Relaxation of diaphragm muscle

CATHERINE COIRAULT,¹ DENIS CHEMLA,² AND YVES LECARPENTIER²

¹Laboratoire d’Optique Appliquée, École Nationale Supérieure des Techniques Avancées, École Polytechnique, Institut National de la Santé et de la Recherche Médicale U 451, Batterie de l’Yvette, 91761 Palaiseau Cedex; and ²Service d’Explorations Fonctionnelles, Centre Hospitalier et Universitaire de Bicêtre, Assistance Publique-Hôpitaux de Paris, 94275 Le Kremlin-Bicêtre, France

Coirault, Catherine, Denis Chemla, and Yves Lecarpentier. Relaxation of diaphragm muscle. J. Appl. Physiol. 87(4): 1243–1252, 1999.—Relaxation is the process by which, after contraction, the muscle actively returns to its initial conditions of length and load. In rhythmically active muscles such as diaphragm, relaxation is of physiological importance because diaphragm must return to a relatively constant resting position at the end of each contraction-relaxation cycle. Rapid and complete relaxation of the diaphragm is likely to play an important role in adaptation to changes in respiratory load and breathing frequency. Regulation of diaphragm relaxation at the molecular and cellular levels involves Ca²⁺ removal from the myofilaments, active Ca²⁺ pumping by the sarcoplasmic reticulum (SR), and decrease in the number of working cross bridges. The relative contribution of these mechanisms mainly depends on sarcomere length, muscle tension, and the intrinsic contractile function. Increased capacity of SR to take up Ca²⁺ can arise from increased density of active SR pumping sites or in slow-twitch fibers from phosphorylation of phospholamban, whereas impaired coupling between ATP hydrolysis and Ca²⁺ transport into the SR or intracellular acidosis reduces SR Ca²⁺ pump activity. In experimental conditions of decreased contractile performance, slowed, enhanced, or unchanged relaxation rates have been reported in vitro. In vivo, a slowing in the rate of decline of the respiratory pressure is generally considered an early reliable index of respiratory muscle fatigue. Impaired relaxation rate may, in turn, favor mismatch between blood flow and metabolic demand, especially at high breathing frequencies.

in vivo; diaphragm muscle; in vitro

MECHANICAL RELAXATION is the process by which the muscle actively returns, after contraction, to its initial conditions of length and load (47, 56). The diaphragm, like the heart, contracts and relaxes continuously throughout life and must return to a relatively constant resting position at the end of each contraction-relaxation cycle. For a long time, the relationship between the relaxation phase and diaphragmatic function was not investigated. However, some studies have provided new insights into the physiological regulation of diaphragm relaxation (21, 22, 24, 35, 36, 46). Evidence increasingly supports the hypothesis that rapid and complete relaxation of the diaphragm plays an important role in adaptation to changes in respiration load and breathing frequency. Abnormalities in diaphragm relaxation have been reported in diseased states affecting respiratory muscles, e.g., fatigue, myopathy, and congestive heart failure (23, 35, 66). Relaxation abnormalities may contribute, at least in part, to impaired contractile performance (23, 66).

The present review focuses on recent developments in the understanding of diaphragm relaxation. We shall first briefly summarize present knowledge of relaxation at the molecular and cellular levels. We will then describe the mechanical properties of diaphragm relaxation at the multicellular level. Finally, we will consider the diagnosis, significance, and pathophysiological implications of relaxation in the in vivo diaphragm.

REGULATION OF RELAXATION AT THE MOLECULAR AND CELLULAR LEVELS

Relaxation is controlled by a complex interplay between inactivation (i.e., the overall processes leading to the disappearance of force-generating sites) and loading conditions (forces affecting muscle length and tension). Nonuniform distribution in time and space of inactivation and load may be a third control mechanism. The rate of inactivation is limited mainly by 1) active Ca²⁺ pumping by the sarcoplasmic reticulum (SR); 2)
Ca^{2+} removal from troponin C (TnC); and 3) the instantaneous number of working cross bridges (Fig. 1).

