Sarcolipin Overexpression in Rat Slow Twitch Muscle Inhibits Sarcoplasmic Reticulum Ca\(^{2+}\) Uptake and Impairs Contractile Function*

Received for publication, June 20, 2002, and in revised form, September 11, 2002

Published, JBC Papers in Press, September 16, 2002, DOI 10.1074/jbc.M206171200

A. Russell Tupling‡§, Michio Asahi, and David H. MacLennan¶

From the Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6, Canada

Sarcolipin (SLN)1 is an inhibitor of sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPases (SERCAs) in vitro, but its function in vivo has not been defined. NF-SLN cDNA (SLN tagged N-terminally with a FLAG epitope) was introduced into rat soleus muscle in one hindlimb by plasmid injection and electrotorn. Western blotting showed expression and co-immunoprecipitation showed physical interaction between NF-SLN and SERCA2a. Contractile properties and SERCA2a function were assessed and compared with vector-injected contralateral soleus muscles. NF-SLN reduced both peak twitch force (\(P_t\)) (123.9 ± 12.5 \text{versus} 69.8 ± 8.9 \text{millinewtons}) and tetanic force (\(P_{st}\)) (562.3 ± 51.0 \text{versus} 300.7 ± 56.9 \text{millinewtons}) and reduced both twitch and tetanic rates of contraction (\(+dF/dt\)) and relaxation (\(-dF/dt\)) significantly. Repetitive stimulation (750-ms trains at 50 Hz once every 2 s for 3 min) showed that NF-SLN increased susceptibility to fatigue. These changes in contractile function were observed in the absence of endogenous phospholamban, and NF-SLN had no effect on either SERCA2a or SERCA1a expression levels. NF-SLN also decreased maximal Ca\(^{2+}\) transport activity at pCa 5 by 31% with no significant change in apparent Ca\(^{2+}\) affinity (6.38 ± 0.07 \text{versus} 6.39 ± 0.08 \text{pCa units}). These results show that NF-SLN expression impairs muscle contractile function by inhibiting SERCA function and diminishing sarcomplasmic reticulum Ca\(^{2+}\) stores.

Our initial studies indicated that SLN decreased the apparent affinity of SERCA1a for Ca\(^{2+}\) and increased maximal transport activity at high Ca\(^{2+}\) concentrations (6). Our more recent studies have confirmed that co-expression of NF-SLN with SERCAs in HEK-293 cells decreases the apparent affinity of both SERCA1a and SERCA2a for Ca\(^{2+}\) but did not confirm that NF-SLN increases maximal transport activity of SERCA1a in high Ca\(^{2+}\) (7). The apparent increase in maximal transport activity of SERCA1a observed earlier (6) could be explained by an underestimate in the enzyme-linked immunosorbent assay used in that study to measure the amount of SERCA1a (7). NF-SLN was found to be a more effective inhibitor of SERCA2a than of SERCA1a, being almost equal to PLN in its ability to decrease apparent Ca\(^{2+}\) affinity and, in contrast to PLN, even decreasing maximal transport activity at pCa 5 (7).

PLN is a well-characterized regulator of SERCA2a activity in cardiac muscle (8). Inhibitory interactions between PLN and SERCA2a result in a decrease in the apparent affinity of SERCA2a for Ca\(^{2+}\) (9) with no effect on the maximal rate of Ca\(^{2+}\) uptake by SERCA2a (7, 10, 11). Inhibitory interactions can be reversed by elevation of cytosolic Ca\(^{2+}\) or by phosphorylation of Ser16 and Thr17 in PLN cytosolic domain 1A (12). Several studies have shown that PLN is a major regulator of left ventricular basal contractile parameters and their responses to \(\beta\)-agonists (13–16). Ablation of PLN is associated with significant increases in cardiac contractility and left ventricular systolic function (14, 15), whereas the 2-fold overexpression of wild-type PLN in transgenic mice had an inhibitory effect on both the kinetics of Ca\(^{2+}\) transients and contractile parameters in ventricular myocytes and impaired basal left ventricular systolic function in vivo (16). Structural similarities between SLN and PLN genes and PLN and PLN protein sequences indicate that the two genes are members of a family (4).

