Review

Signal Transduction and Ca^{2+} Signaling in Intact Myocardium

Masao Endoh^{1,*}

¹*Yamagata University, Yamagata 990-8045, Japan*

Received March 22, 2006

Abstract. The experimental procedures to simultaneously detect contractile activity and Ca^{2+} transients by means of the Ca^{2+} sensitive bioluminescent protein aequorin in multicellular preparations, and the fluorescent dye indo-1 in single myocytes, provide powerful tools to differentiate the regulatory mechanisms of intrinsic and external inotropic interventions in intact cardiac muscle. The regulatory process of cardiac excitation-contraction coupling is classified into three categories; upstream (Ca^{2+} mobilization), central (Ca^{2+} binding to troponin C), and/or downstream (thin filament regulation of troponin C property or crossbridge cycling and crossbridge cycling activity itself) mechanisms. While a marked increase in contractile activity by the Frank-Starling mechanism is associated with only a small alteration in Ca^{2+} transients (downstream mechanism), the force-frequency relationship is primarily due to a frequency-dependent increase of Ca^{2+} transients (upstream mechanism) in mammalian ventricular myocardium. The characteristics of regulation induced by β - and α -adrenoceptor stimulation are very different between the two mechanisms: the former is associated with a pronounced facilitation of an upstream mechanism, whereas the latter is primarily due to modulation of central and/or downstream mechanisms. α -Adrenoceptor-mediated contractile regulation is mimicked by endothelin ET_A - and angiotensin II AT_1 -receptor stimulation. Acidosis markedly suppresses the regulation induced by Ca^{2+} mobilizers, but certain Ca^{2+} sensitizers are able to induce the positive inotropic effect with central and/or downstream mechanisms even under pathophysiological conditions.

Keywords: Ca^{2+} sensitizer, Ca^{2+} transient, force-frequency relationship, Frank-Starling mechanism, acidosis

1. Introduction

The cardiac pump is able to alter its function immediately in response to the requirement for blood supply to vital organs in the body by means of alteration of myocardial contractility. Cardiac myocytes act as a functional syncytium and all cells are excited simultaneously to contribute to maintenance of pump function, which is in strong contrast to skeletal muscle fibers. Therefore, the regulation of contractile function of individual myocytes achieved by modulation of intracellular Ca^{2+} signaling in individual cardiac myocytes plays a crucial role in adaptation of cardiac pump func-

tion in vivo.

In cardiac myocytes, Ca^{2+} influx induced by activation of voltage-dependent L-type Ca^{2+} channels (DHP receptors) upon membrane depolarization triggers the release of Ca^{2+} via Ca^{2+} release channels (ryanodine receptors) of sarcoplasmic reticulum (SR) through a Ca^{2+} -induced Ca^{2+} release (CICR) mechanism. This in turn elevates the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) to a level of 10^{-6} – 10^{-5} mol/L from diastolic levels of approximately 10^{-7} mol/L. In the presence of diastolic levels of $[\text{Ca}^{2+}]_i$, troponin I inhibits the interaction of thin (actin, troponin subunits, tropomyosin) and thick (myosin) filaments. Ca^{2+} ions released via the CICR mechanism diffuse through the cytosolic space to contractile proteins to bind to troponin C resulting in the release of inhibition induced by troponin I. The Ca^{2+} binding to troponin C thereby triggers the sliding of thin and thick filaments, that is, the activation of a crossbridge and subsequent

*Corresponding author. mendou@med.id.yamagata-u.ac.jp

Published online in J-STAGE

DOI: 10.1254/jphs.CPJ06009X

cardiac force development and/or cell shortening. Subsequent to the elevation and contractile activation, the $[Ca^{2+}]_i$ is returned to diastolic levels mainly by activation of the SR Ca^{2+} pump (SERCA2a) and sarcolemmal (SL) Na^+/Ca^{2+} exchanger (NCX), and partly by the SL Ca^{2+} pump. Thus, the transient increase in $[Ca^{2+}]_i$ (Ca^{2+} transient), which occurs subsequent to membrane excitation (the fast rising phase of an action potential) preceding force development or cell shortening, plays a key role in cardiac excitation-contraction (E-C) coupling by determining the amount of Ca^{2+} ions to bind to troponin C (Fig. 1).

Measurement of Ca^{2+} transients simultaneously with alteration of contractile function in intact contracting myocardium and/or single cardiac myocytes is an excellent procedure for differentiating the site of action of inotropic interventions. The regulatory mechanisms of cardiac E-C coupling in respect to Ca^{2+} signaling are classified into three processes: an *upstream mechanism* that increases intracellular Ca^{2+} mobilization and thereby Ca^{2+} transients; a *central mechanism* that plays a key role in cardiac E-C coupling by triggering crossbridge cycling by Ca^{2+} binding to troponin C; and a *downstream mechanism* that involves the thin filament regulation of troponin C or crossbridge cycling and direct activation of the crossbridge (Fig. 1). It should be noted, however, that these mechanisms interact with each other to regulate myocardial contractility in intact myocardium. For example, activation of the upstream mechanism results in the activation of a central mechanism because the increase in force by increased number of crossbridges leads to an increase in Ca^{2+} binding affinity of troponin C, which constitutes a positive feedback mechanism. Therefore, the alteration of Ca^{2+} transients induced by a pure elevation of extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) can be used as the standard to compare with the effects of various inotropic interventions (1).

Cardiac myocytes are equipped with intrinsic regulatory mechanisms and regulated also by external regulatory mechanisms triggered via SL receptor activation induced by neurohumoral interventions. Intrinsic regulatory mechanisms are exerted by the property pertained by the myocardial cell itself, including the Frank-Starling mechanism and the force-frequency relationship, while the external regulatory mechanisms involve neurohumoral regulation induced by activation of SL receptors, for example, β - and α -adrenoceptors, angiotensin II (Ang II), endothelin (ET), cytokine receptors, and cardiotoxic agents such as Ca^{2+} mobilizers and Ca^{2+} sensitizers. A pronounced dissociation of contractile function from Ca^{2+} transients occurs by certain cardiotoxic agents such as α -adrenoceptor agonists, Ang II- and ET-receptor agonists, Ca^{2+} sensitizers, and under

pathophysiological conditions such as acidosis, stunned myocardium, and heart failure.

2. Experimental procedures

In 1978, Allen and Blinks succeeded in detecting intracellular Ca^{2+} transients by means of the Ca^{2+} sensitive bioluminescent protein aequorin in intact frog ventricular myocardium (2). Aequorin is contained in the vesicles of the tentacles of the jellyfish named *Aequorea folskålea* or *Aequorea aequorea*, which can be caught on the deck of the Friday Harbor laboratories, where once or twice a day, thousands of jellyfishes cluster. Aequorin is an apoprotein with a molecular size of about 20 kDa associated with the chromophore coelenterazine. An aequorin molecule has three Ca^{2+} binding sites, and upon binding of three Ca^{2+} , it emits light. Experimentally, the intensity of bioluminescence emitted by aequorin upon Ca^{2+} binding is proportional to the power of 2.5, and therefore, the relationship of the power of -2.5 of aequorin light intensity and Ca^{2+} concentration is linear over the range of the physiological $[Ca^{2+}]_i$ (10^{-7} – 10^{-5} mol/L) in cardiac and skeletal muscle (3). Since the rate of light emission is rapid enough to follow the alteration of $[Ca^{2+}]_i$, the intensity of aequorin light signals detected from the myocardium by means of a photomultiplier in the dark room can be used as an indicator of $[Ca^{2+}]_i$ in intact myocardium. A multicellular preparation of cardiac muscle loaded with aequorin by a microinjection or chemical loading procedure can be fixed in an organ bath in the dark room, and superfused with oxygenated Krebs-Henseleit solution at 37°C. Contractile force and aequorin light signals are recorded simultaneously. An aequorin-loaded multicellular preparation has advantages in that the aequorin light signals can be sensitively and stably detected in intact muscle preparations with minimal movement artifact and with intact physiological regulatory processes modulated through physiological pathways or activated by external inotropic interventions. The contractile response can be detected under ideal experimental conditions in which the muscle is stretched to a length close to the muscle length at which the contractile force is maximal (L_{max}) and the drug response is not altered by aequorin loading. On the other hand, disadvantages include hazardous procedures of aequorin loading and requirement of signal averaging. It should be noted that much larger numbers of cells contribute to the force development than the cells from which the aequorin signals are measured. Furthermore, the aequorin light signals do not directly reflect the Ca^{2+} concentration in the vicinity of troponin C but do reflect the alteration of cytosolic Ca^{2+} concentration,

Fig. 1. Role of Ca^{2+} ions in regulation of cardiac E-C coupling and classification of Ca^{2+} sensitizers. DHPR: voltage-dependent L-type Ca^{2+} channels, RyR: ryanodine receptors (sarcoplasmic reticulum (SR) Ca^{2+} release channels), NCX: Na⁺/Ca²⁺ exchanger, SERCA 2a: SR Ca^{2+} pump ATPase, CICR: Ca^{2+} -induced Ca^{2+} release mechanism, PLB: phospholamban, TnC: troponin C, TM: tropomyosin. Arrows from crossbridge to Ca^{2+} binding of TnC indicates the feedback regulation of Ca^{2+} binding affinity of TnC (central mechanism) by force generation (attachment) exerted by crossbridge cycling (downstream mechanism).

