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Review

# Force–frequency relationship in intact mammalian ventricular myocardium: physiological and pathophysiological relevance

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**Abstract**

The force–frequency relationship (FFR) is an important intrinsic regulatory mechanism of cardiac contractility. The FFR in most mammalian ventricular myocardium is positive; that is, an increase in contractile force in association with an increase in the amplitude of $Ca^{2+}$ transients is induced by elevation of the stimulation frequency, which reflects the cardiac contractile reserve. The relationship is different depending on the range of frequency and species of animal. In some species, including rat and mouse, a 'primary-phase' negative FFR is induced over the low-frequency range up to approximately 0.5–1 Hz (rat) and 1–2 Hz (mouse). Even in these species, the FFR over the frequency range close to the physiological heart rate is positive and qualitatively similar to that in larger mammalian species, although the positive FFR is less prominent. The integrated dynamic balance of the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) is the primary cellular mechanism responsible for the FFR and is determined by sarcoplasmic reticulum (SR) $Ca^{2+}$ load and $Ca^{2+}$ flux through the sarcolemma via L-type $Ca^{2+}$ channels and the $Na^+$-$Ca^{2+}$ exchanger. Intracellular $Na^+$ concentration is also an important factor in $[Ca^{2+}]_i$ regulation. In isolated rabbit papillary muscle, over a lower frequency range (<0.5 Hz), an increase in duration rather than amplitude of $Ca^{2+}$ transients appears to be responsible for the increase in contractile force, while over an intermediate frequency range (0.5–2.0 Hz), the amplitude of $Ca^{2+}$ transients correlates well with the increase in contractile force. Over a higher frequency range (>2.5 Hz), the contractile force is dissociated from the amplitude of $Ca^{2+}$ transients probably due to complex cellular mechanisms, including oxygen limitation in the central fibers of isolated muscle preparations, while the amplitude of $Ca^{2+}$ transients increases further with increasing frequency ('secondary-phase' negative FFR). Calmodulin (CaM) may contribute to a positive FFR and the frequency-dependent acceleration of relaxation, although the role of calmodulin has not yet been established unequivocally. In failing ventricular myocardium, the positive FFR disappears or is inverted and becomes negative. The activation and overexpression of cardiac sarcoplasmic reticulum $Ca^{2+}$ ATPase (SERCA2a) is able to reverse these abnormalities. Frequency-dependent alterations of systolic and diastolic force in association with those of $Ca^{2+}$ transients and diastolic $[Ca^{2+}]_i$ levels are excellent indicators for analysis of cardiac excitation-contraction coupling, and for evaluating the severity of cardiac contractile dysfunction, cardiac reserve capacity and the effectiveness of therapeutic agents in congestive heart failure.


*Keywords:* Bay K 8644; $Ca^{2+}$ transient; Intracellular $Ca^{2+}$ concentration; Cyclopiazonic acid; Force–frequency relationship (FFR); Forskolin; Gingerol; Intracellular $Na^+$ concentration; L-type $Ca^{2+}$ channel; $Na^+$–$Ca^{2+}$ exchanger; Ryanodine channel; SERCA2a; Thapsigargin; Trifluoperazine; W-7

**Contents**



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## 1. Introduction

The force–frequency relationship (FFR) as well as Frank–Starling's mechanism plays an essential role in adjusting mechanical performance of the heart, allowing the cardiac pump to respond to the continually altering hemodynamic needs of the body. Endogenous regulators, such as neurotransmitters, hormones, autacoids, and cytokines, trigger receptor-mediated signal transduction processes which contribute to the adjustment of cardiac contractile function partly through modulation of the FFR.

Mechanisms underlying contractile regulation induced by alteration of the frequency and/or rhythm of cardiac contraction have long been of widespread interest in cardiac physiology and pathophysiology since Bowditch (1871) and Woodworth (1902) described that the cardiac contractile force depends on the rate and/or rhythm of the heart. A number of studies attempted to elucidate the cellular mechanisms underlying the frequency-dependent alteration of contractility in cardiac muscle (for reviews, see Bers, 2001; Blinks and Koch-Weser, 1961; Edman and Johannsson, 1976; Lewartowski and Pykowski, 1987; Schouten et al., 1987). It has long been believed that the frequency-dependent increase in myocardial contractility is associated with an increase in the amount of $Ca^{2+}$ ions entering the myocardial cells with each beat because cardiac excitation–contraction coupling essentially depends on $Ca^{2+}$ influx during the plateau phase of action potential (e.g., see Koch-Weser and Blinks, 1963 or the recent review by Bers, 2002). The experimental evidence, however, to support this postulate had been lacking until Allen and Blinks (1978) first showed in intact cardiac muscle loaded with the $Ca^{2+}$ sensitive bioluminescent protein aequorin (Blinks et al., 1982) that the amplitude of intracellular $Ca^{2+}$ transients increased when the frequency of electrical stimulation was elevated.

Myocardial contractility deteriorates in heart failure and dysfunction of important regulatory mechanisms occurs:

(1) Frank–Starling's mechanism loses its potential to increase the strength of cardiac contractile force that is exerted depending on the extent of stretching of myofibrils over the physiological range of fiber lengths;
(2) the FFR loses its physiological functional operation to increase the amplitude of contraction depending on the elevated frequency of contraction over the physiological range (positive FFR); and
(3) facilitatory regulation, e.g., induced by activation of β-adrenoceptors is suppressed (for reviews, see Bers, 2001; Endoh, 1998; Houser et al., 2000; Katz, 2000).

An inversed FFR in heart failure has pathophysiological significance in respect to the therapeutic basis of contractile dysfunction, in addition to the fundamental research interest in the cellular mechanisms involved in the abnormal FFR. It is a matter of course that the reversal of these dysfunctions in mechanical performance by means of physical and pharmacological procedures is of extreme importance in cardiovascular therapy.

In this article, I will review the role of the FFR in physiological and pathophysiological regulation of myocardial contractility. I will focus on the characteristics of frequency-dependent regulation of cardiac contractility,

namely the cellular mechanisms involved in the frequency-dependent regulation of $Ca^{2+}$ signaling processes and the clinical relevance of the FFR.

## 2. Characteristics of frequency-dependent regulation of myocardial contractility

While a positive FFR is elicited in ventricular myocardium of most mammalian species, negative FFRs are also induced in some species of animals, including rat and mouse, and in most species at very high frequencies of stimulation. In failing ventricular myocardium of experimental animals and humans, a negative FFR is elicited over the entire range of frequencies.

### *2.1. Positive force–frequency relationship*

As a representative example of positive FFR in mammalian ventricular myocardium, the observations in the rabbit ventricular myocardium will be described in some detail. In isolated rabbit papillary muscle loaded with aequorin, the FFR in association with $Ca^{2+}$ transients was first investigated in detail in 1989 (Endoh, 1989). Fig. 1 shows the influence of frequency on the $Ca^{2+}$ transients, contractile force and their relationship in the aequorin-loaded rabbit papillary muscle.

