Effects of Sustained Length Dependent Activation on *In Situ* Cross-bridge Dynamics in Rat Hearts

Pumping of blood out of the healthy heart is regulated on a beat-to-best basis so that the blood volume ejected from the left ventricle (LV) matches the venous blood volume returning to the heart. When venous blood return is increased acutely the force developed by the LV must increase to enable it to eject more blood. This homeostatic mechanism is the basis of the Frank-Starling law of the heart.

Ca^{2+} release from internal stores is an important determinant of force developed by cardiac muscle. Nevertheless, it has been established that acute stretch of fibers increases force without an increase in the systolic (force generating phase) Ca^{2+} transient. This phenomenon is called length-dependent activation (LDA). Studies with isolated cardiac fibers have shown that Ca^{2+} sensitivity increases with muscle sarcomere length, but the mechanism for the increase in force development remains unclear.

One frequently reported consequence of stretch is that the probability of cross-bridge binding and their transition to strong attachments (force producing state) increases as the distance between myosin and neighbouring actin filaments (interfilament spacing) decreases, as the sarcomere length increases.

We have been applying small angle X-ray scattering to studies of cardiac muscle contractions in the beating hearts of rodents [1,2]. With higher photon fluxes at **BL40XU** beamline it is possible to record very rapid diffraction patterns to analyze events within the cardiac cycle, and therefore also the cross-bridge cycle. This allows us to probe how force generation is regulated *in vivo*. This is important as the beating heart performs 'work', in other words, contraction occurs under a physical load during systole in contrast to isolated muscle in studies implementing isometric contractions. Therefore our objective was to investigate how LDA affects cross-bridge dynamics in the *in situ* heart [3].

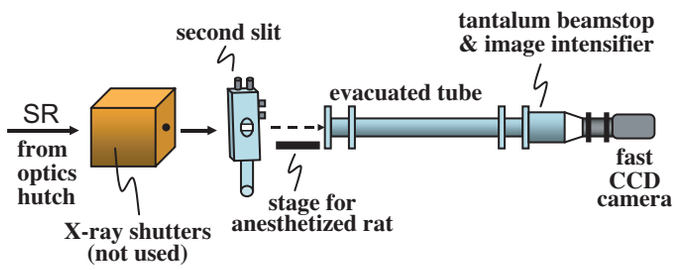

Fig. 1. Experimental set up at the BL40XU hutch for *in situ* whole-heart X-ray diffraction recordings. V5445P image intensifier and C4880-80-24A CCD camera (Hamamatsu Photonics) were used. Camera distance from the beating hearts was *ca*. 3 m during all experiments.

The aim of this study was to determine if sustained increased in venous return to the heart significantly decreases myosin interfilament spacing and increases cross-bridge formation within a localized region of the surface layer of LV muscle (anterior free-wall) of rat hearts. To achieve this we recorded diffraction patterns under baseline conditions and again in the same region during acute volume loading; i.e. an intravenous infusion was given to increase venous return to the heart. Recorded diffraction patterns indicate the proximity of myosin thick filaments to actin thin filaments within the filament lattice of myofibers, due to the large size, abundance and highly ordered arrangement.

We used a narrow collimated quasi-monochromatic beam (0.2 mm × 0.2 mm) provided at BL40XU beamline for X-ray diffraction recordings of spontaneously beating Sprague-Dawley rat hearts (Fig. 1). Beam flux was ~10^{12} photons/s (reduced with 3 mm thick Al bar, 15 keV and ring current 60-100 mA). Beating hearts were continuously exposed for *ca*. 2 s to the X-ray beam and diffraction images recorded at a 15-ms sampling interval (about 8-11 consecutive heart beats per recording). Beam orientation was perpendicular to the fiber direction in the outermost layer of the LV (equatorial position reflections). Anesthetized rat models were prepared as detailed elsewhere [2]. Simultaneous macro-level determinations of LV performance were made using intracardiac catheters to determine LV pressure (LVP) and volume (LVV) changes. Indices of cardiac function and heart work were determined with the aid of pressure-volume (P-V) loops and compared with *in situ* indices of crossbridge dynamics.

An index of myosin mass transfer to actin was determined as the decrease in intensity ratio (intensity of 1,0 reflection over 1,1) during the cardiac cycle (beat-to-beat interval identified from P-V loops). The distance between 1,0 reflections was converted to a lattice spacing between myosin filaments ($d_{1,0}$, nm) using a pixel calibration factor determined from a collagen sample.

Indices of cardiac function and cross-bridge cycling from a typical heart under baseline and volume loading conditions are shown (Fig. 2). As can be seen LVP (force developed) did not increase with stretch, but the volume changes during the cardiac cycle and the rate of pressure development (LVP dP/dtmax) increased significantly. At the same time,

interfilament spacing $d_{1,0}$ and equatorial intensity ratio ($I_{1,0}/I_{1,1}$) changes during the cardiac cycle were larger during volume loading. Notably, not only was the minimum intensity ratio decreased (more myosin heads in close proximity to actin), but the interfilament spacing was decreased by ~0.5 nm. From these 2-s recordings we obtained mean values for each rat. In all the hearts examined we found that the maximum rate of LVP development was significantly correlated with myosin mass transfer index (Fig. 3, top panel).

