Impaired load dependence of diaphragm relaxation during congestive heart failure in the rabbit

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Lecarpentier, Y., C. Coirault, O. Langeron, F. X. Blanc, S. Salmeron, P. Attal, B. Riou, and D. Chemla. Impaired load dependence of diaphragm relaxation during congestive heart failure in the rabbit. J. Appl. Physiol. 87(4): 1339–1345, 1999.—The load dependence (LD) of relaxation was studied in the diaphragm of rabbits with congestive heart failure (CHF). CHF (n = 15) was induced by combined chronic volume and pressure overload. Aortic insufficiency was induced by forcing a catheter through the aortic sigmoid valves, followed 3 wk later by abdominal aortic stenosis. Six weeks after the first intervention, animals developed CHF. Sham-operated animals served as controls (C; n = 12). Diaphragm mechanics were studied in vitro on isolated strips, at 22°C, in isotonic and isometric loading conditions. Contractility was lower in the CHF group, as reflected by lower total tension: 1.11 ± 0.10 in CHF vs. 2.38 ± 0.15 N/cm² in C in twitch (P < 0.001) and 2.46 ± 0.22 in CHF vs. 4.90 ± 0.25 N·cm⁻² in C in tetanus (P < 0.001). The index LD was used to quantify the load dependence of relaxation: LD is <1 in load-dependent muscles and tends toward 1 in load-independent muscles. LD was significantly higher in CHF than in C rabbits, in both twitch (0.99 ± 0.01 vs. 0.75 ± 0.03; P < 0.001) and tetanus (0.95 ± 0.02 vs. 0.84 ± 0.02; P < 0.001). In the CHF rabbits' diaphragm, the fall in total tension was linearly related to the fall in load dependence of relaxation. The decrease in load dependence of relaxation in CHF animals suggests sarcoplasmic reticulum abnormalities. Impairment of the sarcoplasmic reticulum may also partly account for the decrease in contractile performance of diaphragm in CHF animals.

sarcolemmal reticulum; cardiac hypertrophy

The load dependence (LD) of relaxation is a basic mechanical property of most striated muscles. First described in mammalian heart muscle (2, 21), this property was later observed in diaphragm muscle by Hervé et al. (17). This mechanical property requires the presence of effective Ca²⁺-sequestering systems, particularly the sarcoplasmic reticulum (SR). Load-dependent relaxation is typical of adult mammalian myocardium and diaphragm in which the SR is well developed (3, 17, 21). Load-independent relaxation is typical of striated muscles, in which the SR is poorly developed or nonfunctional (3, 4, 14, 17, 21). Like the heart, the diaphragm contracts rhythmically throughout life and must return to its initial resting length in time for the next contraction. The LD of diaphragm relaxation is particularly important during high-frequency breathing, as it allows the diaphragm to return rapidly to its initial length.

Mechanical diaphragm performance has been investigated in numerous studies during congestive heart failure (CHF). A fall in diaphragm strength has been observed in both clinical and experimental heart failure (5, 11, 16, 18, 20, 22, 25, 26, 30). Moreover, CHF diaphragm relaxation has been less extensively studied (5, 18, 30). In addition, since these studies were conducted in isometric conditions, the isotonic mechanical properties and LD of relaxation were not investigated. Therefore, we studied the mechanical properties of relaxation, especially LD, in the diaphragm of rabbits submitted to combined chronic volume and pressure overload. We tested the following hypotheses: 1) that LD of diaphragm relaxation was impaired in CHF; and 2) that the potential fall in LD of diaphragm relaxation was related to the decrease in diaphragm contractile performance in CHF.