The physiological function of SLN has not been evaluated, either in skeletal muscle or in cardiac muscle. Because NF-SLN and PLN affect SERCA2a function in a similar fashion in vitro, it is likely that SLN and PLN would affect muscle contractility in a similar fashion. In this study, we expressed NF-SLN in rat soleus muscle by intramuscular injection and electrotomentum of rabbit NF-SLN cDNA to explore the possibility that SLN can regulate SERCA2a activity and slow twitch soleus muscle contractility just as PLN can regulate cardiac contractility. We found that NF-SLN reduces peak isometric force, slows the rates of contraction and relaxation, and increases susceptibility to fatigue. Ca\(^{2+}\) uptake in postnuclear homogenates from these muscles was also impaired. We propose that NF-SLN can impair muscle contractile function indirectly by inhibiting SERCA function and thus lowering basal Ca\(^{2+}\) stores in the sarcoplasmic reticulum.

---

* This work was supported by Grant MT12545 from the Canadian Institutes of Health Research and Grant T5042 from the Heart and Stroke Foundation of Canada (to D. H. M.).
† These authors contributed equally to this work.
‡ To whom correspondence should be addressed: Banting and Best Department of Medical Research, University of Toronto, Charles H. Best Inst., 112 College St., Toronto, ON M5G 1L6, Canada. Tel.: 416-978-5008; Fax: 416-978-8528; E-mail: david.maclennan@utoronto.ca.
§ Postdoctoral Fellow of the Heart and Stroke Foundation of Canada.
¶ This paper is available online at http://www.jbc.org.

1 The abbreviations used are: SLN, sarcolipin; SERCA, sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase; PLN, phospholamban; NF-SLN, sarcolipin tagged N-terminally with a FLAG epitope; MOPS, 4-morpholinepropanesulfonic acid.
**EXPERIMENTAL PROCEDURES**

**Materials—**Enzymes for DNA manipulation were from New England Biolabs and Pharmacia Corp., and the expression vector pcDNA3.1 was from Invitrogen. G-Sephrose 4B and Bio-Rad Chemiluminescence were used for measurement of co-immunoprecipitation and immunoblotting were from Pierce. FLAG antibody M2 and the monoclonal antibody 5C5 against α-sarcomeric actin were from Sigma; the anti-PLN antibody 1D11 (17) was a gift from Dr. Robert Johnson (Merck Research Laboratories); the monoclonal antibody 2A7-A1 against SERCA2a and SERCA2a/2b was from Pierce Bioreagents Inc. The Ai2 monoclonal antibody against SERCA1 was produced in our laboratory (18).

**Reverse Transcription-PCR for Estimation of SLN Expression in Total mRNA—**Reverse transcription-PCR was performed on total RNA isolated from soleus and heart using guanidine-isothiocyanate-pheophorbide chloroform extraction (19), the latter being included as a positive marker for SLN because SLN is expressed highly in rat heart (5). RNA quality and quantity were determined by absorbance at 260 and 280 nm.

A 5-µg aliquot of total RNA was reverse transcribed in 20 µl of a reaction mix (Sigma Enhanced Avian HS reverse transcription-PCR kit) containing 20 units of eAMV reverse transcriptase at 42 °C in the presence of RNase inhibitor and 1 µM specific SLN reverse primer (SLN, 5′-GGG ACT GAC TCG TGT GTG CCC T-3′). The polymerase chain reaction was carried out in a total volume of 50 µl with JumpStart AccuTaq LA DNA polymerase mix (Sigma), with 200 µM dNTPs, 0.4 µM each of two 5′ and 3′ primers (SLN, forward, 5′-GGT GTG CAC TCA GAA GTC CTC CT-3′ and reverse, 5′-GGG ACT GAC TCG TGT GTG CCC T-3′) and 5 µl of first strand cDNA. Denaturation was for 30 s at 94 °C for 1 min, and 35 cycles were at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. The amplified products were separated on 1.2% agarose gel containing ethidium bromide.

**Cell Culture and Heterologous Expression—**The culture of HEK-293 cells, their transfection with cDNAs encoding SERCA1a, SERCA2a, and NF-SLN, and the isolation of microsomal fractions from transfected cells expressing these proteins have been described in earlier publications (6, 20, 21).

