Eur Respir J 1998; 11: 758–766
DOI: 10.1183/09031936.98.11030758

European Respiratory Journal
ISSN 0903 - 1936

SERIES 'CELL BIOLOGY OF RESPIRATORY MUSCLES'
Edited by M. Decramer and M. Aubier
Number 4 in this Series

Calcium ATPase and respiratory muscle function

M. Aubier, N.Viires

Calcium ATPase and respiraory muscle function. M. Aubier, N. Viires. ©ERS Journals Ltd 1998.
ABSTRACT: The sarcoplasmic reticulum (SR) of striated muscle is a highly specialized intracellular membrane system that plays a key role in the contraction-relaxation cycle of muscle. Its primary function is the regulation of cytoplasmic Ca^{2+} concentration. A key element in this regulation is the Sarco(endo)plasmic reticulum Ca^{2+}-adenosine triphosphatase (SERCA), which by sequestering Ca^{2+} into the SR, induces and maintains relaxation. It has been extensively studied with respect to structure and mechanism of action, and more recently to gene expression. Three separate genes encode five SERCA isoforms, two of which, SERCA 1 and SERCA 2, are expressed in skeletal muscle.

In the first part of this review we focus on the general properties of the Ca^{2+} pump (structure and function and regulation of activity). In the second part we describe variations in SERCA expression in various physiological and pathological situations. These have essentially been studied in the heart and skeletal muscles, with data in respiratory muscles being very limited.
Eur Respir J 1998; 11: 758–766.

INSERM U 408, Unité de Pneumologie, Hôpital Bichat, 46, rue H. Huchard, 75018 Paris, France.

Correspondence: M. Aubier
Unité de Pneumologie
46, rue Henri Huchard
75018 Paris
France
Fax: 33 140258818

Keywords: Diaphragm
respiratory muslces
sarcoplasmic reticulum
SERCA pumps

Received: September 29 1997
Accepted after revision November 15 1997

The major proteins responsible for contraction and relaxation in skeletal muscle are myosin and the sarcoplasmic reticulum (SR) Ca^{2+}-adenosine triphosphatase (ATPase), respectively. Both these proteins exist as multiple isoforms and contribute to defining skeletal muscle phenotype. While changes in myosin isoform composition have been extensively studied in physiopathological situations, comparatively little is known of the expression or regulation of the Ca^{2+}-ATPase isoforms.

Ca^{2+}-ATPases constitute a large family of proteins that fall into two distinct groups, the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), and the plasma membrane Ca^{2+}-ATPase (PMCA). Most eukaryotic cells coexpress, in a tissue-specific and differentiation stage-specific manner, one or more types of SERCA and PMCA pumps. This review will focus on the SERCA pumps.

The skeletal muscle SR Ca^{2+}-ATPase is part of the SERCA family of calcium pumps involved in the transport of calcium from the cytosol to various intracellular stores such as the SR, the endoplasmic reticulum (ER) and calciosomes. It is present in several cell types and plays an important role in controlling cellular functions such as relaxation and secretion. In skeletal muscle it is localized in the SR.

Sarcoplasmic reticulum

The SR is an intracellular membrane network that is in close contact with the myofibrils and couples with the sarcolemma through transverse tubules (T tubules). Depolarizing currents in the transverse tubule culminate in a signal for Ca^{2+} release from the SR, which in turn initiates muscle contraction. The SR has two additional functions essential to excitation-contraction coupling, namely Ca^{2+} reuptake to initiate muscle relaxation, and Ca^{2+} storage to maintain relaxed muscle in a quiescent state. The ability of this system to regulate cytoplasmic Ca^{2+} concentrations plays a central role in the contraction-relaxation cycle of skeletal, cardiac and, to a lesser degree, smooth muscle [1–5].

In recent years, an understanding of the molecular events involved in Ca^{2+} regulation by the SR has come about through resolution of the sarco-tubular system into its component membrane domains and through isolation, reconstitution and biochemical analysis of individual proteins in these domains.

In skeletal muscles, the SR membrane system *in situ* is composed of two distinct portions: 1) voluminous, matrix filled terminal cisternae which are associated with the transverse tubule; and 2) the longitudinal SR,which contains very little lumenal structure and connects medially with the two terminal cisternae [6].

It is now clear that certain functions of the SR are restricted to specific regions of this membrane system [7] (fig. 1).

Early fractionation of the SR by sucrose gradient centrifugation and subsequently freeze fracture techniques, showed that two distinct heavy and light fractions could be isolated [6, 8].

Previous articles in this series: No. 1: G.C. Sieck, Y.S. Prakash. Cross bridge kinetics in respiratory muscles. *Eur Respir J* 1997; 10: 2147–2158. **No. 2:** J.G. Gea. Myosin gene expression in the respiratory muscles. *Eur Respir J* 1997; 10: 2404–2410. **No. 3:** B.J. Petrof. Respiratory muscles as a target for adenovirus-mediated gene therapy. *Eur Respir J* 1998; 11: 492–497.

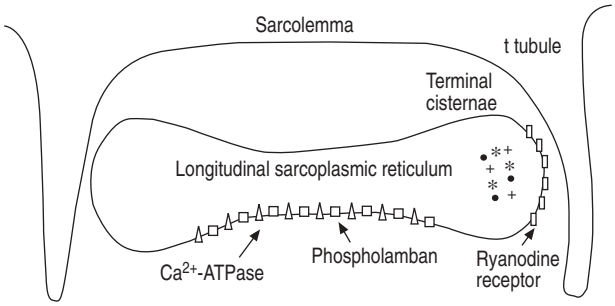

Fig. 1. – Schematic representation of the sarcoplasmic reticulum showing the arrangement of constituent proteins. Within the longitudinal membrane the major protein is the Ca^{2+}-adenosine triphosphatase (ATPase). Phospholamban is also present with a similar distribution to the Ca^{2+} pump. The terminal cisternae contains the acidic calcium binding proteins calsequestrin (●), calreticulin (+) and a 170 kDa protein, now referred to as the histidine rich Ca^{2+} binding protein (*).

The light fraction, which corresponds predominantly to the longitudinal SR, is primarily concerned with the uptake of calcium and contains the 110 kDa Ca^{2+}-ATPase as it's major contituent. Lumenal glycoproteins of 53 and 160 kDa are also present in this fraction. In cardiac muscle and in slow twitch skeletal muscle, the regulatory protein phospholamban (a homomeric pentamer of 6 kDa subunits), which is thought to interact with the calcium pump and mediate the effects of catecholamines on Ca^{2+} transport, is present with a similar distribution to the Ca^{2+} pump.

The heavy fraction, corresponding to the terminal cisternae, is the site of calcium release and storage. It contains the calcium release channel or ryanodine receptor, which is a high molecular weight tetramer made up of 565 kDa subunits. The acidic calcium binding proteins calsequestrin, calreticulin and a 170 kDa protein now referred to as the histidine rich Ca^{2+} binding protein, are also located in this fraction [9–12].

