Mechanoenergetic Estimation of Multiple Cross-Bridge Steps per ATP in a Beating Heart

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Abstract: The efficiency from the ventricular O_2 consumption (VO_2) to the total mechanical energy (TME) generated by ventricular contraction has proved relatively constant at $\sim 35\%$, independent of the loading and contractile conditions in a canine heart. TME is the sum of the external mechanical work for ejecting a stroke volume against the afterload and of the mechanical potential energy for developing ventricular pressure in each beat. The $\sim 35\%$ VO_2 -to-TME efficiency indicates an also constant $\sim 60\%$ ATP-to-TME efficiency in a beating heart, based on the nominal $\sim 60\%$ VO_2 -to-ATP efficiency in the myocardial oxidative phosphorylation. I newly attempted to explain the load-independent $\sim 60\%$ ATP-to-TME efficiency by the recently reported ~ 7 – 10 nm unitary step size and ~ 0.8 – 1.5 pN unitary force of a

cross-bridge (CB) at the molecular level in *in vitro* motility assays. This single CB behavior suggests that its unitary cycle could generate a mechanical energy of ~ 0.6 – 1.5×10^{-20} J at most. From the nominal free energy of $\sim 10 \times 10^{-20}$ J per ATP, the efficiency from one ATP to the CB unitary cycle would then be ~ 6 – 15% . This low efficiency is only $\sim 1/10$ – $1/4$ of the $\sim 60\%$ ATP-to-TME efficiency at the heart level. This discrepancy suggests that each CB would repeat the unitary cycle at least ~ 4 – 10 times per ATP to achieve the high constant ATP-to-TME efficiency in a beating heart. This seems to represent a considerable mechanoenergetic advantage of the heart at the integrative heart level as compared to the molecular CB level. [The Japanese Journal of Physiology 54: 103–108, 2004]

Key words: myocardium, mechanics, energetics, unitary cycle, sliding filament.

The efficiency from the ventricular O_2 consumption (VO_2) to the total mechanical energy (TME) generated by ventricular contraction has proved to be relatively constant at $\sim 35\%$, independent of the loading and contractile conditions in a beating canine heart [1–6]. This suggests a high constant efficiency of $\sim 60\%$ from ATP to TME in a beating heart [5, 6], based on the $\sim 60\%$ efficiency of the myocardial oxidative phosphorylation to generate ATP from VO_2 at the nominal 3P:O atomic stoichiometry (i.e., $\sim 0.35 = \sim 0.60 \times \sim 0.60$) [5–7]. The load-independence of this ATP-to-TME efficiency has been supported by other investigators measuring heat as the energy output [7, 8]. Furthermore, this organ-level mechanoenergetics framework established first in the dog heart has been confirmed in the heart preparations of different mammalian species such as the rabbit and rat [9, 10].

At the molecular level, recent *in vitro* motility as-

says have shown that a cross-bridge (CB) made of myocardial actin and myosin molecules produces per an ATP molecule a ~ 7 – 10 nm unitary step under no load and a ~ 0.8 – 1.5 pN unitary force under no shortening for both V1 and V3 myosin isoforms of the rat and rabbit [11, 12]. Their product, i.e., step size times force, suggests that the CB unitary cycle per ATP could generate a mechanical energy of at most ~ 0.6 – 1.5×10^{-20} J. Based on the nominal free energy of $\sim 10 \times 10^{-20}$ J per ATP [7], the efficiency from one ATP to the CB unitary cycle would then be ~ 6 – 15% . This efficiency is only $\sim 1/10$ – $1/4$ of the $\sim 60\%$ ATP-to-TME efficiency in a beating heart.

In the present review, I considered theoretically on these already reported myocardial mechanoenergetics at both the organ level and the molecular level. Based on this consideration, I could estimate that each CB would repeat its unitary cycle several to a few dozen

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times per ATP to achieve the high constant ATP-to-TME efficiency in a beating heart. This seems to represent a considerable mechanoenergetic advantage of the heart at the integrative level in comparison to the elemental level.

Background

In cardiac mechanoenergetics, I proposed for the first time a specific area (systolic pressure–volume area, PVA) as a measure of TME generated by each left ventricular (LV) contraction in the pressure–volume (P – V) diagram (Fig. 1A) [5, 6, 13]. PVA consists of the P – V area representing the external mechanical work (EW) and that representing the mechanical potential energy (PE) generated in the ventricular wall. Namely, TME is the sum of EW and PE (Fig. 1A) [5, 6, 13].