While a positive FFR is consistently elicited over the wide frequency range examined, until the 'secondary-phase' negative FFR is induced at very high frequencies, the influence of increases in frequency on the amplitude and duration of $Ca^{2+}$ transients is different depending on the frequency range. At lower frequencies of 0.06–0.5 Hz (Fig. 1A, left), the prolonged duration of $Ca^{2+}$ transients appears to be related more closely to the increase in contractile force than to the amplitude of $Ca^{2+}$ transients, whereas at higher frequencies of 1.0–1.67 Hz (Fig. 1A, right), the increase in contractile force was associated with a parallel frequency-dependent increase in the amplitude of $Ca^{2+}$ transients. The prolonged duration of $Ca^{2+}$ transients at lower frequencies may not be due to alteration of the action potential duration because in rabbit ventricular muscle, the action potential duration was altered little over the frequency range of 0.5 Hz or lower (Baudet et al., 1996). The action potential duration increased gradually when the stimulation frequency was elevated between 0.5 and 5 Hz (Baudet et al., 1996) which may further increase the $Ca^{2+}$ influx via the voltage-dependent L-type $Ca^{2+}$ channel to contribute to the positive FFR essentially by an increase in sarcoplasmic reticulum (SR) $Ca^{2+}$ load.

Summarized data determined over a wide frequency range are presented in Fig. 1B, and the steady-state relationship between the amplitude of $Ca^{2+}$ transients and contractile force during alteration of the stimulation frequencies is shown in Fig. 1C. The increase in contractile force correlates well with the increase in the amplitude of $Ca^{2+}$ transients induced by elevation of the frequency over the range of 0.5–2.0 Hz (Fig. 1C). When the frequency is 2.5 Hz and higher, the increase in contractile force became less, shifted gradually from the increase in the amplitude of $Ca^{2+}$ transients and became negative even when the amplitude of $Ca^{2+}$ transients rose further by increasing the frequency. This 'secondary-phase' negative FFR induced over a very high-frequency range is observed consistently in mammalian ventricular myocardium and is probably due to complex cellular mechanisms, including oxygen limitation in the central fibers of the isolated rabbit papillary muscle (Fig. 1B and C). Because the diastolic $[Ca^{2+}]_i$ level was not altered even at the highest frequencies examined in these experiments, the diastolic $Ca^{2+}$ overload may play little role in the induction of 'secondary-phase' negative FFR. As will be considered later, some mechanistic processes have been proposed as the mechanism underlying the 'secondary-phase' negative FFR in rats.

The FFR and the influence of rhythm on the contractile force (the post-rest and post extrasystolic potentiation and/or depression) in isolated ventricular myocardium reflect an essential contractile regulation in the heart in vivo under physiological and pathophysiological conditions (Mulieri et al., 1992; Zaugg et al., 1995). In perfused rat heart, a positive FFR was observed over a frequency range of 3 to 5 Hz which flattened after heart failure (Narayan et al., 1995). In isolated rat ventricular muscle, a flat (Litwin and Morgan, 1999) or weakly positive (Schouten and Ter Keurs, 1991; Vornanen, 1992) FFR was elicited up to 3–4 Hz, until the 'secondary-phase' negative FFR became evident at the higher frequency range. Taylor et al. (2004) have also shown that the rat left ventricular muscle displayed a positive FFR over the frequency range of 1–4 Hz without significant qualitative differences to the dog and human ventricular muscle.

The intact mouse ventricular muscle likewise showed a positive FFR albeit the relation is less steep than in other species (Bluhm et al., 2000; Stull et al., 2002). On the other hand, in the mouse ventricular myocardium, the increase in contractile force induced by elevation of the frequency was so disproportionately high compared to the increase in $Ca^{2+}$ transients that the authors postulated that the positive FFR may involve a frequency-dependent increase in myofilament $Ca^{2+}$ sensitivity in this species (Gao et al., 1998). It appears to be evident, therefore, that the shape of the FFR in mouse ventricular myocardium is positive over the range of frequencies close to the physiological heart rate.

Various factors that affect the intracellular $Ca^{2+}$ handling are postulated to contribute to the observed relationship (Bers, 2000). In the rabbit ventricular muscle, it is postulated that the SR $Ca^{2+}$ uptake relative to the $Ca^{2+}$ efflux via the $Na^{+}$–$Ca^{2+}$ exchange increases progressively with frequency and may primarily contribute to the positive FFR in this species (Maier et al., 2000a). $Ca^{2+}$ influx via L-type $Ca^{2+}$ channels during the action potential plateau also plays a