MATERIALS AND METHODS

Preparation of Animals and Surgical Procedure

Care of the animals and the performance of all experiments were in accordance with the Helsinki recommendations. We used 27 female New Zealand rabbits. All surgical procedures were performed in sterile conditions after anaesthesia with pentobarbital sodium (30 mg/kg iv) while the animals were spontaneously breathing room air. CHF (n = 15 rabbits) was induced by combined chronic volume and pressure overload. Aortic insufficiency was induced by introducing a catheter into the carotid artery and forcing it into the aortic sigmoid valves. Aortic pulse pressure (APP) = systolic minus diastolic aortic blood pressure) was determined before (APPb) and after (APPa) the procedure. The APPa/APPb ratio had to be >1.50 for inclusion in the CHF group. Three weeks after the first surgical procedure, the abdominal aorta was exposed, and a catheter was positioned alongside it. The catheter and aorta were ligated together just above the right renal artery, and the catheter was then gently removed, thereby reducing the abdominal aortic lumen by 45%. The model reproducibly induced cardiac hypertrophy with physical signs of CHF within 3 wk of aortic banding, i.e., 6 wk after aortic incompetence. Control animals (C; n = 12) underwent the same surgical operations but without induction of aortic insufficiency and constriction.
Following brief anesthesia with pentobarbital sodium, animals were thoracotomized and laparotomized. The heart was quickly removed, and a left ventricular (LV) papillary muscle was carefully excised and mounted on a force transducer in a tissue chamber containing 60 ml of a Krebs-Henseleit solution (in mM): 118 NaCl, 4.7 KCl, 1.2 MgSO_4·7H_2O, 1.1 H_3PO_4, 25 NaHCO_3, 2.5 CaCl_2·H_2O, and 4.5 glucose. The solution was bubbled with 95% O_2-5% CO_2.

The LV papillary muscle was electrically stimulated by means of two platinum electrodes delivering 5-ms rectangular pulses at 0.17 Hz. A strip of the ventral part of the costal diaphragm was carefully dissected free from the muscle in situ. The insertions on both the central tendon and the ribs were kept intact. The diaphragm strip was rapidly mounted in another tissue chamber, containing 60 ml of the same Krebs-Henseleit solution bubbled with 95% O_2-5% CO_2 and maintained at 22°C and pH 7.40. The costal end of the strip was held in a stationary clip at the bottom of the chamber, whereas the central tendon end was maintained by means of another clip attached to a second electromagnetic force transducer. The diaphragm strip was electrically stimulated in twitch mode by means of two platinum electrodes delivering rectangular pulses of 5-ms duration at a rate of 10 pulses/min. The muscle strip was also stimulated in tetanic conditions as follows. Electrical stimulus: 5-ms duration; stimulation frequency: 33 Hz; train duration: 400 ms, 10 trains/min. The 33-Hz stimulation frequency induced the maximum isometric tetanic tension in rabbit diaphragm at 22°C (20). In both diaphragm and heart muscles, experiments were carried out at the resting tension level at which maximum isometric active tension was achieved (L_o). Body weight and LV weight were noted. At the end of the experiment, cross-sectional area (in cm^2) of the heart muscles was calculated from the ratio of fresh muscle weight to muscle length at L_o, assuming a muscle density of 1.056.

Electromagnetic Lever Systems

The electromagnetic device consisted of an aluminum lever cemented to a coil suspended in the field of a permanent magnet (8). A force couple developed when an electrical current was passed through the coil. Lever displacement was measured by means of a photoelectric transducer composed of an incandescent lamp, a miniature photodiode, and a preamplifier acting as a current-to-voltage converter. The light emitted by the lamp was modulated by the lever displacement, and current alterations in the photodiode were converted into voltage alterations. The two electromagnetic lever systems were linear in the range of 0–5 mm of muscle shortening.

Mechanical Protocol

For the diaphragm muscle strip, classic mechanical parameters were measured from four contractions starting from L_o (Fig. 1). The first contraction was abruptly clamped to zero load by means of the zero-load clamp technique just after the electrical stimulus (2). This technique enables direct measurement of the maximum unloaded shortening velocity (V_{ZL}), under conditions in which the total tension was forced to zero. The second contraction was isotonic and loaded with preload only. The third contraction was fully isometric. The fourth contraction was isometric and after loaded to one-half the active isometric tension (i.e., total isometric tension minus resting tension). Different techniques have been used to determine unloaded shortening velocity: 1) the zero-load clamp technique (V_{ZL}); 2) the mathematical extrapolation to zero load (V_{max}); 3) the slack test (V_o). It has been shown that values of V_{ZL} and V_{max} do not significantly differ (2). Conversely, the ratio V_o/V_{max} has been shown to be ~1.6 (6). Thus V_{ZL} is expected to be lower than V_o.

Mechanical Parameters

Contraction phase V_{ZL} was determined from the first contraction. Maximum shortening (V_{cmax}) and lengthening (V_{rmax}) velocities were determined from the second contraction. V_{ZL} and V_{cmax} were expressed in L_o·s^{-1}. Total isometric tension was taken as the peak value of the third contraction. Total isometric tension was expressed in N·cm^{-2}, and positive peak of isometric tension derivative (+dP/dt) in N·cm^{-2}·s^{-1}.