Recent molecular cloning analysis have demonstrated the existence of distinct isoforms of many of these proteins. Skeletal muscle isoforms of the ryanodine receptor, the calcium ATPase (SERCA) and calsequestrin have been identified, although very little is known about the inter-relations between these isoforms or about their regulation.

Structure and function

The SERCA of the SR plays a key role in regulation of skeletal muscle function. By pumping calcium from the sarcoplasm to luminal spaces in the organelle it lowers sarcoplasmic Ca^{2+} concentration (to the range of 100 nM), thereby inducing and maintaining muscle relaxation. It represents 60–80% of the total protein content in the SR of adult animals and has been extensively studied with respect to its structure, reaction kinetics and gene expression [13–25].

The Ca^{2+}-ATPase is a single large polypeptide with a molecular weight of 100 kDa. Electron microscopic and x-ray diffraction studies have revealed that it is comprised of a cytoplasmic headpiece and stalk sectors and a transmembrane basepiece, making up a tripartite structure (fig. 2). The enzyme is asymetrically oriented in the membrane with virtually all of its extramembranous mass in

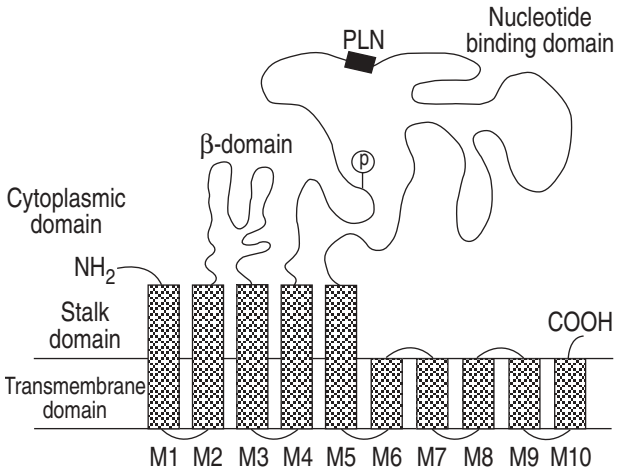

Fig. 2. – Structural diagram of the Ca^{2+}-adenosine triphosphatase (ATPase) molecule. Ten putative transmembrane segments (M1–M10) are shown. Sites for the binding of phospholamban (PLN) and for regulatory serine phosphorylation of the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) 2 isoform (P) are also indicated. Modified from [1, 24].

the cytoplasm. It catalyses Ca^{2+} transport to the lumen of the SR by an active process that requires adenosine triphosphate (ATP). Enzyme phosphorylation and ATP hydrolysis result in translocation of the two Ca^{2+} ions bound to the enzyme from a high affinity site to a low affinity site. The two calcium ions are then released into the lumen of the SR (fig. 3).

The Ca^{2+}-ATPase has been purified and its primary structure determined by direct amino acid sequence determination and by complementary deoxyribonucleic acid (DNA) cloning (fig. 2). Analysis of hydrophobic sequences led to the assignment of 10 transmembrane helices (M1 to M10): four in the NH_2 terminal quarter and six in

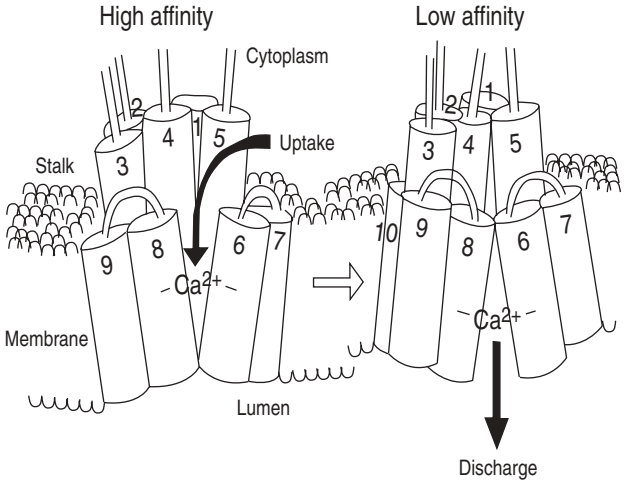

Fig. 3. – Model illustrating the mechanism of Ca^{2+} transport by the Ca^{2+}-adenosine triphosphatase (ATPase). In the high affinity state, high affinity Ca^{2+} binding sites located near the centre of the transmembrane domain are accessible to cytoplasmic calcium but not to luminal calcium. The sites are made up from amino acid residues located in proposed transmembrane sequences M4, M5, M6 and M8. Conformational changes induced by adenosine triphosphate (ATP) hydrolysis lead to the low affinity state, in which high affinity calcium binding sites are disrupted, access to the sites by cytoplasmic Ca^{2+} is closed off and access to the sites by luminal calcium is gained. From [1].

the COOH terminal quarter to make up a basepiece. The stalk sector is made up of five α-helices that are contiguous with transmembrane helices. The large globular cytoplasmic headpiece is composed of three segments: the β-strand, between transmembrane segments 2 and 3, the phophorylation and phospholamban binding sites, attached to segment 4 and the nucleotide binding domain attached to segment 5. Structural interactions between the nucleotide binding region and the COOH-terminal transmembrane domains appear to determine isoform-specific calcium dependances [26].

The structure-function relationships have beeen extensively studied by MACLENNAN and co-workers [27–29] and others [24] using site directed mutagenesis.

To date more than 200 different point mutants of the SERCA have been expressed transiently in mammalian cell lines and analysed for function by a panel of assays comprising measurement of the rates of Ca^{2+} uptake in microsomal vescicles and ATP hydrolysis, phosphorylation from ATP or inorganic phosphate (Pi), as well as Ca^{2+} occlusion stabilized with β,γ-bidentate chromium (III) complex of ATP (CrATP) (reviewed by ANDERSEN *et al.* [16]). Mutations performed on amino acids in transmembrane sequences M4, M5, M6 and M8 have identifed this region as the calcium binding and translocation domain (although M8 plays a peripheral role in these functions as compared to the other three residues [29]. Five residues, glutamic acid $(Glu)^{309}$, Glu^{771}, asparagine $(Asn)^{796}$, threonine $(Thr)^{799}$, and aspartic acid $(Asp)^{800}$, located in transmembrane segments M4, M5 and M6, appear to have unique importance since it was impossible to alter one of these residues without a complete loss of the ability to occlude calcium [24]. Aspartic acid-351 has been identified as the site of catalytic phosphorylation, while lysine $(Lys)^{515}$ is involved in ATP binding (fig. 2).

Isoforms

Three separate genes encode the SERCA family of calcium pumps.

The SERCA 1 gene is exclusively expressed in fast twitch skeletal muscle. Developmentally regulated alternative splicing of SERCA 1 results in an adult isoform (SERCA 1a) and a neonatal isoform (SERCA 1b) [30–32].

The SERCA 2 gene is expressed in slow-twitch skeletal muscle, cardiac muscle, smooth muscle and nonmuscle tissues. Tissue-dependant processing of the SERCA 2 gene transcript yields four SERCA 2 messenger ribonucleic acids (mRNAs) (classes 1–4). Class 1 mRNA encodes the SERCA 2a isoform, found in cardiac, smooth and slow twitch skeletal muscles. Class 2, 3 and 4 mRNAs encode the SERCA 2b "housekeeping" isoform, ubiquitously expressed at low levels in all cell types, but mainly in smooth muscle and nonmuscle tissues [17, 33–37].