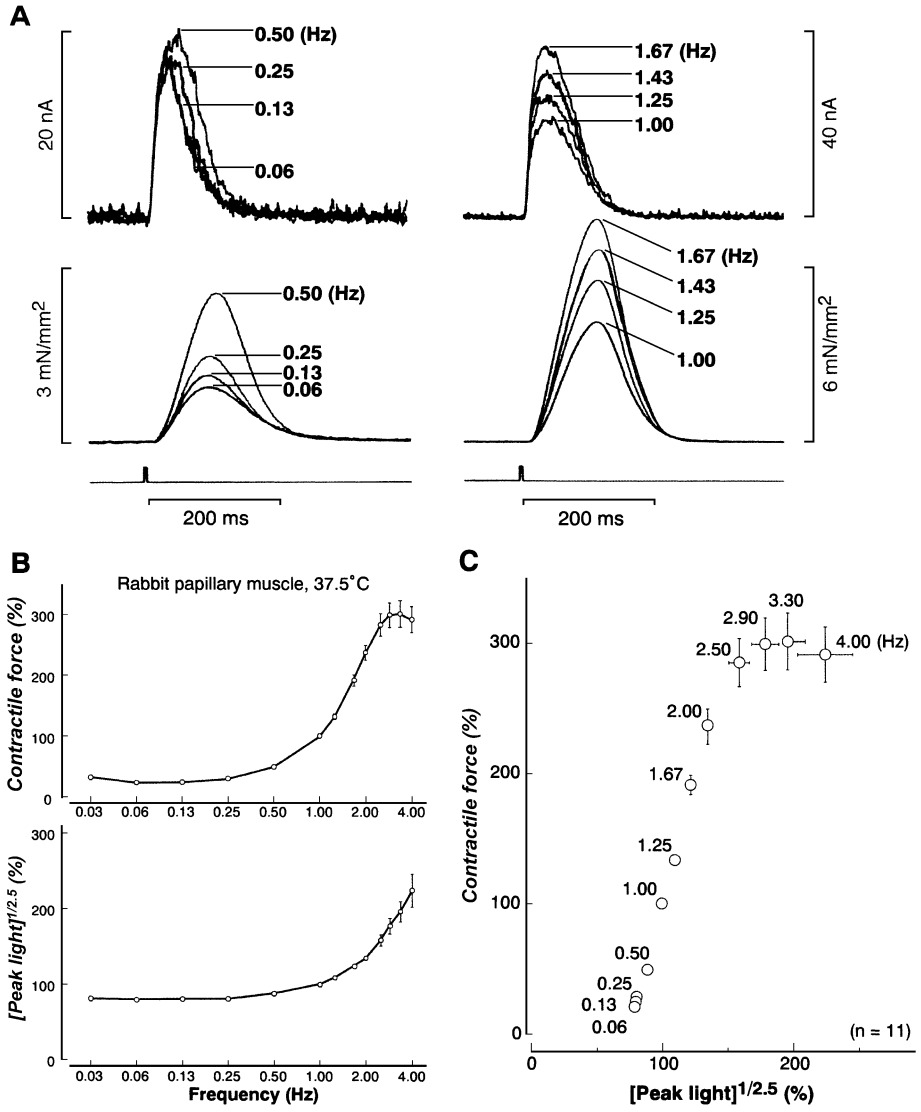


Fig. 1. Force–frequency relationship determined in isolated rabbit papillary muscles loaded with the $Ca^{2+}$-sensitive bioluminescent protein aequorin at 37.5 °C. (A) Actual representative tracings of aequorin light transients (upper tracings) and contractile force (lower tracings) over a range of lower frequencies (left-hand side panel) and of higher frequencies (right-hand side panel). (B) Force–frequency (upper panel) and $Ca^{2+}$ transient–frequency (lower panel) relationships ($n$=11 each). (C) Steady-state relationship between the amplitude of $Ca^{2+}$ transients and contractile force during alteration of the stimulation frequency.

crucial role in the frequency-dependent regulation of contraction.

### 2.2. Negative force–frequency relationship

In mammalian ventricular myocardium, a negative FFR is induced in some species and under certain experimental conditions. Negative FFRs can be classified into at least three classes according to the potential mechanisms underlying the individual cases, and will be discussed further in detail.

#### 2.2.1. '*Primary-phase*' *negative force–frequency relationship*

The 'primary-phase' negative FFR is induced when the stimulation frequency is increased over a low-frequency range, up to 0.5–1 Hz in the rat (Mubagwa et al., 1997; Orchard and Lakatta, 1985; Schouten and Ter Keurs, 1991; Vornanen, 1992) or to 1–2 Hz in the mouse (Namekata et al., 2004; Redel et al., 2002), but not in larger mammalian species (e.g., not in the rabbit as shown in Fig. 1). This 'primary-phase' negative FFR in the ventricular myocardium is likewise induced in atrial muscle, and therefore, the shape of the FFR in mouse and rat is essentially similar to that observed in mammalian atrial myocardium, while the frequency dependence is different depending on individual tissues. When the frequency is further increased, the contractile force reaches the lowest level, i.e., achieves a trough, and then the FFR becomes positive over the range close to the physiological heart rate in individual species. The mechanism underlying the 'primary-phase' negative FFR is different from the mechanism underlying the

'secondary-phase' negative FFR and the 'overall' negative FFR induced in the failing myocardium. In rat and mouse ventricular myocardium, SR $Ca^{2+}$ content is often relatively high even at very low stimulation frequencies. This may be due in part to a relatively high $[Na^+]_i$ that limits $Ca^{2+}$ extrusion via $Na^+$–$Ca^{2+}$ exchange (Bers, 2001). The characteristics of intracellular ion balance in these species may be involved in inducing the 'primary-phase' negative FFR.

#### 2.2.2. '*Secondary-phase*' *negative force–frequency relationship*

The 'secondary-phase' negative FFR induced over the very high frequency range is consistently observed in mammalian ventricular myocardium (e.g., in the rabbit as shown in Fig. 1B and C). It may result from complex cellular mechanisms, such as oxygen limitation in the central fibers of isolated muscle preparations, altered $Ca^{2+}$ handling, including potential $Ca^{2+}$ overload, and alteration of intracellular pH (Antoons et al., 2002; Layland and Kentish, 1999; Maier et al., 2000a; Morii et al., 1996).

#### 2.2.3. '*Overall*' *negative force–frequency relationship*

In hamster ventricular myocardium, an 'overall' negative FFR is consistently induced over the entire range of stimulation frequencies (Finkel et al., 1995). In this species, nitric oxide (NO) released in a frequency-dependent manner appears to be crucial to the underlying mechanism. In addition, it has been well documented that the positive FFR disappears and/or becomes inverted to an 'overall' negative FFR in failing myocardium due to abnormalities in $Ca^{2+}$ handling processes (Ezzaher et al., 1992; Gwathmey et al., 1987; Mulieri et al., 1992).

### 2.3. *Characteristics of the force–frequency relationships in rat, mouse and hamster*

The FFRs in rat, mouse and hamster show characteristic differences compared to larger mammalian species.

#### 2.3.1. *Rat ventricular myocardium*

In rat ventricular myocardium, the FFR is triphasic over the frequency range of examined. The contractile force is minimal at 0.3–0.66 Hz, and at this lower frequency range, a 'primary-phase' negative FFR is induced (Mubagwa et al., 1997; Orchard and Lakatta, 1985; Schouten and Ter Keurs, 1991; Vornanen, 1992). Schouten and Ter Keurs (1991) showed that the 'primary-phase' negative FFR is inhibited by caffeine and theophylline that suppress SR $Ca^{2+}$ uptake but not by $Ca^{2+}$ antagonists, nifedipine and $Mn^{2+}$, that suppress $I_{Ca}$, whereas the positive FFR over the higher frequency range was abolished by the $Ca^{2+}$ antagonists but not by caffeine and theophylline. These findings imply that different mechanisms are responsible for the increase in contractile force induced by decreasing or increasing stimulation frequency.

Several different cellular mechanisms have been proposed for the 'secondary-phase' negative FFR. The 'secondary-phase' negative FFR at frequencies of 3–5 Hz may be partly due to a decrease in $pH_i$, which decreases the myofilament $Ca^{2+}$ sensitivity (Morii et al., 1996). On the other hand, in isolated rat thin ventricular trabeculae, a positive FFR was caused by frequency-dependent loading of the SR with $Ca^{2+}$, which may be saturated at high frequencies or under $Ca^{2+}$ overload conditions and may contribute in part to the 'secondary-phase' negative FFR (Layland and Kentish, 1999). Maier et al. (2000a) suggested that the 'secondary-phase' negative FFR over a high-frequency range (3 Hz) in rat ventricular muscle strips might be due to the refractoriness of SR $Ca^{2+}$ release channels. Taylor et al. (2004) have recently postulated that the negative FFR observed in rats in the previous studies may in part be due to an artifact, i.e., an increase in SR $Ca^{2+}$ ATPase (SERCA2a) activity early after the excision and preparation of the muscle strips, based on the observations that the rat left ventricular muscle displayed a positive FFR over the frequency range of 1–4 Hz without significant qualitative differences to the dog and human ventricular muscles. It appears evident, however, that the mechanisms involved in determining the frequency-dependent regulation of $[Ca^{2+}]_i$ in the rat ventricular myocardium are quite different from those in the rabbit, even when they are compared under the same experimental conditions (Maier et al., 2000a). Experimental conditions, which affect the potential extent of activated state of SERCA2a (Taylor et al., 2004), may have a stronger impact on the expression of characteristic FFR in rats than in larger mammalian species. While the 'primary-phase' negative FFR is induced consistently in the rat ventricle over the lower frequency range up to 0.5–1 Hz, the characteristics of the FFR over the higher frequency range close to the physiological heart rate may be determined by contribution of various labile factors leading to the flat, positive and/or enhanced 'secondary-phase' negative FFR, which have not yet been fully defined and remain for future study.