Fig. 1. Mechanical parameters of contraction, relaxation, and load sensitivity. Top: muscle shortening length (L/L_o) vs. time. Bottom: tension vs. time. Contraction 1 was loaded with preload only and was rapidly clamped to zero load with critical clamping just after stimulation. Contraction 2 was loaded with preload only. Contraction 3 was fully isometric. Contraction 4 was loaded at 50% of isometric active tension. Two indexes were used to quantify load sensitivity of relaxation. 1) Ratio of load dependence (LD) of 2 areas LD = A_{iso}/A_{isom}, where A_{iso} was isometric area and was limited by tension vs. time curve of contraction 4; and where A_{isom} was isometric area and was limited by tension vs. time curve of contraction 3 below 50% of active tension level. LD = A_{iso}/A_{isom} approaches 1 when load sensitivity of relaxation disappears. 2) Ratio of 2 times (t_1 = t_2), where t_1 was time to the end of isometric relaxation phase of the contraction after loaded at 50% of active tension at L_o, and t_2 was time at which the full isometric contraction was relaxed to the same level of load. Vertical arrow indicates time at which unloaded shortening velocity at zero load (V_{ZL}) was measured.
relaxation phase of different afterloaded contractions almost coincides with relaxation of the full isometric contraction (Fig. 2, right). Two indexes have been proposed to quantify the LD of heart and diaphragm muscles (Fig. 1) (17, 21): 1) the ratio LD of two areas, i.e., isotonic area (A\text{isot}) to isometric area (A\text{isom}), A\text{isot}/A\text{isom}; A\text{isot} is limited by the isometric tension vs. time curve at 50% of the active isometric tension; A\text{isom} is limited by the full isometric tension vs. time curve below the same level of load; 2) the ratio of two times (t\text{i}/t\text{j}), where t\text{i} is the time at the end of the isotonic relaxation phase of the contraction afterloaded at 50% of the active isometric tension and t\text{j} is the time at which the full isometric contraction is relaxed to the same load level. These ratios range from 0.70 to 0.85 in typical load-sensitive relaxation and tend toward 1 in typical load-insensitive relaxation.

For the LV papillary muscle, maximum unloaded shortening velocity of contraction (V\text{2L}, in L·s\textsuperscript{-1}) was produced and measured in the same fashion as described for the diaphragm muscle above (2). Peak isometric tension, i.e., peak isometric force normalized per cross-sectional area (in N·cm\textsuperscript{-2}), was measured from the fully isometric contraction.

Statistical Analysis

Data are means ± SE. Controls were compared with CHF animals by using Student's unpaired t-test after ANOVA. Comparisons in twitch and tetanus modes were performed successively; P values of <0.05 were required to rule out the null hypothesis. Linear regressions were based on the least squares method.

RESULTS

Cardiac Hypertrophy and CHF

LV weight was twice as high in CHF than in C animals (6.9 ± 0.4 vs. 3.2 ± 0.1 g; P < 0.001). The LV weight-to-body weight ratio was higher in CHF than in C rabbits (2.14 ± 0.12 vs. 1.26 ± 0.03; P < 0.001). These results reflected the marked cardiac hypertrophy induced by chronic overload. At the time of the study, the physical signs of heart congestion observed in the CHF animals were as follows: ascitis (13/15); pericarditis (11/15); pleuritis (9/15); subcutaneous edema (8/15); pulmonary congestion (8/15); and hepatic congestion (9/15). In CHF group, the mechanical performance of LV papillary muscles fell dramatically. Both total isometric tension (2.1 ± 0.3 vs. 1.2 ± 0.2 N·cm\textsuperscript{-2}; P < 0.05) and V\text{2L} (0.68 ± 0.05 vs. 0.29 ± 0.04 L\textsubscript{o}·s\textsuperscript{-1}; P < 0.001) were reduced by half in CHF group compared with controls.

Diaphragm Contraction and Relaxation

In both twitch and tetanus modes, contraction parameters (i.e., V\text{2L}, V\text{cmax}, total isometric tension, and +dP/dt) were markedly lower in CHF than in C rabbits (Fig. 3). In twitch and tetanus modes, the two relaxation parameters (V\text{rmax} and −dP/dt) were significantly lower in CHF than in C rabbits (Fig. 4). In twitch mode, the ratio of V\text{rmax} in CHF to V\text{rmax} in C animals (~25%) was lower than the ratio of V\text{cmax} in CHF to V\text{cmax} in C animals (~46%) (Figs. 3 and 4). In tetanus mode, the ratio of V\text{rmax} in CHF to V\text{rmax} in C group (~50%) was lower than the ratio of V\text{cmax} in CHF to V\text{cmax} in C rabbits (~55%) (Figs. 3 and 4).

