Explaining Load Dependence of Ventricular Contractile Properties with a Model of Excitation–Contraction Coupling

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D. Burkhoff. Explaining Load Dependence of Ventricular Contractile Properties With a Model of Excitation–Contraction Coupling. *Journal of Molecular and Cellular Cardiology* (1994) **26**, 959–978. A theory is present which accounts for a very broad range of ventricular properties that have been noted in recent experiments. The theory is based upon a four-state biochemical scheme that accounts for the dynamic interaction between calcium, actin and myosin which includes a calcium-free force generating complex between actin and myosin. This original scheme was supplemented by incorporating two additional basic properties of cardiac muscle: length dependence of calcium binding affinity and load dependence of force generation. The biochemical scheme was used to provide the force-length-time properties of cardiac muscle which were used to construct a ventricle via a spherical geometry. In addition to being able to accurately interrelate previously measured calcium and muscle force transients, this theory was able to account for many fundamental aspects of ventricular performance including: a realistic contractility dependent curvilinearity of the end-systolic pressure–volume relationship; enhancement of contractile strength on ejecting compared to isovolumic beats; improved contractile efficiency on ejecting as compared to isovolumic beats; appropriate load-dependent changes in time to peak pressure, time constant of relaxation and duration of contraction on isovolumic and ejecting beats; realistic estimated time course of tension-dependent heat generation. The explanation for these phenomena were explored within the context of the theory and presented in detail.

KEY WORDS: Length-dependent activation; Muscle mechanics; Calcium binding; Ventricular mechanics.

Introduction

Knowledge of the fundamental laws which govern the dynamics of ventricular contraction is of primary importance for advancing understanding of heart function in health and disease. Currently, ventricular pump function is commonly characterized in terms of indices derived from analysis of pressure–volume loops measured under different loading conditions such as the end-systolic pressure–volume relationship (Sagawa, 1978) or pre-load recruitable stroke work (Glower *et al.*, 1985). However, because of their phenomenologic nature, these approaches provide very limited information about myocardial muscle properties. Furthermore, in many instances, the basic assumptions of the theories of ventricular pump function contradict observations made in isolated cardiac muscle. On the other hand, it is unknown whether many of the fundamental concepts of cardiac muscle contraction derived from observations made in isolated superfused muscle preparations (either intact or skinned) pertain to the more physiological conditions of muscle in the wall of the ventricle. Thus, there are a number of major conceptual gaps between how we think about muscle mechanics and how we think about ventricular mechanics.

The main purpose of this theoretical study is to test the feasibility of an alternate theory of ventricular mechanics that is based on a simple model of excitation–contraction coupling that describes the kinetic interactions between calcium and the myofilaments. The model has its basis, a four-state biochemical scheme derived from previous theoretical considerations (Hill, 1983) which has already been shown to account for some basic aspects of

cardiac muscle mechanics (Yue, 1987; Peterson et al., 1991).

A brief summary of the limitations of the currently popular theory of ventricular contraction, which is based on the time-varying elastance model, will be presented; this will be followed by an explanation of the new theory with development of a mathematical model of ventricular properties. The model will then be subjected to simulated changes in loading conditions and the behaviour of the model will be compared to previously published results obtained from isolated canine hearts (Burkhoff et al., 1987, 1991, 1993). It will be shown that the proposed theory can account for complex load dependent changes in ventricular pressure generating capacity, time course of contraction, time course of relaxation and energy consumption. The limitations of the theory are discussed.

Theoretical Considerations and Methods

Current theory of ventricular contraction

The course of research in cardiovascular physiology has been influenced greatly by the time-varying elastance theory of ventricular contraction which was introduced in the early 1970s by Suga and Sagawa (1974). The success of this theory likely relates to two factors. First, the theory permitted description of ventricular mechanical pump properties throughout the cardiac cycle by interrelating instantaneous ventricular pressure and volume [LVP(t) and LVV(t), respectively] with a simple equation: $[LVP(t) = E(t)[LVV(t)-V_o]$. In principle, the $E(t)$ function describes the time variations in instantaneous ventricular volume-elastance during a beat and was proposed as a load-independent characterization of dynamic ventricular pump properties. Thus, $E(t)$ could be used to understand many aspects of ventricular–vascular coupling in quantitative terms and the availablity of this theory has resulted in many advances along this line (Sagawa et al., 1988). Second, the value of $E(t)$ at end systole (E_{es}) varies directly with inotropic background and it was therefore proposed that E_{es} could serve as a load-independent index of ventricular contractility. The theoretical load-independence of this index set it apart from other available contractile indices at that time and thus E_{es} emerged, and remains, one of the most commonly used indices of ventricular contractility in basic and clinical research of ventricular mechanics.

However, several shortcomings of the $E(t)$ theory

have been identified over the past several years. First, it was demonstrated that there is a flow-dependent reduction in ventricular pressure below what would be expected by the $E(t)$ theory. This led to modification of the theory to include what is referred to as an internal resistance (R_i) (Hunter et al., 1983; Shroff et al., 1985) such that ventricular pressure and volume could be interrelated by the following equation: $LVP(t) = E(t)[LVV(t)-V_o]-R_iF(t)$, where $F(t)$ is the instantaneous flow out of the ventricle. Second, it was shown in isolated papillary muscles that the time from the start of a contraction to the time of peak contraction is highly dependent upon the loading conditions imposed on the muscle (Elzinga and Westerhof, 1981; Hisano and Cooper, 1987). This finding has since been confirmed in isolated canine hearts (Hunter, 1989; Burkhoff et al., 1993). Third, the theory never accounted for well known afterload dependent changes in the rate of relaxation (Brutsaert and Sys, 1989). Fourth, while the elastance theory only accounts for a linear relationship between pressure and volume at any instant during the cardiac cycle, it is known that these relations can be non-linear, even at the end of systole (Burkhoff et al., 1987; Kass et al., 1989). Fifth, results of recent studies have indicated that generated pressures can be greater when the ventricle is allowed to eject as compared to when it contracts isovolumically; this has been demonstrated in a variety of experimental models (Hunter, 1989; Burkhoff et al., 1991, 1993; Yasumura et al., 1988; Igarashi et al., 1991; Campbell et al., 1986, 1990; Vaartjes and Boom, 1987). Indeed, it has been shown that neither the $E(t)$ nor the $E(t)$-R_i theory could account for the behaviour of intact hearts (Campbell et al., 1986, 1990). Sixth, the time-varying elastance theory leads to the notion of a load-independent metabolic-to-mechanical energy transduction efficiency whereas recent findings (Burkhoff et al., 1991; Yasumura et al., 1988) suggest that this efficiency may vary with load. Finally, several researchers have objected to the fact that the $E(t)$ theory is a phenomenological description of ventricular behavior which lacks a foundation in basic principles of muscle contraction (Elzinga et al., 1989; Cooper, 1990).

Thus, there are several important limitations of the $E(t)$ theory for describing the load dependence of ventricular behaviour. While there have been numerous suggestions that at least some of these observations could relate to length dependence of myofilament activation (Tucci et al., 1984; Sugiura et al., 1989; Hunter 1989; Burkhoff et al., 1991, 1993), no unified theory has been proposed thus

far which is able to account for the observed phenomena.

It is the premise of this study that basic understanding of cardiac muscle physiology, with particular emphasis on length dependence of activation, can provide the foundation for developing a new theory of ventricular contraction. Accordingly, a model of muscle contraction will be presented. Then, a simple geometric transformation will be used to construct a ventricle from the model muscle in order to interrelate muscle length and force to ventricular volume and pressure (which involves non-linear transformations). Finally, the ventricle so constructed will be mathematically coupled to a simulated arterial system so that experiments previously performed on real hearts can be reproduced for the model ventricle. Results from real hearts will then be compared to behaviour predicted from the new theory.