Active Ca^{2+} Pumping by the SR

The SR is an intracellular membrane system that plays a critical role in relaxation by actively pumping Ca^{2+}, thus decreasing the cytosolic Ca^{2+} concentration. The transport of 2 Ca^{2+} from the cytoplasm into the SR is coupled with the hydrolysis of 1 ATP through Mg^{2+}-Ca^{2+}-activated ATPase. The rate of Ca^{2+} uptake into the SR depends on the density of active pumping sites and the rate at which each pump sequesters Ca^{2+}. By actively loading the SR with Ca^{2+}, this mechanism plays also a key role in regulating the amount of Ca^{2+} released during contraction.

Physiological diversity of the SR. In mammalian muscles composed of different fiber types, such as diaphragm, relaxation performance reflects the contribution of the different fiber types. It is generally agreed that the capacity of the SR to take up Ca^{2+} is an indication of muscle fiber-type specificity. The higher capacity of SR Ca^{2+} uptake in fast- compared with slow-twitch fibers is associated with a higher relaxation rate, which largely arises from differences in the density of active pumping sites (28, 114). In addition to these quantitative differences, studies have provided evidence of the molecular diversity of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA). At least two isoforms of the SR Ca^{2+} pump, SERCA 1 and SERCA 2a, have been detected in adult diaphragm (2, 92), as previously reported in other adult skeletal muscles (76). SERCA 1 is exclusively expressed in fast-twitch skeletal fibers, whereas the SERCA 2a isoform is expressed in slow-twitch skeletal fibers as well as in cardiac and some smooth muscles (76). It has been shown that SERCA 1 and 2a isoforms display qualitatively similar enzymatic properties (i.e., their transport capacities and ATP affinities are similar) and are activated by Ca^{2+} in a similar cooperative manner (75, 76). Perhaps the most interesting difference between

Fig. 1. Major molecular and cellular mechanisms regulating relaxation processes. A: role of sarcoplasmic reticulum (SR) in contraction-relaxation coupling. After depolarization of sarcolemmal membrane, Ca^{2+} release to trigger contraction occurs at triads, i.e., specialized junctions where SR channels are opened by voltage sensors in transverse tubule. Ca^{2+}-ATPase of SR transports cytoplasmic Ca^{2+} into SR lumen to lower intracellular Ca^{2+} concentration, resulting in myofibrillar relaxation. In slow-twitch fiber, this Ca^{2+} uptake by Ca^{2+} pump is regulated by another SR protein, termed “phospholamban.” Phosphorylation of phospholamban stimulates activity of the Ca^{2+} pump. B: in isometric conditions, muscle develops high tension, and sarcomere shortening is minimal. High sensitivity of troponin C (TnC) to Ca^{2+} and cooperative interactions between cross bridges impede Ca^{2+} removal from myofilaments. In these conditions, inactivation rate is limited mainly by the rate of Ca^{2+} dissociation from the myofilaments. C: low loading conditions: sarcomere shortens substantially, lattice spacing increases, and the low sensitivity of TnC to Ca^{2+} promotes dissociation of Ca^{2+}. Consequently, at low load levels, inactivation rate is limited mainly by active Ca^{2+} pumping by SR pump.
these isoenzymes is the regulation of the SERCA 2 isoenzyme by phospholamban, another SR protein present in slow-twitch and cardiac muscles but not in fast-twitch muscle (59). In vitro studies have shown that unphosphorylated phospholamban decreases the affinity of SERCA 2a for Ca^{2+}, whereas phosphorylation relieves the inhibitory effects (see below, Short-term regulation of SR function).

