REAL TIME *IN VIVO* MEASUREMENTS OF
CROSSBRIDGE DYNAMICS IN CARDIAC MUSCLE

Small angle X-ray scattering is one technique that is increasingly been used to investigate crossbridge cycling (formation of strong bonds between the major protein thick and thin filaments) in cardiac, skeletal and insect flight muscles. 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.

In spite of the interest in investigating the cellular basis of heart function and dysfunction, whole-heart investigations at the fiber level had not been performed until recently [1,2]. Since the heart beats continuously throughout life, in contrast to skeletal muscle the heart alternates between rapid periods of contraction (referred to as systole) and relaxation (diastole, including LV filling) and has high metabolic demands, making it vulnerable to ischemia. As the heart, and in particular the left ventricle (LV), is controlled both by intrinsic and extrinsic factors it is of scientific interest and medical value to investigate how the cellular components of myofibers are modulated by endogenous hormones and hemodynamic changes (increased filling or heart rate).

There are several reasons why *in situ* cardiac measurements under physiological conditions are needed. First, in contrast to isolated muscle studies during isometric contractions, the beating heart performs work, i.e. contraction occurs under a load during systole. Factors such as temperature, fiber operating range (length is influenced by structure of the whole heart) and the extracellular chemical environment have major impacts on crossbridge formation *in vivo*. Finally, there is no *a priori* reason to assume that crossbridge dynamics will be uniform throughout the heart. It is for these reasons that we investigated how crossbridge dynamics are influenced by local ischemia in the anterior wall of the LV of rat hearts.

We used a narrow collimated quasi-monochromatic beam (0.2 mm $\times$ 0.2 mm) provided at beamline **BL40XU** 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. Thus the contribution of a specific LV region to the work performed by the whole LV (in addition to other important hemodynamic information) can be determined with the aid of pressure-volume (P-V) loops and *in situ* indices of crossbridge dynamics [3].

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.

Increases in LVP during systolic contraction of the hearts correlated with a decrease in intensity ratio (i.e. mass transfer to actin during crossbridge formation) and an increase in $d_{1,0}$ spacing (Fig. 2). During relaxation, intensity ratio increased rapidly and $d_{1,0}$ spacing remained elevated until ventricular filling (LV refills with blood), when $d_{1,0}$ spacing decreased to a minimum, while intensity ratio remained stable. Thus under normal physiological conditions intensity ratio was high when the strong crossbridges were detached
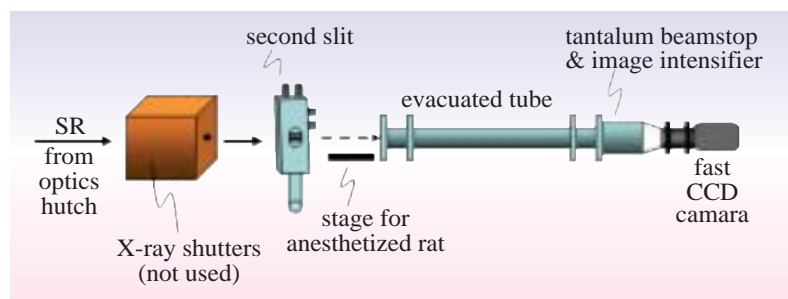

Fig. 1. Experimental set up for *in situ* whole-heart X-ray diffraction recordings. V5445P image intensifier and C4880-80-24A CCD camera (Hamamatsu Photonics) were used. Camera distance from the beating hearts was *ca*. 3 m during all experiments.

from actin filaments, but decreased in direct proportion with the pressure formed during contraction (left panel *black line* in Fig. 3); i.e. the change in intensity ratio is proportional to mass transfer of crossbridges *in situ*, consistent with past studies in isolated cardiac muscle [4].