**Construction of NF-SLN—**The preparation of the rabbit NF-SLN cDNA construct was described previously (6). NF-SLN is a fusion protein of SLN with a FLAG epitope at its N terminus that does not alter its function (6). The NF-SLN construct was cloned into the XbaI and XhoI sites of the expression vector pcDNA3.1.

**DNA Injection and Electric Pulse Delivery—**Injection and electric pulse delivery of the NF-SLN cDNA construct into rat soleus muscle was achieved using the protocol of Mir et al. (22). Male Sprague-Dawley rats weighing 539 ± 12 g (n = 5) were fully anesthetized with a mixture of ketamine (50–100 mg/kg) and xylazine (5–10 mg/kg). After achieving full anesthesia, the soleus muscle was dissected, and immediately before injection a total of 150 µg of DNA dissolved in 0.9% sterile NaCl (0.5 µg/µl) using a 26G 0.5 stainless steel needle. For each animal, the soleus muscle from the experimental hindlimb was injected with NF-SLN, and the soleus muscle from the contralateral control hindlimb was injected with an equal concentration of the expression vector pcDNA3.1. Approximately 1 min after DNA injection, electric pulses (9 pulses, 2 Hz, 200 V/cm, 20 ms/pulse) were applied by two implanted stainless steel needle electrodes (21G 1.5) connected to a Grass stimulator (Grass S88). Following electroporation, incisions in the skin directly covering each soleus muscle were closed using surgical staples, and the animals were allowed to recover under a heat lamp.

**Electrical Stimulation and Muscle Contractile Measurements—**Three days after DNA injection, electrically evoked muscle force was measured in situ from the soleus muscle from each hindlimb across a range of stimulation frequencies from 1 to 70 Hz. The rats were anesthetized initially with a mixture of ketamine (50–100 mg/kg) and xylazine (5–10 mg/kg) and given maintenance intraperitoneal injections of pen-tobarbital sodium (40 mg/kg), as required. All other details pertaining to the surgical preparation and the animal stimulation apparatus have been described elsewhere (23, 24). Soleus muscles contracted isometrically (i.e. length remained constant), and both twitch (1 Hz) and tetanic (10–70 Hz) force were obtained via direct muscle stimulation, with the use of stainless steel electrodes and with a single 0.2-ms pulse at 70 V. Muscle stimulation was performed using a Grass S88 stimulator, and force data were collected on-line using a 640A signal interface (Aurora Scientific Inc.) connected to a National Instruments 16-bit A/D card and analyzed using the Dynamic Muscle Control and Data Acquisition (DMC) and Dynamic Muscle Analysis (DMA) Software (Aurora Scientific Inc.). An independent calibration was performed daily for each force transducer (Grass, model FT 10). At least 5 min before commencing data collection, optimal length (L0) for peak twitch force (P0) was established. Peak tetanic force (P) occurred at a stimulation frequency of 50 Hz, and only P0 and P were analyzed because peak force amplitude and peak rates of contraction (+dF/dt) and relaxation (−dF/dt). To assess the effects of NF-SLN on soleus susceptibility to fatigue, each muscle was stimulated repeatedly for 3 min using a fatigue protocol consisting of 700-ms contractions at 50 Hz, once every 2 s. Fatigue data are expressed as percentages of resting data. Experimental protocols were approved by the Animal Care Committee of the University of Toronto.

**Preparation of Postnuclear Homogenates—**Immediately after muscle contractile measurements, soleus muscles were excised, weighed, and embedded in a 10-mold (w/v) in homogenizing buffer containing 5 mM HEPES, pH 7.5, 250 mM sucrose, 0.2 mM phenylmethylsulfonyl fluoride, and 1 mM diithiothreitol and homogenized with a Tissumizer (Tekmar Company, TR-10) at 90% maximal power with two 30-s bursts. The homogenates were frozen immediately in liquid nitrogen for later analysis. Just prior to analysis, the homogenates were thawed on ice and centrifuged at 1,700 × g in an Eppendorf 5415C centrifuge (Brinkman Instruments Ltd.) for 5 min to sediment nuclei and cell debris. The postnuclear homogenate was assessed for protein concentration using the Bio-Rad method with bovine serum albumin as a standard and was used for all subsequent analyses, unless otherwise stated.