Fig. 2. Influence of acidosis on activities of regulatory proteins involved in cardiac E-C coupling in mammalian ventricular myocardium. Crosses attached to the proteins and function represent the suppression by acidosis, the size of which imply the strength of suppression. Abbreviations are the same as those in Fig. 1.

which is supposed to represent the former; however, contributions of unknown processes including hindrance to Ca^{2+} diffusion cannot be excluded. While in myocardial cells aequorin may be distributed freely and evenly throughout the cytosolic space, it is postulated in smooth muscle cells that aequorin distribution is limited to a space narrower than that where indo-1 is distributed (4).

Single cardiac myocytes loaded with Ca^{2+} sensitive fluorescent dyes, such as indo-1, fura-2, fluo-3, and so on, are currently used more and more because of the ease of loading procedures. Although these experimental methods provide elegant experimental preparations, the signals obtained from these preparations have to be analyzed with careful consideration. Fluorescent signals are unstable and the diastolic signal levels exceed the

peak of Ca^{2+} transients when the frequency of stimulation is raised, which occurs only rarely with aequorin-loaded preparations. Furthermore, single myocytes are contracting (shortening) from the slack length, which is far from an ideal condition for measurement of mechanical responses to inotropic interventions. To overcome these disadvantages, it is useful to carry out the study by simultaneously comparing the drug response of Ca^{2+} signals in single and multicellular preparations (5–8).

3. Influence of alteration of $[\text{Ca}^{2+}]_o$

The effect of an elevation of $[\text{Ca}^{2+}]_o$ is considered to be a standard of the regulatory mechanism because the relationship between Ca^{2+} transients and contractile function is altered by pure modulation of an upstream mechanism, although modulation by the feedback regulation of Ca^{2+} binding affinity of troponin C (central mechanism) induced by force development (downstream mechanism) is operative. If other inotropic interventions employed can shift the relationship of Ca^{2+} transients and contractile force to the left or right of the relationship induced by an elevation of $[\text{Ca}^{2+}]_o$ without altering the time course of Ca^{2+} transients, it is considered that these inotropic interventions are able to cause an increase or decrease in myofilament Ca^{2+} sensitivity, respectively. When $[\text{Ca}^{2+}]_o$ is elevated stepwise, the amplitude of Ca^{2+} transients and contractile force are increased in parallel and the relationship of both parameters is linear as demonstrated in the rabbit as well as dog ventricular myocardium (9, 10).

4. Intrinsic regulatory mechanisms

Two major intrinsic regulatory mechanisms that are essential for the immediate adjustment of cardiac contractile function to rapidly changing requirements of blood supply to the body are the Frank-Starling mechanism and the force-frequency relationship. The contrasting processes of cardiac Ca^{2+} signaling are responsible for the respective regulation: the former is primarily achieved by the downstream mechanism, whereas the latter is due to activation of the upstream mechanism in intact myocardial cells (11, 12).

4-1. Frank-Starling mechanism

The Frank-Starling mechanism reflects basic myocardial contractility represented by the length-tension relationship in isolated cardiac muscle and the ventricular function curve in experimental animals and patients. When the contractile force is markedly altered depending on the extent of stretch of the cardiac muscle,

the aequorin light signal at $L_{\max}$ is slightly abbreviated compared with that at slack length, probably because of the feedback mechanism, that is, an increase in Ca^{2+} binding affinity induced by the force development (1). However, the amplitude of Ca^{2+} transients is essentially identical during operation of the Frank-Starling mechanism. It is generally accepted that the ventricular function curve reflects cardiac pump function, which fluctuates widely with various pathophysiological situations from the super-normal in hyperthyroidism to an aggravated state depending on the extent of severity of contractile dysfunction. Two potential mechanisms have been proposed to be underlying the Frank-Starling mechanism. First, the optimum extent of thin and thick filaments overlapping for force development is believed to play an essential role (longitudinal hypothesis). The second hypothesis of an abbreviated distance between actin and the myosin head with stretch (lateral hypothesis) has also been proposed. In addition, aggravation of the Frank-Starling mechanism in muscle isolated from the heart failure model animal or patients may be ascribed to the dysfunction of the upstream mechanism because cardiotonic agents such as digitalis can reverse the deteriorated Frank-Starling mechanism.

4-2. Force-frequency relationship

When the rate of contraction in mammalian ventricular myocardium is increased over the physiological range, the contractile force is increased in most species, which has been recognized and documented as the force-frequency relationship. It is another important intrinsic regulatory mechanism of myocardial contractility (13). In contrast to the Frank-Starling mechanism, the positive force-frequency relationship in ventricular myocardium observed in most mammalian species is primarily due to a frequency-dependent increase in the amplitude of Ca^{2+} transients (14). The increase in Ca^{2+} transients is considered to be caused by 1) the prolonged duration of depolarization per unit time, resulting in longer activation in L-type Ca^{2+} influx, and 2) the increased accumulation of Na^+ ions by repetitive excitation per unit time that leads to the elevation of $[\text{Ca}^{2+}]_i$ by NCX (14).

The positive force-frequency relationship in ventricular myocardium disappears or is inverted to become negative in failing ventricular myocardium in the late stage of congestive heart failure. This phenomenon is ascribed to the dysfunction of Ca^{2+} handling, that is, disappearance or inversion of the positive Ca^{2+} transient-frequency relationship, which is directly reflected to that of the force-frequency relationship induced by hemodynamic as well as neurohumoral interventions during the course of progress of heart failure. Concerning the subcellular mechanisms of dysfunction of Ca^{2+} handling,

namely, the essential cause, there are controversial observations supporting the shortage of SR Ca^{2+} stores due to down-regulation of Ca^{2+} uptake via SERCA2a (15). On the other hand, some studies realize that the dysfunction of the ryanodine receptor in relation to hyper-phosphorylation of this and other regulatory proteins plays a crucial role in the inverted force-frequency relationship in heart failure (16). It has been shown that the agent that increases cAMP such as forskolin can reverse the inverted force-frequency relationship, which means that the upstream mechanism is crucial for the relationship (17).

5. External regulatory mechanisms

Among diverse physiological and pathophysiological regulatory processes of cardiac Ca^{2+} signaling, regulation mediated by sympathetic nerve stimulation (18, 19) and β -adrenoceptors plays the most crucial role as it is activated frequently in physiological conditions, prominently modulates the pathological process of congestive heart failure, and is down-regulated in severe heart failure. Most sympathomimetic amines act predominantly by activation of β -adrenoceptors through accumulation of cAMP, subsequent activation of protein kinase A (PKA), and partially through a cAMP-independent mechanism mediated by activation of cardiac α -adrenoceptors (20–26). Phosphodiesterase (PDE) inhibitors elicit the positive inotropic effect via a mechanism similar to that of β -adrenoceptor stimulation because they act by activation of the cAMP/PKA signaling pathway (27–43). A number of agents such as papaverine (27, 28), cilostamide and isobutylmethylxanthine (29), amrinone (30), milrinone (32), vesnarinone (31), OPC-8490 (36), ZSY-39 (33), Ro-1724 (34), olprinone (35), Y-20487 (37), and UK-1745 (38) belong to the same family, which induce a positive inotropic effect by themselves and are able to enhance the effect of β -adrenoceptor stimulation (except UK-1745). Some of them are very effective in the treatment of cardiac pump dysfunction in acute and decompensated heart failure (39–43).

The regulation of Ca^{2+} signaling induced by the activation of the receptor family that is coupled to the stimulation of phosphoinositide hydrolysis is characteristically different from that of β -adrenoceptor stimulation. Activation of α -adrenoceptors (44–64), as well as Ang II AT_1 (65–69) and ET (70–83) receptors, causes a characteristic modulation of Ca^{2+} signaling including facilitation of intracellular Ca^{2+} mobilization in association with an increase in myofilament Ca^{2+} sensitivity (9), along with the long-term regulation of cardiac remodeling. In dog ventricular myocardium, ET-1 alone elicited

only a small negative inotropic effect, but it modulated Ca^{2+} signaling and contractility in a complex manner by crosstalk with norepinephrine (84–90).

The activation of muscarinic cholinergic (91–97) and adenosine (98, 99) receptors induces an important inhibitory regulation of cardiac Ca^{2+} signaling.