A third isoform, SERCA 3, is less well documented, but like the SERCA 2 gene, shows widespread tissue distribution [38]. Recent studies have demonstrated SERCA 3 mRNA in endothelial and epithelial cells, platelets, the T-lymphoblastoid Jurkat cell line and in the heart tube at early stages of development [39–41]. It has also been detected in soleus and diaphragm muscle, but at very low levels.

At the protein level, SERCA 1 and SERCA 2 show 84% sequence homology. SERCA 3 is 75% identical to SERCA 1 or to SERCA 2 [42]. The isoforms of the SERCA 2 gene differ only with respect to their C-terminal part. The last four amino acids in the SERCA 2a isoform are replaced by an extended sequence of 49 amino acids in SERCA 2b. The C-terminus of SERCA 2a is located in the cytosol whereas that of SERCA 2b is in the lumen. As a consequence of their conserved primary structure, all of the known SERCA isoforms are predicted to have essentially identical transmembrane topologies and tertiary structure. Site-directed mutagenesis studies have also revealed that residues that are critical for normal functioning of the enzyme and pump are conserved among all the isoforms.

Despite these similarities, it seemed likely that there would be functional differences among isoforms, which combined with tissue- or cell-specific expression might impart unique properties of calcium homeostasis to certain cells seemed likely [42]. Functional comparisons between isoforms of the SERCA pumps were, thus, carried out by LYTTON *et al.* [42]. A COS-1 cell (a monkey kidney cell line) expression system was used to examine the biochemical properties of SERCA 1, 2a, 2b and 3. All isoforms displayed qualitativley similar enzymatic properties and were activated by calcium in a co-operative manner. The quantitative properties of SERCA 1 and SERCA 2 were identical in all respects. SERCA 2b, however, appeared to have a lower turnover rate for both calcium transport and ATP hydrolysis. SERCA 3 displayed a reduced appparent affinity for calcium, and increased affinity for vanadate and an altered pH dependance as compared to the other isoforms.

It has been demonstrated that the denisity of pumping sites is increased when the fast (SERCA 1) *versus* the slow (SERCA 2) isoform is expressed and, thus, total SERCA protein density largely accounts for the different Ca^{2+} uptake capacities in fast- and slow-twitch muscles [43–51].

Evidence for variations in intrinsic functional properties between SERCA 1 and SERCA 2 isoforms has been provided by observations demonstrating the inability of the slow (SERCA 2) as opposed to the fast (SERCA 1) muscle enzyme to utilize guanine triphosphate (GTP) as a substrate for Ca^{2+}-dependant phosphoenzyme formation and Ca^{2+} transport, and by the fact that the activity of SERCA 2 and not that of SERCA 1 can be regulated by the intrinsic membrane proteins phospholamban or CaM kinase (see below) [51].

Regulation of SERCA pump activity

The activity of the Ca^{2+}-ATPase in cardiac and slow-twitch skeletal muscles is regulated by interaction with phospholamban (PLN). Phospholamban, a small transmembrane homopentamer of 52 amino acids, is co-localized with SERCA 2 in the longitudinal SR membrane. The NH_2-terminal half of each monomer is hydrophilic and positively charged, whereas the hydrophobic COOH-terminal half is responsible for anchoring the protein into the SR membrane. Current models of Ca^{2+}-ATPase regulation by phospholamban depict unphosphorylated phospholamban as an inhibitor of the Ca^{2+}-ATPase. Inhibition is exerted by association of the two proteins [13, 51–54].

Phosphorylation of PNL by Ca^{2+}/calmodulin-dependant or cyclic adenosine monophosphate (cAMP) dependant protein kinases, at adjacent residues, leads to the expression of full ATPase activity, presumably as a result of dissociation of PLN from the ATPase. The effect of phosphorylation of PLN is to increase the affinity of the ATPase for calcium, thus resulting in an increased rate of calcium transport [55–58].

In vivo only SERCA 2 activity is inhibited by unphosphorylated phospholamban [49]. However when expressed in COS-1 cells, the activities of SERCA 1, SERCA 2a and SERCA 2b were all affected by phospholamban, whereas SERCA 3 conserved its sensitivity for Ca^{2+} [59]. The absence of sensitivity of SERCA 1 to phospholamban *in vivo* is not due to differences in the sequence of the phospholamban binding site, but rather to the absence of expression of the phospholamban gene in this tissue. The phospholamban binding site in SERCA 3 is very different from that in the other Ca^{2+}-ATPases, explaining why SERCA 3 is not inhibited by phospholamban [59].

Recent studies have demonstrated that the expression of SERCA 2 and phospholamban can be differentially regulated [60]. For example, SERCA 2 is expressed before phospholamban during muscle development [61]. In animals treated with thyroid hormone there is an increase in SERCA 2 mRNA and a decrease in phospholamban mRNA [62]. On the other hand, under some circumstances the transcription of the two genes can be co-ordinated [63].

A recent study using monoclonal antibodies against phospholamban showed little effect on calcium uptake in fast or slow skeletal muscle SR vesicles, whereas there was a significant stimulatory effect on calcium uptake with the antibody in cardiac SR [64]. Thus, the *in vivo* role of phospholamban in slow-twitch skeletal muscle is unclear.

It has, thus, been suggested that phospholamban in slow-twitch muscle has a different function to that in cardiac muscle [65]. Whether or not the calcium channel forming property of phospholamban has a role in any of these processes remains to be investigated [64].

In addition to phospholamban phosphorylation, recent studies have demonstrated direct phosphorylation of SERCA 2 by membrane-associated CaM kinase 11 [51], resulting in an increased maximum velocity (V_{max}). This may provide a novel mechanism for the modulation of the enzymatic and Ca^{2+} transport functions of this enzyme in cardiac and slow-twitch skeletal muscle. This finding has not been confirmed by the team of MACLENNAN and coworkers [66].

SERCA pump activity can be negatively modulated by reactive oxygen species (ROS) [67–73]. This has important consequences (besides alteration in muscle relaxation rates) in view of the fact that an early biological event associated with oxidative stress is the loss of calcium homeostasis [74–77]. Indeed, changes in intracellular calcium levels have been implicated in mechanisms of oxidative cell injury in pathophysiological conditions (reviewed in [68]). One of the early events attendant on an elevated Ca^{2+} concentration is an impairement of mitochondrial function. Therefore, impairement of SR function (decreased SERCA activity) may be a requirement for calcium induced mitochondrial damage and subsequent cell death [69].

