Effect of chronic obstructive pulmonary disease on calcium pump ATPase expression in human diaphragm

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1Surgical and Research Services, Philadelphia Veterans Affairs Medical Center, and 2Departments of Surgery and Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia; 3National Eye Institute, National Institutes of Health, Bethesda, Maryland; and 4Department of Biology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, Pennsylvania

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Nguyen, Taitan, Neal A. Rubinstein, Camasamudram Vijayasarathy, Lawrence C. Rome, Larry R. Kaiser, Joseph B. Shrager, and Sanford Levine. Effect of chronic obstructive pulmonary disease on calcium pump ATPase expression in human diaphragm. J Appl Physiol 98: 2004–2010, 2005. First published February 17, 2005; doi:10.1152/japplphysiol.00767.2004.—We have previously demonstrated that human diaphragm remodeling elicited by severe chronic obstructive pulmonary disease (COPD) is characterized by a fast-to-slow myosin heavy chain isoform transformation. To test the hypothesis that COPD-induced diaphragm remodeling also elicits a fast-to-slow isoform shift in the sarcoplasmic reticulum Ca2+ ATPase (SERCA), the other major ATPase in skeletal muscle, we obtained in vitro preparations of the costal diaphragm from 10 severe COPD patients and 10 control subjects. We then used isoform-specific monoclonal antibodies to characterize diaphragm fibers with respect to the expression of SERCA isoforms. Compared with control diaphragms, COPD diaphragms exhibited a 63% decrease in fibers expressing only fast SERCA (i.e., SERCA1; \( P < 0.001 \)), a 190% increase in fibers containing both fast and slow SERCA isoforms (\( P < 0.001 \)), and a 19% increase (\( P < 0.05 \)) in fibers expressing only the slow SERCA isoform (i.e., SERCA2). Additionally, immunoblot experiments carried out on diaphragm homogenates indicated that COPD diaphragms expressed only one-third the SERCA1 content noted in control diaphragms; in contrast, COPD and control diaphragms did not differ with respect to SERCA2 content. The combination of these histological and immunoblot results is consistent with the hypothesis that diaphragm remodeling elicited by severe COPD is characterized by a fast-to-slow SERCA isoform transformation. Moreover, the combination of these SERCA data and our previously reported myosin heavy chain isoform data (Levine S, Nguyen T, Kaiser LR, Rubinstein NA, Maislin G, Gregory C, Rome LC, Dudley GA, Sieck GC, and Shrager JB. Am J Respir Crit Care Med 168: 706–713, 2003) suggests that diaphragm remodeling elicited by severe COPD should decrease ATP utilization by the diaphragm. SERCA1; SERCA2; myosin heavy chain

THE SARCOENDOPLASMIC RETICULUM Ca2+ ATPase (SERCA) pumps Ca2+ from the myoplasm into the sarcoplasmic reticulum and thereby initiates relaxation of striated muscle; therefore, it is an essential protein for the excitation-contraction-relaxation cycle of skeletal muscle. The human diaphragm expresses both a fast SERCA isofom (SERCA1) and a slow SERCA isofom (SERCA2). Despite the obvious importance of SERCA to diaphragm function, the literature does not contain any reports regarding the effect of chronic obstructive pulmonary disease (COPD) (or any other chronic pulmonary disease) on diaphragm SERCA expression.