Under pathological conditions, such as myocardial infarction and heart failure, diverse signaling processes triggered by activation of cytokine receptors come into play to regulate pathological features partly through modulation of cardiac Ca^{2+} signaling.

Cardiotonic agents are of extreme importance to reverse contractile dysfunction in acute and decompensated heart failure, acting through facilitation of Ca^{2+} mobilization and/or an increase in myofilament Ca^{2+} sensitivity (100–110). Some Ca^{2+} sensitizers are able to elicit a positive inotropic effect even under acidotic conditions and in the failing heart (111–116).

5-1. β -Adrenoceptor stimulation

β -Adrenoceptor stimulation elicits the most effective increase in contractile function in association with a marked facilitation of Ca^{2+} transients and an abbreviation of the Ca^{2+} transient that is reflected in a prominent abbreviation of the duration of contraction and an acceleration of relaxation (a positive lusitropic effect). While the β_1 -adrenoceptor, which is the predominant subtype in cardiac muscle, is coupled to activation of adenylyl cyclase through G_s proteins, the β_2 -adrenoceptor is coupled to both G_s and G_i proteins, and thus has regulatory profiles different from those of β_1 -adrenoceptor stimulation. Ca^{2+} signal regulation is achieved by cAMP/PKA signaling, namely serine/threonine phosphorylation of a series of cardiac Ca^{2+} regulatory proteins. Facilitation of Ca^{2+} transients involves phosphorylation of the following proteins: 1) L-type Ca^{2+} channels (an increase in availability of Ca^{2+} channels that results in an increased Ca^{2+} influx upon membrane depolarization), 2) phospholamban (an activation of SERCA2a due to disinhibition induced by dephosphorylated phospholamban to increase SR Ca^{2+} stores and acceleration of decline of Ca^{2+} transients), 3) ryanodine receptors (a facilitation of Ca^{2+} release from SR). Abbreviation of Ca^{2+} transients and acceleration of relaxation are ascribed to phosphorylation of 1) phospholamban and 2) troponin I (a decrease in myofilament Ca^{2+} sensitivity). In addition, it has been shown that other functional proteins such as myosin binding protein C and NCX are phosphorylated to potentially contribute to the regulation of Ca^{2+} handling (11, 12).

Most sympathomimetic amines such as phenylephrine (20, 23, 24), dopamine (21, 46), norepinephrine, epinephrine (46), isoproterenol (22), dobutamine (25),

and denopamine (26) increase Ca^{2+} transients in a concentration-dependent manner mainly via activation of β_1 -adrenoceptors and subsequent accumulation of cAMP. When the effects of norepinephrine, epinephrine, and isoproterenol are compared with those of elevation of $[\text{Ca}^{2+}]_o$, catecholamines can increase the amplitude of Ca^{2+} transients to a level much higher than the elevation of $[\text{Ca}^{2+}]_o$. The relationship between the amplitude of Ca^{2+} transients and contractile force is shifted by catecholamines to the right of that with elevation of $[\text{Ca}^{2+}]_o$ (9). This may be partly due to a decrease in myofilament Ca^{2+} sensitivity in relation to troponin I phosphorylation and abbreviation of Ca^{2+} transients due to activation of SERCA2a. Abbreviation of twitch contraction is much more pronounced than that of Ca^{2+} transients, which implies a significant contribution of the decrease in Ca^{2+} sensitivity induced by catecholamines in relation to troponin I phosphorylation (9).

5-2. α -Adrenoceptor stimulation

α -Adrenoceptors have long been supposed to play an insignificant role in cardiac Ca^{2+} signal and functional regulation. However, during the past three decades, α -adrenoceptors have been recognized to play an important role in cardiac regulation including contractile function and remodeling through the cAMP/PKA-independent process. α -Adrenoceptor stimulation has been shown to modulate cardiac Ca^{2+} signaling by stimulation of phosphoinositide hydrolysis, resulting in the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol that activates protein kinase C (PKC) (54, 55, 60).

α -Adrenoceptor-mediated regulation of Ca^{2+} signaling and cardiac contractility shows characteristics completely different from those mediated by β -adrenoceptors. α -Adrenoceptor-mediated positive inotropic effects show a wide range of species-dependent variation (54, 55, 60). They are pronounced in the rabbit (45, 50); moderate in rat, guinea pig, and monkey (49); least in the ferret (52); and absent in dog ventricular myocardium (47, 48, 63). In single mouse ventricular myocytes, α -adrenoceptor stimulation decreases Ca^{2+} transients and cell shortening (64).

The α -adrenoceptor-mediated effect is more susceptible to the inhibitory action of Ca^{2+} antagonists (53) and is suppressed more easily by elevation of stimulation frequency and experimental temperature than the β -adrenoceptor-mediated effect (44, 55, 60). Concerning the α -adrenoceptor subtype involved in regulation of Ca^{2+} signaling and contractile activity in the rabbit ventricular myocardium, the α_{1B} -subtype is predominant (approximately 80%), which is susceptible to chlorethyl-clonidine (56, 59). Other subtypes, including the α_{1A} -

subtype, which is inhibitable with WB 4101, 5-methylurapidil (57), (+)-niguldipine (58), and the α_{1D} -subtype, which is susceptible to BMY 7378 (62), contribute partially to the contractile regulation in the rabbit. Activation of PKC induced by endogenously generated diacylglycerol may play a crucial role in the α -adrenoceptor-mediated positive inotropic effect. However, externally administered phorbol esters that have an effect to activate PKC in vitro do not mimic, but rather suppress the α -mediated positive inotropic effect by inhibiting the receptor-mediated stimulation of phosphoinositide hydrolysis (51). Furthermore, the PKC inhibitors suppress only partially the α -mediated effect (60, 61).

In the rabbit papillary muscle, phenylephrine in the presence of a β -blocker elicits a positive inotropic effect in association with no change in cAMP levels (22, 23). However, there is an increase in Ca^{2+} transients, but much less than that induced by isoproterenol (9). In contrast to the effect of β -adrenoceptor stimulation, the positive inotropic effect of α -stimulation is accompanied by an increase in Ca^{2+} transients much lower than that induced by elevation of $[\text{Ca}^{2+}]_o$ (9). The relationship of Ca^{2+} transients and contractile force is shifted to the left, indicating that α -adrenoceptor stimulation increases myofilament Ca^{2+} sensitivity in addition to a moderate increase in Ca^{2+} transients (9). α -Adrenoceptor stimulation elicits a reciprocal effect on the duration of Ca^{2+} transients and twitch contraction (9). This indicates that an increased Ca^{2+} binding affinity to troponin C may lead to a prolongation of the duration of contraction, simultaneously decreasing the amount of Ca^{2+} ions available for aequorin binding (9). The maximum contractile response to α -adrenoceptor stimulation is approximately 60% of the maximal response to isoproterenol (ISO_{max}), whereas that of Ca^{2+} transients is only 10% of ISO_{max} , which indicates an increase in Ca^{2+} sensitivity induced by α -adrenoceptor stimulation (9). The dopamine-induced positive inotropic effect is ascribed to activation of both β - and α -adrenoceptors (9, 55, 60).

5-3. Ang II

Ang II exerts a positive inotropic effect through activation of AT_1 receptors and subsequent acceleration of phosphoinositide hydrolysis (65, 66). Activation of PKC by phorbol 12,13-dibutyrate inhibits the Ang II-induced stimulation of phosphoinositide hydrolysis and thereby the positive inotropic effect of Ang II in rabbit ventricular myocardium (65). The positive inotropic effect of Ang II shows a wide range of species-dependent variation, being pronounced in the rabbit ventricular myocardium (66) and is associated with

activation of L-type Ca^{2+} channels (67, 69), the Na^+/H^+ exchanger (67, 68), and NCX (68). The positive inotropic effect of Ang II is similar to the α -adrenoceptor-mediated effect, and it is associated with a moderate increase in Ca^{2+} transients and an increase in myofilament Ca^{2+} sensitivity in rabbit papillary muscle (7) and single ventricular myocytes (68).

5-4. ET

ET isopeptides induce a pronounced positive inotropic effect associated with stimulation of phosphoinositide hydrolysis (71) via predominant activation of ET_A receptors (73) in mammalian ventricular myocardium of most species (70, 74, 76). The positive inotropic effect of ET isopeptides shows a wide range of species-dependent variation. The most pronounced positive inotropic effect is observed in rabbit ventricular myocardium among the species examined; and ET-1, ET-2, and ET-3 exert the effect with an identical efficacy and potency (70). While the positive inotropic effect of ET-3 (73), and ET-1 at lower concentrations ($<10^{-8}$ M) (72), is susceptible to ET_A -receptor antagonists, the ET_A -receptor antagonists are unable to shift the main portion of the concentration-response curve for ET-1 in rabbit papillary muscle (72, 76, 81). The potent ET_A -receptor antagonist TAK-044 can inhibit the positive inotropic effect of ET-1 in the rabbit, but a concentration one hundred times higher than that which inhibits the ET-3-induced response is required (83). ET-1, but not ET-3, shows much higher potency for induction of the positive inotropic effect in rabbit single ventricular myocytes than papillary muscles, the reason for which is currently unknown (81). The positive inotropic effect of ET-1 is associated with a moderate increase in L-type Ca^{2+} current (78) and an increase in myofilament Ca^{2+} sensitivity (77, 81), the latter being susceptible to the inhibitors of the Na^+/H^+ exchanger (80), PKC and tyrosine kinase (82).