It has recently been demonstrated that SERCA constitutes a major target for ROS both *in vitro* and *in vivo* [78, 79]. In addition, peroxynitrite has recently been shown to inactivate the calcium pump *in vitro* [67]. Peroxynitrite has been identified as a potentially harmful reactive oxygen species due to the high reactivity and selectivity in its reaction with biomolecules such as lipids and proteins. Peroxynitrite forms under conditions of simultaneous generation of superoxide and nitric oxide. Its reaction with tyrosine leads to oxidation, hydroxylation and to ortho-nitrotyrosine. The latter has been discussed as a biological marker for the assessement of the exposure of tissue to oxidative stress, and in particular NO-derived ROS. In this connection it is interesting to cite a recent study, which demonstrates that during biological aging, significant amounts of nitrotyrosine accumulate on the skeletal muscle ATPase and that this modification is selective to the SERCA 2a isoform [80]. However, the physiological significance of this finding remains to be discerned.

Changes in SR function and SERCA gene expression in different physiological or pathological situations

Cardiovascular

Changes in SR function and SERCA gene expression have been extensively studied in the cardiovascular system (myocardium and vessels) as alterations in myocardial relaxation are associated with most cardiac diseases. In cardiac muscle the SERCA 2 gene has been shown to be regulated by a number of factors shown in table 1 [59]. SERCA 2 levels are increased by thyroid hormone and decreased by pressure overload and during end-stage heart failure.

Recently, knock out of the phospholamban gene was carried out in an elegant study by the team of KRANIAS and co-workers [80]. The phospholamban deficient anim-als (mice) showed no gross developmental abnormalities, but

Table 1. – Changes in sarcoplasmic reticulum (SR) function and sarco(endo)plasmic reticulum Ca^{2+}-adenosine triphosphatase (SERCA) gene expression

Physiological or pathological situation	SR function	SERCA gene expression
Heart		
Birth *versus* foetus	↑	↑
Senescent *versus* adult	↓	↓
Pressure overload in rat and rabbit	↓	↓
Cardiomyopathy of Syrian hamster		
Hypertrophic strain	→ or ↓	↓
Dilated strain	→ or ↓	
Human heart failure	→ or ↓	↓
Thyroid hormone		
Hypothyroidism	↓	↓
Hyperthyroidism	↑	↑
Conditioning swimming	↑	↑
Vessels		
Development (from 5 W to 17 W)	→	2a ↑ 2b →
Hypertension	↑	2a ↑ 2b ↑

↑: increased; →: unchanged; ↓: decreased. From [60]. 2a, 2b: SERCA 2a and 2b isoforms, respectively.

exhibited enhanced myocardial performance without changes in cardiac frequency. This resulted in enhanced cardiac performance, SR function and Ca^{2+} uptake. These findings indicate that phospholamban acts as a critical repressor of basal myocardial contractility and may be a key phosphoprotein in mediating the heart's contractile responses to β-adrenergic agonists.

Skeletal muscle

Comparatively little is known regarding SERCA expression and regulation in skeletal muscle. The results of recent studies are summarized in table 2 [82–89].

Collectively, these studies show that the expression of SERCA isoforms (and related functional properties) can be regulated by a number of factors, often in a tissue specific manner. They further demonstrate that pre- as well as post-translational levels of regulation exist.

Respiratory muscles (diaphragm)

The remainder of this review will be devoted to the respiratory muscles and, more specifically, to the diaphragm.

The diaphragm, like the heart, contracts rhythmically for life and must return at the end of each relaxation phase to a relatively constant resting position. While numerous studies have elucidated its contractile process, the mechanical properties of diaphragmatic relaxation were virtually ignored until the recent elegant studies carried out by the team of LECARPENTIER and co-workers [90, 91].

Mechanical indices of relaxation reflect the abilites of the Ca^{2+}-ATPase to sequestrate calcium into the SR. Thus, an interesting finding of their studies was the demonstration that the diaphragm, like the heart, shows "load sensitivity of relaxation". This mechanical property reflects the diaphragm's intrinsic capacity to control relaxation according to the level of load. In the heart, this property has been shown to imply a well functioning SR, and is absent under various conditions in which the SR is poorly developed, nonfunctional, destroyed or inhibited [92].

The authors further examined diaphragmatic fatigue, and found marked alterations in relaxation consisting of an inhibition of load dependance in addition to an increased half relaxation time (1/2 RT). These findings strongly implicate the Ca^{2+}-ATPase pumps in the fatigue process. The load sensitivity of relaxation might be of particular benefit during high-frequency breathing when the diaphragm muscle must return rapidly to its resting length. If such a mechanism ever fails, incomplete relaxation will shift the diaphragm along its passive length-tension curve, thus placing it at a mechanical disadvantage for optimal force generation.

Studies in humans have also demonstrated slowing of diaphragmatic relaxation rate during fatiguing contractions [93, 94]. Fatigue has also been demonstrated in the respiratory muscles of patients with chronic airway obstruction. In these patients the rate of relaxation of the diaphragm decreases while it becomes fatigued [95, 96].

Despite the important clinical implications of altered diaphragmatic relaxation, the molecular mechanisms involved have not been studied. Only three studies have examined SERCA expression in the diaphragm and, thus, virtually nothing is known regarding regulatory mechanisms.

A study by DILLMANN and co-workers [97] compared thyroid hormone responses of Ca^{2+}-ATPases of various muscles (including the diaphragm). Their results, shown in figure 4, clearly differentiate the diaphragm from other muscles in that SERCA expression is not under the control of thyroid hormones.

ANGER *et al.* [98] have recently investigated the ex-pression of the genes encoding the SERCA pumps in the heart and diaphragm of the cardiomyopathic Syrian hamster (CSH) of the dilated Bio53-58 strain. The myopathy of the CSH is characterized by cellular necrosis, which affects several tissues including the diaphragm. Myocardial contractility is depressed and is associated with impairment of diaphragmatic mechanics (including prolonged relaxation rates) at a stage when congestive heart failure is not yet observed. The impairment of diaphragm function is partly responsible for alveolar hypoventilation.

Table 2. – Changes in SERCA gene and protein expression in skeletal muscle in different pathophysiological situations

Situation/muscle	SERCA (mRNA)	SERCA (protein)	First author [Ref.]
Notexin induced necrosis	SERCA 2 ↓↓		ZÁDOR [83]
(*soleus*)	SERCA 1 ↓↓		
Functional overload		SERCA 1 ↓	TALMADGE [84]
(*cat plantaris*)		SERCA 2 ↑	
Thyroid hormone (rat)			
soleus	SERCA 1 ↑↑	SERCA 1 ↑↑	VANDER LINDEN [85]
	SERCA 2 -	SERCA 2 ↓	
EDL	SERCA 2 ↓↓	SERCA 2 ↓↓	
Heart failure	SERCA 2 ↓	SERCA 2 ↓	SIMONN [86]
(*rat soleus*)			
Overload	SERCA 1 ↓	SERCA 1 ↓	KANDARIAN [87]
(*rat plantaris*)	SERCA 2 ↑	SERCA 2 ↑	
Denervation - 28 days (rat)			
soleus	SERCA 2 ↓	SERCA 2 ↓	SCHUTTE [88]
EDL	SERCA 2 ↓	SERCA 1 ↓	
Unloading (rat)			
soleus	SERCA 1 ↑↑	SERCA 1 ↑	SCHUTTE [89]
EDL	SERCA 2 ↓	SERCA 2 ↓	
Chronic electrical stimulation		SERCA 1 ↓	BRIGGS [90]
(*dog latissimus dorsi*)		SERCA 2 ↑	

mRNA: messenger ribonucleic acid; EDL: extensor digitorum longus. For further definitions, see table 1.