New theory of ventricular contraction

The development of techniques to measure instantaneous intracellular calcium concentration $\{[Ca^{2+}]_i(t)\}$ in beating muscle cells (Allen and Blinks, 1978) has led to an understanding of the temporal relations between free calcium concentration and force generation. A typical example taken from the literature (Yue, 1987), shown in Fig. 1, demonstrates that as measured with aequorin there is a rapid rise in calcium concentration early in the beat which is followed by a slower rise in force. It is clear that by the time force is at its maximal level, $[Ca^{2+}]_i$ has fallen significantly and that the muscle maintains significant force past the time when $[Ca^{2+}]_i$ has fallen to near its diastolic level. This *delay* between free calcium transient and force generation has been considered to arise from the kinetics of calcium binding to troponin and of crossbridge cycling as has been explained by different models (Yue, 1987; Lee and Allen, 1993). In addition, experimental findings suggest that there is also a time lag between the fall of cytosolic calcium and the fall of calcium bound to the myofilaments. Results of two studies (Yue, 1987; Peterson *et al.*, 1991) suggest that a four-state biochemical scheme originally proposed by Hill for skeletal muscle (Hill, 1983), which includes a calcium-free force generating state, can account for the temporal relations between free calcium, bound calcium and force in cardiac muscle. This scheme, shown in Fig. 2, provides one plausible means of accounting for the kinetic interactions between actin (A), myosin (M), troponin C (Tn) and calcium (Ca^{2+}) during a con-
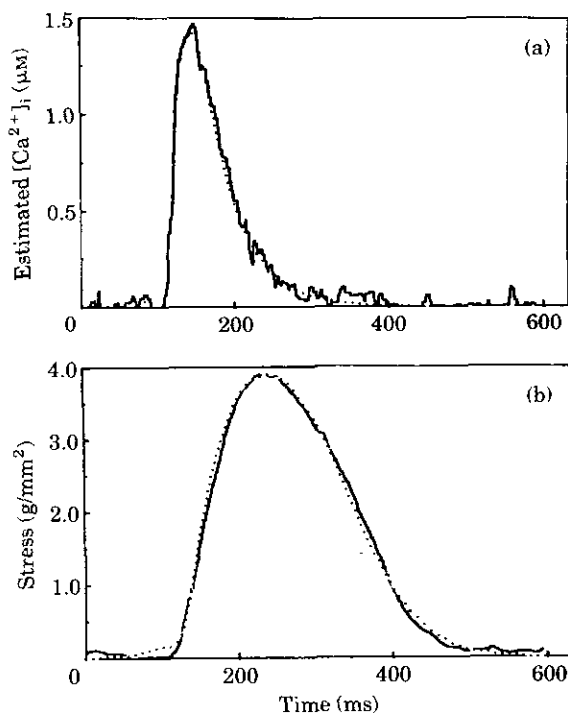
Figure 1 Calcium transient (a) and simultaneously measured force generation (b) during an isometric muscle contraction at L_{max} (——). These curves were taken from the literature (Yue, 1987). The dotted line in (a) shows the smoothed calcium transient; this was used as the driving function for the system of differential equations describing muscle contractile properties. The dotted line in (b) shows the model prediction of muscle force from the calcium transient with optimally adjusted rate constants (see Table 1). This shows the excellent concordance between measured force and force predicted by the model.
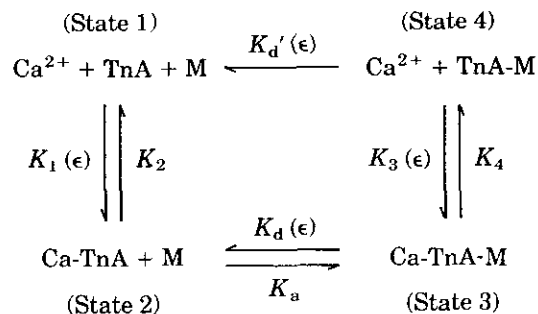
Figure 2 Four-state biochemical scheme that forms the basis of the model used to interrelate instantaneous calcium transient and muscle force production and, ultimately, left ventricular pressure. Rate constants of different reactions denoted by the K's; ϵ denotes length dependence of the respective rate constant. Ca, calcium; Tn.A, troponin complexed with actin; M, myosin.

centration cycle. In brief, when Ca^{2+} concentration is low (diastolic state) actin and myosin are essentially unbound (State 1) and no active force is generated. After Ca^{2+} is released from the sarcoplasmic reticulum, calcium binds to TnC and the system shifts towards State 2. Once Ca^{2+} is bound, a conformational change in the actin–troponin–tropomyosin complex permits actin-myocin binding to occur and there is appearance of State 3, a force generating state. There are two pathways by which actin–myosin uncoupling (relaxation) can occur. The first is simply back to State 2. The second pathway is a state in which calcium can be released first with persistence of the actin–myosin bond and force generation (State 4). From State 4, calcium can be rebound (back to State 3) or actin–myosin bonds can break and the system returns to State 1 (relaxation); this later step is an irreversible process. In this simple view of excitation–contraction coupling, muscle force (or stress, σ) generation would be related to the number of force generating units so that:

$$\sigma = \alpha([Ca^{2+}\text{-Tn·A-M}] + [Tn\text{·A-M}]) \qquad (1)$$

where α is a proportionality constant.

This construct, however, has no provisions for explaining why force production varies with the preload and afterload conditions imposed on the muscle cell. Studies performed on isometrically contracting cardiac muscles have suggested several factors which may underlie the load dependence of cardiac muscle strength, though the mechanisms are not known with certainty. First, there is evidence suggesting that the affinity of the myofilaments for calcium increases as the length (ϵ) of the muscle cell is increased (Hibberd and Jewell, 1982). Increased binding affinity would lead to a greater amount of calcium binding, resulting in a greater number of actin–myosin interactions and ultimately leading to greater force production. This concept which as discussed below, is not universally accepted (Hofmann and Fuchs, 1988, 1987a,b,), can be incorporated by introducing length-dependence to the rate constants of the biochemical scheme, as symbolized by the ϵ associated with the various Ks. Second, length may affect the ability of the myofilaments to generate force. Even with saturating concentrations of calcium supplied to the myofilaments of skinned fibres, force has been shown to decrease by as much as 60% when sarcomere length is decreased from 2.3 and 1.5 μM (Allen and Kentish, 1985; Kentish et al., 1986; Fabiato and Fabiato, 1975). Despite the central importance of this fundamental observation in car-

diac physiology, the mechanism is not known, though several factors have been proposed (Allen and Kentish, 1985). These include a direct effect of length on force generating capacity of individual crossbridges, *restoring forces* (due to myofilament interactions, sarcolemmal deformations, extracellular matrix constraints, etc.) or length-dependent variations in the number of potential actin–myosin binding sites. One way to account for such factors in the scheme is by assuming that α, the proportionality factor relating force to the number of actin–myosin bonds, varies with muscle length [i.e. $\alpha = \alpha(\epsilon)$]; other approaches are possible and the advantages and disadvantages of these will be discussed further (see Limitations).

It has been shown during contractions in which cardiac muscle is allowed to shorten, that the calcium transient is prolonged as compared to that measured when it is contracting isometrically from the same end-diastolic length (Housmans et al., 1983). This finding has been interpreted as indicating that as the muscle shortens calcium is being released from the myofilaments due to the instantaneous decrease in calcium affinity. This observation suggests, within the context of the proposed biochemical scheme (Fig. 2), that the length dependent changes in the rate constants occur instantaneously.

There is at least one other basic observation pertaining to the impact of shortening on muscle force generation: the force–velocity relationship (Sonnenblick, 1962, 1965) which is the phenomenon in cardiac muscle whereby muscle force generating capability decreases in proportion to the velocity with which the muscle is shortening. There is an analogous effect in the ventricle (Hunter et al., 1979, 1983; Shroff et al., 1985) whereby the pressure generating capability of the ventricle decreases in proportion to the flow out of the ventricle. This could be accounted for in the biochemical scheme, in a phenomenological manner, by adding a term to eqn (1) which decreases force in proportion to the velocity of shortening:

$$\sigma(t,\varepsilon) = \alpha(\varepsilon)\{[Ca^{2+}\text{-Tn·A-M}] + [Tn\text{·A-M}]\} - vR(\sigma) \quad (2)$$

where v is the velocity of shortening ($v \equiv d\epsilon/dt$) and $R(\sigma)$ is the proportionality constant that varies as a function of the amount of force being generated in accordance with previous observations (Shroff et al., 1985) such that $R(\sigma) = \sigma/v_{max}$, where v_{max} is the velocity of shortening that abolishes force development.

In order to test the feasibility of this theory, numerical techniques were used to determine

Table 1 Rate constants and proportionality constants used in simulation

$K_{1,ref}$	40/M/s
K_2	20/s
$K_{3,ref}$	2000/M/s
K_4	37/s
K_a	0.4/M/s
$K_{d,ref}$	0/s
$K'_{d,ref}$	37/s
α_o	8.5 mmHg/M
v_{max}	800 ml/s

whether the proposed model can account for the diverse phenomena observed in the ventricle reviewed above. The following differential equations governing the four-state scheme were programmed on a computer.

$$d[Tn \cdot A]/dt = - K_i[Ca^{2+}]_i(t)[Tn \cdot A] + K_d'[Tn \cdot A-M] + K_2[Ca-Tn \cdot A]$$

$$d[Ca-Tn \cdot A]/dt = K_i[Ca^{2+}]_i(t)[Tn \cdot A] - K_a[Ca-Tn \cdot A] - K_2[Ca-Tn \cdot A][M] + K_d[Ca-Tn \cdot A-M]$$

$$d[M]/dt = K_d'[Tn \cdot A-M] + K_d[Ca-Tn \cdot A-M] - K_2[Ca-Tn \cdot A][M] \quad (3)$$

$$d[Ca-Tn \cdot A-M]/dt = K_3[Ca^{2+}]_i(t)[Tn \cdot A-M] + K_a[Ca-Tn \cdot A][M] - (K_d + K_4)[Ca - Tn \cdot A-M]$$

$$d[Tn \cdot A-M]/dt = - \{[Ca^{2+}]_i(t)K_3 + K_d'\}[Tn \cdot A-M] + K_a[Ca-Tn \cdot A][M]$$

The driving function for these equations is the instantaneous concentration of free calcium $\{[Ca^{2+}]_i(t)\}$ which, for purposes of this analysis, was obtained from previously published data. Figure 1 (a) shows a calcium transient from the literature (Yue, 1987) that was hand-digitized (solid line) and smoothed (dotted line). Initial values for the rate constants were taken from the literature (Peterson et al., 1991). These constants were adjusted slightly in order to provide a good fit between the muscle stress measured simultaneously with the $[Ca^{2+}]_i(t)$ transient of Figure 1 (Yue, 1987) and muscle stress predicted from the model; final values of the rate constants are shown in Table 1 and discussed further below. The solid line in Figure 1(b) shows stress measured from the isometrically contracting muscle (Yue, 1987); the dotted line in this panel shows the time course of muscle stress predicted from eqn (3) with optimally adjusted rate constants. The striking similarity of these two curves suggests that this four-state biochemical scheme can be used to interrelate the calcium estimated from the aequorin signal and the force measured

under isometric conditions (conditions under which v equals 0). On the other hand, the four-state model, with seven adjustable rate constants, has a rather high number of degrees of freedom with which to fit the relatively simple force curve, so this concordance provides no proof of the construct. Also, note that the best fit was obtained when K_d was set to a value of 0.