5-5. Crosstalk of ET with norepinephrine

In the dog ventricular myocardium, ET-1 alone induces only a small transient negative inotropic effect (8). However, ET-1 exerts a complex contractile regulation by crosstalk with β -adrenoceptor stimulation induced by sympathomimetic amines such as norepinephrine and isoproterenol (8). The crosstalk involves diverse signaling pathways (8). In the presence of weak β -adrenoceptor stimulation induced by low [10^{-10} M (sub-threshold) – 10^{-9} M (threshold)] concentrations of norepinephrine, ET-1 elicits a long-lasting positive inotropic effect in a concentration-dependent manner in association with a moderate increase in Ca^{2+} transients and an increase in myofilament Ca^{2+}

sensitivity (8, 84). α -Adrenoceptor stimulation and Ang II likewise elicit a positive inotropic effect in the presence of low concentrations of norepinephrine in canine ventricular myocardium, but the extent of the effect of Ang II is less than ET-1 (86). The positive inotropic effect of ET-1 requires activation of both PKC and PKA (8), and it is inhibited by genistein (a tyrosine kinase inhibitor) (87), wortmannin (an MLCK inhibitor at μM concentration) (88), and Y-27632 (a Rho kinase inhibitor) (90).

In the presence of high concentrations (approximately 10^{-6} M) of norepinephrine, ET-1 elicits a pronounced and long-lasting negative inotropic effect that is associated with a decrease in L-type Ca^{2+} current (79) and Ca^{2+} transients (8), but with no change in cAMP levels (75) and Ca^{2+} sensitivity (8). The negative inotropic effect of ET-1 is suppressed by treatment with pertussis toxin, a guanylyl cyclase inhibitor (LY83583), a PKG inhibitor (KT5823), and a phosphatase inhibitor (cantharidin) (85).

The long-lasting positive and transient negative inotropic effect of ET-1 in dog ventricular myocardium is mediated by ET_A -receptor activation, while the long-lasting negative inotropic effect involves both predominant ET_A - and partial ET_B -receptor activation (89).

5-6. Muscarinic and adenosine receptor stimulation

Acetylcholine (ACh) acts on muscarinic and nicotinic receptors, resulting in a negative and positive inotropic effect in mammalian ventricular myocardium (91). Muscarinic receptor stimulation induces activation of dual pathways through activation of G_i proteins that are susceptible to pertussis toxin (96). The activation of potassium channels ($I_{K\text{ACh}}$) leads to an abbreviation of action potential duration and subsequent decrease in the amplitude of Ca^{2+} transients, which results in a pronounced negative inotropic effect in atrial muscle but not in mammalian ventricular muscle (8, 97). This direct negative inotropic effect is associated with an increase in cGMP levels, mimicked by an increase in cGMP and cGMP analogues (93, 95). In ferret ventricular myocardium, this inhibitory regulation is exceptionally pronounced among mammalian species and leads to a marked decrease in Ca^{2+} transients and a negative inotropic effect even in the absence of β -adrenoceptor stimulation (97). For a given decrease in Ca^{2+} transients, the attenuation of contractile force induced by carbachol is less than that caused by lowering $[\text{Ca}^{2+}]_o$. This is an indication that muscarinic receptor stimulation decreases Ca^{2+} transients, but may simultaneously increase myofilament Ca^{2+} sensitivity (97).

In the presence of β -adrenoceptor stimulation,

muscarinic receptor stimulation elicits a pronounced negative inotropic effect in ventricular myocardium, which has been well documented as an “anti-adrenergic effect” or “accentuated antagonism” in mammalian ventricular myocardium (97). This indirect inhibitory effect of muscarinic receptor stimulation is exerted selectively on β -, but not α -adrenoceptor-mediated positive inotropic effect (94), and is associated with a decrease in cAMP levels in dog ventricular myocardium (92) and a parallel decrease in Ca^{2+} transients (8, 97). The subcellular mechanism is ascribed either to a decrease in cAMP levels through coupling to G_i proteins (96) and/or to activation of phosphatase, which dephosphorylates the functional proteins that have been phosphorylated previously by activation of PKA (8).

Adenosine elicits a differential inhibitory action on the β -mediated effect without affecting the α -adrenoceptor-mediated positive inotropic effect (98, 99), which is mediated by activation of pertussis toxin sensitive G_i proteins (96).

5-7. Cardiotonic agents

Cardiotonic agents, such as catecholamines (e.g., norepinephrine, dobutamine, dopamine, and denopamine) and PDE III inhibitors (e.g., milrinone, amrinone, olprinone, vesnarinone, enoximone, and piroximone), are currently used for the treatment of cardiac contractile dysfunction in patients with acute and decompensated heart failure. Cardiotonic agents increase contractile function by acting at three different steps of cardiac E-C coupling, that is, the upstream, central, and downstream mechanisms (1). Most of the cardiotonic agents currently available for clinical use induce a positive inotropic effect via the upstream mechanism by facilitating mobilization of $[\text{Ca}^{2+}]_i$ through activation of different subcellular processes leading to an increase in Ca^{2+} transients in intact myocardial cells (Ca^{2+} mobilizers). Other classes of agents (Ca^{2+} sensitizers) are capable of inducing a positive inotropic effect by increasing the binding affinity of troponin C for Ca^{2+} (central) and/or facilitating the thin filament regulation of contractile proteins (troponin C or crossbridge) or direct activation of crossbridge cycling itself (downstream mechanism) (Fig. 1).

5-7-1. Ca^{2+} mobilizers

Ca^{2+} mobilizers increase the amplitude of Ca^{2+} transients and contractility in parallel similar to the effect induced by an elevation in $[\text{Ca}^{2+}]_o$. The relationship of increases in Ca^{2+} transients and contractile force induced by Ca^{2+} mobilizers and elevation of $[\text{Ca}^{2+}]_o$ are superimposable if Ca^{2+} mobilizers exert no additional action other than increasing Ca^{2+} transients. In the case

of β -adrenoceptor agonists and PDE III inhibitors, acceleration of a decline in Ca^{2+} transients due to an increased rate of Ca^{2+} uptake into the SR by SERCA2a activation and a decrease in Ca^{2+} sensitivity of troponin C due to troponin I phosphorylation produce a shift of the relationship to the right. This shift to the right indicates an apparent decrease in Ca^{2+} sensitivity (9). Ca^{2+} mobilizers generally suffer from the potential risk of Ca^{2+} overload leading to cardiac arrhythmias, cell injury, and ultimate cell death (110).

5-7-2. Ca^{2+} sensitizers

Current research interests have been focused more on Ca^{2+} sensitizers, which could overcome the disadvantages associated with Ca^{2+} mobilizers in the therapy of heart failure patients as follows: 1) Ca^{2+} sensitizers do not increase the activation energy required for $[\text{Ca}^{2+}]_i$ handling; 2) they are free from potential risks of arrhythmias, cell injury, apoptosis, and cell death due to Ca^{2+} overload; and 3) they have the potential to reverse contractile dysfunction under pathological conditions, such as acidosis, myocardial stunning, and congestive heart failure (104, 110). Ca^{2+} sensitizers can be classified into three classes: class I acting on the central mechanism; class II acting on the thin filament regulation of troponin C and/or crossbridge cycling; and class III directly acting on the crossbridge cycling, depending on the sites and/or mechanisms of action as shown in Fig. 1. Differentiation of class I and class II agents require careful analysis because certain agents and inotropic interventions (e.g., EMD 57033 and acidosis) affect the Ca^{2+} binding affinity of troponin C (central mechanism) by allosteric regulation via troponin I (Fig. 1).

It is noteworthy that the currently available cardiotonic agents, such as pimobendan and levosimendan, which act to increase myofilament Ca^{2+} sensitivity, also possess PDE III inhibitory action (39 – 43).

In mammalian ventricular myocardium, the muscarinic receptor agonist carbachol is able to abolish the positive inotropic effect of the pure PDE inhibitors including amrinone, milrinone, and olprinone (32). However, the positive inotropic effects of certain cardiotonic agents such as sulmazole (100), pimobendan (101), pimobendan's active metabolite UD-CG 212 Cl (109), Org 30029, Org 9731 (102, 103), SCH 00013 (105), and EMD 57033 are partially resistant to the inhibitory action of carbachol. These agents exert a positive inotropic effect without an increase in Ca^{2+} transients acting via central and/or downstream mechanisms in the presence of carbachol (32, 104, 110).