#### 2.3.2. *Mouse ventricular myocardium*

In isolated mouse ventricular myocardium, a prominent 'primary-phase' negative FFR is induced over the lower frequency range when the frequency is increased up to 1 Hz (Redel et al., 2002) or 2 Hz (Namekata et al., 2004), depending on the experimental temperature and $[Ca^{2+}]_o$. The findings that a novel $Na^+$–$Ca^{2+}$ exchange inhibitor, SEA0400, is capable of inhibiting the 'primary-phase' negative FFR under these experimental conditions imply that the frequency-dependent increase in $Ca^{2+}$ efflux via $Na^+$-$Ca^{2+}$ exchange may be responsible for the 'primary-phase' negative FFR at lower frequencies up to 1–2 Hz in mouse ventricular myocardium (Namekata et al., 2004). In addition, it has also been reported that the FFR is altered depending on the experimental conditions. In mouse

papillary muscle, at low temperature and low $[Ca^{2+}]_o$, the FFR was strongly positive, whereas, at high temperature and high $[Ca^{2+}]_o$, it became negative (Redel et al., 2002). It is postulated that the mechanism of 'secondary-phase' negative FFR in mouse ventricular myocytes is that $[Ca^{2+}]_i$–frequency relationship depends on a balance between the increase in SR $Ca^{2+}$ content and the loss of trigger L-type $Ca^{2+}$ current, with the latter being enhanced at higher frequencies of 2–4 Hz due to slow recovery from inactivation (Antoons et al., 2002).

*2.3.3. Hamster: influence of NO on the force–frequency relationship*

Modification by NO of the frequency-dependent regulation of contractile force in mammalian ventricular myocardium appears to be markedly different depending on the species of animals. In isolated hamster papillary muscle, $N^G$-monomethyl-L-arginine (L-NMMA, a competitive inhibitor of NO synthase) reversed the 'overall' negative FFR in the control making it positive (Finkel et al., 1995). However, NO synthase inhibition and/or the NO donor had little or only marginal effects on the FFR in human and rat ventricular myocardium (Cotton et al., 2001; Prabhu et al., 1999).

## 3. Role of the sarcoplasmic reticulum

The SR $Ca^{2+}$ load plays the central role in determining the characteristics of the FFR. When the frequency of stimulation is elevated, SR $Ca^{2+}$ uptake relative to the $Ca^{2+}$ extrusion via $Na^+$–$Ca^{2+}$-exchange increases significantly in nonfailing human myocardium, which may be responsible for the positive FFR (Pieske et al., 1999). In failing human myocardium, a reduction in SERCA2a activity appears to contribute significantly to impairment of both systolic and diastolic function (Schmidt et al., 1998). Hashimoto et al. (2000) have found that SERCA2a over-expressing transgenic mice exhibit a positive FFR, whereas the relation is flat in age- and strain-matched controls, supporting the postulate that SERCA2a plays a crucial role in induction of the positive FFR. The authors postulate that an increase in SERCA2a expression increases the ability of the SR to store $Ca^{2+}$, such that more $Ca^{2+}$ is available to be released during each heartbeat at higher stimulation rates. Münch et al. (2000) have shown that protein expression of SERCA2a is unchanged in failing human left ventricular myocardium, but the suppressed function of SERCA2a correlates well with the abnormalities in the FFR. SERCA2a activity is regulated by the extent of phosphorylation of phospholamban at $Ser^{16}$, mainly induced by protein kinase (PKA) and $Thr^{17}$, primarily phosphorylated by $Ca^{2+}$/calmodulin-dependent protein kinase (CaMKII; Hagemann and Xiao, 2002). Bluhm et al. (2000) identified phospholamban as a major determinant of the cardiac FFR by comparing the FFR in wild-type and phospholamban knockout mice. Isoda et al. (2003) have likewise collected the evidence to support the importance of phospholamban phosphorylation using CREM (cyclic AMP response element modulator) knockout mice where the FFR was markedly depressed in association with a decrease in phospholamban phosphorylation due to an increased activity of protein phosphatase-1 (PP-1) because of the absence of CREM-induced inhibitory regulation of PP-1.

On the other hand, Meyer et al. (1999) suggest that the ratio of phospholamban to SERCA2a, but not the absolute amount of SERCA2a expressed, is important for determining the characteristics of the FFR based on their findings in transduction experiments. In these experiments, adult rabbit ventricular myocytes over-expressing phospholamban showed an augmented positive FFR, whereas SERCA2a-transduced myocytes and papillary muscles displayed a negative FFR (Meyer et al., 1999). The cellular mechanism causing overexpression of SERCA2a, resulting in a negative FFR, is not clear and awaits further study.

The importance of SR $Ca^{2+}$ ATPase in determining the FFR has been also supported by the pharmacological inhibition of the SR $Ca^{2+}$ ATPase activity, but the detailed results have been controversial, suggesting that differences in experimental conditions might markedly affect the experimental outcome. Findings with 2,5 di-(*tert*-butyl)-1,4-benzohydroquinone, a putative inhibitor of the SR $Ca^{2+}$ pump in rat and rabbit ventricular myocardium indicate that SR $Ca^{2+}$ uptake plays a crucial role in the FFR (Baudet et al., 1996). Cyclopiazonic acid, a SR $Ca^{2+}$ ATPase inhibitor, depressed the amplitude of the positive FFR without changing the shape of the relationship in dog and human ventricular myocardium (Taylor et al., 2004). By contrast, it has been reported that in nonfailing human myocardium cyclopiazonic acid reversed the FFR from positive to negative (Schwinger et al., 1997). Thapsigargin, another SERCA2a inhibitor, also significantly suppressed the positive FFR in guinea pig ventricular cardiomyocytes (Money-Kyrle et al., 1998). The difficulties associated with these experiments are that the extent of SR $Ca^{2+}$ ATPase inhibition in intact myocardium is not known. Provided that SR function is pharmacologically abolished, the positive FFR may be induced by the frequency-dependent increase in $Ca^{2+}$ influx through sarcolemma as has been shown in the presence of ryanodine in ventricular myocardium of experimental animals and humans with heart failure (Schlotthauer et al., 1998; Stemmer and Akera, 1986).