**Western Blot Analysis—**Western blotting was performed to determine the relative expression levels of NF-SLN, PLN, SERCA2a, and SERCA1a (25) in rat soleus muscles that were either injected with NF-SLN or the expression vector pcDNA3.1. After assuring linearity of band density, 20 µl (20 µg) of the postnuclear homogenate were applied to 12.5% NF-SLN and PLN or 8% (SERCA2a and SERCA1a) acrylamide gels, separated by SDS-PAGE, and transferred to nitrocellulose membranes. After blocking with 5% skim milk, the membranes were incubated with both anti-α-sarcomeric actin antibody 5C5 to control for protein loading and either anti-FLAG antibody M2, anti-PLN antibody 1D11, anti-SERCA2a antibody 2A7-A1, or anti-SERCA1a antibody A52 for 1 h at room temperature and then washed with Tris-buffered saline with 0.1% Tween 20. They were then treated with horseradish peroxidase–conjugated goat secondary antibodies, washed, and stained with an enhanced chemiluminescence kit (Pierce Super Signal). Densitometric analysis was performed using NIH Image 16.1 software. Both samples for a given animal were run in duplicate on separate gels along with a standard. Microsomes prepared from HEK-293 cells that had been transfected with either NF-SLN or PLN cDNAs (7) served as positive controls and molecular weight standards for NF-SLN and PLN, respectively.

**Comparison of NF-SLN Synthesis in HEK-293 Cells and in Soleus Muscle—**Western blotting was performed on post-nuclear homogenates from soleus muscles that expressed NF-SLN and microsomes prepared from HEK-293 cells that expressed both NF-SLN and either SERCA1a or SERCA2a. Soleus and HEK-293 samples were applied to the same 8% gel, separated by SDS-PAGE, transferred to nitrocellulose, and stained with an appropriate monoclonal antibodies. Densitometric analysis was performed to determine the relative densitometric ratios of SERCA1a and SERCA2a to NF-SLN in both rat soleus and HEK-293 cells.

**Co-immunoprecipitation from Postnuclear Homogenates—**To confirm association between SERCA2a, the predominant SERCA isoform expressed in rat soleus (26), and NF-SLN, co-immunoprecipitation of SERCA2a and NF-SLN was carried out as described previously (21) using aliquots of postnuclear homogenates at 1 mg protein/ml and a protein G-Sepharose/NF-SLN FLAG antibody M2 complex. The presence of SERCA2a associated with NF-SLN was then detected by Western blotting and detection procedures using the anti-SERCA2a monoclonal antibody 2A7-A1.

Ca2+ Transport Assay—Ca2+ transport activity in postnuclear homogenates at 1 mg protein/ml was assayed in 150 µl of a reaction mixture containing 20 mM MOPS-Tris-HCl, pH 6.8, 100 mM KC1, 5 mM MgCl2, 0.5 mM EGTA, 5 mM potassium oxalate, and about 10 µg of protein per reaction mixture. The maximal transport activity values are reported as percentages of control (pCDNA), which was set to 100%. On a given analytical day, the samples from all conditions were analyzed in duplicate.
Expression and Localization of NF-SLN cDNA in Rat Soleus

Rabbit NF-SLN cDNA was cloned into the expression vector pcDNA3.1, dissolved in 0.9% sterile NaCl, and injected and electrotransferred into rat soleus. Postnuclear homogenates were prepared 3 days later. A, representative 20-µg samples prepared from soleus muscles injected with NF-SLN and controls injected with vector only were stained with antibody M2 to determine relative expression levels of NF-SLN. Left lane, 20 µg of microsomal protein from HEK-293 cells transfected with NF-SLN was used as a positive control and as a molecular weight standard (NF-SLN std). B, relative expression levels of NF-SLN in soleus muscles injected with NF-SLN from all eight animals used in this study were determined by staining with antibody M2. Staining with antibody 5C5 against NF-SLN from all eight animals used in this study were determined by Western blotting on postnuclear homogenates from experimental muscles injected with NF-SLN cDNA and controls. Overall, there were no differences (p > 0.05) in either SERCA2a or SERCA1a expression levels between NF-SLN and control muscles (Fig. 3C). We confirmed an earlier report (27) in which immunoblot and Northern blot analysis were used to show that PLN is not expressed in rat soleus muscle (Fig. 3D).