Over the past several years, our laboratory (21–24) has put forward the overall hypothesis that diaphragm remodeling elicited by severe COPD is characterized by 1) an increase in the capacity for aerobic generation of ATP and 2) a decrease in the rate of ATP utilization. The evidence for the increase in ATP generating capacity is 1) the finding by Orozco-Levi et al. (30) that the mitochondrial volume fraction increases as the severity of COPD increases; and 2) our finding that severe COPD elicits a twofold increase in the oxidative capacity of succinic dehydrogenase, a mitochondrial oxidative enzyme (21). Regarding the rate of ATP utilization, we have presented evidence that the myosin heavy chain ATPase activity (i.e., the cross-bridge ATPase) is decreased at both the individual myofiber level as well as the organ level (i.e., whole diaphragm). However, in some human myofibers, the rate of ATP utilization by SERCA (i.e., the Ca2+ pump) approaches that of the myosin ATPase, whereas in other fibers, the rate of ATP utilization by SERCA exceeds that of the myosin ATPase. Admittedly, these observations made on the single permeabilized fiber during isometric contraction under maximum activation may overestimate the contribution of SERCA to ATP utilization under nonisometric contractions (see Ref. 41 and discussion). Nonetheless, at the present time, there is general agreement that ATP utilization by SERCA plays a major role in ATP supply-demand relationships in the diaphragm. Therefore, we hypothesized that severe COPD elicits changes in diaphragm SERCA expression that would be expected to decrease ATP utilization, and we carried out the present study to test this hypothesis.

METHODS

Subjects

Our study cohort consisted of 10 control subjects who were undergoing surgery for resection of solitary pulmonary nodules and 10 patients with severe COPD (of the heterogenous emphysema type) who were undergoing lung volume reduction surgery. Informed consent for diaphragm biopsies was obtained from each of the subjects, and our protocol was approved by the Institutional Review Boards of the Philadelphia Veterans Affairs Medical Center and the University of Pennsylvania.

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Pulmonary Function Tests

Before surgery, subjects underwent spirometry and measurement of lung volumes by plethysmography, and values were compared with predicted normal values (13, 17). Prior workers have described artifically high values for lung volumes in patients with COPD, and we used strategies for avoiding these errors described by others (34, 39).

Biopsies

Full thickness biopsies (~15–25 mm by 6–8 mm) were obtained from the same region of the right anterior costal diaphragm lateral to the insertion of the phrenic nerve and prepared for immunohistochemistry by the method of Larsson and colleagues (19, 20) and for biochemical assays as previously described (23, 29). All diaphragm samples were then stored at −70°C until analyzed.

Immunohistochemistry

Methodology. Immunohistochemistry was carried out as previously described (29) with some modifications. Briefly, a series of five adjacent transverse sections from each of the 12 diaphragm samples was used. One section from each series was stained with hematoxylin and eosin, and the remaining four sections were separately stained with one of the following specific mouse monoclonal antibodies for SERCA or myosin heavy chain (MHC) isoforms. The antibodies used for this study were clone I1D8 for SERCA2 (12), clone VE121G9 for SERCA1 (16), NOQ7.5.4D for MHC I (28), and SC-71 for MHC IIa (38). SERCA antibodies (Affinity Bioreagents, Golden, CO) were used at 1:40 dilutions, respectively. MHC IIa antibodies (kindly provided by Dr. Schiaffino, University of Padova, Padova, Italy) were used at 1:40 dilutions, respectively. Localization of the bound monoclonal antibodies was visualized with a fluorescent secondary antibody (i.e., Cy3-conjugated goat anti-mouse IgG, diluted 1:500). Each of the above sections was then digitally captured using our image analysis system (see Ref. 29 for a complete description).

Fiber-type proportions. First, we outlined the perimeter of individual fibers using Scion Image (Scion, Frederick, MD) software and an Intuos graphics tablet (Wacom Technology, Vancouver, WA). We then traced each of these fibers through the next four serial sections that were stained with either SERCA or MHC antibodies. On the basis of immunoreactivity pattern to SERCA antibodies, each of the fibers was categorized as SERCAI (i.e., fibers expressing only fast SERCA isoform), SERCA2 (i.e., fibers expressing only slow SERCA isoform), or hybrid SERCA fibers (i.e., fibers containing both SERCA isoforms). Similarly, fibers expressing only MHC I, MHC IIa, or both MHCs were categorized as types I, IIa, and hybrid fibers, respectively. We then examined the relationship between SERCA and MHC expression patterns in each of the fibers. We analyzed a minimum of 400 fibers (means ± SE, 600 ± 40) for each of the 12 subjects to obtain mean fiber-type proportion as previously described (23, 24).