More recently, it has been found that carbachol abolishes the positive inotropic effects of levosimendan

(5, 116) and its active metabolite OR-1896 (107), as well as the effect elicited by crosstalk of ET-1 and norepinephrine (8). Since the positive inotropic effects of these cardiotoxic interventions are associated with a shift of the relationship between $[\text{Ca}^{2+}]_i$ and contractile activity (force or cell shortening), it is postulated that the Ca^{2+} sensitization induced by these agents involves cAMP/PKA-mediated signal transduction. The regulatory proteins that are phosphorylated by PKA, which lead to the Ca^{2+} sensitizing mechanism remain to be identified.

The positive inotropic effect of Ca^{2+} mobilizers is suppressed under pathophysiological conditions, including acidosis, stunned myocardium, and heart failure. Certain Ca^{2+} sensitizers are able to induce an effective positive inotropic effect even under such conditions.

Various processes of cardiac E-C coupling are affected under pathophysiological conditions. For example, acidosis is a major component of cardiac ischemia, which suppresses cardiac contractile function by affecting diverse processes of cardiac E-C coupling, including both Ca^{2+} mobilization and Ca^{2+} sensitivity as shown with crosses in Fig. 2 (the sizes of crosses imply the extent of influence on individual processes). The conformational changes exerted by Ca^{2+} through interaction of troponin C with troponin I play a key role in the acidosis-induced modulation of contractile proteins. The C-terminal domain of troponin I, downstream of the inhibitory peptide, is critical for the inhibitory effect of acidosis on Ca^{2+} activation in cardiac myofilaments (113). When the extracellular pH is lowered from 7.4 to 6.6, a marked dissociation of contractile function from Ca^{2+} transients, that is, an increase in Ca^{2+} transients in association with a decrease in contractile force is induced (Fig. 3). This indicates that the decrease in Ca^{2+} binding affinity of troponin C plays a predominant role in the influence of acidosis in aequorin-loaded dog

ventricular myocardium (111). Org-30029 elicits a pronounced positive inotropic effect by an increase in myofilament Ca^{2+} sensitivity even under acidotic conditions, in which the positive inotropic effect induced by the elevation of $[\text{Ca}^{2+}]_o$ is markedly suppressed (111). In contrast, the increase in Ca^{2+} sensitivity induced by pimobendan and its active metabolite DD-CG 212 Cl is suppressed under acidotic conditions (112). While the increase in Ca^{2+} sensitivity induced by levosimendan and its active metabolite OR-1896 is essentially unaltered by acidosis, the positive inotropic effect of these compounds is decreased due to an attenuation of the increase in Ca^{2+} transients induced by these agents (108, 114, 115). This indicates that the increase in Ca^{2+} transients induced by levosimendan and OR-1896 is more susceptible to acidosis than the increase in myofilament Ca^{2+} sensitivity produced by these compounds. Hence, the effectiveness of levosimendan as a cardiotoxic agent is markedly decreased by acidosis (115).

In failing heart, the activation of contractile proteins by intrinsic regulation, including the Frank-Starling mechanism and the force-frequency relationship, as well as external regulation induced by β -adrenoceptor stimulation, deteriorates during the course of congestive heart failure. EMD 57033 is able to increase the contractility by an increase in Ca^{2+} sensitivity even when the β -adrenoceptor-mediated positive inotropic effect is markedly decreased in ventricular myocytes isolated from the volume-overload rabbit heart (116).

6. Conclusion

In intact cardiac muscle, the contractile activity is widely altered by the intrinsic property of cardiac myocytes, such as the Frank-Starling mechanism and the force-frequency relationship. In addition, neurohumoral factors, such as β - and α -adrenoceptor stimulation, ET, Ang, and cytokines, are involved in contractile regulation under various physiological and pathophysiological conditions. Pathophysiological conditions, including acidosis, stunned myocardium, and heart failure, markedly abrogate the intrinsic and neurohumoral regulation. Application of the experimental procedures to simultaneously detect Ca^{2+} transients and contractile activity by means of the Ca^{2+} sensitive bioluminescent protein aequorin and fluorescent dyes, such as indo-1, provides powerful tools for differentiating the mode of regulation induced by intrinsic and external inotropic interventions in intact cardiac muscle. While the Frank-Starling mechanism is associated with only small changes in Ca^{2+} transients, the force-frequency relationship is primarily due to an alteration of Ca^{2+} transients. The regulatory activities induced by β - and α -adreno-

Fig. 3. Influence of acidosis on Ca^{2+} transients and force of isometric contractions in an aequorin-loaded dog ventricular trabecula (stimulation frequency, 1 Hz; temperature, 37°C). Reproduced from ref. 111 with permission of the American Physiological Society ©1996.

ceptor stimulation are very different between the two mechanisms: the former decreases, but the latter increases Ca^{2+} sensitivity. α -Adrenoceptor-mediated regulation is mimicked by endothelin ET_A - and angiotensin II AT_1 -receptor stimulation. However, the receptor-mediated regulation of Ca^{2+} signaling induced by stimulation of phosphoinositide hydrolysis shows a wide range of species-dependent variation. In addition, α -adrenoceptor stimulation, and ET and Ang II induce a negative inotropic effect in association with a decrease in Ca^{2+} transients and Ca^{2+} sensitivity in single mouse ventricular myocytes (64). These agents elicit a positive inotropic effect on Langendorff perfused mouse heart, the mechanism underlying which remains unclear at the moment. Acidosis markedly suppresses the regulative activities induced by Ca^{2+} mobilizers, but certain Ca^{2+} sensitizers are able to induce an inotropic effect even under such pathophysiological conditions. The experimental procedures to detect Ca^{2+} transients simultaneously with contractile activity in intact cardiac muscle and myocytes provide powerful tools for insight into the mode of regulation induced by diverse inotropic interventions under physiological and pathophysiological conditions with respect to Ca^{2+} signaling processes.

Acknowledgment

The author is grateful for the technical assistance of Mr. Ikuro Norota, who prepared the figure.

References

- 1 Blinks JR, Endoh M. Modification of myofibrillar responsiveness to Ca^{++} as an inotropic mechanism. *Circulation*. 1986;73: III85–III98.
- 2 Allen DG, Blinks JR. Calcium transients in aequorin-injected frog cardiac muscle. *Nature*. 1978;273:509–513.
- 3 Blinks JR, Wier WG, Hess P, Prendergast FG. Measurement of Ca^{2+} concentrations in living cells. *Prog Biophys Mol Biol*. 1982;40:1–114.
- 4 Abe F, Mitsui M, Karaki H, Endoh M. Calcium compartments in vascular smooth muscle as detected by aequorin signal. *Br J Pharmacol*. 1995;116:3000–3004.
- 5 Sato S, Talukder MAH, Sugawara H, Sawada H, Endoh M. Effects of levosimendan on myocardial contractility and Ca^{2+} transients in aequorin-loaded right-ventricular papillary muscles and indo-1-loaded single ventricular cardiomyocytes of the rabbit. *J Mol Cell Cardiol*. 1998;30:1115–1128.
- 6 Fujita S, Endoh M. Effects of endothelin-1 on $[\text{Ca}^{2+}]_i$ -shortening trajectory and Ca^{2+} sensitivity in rabbit single ventricular cardiomyocytes loaded with indo-1/AM: comparison with the effects of phenylephrine and angiotensin II. *J Card Fail*. 1996;2: S45–S57.
- 7 Watanabe A, Endoh M. Relationship between the increase in Ca^{2+} transient and contractile force induced by angiotensin II in aequorin-loaded rabbit ventricular myocardium. *Cardiovasc Res*. 1998;37:524–531.
- 8 Chu L, Takahashi R, Norota I, Miyamoto T, Takeishi Y, Ishii K, et al. Signal transduction and Ca^{2+} signaling in contractile regulation induced by crosstalk between endothelin-1 and norepinephrine in dog ventricular myocardium. *Circ Res*. 2003;92:1024–1032.
- 9 Endoh M, Blinks JR. Actions of sympathomimetic amines on the Ca^{2+} transients and contractions of rabbit myocardium: reciprocal changes in myofibrillar responsiveness to Ca^{2+} mediated through α - and β -adrenoceptors. *Circ Res*. 1988;62:247–265.
- 10 Endoh M. The effects of theophylline on aequorin light transients and force in the isolated dog right ventricular myocardium. *J Mol Cell Cardiol*. 1994;26:87–97.
- 11 Endoh M. The effects of various drugs on the myocardial inotropic response. *Gen Pharmacol*. 1995;26:1–31.
- 12 Endoh M. Changes in intracellular Ca^{2+} mobilization and Ca^{2+} sensitization as mechanisms of action of physiological interventions and inotropic agents in intact myocardial cells. *Jpn Heart J*. 1998;39:1–44.
- 13 Endoh M, Hashimoto K. Frequency-force relationship in the blood-perfused canine papillary muscle preparation. *Jpn J Physiol*. 1970;20:320–331.
- 14 Endoh M. Frequency-dependent inhibition of the intracellular calcium transients by calmodulin antagonists in the aequorin-injected rabbit papillary muscle. *Adv Exp Med Biol*. 1989; 255:461–470.
- 15 Houser SR, Margulies KB. Is depressed myocyte contractility centrally involved in heart failure? *Circ Res*. 2003;92:350–358.
- 16 Yano M, Okuda S, Oda T, Tokuhisa T, Tateishi H, Mochizuki M, et al. Correction of defective interdomain interaction within ryanodine receptor by antioxidant is a new therapeutic strategy against heart failure. *Circulation*. 2005;112:3633–3643.
- 17 Endoh M. Force-frequency relationship in intact mammalian ventricular myocardium: physiological and pathophysiological relevance. *Eur J Pharmacol*. 2004;500:73–86.
- 18 Endoh M, Hashimoto K. Pharmacological evidence of autonomic nerve activities in canine papillary muscle. *Am J Physiol*. 1970;218:1459–1463.
- 19 Endoh M, Hashimoto K, Kimura T. Effects of perivascular nerve stimulation on the contraction and automaticity of the blood-perfused canine papillary muscle. *Br J Pharmacol*. 1972;45:603–615.
- 20 Schümann HJ, Endoh M, Wagner J. Positive inotropic effects of phenylephrine in the isolated rabbit papillary muscle mediated both by α - and β -adrenoceptors. *Naunyn Schmiedeberg Arch Pharmacol*. 1974;284:133–148.
- 21 Endoh M. Effects of dopamine on sinus rate and ventricular contractile force of the dog heart in vitro and in vivo. *Br J Pharmacol*. 1975;55:475–486.
- 22 Schümann HJ, Endoh M, Brodde O-E. The time course of the β - and α -adrenoceptor stimulation by isoprenaline and methoxamine on the contractile force and cAMP level of the isolated rabbit papillary muscle. *Naunyn Schmiedeberg Arch Pharmacol*. 1975;289:291–302.
- 23 Endoh M, Brodde O-E, Schümann HJ. Relationship between the level of cAMP and the contractile force under stimulation of α - and β -adrenoceptors by phenylephrine in the isolated rabbit papillary muscle. *Naunyn Schmiedeberg Arch Pharmacol*. 1976;295:109–115.
- 24 Endoh M. Inhibition of the positive inotropic effect of dopamine