It was of interest to obtain a measure of the amount of NF-SLN synthesis that occurred in soleus muscle following injection of NF-SLN cDNA. It was not feasible to determine absolute amounts, because purified NF-SLN was not available to us. It was, however, possible to determine the relative level of NF-SLN in relation to the amount of SERCA in soleus muscle and to compare the results with the ratio between NF-SLN and SERCA expressed in HEK-293 cells under conditions where optimal physiological effects are obtained.

Western blotting was carried out on post-nuclear homogenates from soleus muscles that expressed NF-SLN and on microsomes prepared from HEK-293 cells that expressed NF-SLN together with either SERCA1a or SERCA2a. Soleus and HEK-293 samples were applied to the same 8% gel, separated by SDS-PAGE, transferred to nitrocellulose, and stained for NF-SLN and either SERCA1a or SERCA2a using the appropriate monoclonal antibodies. Densitometric analysis was performed to determine the ratios of SERCA1a and SERCA2a to NF-SLN in the transfected rat soleus homogenate. Densitometric analysis was also used to determine ratios of SERCA1a to NF-SLN and SERCA2a to NF-SLN in HEK-293 cells that expressed NF-SLN and either SERCA1a or SERCA2a.

Analysis of all eight soleus samples that expressed NF-SLN distinguished three samples that expressed the highest amount of NF-SLN relative to SERCA1a and SERCA2a (Fig. 1B, lanes 1, 5, and 8). If we set the average SERCA:NF-SLN ratio in HEK-293 cells at 1, then we could calculate that the average densitometric ratio of SERCA2a to NF-SLN expression in soleus muscle was 3.75, and the ratio of SERCA1a to NF-SLN was 8.5. If we assume that SERCA1a and SERCA2a each account for about 50% of the total SERCA in soleus muscle (28), then the average densitometric ratio of SERCA1a plus SERCA2a to NF-SLN would be about 6. On the basis of endogenous SERCA expression in soleus muscle, we are expressing only about ⅛ of the amount of NF-SLN that has optimal effects on SERCA function in HEK-293 cells. However, SLN is expressed endogenously in rat soleus muscle, presumably at an optimal level. Thus, in our experiments, it is the effect of NF-SLN overexpression on top of an optimal base line of SLN expression that we are measuring in our physiological studies of transfected soleus muscle. We present the analysis of muscle
contractile function and SERCA function for the three experiments in which NF-SLN was most highly expressed.

**Effects of NF-SLN Expression on Slow Twitch Muscle Contractility and Susceptibility to Fatigue**—To evaluate the physiological effects of NF-SLN expression in slow twitch skeletal muscle, isometric contractile characteristics, including both twitch (Fig. 4A) and tetanus (Fig. 5A), from soleus muscles injected with NF-SLN cDNA were measured in situ and compared with contractile characteristics assessed simultaneously in controls. NF-SLN reduced (p < 0.05) peak twitch force (P₀) by 44% compared with control (Fig. 4B). The maximal twitch rate of contraction (+dF/dt) was reduced (p < 0.05) by 34%, and the maximal twitch rate of relaxation (-dF/dt) was reduced (p < 0.05) by 25% with NF-SLN expression (Fig. 4C). Maximum tetanic force (Pₐ), which occurred at a stimulation frequency of 50 Hz, was reduced (p < 0.05) by 47% (Fig. 5B), tetanic +dF/dt was reduced (p < 0.05) by 50%, and tetanic -dF/dt was reduced (p < 0.05) by 40% with NF-SLN expression (Fig. 5C).

Soleus susceptibility to fatigue was assessed by repetitive stimulation of the muscle using the protocol outlined under “Experimental Procedures.” The force loss and slowing of contraction that are characteristic of fatigue were greater (p < 0.05) in soleus muscles expressing NF-SLN compared with control. When expressed as a percentage of normal resting values, P₀ was reduced (p < 0.05) by 71 ± 3.9%, and +dF/dt was reduced by 45 ± 6.6% in NF-SLN-expressing muscles compared with reductions in P₀ (p < 0.05) of 65 ± 2.8 and reductions in +dF/dt of 32 ± 7.6% in controls. In contrast, the peak relaxation rate was slowed with fatigue to a similar extent: 63 ± 5.1% in NF-SLN muscle and 71 ± 7.3% in control muscle.