Long-term regulation of SR function. Changes in the expression of the genes encoding the SR Ca^{2+}-pumps can be associated with a coordinated shift in myosin heavy chain isoforms, such as observed during matura-
tion and in chronically stimulated muscles (4, 12). An age-related decline in SR Ca^{2+} pump function has been reported in skeletal muscles (63, 85). This decline, presumably due to impaired coupling between ATP hydrolysis and Ca^{2+} transport into the SR (57, 85), may contribute to the slowing of the diaphragm relaxation rate in aged animals (113).

Expression of the SERCA isoforms has so far rarely been studied in diseases affecting the diaphragm (2, 92). In the cardiomyopathic Syrian hamster, impaired contraction and relaxation phases of the diaphragm have been observed at an early stage, before congestive heart failure is observed (16, 18, 23, 69). The etiology of the disease is unknown, but the prevailing hypothesis is that focal cellular necrosis reflects abnormal Ca^{2+} handling and intracellular Ca^{2+} overload (74). In dia-
phragm from cardiomyopathic Syrian hamster of the dilated Bio 53–58 strain, expression of the fast SERCA 1 isoform is significantly decreased, whereas the expres-
sion of the gene encoding the slow isoform SERCA 2a is unchanged (2).

Several studies have indicated that SERCA expres-
sion can be regulated by a number of factors, often in a tissue-specific manner. In the cardiomyopathic ham-
ster, abnormal expression of SERCA genes is observed at an earlier stage in diaphragm than in myocardium (2). Moreover, long-term therapy with an angiotensin-converting enzyme inhibitor has no effect on the expres-
sion of the SERCA genes in the diaphragm, whereas it prevents the relative decrease in Ca^{2+}-ATPase mRNA in the heart (2). In rats, thyroid hormones do not modify SERCA expression in diaphragm, contrary to what has been observed in other skeletal muscles (92).

Short-term regulation of SR function. Short-term regulation of SR Ca^{2+} uptake is influenced, at least in part, by changes in intracellular metabolite concentra-
tion or in neurohumoral state. In the case of diaphragm muscle, little information is available about the regulatory effects of phospholamban on SR function and relaxation rate. In skeletal muscles other than dia-
phragm, phospholamban can be phosphorylated in vivo during β-adrenergic stimulation by cAMP-dependent protein kinases and Ca^{2+}-calmodulin-dependent pro-
tein kinases. This phosphorylation enhances SR Ca^{2+} uptake and SR Ca^{2+}-dependent ATPase activity by ∼23–27% (55, 59). In contrast, reduction in SR Ca^{2+} pump activity is a common feature during muscle fatigue (17, 110). This effect is presumably due to intracellular acidosis, given that H^{+} inhibits SR ATPase (38, 54).

Relaxation Regulation at the Myofilament Level

Ca^{2+} removal from TnC. Changes in myofilament activation and inactivation kinetics are two important mechanisms that can influence the time course of contraction and relaxation. Binding of Ca^{2+} to TnC acts as a switch that allows cross-bridge interactions, whereas Ca^{2+} removal from TnC inhibits cross-bridge attachment (see Ref. 98 for review). Movements of Ca^{2+} to and from TnC depend, at least in part, on the affinity of Ca^{2+} for TnC, which increases with sarcomere length (1, 33, 99) and decreases with a decline in intracellular pH (38). It has been proposed that both the capacity of TnC to release Ca^{2+} and that of the SR to reuptake it help explain the length dependence of relaxation in heart (14, 68) and diaphragm muscle (21, 23, 46).

Indeed, at heavy load, sarcomere shortening is moder-
ated (23, 24, 68), so that high sensitivity of TnC to Ca^{2+} impedes Ca^{2+} removal from the myofibrillar apparatus. Conversely, at short sarcomere length and/or low load, the low sensitivity of TnC to Ca^{2+} promotes dissociation of Ca^{2+} from TnC (Fig. 1). Provided Ca^{2+} is rapidly sequestered into the SR, relaxation is expected to be faster as sarcomere shortening increases.