- by YM-09538 with α - and β -adrenoceptor antagonistic action in the rabbit papillary muscle. *Jpn J Pharmacol.* 1983; 33:897–899.
- 25 Ishihata A, Kushida H, Endoh M. Enantiomers of dobutamine increase the force of contraction via β adrenoceptors, but antagonize competitively the positive inotropic effect mediated by α -1 adrenoceptors in the rabbit ventricular myocardium. *J Pharmacol Exp Ther.* 1988;246:1080–1087.
 - 26 Kohi M, Norota I, Takanashi M, Endoh M. On the mechanism of action of the β -1 partial agonist denopamine in regulation of myocardial contractility: effects on myocardial α adrenoceptors and intracellular Ca⁺⁺ transients. *J Pharmacol Exp Ther.* 1993;265:1292–1300.
 - 27 Endoh M, Brodde O-E, Schümann HJ. Accumulation of cAMP and positive inotropic effect evoked by isoproterenol under the graded inhibition of phosphodiesterase by papaverine in the isolated rabbit papillary muscle. *J Mol Cell Cardiol.* 1975;7: 703–711.
 - 28 Endoh M, Schümann HJ. Effects of papaverine on isolated rabbit papillary muscle. *Eur J Pharmacol.* 1975;30:213–220.
 - 29 Endoh M, Satoh K, Yamashita S. Inhibition of cyclic AMP phosphodiesterase activity and myocardial contractility: effects of cilostamide, a novel PDE inhibitor, and methylisobutylxanthine on rabbit and canine ventricular muscle. *Eur J Pharmacol.* 1980;66:43–52.
 - 30 Endoh M, Yamashita S, Taira N. Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. *J Pharmacol Exp Ther.* 1982;221: 775–783.
 - 31 Yanagisawa T, Endoh M, Taira N. Involvement of cyclic AMP in the positive inotropic effect of OPC-8212, a new cardiotonic agent, on the canine ventricular muscle. *Jpn J Pharmacol.* 1984;36:379–388.
 - 32 Endoh M, Yanagisawa T, Taira N, Blinks JR. Effects of new inotropic agents on cyclic nucleotide metabolism and calcium transients in canine ventricular muscle. *Circulation.* 1986;73: III117–III133.
 - 33 Endoh M, Satoh H, Norota I, Hirano K. Effects of a new 1,3-thiazole derivative ZSY-39 on force of contraction and cyclic AMP content in canine ventricular muscle. *Cardiovasc Drugs Ther.* 1990;4:1127–1134.
 - 34 Katano Y, Endoh M. Differential effects of Ro 20-1724 and isobutylmethylxanthine on the basal force of contraction and β -adrenoceptor-mediated response in the rat ventricular myocardium. *Biochem. Biophys Res Commun.* 1990;167:123–129.
 - 35 Satoh H, Endoh M. Effects of a new cardiotonic agent 1,2-dihydro-6-methyl-2-oxo-5-[imidazo(1,2-a)pyridin-6-yl]-3-pyridine carbonitrile hydrochloride monohydrate (E-1020) on contractile force and cyclic AMP metabolism in canine ventricular muscle. *Jpn J Pharmacol.* 1990;52:215–224.
 - 36 Endoh M, Satoh H, Norota I, Hirano K, Hosokawa T. Subcellular mechanism of the positive inotropic effect of a new quinolinone derivative OPC-8490 on the dog ventricular myocardium. *Heart Vessels.* 1991;6:158–167.
 - 37 Katano Y, Endoh M. Effects of a cardiotonic quinolinone derivative Y-20487 on the isoproterenol-induced positive inotropic action and cyclic AMP accumulation in rat ventricular myocardium: comparison with rolipram, Ro 20-1724, milrinone, and isobutylmethylxanthine. *J Cardiovasc Pharmacol.* 1992;20: 715–722.
 - 38 Sawada H, Endoh M. Pharmacological characterization of effects of UK-1745, a novel cardiotonic agent with β -adrenoceptor-blocking action, in aequorin-loaded canine right ventricular muscle. *J Mol Cell Cardiol.* 1999;31:1047–1062.
 - 39 Endoh M. Physiological and pathophysiological modulation of calcium signaling in myocardial cells. *Jpn Circ J.* 1991;55:1108–1117.
 - 40 Endoh M, Hori M. Basic pharmacology and clinical application of new positive inotropic agents. *Drugs of Today.* 1993;29:29–56.
 - 41 Endoh M. Mechanism of action of Ca²⁺ sensitizers – update 2001. *Cardiovasc Drugs Ther.* 2001;15:397–403.
 - 42 Endoh M. Mechanisms of action of novel cardiotonic agents. *J Cardiovasc Pharmacol.* 2002;40:323–338.
 - 43 Endoh M. The therapeutic potential of novel cardiotonic agents. *Expert Opin Invest Drugs.* 2003;12:735–750.
 - 44 Endoh M, Schümann HJ. Frequency-dependence of the positive inotropic effect of methoxamine and naphazoline mediated by α -adrenoceptors in the isolated rabbit papillary muscle. *Naunyn Schmiedebergs Arch Pharmacol.* 1975;287:377–389.
 - 45 Endoh M, Schümann HJ, Krappitz N, Hillen B. α -Adrenoceptors mediating positive inotropic effects on the ventricular myocardium: some aspects of structure-activity relationship of sympathomimetic amines. *Jpn J Pharmacol.* 1976;26:179–190.
 - 46 Schümann HJ, Motomura S, Endoh M, Brodde O-E. Comparison of the mechanisms underlying the positive inotropic actions of dopamine, adrenaline and isoprenaline on the isolated rabbit papillary muscle. *Naunyn Schmiedebergs Arch Pharmacol.* 1977;297:257–267.
 - 47 Endoh M, Shimizu T, Yanagisawa T. Characterization of adrenoceptors mediating positive inotropic responses in the ventricular myocardium of the dog. *Br J Pharmacol.* 1978; 64:53–61.
 - 48 Yamashita S, Endoh M. Adrenoceptors mediating the positive inotropic effect in the canine atrial myocardium. *Jpn J Pharmacol.* 1981;31:649–651.
 - 49 Endoh M, Satoh K, Taira N. α -Adrenoceptors mediating the positive inotropic effect of phenylephrine in the right ventricular muscle of the monkey (*Macaca fuscata*). *Naunyn Schmiedebergs Arch Pharmacol.* 1982;318:370–373.
 - 50 Hiramoto T, Kushida H, Endoh M. Further characterization of the myocardial α -adrenoceptors mediating positive inotropic effects in the rabbit myocardium. *Eur J Pharmacol.* 1988;152: 301–310.
 - 51 Kushida H, Hiramoto T, Satoh H, Endoh M. Phorbol ester does not mimic, but antagonizes the α -adrenoceptor-mediated positive inotropic effect in the rabbit papillary muscle. *Naunyn Schmiedebergs Arch Pharmacol.* 1988;337:169–176.
 - 52 Endoh M, Hiramoto T, Kushida H. Preponderance of β - over α -adrenoceptors in mediating the positive inotropic effect of phenylephrine in the ferret ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol.* 1989;339:362–366.
 - 53 Kushida H, Hiramoto T, Endoh M. The preferential inhibition of α 1- over β -adrenoceptor-mediated positive inotropic effect by organic calcium antagonists in the rabbit papillary muscle. *Naunyn Schmiedebergs Arch Pharmacol.* 1990;341: 206–214.
 - 54 Endoh M, Hiramoto T, Ishihata A, Takanashi M, Inui J. Myocardial α 1-adrenoceptors mediate positive inotropic effect and changes in phosphatidylinositol metabolism. Species differences in receptor distribution and the intracellular coupling process in mammalian ventricular myocardium. *Circ Res.*