**Ca²⁺ Uptake by Sarcoplasmic Reticulum**—Postnuclear homogenates were adjusted to a concentration of 1 mg protein/ml and assayed for Ca²⁺ dependence of Ca²⁺ uptake to assess the effects of NF-SLN expression on SERCA function. NF-SLN reduced absolute Ca²⁺ uptake across a range of free Ca²⁺ levels from pCa 7 to pCa 5 (Fig. 6). NF-SLN reduced Ca²⁺ uptake at pCa 5.5 to pCa 5 such that maximal transport activity was reduced (p < 0.05) by ~31% compared with control. There was no significant change, however, in apparent Ca²⁺ affinity, expressed as K_Ca in pCa units (6.36 ± 0.07 versus 6.39 ± 0.08, mean ± S.E.).

These data differ from those obtained in studies of co-expression of NF-SLN with SERCA1a or SERCA2a in HEK-293 cells, where a significant change was observed in apparent Ca²⁺ affinity (7). This difference is most likely due to differences in the molar ratios of NF-SLN expression compared with the expression of SERCA. In our studies with HEK-293 cells (7), we noted changes in ΔK_Ca of ~0.17 to ~0.34 pCa units when the molar ratios of NF-SLN to SERCA1a or SERCA2a cDNAs used in transfection were 4:1. However, if the ratios were reduced to 1:1, then no differences in ΔK_Ca were observed in the presence and absence of NF-SLN expression. It is probable that the ratio of SERCA to SLN remains high, even after overexpression of NF-SLN.
To assess the physiological function of SLN in vivo, we expressed NF-SLN in rat soleus muscles from one hindlimb while the contralateral limb served as a control. Our results confirm our most recent findings from in vitro studies that SLN acts as an inhibitor of SERCA function over a concentration range from pCa 7 to pCa 5, resulting in a decreased apparent Ca\(^{2+}\) affinity and lower maximal transport activity (7). This decrease in Ca\(^{2+}\) uptake is reflected in muscle contractile performance.
Physiological Effects of NF-SLN Expression

which is impaired following expression of NF-SLN in slow twitch skeletal muscle. In resting soleus, both the kinetics and amplitude of contraction were reduced with NF-SLN expression, and when soleus muscles were stimulated repeatedly, fatigue was more pronounced in muscles expressing NF-SLN compared with controls. Thus, NF-SLN expression in slow twitch skeletal muscle is similar to a model of PLN overexpression in the heart, at least in terms of its effects on muscle contractility.

We were able to express NF-SLN in rat soleus using a protocol of injection and electrotransfer of plasmid DNA. Electroporation combined with cDNA injection was necessary to achieve NF-SLN expression, because we were unable to detect NF-SLN in rat soleus muscles that were injected with NF-SLN cDNA, without electroporation (data not shown). This finding is in agreement with other studies that employed in vivo electroporation to transfer plasmid DNA into skeletal muscles (22, 29–31). Our co-immunoprecipitation experiments and Ca\(^{2+}\) transport assays in postnuclear homogenates indicate that expressed NF-SLN interacts both functionally and physically with SERCA2a in rat soleus sarcoplasmic reticulum membranes.

We confirmed that rat soleus does not express PLN, which is important given that co-expression of PLN and NF-SLN with either SERCA2a or SERCA1a is superinhibitory for SERCA function (7). We have also shown that rat soleus is a useful model system in which to assess the function of SLN co-expressed with SERCA2a and SERCA1a. The design of this study incorporated an internal paired control, and because muscle function and sampling occurred just 3 days following DNA injection, at the earliest time point where peak expression of a typical introduced cDNA is observed (22), compensatory changes in the expression of other functionally related Ca\(^{2+}\) regulatory proteins would be minimal. In fact, we did not find any significant changes in the expression levels of either SERCA2a or SERCA1a with NF-SLN expression.