It has been demonstrated that agents, such as forskolin that increases cyclic AMP (Mulieri et al., 1992; Pieske et al., 1998), and the cyclic AMP derivative 8-(4-chlorophenylthio) adenosine cyclic 3',5'-monophosphate (Money-Kyrle et al., 1998) are able to reverse the negative FFR and make it positive by a frequency-dependent increase in SR $Ca^{2+}$ load mediated by phospholamban phosphorylation. However, the effects of these agents are markedly dependent on the concentration that affects the extent of accumulation of cyclic AMP resulting in activation of PKA. These agents

at low concentrations reversed the negative FFR, whereas at high concentrations, e.g., isoproterenol at $10^{-7}$ M, enhanced the negative FFR in failing human myocardium (Schwinger et al., 1993). The effects of epinephrine on the FFR in rabbit papillary muscle are essentially consistent with these previous observations, and in addition, indicate that the alteration of $Ca^{2+}$ kinetics causes a complete dissociation of contractile force from $Ca^{2+}$ transients (Fig. 2). In the presence of epinephrine at $10^{-4}$ M, the amplitude of $Ca^{2+}$ transients was increased (positive $Ca^{2+}$ transient–frequency relationship), in association with a marked abbreviation of $Ca^{2+}$ transients (Fig. 2, upper panel) when the frequency was elevated stepwise from 0.13 to 1.0 Hz. A prominent negative FFR was induced, however, by increasing the frequency probably due to the abbreviation of twitch contraction (Fig. 2, lower panel).

## 4. The role of L-type $Ca^{2+}$ channels

$Ca^{2+}$ influx via L-type $Ca^{2+}$ channels in sarcolemma is essential for cardiac force development by contributing to at least two important processes, i.e., to trigger $Ca^{2+}$ release from ryanodine channels in SR membrane (the $Ca^{2+}$-induced $Ca^{2+}$-release mechanism) and to supply $Ca^{2+}$ to SR $Ca^{2+}$ stores. Thus, L-type $Ca^{2+}$ currents play a crucial role also in determining the characteristics of FFR. The $Ca^{2+}$ influx via L-type $Ca^{2+}$ channels is increased when the stimulation frequency is elevated. At higher stimulation frequencies, the fraction of time of action potential plateau per unit time increases, which leads to a prolongation of depolarization duration, where the $Ca^{2+}$ influx via voltage-dependent L-type $Ca^{2+}$ channels is increased. In fact, when the SR function is depressed by ryanodine, the positive FFR mainly supported by the frequency-dependent increase in L-type $Ca^{2+}$ currents is induced in all the species examined, including rat, mouse, ferret and guinea pig (Stemmer and Akera, 1986).

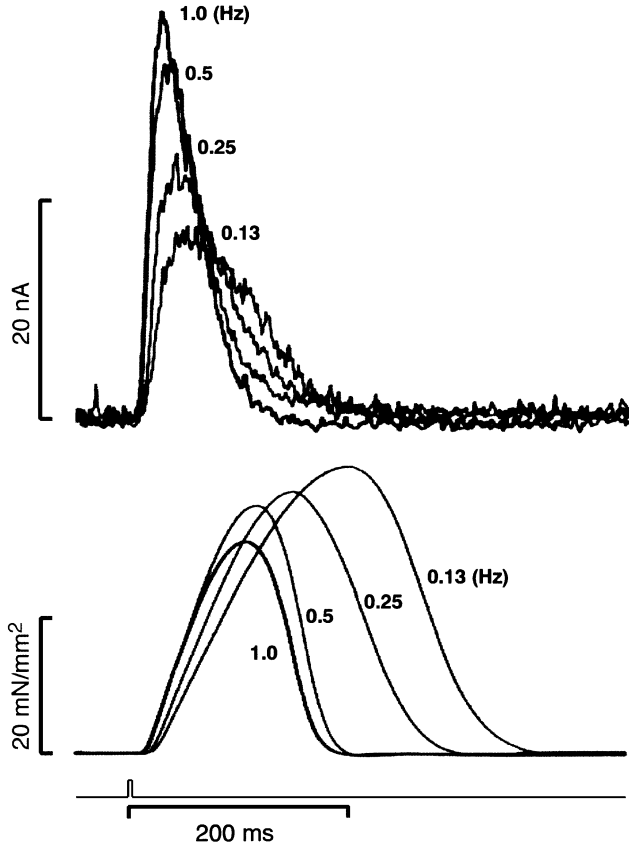


Fig. 2. Influence of epinephrine ($10^{-4}$ M) on the $Ca^{2+}$ transient–frequency and force–frequency relationships in isolated rabbit papillary muscle loaded with aequorin. Experiential conditions are the same as those in Fig. 1.

Alteration of L-type $Ca^{2+}$ currents is not considered to play an important role in the negative FFR that is observed in failing human ventricular myocardium. The increase in $Ca^{2+}$ influx by elevating $[Ca^{2+}]_o$ or Bay K 8644 and FPL 64176, the L-type $Ca^{2+}$ channel agonists, increased the contractile force at lower frequencies but did not normalize the negative FFR, in contrast to a drug that increased SR $Ca^{2+}$ pump activity and reversed the negative FFR in failing human ventricular myocardium (Rossman et al., 2004). However, the effects of such agents that modulate L-type $Ca^{2+}$ channel activity on the FFR are still controversial. Reuter et al. (1999) showed in failing human myocardium that Bay K 8644 ($10^{-7}$ M) and nifedipine ($10^{-8}$ M) restored a positive FFR, which had been negative before the application of these agents, an indication that the altered FFR and $Ca^{2+}$ homeostasis in failing human myocardium may result from the changes in sarcolemmal $Ca^{2+}$ influx and/or ensuing alteration of SR $Ca^{2+}$ load. The extent of contributions by these processes, i.e., sarcolemmal $Ca^{2+}$ flux and SR $Ca^{2+}$ load, is probably crucial to the cellular mechanisms involved in determining the characteristics of FFR.

## 5. The role of $Na^+$ ions

Langer (1971) suggested that increasing frequency may result in an increase in intracellular $Na^+$ ion concentration ($[Na^+]_i$) and that this, in turn, may elevate the $[Ca^{2+}]_i$ via $Na^+$–$Ca^{2+}$ exchange. This may in part be the mechanism causing the increase in $Ca^{2+}$ transients induced by increasing the stimulation frequency. Experiments by means of $Na^+$-sensitive microelectrodes have revealed that in cardiac Purkinje fibers, increasing the frequency resulted in a frequency-dependent increase in the intracellular $Na^+$ activity ($a^i_{Na}$; Boyett et al., 1987; Cohen et al., 1982). Subsequently, Wang et al. (1988) analyzed the relationship between the twitch force and $a^i_{Na}$ in detail and demonstrated that in guinea pig papillary muscle, the relation between the twitch force and $a^i_{Na}$ was sigmoidal over the range of 0.5–5 Hz and was steeper in the range of 1–4 Hz. From these studies, it is evident that an increase in $a^i_{Na}$ is an important factor involved in the positive FFR. In rat ventricular myocytes showing a negative FFR, increasing the frequency

had less effect on the $a^{i}_{Na}$ compared with myocytes that showed a positive FFR (Frampton et al., 1991).