Fiber-type area fraction. We calculated the area fraction of the diaphragm occupied by a particular fiber type as the quotient of the cross-sectional area of all the fibers analyzed in a given diaphragm and the cross-sectional area of the total diaphragm sample. The COPD subjects did not differ significantly from the control subjects with respect to data sets (27). If the MANOVA indicated statistically significant differences in data sets between the groups, we used group t-tests to compare COPD and control groups with respect to individual measurements. Differences not significant at the 0.05 level were attributed to chance.

RESULTS

Vital Statistics and Pulmonary Function Measurements

The COPD subjects did not differ significantly from the control subjects with respect to age, height, weight, or body mass index (Table 1). The COPD group consisted of 8 men and 2 women, and our control group consisted of 5 men and 5 women.

Table 1 indicates that the COPD subjects had higher values for residual volume, functional residual capacity, and total lung capacity than the control subjects, whereas COPD subjects had lower values with respect to forced expiratory volume in 1 s,
forced vital capacity, and the ratio of the forced expired volume in 1 s to forced vital capacity than the control subjects. The spirometric measurements carried out on the COPD subjects indicated that three had severe COPD and seven had very severe COPD according to the Global Initiative for Chronic Obstructive Lung Disease criteria (5).

SERCA Immunohistochemistry

Comparison of representative COPD and control diaphragms. Figure 1 compares serial sections from a representative COPD and a representative control diaphragm. The figure shows that both control and COPD diaphragms contained the following three types of fibers: 1) those expressing only SERCA1 (i.e., SERCA1 fibers); 2) those expressing only SERCA2 (i.e., SERCA2 fibers); and 3) those expressing both SERCA isoforms (i.e., hybrid fibers). Importantly, the figure shows that the COPD diaphragm contains a lower proportion and a lower area fraction of SERCA1 fibers than the control diaphragm. In contrast, the COPD diaphragm exhibits a higher proportion and a higher area fraction of SERCA2 fibers than the control diaphragm.

Quantitative comparison of COPD and control groups with respect to expression of SERCA isoforms. Figure 2 compares COPD and control diaphragms with respect to fiber types

**Table 1. Comparison of COPD and control subjects with respect to vital statistics and pulmonary function measurements**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>COPD (n = 10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vital statistics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>58±5</td>
<td>59±3</td>
<td>0.44</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166±3</td>
<td>169±3</td>
<td>0.16</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75±4</td>
<td>75±5</td>
<td>0.48</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27±1</td>
<td>26±1</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Spirometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁,₀, Liters</td>
<td>2.34±0.17</td>
<td>0.74±0.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Percent predicted</td>
<td>94±5</td>
<td>25±2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>98±4</td>
<td>71±4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEV₁,₀/FVC × 100</td>
<td>78±4</td>
<td>29±2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Lung volumes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual volume, % predicted</td>
<td>103±8</td>
<td>240±10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Functional residual capacity, % predicted</td>
<td>101±1</td>
<td>179±9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total lung capacity</td>
<td>102±3</td>
<td>131±4</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE. COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV₁,₀, forced expired volume in 1 s; FVC, forced vital capacity. Group t-tests were used to compute the statistical significance of differences between control and COPD groups with respect to vital statistics and pulmonary function tests. Nominal P values for one-tail are reported. In this study, spirometry was measured in all control and COPD patients, whereas lung volumes and capacities were only measured in 5 of the 10 controls and all of the COPD patients.

Serum vital capacity, and the ratio of the forced expired volume in 1 s to forced vital capacity than the control subjects. The spirometric measurements carried out on the COPD subjects indicated that three had severe COPD and seven had very severe COPD according to the Global Initiative for Chronic Obstructive Lung Disease criteria (5).
Table 2. Comparison of COPD and control diaphragms with respect to SERCA-determined area fractions

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>COPD (n = 6)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area fraction, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERCA2</td>
<td>48 ± 2</td>
<td>57 ± 3</td>
<td>0.010</td>
</tr>
<tr>
<td>SERCA1</td>
<td>40 ± 1</td>
<td>15 ± 4</td>
<td>0.0006</td>
</tr>
<tr>
<td>Hybrid</td>
<td>12 ± 3</td>
<td>29 ± 4</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Values are means ± SE. SERCA, sarcoplasmic reticulum Ca^{2+} ATPase.