Aside from the central role of TnC, recent findings suggest that Ca^{2+} regulation of contractile proteins in intact muscle is modulated by cooperative interactions between cross bridges and the troponin-tropomyosin complex (see Ref. 80 for review). In addition, changes in muscle length may induce a change in interfilament spacing, which modulates the ability of cross bridges to react with thin filaments (80). Last, phosphorylation of myofilament proteins, e.g., by adrenergic agonists, can modulate myofilament activation, although the precise impact of functional changes on the dynamics of contraction and relaxation remains poorly documented in diaphragm.

Instantaneous number of cross bridges during relax-
ation. During the contraction-relaxation cycle, myosin heads attach to and detach from actin filament. Studies involving structural biology (89), optical tweezers (40), glass needle techniques (111), molecular genetics (97, 104), and theoretical models (52, 53, 64) have provided insights into the link between biochemical events of the actomyosin ATPase cycle and myosin molecular motor mechanics. Major steps in the cross-bridge cycle are shown in Fig. 2. The binding of ATP to the myosin head induces rapid dissociation of the myosin head from the actin filament (step 1). ATP is rapidly hydrolyzed to ADP and P_i, which remain tightly bound to the myosin (step 2). Attachment of the myosin head with actin leads to the formation of an active cross bridge (step 3). This step triggers the release of P_i, which in turn triggers the power stroke with the production of force and displacement of the myosin head relative to actin (step 4). After the power stroke, ADP dissociates from the myosin head (step 5), which in turn allows the binding of a new ATP molecule (step 1). In resting
striated muscle, the myosin heads remain in an unattached but energized state.

At any moment during the contraction-relaxation cycle, muscle tension depends on the instantaneous number of cross bridges in the power stroke (step 4) (20, 52, 53). In rabbit diaphragm, duration of the power stroke (~1 ms) is short compared with the total duration of the cycle (~300 ms) (64). Accordingly, it is expected that each cross bridge operates a limited number of cycles during the overall contraction-relaxation process (Fig. 2). One possibility could be that cross-bridge kinetics have a limited influence on the overall time course of diaphragm relaxation, a point that deserves further studies.

Other Suggested Mechanisms

It has been proposed that parvalbumin, a high-affinity Ca^{2+}-binding protein, may modulate the relaxation rate in mammalian skeletal muscle by facilitating Ca^{2+} transport from the myofibrils to the SR (45, 84). Parvalbumin is highly concentrated in fast skeletal muscles of fish and amphibians but also exists, generally at lower concentrations, in mammalian fast-contracting skeletal muscles. In mammalian muscles, the relaxation rate has been found to correlate with parvalbumin concentration (45, 84). Direct parvalbumin gene transfer significantly shortens twitch half relaxation time in soleus muscle but not in fast-twitch muscle (84). The function of parvalbumin in mammalian skeletal muscle has not yet been established with any certainty. Tetanus relaxation time is not affected by transfection of soleus with parvalbumin cDNA (84). The functional role of parvalbumin has also been questioned by other researchers, given that the rate of Ca^{2+} binding and release is too slow to allow rapid removal of Ca^{2+} from the myofibrils (90). Clearly, further studies are needed to determine the precise functional role of parvalbumin in diaphragm relaxation.

It has also been suggested that changes in membranous ionic conductances play a role in the slowing of relaxation in fatigued diaphragm by slowing of action potential repolarization (37, 106, 107). It remains to be determined whether slowing of action potential repolarization directly regulates tetanic contraction-relaxation phases by setting the activating Ca^{2+} levels.

In heart muscle, the role of muscular/endothelium-derived factors have been involved in regulating relaxation (14), and this field remains to be explored in skeletal muscles.

MECHANICAL ASPECTS OF RELAXATION IN ISOLATED DIAPHRAGM MUSCLE

Relaxation in Isometrically Contracting Diaphragm

The vast majority of experiments on diaphragm relaxation have been conducted on isometrically contracting muscle. In this type of contraction, maximal tension is developed, but essentially no shortening occurs. The diaphragmatic relaxation rate is usually quantified by the twitch half relaxation time (t_{1/2}), defined as the time it takes for peak tension to decrease by 50%.