On the other hand, the ionophore monensin increased the $[Na^+]_i$ and reversed the positive FFR, making it negative in rabbit and guinea pig ventricular myocardium (Mubagwa et al., 1997). The authors postulated that in the presence of high $[Na^+]_i$, the SR $Ca^{2+}$ loading may increase during diastole, possibly via reverse-mode $Na^+$–$Ca^{2+}$ exchange, providing higher $Ca^{2+}$ transients at slower rates of stimulation. This provides an excellent explanation for the rest potentiation of contraction. It was further shown that some secondary factor might modify the relation between the twitch force and $a^{i}_{Na}$. As the frequency of stimulation increased and the $a^{i}_{Na}$ rose, the diastolic membrane potential hyperpolarized probably due to an increase in $a^{i}_{Na}$, leads to an enhancement of the electrogenic $Na^+$–$K^+$ pump to hyperpolarize the cell membrane (Wang et al., 1988).

In human myocardium, $Na^+$ influx and $Na^+$ homeostasis may also play a crucial role in determining the FFR (Müller-Ehmsen et al., 1997). In failing human myocardium, the $[Na^+]_i$ homeostasis may be altered in a manner as seen when higher $[Na^+]_i$ in failing myocardium enhances the $Ca^{2+}$ influx through the $Na^+$–$Ca^{2+}$ exchanger at lower frequencies and maintains the SR $Ca^{2+}$ load and the contractile force; while at higher frequencies, failing myocytes with a high $[Na^+]_i$ cannot further increase the SR $Ca^{2+}$ load and are prone to the diastolic $Ca^{2+}$ overload (Pieske et al., 2002). Flesch et al. (1996) have shown in failing human myocardium (NYHA class IV) that BDF 9148, a $Na^+$ channel activator, restored the positive FFR and reduced the frequency-dependent increase in diastolic tension. They postulated that the increased $Na^+$–$Ca^{2+}$ exchange activity in severely failing human myocardium may be responsible for the beneficial effects of BDF 9148 (Flesch et al., 1996). The role of $[Na^+]_i$ in the contractile regulation in failing myocardium may be dependent on the severity of heart failure and is modified by the experimental conditions.

## 6. The role of calmodulin

Calmodulin (CaM) and $Ca^{2+}$-CaMKII are of special interest in cardiac contractile regulation because of their role modulating $Ca^{2+}$ influx, SR $Ca^{2+}$ uptake and SR $Ca^{2+}$ release during cardiac excitation–contraction coupling (Maier and Bers, 2002). The CaM antagonists, W-7 (Tanaka et al., 1982; Fig. 3A) and trifluoperazine (TFP; Babu and Gulati, 1990; Fig. 3B) exerted more pronounced inhibitory actions on the contractile force (left panel) and aequorin peak light transients (right panel) at higher frequencies in the isolated rabbit papillary muscle (Endoh, 1989). While these observations support the role of CaM in the frequency-dependent regulation of contractile force and $Ca^{2+}$ transients, the contributions of factors other than CaM could not be excluded in these experiments. These agents have various direct actions on ion channels, SR and contractile proteins (Bkaily et al., 1984; Kimura, 1993; Klockner and Isenberg, 1987).

Frequency-dependent acceleration of relaxation (FDAR) of twitch contraction is an important intrinsic physiological mechanism because it allows more rapid ventricular filling at higher heart rates, which has been shown also to be apparent in single myocytes and to depend on the SR $Ca^{2+}$ transport and CaMKII activation (Bassani et al., 1995). The frequency-dependent acceleration of $[Ca^{2+}]_i$ decline was abolished by the selective CaMKII inhibitor, KN-93 (Li et al., 1997). Furthermore, the phospholamban knockout myocytes have higher SERCA2a, higher SR $Ca^{2+}$ loading and reduced $Na^+/Ca^{2+}$ exchange activity and retain CaM-

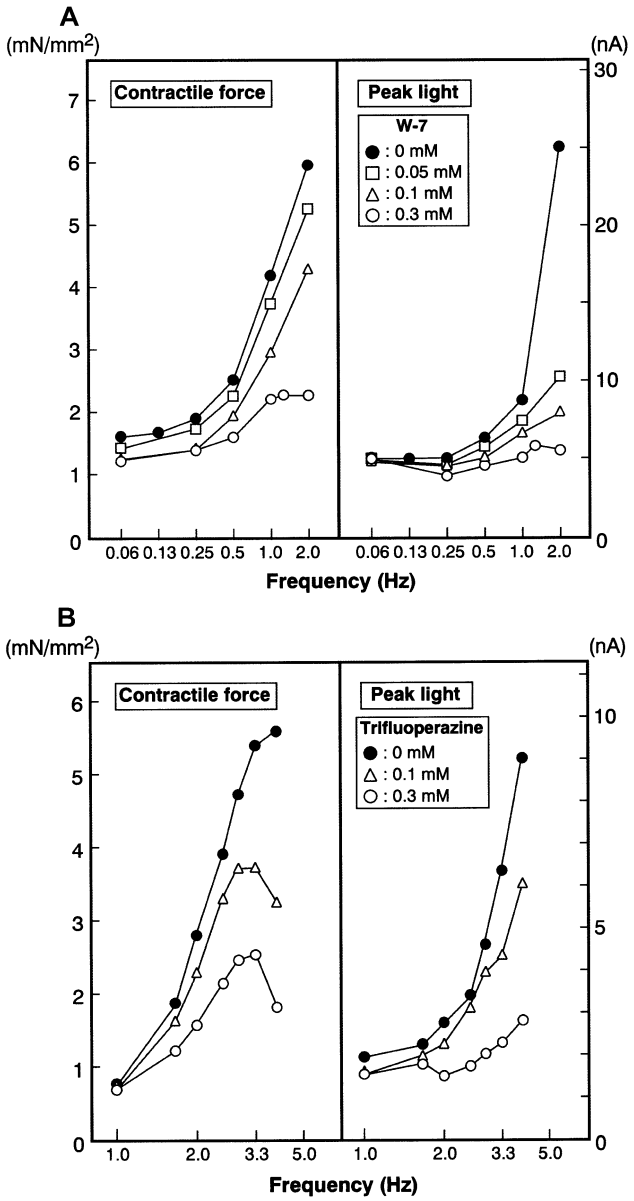


Fig. 3. Frequency-dependent inhibition of the steady-state force–frequency and $Ca^{2+}$ transient–frequency relationships by the calmodulin inhibitors, W-7 (A) and trifluoperazine (B), in rabbit papillary muscles loaded with aequorin (modified from Endoh, 1989).