A limitation in this study was that we were unable to quantify SLN and NF-SLN expression in each muscle. Despite several attempts, an antibody against SLN has not been generated to date, so we could not determine total SLN protein levels or compare expression between control and muscles expressing NF-SLN. The lack of highly purified NF-SLN also made it difficult to carry out measurements of NF-SLN transport activity in postnuclear homogenates that were prepared from soleus muscles injected with NF-SLN (●) and controls injected with vector only (■). Mean data from three muscles that expressed the highest levels of NF-SLN and paired control muscles are shown.

**FIG. 6.** Effect of NF-SLN on SERCA Ca\(^{2+}\) transport activity. Ca\(^{2+}\) dependence of Ca\(^{2+}\) transport activity was assessed in post-nuclear homogenates that were prepared from soleus muscles injected with NF-SLN (●) and controls injected with vector only (■). Mean data from three muscles that expressed the highest levels of NF-SLN and paired control muscles are shown.
and a reduced Ca\(^{2+}\) store could alter excitation-contraction coupling in skeletal muscle (35), accounting for the overall negative effect of NF-SLN on isometric twitch and tetanic contractile properties observed in this study. Because SLN is normally expressed in the heart, an obvious implication from this study and an earlier study (7) is that overexpression of SLN in the heart would have the potential to impair cardiac contractile function.

In a comparable study, the overexpression of PLN in mouse fast twitch skeletal muscle impaired only the relaxation rate with no effect on the rate of contraction or peak twitch amplitude (32), in contrast to the effects of overexpression of PLN in the heart (16). This is surprising, because mouse fast twitch skeletal muscle should also express SLN, and we have shown that co-expression of NF-SNL and PLN is superinhibitory for SERCA1a function (7), the predominant isoform expressed in fast twitch skeletal muscle (28). In our study it is not possible to define the percentage of SERCA molecules that were inhibited by the sum of NF-SLN plus endogenous SLN, nor was it possible to define the percentage of SERCA molecules that were inhibited by the sum of ectopically expressed PLN plus endogenously expressed SLN in the study of Slack et al. (32).

SERCA pumps are much more abundant in fast twitch muscle compared with slow twitch skeletal muscle (36). Therefore, a limitation in both studies is that the systems were not manipulated to achieve optimal ratios of SERCA to inhibitor.

We also assessed the effects of NF-SNL expression on soleus susceptibility to fatigue, an important characteristic that distinguishes slow twitch from fast twitch fibers. Compared with control, resting tetanic force was 47% lower in soleus muscles that expressed NF-SNL. Nevertheless, our fatigue protocol indicated that there was a relatively greater loss of force and reduced rate of contraction in soleus muscles expressing NF-SNL. However, the relative change in relaxation rate was similar between soleus muscles that expressed NF-SNL and control, suggesting that there is no interaction between NF-SNL expression and fatigue in relation to the slowing of relaxation that normally occurs with fatigue (37).

These results suggest that NF-SNL expression was only indirectly responsible for the observed differences in susceptibility to fatigue. In the relationship between force and Ca\(^{2+}\), there is a range over which small changes in pCa lead to large changes in force (38). It is possible that resting soleus muscles expressing NF-SNL, as opposed to control, were already in that sensitive range where small reductions in Ca\(^{2+}\) release would lead to reductions in force, especially given that basal sarcoplasmic reticulum Ca\(^{2+}\) stores and Ca\(^{2+}\) release are suspected to be lower in soleus muscles that express NF-SNL.

Brody disease is an inherited disorder of skeletal muscle function characterized by exercise-induced impairment of muscle relaxation (39). We have associated mutations in the ATP2A1 gene encoding SERCA1 with autosomal recessive inher- itance of Brody disease but not with autosomal dominant inheritance (4, 40). A search for mutations in the SLN gene in five Brody families that were not linked to ATP2A1 has not revealed any alterations in coding, splice junction, or promoter sequences in the SLN gene (4). On the basis of the results of this study, however, abnormal SLN expression levels should not be ruled out in evaluating SLN as a candidate gene for Brody disease.

In summary, we have found that expression of NF-SNL in rat soleus results in a significant depression in muscle contractility and increased susceptibility to fatigue. Ca\(^{2+}\) uptake in postnuclear homogenates from these muscles was also depressed, confirming that NSF-SNL acts as an inhibitor of SERCA function in vivo. These results imply that overexpres-

sion of SLN has the potential to impair skeletal muscle relaxation.