An impaired relaxation rate can result from a variety of physiological and pathological conditions, including aging (113), maturation (103), hemidiaphragmatic pa-
ralysis (112), denervation and/or prolonged malnutrition (71), cardiomyopathy (66, 69), and fatigue (34, 46, 106, 107). In experimental conditions where contractile performance is altered, the relaxation rate can be slowed, enhanced, or unchanged. In fatigued diaphragm, combined hypoxia and hypercapnia (34) or severe hypoxia (107) accentuate the slowing of relaxation. Conversely, the depressive effects of streptozotocin-induced diabetes on the contractile performance of rat diaphragm has been associated with an accelerated relaxation rate (79). No significant changes in half-relaxation time of isolated diaphragm have been found in emphysema (39, 58, 72), except in one study (102). The precise molecular and cellular mechanisms responsible for changes in relaxation in pathological conditions remain to be determined. Finally, whereas inhibition of the SR function by ryanodine prolongs $t_{1/2}$ (46), pharmacological interventions such as corticotherapy (27, 103, 108), salbutamol (105), or theophylline (43) have had a limited influence on the isometric relaxation rate.

**Diaphragm Relaxation at Different Load Levels**

Because the in vivo diaphragm contracts and relaxes against various levels of loads, it is of interest to analyze the influence of both muscle length and load on diaphragm relaxation. During afterloaded contractions, the muscle relaxation phase classically consists of isotonic shortening (i.e., muscle length increases while muscle tension remains constant), followed by isometric tension decay (i.e., muscle tension falls at fixed muscle length) (47, 56).

**Load-dependence of relaxation.** It has been shown that loading conditions finely modulate diaphragmatic relaxation processes (21, 22, 24, 46). Diaphragm relaxation is load sensitive (46), as also observed in myocardium (14, 65). This general property is characterized by an overall time course of relaxation that is strongly affected by the afterload level. A contraction loaded with light or moderate load terminates earlier than a full isometric contraction (46). Thus the more the muscle is allowed to shorten, the shorter the overall duration of the contraction-relaxation cycle. This can be considered a manifestation of the shortening-induced deactivation phenomenon. In diaphragm, the load sensitivity of relaxation disappears after fatigue or after inhibition of the SR by ryanodine (46). It is markedly impaired in rabbit diaphragm with experimental chronic congestive heart failure (66). In this animal model, alteration of $Ca_{2+}$ sequestration by the SR is thought to account, at least in part, for the decreased load dependence of relaxation.

**Isometric relaxation.** Recent study has focused on the effects of loading conditions and stimulation mode on the peak rate of tension decline. When tension decay occurs at initial length, the peak rate of tension decline is mainly determined by afterload, regardless of preload, time, and stimulation mode (22). Except at approximately isometric load levels, an increase in afterload linearly accelerates the peak rate of tension decline (22). Similar results were obtained from phase-plane analysis of instantaneous rate of tension decline as a function of instantaneous tension (instantaneous $–dP/dt$ vs. instantaneous tension phase-plane) (22).