characterized by SERCA expression. The figure indicates that COPD diaphragms contained an appreciably lower proportion of SERCA1 fibers and greater proportions of SERCA2 and hybrid fibers. Additionally, Table 2 indicates that COPD diaphragms exhibited an appreciably lower area fraction for SERCA1 fibers than controls. In contrast, COPD diaphragms exhibited higher area fractions of SERCA2 and hybrid fibers than controls (Table 2).

MHC Immunohistochemistry

Table 3 compares the COPD and control diaphragms with respect to MHC expression. As expected from our laboratory’s previous work (21–24, 29), COPD diaphragms exhibited higher proportions and area fractions of slow (i.e., type I) fibers. In contrast, COPD diaphragms exhibited higher area fractions of SERCA2 and hybrid fibers (Table 3).

Coexpression Patterns of SERCA and MHC Isoforms

Figure 3 shows the five SERCA and MHC coexpression patterns that we noted in COPD and control diaphragms. The figure shows that the two major coexpression patterns in the control diaphragms were fibers containing SERCA2 and MHC I and those containing SERCA1 and MHC Ila; together, these fiber types accounted for 89% of the fibers in the control diaphragms. The remainder of the control fibers was accounted for by fibers containing both SERCA isoforms in combination with either MHC I, MHC Ila, or both of these MHC isoforms (i.e., hybrid MHC and hybrid SERCA fibers).

Importantly, Fig. 3 indicates that the COPD diaphragms showed the same five coexpression patterns that we noted in control diaphragms. A MANOVA indicated that the COPD and control diaphragms differed with respect to these coexpression data sets. With respect to the two major fiber types in the control diaphragms, COPD diaphragms exhibited a somewhat larger proportion (P < 0.05) of fibers containing SERCA2 and MHC I as well as an appreciably smaller proportion (P < 0.001) of fibers containing SERCA1 and MHC Ila. Unlike the control group, these two coexpression patterns accounted for only 68% of the fibers in COPD diaphragms; therefore, hybrid SERCA fibers constituted 32% of the diaphragm fibers in COPD diaphragms.

With respect to the subsets of hybrid SERCA fibers, Fig. 3 indicates that the COPD diaphragms exhibited a greater proportion of hybrid SERCA fibers coexpressing either MHC I or MHC Ila (P = 0.01 and 0.006, respectively) than control diaphragms; in contrast, COPD and control diaphragms did not differ with respect to the small proportion of hybrid SERCA fibers that contained both MHC isoforms (i.e., I and Ila).

SERCA Protein Isoform Analyses

Electrophoretic mobility. Under the conditions of our experiments (see Methods), we noted that SERCA1 and SERCA2 isoforms had a molecular mass of ~116 kDa in both COPD and control diaphragms.

SERCA1 immunoblots. Figure 4A compares COPD and control diaphragms with respect to expression of SERCA1 on immunoblots; inspection of this panel indicates that COPD diaphragms express less SERCA1 than control diaphragms. Figure 4B compares COPD and control diaphragms with respect to BAP measurements of the blots shown in A; it shows that mean SERCA1 BAP measurements in COPD diaphragms is approximately one-third that noted in control diaphragms (P < 0.01).

SERCA2 immunoblots. Figure 5A compares COPD and control diaphragms with respect to expression of SERCA2 on immunoblots; inspection indicates that COPD and control diaphragms appear to express similar amounts of SERCA2. Figure 5B compares COPD and control diaphragms with respect to BAP measurements of the blots shown in A; it shows that COPD and control diaphragms do not differ with respect to mean BAP measurements of SERCA2.
DISCUSSION

Major Findings

The major histological findings of our study are that the diaphragms of patients with severe COPD exhibit a smaller proportion of fibers containing only SERCA1, a greater proportion of fibers containing only SERCA2, and a greater proportion of fibers containing both SERCA isoforms (i.e., hybrid SERCA fibers) than control diaphragms. Additionally, we noted that fibers containing only SERCA2 express MHC I exclusively; fibers containing only SERCA1 express MHC IIa exclusively; and fibers containing both SERCA isoforms can either express only MHC I, only MHC IIa, or both MHC I and MHC IIa. Last, our immunoblot experiments indicate that COPD diaphragms express appreciably less SERCA1 than control diaphragms; in contrast, COPD and control diaphragms do not differ with respect to SERCA2 expression.