The next step in formulating the new theory was to construct a ventricle out of muscles with properties described by eqns (2) and (3) using a simple geometrical model. In order to interrelate muscle stress (σ in eqn 2) and strain (ϵ) to ventricular pressure (LVP) and volume (LVV), the ventricle was assumed to be constructed as a thick-walled sphere with uniform muscle properties and with σ assumed to represent the average stress across the wall. Accordingly, σ was transformed into LVP based upon inner LV chamber radius (r_i), outer LV chamber radius (r_o), LVV, and left ventricular wall volume (LVWV) (Grossman et al., 1977; Hunter and Baan, 1980):

$$r_i = (3LVV/4\pi)^{1/3}$$
$$r_o = [(3/4\pi) (LVV + LVWV)]^{1/3} \quad (4)$$
$$LVP = \sigma(r_o^2 - r_i^2)/r_i^2$$

LVWV was assumed to be equal to 100 ml, a typical value for hearts from 20 kg dogs. LVV was then related to muscle length (L) by assuming that the latter was related to the mid-wall circumference of the sphere: $L = 2\pi[(r_i + r_o)/2]$. L was then converted into a strain value (ϵ) to provide a value of 0 when LVV was 0, and 1 when LVV attained an arbitrary reference volume of 50 ml (a typical upper limit for hearts of 20 kg dogs) according to the following system of equations:

$$r_{o,min} = (3 \; LVWV/4\pi)^{1/3}$$
$$r_{i,min} = 0$$
$$L_{min} = 2\pi(r_{o,min} + r_{i,min})/2 \quad (5)$$

$$r_{o,ref} = [(3/4\pi) (50 + LVWV)]^{1/3}$$
$$r_{i,ref} = (3 \cdot 50/4\pi)^{1/3}$$
$$L_{ref} = 2\pi(r_{o,ref} + r_{i,ref})/2$$

$$\epsilon = (L-L_{min})/(L_{ref}-L_{min})$$

For the final computations of ventricular performance, the values of K_1, K_3, K_d and K_d' were assumed to vary with ϵ as summarized in the following equations:

$$K_1 = K_{1,ref}\epsilon^{\gamma}$$
$$K_3 = K_{3,ref}\epsilon^{\gamma} \quad (6)$$
$$K_d = K_{d,ref}(3-\epsilon^{\gamma})/2$$
$$K_d' = K'_{d,ref}(3-\epsilon^{\gamma})/2$$

The length dependence of the rate constants for action myosin uncoupling (i.e. K_d and K_d', with these values increasing as length decreases) were introduced in concordance with suggestions derived from the observation that the rate of isometric relaxation decreases as muscle length increases (Allen and Kentish, 1985); note that since K_d was set to 0 above, so too was $K_{d,ref}$, and this equation is listed above for the general case. The values of the rate constants at $\epsilon = 1$ were set so that they equalled the values used in the analysis presented above which provided a good fit between measured and predicted muscle force. The exponent, γ, was set equal to 5, a value chosen empirically. The remaining parameter which needs to be specified is the ϵ-dependence of α (eqn 2) which was assumed to take on the following form:

$$\alpha = \alpha_0(0.4 + 0.6\epsilon) \qquad (7)$$

according to the theoretical considerations outlined above (Allen and Kentish, 1985; Kentish et al., 1986; Fabiato and Fabiato, 1975), where α_0 is a proportionality factor relating the number of cross-bridges to total stress. A summary of all the parameter values used in the simulation is shown in Table 1. It is to be noted that the over-simplified manner in which the steady-state length–tension relation, the length-dependence of calcium binding and the force–velocity relation are dealt with are for computational and conceptual simplicity rather than for a lack of more elegant models (discussed further below).

Diastolic chamber properties were assumed to be totally passive and were characterized by the following relationship between pressure ($P_{passive}$) and volume (LVV):

$$P_{passive} = B(e^{A.LVV} - 1) \qquad (8)$$

where $A = 0.08$ ml and $B = 0.25$ mmHg, values which provide an end-diastolic pressure–volume relationship typical of a canine heart from a 20 kg dog. Total ventricular pressure was set equal to the sum of the $P_{passive}$ and the active pressure generated by muscle contraction (eqn 2).

From the set of differential equations (eqn 3) with assumed length-dependence of rate constants (eqn 6), free calcium transient taken from the literature (Fig. 1), and assumed relationship between actin-myosin bonds, velocity of shortening and ventricular pressure (eqns 2 and 6) it is possible to define the instantaneous relationship between ventricular volume and pressure. It is to be emphasized that the

calcium transient was assumed to be invariant, i.e. independent of the loading conditions; the limitations introduced by this assumption will be discussed further below. The final step in setting up the analysis was to couple the model ventricle to a Windkessel model of arterial afterload impedance so that the model ventricle can be physiologically loaded and the loading conditions can be varied. In brief, the Windkessel model can be described by a set of non-linear differential equations which relate instantaneous ventricular pressure to ventricular outflow; this has been described in detail previously (Sunagawa et al., 1982).

Some of the phenomena to be examined deal with relationships between energy consumption and mechanical work done by the ventricle. In real hearts, energy consumption is indexed by either myocardial oxygen consumption (mVO_2, since approximately 90% of ATP generation is derived from aerobic metabolism) or heat production (since heat is derived from ATP hydrolysis). ATP is used to support many different processes, including basal metabolism, calcium cycling and force generation. The issue of main concern in the present study is in assessing the energy cost of force (pressure) generation at different loading conditions. It was assumed that basal metabolism and the energy for calcium cycling are independent of loading conditions. The principal energy consuming process during contraction is the hydrolysis of ATP required to uncouple the actin–myosin bond. Therefore, energy consumption for force generation was assumed to be proportional to the number of actin–myosin bonds broken during the course of a contraction and was expressed in these arbitrary units. Instantaneous heat production related to tension development during the beat was assumed to be equal to the accumulated number of bonds broken since the start of the beat. Mechanical work done by the ventricle was quantified, as has been done for real ventricles, by the pressure–volume area (PVA) which has been described in detail previously (Suga, 1990). Briefly, PVA is the area on the ventricular pressure–volume diagram circumscribed by the end-systolic pressure-volume relation, the end-diastolic pressure–volume relation and the systolic portion of the pressure–volume trajectory. It was originally proposed that PVA is the sum of the stroke work (i.e. the external work done by the ventricle) plus a quantity of mechanical energy stored within elastic elements of the contractile proteins at end systole termed the end-systolic potential energy. Accordingly, this index has been proposed as a measure of total mechanical energy liberation of the ventricle and has been
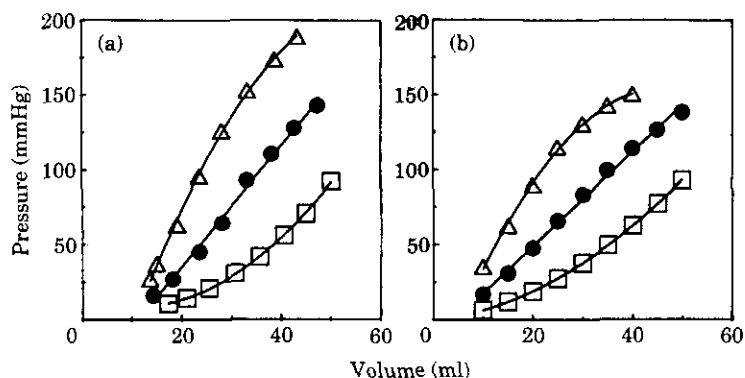
Figure 3 Contractility dependent curvilinearity of the isovolumic end-systolic pressure volume relations (ESPVR). (a) Data obtained from an isolated canine heart at three different contractile states: control (●), enhanced by the calcium channel agonist Bay K 8644 (△) and depressed by the calcium channel antagonist nifedipine (□) (Burkhoff et al., 1987). ESPVR at baseline was linear, at enhanced contractile state was concave to the volume axis and at depressed contractile state was concave to the volume axis. (b) Similar results were obtained from the theoretical ventricle. For these curves, contractile state was altered by either increasing or decreasing the amplitude of the calcium transient (from Fig. 1) by a multiplication factor of 1.5 or 0.5, respectively.

shown to be linearly correlated with mVO_2 over a wide range of loading conditions (Suga, 1990).

Protocols

The main goal of this study was to determine whether previously observed load and contractility dependent changes in end-systolic pressure, mechanical efficiency, time course of contraction and time course of relaxation could be explained by the relatively simple theory of muscle contraction presented above. Detailed sets of data have been obtained previously in isolated canine hearts which summarize these observations in detail (Burkhoff et al., 1987, 1991, 1993). In every case, protocols previously executed in the real experiments were simulated as closely as possible in the theoretical analysis. The analyses of the data obtained from the theoretical analysis were identical to those performed previously on data obtained from the real hearts (i.e. the exact same computer programs were used for both analyses). The details of each protocol and accompanying data analysis scheme are provided below.

