac performance is increased as part of the fight-or-flight stress response. The increase in contractility with sympathetic stimulation is an orchestrated combination of intrinsic inotropic, lusitropic, and chronotropic effects mediated in part by activation of β-adrenergic receptors and protein kinase A. This activation causes phosphorylation of several Ca cycling proteins in cardiac myocytes (increasing Ca entry via L-type Ca channels, sarcoplasmic reticulum Ca pumping, and the dissociation rate of Ca from the myofilaments). Here, we demonstrate how Na/K-pump stimulation, mediated by phospholemman phosphorylation, is an additional important player in the sympathetic fight-or-flight response. Enhancement of Na/K-ATPase activity limits the rise in intracellular Na caused by the higher level of Na influx and, by doing so, limits the rise in cellular and sarcoplasmic reticulum Ca load. Mutations that would prevent phospholemman phosphorylation and thus make Na/K-ATPase insensitive to sympathetic stimulation could lead to excessive elevation of intracellular Na during stress. Although this could enhance inotropy (like Na/K-ATPase inhibition by cardiac glycosides), it also could contribute to arrhythmogenesis (again, like glycosides) during stress or exercise when sympathetic tone is elevated.

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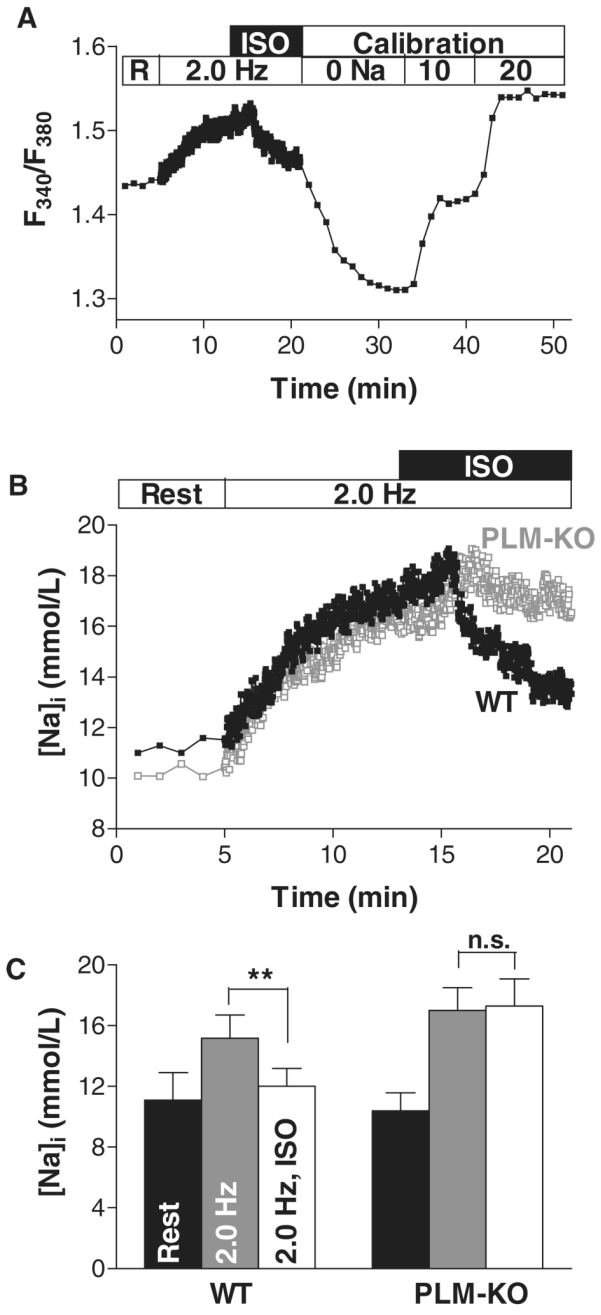

Figure 1.

Effect of pacing (2-Hz field stimulation) and isoproterenol (ISO; 1 μmol/L) on $[Na]_i$ in myocytes from WT and PLM-KO mice. A, SBFI fluorescence ratio (F_{340}/F_{380}) in a myocyte from a WT mouse. A 3-point calibration was done at the end of the experiment. B, Representative Na traces. C, Mean data for WT (7 myocytes from 4 hearts) and PLM-KO mice (9 myocytes from 5 hearts). $^{**}P<0.01$.