**Isotonic relaxation.** Mechanical determinants of diaphragmatic shortening have been analyzed over the whole load continuum. It has been demonstrated that maximum extent of muscle shortening ($\Delta L$) is the main mechanical determinant of peak shortening velocity (21). Peak shortening velocity ($VL$) physiologically increases with the extent of muscle shortening, irrespective of initial muscle length and of the load imposed on the muscle during the shortening process (21). Stimulation mode and time influence the isotonic shortening velocity, the slope of the $VL–\Delta L$ relationship being lower in twitch than in tetanus mode and/or when the shortening process is delayed (21, 25). Similar regulation of the isotonic relaxation rate has been observed in isolated human diaphragm (25), isolated rat diaphragm (21), and in human quadriceps muscle (26). Two mechanisms may explain the physiological coupling between the rate of muscle lengthening and the extent of shortening. First, the decay of mechanical activity can be accelerated at small end-shortening length. Second, the amount of potential energy stored during contraction and released during relaxation is negatively related to end-shortening length. In the cardiomyopathic Syrian hamster, a reproducible model of progressive skeletal and cardiac muscle disease (16, 67, 100), $VL$ is slowed and the overall duration of isotonic shortening is prolonged (23). The myopathic process affecting the diaphragm modifies the coupling between $\Delta L$ and $VL$.

**Auxotonic Relaxation of Sarcomeres**

Sarcomeres relax auxotonically, i.e., changes in sarcomere length and tension occur simultaneously. Because loading conditions have opposite effects on isotonic and isometric relaxation rates, it has been suggested that different intracellular mechanisms regulate the diaphragmatic isometric relaxation rate on the one hand and the muscle shortening rate on the other hand (22). It has been proposed that, among these mechanisms, differences in sarcomere length ($SL$) at the onset of the relaxation phase play a critical role (24).

**Laser-diffraction techniques** have been extensively used to analyze $SL$ at rest and during active contractions in both diaphragm (23, 24, 102) and other striated muscles (19, 31, 32, 68). Sarcomere motion during relaxation of frog skeletal muscle has been investigated by Cleworth and Edman (19) and Edman and Flitney (31, 32). These authors analyzed auxotonic changes in $SL$ during tension decay in isometrically contracting muscles (19, 31, 32). In hamster diaphragm, the sarcomere relaxation process has been investigated at various external-load levels (Fig. 3) (24). As observed in myocardium (68), sarcomere relaxation in afterloaded diaphragm displays two consecutive phases: an initial phase of rapid sarcomere shortening corresponding in time to the isometric relaxation phase of the whole
diaphragm muscle strip, followed by a second, slower relaxation phase corresponding in time to the whole isometric relaxation phase (Fig. 3) (24). As the load level increases, isotonic muscle lengthening occurs at progressively longer SL, corresponding to the SL at peak shortening. In contrast, isometric relaxation begins at an almost constant SL, regardless of the external load levels (24). Thus, SL may play an important role in regulating the isotonic lengthening rate of isolated diaphragm strip but not the peak rate of tension decline.

A complex equilibrium, modulated by the capacity of TnC to liberate Ca²⁺ and SR to recapture Ca²⁺ by length-dependent changes in myofilament lattice spacing, may help explain why relaxation occurs earlier and faster at low loads than it does at heavy loads. Provided SR is efficient, length-dependent changes in the affinity of TnC and in myofilament lattice spacing may favor rapid and precocious muscle lengthening when the muscle length is notably shortened (i.e., at low loads) (Fig. 1). Increases in potential energy stored during shortening may also help accelerate sarcomere relaxation.

**RELAXATION OF IN VIVO DIAPHRAGM**

Assessment of Relaxation in In Vivo Studies

Assessment of the diaphragmatic relaxation rate is of importance in clinical situations involving respiratory muscle fatigue. Indeed, inspiratory muscle fatigue may precipitate or intensify respiratory distress (91). Moreover, in patients satisfying the usual criteria for weaning, unsuccessful weaning trials have been associated with diaphragmatic fatigue (13, 88). It is thus of interest to develop a reliable, precocious index of respiratory muscle fatigue. Esau et al. (35, 36) have proposed that the rate of decline of transdiaphragmatic pressure (Pdi) after voluntary sniff maneuvers and/or phrenic nerve stimulation reflects the diaphragm relaxation rate. In subsequent studies, the relaxation rate has been calculated from different pressure curves, i.e., Pdi (5, 109), esophageal (Pes) (62), mouth (60, 70) and nasal (60, 61, 81) pressure curves. In all these studies, it was assumed that changes in pressure curves reflected changes in diaphragm tension and length. This hypothesis is based on two arguments. First, the time constants of stress adaptation of the lungs and chest wall are thought to be several orders of magnitude greater than the time constant of relaxation of the inspiratory muscles (93). Thus stress adaptation of the lungs and chest wall is not expected to modify pressure decay (70). Second, the electrical activity of the diaphragm normally ceases when pressure decay begins, so that the onset of pressure decay coincides with the beginning of the diaphragm relaxation phase (5, 35, 60). However, many studies have indicated that the diaphragm continues to receive motor output during the early portion of expiration. Whether this so-called postinspiratory activity changes in response to fatiguing load is not known, but it is certain that it can be prolonged by hypoxia.