Results

Contractility dependence of end-systolic pressure–volume relationship

The first phenomenon examined was the linearity of the end-systolic pressure–volume relationship

(ESPVR) and how this linearity was altered by changes in contractile state (Burkhoff et al., 1987). For this entire protocol, the ventricle was constrained to contract isovolumically and the peak pressure was recorded at many volumes spanning a range between 5 and 50 ml. In the previously published study, among the interventions used to alter contractile state were infusions of Bay K 8644 (a calcium channel agonist) and nifedipine (a calcium channel antagonist). In both cases, the predominant mechanism whereby contractile state is affected is by changing the amplitude of the calcium transient. Therefore, for the model ventricle, contractile state was increased by multiplying the calcium transient [i.e. the curve in Fig. 1(a)] by a scaling factor of 1.5, and contractile state was decreased by multiplying the calcium transient by a scaling factor of 0.5.

Results are shown in Figure 3. For the real ventricle (left panel) the ESPVR at baseline contractile state was linear as determined by statistical means. However, when contractile state was enhanced the ESPVR became concave to the volume axis and it became convex to the volume axis when contractile state was decreased below baseline. As shown in Figure 3(b), the model ventricle exhibited the same behaviour. Further analysis indicated that, as for the real ventricle (Burkhoff et al., 1987), the degree of ESPVR convexity or concavity was proportional to the decrease or increase, respectively, in contractile state achieved by scaling the calcium transient.
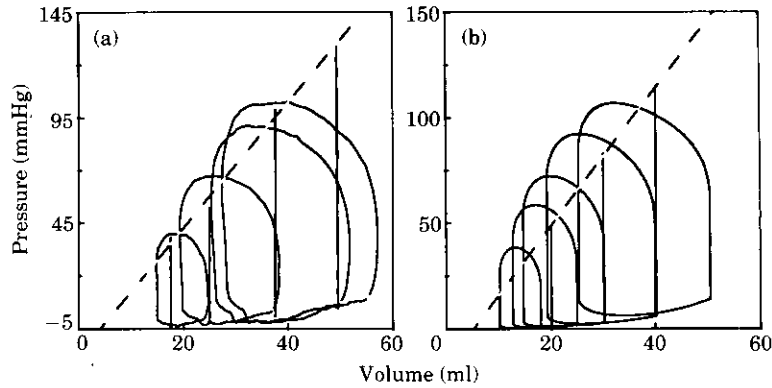
Figure 4   ESPVRs measured under isovolumic and ejecting conditions. On the left, data obtained from an isolated canine heart in which the heart initially contracted isovolumically and then ejected with afterload set so that the ejection fraction was approximately 50%. The ESPVR measured during ejection lies above that of the isovolumic contractions suggesting an ejection mediated enhancement of contractile state (Burkhoff *et al.*, 1991). Similar results were obtained from the theoretical ventricle (b).
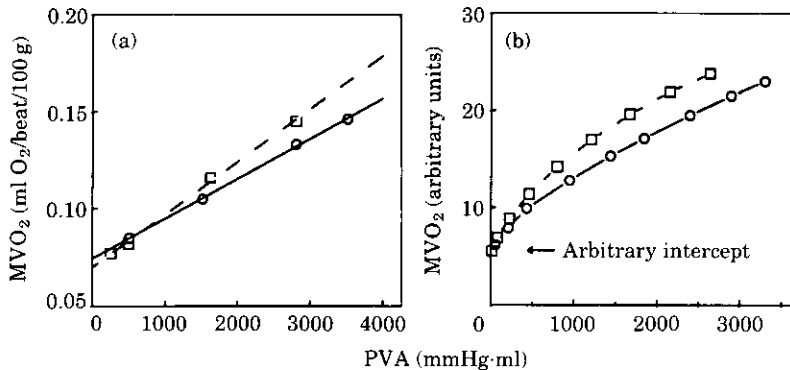
Figure 5 mVO_2-PVA relations measured under isovolumic and ejecting conditions. (a) Data obtained from an isolated canine heart in which the heart initially contracted isovolumically (□) and then ejected (○) with afterload set so that the ejection fraction was approximately 50% (measured simultaneously with data shown in Fig. 4). The mVO_2-PVA relation measured during ejection lies below that of the isovolumic contractions indicating that despite the apparent ejection-mediated enhancement of contractile state the energy consumption is less for the same PVA (Burkhoff *et al.*, 1991). Similar results were obtained from the theoretical ventricle (b).

Load dependence of end-systolic pressure–volume relations

The next phenomenon examined was the load dependence of the ESPVR (Burkhoff *et al.*, 1991). The parameters of the Windkessel afterload were set to provide a ventricular ejection fraction of approximately 50%. Pressure–volume loops were recorded at several different preload volumes ranging between 60 and 20 ml. Then, the ventricle was constrained to contract isovolumically and pressure waves were recorded over the same range of ventricular volumes. From these loops, the ESPVR could be constructed by determining the line of regression through the upper left corner of the pressure–volume loops.

A comparison between ESPVRs measured under isovolumic and ejecting conditions obtained from an isolated heart is shown on the left side of Figure 4; the dashed line shows the ESPVR from the isovolumic conditions. As shown, the pressure–volume loops of the ejecting contractions break through the isovolumic ESPVR and constitute an ESPVR that falls above that of the isovolumic contractions. Thus, for any given end-systolic volume, end-systolic pressure is greater when the heart is allowed to eject than when it contracts isovolumically. The behaviour of the theoretical ventricle, shown in Figure 3(b), exhibits the same property. Further analysis indicated that the greater the ejection fraction the greater the increase in the ESPVR on ejecting beats as was shown

previously to be the case in the real ventricle (Burkhoff et al., 1991).

Load dependence of mVO$_2$-PVA relations

The effect of afterload on the relationship between PVA and mVO$_2$ is shown in Figure 5. The data from the real heart (Burkhoff et al., 1991) [Fig. 5(a)] show that for any given PVA, the heart consumes less oxygen during ejecting as compared to isovolumic contractions; this is despite the fact that the apparent contractile state (as judged by the ESPVR) is greater on the ejecting beats (Fig. 4). For the model LV, the mVO$_2$-PVA relation was nearly linear except at very low values of PVA where it became concave to the PVA axis. The impact of load on the model ventricle [Fig. 5(b)] was similar in that the slope of the relationship was decreased on the ejecting beats. Recall that the O$_2$ axis-intercepts for the theoretical relations were set arbitrarily. Further analysis showed, as was shown to be the case in the real ventricle (Burkhoff et al., 1991), the greater the ejection fraction the greater the decrease in the slope of the mVO$_2$-PVA relationship.

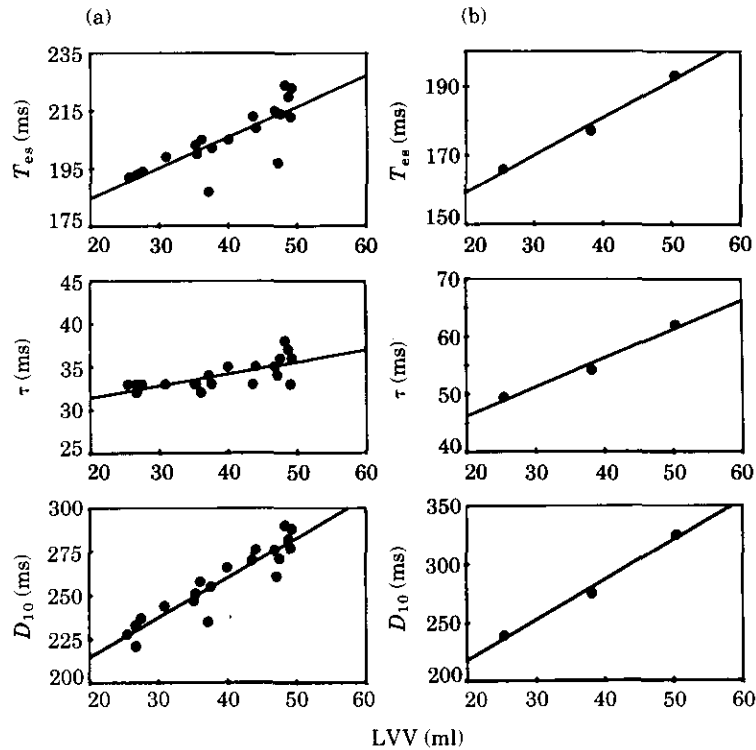## Impact of volume on isovolumic pressure waveform

The next test of the theory was to examine the influence of ventricular volume on the characteristics of the isovolumic pressure waveform. Isovolumic pressure waveforms were recorded from real ventricles over a wide range of volumes (Burkhoff et al., 1993) as follows. The afterload was adjusted to provide an ejection fraction of about 50%, preload adjusted for an end-diastolic volume of approximately 50 ml and the heart allowed to attain a steady contractile state. Then, ventricular volume was clamped at a predetermined time during the filling phase of the contraction and the pressure wave recorded on the first isovolumic beat. This procedure was repeated many times with varying values for the clamp time resulting in a series of isovolumic beats at different volumes but each of which has the same history of contraction mode immediately prior to the isovolumic beat (i.e. all isovolumic beats were preceded by ejecting beats with the exact same afterload and preload). The protocol was executed in this manner in the real hearts to ensure that the calcium transient, which is believed to change with steady state but not abrupt changes in loading condition, was as close as possible on the isovolumic beats at the different volumes.