We have then found that TME measured by the PVA linearly correlates with $\dot{V}O_2$ in canine LVs (Fig. 1B) [5, 6, 13]. The slope of the $\dot{V}O_2$ -to-TME relation above the sum of the basal metabolism and excitation-contraction coupling $\dot{V}O_2$ components is constant regardless of the LV loading and contractile conditions [5, 6, 13]. This mechanoenergetic framework reasonably matches with the already established time-varying elastance model of the LV (Fig. 1C) [5, 14].

This unique cardiac mechanoenergetics is consistent with the cardiac Fenn effect [1, 3, 5, 8], which is significantly different from the original Fenn effect established in the skeletal muscle [7, 15]. The heart requires much less shortening heat or energy than the skeletal muscle [5, 7]. This difference leads to a higher mechanical efficiency in shortening and ejecting contractions of the heart [5, 7]. Moreover, the time-varying elastance model I established in the heart in the 1970s does not hold for the skeletal muscle [7]. In the skeletal muscle, the so-called cocked spring model with an instantly rising elastance was discarded because its energetic prediction did not match the skeletal muscle Fenn effect [15]. In contrast, the energetic prediction of the time-varying elastance model of the heart reasonably matches the cardiac Fenn effect [1, 3, 4, 7].

The linear relationship between $\dot{V}O_2$ and TME (Fig. 1B) above the $\dot{V}O_2$ components for both the basal metabolism and the excitation-contraction coupling indicates a load- and contractility-independent constant O_2 cost of TME [5, 6]. Its reciprocal indicates a constant contractile efficiency of $\sim 35\%$ (Fig. 1B) [5, 6]. Note that this efficiency is different from and greater than the conventional mechanical efficiency from $\dot{V}O_2$ to the external mechanical work which is part of TME

Fig. 1. Cardiac mechanoenergetics framework at the heart level. **A:** Total mechanical energy (TME) corresponds to the specific area (shaded, designated as pressure-volume area, and abbreviated as PVA) under the end-systolic pressure–volume (P – V) relation (ESPVR) in the left ventricular P – V diagram. TME consists of external mechanical work (EW) and mechanical potential energy (PE). **B:** Experimentally established a linear relationship between the left ventricular O_2 consumption and TME regardless of its loading conditions, whether isovolumic or ejecting at a constant contractile condition. The slope of the diagonal linear relation indicates O_2 cost of TME, and its reciprocal indicates the contractile efficiency from O_2 to TME. The increasing O_2 consumption with TME is for cross-bridge (CB) cycling (shaded). The other part of O_2 consumption independent of TME is used for the non-mechanical activities such as basal metabolism and calcium (Ca) handling for excitation-contraction coupling. Changes in contractile state (E_{max}) affect only the O_2 consumption for Ca handling and then the elevation of the linear relation between left ventricular O_2 consumption and TME. **C:** The concept of TME was deduced from the time-varying elastance model of the ventricle. When the chamber increases its volume elastance to its maximum E_{max} by contraction, the increasing elastance generates not only EW to the afterload when ejection occurs, but also PE within the stiffening wall.

(Fig. 1A) [5, 6]. Therefore, although the mechanical efficiency varies between 0 and $\sim 30\%$ depending on the fraction of the mechanical work in TME, the contractile efficiency remains constant at $\sim 35\%$ [5]. Since the oxidative phosphorylation in myocardium has a nominal 3P:O atomic stoichiometry equivalent to a chemical efficiency of $\sim 60\%$, the $\sim 35\%$ efficiency from $\dot{V}O_2$ to TME means a constant efficiency

of $\sim 60\%$ from ATP to TME (Fig. 2A) (i.e., $\sim 0.6 = \sim 0.35 / \sim 0.6$) [5–7].

At the molecular level, the *in vitro* motility assay of skeletal muscle actin-myosin interactions has shown that a load-free CB could slide over multiple unitary steps per ATP [16–19]. A tight coupling between ATP and CB sliding, though, is not fully negated for skeletal muscle CBs [20, 21]. Apart from the skeletal muscle, Sugiura *et al.* discovered that each attached CB produces a unitary step sliding of a gaussian peak of ~ 10 nm under no load and a unitary force of a mean peak of ~ 1.5 pN under no sliding, using rat myocardial actin and myosin (V1 and V3 isoforms) molecules in an *in vitro* motility assay [11]. Palmiter *et al.* reported a unitary step size of ~ 7 nm and a unitary force of ~ 0.8 pN, using rabbit actin and myosin (V1 and V3 isoforms) molecules [12]. These step sizes are largely comparable to the myosin-head length and seem reasonable if the CB sliding is driven by the tilting of a myosin head attached to an actin filament (Fig. 2B). These unitary values fall in the lower range of the step size (~ 6 – 20 nm) and are smaller than or comparable to the force (~ 1.5 – 6 pN) of skeletal muscle CBs in the literature [16–21]. However, unlike a

skeletal muscle CB, no group has yet reported evidence of multiple steps of a cardiac CB cycling per ATP.