Critique of Experimental Design and Methodology

First, the possibility exists that the SERCA as well as the SERCA-myosin coexpression patterns were due to longitudinal variation in the diaphragm biopsies. To test this hypothesis, we obtained longitudinal sections on representative COPD and control diaphragms. We noted that for any given set of longitudinal sections, there was no variation in SERCA or MHC staining. These observations indicate that longitudinal variations do not account for the SERCA and MHC coexpression patterns noted in RESULTS.

Second, our immunohistochemical experiments only provide data on the presence or absence of particular SERCA or MHC isoforms in any given diaphragm fiber. Therefore, these data do not contain any information about the relative amounts of the two SERCA isoforms in hybrid SERCA fibers or of the relative amounts of the two MHC isoforms in hybrid MHC fibers.

Third, our use of the BAP as a measure of SERCA isoform expression in diaphragm fibers warrants comment. The reason we expressed both SERCA1 and SERCA2 immunoblot measurements in this way is because we did not have pure SERCA isoform standards to obtain the relationship between BAP and SERCA isoform content. We recognize that the validity of these measurements is dependent on loading the same amount of myofiber protein in each lane of the SDS-PAGE; therefore, as noted in METHODS, we can assure the reader that the diaphragm homogenates loaded in each of the electrophoresis lanes (i.e., wells) had less than a 10% variation in myofiber protein concentration; moreover, when normalized for these small variations in protein, the results of our immunoblot experiments remained the same.

Interpretation of Results

Changes in proportions of pure SERCA fibers elicited by COPD. Although SERCA1 and SERCA2 have the same Ca^{2+} ATPase activity in vitro, many studies (in experimental animals) have demonstrated that fibers containing only SERCA1 exhibit two to seven times the Ca^{2+} ATPase activity as fibers containing only SERCA2 (4, 15, 44). In human vastus lateralis and pectoralis muscles, the SERCA activity of fast fibers is 1.3- to 3.4-fold higher than that of slow fibers (37, 41). Electron microscopy experiments indicate that the reason for higher SERCA activity in SERCA1 fibers is that these fibers have a greater volume fraction of SR as well as a greater density of SERCA molecules per unit mass of SR than fibers containing only SERCA2 (2, 3, 6, 7, 44). Therefore, the marked decrease in pure SERCA1 fibers coincident with the small increase in pure SERCA2 fibers would be expected to
decrease total SERCA content per unit mass of diaphragm muscle.

**SERCA isoform changes elicited by severe COPD.** Our immunoblot findings that severe COPD elicits marked decreases in SERCA1 isoform content and no change in SERCA2 isoform content is consistent with our hypothesis that severe COPD elicits decreases in diaphragm SERCA content.

**Increased proportion of hybrid SERCA fibers in COPD diaphragm.** On the basis of the data in Fig. 3, we hypothesize that the complete fast-to-slow transformation of SERCA and MHC isoforms involves the transformation of a SERCA1/MHC IIa fiber into a SERCA2/MHC I fiber. Therefore, we suggest that hybrid fibers for either SERCA and/or MHC represent intermediates in this transformation. In the subjects with severe COPD, 32% of the fibers were hybrid for SERCA, whereas only 7% were hybrid for MHC. Therefore, our data suggest that in diaphragm adaptations elicited by severe COPD, there is some degree of discoordination in the fast-to-slow transformation processes involving SERCA and MHC. This latter finding differs from those of previous workers who reported that chronic low-frequency stimulation of limb muscles is associated with coordinate fast-to-slow isoform transformations of both SERCA and MHC (8, 9).