Decay of pressure has been described in terms of time constant of the monoexponential phase of pressure decline (τ), maximum relaxation rate (MRR), and t₁/₂ (35). MRR is defined as the negative peak of the pressure derivative as a function of time and measures the initial part of the pressure decay. It has been shown that MRR varies with Pdi, i.e., that relaxation is accelerated when Pdi increases (36, 70, 83). The τ analyzes the latter portion of the pressure decay curve, i.e., after MRR. In normal subjects, pressure decay is generally monoexponential over the lower 50–70% of the pressure decay curve (35). The time constant of this exponential function is obtained from the equation for an exponential function \( P = P_0 e^{-\frac{t}{\tau}}, \) where \( P_0 \) is Pdi at MRR, and assuming a zero-pressure asymptote.

In the in vivo diaphragm, numerous studies have shown that respiratory muscle fatigue slows the relaxation rate, as attested by an increase in \( \tau \) and/or a decrease in MRR (5, 35, 36, 60, 61, 70, 77, 81–83). Moreover, slowing of relaxation has been shown to be an early signal of the onset of fatigue because it precedes failure of the diaphragm to generate a previously attainable Pdi (35). A slowing in relaxation rate...
has been reported in normal subjects after fatiguing contractions (35, 36, 77, 82) and in patients with chronic obstructive pulmonary disease (COPD) walking until they are in a state of severe dyspnea (62). It has been proposed that slowing of inspiratory muscle relaxation is a predictive index of weaning failure in mechanically ventilated patients (44).

Apart from fatigue, physiological and/or disease-related changes in diaphragm relaxation have not been extensively investigated. An increase in initial lung volume accelerates the relaxation rate, suggesting that initial muscle length modulates the in vivo relaxation performance (70, 96). This would tend to accelerate the relaxation rate in COPD patients, particularly during exercise, so that the slowing of relaxation after exercise would tend to be underestimated (62). In COPD patients, alterations in lung mechanics impair the transmission of pleural pressure to the upper airways (94), thus limiting the usefulness of the relaxation rate as an indicator of fatigue in clinical studies.

In animal studies, length-derived parameters have also been used to analyze diaphragmatic relaxation. Sonomicrometer techniques have made it possible to measure movements of the diaphragm in spontaneously breathing dogs (29, 30, 41, 86, 87). Newman and colleagues (86) showed that decay of pleural pressure (P_{es}) ends before the crural and costal parts of the diaphragm have returned to initial muscle length. Thus P_{es} swings during relaxation do not totally coincide in time with diaphragm muscle relaxation. In supine animals, peak lengthening velocity of the crural diaphragm, which has a greater extent of shortening, exceeds that of the costal part (29, 86, 87). This suggests that in the living animal V_{l} is related to Δ L, as also observed in vitro (21, 46).

ROLE OF DIAPHRAGM RELAXATION IN RESPIRATORY FUNCTION

Initial Resting Length

The ventilatory performance of the diaphragm depends, at least in part, on its initial resting length. For a given posture, the initial muscle length depends in turn on intrinsic muscle properties of relaxation and tissue compliance. The optimal resting length (L_{o}) of the diaphragm for tension generation is at or slightly below functional residual capacity (78). During inspiration between resting volume and total lung capacity, the diaphragm is then placed at a mechanical disadvantage for optimal force generation, and its inspiratory shortening capacity is severely curtailed. In diaphragm, the potential interplay between relaxation and muscle compliance remains to be documented.