Each isovolumic pressure waveform was characterized by three parameters: T_{es}, time to end systole, which for isovolumic beats is equivalent to the time from the start of contraction to the time of peak pressure; τ, the time constant of relaxation, determined by fitting a mono-exponential function to the pressure wave during the diastolic pressure decay (Yellin et al., 1986); and D_{10}, an index of the overall duration of contraction, defined as the width of the pressure wave (in ms) at a level 10% of the developed pressure.

Data from a real isolated ventricle are shown in Figure 6(a). Each of these parameters increased as the volume of the isovolumic contraction was increased. The same protocol was executed in the theoretical ventricle and the results, shown in Figure 6(b), exhibit the same behaviour (though the absolute values of the parameters differ between real and model ventricle).

Comparing pressure waveforms between isovolumic and ejection conditions

The characteristics of the pressure waveform measured under isovolumic and ejecting conditions were compared. In this protocol, which was similar to that described in the previous section, pressure waveforms were measured under three conditions. First, the ventricle was allowed to eject against a specified afterload impedance; under these conditions, the end-diastolic and end-systolic volumes were noted. Next, the ventricle was forced to contract isovolumically at the end-diastolic volume of the ejecting beat. Third, the ventricle was forced to contract isovolumically at the end-systolic volume of the ejecting beat. As in the previous protocol, and with the same rationale, each isovolumic beat was the very first beat after the switch from the ejecting to isovolumic mode of contraction. The pressure waveforms measured under each condition were characterized by the parameters described above (T_{es}, τ and D_{10}). Note that for the ejecting heart, T_{es} was taken in the currently accepted manner as the time from the onset of contraction to the point of maximal instantaneous elastance {i.e. maximum value of LVP(t)/[LVV(t)-V$_o$]}. The results are shown in Figure 7. EDV and ESV denote the parameter values determined under isovolumic conditions at the end-diastolic and end-systolic volumes, respectively; EJ denotes the value determined under ejecting conditions. Data from the real ventricles represent the average results from six hearts (Burkhoff et al., 1993) with afterload set to allow an ejection fraction of approximately 50%, as in the theoretical

Figure 6 Impact of volume on characteristics of isovolumic pressure waveform. (a) Data obtained from an isolated canine heart in which the ventricle was constrained to contract isovolumically at many different volumes from a preceding steady state ejecting condition (so that the history of contraction are the same on all the beats) (Burkhoff *et al.*, 1993). The isovolumic pressure waves were characterized by three parameters as detailed in the text: the time to end systole (defined as the time of peak pressure), T_{es}; the time constant of relaxation, τ; and the duration of the contraction measured at a level of 10% developed pressure, D_{10}. For the real heart, each of these parameters increased over the range of volumes investigated. Similar results were obtained from the theoretical ventricle (b), though the absolute values of these parameters were different between real and model heart (see Discussion).

analysis. The real ventricles exhibits a prolongation of T_{es}, a decrease in τ, and relatively little variation in D_{10} on ejecting as compared to isovolumic beats. As shown in Figure 7(b) the model ventricle exhibits very similar behaviour, with the only deviation being that in the model ventricle, D_{10} on the ejecting beat was closer to that of the isovolumic beat at ESV whereas in the real ventricle D_{10} was closer to that of the isovolumic beat at EDV.

Theoretical explanation for load dependence of ventricular performance

It is possible to delineate, in principle, the events responsible for the load dependence of the time course of ventricular performance within the context of the proposed theory; to aid in this explanation, Figure 8 shows the time varying concentrations of the various elements of the theoretical model on an ejecting beat, on an isovolumic beat at the end-diastolic volume (ISO$_{EDV}$, dotted lines) and

on an isovolumic beat at the end-systolic volume (ISO$_{ESV}$, dashed lines).

On ejecting beats, a large proportion of calcium binding occurs over a time when ventricular volume is at or near EDV and calcium binding affinity is high (first vertical line in Figure 8 shows onset of ejection). Consequently, the early time course of calcium binding and peak calcium binding on the ejecting beat is nearly identical to that of the ISO$_{EDV}$ beat and, furthermore, is much greater than calcium binding on the ISO$_{ESV}$ beat. As ejection proceeds and calcium binding affinity decreases, calcium binding moves towards that which exists during the ISO$_{ESV}$ beat and thus the rate of decline of calcium binding is greater on the ejecting beat than during either ISO$_{EDV}$ or ISO$_{ESV}$. These statements hold for the time course of calcium bound to actin $\{[CaA](t)\}$ and for the total calcium bound {i.e. $[CaA](t) + [CaAM](t)$}.

The early time course of actin–myosin binding {indicated by the initial portion of the curve showing $[CaAM](t)$ in Fig. 8} is much greater on ISO$_{EDV}$
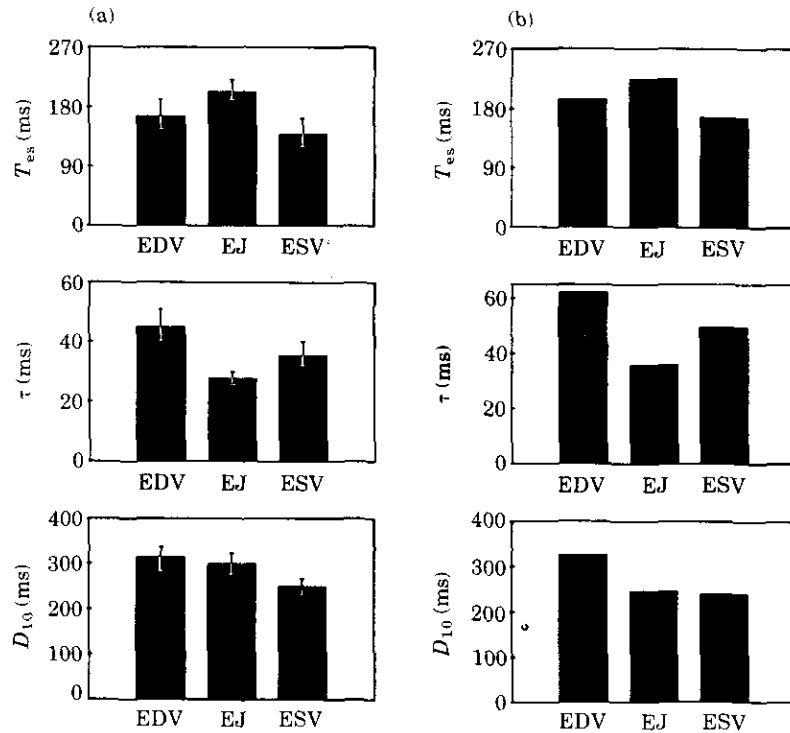
Figure 7 Comparing pressure waveforms between isovolumic and ejection conditions. (a) Data obtained from an isolated canine heart in which the heart was ejecting with an ejection fraction of approximately 50% (EJ), isovolumically at the end diastolic volume (EDV) of the ejecting beat and isovolumically at the end systolic volume (ESV) of the ejecting beat. The pressure waves were characterized by the same three parameters as defined in Figure 6. For ejecting conditions. T_{es} was defined in the conventionally manner as the time to peak chamber elastance (which, in turn, is defined as $LVP(t)/[LVV(t)-V_o]$). As shown, T_{es} is much greater, τ is much shorter and D_{10} is intermediate on the ejecting as compared to the isovolumic contractions. Similar results were obtained from the theoretical ventricle (b) though the absolute values of these parameters were different between real and model heart (see Discussion).

than on ISO_{ESV}. As expected, actin–myosin binding on the ejecting beat initially follows that of ISO_{EDV}, but approaches that of ISO_{ESV} towards the end of ejection (end of ejection shown by second vertical line). Calcium-free actin–myosin bond ([AM]) appearance is delayed compared to the time course of [CaAM]. [AM](t) on the ejecting beat follows the time course of [AM](t) on ISO_{ESV} but has the magnitude of the ISO_{EDV} beat. The earlier appearance of [AM] on the ejecting beat reflects calcium unbinding due to the decreasing calcium affinity as ejection proceeds; the higher magnitude on the ejecting beat compared with ISO_{ESV} reflects the greater amount of binding during the early part of the beat. As shown in Figure 8, the total number of force generating units {[AM](t) + [CaAM](t)} on the ejecting beat follows that of the ISO_{EDV} during the initial portion of the beat, but then shifts towards that of the ISO_{ESV}. Consequently, once again, the rate of decline of total actin–myosin bonds on the ejecting beat is much greater than that on either of the isovolumic contractions.

The time course of pressure generation and total number of force generating units on the isovolumic and ejecting contractions are also compared in Figure 8. For the isovolumic beats, the pressure waves are simply scaled versions of the respective [AM](t) + [CaAM](t) curves (since volume and therefore length are fixed). However, because ventricular volume is changing on the ejecting beat, the visual appearance of [AM](t) + [CaAM](t) and the pressure wave are very different, this being a consequence of length-dependence of force generation (eqn 7) and, to a lesser extent, shortening velocity reduction in force generation (eqn 2). As can be seen, pressure generation during the ejecting beat is substantially less than that on the isovolumic beat despite formation of a similar peak number of force-generating units on the ISO_{EDV} beat. The more rapid fall of [AM](t) + [CaAM](t) during the ejecting beat translates into a more rapid fall in pressure during relaxation and shortening of the overall duration of contraction compared to the ISO_{EDV} beat.