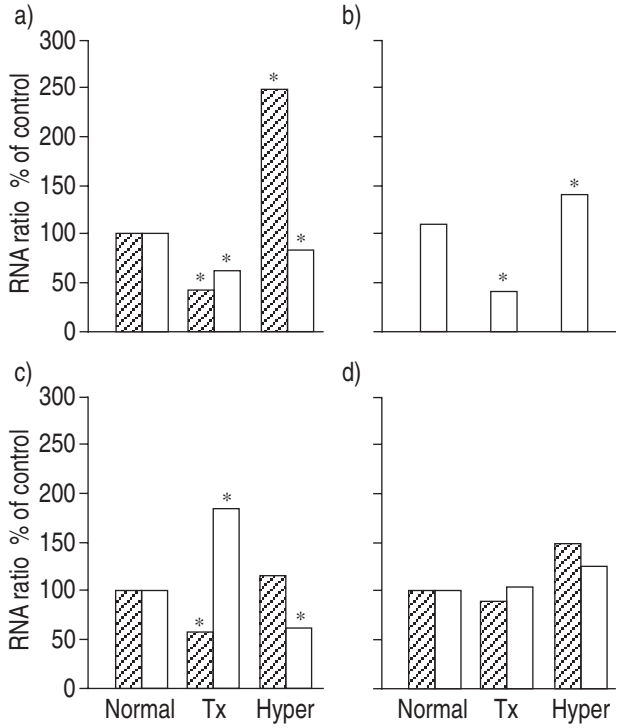

Fig. 4. – Influence of thyroid status on mRNA levels of SERCA 1 (▨) and SERCA 2 (□) in: a) soleus; b) heart; c) extensor digitorum longus; and d) diaphragm. The ratio of Ca^{2+} adenosine triphosphatase (ATPase) mRNA over HSP70c mRNA in control animals was set at 100% and changes induced by alterations in thyroid status expressed in relation to control. *: $p<0.05$ *versus* controls. Tx: hypothyroid status; Hyper: hyperactive thyroid. For further definitions of abbreviations, see tables 1 and 2.

At 6 months, the myopathic process resulted in a decreased expression of SERCA 1 with levels of SERCA 2 being unchanged in the diaphragm. SERCA gene expression was subsequently also altered in the heart (but at 9 months).

We have recently examined the effects of chronic corticosteroid administration on SERCA expression in the diaphragm. The rationale for the hypothesis that corticosteroids modify SERCA expression is the demonstration of increased twitch relaxation times and a leftward shift in force-frequency curves following chronic administration of triamcinolone [99, 100].

Our results demonstrate an increased expression of SERCA 2 mRNA in the diaphragm of steroid treated (ST) animals as detected by northern blot analysis, although this did not reach statistical significance (N. Viires, A-M. Lompré, *et al*. unpublished observations). At the protein level, however, no significant difference between the two experimental groups of animals (ST and controls) was detected. This could be due in part to the relatively long half life of the protein. We further examined the expression of phospholamban in the diaphragm in as much as the expression of these two proteins is not always regulated in a co-ordinated manner. The expression of this regulatory protein was not influenced by steroid treatment. While our results show that corticosteroids do not alter the expression of these proteins in the diaphragm, we cannot conclude that they are not targets for steroid action. In this connection further studies are needed to determine whether the activity of SERCA pumps is modified by these agents.

In summary, in contrast to the situation in cardiac or skeletal muscles, very little is known regarding the regulation of the expression of SERCA and phospholamban levels in the diaphragm. These proteins play an important role in sarcoplasmic reticulum function and may thus be involved in long-term changes in muscle contractility (notably relaxation). Further studies are clearly needed, however, to determine the factors that control the expression of SERCA (phospholamban) genes in the diaphragm.

Acknowledgement: The authors gratefully acknowledge the excellent collaboration of A.M. Lompré in these studies.

References

1. MacLennan DH. Molecular tools to elucidate problems in excitation-contraction coupling. *Biophys J* 1990; 58: 1355–1365.
2. Kirtley ME, Sumbilla C, Inesi G. Mechanisms of calcium uptake and release by sarcoplasmic reticulum. *In*: Intracellular Calcium Regulation. Alan R, ed. Liss, Inc, 1990; pp. 249–270.
3. Fleischer S, Inui M. Biochemistry and biophysics of excitationcontraction coupling. *Annu Rev Biochem Biophys* 1989; 18: 333–364.
4. Ebashi S, Endo M, Ohtsuki T. Control of muscle contraction. *Q Rev Biophys* 1969; 3: 351–384.
5. Hasselbach W. Relaxing factor and relaxation of muscle. *Prog Biophys Mol Biol* 1964; 14: 167–222.
6. Saito A, Seiler S, Chu A, Fleischer S. Preparation and morphology of sarcoplasmic reticulum terminal citernae from rabbit skeletal muscle. *J Cell Biol* 1984; 99: 875–885.
7. Jorgensen AO, Shen ACY, MacLennan DH, Tokuyasu KT. Ultrastructural localization of the Ca^{2+} + Mg^{2+}-dependant ATPase of sarcoplasmic reticulum in rat skeletal muscle by immunoferritin labelling of ultrathin frozen sections. *J Cell Biol* 1982; 92: 409–416.
8. Kelly DE, Kuda AM. Subunits of the triadic junction in fast skeletal muscle as revealed by freeze-fracture. *J Ultrastruc Res* 1979; 68: 220–233.
9. Franzini-Armstrong C. Structure of sarcoplasmic reticulum. *Fed Proc* 1980; 39: 2403–2409.
10. Franzini-Armstrong C, Nunzi C. Junctional feet and particles in the triads of a fast-twitch muscle fiber. *J Muscle Res Cell Motil* 1983; 4: 233–252.
11. Meissner G, Conner G, Fleischer S. Isolation of sarcoplasmic reticulum by zonal centrifugation and purification of Ca^{2+} pump and Ca^{2+}-binding proteins. *Biochem Biophys Acta* 1973; 298: 246–269.
12. Mitchell RD, Saito A, Palade P, Fleischer S. Morphology of isolated triads. *J Cell Biol* 1983; 96: 1017–1029.
13. Lytton J, MacLennan DH. Sarcoplasmic reticulum. *In*: The Heart and Cardiovascular System, second edition. Fozzard HA, *et al*., eds. New York, Raven Press, Ltd., 1990; pp. 1203–1222.
14. Maruyama K, Clarke DM, Fujii J, Loo TW, MacLennan DH. Expression and mutation of Ca^{2+}-ATPase of the sarcoplasmic reticulum. *Cell Motil Cytoskeleton* 1989; 14: 26–34.
15. Martonosi A. The development of sarcoplasmic reticulum membranes. *Annu Rev Physiol* 1982; 44: 337–355.
16. Korczak B, Zarain-Herzberg A, Brandl CJ, Ingles CJ, Green NM, MacLennan DH. Structure of the rabbit fast-twitch skeletal muscle Ca^{2+}-ATPase gene. *J Biol Chem* 1988; 263 (10): 4813–4819.