KII-dependent acceleration of the $[Ca^{2+}]_i$ decline associated with steady-state twitch contraction (Li et al., 1998). In both wild-type and phospholamban knockout mouse myocardium, the FDAR of twitch contraction was prominent but was largely suppressed by KN-93 (DeSantiago et al., 2002). It should be noted, however, that the issue is still controversial because there is evidence indicating in rat myocardium that the frequency-dependent abbreviation and acceleration of $[Ca^{2+}]_i$ decline are independent of PKA- and/or CaMKII-mediated phosphorylation of phospholamban (Hussain et al., 1997; Kassiri et al., 2000).

There is other evidence indicating the potential contribution of CaM to the frequency-dependent regulation of cardiac contractility. CaM is a critical $Ca^{2+}$ sensor for both inactivation and facilitation of L-type $Ca^{2+}$ channels (Yuan and Bers, 1994; Zuhlke et al., 1999). CaMKII enhanced L-type $Ca^{2+}$ currents and reciprocally reduced the $Ca^{2+}$ release from SR (reduction in $Ca^{2+}$ transients) while increasing the SR $Ca^{2+}$ content (Delgado et al., 1999; Wu et al., 2001), which indicates that the activation of CaMKII may elicit differential effects on sarcolemmal L-type $Ca^{2+}$ channels and SR $Ca^{2+}$ release channels. Alternatively, the attenuation of $Ca^{2+}$ release by CaMKII (as a primary action) could induce a secondary increase in L-type $Ca^{2+}$ currents by inhibition of the $Ca^{2+}$-induced inactivation of $Ca^{2+}$ channels (Takamatsu et al., 2003).

On the other hand, it has been reported that enhancement of the $Ca^{2+}$-induced $Ca^{2+}$ release by CaMKII could contribute to the positive FFR observed in cardiac muscle (Maier and Bers, 2002). When the SR $Ca^{2+}$ load and L-type $Ca^{2+}$ currents were controlled at constant in intact cells, SR $Ca^{2+}$ release was increased by CaMKII and FK 506 which interferes with the interaction between the $Ca^{2+}$ release channel and the FK-binding protein (Bers et al., 1998). The increase in $[Ca^{2+}]_i$ induced by an elevation of frequency may stimulate CaMKII, which increases fractional SR $Ca^{2+}$ release and thereby contributes to the positive FFR (Li et al., 1997).

Evidence for CaM-mediated alteration of the myofilament $Ca^{2+}$ sensitivity has also been reported. $Ca^{2+}$ sensitivity tended to progressively decrease with repeated $Ca^{2+}$ activation in the absence of CaM in β-estin-skinned cardiac myocytes from Wistar rat hearts, and the presence of CaM significantly increased the myofilament $Ca^{2+}$ sensitivity under these experimental conditions (Suematsu et al., 2002).

## 7. ‘Overall’ negative force–frequency relationship in heart failure

In failing heart, the normal positive FFR disappears or is replaced by a negative FFR with increased end-diastolic pressure when the heart rate increases (Ezzaher et al., 1992; Gwathmey et al., 1987). In congestive heart failure (idiopathic dilated cardiomyopathy), the positive FFR is markedly depressed which is in strong contrast to the nonfailing heart of healthy subjects (Mulieri et al., 1992). The depressed or ‘overall’ negative FFR in failing human hearts is a reliable physiologic signature of the cardiomyopathic state even when the contractile force at low stimulation frequencies is relatively high (Rossman et al., 2004). The abnormalities of FFR induced in heart failure depend also on the species of animal. In pig ventricular myocardium, the positive FFR was markedly depressed but did not invert and become negative even in severe heart failure (Eising et al., 1994).

### 7.1. Mechanisms: the role of SR $Ca^{2+}$ load

While both the alteration of $Ca^{2+}$ handing and myofilament $Ca^{2+}$ sensitivity could contribute to the deterioration of positive FFR in heart failure, the latter may not be responsible for contractile dysfunction because the $Ca^{2+}$ sensitivity increased in end-stage failing hearts, probably due to the increased percentage of dephosphorylated troponin I found in failing hearts (Van der Velden et al., 2003). The impaired FFR is not due to an inability of contractile proteins to further increase contractility, but it is rather a reflection of an altered functional balance between $Ca^{2+}$ reuptake and $Ca^{2+}$ extrusion (Rossman et al., 2004).

In patients with hypertrophic cardiomyopathy in whom the predominant ‘secondary-phase’ negative FFR is present, SERCA2 mRNA levels were significantly lower than in patients who showed a prevailing positive FFR (Somura et al., 2001). This implies that the down-regulation of SERCA2 mRNA, resulting in altered $Ca^{2+}$ handling, may contribute to alteration of FFR in hypertrophic cardiomyopathy patients with severe hypertrophy. On the other hand, Münch et al. (2000) have found that protein expression of SERCA2a in failing human ventricular myocardium is not different to that in nonfailing myocardium, but the function of SERCA2a that is regulated by phosphorylation of phospholamban correlates well with alteration of the FFR. Supporting the crucial role of SERCA2a in controlling the FFR, Huke et al. (2003) have found that the positive FFR that is observed in control hearts is not induced in SERCA2a$^{+/-}$ gene-targeted mice, even in the absence of heart failure. In addition, the overexpression of SERCA2a in human ventricular myocytes isolated from patients with end-stage heart failure restored the positive FFR (Del Monte et al., 1999).

In mouse myocytes isolated from hypertrophied hearts which failed 7 weeks after pressure overload, the positive FFR that was observed as alterations of cell shortening and peak $Ca^{2+}$ transients in control myocytes were depressed due to impaired augmentation of $Ca^{2+}$ transients and SR $Ca^{2+}$ load induced by raising the frequency (Ito et al., 2000). The SR $Ca^{2+}$ uptake did not increase in failing human myocardium as the stimulation frequency was increased, which may be due to the depressed SR $Ca^{2+}$ ATPase, combined with the enhanced cytosolic $Ca^{2+}$ extrusion via

$Na^+$–$Ca^{2+}$ exchanger overexpressed in failing human myocardium (Pieske et al., 1999).

Schlotthauer et al. (1998) observed in human failing myocardium that the blunted or negative aequorin light–frequency relationship and FFR became positive when ryanodine inhibited the SR function and thereby reduced twitch force and $Ca^{2+}$ transients. These observations imply that the frequency-dependent decrease in ryanodine-sensitive $Ca^{2+}$ release may be responsible for the blunted or negative FFR in human failing heart. The important role of the SR $Ca^{2+}$ load is supported by the findings that the inhibition of SR $Ca^{2+}$ ATPase by thapsigargin abolished the positive FFR in the nonfailing human ventricular myocytes, but had no effect in the failing myocytes (Davia et al., 1997). In these experiments, it was shown that there are no differences in the FFR between nonfailing and failing myocytes after the inhibition of SR $Ca^{2+}$ ATPase.