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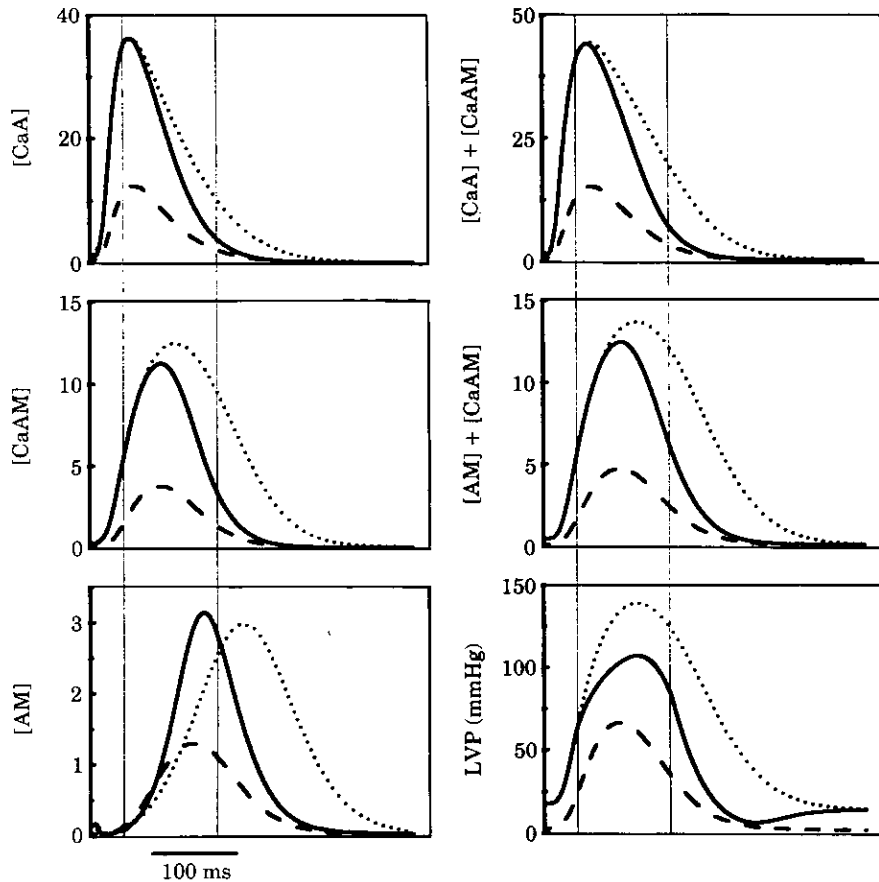
Figure 8   Predicted time course of various elements in the model during ejection (—), isovolumic contraction at the end-diastolic volume (· · · ·) and isovolumic contraction at the end-systolic volume (– – – –). [CaA], time varying concentration of calcium–actin complex (state 2); [CaAM], time varying concentration of calcium–actin–myosin complex (state 3); [CaA] + [CaAM] time varying concentration of total calcium bound to myofilaments. [AM], time varying concentration of actin–myosin complex in absence of bound calcium (state 4); [CaAM] + [AM], time varying concentration of total actin–myosin binding; LVP, left ventricular pressure predicted from the theoretical ventricle. Note different scales used in the various panels. First vertical line shows onset of ejection on the ejecting beat; second vertical line shows end of ejection.

According to current conventions (which are based upon the time-varying elastance theory of contraction), end-systole is defined as the time at which ventricular chamber elastance {i.e. LVP(t)/[LVV(t)-V_o]} reaches its maximum value during the beat. For isovolumic contractions [with LVV(t) = constant], end-systole is coincident with the occurrence of peak ventricular pressure which, in turn, occurs at the time of peak concentration of force-generating units ([AM] + [CaAM]). However, neither of these two conditions hold for the conventionally defined end-systolic point during the ejecting contraction, which occurs very close to the time of end ejection (denoted by the second vertical line in Figure 9). As can be seen, peak actin–myosin bonding occurs in mid-ejection (near the time of peak [AM] + [CaAM] on the isovolumic beats), while the designated time of end-systole occurs

during a time when actin–myosin bond concentration is falling rapidly, a point which seems arbitrary within the time course of biochemical events occurring during the beat.

Theoretical explanation for load dependence of ventricular energetics

The explanation for the load dependence of myocardial energetics is explored in Figure 9. The graph in Figure 9(b) shows the pressure–volume loop of an ejecting beat with ejection fraction 50% (solid line) and an isovolumic beat (dotted line) set at a volume which provides the exact same PVA as on the ejecting beat (see figure legend); the isovolumic volume is slightly less than the EDV of the ejecting beat. The dashed line in the figure connects the
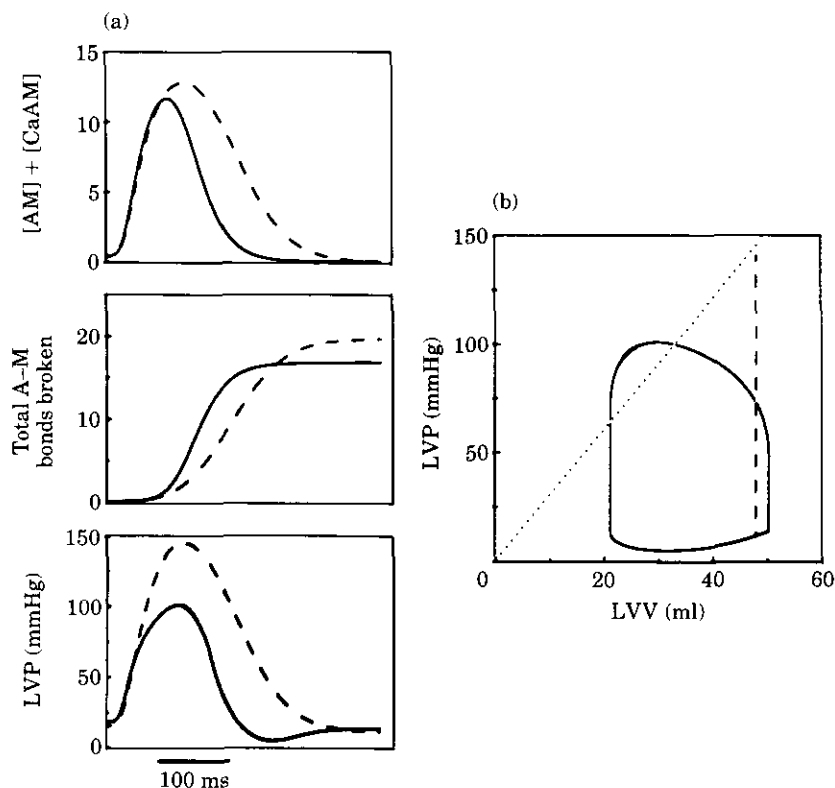
Figure 9 Load dependence of energetics. Pressure–volume loops from an ejecting beat (—) and an isovolumic beat (– – –) with preload adjusted to provide same pressure volume area (3345 mmHg ml) as on the ejecting beat (b); the dotted line shows the approximate ESPVR under the isovolumic condition, demonstrating that the ejecting beat has a greater apparent contractile state (as in Fig. 4). The time course of total actin–myosin bond concentration {[AM] + [CaAM]}, the accumulated total number of actin–myosin bonds broken (a surrogate for tension dependent heart) and the left ventricular pressure (LVP) on the ejecting beat (—) and the isovolumic beat (– – –) are shown in (a). The tension dependent heat increases quicker but reaches a lower plateau value on the ejecting than on the isovolumic contraction due in part to the prolonged contraction duration on the isovolumic beat. The plateau value of this curve is hypothesized to be proportional to the oxygen consumption related to crossbridge interactions; thus, the theory predicts in accordance with previous observations that despite the same PVA, oxygen consumption is greater on the isovolumic than the ejecting contraction.

origin with the peak pressure on the isovolumic beat and serves as one of the boundaries for determining the PVA. Note that the pressure–volume loop on the ejecting beat breaks through this line, exhibiting contraction associated enhancement of the ESPVR as discussed above. The time course of total actin–myosin bond concentration {[AM](t) + [CaAM](t)}, the time course of accumulated number of actin–myosin bonds broken and the associated ventricular pressure traces are shown in the left side of the figure. As shown, while the initial time course of total actin–myosin bond formation and its peak concentration are similar between the two beats, the time course of decline commences sooner on the ejecting beat and is slightly more rapid. As shown in the middle panel, actin–myosin uncoupling begins slightly before peak [AM] + [CaAM] is reached. There are initially a greater

number of actin–myosin bonds being broken on the ejecting beat but, due to the prolongation of the contraction on the isovolumic beat, the total number of bonds broken is greater on the isovolumic than the ejecting contraction. Estimated energy consumption, which is assumed to be proportional to the total number of actin–myosin bonds broken at the end of the beat (i.e. the plateau value of the curve) is therefore predicted to be higher on the isovolumic than ejecting contraction despite the fact that both contractions have exactly the same PVA. It should be pointed out that the graph showing the time course of accumulated number of actin–myosin bond breakages, which has been hypothesized above to indicate the time course of heat production, bears a striking resemblance to the experimentally recorded time course of tension-dependent heat generation (Alpert et al., 1989)

which has been related to myosin ATPase activity (discussed below).