Regulation of Diaphragmatic Blood Flow

As in other skeletal muscles, diaphragm contractile performance is dependent on an adequate energy supply (9, 10). An adequate blood flow is necessary to maintain constant continuous regeneration of high-energy phosphate compounds (95) as well as to ensure exchanges of metabolites with the extracellular space (6). The importance of the relaxation phase in regulating diaphragmatic blood flow has been discussed in numerous studies (see Ref. 49 for review). It has been shown that the relaxation phase is responsible for accommodating most of the changes in blood perfusion brought about by increased diaphragmatic work (48, 50). Diaphragmatic blood flow is reduced during inspiration (3, 10, 50) and can be completely abolished during forceful contractions (8, 10). Blood flow restriction has been attributed mainly to intramuscular pressure acting on the blood vessels between muscle fibers (10, 101). This pressure in turn depends on the load to which the diaphragm is subjected and the degree of muscle shortening (49, 50). During relaxation, intramuscular pressure declines to baseline, thus allowing diaphragmatic perfusion to occur (7, 48, 51). At low duty cycle (i.e., the ratio of inspiratory time to the total time taken for one respiratory cycle), the duration of relaxation is long, and the blood flow can increase to meet the metabolic demands (7, 15, 48, 51). Conversely, at duty cycle higher than 0.3, the relaxation period may not be long enough to meet diaphragmatic oxygen demand (48). Given that diaphragmatic blood flow is diminished by intramuscular pressure, delayed or slowed relaxation may further limit diaphragmatic perfusion, especially in cases of high breathing frequencies.

CONCLUSIONS

The importance of relaxation in the regulation of breathing is crucial, because the diaphragm must return to its optimal muscle length between each inspiration and because adequate diaphragmatic perfusion depends in part on rapid and efficient muscle relaxation, at least during loaded breathing.

The molecular and cellular mechanisms involved in the regulation of diaphragm relaxation are complex, and their relative contribution varies depending on muscle tension, sarcomere length, and the intrinsic contractile function. A number of factors alter Ca^{2+} uptake capacity, myofilament sensitivity, and/or crossbridge numbers, thereby exerting positive or negative effects on diaphragm relaxation performance. Future studies will provide more details about how these factors interact and influence diaphragm relaxation in both normal and pathological conditions. Some of the important questions to be addressed with regard to the molecular and cellular mechanisms involved in the regulation of diaphragm relaxation are: What is the
relative importance of SR dysfunction vs. changes in contractile protein characteristics in the different diseases that alter diaphragmatic relaxation? Do muscular/endothelium-derived factors influence diaphragmatic relaxation? If so, which pathways are involved? Are the mechanically relaxant effects of increased intracellular phosphorylation dependent on SR function or on myofilament activation? Answers to these questions will provide important information needed for the development of therapeutic agents that can specifically enhance diaphragmatic relaxation.

In the in vivo diaphragm, numerous studies have shown that respiratory muscle fatigue slows the relaxation rate. Aside from fatigue, studies on the diaphragmatic relaxation rate will likely be an important area of research in clinical situations involving respiratory muscle weakness. Methods will need to be developed to enable direct measurement of the diaphragmatic relaxation rate, particularly in patients in whom alterations in lung mechanics impair the transmission of pleural pressure to the upper airways, or when postinspiratory activity of the diaphragm is present.

Address for reprint requests: C. Coirault, INSERM U 451-LOA-ENSTA-Ecole Polytechnique, Batterie de l’Yvette, 91761 Palaiseau Cedex, France (E-mail: coirault@enst.u-stras.fr).

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