**Functional Implications of Our Findings**

**Rate of relaxation.** There are three major determinants of relaxation at the myofiber level and they represent the three sequential processes that comprise relaxation; i.e., 1) uptake of myoplasmic Ca$^{2+}$ by SERCA (and temporary binding to parvalbumin); 2) the off-rate of Ca$^{2+}$ from troponin; and 3) the cross-bridge detachment rate constant. In studies of three fiber types in toadfish whose twitch speed varied by 50-fold, we found that all three processes speed up as twitch speed increases (35, 36). We found that in two of the fiber types, cross-bridge detachment was rate limiting, but in the third, Ca$^{2+}$ uptake by SERCA appeared to be rate limiting. These results are consistent with the concept that any of the three above-noted processes can become rate limiting, if that process is slowed while the rate of the other two remains constant.

Indeed, slowing of the SERCA pumping rate by the administration of cyclopiazonic acid in fish muscle results in a slowed relaxation rate (40). In mammals, the combination of the rodent experiments of Dulhunty (1) and Pette and Heilmann (32) and the clinical observations of Karpati et al. (14) in patients with Brody’s disease (a congenital myopathy characterized by very low levels of SERCA content in type II fibers) demonstrate that decreases in SERCA content are associated with decreases in the rate of muscle relaxation. However, Westerblad and Allen (42, 43) and others (10, 25, 26, 33) have convincingly demonstrated that under certain experimental conditions (which presumably have clinical counterparts), processes involving other muscle proteins (e.g., cross-bridge kinetics, parvalbumin, etc.) constitute the rate-limiting factor for muscle relaxation.

**Bioenergetics.** First, as noted in the introduction, the ATP utilization rate of SERCA is appreciable and in some circumstances exceeds that of the cross-bridge ATPase (i.e., MHC ATPase). For example, Szentesi and colleagues (41) compared the ATPase activity of SERCA and the MHC in human myofibers from the vastus lateralis; these fibers were studied under conditions characterized by maximum chemical activation (i.e., Ca$^{2+}$ concentration of 10$^{-4.4}$ mol/l) and isometric contraction. Their results indicate that under these conditions the ratios of maximum ATPase activity of SERCA to the MHC for fiber types I, IIA, IIX, and IIx were 1.1, 0.5, 1.3, and 1.6, respectively. These data indicate that SERCA plays a major role in ATP utilization; therefore, the hypothesized decreases in SERCA content of the COPD diaphragms would be expected to decrease ATP utilization at the organ level (i.e., whole diaphragm).

Second, reduced SERCA pumping and the accompanying slower relaxation will likely change the force-frequency characteristics of diaphragm fibers. This will permit a given level of force generation with a lower frequency of stimulation, which in turn will reduce the energetic cost of Ca$^{2+}$ pumping in the diaphragm fibers and thereby increase efficiency. In intact fibers, as opposed to the skinned fibers discussed above (where Ca$^{2+}$ is pumped at a constant rate), the amount of ATP utilized for a given tetanic contraction depends on the amount of Ca$^{2+}$ released during the contraction (11, 31). There is usually a large amount of Ca$^{2+}$ released during the first stimulus, and smaller amounts are released during the subsequent stimuli within the contraction to maintain the level of activation. If Ca$^{2+}$ is removed from the myoplasm very quickly, then Ca$^{2+}$ must be released at a high rate to maintain activation. By contrast, if Ca$^{2+}$ is removed from the myoplasm slowly, then Ca$^{2+}$ needs to be released at only a low rate to maintain activation. Ultimately, at the end of the contraction, all of the Ca$^{2+}$ that was released is pumped back into the SR; hence the fiber with a slower total SERCA pumping rate will use less ATP during a contraction of a given duration and hence will be more economical.

In summary, the combination of the SERCA immunohistochemical and immunoblot measurements in this manuscript is consistent with our hypothesis that diaphragm remodeling elicited by severe COPD should decrease ATP utilization by the diaphragm.

**GRANTS**

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