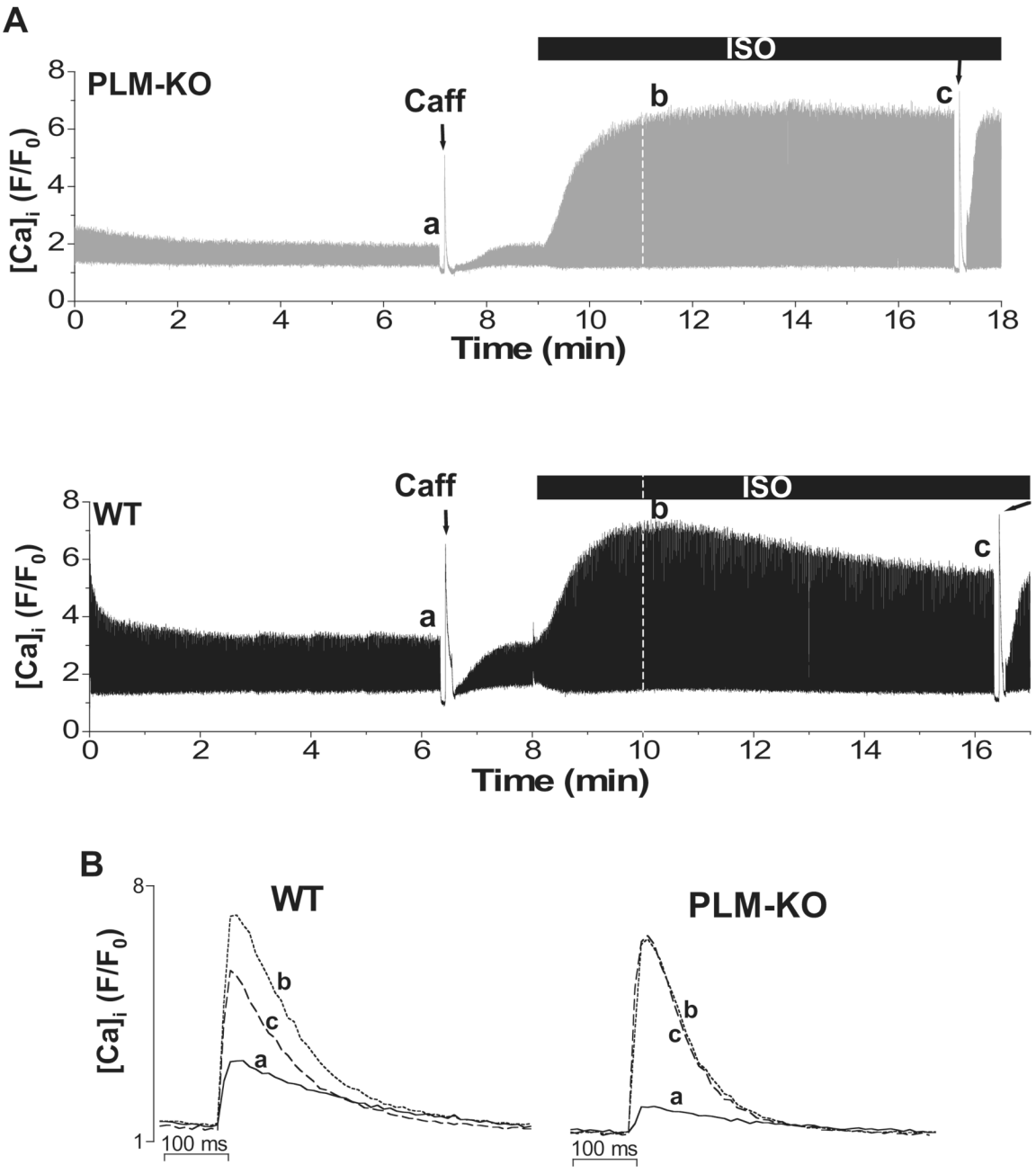

Figure 2.

Effect of isoproterenol (ISO; 1 μmol/L) on Ca transients (2-Hz stimulation) in myocytes from WT and PLM-KO mice. A, Representative experiments on a long time scale. B, Individual Ca transients under control conditions (a), early (2 minutes) during isoproterenol application (b), and at steady state in the presence of isoproterenol (c). Caff indicates caffeine.