As expected, we found an inverse relation between $d_{1,0}$ and LV volume over the contractile phase of the cardiac cycle (Fig. 3, bottom panel). This suggests that interfilament spacing increases during fiber shortening in a predictable manner. Interestingly, diastolic relaxation of the LV caused a further 1 nm increase in $d_{1,0}$ at a time when sarcomeres are expected to be lengthening. X-ray diffraction techniques showed that the reduction in interfilament spacing caused by stretch initiated a greater activation of force-producing cross-bridges in early contraction. These findings support a role for interfilament spacing in modulating LDA.

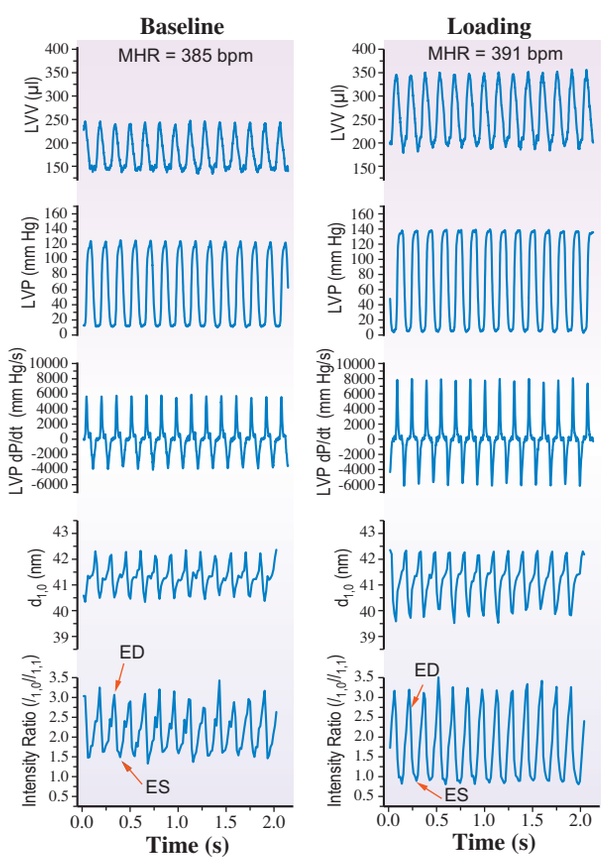

Fig. 2. Examples of the calculated beat-to-beat changes in intensity ratio ($I_{1,0}/I_{1,1}$), $d_{1,0}$, and measures of cardiac function over a series of heart beats under normal baseline conditions and volume loading (60 ml/h intravenous lactate solution).

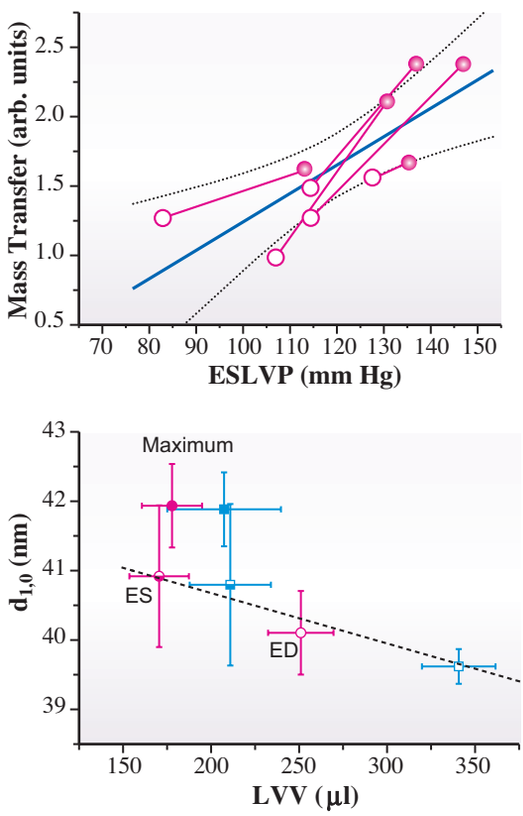

Fig. 3. Relation between the number of cross-bridges (mass transfer) developed locally in the heart wall and the rate of pressure development during contractions of the beating hearts (upper panel) and myosin spacing ($d_{1,0}$) changes in relation to LV volume (LVV) (lower panel). In the upper panel, baseline values are indicated as open circles, connected with volume loading means for the same hearts. In the lower panel, interfilament spacing increased linearly from end-diastole (ED) to end-systole (ES) for baseline (circles) and loading (squares) conditions, but was ~1 nm less than the maximum recorded shortly after the contraction finished.

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