The molecular basis of cardiac mechanics: Regulation of motor unit recruitment.

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*Abstrac*t-Calcium binding to the regulatory proteins of the contractile filaments allows cross-bridge recruitment and force generation. The study tests the hypothesis that cross-bridge recruitment is regulated by a positive feedback mechanism whereby the number of force generating cross-bridges determines the affinity of the regulatory proteins for calcium. The force response to sarcomere length oscillations was measured at constant free Ca^{2+} concentration, during steady tetanus contraction of isolated rat trabeculae, obtained from rat right ventricles (n=5). Tetanus was achieved by using cyclopiazonic acid (CPA) and 8Hz stimulation. SL was measured by laser diffraction techniques. The Force was measured by silicon strain gauge. Sarcomere oscillations were imposed with a fast servomotor. The force response lagged the sarcomere length oscillations at frequency smaller than 4Hz (99.3±39.9 msec at 1Hz). A counter clockwise hysteresis was obtained between the force and the SL. There was no unique force-length relation at constant activation since the force was depended on a short-term history of contraction. The study establishes the existence of a positive feedback that regulates cross-bridge recruitment, cardiac mechanics and energetics: the steep force-length relation and the adaptive control of energy consumption by the prevailing loading conditions.

Keywords - Excitation contraction coupling; Frank-Starling law, Length dependent Ca sensitivity, Cooperativity.

I. INTRODUCTION

The biological linear motor, that produces cardiac and skeletal muscle contraction, is by far smaller than all human made nano-motors: its length is 19nm, its width is 5nm and it generates stroke steps of 5nm. The biological motor unit is composed of the myosin head that projects form the myosin filament and interacts with the actin filament to form the actomyosin cross-bridge. One of the most intriguing features of the actomyosin cross-bridge (motor unit) is its high biochemical to mechanical energy conversion efficiency. About 70% of the free energy liberated from ATP hydrolysis is converted into mechanical energy (External work and potential energy), and all this is done within the actomyosin motor unit, between the ATPase domain, the actin binding site and the neck-or hinge domain, which are only few nano-meter apart.

The unitary force generated by a single actomyosin motor unit is around 2pN (10^{-13} Kg). Cardiac and skeletal muscle can generates stresses of 2 Kg/cm^2 ($200mN/mm^2$) since each cubic millimeter of the cardiac or skeletal muscle contains $100 \bullet 10^{12}$ motor units (The length of half sarcomere is around 1.0μm). The muscle has precise intracellular switching and control mechanisms that allow the muscle to regulate cross-bridge recruitment and to accommodate for the different loading conditions. The importance of the intracellular control mechanism was established by Otto Frank (1899) and starling (1918) who have described the role of the mechanical initial loading condition (Preload) on muscle contraction and by Fenn (1923) who has showed that the muscle possesses a fundamental property that enable it to adjust its energy cost to prevailing mechanical constraints, after stimulation and during the contraction [1].

The present study concentrates on the regulation of XB recruitment. The troponin regulatory proteins situated along the actin filament regulate XB recruitment. The regulated actin exists in two main conformations [2]: off actin, which inhibits XB cycling, and on actin, which allows ATP hydrolysis by the XB ATPase and XB turnover to the force generation (Strong) conformation [3,4]. Calcium binding to troponin turns the regulated actin from the off to the on state [2].

Our recent studies have established the existence of a dominant positive feedback mechanism, denoted as the cooperativity mechanism, which regulates XB recruitment [5][6]. The cooperativity mechanism suggests that the number of cross bridges in the strong conformation determines the affinity of the regulatory protein, the troponin complex, for calcium. An increase in the afterload increases the affinity of calcium binding to troponin, the number of bound calcium to troponin and hence the rate of energy consumption by the sarcomere. The existence of this mechanism was substantiated from the analysis of the force-length – free calcium relationship in the skinned rat trabeculae [5].