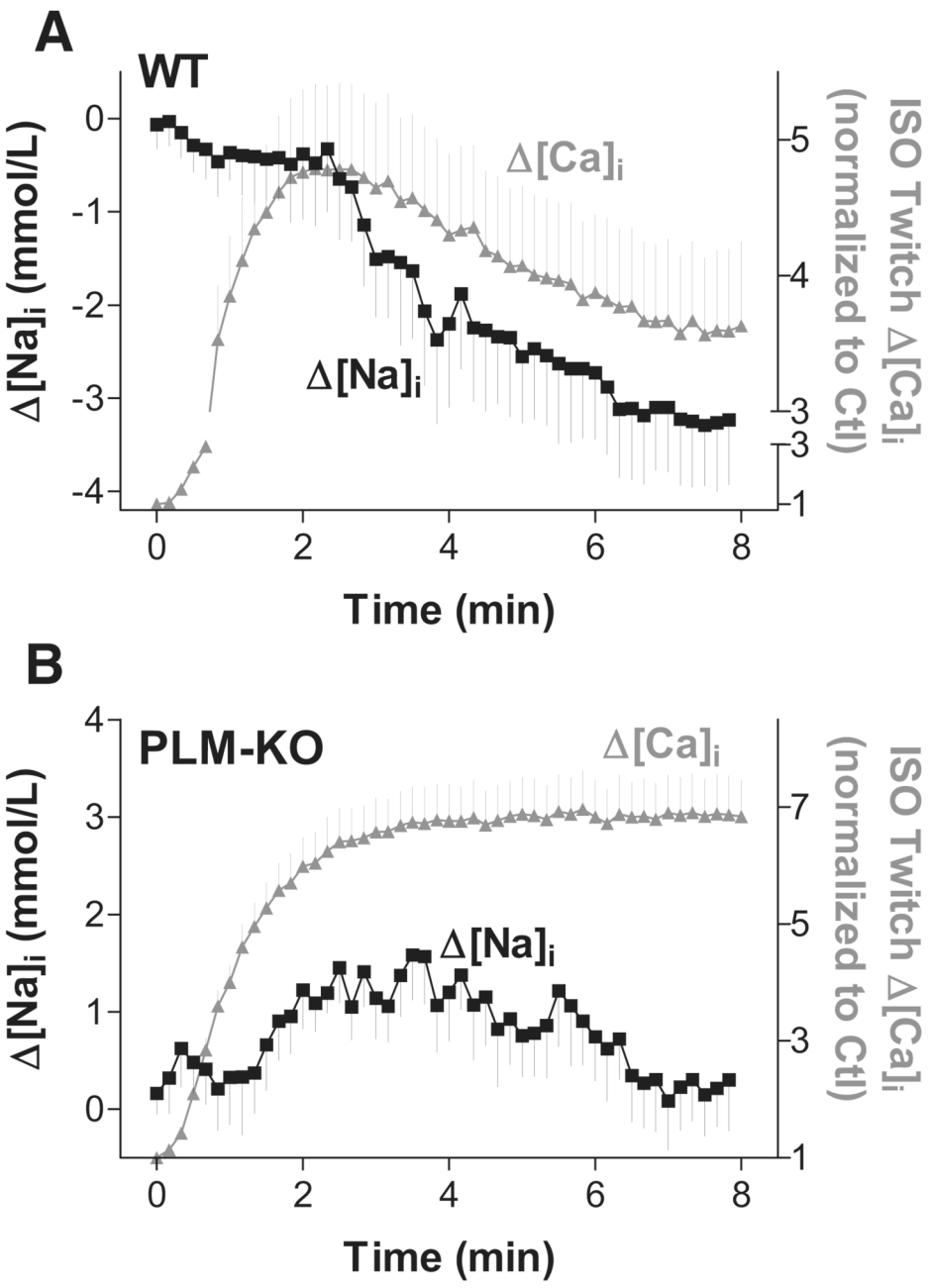

Figure 3.

Time course of isoproterenol (ISO)-induced changes in $[Na]_i$ (black) and amplitude of Ca transients (gray) in myocytes from WT (A) and PLM-KO mice (B). For the WT mice, mean data were calculated for the 7 myocytes in which we measured $[Na]_i$ and the 12 myocytes in which Ca transients increased to a peak and then declined to a new steady-state level in the presence of isoproterenol. For the PLM-KO mice, we averaged $[Na]_i$ over 9 cells and Ca transient amplitude over 18 myocytes. Ctl indicates control.