- 1991;68:1179–1190.
- 55 Endoh M. Signal transduction of myocardial α_1 -adrenoceptors: regulation of ion channels, intracellular calcium, and force of contraction – a review. *J Appl Cardiol.* 1991;6:379–399.
 - 56 Takanashi M, Norota I, Endoh M. Potent inhibitory action of chlorethylclonidine on the positive inotropic effect and phosphoinositide hydrolysis mediated via myocardial α_1 -adrenoceptors in the rabbit ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol.* 1991;343:669–673.
 - 57 Endoh M, Takanashi M, Norota I. Role of α_1 adrenoceptor subtype in production of the positive inotropic effect mediated via myocardial α_1 adrenoceptors in the rabbit papillary muscle: influence of selective α_1 subtype antagonists WB 4101 and 5-methylurapidil. *Naunyn Schmiedebergs Arch Pharmacol.* 1992;345:578–585.
 - 58 Endoh M, Takanashi M, Norota I. Effect of (+)-niguldipine on myocardial α_1 -adrenoceptors in the rabbit. *Eur J Pharmacol.* 1992;223:143–151.
 - 59 Yang H-T, Endoh M. Dissociation of the positive inotropic effect of methoxamine from the hydrolysis of phosphoinositide in rabbit ventricular myocardium: a comparison with the effects of phenylephrine and the subtype of the α_1 -adrenoceptor involved. *J Pharmacol Exp Ther.* 1994;269:732–742.
 - 60 Endoh M. Cardiac α -adrenoceptors that regulate contractile function: subtypes and subcellular transduction mechanisms. *Neurochem Res.* 1996;21:217–229.
 - 61 Talukder MAH, Endoh M. Differential effects of protein kinase C activators and inhibitors on α - and β -adrenoceptor-mediated positive inotropic effect in isolated rabbit papillary muscle. *J Cardiovasc Pharmacol Ther.* 1997;2:159–170.
 - 62 Yang H-T, Endoh M. Pharmacological evidence for α -adrenoceptors in the rabbit ventricular myocardium: analysis with BMY 7378. *Br J Pharmacol.* 1997;122:1541–1550.
 - 63 Chu L, Endoh M. Positive inotropic effect of α_1 -adrenoceptor stimulation in dog ventricular myocardium. *J Cardiovasc Pharmacol.* 2001;38 Suppl 1:S13–S16.
 - 64 Sakurai K, Norota I, Tanaka H, Kubota I, Tomoike H, Endoh M. Negative inotropic effects of angiotensin II, endothelin-1 and phenylephrine in indo-1 loaded adult mouse ventricular myocytes. *Life Sci.* 2002;70:1173–1184.
 - 65 Ishihata A, Endoh M. Pharmacological characteristics of the positive inotropic effect of angiotensin II in the rabbit ventricular myocardium. *Br J Pharmacol.* 1993;108:999–1005.
 - 66 Ishihata A, Endoh M. Species-related differences in inotropic effects of angiotensin II in mammalian ventricular muscle: receptors, subtypes and phosphoinositide hydrolysis. *Br J Pharmacol.* 1995;114:447–453.
 - 67 Talukder MAH, Endoh M. Pharmacological differentiation of synergistic contribution of L-type Ca^{2+} channels and Na^+/H^+ exchange to the positive inotropic effect of phenylephrine, endothelin-3 and angiotensin II in rabbit ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol.* 1997;355:87–96.
 - 68 Fujita S, Endoh M. Influence of a Na^+/H^+ exchange inhibitor ethylisopropylamiloride, a $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibitor KB-R7943 and their combination on the increases in contractility and Ca^{2+} transient induced by angiotensin II in isolated adult rabbit ventricular myocytes. *Naunyn Schmiedebergs Arch Pharmacol.* 1999;360:575–584.
 - 69 Ichihayagi O, Ishii K, Endoh M. Angiotensin II increases L-type Ca^{2+} current in gramicidin D-perforated adult rabbit ventricular myocytes: comparison with conventional patch-clamp method. *Pflugers Arch.* 2002;444:107–116.
 - 70 Takanashi M, Endoh M. Characterization of positive inotropic effect of endothelin on mammalian ventricular myocardium. *Am J Physiol.* 1991;261:H611–H619.
 - 71 Takanashi M, Endoh M. Concentration- and time-dependence of phosphoinositide hydrolysis induced by endothelin-1 in relation to the positive inotropic effect in the rabbit ventricular myocardium. *J Pharmacol Exp Ther.* 1992;262:1189–1194.
 - 72 Kasai H, Takanashi M, Takasaki C, Endoh M. Pharmacological properties of endothelin receptor subtypes mediating positive inotropic effects in rabbit heart. *Am J Physiol.* 1994;266:H2220–H2228.
 - 73 Endoh M, Norota I, Yang H-T, Fujita S. The positive inotropic effect and the hydrolysis of phosphoinositide induced by endothelin-3 in rabbit ventricular myocardium: inhibition by a selective antagonist of ET_A receptor, FR139317. *J Pharmacol Exp Ther.* 1996;277:61–70.
 - 74 Yang H-T, Zhu Y, Endoh M. Species-dependent differences in inotropic effects and phosphoinositide hydrolysis induced by endothelin-3 in mammalian ventricular myocardium. *Br J Pharmacol.* 1997;120:1497–1504.
 - 75 Zhu Y, Yang H-T, Endoh M. Negative chronotropic and inotropic effects of endothelin isopeptides in mammalian cardiac muscle. *Am J Physiol.* 1997;273:H119–H127.
 - 76 Endoh M, Fujita S, Yang H-T, Talukder MAH, Maruya J, Norota I. Endothelin: receptors subtypes, signal transduction, regulation of Ca^{2+} transients and contractility in rabbit ventricular myocardium. *Life Sci.* 1998;62:1485–1489.
 - 77 Yang H-T, Sakurai K, Sugawara H, Watanabe T, Norota I, Endoh M. Role of $\text{Na}^+/\text{Ca}^{2+}$ exchange in endothelin-1-induced increases in Ca^{2+} transient and contractility in rabbit ventricular myocytes: pharmacological analysis with KB-R7943. *Br J Pharmacol.* 1999;126:1785–1795.
 - 78 Watanabe T, Endoh M. Characterization of the endothelin-1-induced regulation of L-type Ca^{2+} current in rabbit ventricular myocytes. *Naunyn Schmiedebergs Arch Pharmacol.* 1999;360:654–664.
 - 79 Watanabe T, Endoh M. Antiadrenergic effects of endothelin-1 on the L-type Ca^{2+} current in dog ventricular myocytes. *J Cardiovasc Pharmacol.* 2000;36:344–350.
 - 80 Wang H, Sakurai K, Endoh M. Pharmacological analysis by HOE642 and KB-R9032 of the role of Na^+/H^+ exchange in the endothelin-1-induced Ca^{2+} signalling in rabbit ventricular myocytes. *Br J Pharmacol.* 2000;131:638–644.
 - 81 Talukder MAH, Norota I, Sakurai K, Endoh M. Inotropic response of rabbit ventricular myocytes to endothelin-1: difference from isolated papillary muscles. *Am J Physiol.* 2001;281:H596–H605.
 - 82 Wang H, Endoh M. Chelerythrine and genistein inhibit the endothelin-1-induced increase in myofilament Ca^{2+} sensitivity in rabbit ventricular myocytes. *Eur J Pharmacol.* 2001;424:91–96.
 - 83 Yomogida S, Maruya J, Norota I, Ishii K, Endoh M. Differential inhibition by TAK-044 of the inotropic effects of endothelin-1 and endothelin-3. *Eur J Pharmacol.* 2004;492:217–224.
 - 84 Chu L, Endoh M. Biphasic inotropic response to endothelin-1 in the presence of various concentrations of norepinephrine in dog ventricular myocardium. *J Cardiovasc Pharmacol.* 2000;36 Suppl 2:S9–S14.
 - 85 Chu L, Norota I, Ishii K, Endoh M. Inhibitory action of the phosphatase inhibitor cantharidin on the endothelin-1-induced and the carbachol-induced negative inotropic effect in the canine ventricular myocardium. *J Cardiovasc Pharmacol.* 2003;41