II. HYPOTHESIS and AIMS

It is commonly assumed that there is a unique force length relationship, at any given constant free calcium, which may be described by some constitutive law. The suggested existence of a positive feedback mechanism that regulates XB recruitment leads to a contradictory prediction, that there is no unique force-length relation, and the instantaneous force depends not only on the instantaneous length but also on the history of contraction. The cooperativity mechanism leads to the predictions that the generated force should lag

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Abstract

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the sarcomere length (SL) changes, even at constant calcium concentrations. Therefore a hysteresis in the force-length relationship is anticipated when the force response to length oscillations is plotted against the sarcomere length. Sarcomere lengthening increases calcium affinity and the bound calcium thereafter increases the rate of energy consumption and XB recruitment, and vise versa for sarcomere shortening.

The aims of the study are to further validate the existence of the cooperativity mechanism by testing the predicted hysteresis in the force response to sarcomere length oscillations and to quantify the response time of this feedback loop.

III. Methodology

Thin trabeculae from the rat right ventricle were studied (Spargue Dawley, 2-3 month old). The rats were anaesthetized with diethyl ether, the hearts were quickly removed and transferred to a dissection dish. Under a binocular microscope the right ventricle was opened and long, thin and unbranched trabeculae, that were seen running between the atrium-ventricle ring and the right ventricle free wall were dissected. After dissection the trabeculae were mounted in the experimental setup. The trabeculae were mounted to the force transducer basket at the ventricular end and attached to the motor arm at the valvular end. The muscles were superfused with modified Krebs-Henseleit solution and gassed with 95% O_2 and 5% CO_2. The temperature was 25 ^oC, PH was 7.40. The muscle was stimulated with wire electrodes. The stimulation amplitude was one and a half fold of the stimulus threshold, with duration of 2ms.

Force was measured with silicon strain gauge (Snsonor 801) with a sensitivity of 80 mN/V and resonance frequency of 1300Hz. Sarcomere length was measured by a laser diffraction technique. The trabecula can be treated as a grating, therefore it generates a diffraction pattern when it is expose to a beam of parallel light. The angle (α) between the first-order diffraction beam and the transmitted light (zero order) is inversely related to the grading spacing, i.e. to the sarcomere length. Thus, SL is calculated by: $SL = \lambda/\sin\alpha$ where λ is wavelength of the light. SL is measured by a unique system (University of Calgary) that detects the position of the mean of the gaussian of the first order diffraction and converts the position to a voltage output that is proportional to the precise sarcomere length, by using a nonlinear amplifier. The trabecula was illuminated by monochromatic light from a HeNe laser (λ=0.633μm). The diffraction pattern was detected by a fast single line array CCD (Dalsa) with 2048 pixels and frame rate of 7500 per second. The muscle length was controlled by a fast servomotor (Aurora 308B) with a time response of 250μsec. The motor unit includes a precise capacitor position sensor that allows closing the length closed loop control.

Tetanus was elicited in the presence of cyclopiazonic acid (CPA). CPA blokes Ca^{2+} uptake by the Sarcoplasmic Reticulum calcium ATPase and therefore prolongs the twitch. The CPA concentration was 30μM. Tetanus was elicited for 3.5sec every ten regular twitches. The regular twitch stimulation frequency was 0.2Hz. The tetanus stimulation frequency was 8Hz with pulse width of 40ms. Calcium concentration in the solution was 6mM.

To quantify the phase delay between the force response and the sarcomere length perturbations – slow sinus oscillations of various frequencies (1, 2, and 4Hz) were imposed, as shown in Fig. 1. The magnitude of the slow oscillations was 50±26nm (peak to peak).

The number of attached force generating XBs determines the stiffness of isolated fiber. The slow oscillations might affect the force per XB (unitary force) and the number of strong XBs. To differentiate between the two and to quantify the number of strong XBs - fiber stiffness was measured by imposing additional high frequency (50Hz) and small amplitude oscillations (Fig. 1) on top of the slow and large oscillations. The amplitude of the secondary small oscillation was smaller than 5nm and within the order of the XB stroke length. The stiffness was calculated as the ratio between the high frequency oscillation in the force and the sarcomere length. Each perturbation was repeated at last 5 times and was averaged. The sampling rate was 5000Hz.