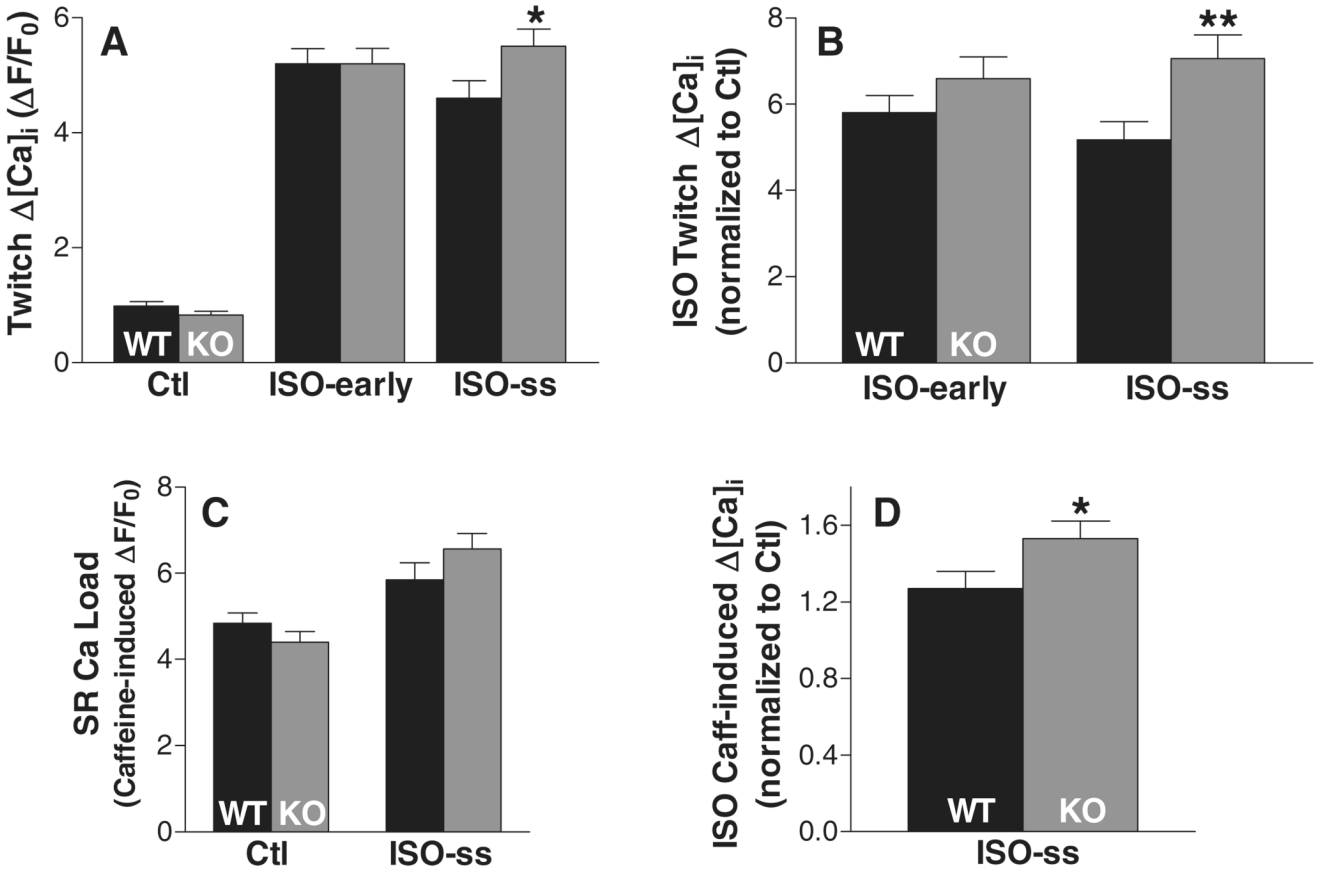

Figure 4.

Inotropic effect of isoproterenol (1 μmol/L) in myocytes from WT and PLM-KO mice. A, Amplitude of Ca transients under control conditions (Ctl; at time point a in Figure 2A), early (2 minutes) during isoproterenol treatment (ISO-early; time point b in Figure 2A), and at steady state in the presence of isoproterenol (ISO-ss; time point c in Figure 2A). Mean data for 20 myocytes from WT mice (5 hearts) and 18 myocytes from PLM-KO mice (6 hearts). B, Ratio of Ca transient amplitude in the presence of isoproterenol vs control conditions. The ratio was calculated after 2 minutes in isoproterenol (ISO-early) and after reaching steady state in the presence of isoproterenol (ISO-ss). C, Amplitude of caffeine (Caff) -induced Ca transients under control conditions and at steady state in the presence of isoproterenol. D, Ratio of the amplitude of caffeine-induced Ca transients at steady state in the presence of isoproterenol and under control conditions. *P<0.05, **P<0.01.