### 7.2. Influence of pharmacological agents

There are agents that are able to reverse the 'overall' negative FFR acting through different signaling pathways. The effect of the cyclic AMP-accumulating agents is complex, which depends on the combination of the extent of cyclic AMP accumulation and the frequency-dependent $[Ca^{2+}]_i$ regulation.

#### 7.2.1. Agents that increase cyclic AMP

It has been reported that pharmacological interventions, such as gingerol (SR $Ca^{2+}$ ATPase activator), isoproterenol and ouabain fail to restore the positive FFR in failing human ventricular myocardium (Maier et al., 2000b), while forskolin, an adenylyl cyclase activator, at a low concentration of $3\times10^{-7}$ M, that had only marginal inotropic effects by itself, partially normalized the inverse FFR in end-stage failing human myocardium (Pieske et al., 1998). These observations are consistent with those that the agents which increase cyclic AMP levels are able to reverse the abnormalities of FFR seen in end-stage heart failure (Phillips et al., 1990), and that a moderate stimulation by isoproterenol ($10^{-8}$ M) partly reversed the negative FFR in NYHA class IV and preserved the positive FFR in nonfailing myocardium (Schwinger et al., 1993). In contrast, a high concentration of isoproterenol ($10^{-7}$ M) enhanced the decline in contractile force at higher stimulation frequencies (Schwinger et al., 1993). The observations with epinephrine in the rabbit papillary muscle (Fig. 2) are essentially similar to those with the high concentration of isoproterenol in human ventricular myocardium, and these findings indicate that the extent of cyclic AMP accumulation may play a crucial role in characterizing the FFR. In transgenic mice expressing a $G_i$-coupled receptor (Ro1) targeted to the heart, the positive FFR is significantly suppressed, which implies that $G_i$-proteins that are increased in failing myocardium to suppress the cyclic AMP accumulation are likely to contribute in part to the abnormal FFR in heart failure (Baker et al., 2001). In the rabbit heart failure model produced by rapid atrial pacing, the normal β-adrenergic amplification of the ascending limb of the positive FFR by dobutamine was absent, but an enhanced 'secondary-phase' negative FFR was prevented by dobutamine (Ryu et al., 1997), supporting the view that the effects of cyclic AMP accumulation may be exerted in a frequency-dependent manner.

A frequency-dependent increase in $Ser^{16}$ phosphorylation of phospholamban, which is stimulated via the cyclic AMP-dependent process to lead to an activation of SERCA2a and is associated with the positive FFR in nonfailing human heart, is not induced in muscles from dilated cardiomyopathy (Brixius et al., 2003). The frequency-dependent increase in $Ser^{16}$ phosphorylation of phospholamban catalyzed by PKA via forskolin- or isoproterenol-induced cyclic AMP accumulation may be responsible for the reversal of the negative FFR.

#### 7.2.2. Agents that increase the $[Na^+]_i$

In combination, ouabain and BDF 9148, a $Na^+$ channel activator, which elevates the $[Na^+]_i$, completely restored the positive FFR in muscles from NYHA class IV heart failure (Schwinger et al., 1993). BDF 9148 ($10^{-7}$ M) alone could also restore the positive FFR in failing human myocardium (Flesch et al., 1996). It has been reported that BDF 9148 reduced the frequency-dependent increase in diastolic tension in severely failing human myocardium probably via the overexpressed $Na^+$–$Ca^{2+}$ exchanger (Flesch et al., 1996). The authors suggested that overexpressing the $Na^+$–$Ca^{2+}$ exchanger in failing human myocardium might be responsible for the beneficial effects of BDF 9148; however, the detailed mechanism is not understood and awaits further study. These findings support a crucial role of $Na^+$ in the induction of the positive FFR, in contrast to an elevation of $[Ca^{2+}]_o$ that produced a more pronounced increase in contractile force at low frequencies, but enhanced further the negative FFR in end-stage failing human myocardium (Schwinger et al., 1993).

#### 7.2.3. Effects of levosimendan

Levosimendan, an agent that causes $Ca^{2+}$ sensitization and PDE III inhibition, improves both systolic and diastolic functions in end-stage failing human myocardium, and the effects are more pronounced at higher frequencies under prevailing diastolic dysfunction (Janssen et al., 2000). It has also been reported that levosimendan improves the FFR without affecting $Ca^{2+}$ transients, probably through an increase in myofilament $Ca^{2+}$ sensitivity in terminally failing human (dilated cardiomyopathy) myocardium (Brixius et al., 2002).

### 7.3. Frequency-dependent diastolic dysfunction

Diastolic function is also disturbed in failing hearts where SR $Ca^{2+}$ ATPase activity is decreased. The frequency-dependent rise of diastolic force in association with

an increase in diastolic $[Ca^{2+}]_i$ is induced when the stimulation frequency increases (Gwathmey et al., 1987), which is inversely correlated with the SR $Ca^{2+}$ ATPase activity (Schmidt et al., 1998). Frequency dependence of the diastolic dysfunction in failing human myocardium is inversely correlated with the $Na^+$–$Ca^{2+}$ exchanger expression, indicating that the diastolic function is preserved by increased expression of the $Na^+$–$Ca^{2+}$ exchanger (Hasenfuss et al., 1999). These findings indicate that impairment of either $Ca^{2+}$ uptake via SR $Ca^{2+}$ ATPase, $Ca^{2+}$ extrusion via $Na^+$–$Ca^{2+}$ exchanger or both are responsible for diastolic dysfunction in severe heart failure.

## 8. Summary

In ventricular myocardium of most mammalian species, the positive FFR is induced over the entire frequency range until the 'secondary-phase' negative FFR appears at extremely high frequencies. In some species, including rat and mouse, however, the 'primary-phase' negative FFR is also induced over a low-frequency range up to 0.5–1 Hz (rat) and 1–2 Hz (mouse). Even in these species, the positive FFR is elicited at frequencies near the physiological heart rate. The SR $Ca^{2+}$ load, which is determined by interrelated frequency-dependent modulation induced by SERCA2a, $Na^+$–$Ca^{2+}$ exchanger and L-type $Ca^{2+}$ channels, plays a central role in characterizing the FFR. Phosphorylation of phospholamban to regulate SERCA2a activity and intracellular $Na^+$ ions to affect $[Ca^{2+}]_i$ via $Na^+$–$Ca^{2+}$ exchanger are important as modulators. Calmodulin may contribute to the frequency-dependent regulation of contraction and relaxation, the role of which has not yet been unequivocally defined. The integrated dynamic control of intracellular $Ca^{2+}$ ions determined by the frequency-dependent regulatory processes is responsible for determining the FFR. In failing myocardium, the positive FFR disappears or is inverted and becomes negative. Activation and overexpression of SERCA2a are able to reverse the abnormalities of the FFR in failing heart, supporting the crucial role of the SR $Ca^{2+}$ load. Analysis of the characteristics of FFR in association with $Ca^{2+}$ transients is helpful for elucidating the physiological mechanisms and pathophysiological alterations of cardiac excitation–contraction coupling.

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