LD of Diaphragm Relaxation

The decrease in the LD of relaxation in CHF was demonstrated by applying superimposed afterloaded contractions from preload up to full isometric contraction. In control diaphragm, afterloaded contractions were completed in a shorter period of time than the relaxation phase of the full isometric contraction in twitch (Fig. 2, left) and tetanus (not shown) modes. In contrast, in CHF diaphragm, the time course of tension decrement of different afterloaded contractions almost coincided with the relaxation of the full isometric contraction in twitch (Fig. 2, right) and tetanus (not shown) modes. The two indexes quantifying the LD of relaxation (i.e., LD = A\text{isot}/A\text{isom} and t\text{i}) were significantly higher in CHF than in C rabbits (Fig. 4). These
results reflected the marked loss of the LD of relaxation in CHF diaphragm, in both twitch and tetanus modes. The loss of LD of relaxation ran parallel to impaired diaphragm contractile performance. There was a linear relationship between the total isometric tension and \( \frac{A_{\text{isot}}}{A_{\text{isom}}} \) in both twitch \((r = -0.75; P < 0.001)\) and tetanus \((r = -0.82; P < 0.001)\) modes (Fig. 5). Thus in CHF, the greater the impairment of LD of relaxation, the poorer the contractile performance of the diaphragm. The slope of this relationship was steeper in tetanus than in twitch.

**DISCUSSION**

This study provided evidence of marked abnormalities of isotonic and isometric relaxation in the diaphragm of rabbits with experimental CHF. A combined chronic volume and pressure overload led to a high
degree of cardiac hypertrophy and heart failure, as reflected by physical signs of CHF and impaired intrinsic cardiac performance in the overloaded rabbits. The LD of relaxation was markedly impaired in CHF diaphragm, indicating diaphragm relaxation dysfunction in CHF and possibly suggesting SR abnormalities. The fall in LD of relaxation observed in CHF diaphragm paralleled that in contractile performance.

LD of Relaxation

We found that LD of relaxation was strongly impaired in the CHF diaphragm. This important mechanical property requires a well-functioning SR (3, 21). Indeed, relaxation is load independent in various experimental conditions in which the SR 1) is poorly developed, as in frog myocardium (3, 14, 21); 2) is nonfunctional, as in newborn myocardium (4, 14); 3) is destroyed by Br58, a detergent of the SR membrane (3, 21), or is inhibited by caffeine (21). In diaphragm muscle, LD of relaxation has been shown to disappear after ryanodine treatment (17), which modulates Ca2+ homeostasis through SR Ca2+ channels. LD of relaxation also disappears in fatigued diaphragm (17).

Alterations in Ca2+ sequestration by the SR might partly account for the decrease in LD of relaxation observed in the CHF diaphragm. Molecular biology-based studies have provided evidence of alterations in the diaphragm and heart SR during heart failure, which match mechanical changes in cardiac relaxation. Abnormal regulation of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) has been observed in the diaphragm of cardiomyopathic Syrian hamsters, a well-characterized model of CHF. The diaphragm of normal hamsters contains SERCA 1a and 2a mRNA isoforms (1), in keeping with the presence of fast- and slow-twitch fibers. In cardiomyopathic Syrian hamsters, the expression of diaphragmatic SERCA 1 mRNA is significantly lower, with no modification of SERCA 2 mRNA levels (1). In the heart, LD of relaxation is also impaired during cardiac hypertrophy and failure induced by chronic overload (23) and during hypoxia (23, 31). These mechanical abnormalities observed during heart failure have mainly been attributed to an impairment of Ca2+-sequestering systems, particularly the SR. Abnormalities in the regulation of cardiac SERCA have been described in chronic overload and CHF (27). We cannot exclude the involvement of other mechanisms, such as a decrease in myofilament affinity for Ca2+, which might impair the LD of relaxation (31).