Discussion

A theory is presented which accounts for a very broad range of ventricular properties that have been noted in recent experiments. The theory is based upon a four-state biochemical scheme that models the interactions between calcium and the myofilaments. This scheme was initially proposed by Hill to explain behaviour of skeletal muscle (Hill, 1983). Yue invoked this scheme to explain the temporal relations within a beat between free calcium concentration (estimated from aequorin luminescence) and cardiac muscle force generation (Yue, 1987). Further experimental evidence to support the validity of the scheme was provided by Peterson et al. (1991) who used a bioassay to estimate the time course of calcium bound to the myofilaments. By obtaining results which suggested that the decline of bound calcium concentration significantly precedes the decline in force, Peterson et al. provided support for the existence of the calcium-free force-generating state (i.e. [AM] in Fig. 2). This original scheme was supplemented by incorporating two additional basic properties of cardiac muscle: length dependence of calcium binding affinity (eqn 6). and load dependence of force generation (eqns 2 and 7). The biochemical scheme was used to provide the force–length–time properties of cardiac muscle which were used to construct a ventricle via a simple spherical geometry as has been used on many occasions (Grossman et al., 1977; Hunter and Baan, 1980).

This theory was able to account for many fundamental aspects of ventricular performance including: (1) this model can account for the time course of isometric muscle force generation from the measured free calcium transient (Fig. 1); (2) a realistic contractility dependent curvilinearity of the end-systolic pressure–volume relationship (ESPVR, Fig. 3); (3) enhancement of contractile strength (defined as by the ESPVR) on ejecting compared to isovolumic beats (Fig. 4); (4) improved contractile efficiency (defined by a decrease in slope of the mVO_2-PVA relationship) on ejecting as compared to isovolumic beats (Fig. 5); (5) appropriate volume-dependent changes in time to peak pressure, time constant of relaxation and duration of contraction on ejecting beats (Fig. 6); (6) ejection mediated prolongation of the time to end systole (defined as the time to peak chamber elastance) and speeding of relaxation (Fig. 7); and (7) realistic

estimated time course of tension-dependent heat generation (Fig. 9). The explanation for most of these phenomena were explored within the context of the theory and presented in detail above, and only a few points require further mention.

The first point concerns contractility dependent curvilinearity of the ESPVR. Contractility induced changes in the shape of the muscle force–length relationship have long been touted as evidence for length-dependence of activation in cardiac muscle (Allen and Kentish, 1985; ter Keurs et al., 1980; Kentish et al., 1986; Jewell, 1977). It has been argued that if muscle length simply scaled the force-generating effectiveness of a given quantity of released calcium then end-systolic force–length relations (ESFLR) measured at different calcium levels (i.e. at different inotropic backgrounds) would be scaled versions of each other. Experimental observations, however, show that the shape of the ESFLR changes with inotropic state. Analogous observations have been made regarding the shape of end-systolic pressure–volume relations (ESPVR) in intact hearts (Burkhoff et al., 1987; Kass et al., 1989). Such shape changes have been interpreted as indicating that muscle length, like calcium itself, is an independent factor which regulates the cascade of biochemical reactions resulting in force generation. Within the context of the proposed theory, length-dependent alterations in the calcium binding affinity can account for this phenomenon.

The second point concerns the predicted time course of actin–myosin bond breakages which was related to the time course of heat generation. Total heat production by cardiac muscle has been partitioned, according to the work of Alpert and associates (Alpert and Mulieri, 1982), into heat related to mechanical activity (initial heart) and heat produced to regenerate high energy phosphates used during the contraction (recovery heart). Initial heart, in turn, is made up of two components; heat due to actin myosin crossbridge cycling (tension dependent heat) and heat due to calcium uptake by the sarcoplasmic reticulum (tension independent heat). In the present analysis, we have been concerned only with the tension dependent heat. Tension dependent heat is calculated experimentally by taking the difference between initial heat and tension independent heat; the latter can be measured after suppressing mechanical activity by exposing muscle to a hypertonic solution containing 2,3-butanedione monoxime (Alpert et al., 1989). It was hypothesized above that high energy phosphate consumption, and therefore heat generation, due to muscle force production was related to crossbridge uncoupling. The graph showing the time course of

total actin–myosin bonds broken (Fig. 9) is thus the surrogate for the time course of tension dependent heat. These curves bear a striking resemblance to the experimentally reported time course of tension dependent heat (Alpert et al., 1989). Both are sigmoidal with a slight delay compared with the onset of mechanical activity and reach a plateau value at the time when mechanical activity is returned to the resting state. Thus, in addition to accounting for the mechanical activity of the ventricle and load dependence of overall energy demands, the present theory can account for the time course of energy utilization related to contractile activity within a beat.

One additional interesting and unanticipated finding was that the analysis may provide insight into the events occurring at the time classically defined as end systole (see Fig. 8). For isovolumic contractions, the pressure waveform is simply a scaled version of the curve depicting the time course of total actin–myosin bonds $\{[CaAM] + [AM]\}$. End systole on such contractions is defined as the time of maximal pressure and therefore the time of peak actin–myosin bonding; this is also the point at which the chamber elastance $\{defined\ as\ LVP(t)/[LVV(t)-V_o]\}$ reaches its maximal value. For ejecting contractions, definitions of end systole include the time of peak chamber elastance or the time of end ejection (points which generally occur very close to each other) (Sagawa, 1978). However, note that the theoretical analysis predicts that the time of end systole (defined by these criteria) bears no such relationship to the [CaAM] + [AM] curve. Thus, end systole on isovolumic and ejecting beats occur at very different times when measured on the time scale of the underlying biochemical events. This raises the questions of whether it is appropriate to define end systole, in a biochemical sense, by these criteria on ejecting beats and whether it is appropriate to compare end systolic times measured under different loading conditions as is standardly done.

Limitations

It should be noted for each analysis presented above that while results obtained from the real and theoretical ventricles were qualitatively very similar, there are differences in the actual parameter values between the real ventricle and the model ventricle. This is because in the analysis performed, no attempt was made to vary parameter values to obtain best fits to the data obtained from the ventricle.

Indeed, this is not possible since one of the main factors, namely the calcium transient $\{[Ca^{2+}]_i(t)\}$, was not measured in previous experiments in dog hearts; rather, $[Ca^{2+}]_i(t)$ was assumed to follow a predetermined course obtained from an isolated muscle and the parameter values were set to provide a good fit between that $[Ca^{2+}]_i(t)$ and the simultaneously measured force from that same muscle (Fig. 1). Furthermore, the purpose of this analysis was to determine, using reasonable parameter values and introducing length-dependence to the rate constants in a simple manner, whether the proposed theory could account for the load-dependent behavior of ventricular contraction. The results presented above indicate, at least in qualitative terms, that the proposed theory can account for all aspects of the load dependence of ventricular mechanical behaviour and energetics which were observed experimentally in canine hearts. While the similarity between previously published data and predictions of the proposed theory are striking, it is to be emphasized that these results are derived from model analysis and are thus subject to the limitations of the underlying assumption built into the theory. Therefore, it is important that the major assumptions be examined and an assessment made of how conclusions might be altered if errors exist.

The present theoretical analysis examined one biochemical scheme to interrelate the calcium transient to mechanical and metabolic performance (Fig. 2). However it is possible that this scheme is incorrect and the concordance between model and reality is coincidental. It may be possible that other, perhaps simpler, schemes would suffice to explain the interrelations. In preliminary analysis, the behaviour of a three-state scheme was tested; the scheme was similar to that presented in Figure 2 except that there was no state 4, so that relaxation had to occur via actin–myosin unbinding before calcium unbinding. Using this scheme, it was not possible to interrelate the calcium transient with the time course of force generation as was demonstrated for the four-state model (Fig. 1). Rate constants could be adjusted to fit either the rising phase or the falling phase of force production separately. It was concluded on the basis of this analysis that a minimum of a four-state model would be required.

One of the starting assumptions in the theory is that calcium binding is dependent upon muscle length. While several groups have advocated this notion (Hibberd and Jewell, 1982; Stephenson and Williams, 1982), results from at least one group do not support this idea. Hofmann and Fuchs (1988, 1987a,b) have suggested that muscle force, but not length, modulates myofilaments calcium sensitiv-

ity. Their findings were consistent with the earlier introduced idea of cooperative binding interactions between actin, myosin, calcium, and tropinin-C (Bremel and Weber, 1972). This possibility was explored extensively in preliminary analyses (not presented above) by forcing the rate constants to vary in proportion to the number of actin–myosin bonds; this modification was implemented on both the three-state and four-state models. For the case of the three-state model, these modifications did not improve its ability to account for the temporal relations between isometric force and calcium. For the case of the four-state model, which already provided a good fit, these modifications did not interfere (i.e. they did not make them worse). However, whereas the theory with ε-dependent rate constants readily reproduced essentially all of the phenomena observed in the intact ventricle, the same was not true for the theory with σ-dependent constants despite addition of many additional constraints and assumptions. Having pointed this out, it is recognized and should be emphasized that the concordance between real heart behaviour and theory predictions with ε-dependent rate constants is no proof of that theory; nor is the discordance between real heart behaviour and theory predictions with σ-dependent rate constants disproof of that theory or of the fundamental observation of cooperative binding interactions kinetics. It is possible that the mathematical representations employed to mimic the various physiological process did not faithfully capture the actual biochemical processes. Furthermore, the nature of the length dependence of the rate constants used (eqn 6) was arbitrary and further investigations are warranted to elucidate this phenomenon in quantitative terms.