- Suppl 1:S89–S92.
- 86 Chu L, Norota I, Yomogida S, Ishii K, Endoh M. Differential inotropic effects of endothelin-1, angiotensin II, and phenylephrine induced by crosstalk with cAMP-mediated signaling process in dog ventricular myocardium. *J Pharmacol Sci.* 2004; 96:199–207.
 - 87 Chu L, Zhang J-X, Norota I, Endoh M. Differential action of a protein tyrosine kinase inhibitor, genistein, on the positive inotropic effect of endothelin-1 and norepinephrine in canine ventricular myocardium. *Br J Pharmacol.* 2005;144:430–442.
 - 88 Chu L, Endoh M. Wortmannin inhibits the myofilament Ca²⁺ sensitization induced by endothelin-1. *Eur J Pharmacol.* 2005; 507:135–143.
 - 89 Chu L, Zhang J-X, Norota I, Endoh M. Receptor subtypes mediating the inotropic effects of endothelin-1 induced by crosstalk with norepinephrine in canine ventricular myocardium. *J Pharmacol Sci.* 2005;97:417–428.
 - 90 Chu L, Norota I, Endoh M. Inhibition by the Rho kinase inhibitor Y-27632 of the increases in contractility and Ca²⁺ transients induced by endothelin-1 in rabbit ventricular myocytes. *Naunyn Schmiedebergs Arch Pharmacol.* 2005;371: 185–194.
 - 91 Endoh M, Tamura K, Hashimoto K. Negative and positive inotropic responses of the blood-perfused canine papillary muscle to acetylcholine. *J Pharmacol Exp Ther.* 1970;175:377–387.
 - 92 Endoh M. Correlation of cyclic AMP and cyclic GMP levels with changes in contractile force of dog ventricular myocardium during cholinergic antagonism of positive inotropic actions of histamine, glucagon, theophylline and papaverine. *Jpn J Pharmacol.* 1979;29:855–864.
 - 93 Endoh M, Shimizu T. Failure of dibutyl and 8-bromo-cyclic GMP to mimic the antagonistic action of carbachol on the positive inotropic effects of sympathomimetic amines in the canine isolated ventricular myocardium. *Jpn J Pharmacol.* 1979;29:423–433.
 - 94 Endoh M, Motomura S. Differentiation by cholinergic stimulation of positive inotropic actions mediated via alpha- and beta-adrenoceptors in the rabbit heart. *Life Sci.* 1979;25:759–768.
 - 95 Endoh M, Yamashita S. Differential responses to carbachol, sodium nitroprusside and 8-bromo-guanosine 3',5'-monophosphate of canine atrial and ventricular muscle. *Br J Pharmacol.* 1981;73:393–399.
 - 96 Endoh M, Maruyama M, Iijima T. Attenuation of muscarinic cholinergic inhibition by islet-activating protein in the heart. *Am J Physiol.* 1985;249:H309–H320.
 - 97 Endoh M. Muscarinic regulation of Ca²⁺ signaling in mammalian atrial and ventricular myocardium. *Eur J Pharmacol.* 1999;375: 177–196.
 - 98 Endoh M, Yamashita S. Adenosine antagonizes the positive inotropic action mediated via beta-, but not alpha-adrenoceptors in the rabbit papillary muscle. *Eur J Pharmacol.* 1980;65:445–448.
 - 99 Endoh M, Kushida H, Norota I, Takanashi M. Pharmacological characteristics of adenosine-induced inhibition of dog ventricular contractility: dependence on the pre-existing level of beta-adrenoceptor activation. *Naunyn Schmiedebergs Arch Pharmacol.* 1991;344:70–78.
 - 100 Endoh M, Yanagisawa T, Morita T, Taira T. Differential effects of sulmazole (AR-L 115 BS) on contractile force and cyclic AMP levels in canine ventricular muscle: comparison with MDL 17,043. *J Pharmacol Exp Ther.* 1985;234:267–273.
 - 101 Endoh M, Shibasaki T, Satoh H, Norota I, Ishihata A. Different mechanisms involved in the positive inotropic effects of benzimidazole derivative UD-CG 115 BS (pimobendan) and its demethylated metabolite UD-CG 212 Cl in canine ventricular myocardium. *J Cardiovasc Pharmacol.* 1991;17:365–375.
 - 102 Kawabata Y, Endoh M. Effects of the positive inotropic agent Org 30029 on developed force and aequorin light transients in intact canine ventricular myocardium. *Circ Res.* 1993;72:597–606.
 - 103 Kawabata Y, Endoh M. Effects of a novel cardiotonic agent, Org 9731, on force and aequorin light transients in intact ventricular myocardium of the dog: involvement of a cyclic AMP-mediated mechanism and myofibrillar responsiveness to Ca²⁺ ions. *J Card Fail.* 1995;1:143–153.
 - 104 Endoh M. Regulation of myocardial contractility by a downstream mechanism. *Circ Res.* 1998;83:230–232.
 - 105 Sugawara H, Endoh M. A novel cardiotonic agent SCH00013 acts as a Ca⁺⁺ sensitizer with no chronotropic activity in mammalian cardiac muscle. *J Pharmacol Exp Ther.* 1998;287: 214–222.
 - 106 Sugawara H, Endoh M. (–)-Enantiomers EMD 57439 antagonizes the Ca²⁺ sensitizing effect of (+)-Enantiomers EMD 57033 on diastolic function but not on systolic function in rabbit ventricular cardiomyocytes. *Jpn J Pharmacol.* 1999;80:55–65.
 - 107 Takahashi R, Talukder MAH, Endoh M. Effects of OR-1896, an active metabolite of levosimendan, on contractile force and aequorin light transients in intact rabbit ventricular myocardium. *J Cardiovasc Pharmacol.* 2000;36:118–125.
 - 108 Takahashi R, Talukder MAH, Endoh M. Inotropic effects of OR-1896, an active metabolite of levosimendan, on canine ventricular myocardium. *Eur J Pharmacol.* 2000;400:103–112.
 - 109 Takahashi R, Endoh M. Increase in myofibrillar Ca²⁺ sensitivity induced by UD-CG 212 Cl, an active metabolite of pimobendan, in canine ventricular myocardium. *J Cardiovasc Pharmacol.* 2001;37:209–218.
 - 110 Endoh M, Sugawara H, Mineshima M. Pharmacology of SCH00013: a novel Ca²⁺ sensitizer. *Cardiovasc Drug Rev.* 2001;19:345–368.
 - 111 Watanabe A, Tomoike H, Endoh M. Ca²⁺ sensitizer Org-30029 reverses acidosis-and BDM-induced contractile depression in canine myocardium. *Am J Physiol.* 1996;271:H1829–H1839.
 - 112 Takahashi R, Shimazaki Y, Endoh M. Decrease in Ca²⁺-sensitizing effect of UD-CG 212 Cl, a metabolite of pimobendan, under acidotic condition in canine ventricular myocardium. *J Pharmacol Exp Ther.* 2001;298:1060–1066.
 - 113 Endoh M. Acidic pH-induced contractile dysfunction via downstream mechanism: identification of pH-sensitive domain in troponin I. *J Mol Cell Cardiol.* 2001;33:1297–1300.
 - 114 Takahashi R, Endoh M. Effects of OR-1896, a metabolite of levosimendan, on force of contraction Ca²⁺ transients under acidotic condition in aequorin-loaded canine ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol.* 2002; 366:440–448.
 - 115 Takahashi R, Endoh M. Dual regulation of myofilament Ca²⁺ sensitivity by levosimendan in normal and acidotic conditions in aequorin-loaded canine ventricular myocardium. *Br J Pharmacol.* 2005;145:1143–1152.
 - 116 Sugawara H, Sakurai K, Atsumi H, Nakada S, Tomoike H, Endoh M. Differential alteration of cardiotonic effects of EMD 57033 and β -adrenoceptor agonists in volume-overload rabbit ventricular myocytes. *J Card Fail.* 2000;6:338–349.