Several intracellular dysfunctions may contribute to the decrease in diaphragm strength observed during heart failure in humans and animals (5, 11, 16, 18, 20, 22, 25, 26, 30). The fall in diaphragm strength may be explained both by alterations of myosin molecular motors and by impaired intracellular Ca2+-sequestrer systems. Various myosin isoform patterns have been described in human diaphragm during CHF (24, 32). The decrease in diaphragm strength can be partly explained by alterations occurring at the cross-bridge level. A decrease in the total number of cross bridges has been shown in a similar model of CHF in the rabbit (20) and in the diaphragm of cardiomyopathic animals (22). A decrease in the single force of cross bridges has also been observed in CHF rabbit diaphragm (20), and histological abnormalities (24) have been reported in human diaphragm during CHF. Moreover, the SR is the main source of Ca2+ activator and thus largely determines diaphragm performance. SR impairment may contribute to the observed linear relationship between the fall in contractility and the fall in LD of relaxation during CHF (Fig. 5). This relationship was particularly steep in tetanus, the physiological mode of stimulation.

Intrinsic Abnormalities of Diaphragm Relaxation in CHF

The mechanical determinants of intrinsic diaphragmatic relaxation are different in isotonic (8) and isometric conditions (9). In isotonically relaxing diaphragm muscle, it has previously been demonstrated that, as
the load level is increased above preload, the peak lengthening velocity is linearly related to the maximum extent of muscle shortening, independent of end-shortening length and initial muscle length (8). Moreover, in diaphragm muscle isometrically relaxing at initial muscle length, and for a load below 80% of the peak isometric tension, the negative peak of tension decline is mainly determined by afterload, regardless of stimulation conditions, initial muscle length, or abrupt changes in load (9). In our study, $V_{\text{rmax}}$ was proportionally more impaired than $V_{\text{cmax}}$ (Figs. 3 and 4). These results indicated impaired coupling between contraction and relaxation under low load, with intrinsic abnormalities of isotonic relaxation. Comparable results have been obtained with the diaphragm of the cardiomyopathic Syrian hamster, in which the peak lengthening rate is proportionally more impaired than the peak shortening amplitude (10). This has been partly attributed to abnormal Ca$^{2+}$-sequestration capacity by the SR. These results are consistent with the reduction in the slope of the relationship between peak lengthening velocity and peak shortening amplitude induced by cyclopiiazonic acid, an inhibitor of SR Ca$^{2+}$-ATPase (29).

Diaphragm Relaxation and Initial Length

Initial muscle length is an important determinant of the relaxation rate in respiratory muscles (8, 9, 15, 19). The LD of relaxation decreases in the myocardium when the initial muscle length decreases (4). Relaxation plays a key mechanical role, particularly in rhythmically contracting muscles such as the heart and diaphragm. Efficient relaxation is especially required during high-frequency breathing, to allow the diaphragm to recover its initial length rapidly, thus preventing an increase in functional residual capacity. Incomplete return to initial length could be due to impaired relaxation. Importantly, no increase in functional residual capacity has been observed in human CHF (25). In our study, CHF experiments were carried out at $L_0$. Thus a change in initial diaphragm length cannot account for abnormal LD of relaxation. Other factors, possibly indirectly related to the relaxation process, may play a role in the decrease in CHF diaphragm mechanical performance, without modulation of the LD of relaxation per se.

Possible Clinical Implications

This study may have clinical implications. An impairment of diaphragm performance has pathophysiologic importance in human CHF, because inspiratory muscle strength is a determinant of maximum oxygen consumption (5) and dyspnea during daily activities strongly correlates with inspiratory muscle weakness (25, 26). Moreover, a relative precession of relaxation abnormalities seems to be a common feature of striated muscles submitted to chronic overload. In skeletal and cardiac muscles, slowing of respiratory muscle relaxation is considered to occur early after excessive loading and precedes the fall in tension (12). Excessive loading generally slows the inspiratory muscle relaxation rate (13, 19, 28). In human CHF, respiratory muscle work increases owing to fluid accumulation within the lung tissues (which become stiffer) and to high airway resistance (7). All these factors induce chronic overload of respiratory muscles and gradually lead to muscle weakness accompanied by relaxation abnormalities.

In conclusion, in rabbits with CHF induced by chronic overload, LD of diaphragm relaxation was markedly decreased. These results suggest that Ca$^{2+}$-sequestering systems, especially the SR, are impaired in CHF diaphragm. The fall in contractile performance of CHF diaphragm was related to the fall in LD of relaxation. Impairment of the SR may partly account for the decreased contractile function in CHF rabbit diaphragm.

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