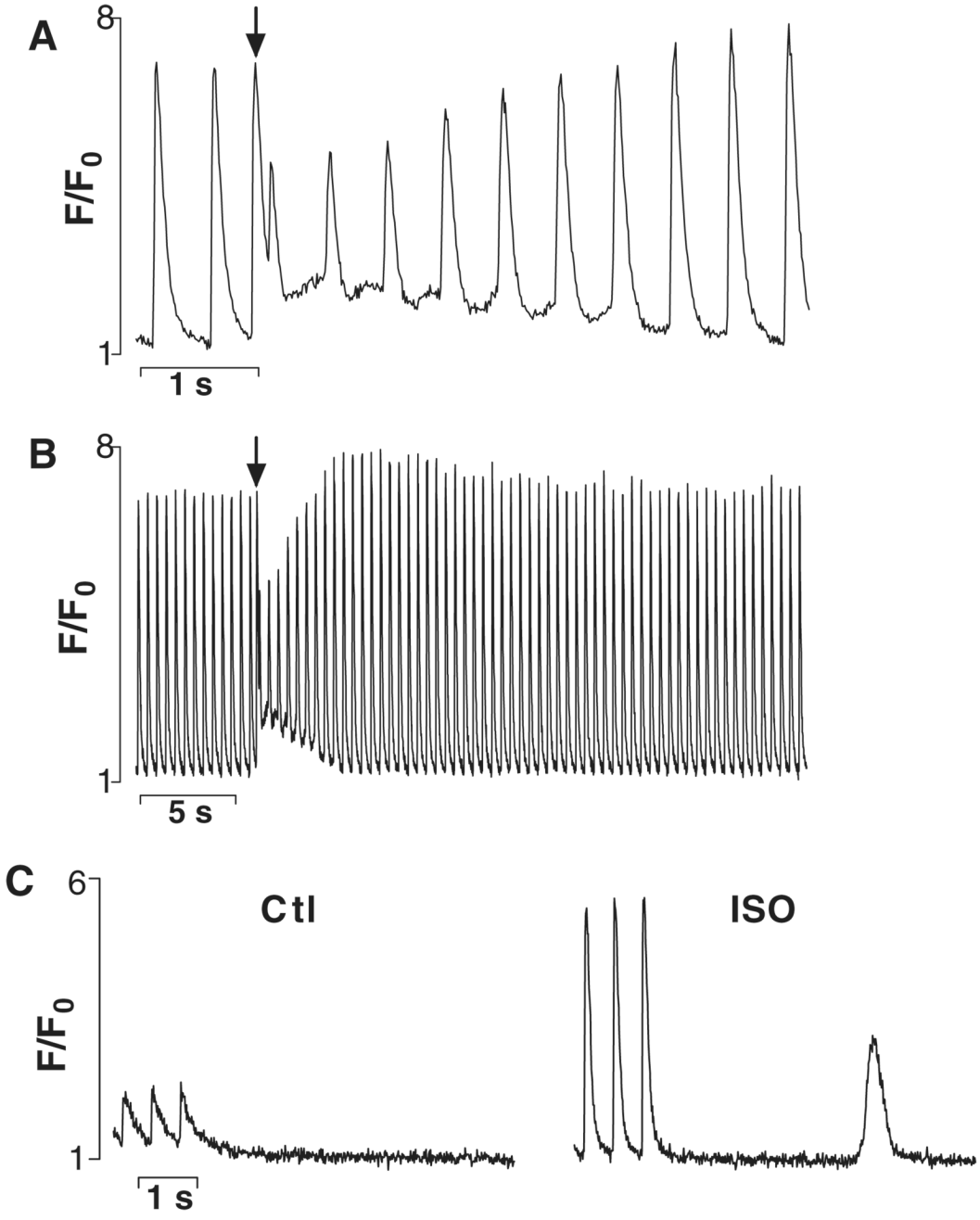

Figure 5.

Spontaneous Ca transients observed in the presence of isoproterenol (ISO). A, A spontaneous Ca transient (black arrow) occurring between triggered beats (field stimulation at 2 Hz) in a myocyte from a PLM-KO mouse. B, A longer time scale of the record in Figure 4A showing the return of Ca transient amplitude to the level before the spontaneous beat (black arrow). C, Spontaneous Ca waves or transients during a 5-second pause in the field stimulation in a myocyte from a PLM-KO mice. No spontaneous Ca transients were observed under control (Ctl) conditions in either PLM-KO or WT mice.