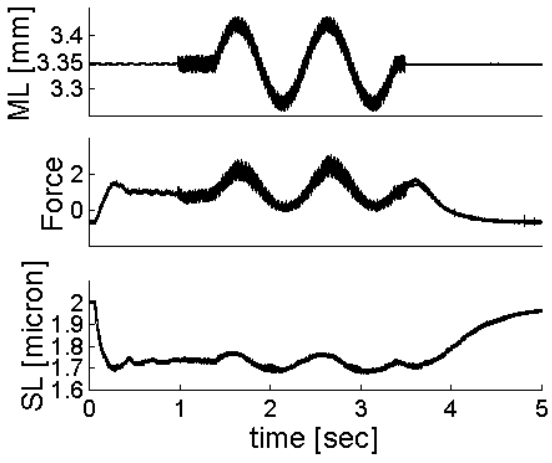

Fig. 1. Muscle length oscillations (1Hz) were imposed during the tetanus contraction and have produced sarcomere and force oscillations. Note the secondary high frequency and low amplitude oscillations imposed 1sec after the time onset of contraction, for the calculation of muscle stiffness.

IV. Results

Figure 2 shows the time course of the oscillations in the sarcomere length (SL), force and stiffness when the muscle length oscillations were at 1 Hz. Note that the SL oscillations preceded the force and the stiffness oscillations. The oscillations of the force and the stiffness were aligned, suggesting that the delay in the force response, at 1 Hz oscillations, resulted primarily from the delay in cross-bridge recruitment.

Figure 3 presents the phase plot of the stiffness and the force against the sarcomere length. Hysteresis was obtained in a counter clockwise direction (CCW) for both the stiffness and the force versus the SL. At the same SL the generated force was larger during muscle shortening and was smaller during muscle lengthening.

The force response lagged the SL oscillation by 99.3±39.9 msec for oscillation at 1Hz. The delay shortened as the oscillation frequency was elevated. At 2 Hz oscillation the delay was 45.93±27.7msec and at 4Hz it decreased to 32.8±24.3msec.

V. Discussion

For SL oscillations below 4Hz the hysteresis between force or the stiffness and the sarcomere length was in the counter clockwise direction. The phenomenon is in accordance with the cooperativity mechanism whereby calcium affinity and the bound calcium are larger when reaching a given SL from a longer SL.

The hysteresis cannot be attributed to some visco-elastic properties of the fibers, since hysteresis due to visco-elasticity is in the clock-wise direction. The area inside the phase plot loop is the work done by the fiber. For a clockwise direction work is done on the fiber, and it is liberated as heat. Counter clockwise direction infers that external work is done by the fiber. Hence, the counter clockwise direction infers that it involves energy consumption by the fiber, which is provided by XB recruitment.

The cooperativity mechanism (fig. 4) is the dominant feedback loop that regulates force generation by the sarcomere [5,6,7]. The cooperativity mechanism underlies four main characteristics of the cardiac muscle:

1. The cardiac muscle steep force-length relationship, where a 10% decrease in the sarcomere length (preload) leads to 30-50% decease in the generated force [5,8]. This feature plays a key role in the regulation of the Frank-Starling Law for the whole heart [9].
2. The length dependent calcium sensitivity [8]. Lower calcium concentrations are required to elicit a given force level as the SL increases. This phenomenon cannot be related to a direct effect of the length on calcium affinity and is mediated by the XBs [10].
3. The linear relationship between the energy consumption and the generated mechanical energy [6]. The cooperativity mechanism provides the adaptive control mechanism whereby changes in the loading conditions affect the rate of energy consumption by the sarcomere.
4.

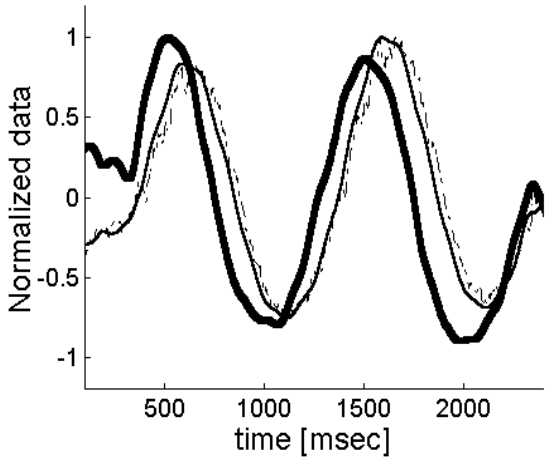

Fig.2. The time course of the sarcomere length (thick solid line), force (thin solid) and stiffness (dashed line), during muscle length oscillation at 1Hz.