Acknowledgments—We are grateful to Dr. Robert Johnson for the kind gift of the anti-PLN antibody 1D11 and to Dr. Nancy H. McKee for access to her laboratory and use of the rat contractile apparatus and Grass S88 stimulator.

REFERENCES

1. MacLennan, D. H., Yip, C. C., Iles, G. H., and Seeeman, P. (1972). Cold Spring Harbor Symp. Quant. Biol. 37, 469–478

2. Wawrzynow, A., Theibert, J. L., Murphy, C., Jona, I., Martinosi, A., and Fischman, J. H. (1992). Arch. Biochem. Biophys. 299, 69–73

3. Fuji, J., Lytton, J., Tada, M., and MacLennan, D. H. (1988). FEBS Lett. 227, 51–55

4. Odermatt, A., Tasher, P. E., Scherrer, S. W., Beatty, B., Kanna, V. K., Cornblath, D. B., Chaudhry, Y., Yee, W. C., Schrank, B., Karpati, G., Breuning, M. H., Knoers, N., and MacLennan, D. H. (1997). Genomics 45, 541–553

5. Gayan-Ramirez, G., Vanzelez, L., Wuytack, F., and Decramer, M. (2000). J. Physiol. 524, 387–397

6. Odermatt, A., Becker, S., Kanna, V. K., Kuryszdowski, K., Leisner, E., Pette, D., and MacLennan, D. H. (1998). J. Biol. Chem. 273, 12960–12969

7. Assali, M., Kuryszdowski, K., Tada, M., and MacLennan, D. H. (2002). J. Biol. Chem. 277, 26725–26728

8. Simmerman, H. K., and Jones, L. R. (1998). Physiol. Rev. 78, 921–947

9. Kimura, Y., Kuryszdowski, K., Tada, M., and MacLennan, D. H. (1996). J. Biol. Chem. 271, 21726–21731

10. Odermatt, A., Tasher, P. E., Kanna, V. K., Busch, H. F., Karpati, G., Jablecki, C., Breuning, M. H., and MacLennan, D. H. (1996). Nat. Genet. 14, 191–194

11. Morris, G. L., Cheng, H. C., Colyer, J., and Wang, J. H. (1991). J. Biol. Chem. 266, 11270–11275

12. Simmerman, H. K., Collins, J. H., Theibert, J. L., Wegener, A. D., and Jones, L. R. (1996). J. Biol. Chem. 271, 15333–15341

13. Shah, J. S., Jones, L. B., and Morad, M. (1991). Am. J. Physiol. 261, H1344–H1349

14. Luo, W., Grupp, I. L., Harrer, J., Ponniah, S., Grupp, G., Duffy, J. J., Harrer, J. M., Hoit, B. D., Lester, J. W., Kranias, E. G., and MacLennan, D. H. (1998). Biochem. Pharmacol. 56, 553–563

15. Mayer, E. J., Huckle, W., Johnson, R. G., Jr., and McKenna, E. (2000). J. Clin. Invest. 106, 1154–1164

16. Kadambi, V. J., Ponniah, S., Harrer, J. M., Hoit, B. D., Dorn, G. W., II, Walsh, A. L., and Kranias, E. G. (1998). Circ. Res. 83, 523–530

17. Sluder, G. L., Cheng, H. C., Colyer, J., and Wang, J. H. (1998). J. Biol. Chem. 273, 26725–26728

18. Allen, D. G., Lannergren, J., and Westerblad, H. (1995). Exp. Physiol. 80, 497–527

19. Brody, I. A. (1969). Engl. J. Med. 281, 187–192

20. Odermatt, A., Kuryszdowski, K., and MacLennan, D. H. (1996). J. Biol. Chem. 271, 14206–14213
Sarcoplasmic Reticulum Ca^{2+} Uptake and Impairs Contractile Function
A. Russell Tupling, Michio Asahi and David H. MacLennan

*J. Biol. Chem.* 2002, 277:44740-44746.
doi: 10.1074/jbc.M206171200 originally published online September 16, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M206171200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 16 of which can be accessed free at
http://www.jbc.org/content/277/47/44740.full.html#ref-list-1