Knowing the multiple steps of a skeletal muscle CB per ATP reported by Yanagida *et al.* [19], we have theoretically indicated the possibility of multiple steps of each CB per ATP to account for the cardiac Fenn effect [22] by use of the Huxley-type CB model [23]. However, we could not relate the resultant values of the multiple steps of CBs [22] with the high constant contractile efficiency at the heart level because unitary step size and force data of a cardiac CB were not available before the work of Sugiura *et al.* [11].

Theoretical Considerations

Knowing the unitary behavior of a cardiac CB [11, 12], I again wondered whether it can account for the load-independent high constant efficiency of $\sim 60\%$ from ATP to TME in a beating heart, and if it can account for it, how this is possible. To this end, I calculated first in the present study the CB unitary energetics, using the CB unitary mechanics reported by Sugiura *et al.* [11]. From the unitary step size of ~ 10 nm

Fig. 2. Unitary cross-bridge cycling per ATP at the molecular and organ levels. **A:** Efficiencies of energy conversions from O_2 to total mechanical energy (TME) via ATP in myocardium. **B:** Unitary step size and force of the attached cross bridge (CB) in the myosin-swinging and filament-sliding model. The unitary values were cited from Sugiura *et al.* [11]. **C:** Efficiency of mechanical energy generated by a unitary step of a CB from the free energy of an ATP molecule at the molecular level. **D:** Efficiency of TME generated by multiple (~ 7 – 20) unitary steps of a CB from the free energy of ATP in cardiac contraction. **E, F, and G:** Average numbers of unitary steps of a CB per ATP estimated in a ventricular contraction under three different loading conditions: isovolumic contraction (**E**); moderately afterloaded, isobarically ejecting contraction (**F**); and preloaded but almost freely ejecting contraction (**G**). The numbers of the unitary steps in these panels will increase reciprocally with the decreases in the unitary step size and force of a CB per ATP from Sugiura *et al.*'s data [11] to Palmiter *et al.*'s data [12].

and the unitary force of ~ 1.5 pN of a single CB [11], the unitary mechanical energy generated by a single CB cycle would be at most their product, i.e., ~ 15 pN nm $= \sim 1.5 \times 10^{-20}$ J (N m = J and 100 pN nm $= 10 \times 10^{-20}$ J) in the swinging CB model (Fig. 2B).

However, the force of a CB attached to an actin molecule will reasonably decrease with sliding or shortening from the maximal reach (~ 10 nm) of the actin-attached myosin head in the attached CB [23]. If the unitary force of an attached CB is assumed to decrease linearly with its shortening, the unitary mechanical energy generated by the single CB cycle could be predicted to decrease to half of $\sim 1.5 \times 10^{-20}$ J, i.e., $\sim 0.75 \times 10^{-20}$ J (Fig. 2C).

Since ATP has nominally a free energy of 57 kJ/mol in myocardium [7], an ATP molecule has a free energy of 9.5 (or ~ 10) $\times 10^{-20}$ J as 57 kJ/mol divided by Avogadro's number of 6.02×10^{23} molecules/mol. From the mechanical energy of the above mentioned $\sim 0.75 \times 10^{-20}$ J generated by a unitary CB cycle and the free energy of the above-mentioned $\sim 10 \times 10^{-20}$ J of an ATP molecule, we can calculate the chemomechanical efficiency from one ATP to a CB unitary cycle to be $\sim 7.5\%$ (Fig. 2C). This is only $\sim 1/8$ of the mechanical efficiency of $\sim 60\%$ from ATP to TME in the ventricle.

To solve this ~ 8 times discrepant efficiencies between the organ and molecular levels, it seems quite reasonable to imagine that each attached CB cycles ~ 8 times on average per ATP, not merely once, to convert the chemical energy of ATP to TME at the high overall efficiency of $\sim 60\%$ in a beating heart (Fig. 2D).

Myocardial sarcomeres usually shorten even in the isometric contraction by stretching the series elasticity that is several times more compliant than that of the skeletal muscle [24]. Moreover, myocardium per se normally shortens more or less in the normally asynchronously contracting ventricular wall, even without ejection in an isovolumic contraction. These mechanical properties of the myocardium should allow the attached CBs on average to slide even when the myocardium contracts isometrically and the ventricle contracts isovolumically (Fig. 2E).