However, for the first time we found that *in vivo* hearts do not maintain constant lattice volume during contractions [2]. In all hearts examined under baseline conditions mass transfer was essentially completed before the maximal extent of the $d_{1,0}$ spacing change (2-5 nm between hearts). Significant lattice expansion occurs after the release of isometric tension, when the aortic valve opens and fiber shortening occurs (see expansion after asterisk in Fig. 3 middle panel). Since $d_{1,0}$ spacing during systole was larger than diastole at any given LVV (Fig. 3 right panel), we suggested that crossbridge formation might cause lattice expansion as the release of radial forces between the filaments has been shown to increase lattice spacing after release from isometric tension in skeletal muscle [see 5].

Left coronary artery occlusion eliminated increases in lattice spacing and severely reduced mass transfer in the ischemic region (Fig. 2 and Fig. 3). The latter is expected as crossbridge formation is dependent on aerobic metabolic pathways, however, the significantly increased intensity ratios

of the ischemic region contrast the findings of *ex vivo* experiments (see ref. [2]).

X-ray diffraction techniques were more sensitive to fiber level differences in crossbridge dynamics than macro-level protocols and therefore have the potential to identify dysfunctional regions of the LV during cardiac disease.
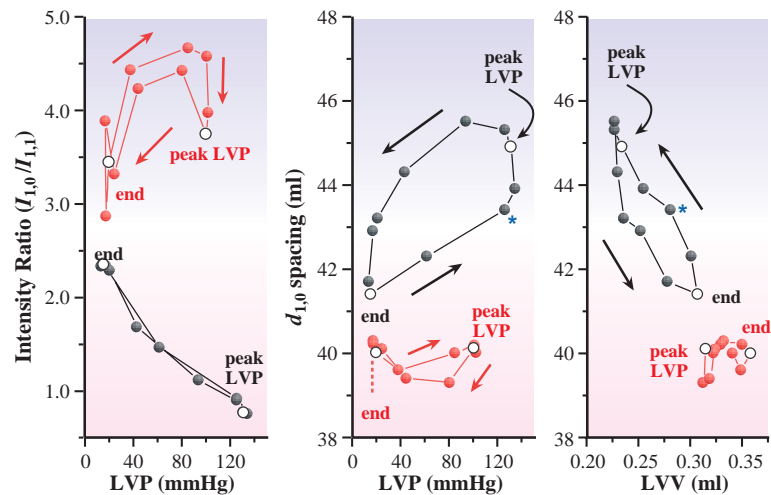
Fig. 3. Changes in the loop relationships between intensity ratio, myosin filament spacing ($d_{1,0}$), LVP and LVV of a typical heart before and after ligation of the left coronary artery (creating ischemia). Baseline conditions (black lines) and during regional ischemia (red lines) in the same heart are presented during average loops. Arrows indicate trajectories of the loops starting at the end of the cardiac cycle, through contraction (and peak LVP) and relaxation. Asterisks indicate the end of isovolumic contraction and the start of fiber shortening (see text).
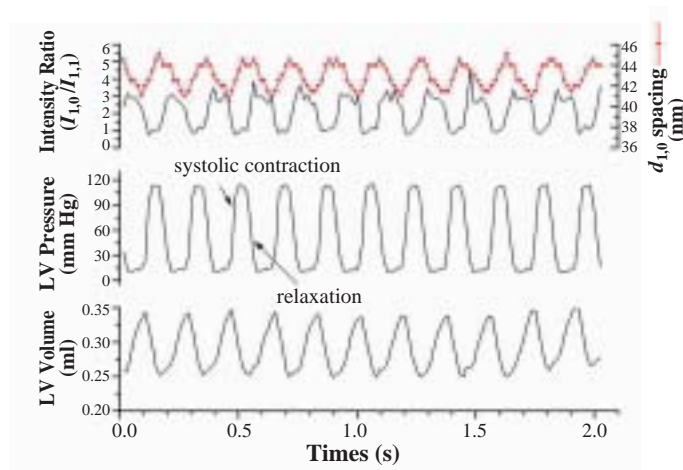
Fig. 2. Real time myosin filament spacing ($d_{1,0}$; red line) and intensity ratio changes calculated from X-ray diffraction patterns in relation to LVP and LVV recorded in a typical spontaneously beating rat heart under normal baseline conditions.

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