17. Zarain-Herzbezrg A, MacLennan DH, Periasamy M. Characterization of rabbit cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase gene. *J Biol Chem* 1990; 265(8): 4670–4677.
18. Toyoshima C, Sasbe H, Stokes DL. Three dimensional cryo-electron microscopy of the calcium ion pump in the sarcoplasmic reticulum membrane. *Nature* 1993; 362: 469–471.
19. Brandl CJ, Green NM, Korzack B, MacLennan DH. Two Ca^{2+} ATPase genes: homologies and mechanistic implications of deduced amino acid sequences. *Cell* 1986; 44: 597–607.
20. Brandl CJ, deLeon S, Martin DR, MacLennan DH. Adult forms of the Ca^{2+}-ATPase of sarcoplasmic reticulum. Expression in developing muscle. *J Biol Chem* 1987; 262: 3768–3774.
21. Eggermont JA, Wuytack F, Casteels R. Characterization of the 3' end of the pig sarcopolasmic/endoplasmic-reticulum Ca^{2+}-pump gene 2. *Biochem Biophys Acta* 1991; 1088: 448–451.
22. Maruyama K, MacLennan DH. Mutation of aspartic acid-351, lysine-352 and lysine-515 alters the Ca^{2+} transport activity of the Ca^{2+}-ATPase expressed in COS-1 cells. *Proc Natl Acad Sci USA* 1988; 85: 3314–3318.
23. Lytton J, MacLennan DH. Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca^{2+}-ATPase gene. *J Biol Chem* 1988; 263: 15024–15031.
24. Andersen JP, Vilsen B. Structure-function relationships of cation translocation by Ca^{2+}-and Na^{+}, K^{+}-ATPases studied by site directed mutagenesis. *FEBS Letters* 1995; 359: 101–106.
25. Wuytack F, Raemaekers L, DeSmedt H, *et al*. Ca^{2+}-transport ATPases and their regulation in muscle and brain. *Ann NY Acad Sci* 82–91,
26. Toyofuko T. Kurzydlowski K, Lytton J, MacLennan DH. The nucleotide binding/hinge domain plays a crucial role in determining isoformspecific Ca^{2+} dependence of organellar Ca(2+)-ATPases. *J Biol Chem* 1992; 267: 14490–14496.
27. MacLennan DH, Toyofuku T. Structure-function relationships in the Ca^{2+} pump of the sarcoplasmic reticulum. *Biochemical Soc Trans* 1992; 20(3): 559–562.
28. MacLennan DH, Clark DM, Loo TW, Skerjanc IS. Site-directed mutagenesis of the Ca^{2+}ATPase of sarcoplasmic reticulum. *Acta Physiol Scand Suppl* 1992; 607: 141–150.
29. Rice WJ, MacLennan DH. Scanning mutagenesis reveals a similar pattern of mutation sensitivity in transmembrane sequences M4, M5, and M6, but not in M8 of the Ca^{2+}ATPase of sarcoplasmic reticulum (Serca 1a). *J Biol Chem* 1996; 271(49): 31412–31419.
30. Korczak B, Zarain-Herzberg A, Brandl CJ, Ingles CJ, Green NM, MacLennan DH. Structure of the rabbit fast-twitch skeletal muscle Ca^{2+}ATPase gene. *J Biol Chem* 1988; 263: 4813–4819.
31. Zhang Y, Phillips MS, Chen HS, *et al*. Characterization of cDNA and genomic DNA encoding SERCA 1, the Ca^{2+}-ATPase of human fast-twitch skeletal muscle sarcoplasmic reticulum, and its elimination as a candidate for Brody's disease. *Genomics* 1995; 30(3): 415–424.
32. MacLennan DH, Brandl CJ, Champaneria S, Holland PC, Powers VE, Willard HF. Fast-twitch and slow-twitch-cardiac calcium ATPase genes map to human chromosomes 16 and 12. *Somatic Cell Mol Genet* 1987; 13: 341–346.
33. DeSmedt H, Eggermont JA, Wuytack F, *et al*. Isoform switching of the sarco(endo)plamsic reticulum Ca^{2+} pump during differentiation of BC_3H1 myoblasts. *J Biol Chem* 1991; 266(11): 7092–7095.
34. Lytton J, MacLennan DH. Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca^{2+}ATPase gene. *J Biol Chem* 1988; 263: 15024–15031.
35. Lompré AM. Characterization and expression of the rat heart sarcoplasmic reticulum Ca^{2+}ATPase mRNA. *FEBS Lett* 1989; 249: 35–41.
36. Zarain-Herzberg A, MacLennan DH, Periasamy M. Characterization of rabbit cardiac sarco(endo)plasmic reticulum Ca^{2+}ATPase gene. *J Biol Chem* 1990; 265: 4670–4677.
37. Van Den Bosch L, Eggermont J, deSmedt H, Mertens L, Wuytack F, Casteels R. Regulation of splicing is responsible for the expression of the muscle-specific 2a isoform of the sarco(endo)plasmic reticulum Ca^{2+}ATPase. *Biochem J* 1994; 302(2): 559–566.
38. Burk SE, Lytton J, MacLennan DH, Shull GE. cDNA cloning, functional expression and mRNA tissue distribution of a third organellar Ca^{2+}pump. *J Biol Chem* 1989; 264: 18561–18568.
39. Anger M, Samuel J-L, Marotte F, Wuytack F, Rappaport L, Lompré A-M. The sarco(endo)plasmic reticulum Ca^{2+}-ATPase mRNA isoform SERCA 3 is expressed in endothelial and epithelial cells in various organs. *FEBS Lett* 1993; 334: 45–48.
40. Wuytack F, Papp B, Verboomen H, *et al*. A SERCA 3-Type Ca^{2+}pump expressed in platelets, in lymphoid cells and in mast cells. *J Biol Chem* 1994; 269: 1410–1416.
41. Anger M, Samuel J-L, Marotte F, Wuytack F, Rappaport L, Lompré A-M. *In situ* mRNA distribution of sarco(endo)-plasmic reticulum Ca^{2+}ATPase isoforms during ontogeny in the rat. *J Mol Cell Cardiol* 1994; 26: 539–550.
42. Lytton J, Westlin M, Burk SE, Shull GE, MacLennan DH. Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J Biol Chem* 1992; 267(20): 14483–14489.
43. Briggs FN, Poland JL, Solaro RJ. Relative capabilities of sarcoplasmic reticulum in fast and slow mammalian skeletal muscles. *J Physiol Lond* 1977; 266: 587–594.
44. Brandl CJ, Green NM, Korzcak B, MacLennan DH. Two Ca^{2+}ATPase genes: homologies and mechanistic implications of deduced amino acid sequences. *Cell* 1986; 44: 597–607.
45. Dulhunty AF, Barnyard MR, Medvecky CJ. Distribution of calcium ATPase in the sarcoplasmic reticulum of fast and slow twitch muscles determined with monoclonal antibodies. *J Membr Biol* 1987; 99: 79–92.
46. Wu KD, Lytton J. Molecular cloning and quantification of sarcoplasmic reticulum Ca^{2+}ATPase isoforms in rat muscles. *Am J Physiol* 1993; 264 (*Cell Physiol* 33): C333–C341.
47. Zubrzycka-Gaarn E, Korczak B, Osinska H, Sarsala MG. Studies on sarcoplasmic reticulum from slow-twitch muscle. *J Muscle Res Cell Motil* 1982; 3: 191–212.
48. Toyofuku T, Kurzydlowski K, Tada M, MacLennan DH. Identification of regions in the Ca^{2+}ATPase of sarcoplasmic reticulum that affect functional association with phospholamban. *J Biol Chem* 1993; 268: 2809–2815.
49. Verboomen H, Wuytack F, deSmedt H, Himpens B, Casteels R. Functional differences between SERCA 2a and SERCA 2b Ca^{2+} pumps and their modulation by phospholamban. *Biochem J* 1992; 286: 591–596.
50. Kandarian SC, Peters DG, Taylor JA, Williams JH. Skeletal muscle overload upregulates the sarcoplasmic reticulum slow calcium pump gene. *Am J Physiol* 1994; 266 (*Cell Physiol* 35): C1190–1197.
51. Hawkins C, Xu A, Narayanan N. Sarcoplasmic reticulum calcium pump in cardiac and slow twitch skeletal muscle