When the series elasticity is stretched by $\sim 7\%$ at the peak of isometric myocardial contraction [24], it allows a half-sarcomere of ~ 1 μ m length to shorten by ~ 70 nm, which is 7 times the CB unitary step of ~ 10 nm. This suggests that the attached CB must slide over ~ 7 unitary steps on average to develop the high peak ventricular pressure in an isovolumic contraction (Fig. 2E).

Furthermore, during ejection with a typical normal

ejection fraction of $\sim 70\%$ against a moderate afterload, myocardium and then half-sarcomeres of ~ 1 μ m length would shorten by $\sim 15\%$ on average, though varying among different layers of the ventricular wall. This shortening corresponds to ~ 150 nm and thus to ~ 15 unitary steps of a CB (Fig. 2F).

As the force generated by each sliding CB decreases with its sliding distance or shortening velocity, the load-independent $\sim 60\%$ efficiency from ATP to TME suggests that the number of unitary steps of a CB per ATP could increase considerably despite the constant ~ 10 nm unitary steps of a CB [11]. If the number of unitary steps of a CB per ATP were always one despite the changing load, the load-independent constant efficiency from ATP to TME could not be maintained. Such a load-dependent efficiency from ATP to TME contradicts the reality [5]; therefore, the multiple unitary steps of a CB on average per ATP are reasonable even in shortening contractions. The multiple unitary steps per ATP would reasonably hold because even the ejecting contraction requires $\dot{V}O_2$ proportional to TME without requiring extra $\dot{V}O_2$ for ejection and muscle shortening [4, 5] in contrast to skeletal muscles [4]. This is the essential difference of the cardiac Fenn effect from the skeletal muscle Fenn effect [7, 15].

As an extreme case, the pre-loaded ventricle can eject its entire volume against a small afterload with almost no extra $\dot{V}O_2$ for a practically zero TME above the $\dot{V}O_2$ of a non-preloaded contraction [2, 5]. In this case, the half-sarcomere would shorten reasonably from ~ 1 to ~ 0.8 μ m by ~ 200 nm, corresponding to ~ 20 unitary steps of a CB per ATP (Fig. 2G).

These ~ 7 to ~ 20 multiple unitary steps on average of each CB per ATP (Fig. 2E, F, and G) may appear contradictory to our previous calculation that each attached CB hydrolyzes ATP once or a little more on average in one contraction of the heart [6, 25, 26]. It was calculated from a reasonably maximum TME of 4,000 mmHg ml $= \sim 0.53$ J per 100 g LV generated by all the ~ 15 μ mol CBs per 100 g LV [6, 25, 26]. This number of CBs is reasonably presumed from a representative myocardial concentration of myosin molecules of ~ 7.5 μ mol per 100 g LV, and the two heavy chains attachable to actin per myosin molecule. The above TME divided by the total number of CBs yields TME of $\sim 6 \times 10^{-20}$ J per CB. Since each ATP has $\sim 10 \times 10^{-20}$ J, as already mentioned, the 60% efficiency of the above TME per ATP leads to $\sim 6 \times 10^{-20}$ J. This is comparable to the above-mentioned TME of $\sim 6 \times 10^{-20}$ J per CB. The coincidence of these two energy values always holds regardless of the loading and contractile conditions because of the

load-independent constancy of the contractile efficiency [5, 6]. This implies that each once-attached CB hydrolyzes ATP once on average regardless of the number of its multiple unitary steps during any cardiac contraction under a variety of loading and contractile conditions.

In these calculations, I adopted the unitary step size and force values reported by Sugiura *et al.* [11]. However, if I use those reported by Palmiter *et al.* [12], ~2.5 times more unitary steps of a CB per ATP would be required to maintain the constant ~60% ATP-to-TME efficiency.

Based on these results, I would propose the possibility that each attached CB slides over several to a few dozen unitary steps by gradually converting the ATP free energy to TME in each of the multiple unitary steps (Fig. 2D–G). This possibility seems to represent a mechanoenergetic advantage of the heart at an integrative level over the mechanoenergetics of individual CB molecules at an elemental level. This possibility is the first reasonably quantitative one to bridge between the integrative (or organ or global) and reductive (or molecular or elemental) experimental findings on cardiac mechanoenergetics. I recently presented this aspect of cardiac contraction in a symposium [27, 28].

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