Force generation in the new theory is dealt with in a phenomenologic rather than mechanistic manner. That is, force is assumed to be proportional to the number of crossbridge attachments minus a term proportional to the velocity of shortening. There is an extensive literature relating muscle length and shortening to crossbridge interactions and force generation dating back to the pioneering work of Huxley (1958). As noted above, previous investigators have suggested that many of the phenomena which have been explained by the new theory are related to length-dependence of activation, which relates in large part to load-dependent alterations in calcium binding. Inclusion of other, even more complicated aspects of muscle biophysics would have added many additional degrees of complexity and many more assumptions and thus was avoided in the present analysis.

Length dependence of force-generation by the myofilaments is another aspect which is dealt with in a phenomenologic manner. Even with saturating concentrations of calcium supplied to the myofilaments of skinned fibres, muscle force decreases significantly when length is decreased (Allen and Kentish, 1985; Kentish et al., 1986; Fabiato and Fabiato, 1975). The fact that these relations are measured in the absence of cell membranes and extracellular elements suggest that mechanisms involving the sarcomere are involved. These may include a direct effect of length on force generating capacity of individual crossbridges, length dependent variations in the number of potential actin–myosin binding sites or forces acting to oppose myofilament actions perhaps due to mechanical interference between myofilaments as length is decreased. The steepness of this relation is markedly increased when intact fibres are studied suggesting a role of forces opposing shortening (so called restoring forces) generated by the sarcolemma or extracellular matrix. There are several different ways in which this factor could be dealt with in the present theory. In the absence of data the choice is somewhat arbitrary. For the present analysis, a simple approach was used by assuming that α, the proportionality factor relating force to the number of actin–myosin bonds, varies with muscle length. It is recognized that the manner in which this phenomenon is dealt with may impact on the physiological conclusions derived from the model analysis.

One factor, believed to be important in describing the properties of cardiac muscle, which has not been included is shortening deactivation. This term refers to the phenomena whereby crossbridges can be uncoupled due to the shortening of the muscle. Generally, this is observed when abrupt, rapid length changes are imposed. Upon considering the finding that the ESPVR of physiologically ejecting beats fell above those of isovolumic beats it was hypothesized that with physiological ejecting patterns (which are generally slow and smooth) this effect was not prominent (Burkhoff et al., 1993). It is for this reason that this phenomenon has been excluded from the present analysis, though it would be simple to account for this in a phenomenologic manner. Consequently, the theory would not account for ventricular performance under conditions where there are rapid, large changes in ventricular volume; it is considered that such changes would not occur in the physiological setting.

An additional limitation relates to load dependence of the calcium transient. It has been demonstrated in isolated papillary muscles that with a

steady-state increase in muscle length there is an immediate increase in force generation that is followed by a further and quite significant gradual increase of force which may take several minutes to reach the steady plateau level (Parmley and Chuck, 1973; Lakatta and Jewell, 1977; Allen and Kurihara, 1982). Paralleling in time and magnitude this gradual increase in force is a gradual increase in peak $[Ca^{2+}]$ (Allen and Kurihara, 1982) suggesting that there is a length-dependent increase in the amount of intracellular calcium released to the myofilaments. The mechanism behind this increase is unknown. At the ventricular level, a gradual increase in pressure generation following a steady-state increase in ventricular pre- and after-loading conditions has also been observed (Tucci et al., 1984; Sugiura et al., 1989; Burkhoff et al., 1991). However, it is unknown whether these also relate to increases in calcium release as in the papillary muscle (though it seems likely that they do). In the present analysis, the calcium transient was assumed to be independent of the loading conditions. Results of three of the analysis would be influenced if calcium were allowed to vary with steady-state changes in load: impact of inotropic state on ESPVR shape (Fig. 3), impact of load on ESPVR (Fig. 4) and impact of load on mVO_2-PVA relation (Fig. 5). In each of these cases, however, such an effect would not alter the principal conclusion drawn from the analysis since both isovolumic and ejecting beats would be affected similarly. It should also be pointed out, as detailed in Results, that the other protocols were executed in a manner in which to essentially eliminate load-dependent changes in the calcium transient.

Ventricular properties were assumed to be derived from muscle force–length properties via a simple spherical model. Muscle properties are assumed to be uniform throughout the ventricle and to be activated simultaneously. Each of these assumptions are known to be over-simplifications of real aspects of ventricular properties. Nevertheless, results of previous studies have supported the notion that despite the complexities of ventricular structure at least some aspects of ventricular excitation–contraction coupling may be quantitatively indicative of the underlying muscle properties (Burkhoff and Hunter, 1992). In order to assess whether the results obtained in the analysis performed were critically dependent upon the techniques used to model the ventricular chamber the entire analysis described above was carried out by assuming that ventricular pressure was linearly proportional to muscle stress and ventricular volume was linearly proportional to muscle length.

Rate constants were also assumed to vary linearly with ventricular volume. The results obtained from that analysis revealed that the general behaviour of the theoretical ventricle and all of the qualitative conclusions arrived at were identical between the two vastly different treaments of the transformation between muscle and ventricular properties. This analysis speaks to the robustness of the underlying biochemical scheme (Fig. 2) to account for the observed phenomena, and the relative insensitivity of how muscle properties are transformed into ventricular properties for determing these qualitative conclusions.

In addition to being able to account for the dynamic relationships between $[Ca^{2+}]_i$ and pressure generation, a new theory should also be able to account for the equilibrium relationships between these two entities. From studies of isolated cardiac muscle it is currently believed that the equilibrium relationship between $[Ca^{2+}]_i$ and force (as can be obtained during tetanic contractions) can be described by a standard enzymatic binding curve with a Hill coefficient that varies with muscle length and ranges between 3 and 6 (Kentish et al., 1986; Yue et al., 1986), indicative of a high degree of cooperativity in binding kinetics within the myofilament system. However, when steady-state isometric (isovolumic) force-pCa and pressure-pCa curves are constructed using the rate constants shown in Table 1, the resultant Hill coefficient was only 2.1. Thus, the degree of cooperativity present in the proposed system which qualitatively accounted for a very broad range of phenomena, was not as high as has been shown previously to exist in isolated cardiac muscle preparations.

Finally, it could be questioned why the focus of this effort was to explain observations in isolated whole ventricles (with the added complexity of ventricular geometry) and not simply to attempt explanation of observations made in linear strips of cardiac muscle. Studies of isolated hearts provide data obtained under condition which may be considered more physiological than those in which isolated muscles are studied. The hearts are blood perfused through the coronaries, whereas muscles are superfused with crystalloid solutions. The haemodynamic loading sequences used in the isolated hearts mimic those encountered physiologically (Sunagawa et al., 1982; Burkhoff et al., 1988) whereas only a few studies of isolated muscle have attempted this (Elzinga and Westerhof, 1981). These two factors may underlie some of the qualitative and quantitative differences in contractile properties between the two types of experimental models. The goal of the present analysis is to test

specifically whether the phenomena observed at the ventricular level all of which have been obtained from a common experimental preparation in which a very broad range of phenomena have been characterized in great detail (Hunter, 1989; Burkhoff et al., 1987, 1991, 1993), could be explained by a unifying theory.

Conclusions

The proposed theory accounts for a very broad range of phenomena related to load dependence of ventricular performance. The two general features which resulted in this success were the inclusion of a calcium-free force-generating state of myofilament interaction and load dependence of biochemical rate constants. The mechanisms of muscle force generation, force–velocity relation and length dependence of force generation have been dealt with in phenomenologic manners and a simple model is used to transform muscle properties into ventricular properties. By dealing with these aspects of cardiac contraction in as simple a manner as possible the properties of the central thesis being tested are more transparent than would otherwise be the case. While the necessary inclusion of several assumptions (required where data is lacking) is a point of criticism for this theoretical work, there have been many insights obtained from the present analysis. It has clearly identified basic questions which require further exploration, not only by exposing their existence but also by pointing out their importance. Furthermore, it is only through such an exercise that many complex hypotheses regarding behaviour of subsystems can be united in an attempt to explain and ultimately understand the behaviour of the macrosystem they comprise. After all, one of the primary reasons for investigating the mechanical properties of individual muscle cells and sarcomeres, as has been done so elegantly over the past decades, is to improve understanding of whole heart function in health and disease.

Several analyses, not presented in detail above, have been carried out to test alternate hypotheses and alternate underlying assumptions (e.g. three-state model, stress-dependent rate constants, assumed linear relation between muscle length and stress and ventricular volume and pressure, respectively) all of which showed that the major conclusions arrived at depended upon the presence of a calcium-free force generating state with length-dependent rate constants but were relatively insensitive to the nature of the ventricular model (linear v spherical).

It is anticipated that techniques which permit measurement of calcium transients in intact hearts (Kihara et al., 1989; Lee et al., 1987) will provide the opportunity to test basic assumptions of the theory. Finally, it will be interesting to determine whether this theory, when applied to analysis of results obtained from abnormal myocardium, can provide any new insights into abnormalities of excitation–contraction coupling present in hypertrophic and cardiomyopathic disease.

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