but not fast twitch skeletal muscle undergoes phosphorylation by endogenous and exogenous Ca^{2+}/calmodulin-dependant protein kinase. *J Biol Chem* 1994; 269(49): 31198–31206.
52. Tada M, Katz AM. Phosphorylation of sarcoplasmic reticulum and sarcolemma. *Annu Rev Physiol* 1982; 44: 401–423.
53. MacLennan DH, Toyofuko T. Regulatory interactions between caclium ATPases and phospholamban. *Soc Gen Physiol Ser* 1996; 51: 89–103.
54. Kimura Y, Kurzydlowski K, Tada M, MacLennan DH. Phospholamban regulates the Ca^{2+}ATPase through intramembrane interactions. *J Biol Chem* 1996; 271(36): 21726–21731.
55. Toyofuko T, Kurzydlowski K, Tada M, MacLennan DH. Identification of regions in the $Ca^{(2+)}$ATPase of sarcoplasmic reticulum that affect functional association with phospholamban. *J Biol Chem* 1993; 268(4): 2809–2815.
56. James P, Inui M, Tada M, Chiesi M, Carafoli E. Nature and site of phospholamban regulation of the Ca^{2+} pump of sarcoplasmic reticulum. *Nature* 1989; 342: 90–92.
57. Kirchberger MA, Tada M, Katz AM. Adenosine 3'5'-monophosphate dependant protein kinase catalyzed phosphorylation reaction and relationship to calcium transport in cardiac sarcoplasmic reticulum. *J Biol Chem* 1974; 249: 6166–6173.
58. Hughes G, Starling AP, Sharma RP, East JM, Lee AG. An investigation of the mechanism of inhibition of the Ca^{2+} ATPase by phspholamban. *Biochem J* 1996; 318: 973–979.
59. Lompré A-M, Anger M, Levitsky D. Sarco(endo)plasmic reticulum calcium pumps in the cardiovascular system: function and gene expression. *J Mol Cell Cardiol* 1994; 26: 1109–1121.
60. Hu P, Yin C, Zhang K-M, *et al*. Transcriptional regulation of phospholamban gene produces coordinate expression of these two sarcoplasmic reticulum proteins during skeletal muscle phenotype switching. *J Biol Chem* 1995; 270(19): 11619–11622.
61. Arai M, Otsu K, MacLennan DH, Periasamy M. Regulation of sarcoplasmic reticulum gene expression during cardiac and skeletal muscle development. *Am J Physiol* 1992; 262: C614–C620.
62. Nagai RN, Zarain-Herzberg A, Brandl CJ, *et al*. Regulation of myocardial Ca^{2+}-ATPase activity and phospholamban mRNA expression in response to pressure overload and thyroid hormone. *Proc Natl Acad Sci USA* 1989; 86: 2966–2970.
63. Leberer E, Hartner K, Brandl CJ, *et al*. Slow/cardiac sarcoplasmic reticulum and phospholamban mRNAs are expressed in chronically stimulated rabbit fast-twitch muscle. *Eur J Biochem* 1989; 185: 51–54.
64. Briggs FN, Lee KF, Wechsler AW, Jones LR. Phospholamban expressed in slow-twitch and chronically stimulated fast-twitch muscles minimally affects calcium affinity of sarcoplasmic reticulum Ca^{2+}-ATPase. *J Biol Chem* 1992; 267(36): 26056–26061.
65. Kovacs RJ, Nelson MT, Simmerman HKB, Jones LR. Phospholamban forms Ca^{2+} selective channels in lipid bilayers. *J Biol Chem* 1988; 263(34): 18364–18368.
66. Odermatt A, Kurzydlowski K, McLennan DH. The V_{max} of the Ca-ATPase of cardiac sarcoplasmic reticulum (SERCA 2a) is not altered by Ca^{2+}/calmodulin dependant phosphorylation or by interaction with phospholamban. *J Biol Chem* 1996; 271: 14206–14213.
67. Viner RI, Hühmer AFR, Bigelow DJ, Schoneich C. The oxidative inactivation of sarcoplasmic reticulum Ca^{2+}-ATPase by peroxynitrite. *Free Rad Res* 1996; 24(4): 243–259.
68. Nicotera P, Kass GEN, Duddy SK, Orrenius S. Calcium and signal transduction in oxidative cell damage. *In*: Calcium, Oxygen Radicals and Cellular Damage. Duncan CJ, ed., Cambridge, Cambridge University Press, 1991; pp. 17–33.
69. Castilho RF, Carvalho-Alves PC, Vercesi AE, Ferreira ST. Oxidative damage to sarcoplasmic reticulum Ca^{2+}-pump induced by Fe^{2+}/H_2O_2 /ascorbate is not mediated by lipid peroxidation or thiol oxidation and leads to protein fragmentation. *Mol Cell Biochem* 1996; 159: 105–114.
70. Astier C, Rock E, Lab C, Gueux E, Mazur A, Rayssiguier Y. Functional alterations in sarcoplasmic reticulum membranes of magnesium-deficient rat skeletal muscle as consequences of free radical-mediated process. *Free Radical Biol Med* 1996; 20(5): 667–674.
71. Scherer NM, Deamer D. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydry groups in the Ca^{2+}-ATPase. *Arch Biochem Biophys* 1986; 246(2): 589–601.
72. Coan C, Ji JY, Hideg K, Mehlhorn RJ. Protein sulfhydryls are protected from irreversible oxidation by conversion to mixed disulfides. *Arch Biochem Biophys* 1992; 295: 369–378.
73. Ritov VB, Goldman R, Stoyanovsky DA, Menshikova EV, Kagan VE. Antioxidant paradoxes of phenolic compounds: peroxyl radical scavenger and lipid antioxidant, etoposide (VP-16), inhibits sarcoplasmic reticulum Ca^{2+}-ATPase *via* thiol oxidation by its phenoxyl radical. *Arch Biochem Biophys* 1995; 321(1): 140–152.
74. Trump BF, Berezesky IK. Role of ion regulation in cell injury, cell death and carcinogenesis. *In*: Cell Calcium Metabolism. Fiskum G, ed. New York, Plenum Press, 1989; pp. 441–449.
75. Reed DJ. Review of the current status of calcium and thiols in cell injury. *Chem Res Toxicol* 1990; 3: 495–502.
76. Nicotera P, Bellomo G, Orrenius S. Calcium-mediated mechanisms in chemical induced cell death. *Ann Rev Pharmacol Toxicol* 1992; 32: 449–470.
77. Gunter TE, Peiffer DR. Mechanisms by which mitochondria transport calcium. *Am J Physiol* 1990; 258: C755–C786.
78. Kukreja RC, Okabe E, Schrier GM, Hess ML. Oxygen radical mediated lipid peroxidation and inhibition of Ca^{2+}-ATPase activity of cardiac sarcoplasmic reticulum. *Arch Biochem Biophys* 1988; 261: 447–457.
79. Kukreja RC, Hess ML. The oxygen free radical system: from equations through membrane protein interactions to cardiovascular injury and protection. *Cardiovascular Res* 1992; 26: 641–655.
80. Viner RI, Ferrington DA, Hümer AFR, Bigelow DJ, Schöneich C. Accumulation of nitrotyrosine on the SERCA 2a isoform of SR Ca^{2+}-ATPase of rat skeletal muscle during aging: a peroxynitrite-mediated process? *FEBS Lett* 1996; 379: 286–290.
81. Luo WL, Grupp IL, Harrer J, Ponniah S, Grupp G, Duffy JL, Doetschman T, Kranias EG. Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and loss of β-agonist stimulation. *Circ Res* 1994; 75(3): 401–409.
82. Zàdor E, Mendler L, Ver Heyen M, Dux L, Wuytack F. Changes in mRNA levels of the sarcoplasmic/endoplasmic-reticulum Ca^{2+}ATPase isoforms in the rat soleus muscle regenerating from notexin-induced necrosis. *Biochem J* 1998; 320: 107–113.
83. Talmadge RJ, Roy RR, Chalmers GR, Eggerton VR. MHC and sarcoplasmic reticulum protein isoforms in