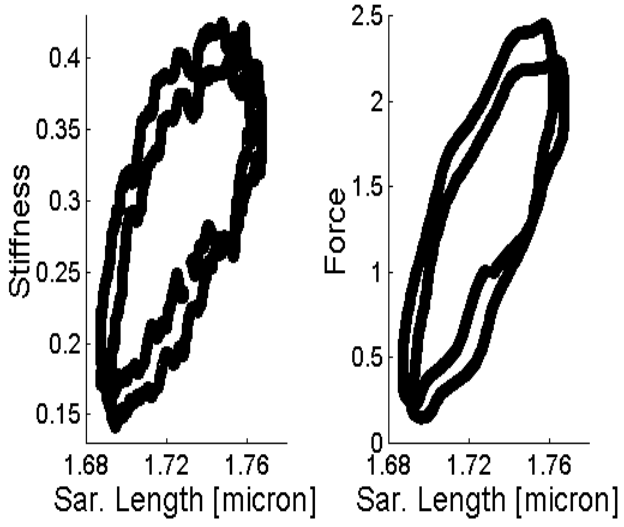

Fig. 3. Phase plot of the stiffness (left) and the force (right) against the sarcomere length, during oscillations at 1Hz.

The switching mechanism [7], in analogue to the positive feedback used in electrical switching circuits (Schmitt Trigger). At diastolic calcium concentration of 0.2μM – all the XBs are at the weak non-force generating state. An increase in the free Calcium by less than 4 folds, To 0.8μM allows the recruitment of enormous (an order of 10^{12}) amount of XBs. The positive feedback provides the steep dependence of the force on the free calcium concentration, within the relative narrow range of the physiological free calcium concentration.

VI. Conclusion

The present experimental study of the force and stiffness responses to sinusoidal sarcomere length oscillations, in the isolated tetanized cardiac trabeculae, validates the existence of the positive feedback (Cooperativity) that regulates cross-bridge recruitment and force generation. The study support the hypothesis that the regulation of XB recruitment can be characterized by the assumptions that:

- Ca^{2+} binding to the troponin regulatory proteins turns the regulated actin into the on-state, and allows ATP hydrolysis by the Actomyosin ATPase and XBs turnover from the weak to the strong force generating conformation.
- The number of force generating XBs feeds back to affinity of Troponin for Ca^{2++}.

Consequently, there is no unique force-length relationship (No constitutive law) even for constant free calcium level or constant activation, and the instantaneous force depends on the history of contraction and on the prevailing loading conditions.

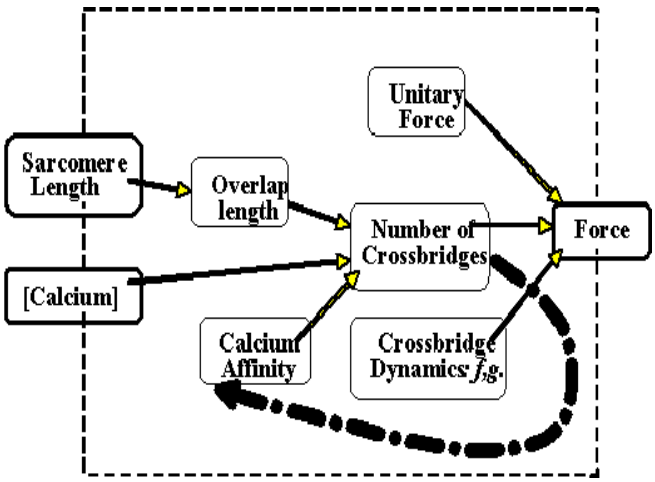

Fig.4. Block diagram describing the relationship between the inputs (the length and the free calcium concentration) and the force output. The cooperativity mechanism describes the dependence of calcium affinity on the number of force generating cross-bridges.

This concept is particularly important in the description of the excitation-contraction coupling of the cardiac muscle, since the cooperativity mechanism plays a key role in the regulation of the Frank-Starling Law and it provides the adaptive control loop whereby cross-bridge recruitment and energy consumption are determined by the prevailing mechanical loading conditions.

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