functionally overloaded cat plantaris muscle fibers. *J Appl Physiol* 1996; 80(4): 1296–1303.
84. Van der Linden CG, Simonides WS, Muller A, *et al*. Fiberspecific regulation of Ca^{2+}-ATPase isoform expression by thyroid hormone in rat skeletal muscle. *Am J Physiol* 1996; 271 (*Cell Physiol* 40): C1908–1919.
85. Simonini A, Lang CS, Dudley GA, Yue P, McElhinny J, Massie BM. Heart failure in rats causes changes in skeletal muscle morphology and gene expression that are not explained by reduced activity. *Circ Res* 1996; 79(1): 128–136.
86. Kandarian SC, Peters DG, Taylor JA, Williams JH. Skeletal muscle overload upregulates the sarcoplasmic reticulum slow calcium pump gene. *Am J Physiol* 1994; 266 (*Cell Physiol* 35): C1190–1197.
87. Schulte L, Peters D, Taylor J, Naavarro J, Kandarian S. Sarcoplasmic reticulum Ca^{2+} pump expression in denervated skeletal muscle. *Am J Physiol* 1994; 267 (*Cell Physiol* 36): C617–C622.
88. Schulte L, Navarro J, Kandarian SC. Regulation of sarcoplasmic reticulum calcium pump gene expression by hindlimb unweighting. *Am J Physiol* 1993; 264 (*Cell Physiol* 33): C1308–C1315.
89. Briggs FN, Lee KF, Feher JJ, Wechslezr AS, Ohlendick K, Campbell K. Ca-ATPase isozyme expression in sarcoplasmic reticulum is altered by chronic stimulation of skeletal muscle. *FEBS Lett* 1990; 259(2): 269–272.
90. Coirault C, Chemla D, Pery-Man N, Suard I, Salmeron S, Lecarpentier Y. Isometric relaxation of isolated diaphragm muscle: influence of load, length, time and stimulation. *J Appl Physiol* 1994; 76(4): 1468–1475.
91. Coirault C, Chemla D, Prey N, Suard I, Lecarpentier Y. Mechanical determinants of isotonic relaxation in isolated diaphragm muscle. *J Appl Physiol* 1993; 75(5): 2265–2272.
92. Herve P, Lecarpentier Y, Brenot F, Clergue M, Chemla D, Duroux P. Relaxation of the diaphragm muscle: influence of ryanodine and fatigue. *J Appl Physiol* 1988; 65(5): 1950–1956.
93. Aubier M, Murciano D, Lecocguic Y, Viires N, Pariente R. Bilateral phrenic stimulation: a simple technique to assess diaphragmatic fatigue in humans. *J Appl Physiol* 1985; 58: 58.
94. Esau SA, Bellemare F, Grassino A, Permutt S, Roussos C, Pardy RL. Changes in relaxation rate with diaphragmatic fatigue in humans. *J Appl Physiol* 1983; 54: 1353–1360.
95. Esau SA, Bye PTP, Pardy RL. Changes in rate of relaxation of sniffs with diaphragmatic fatigue in humans. *J Appl Physiol* 1983; 55: 731–775.
96. Moxham J, Wiles CM, Newhaus D, Edwards RHT. Contractile function and fatigue of the respiratory muscles in man. *In*: Human Muscle Fatigue: Physiological Mechanisms. Roster R, Whelan J, eds. London, Pitman, 1981; pp. 197–205. (Ciba Foundation Symposium).
97. Sayen MR, Rohrer DK, Dillman WH. Thyroid hormone response of slow and fast sarcoplasmic reticulum Ca^{2+} ATPase mRNA in striated muscle. *Mol Cell Endocrinol* 1992; 87: 87–93.
98. Anger M, Lambert F, Chemla D, *et al*. Expression of sarcoplasmic reticulum Ca^{2+} pump of the cardiomyopathic Syrian hamster: the effects of angio-tensin converting enzyme inhibitor. *Am J Physiol (Heart Circ Physiol)* 1995; 268: H1947–1953.
99. Viires N, Pavlovic D, Pariente R, Aubier M. Effects of steroids on diaphragmatic function in rats. *Am Rev Respir Dis* 1990; 142: 34–38.
100. Dekhuijzen PNR, Gayan-Ramirez G, deBock V, Dom R, Decramer M. Triamcinalone and prednisolone affect contractile properties and histopathology of rat diaphragm differently. *J Clin Invest* 1993; 92: 1534–1542.