Cardiac α₁-Adrenoceptors and Inotropy
Myofilament Ca²⁺ Sensitivity, Intracellular Ca²⁺ Mobilization, Signaling Pathway, and Pathophysiological Relevance

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Cardiac α₁-adrenoceptor stimulation elicits a positive inotropic effect because of myofilament Ca²⁺ sensitization with a small increase in Ca²⁺ transients predominantly mediated by α₁₃-adrenoceptors via the intracellular alkalization and potential myosin light chain 2 phosphorylation. However, the α₁-adrenoceptor–mediated inotropy exhibits a wide range of species-dependent variation. In addition, it displays remarkable regional and subtype-dependent variations among species. The signaling pathway for α₁-adrenoceptor–mediated regulation is vulnerable being markedly influenced by experimental and pathophysiological conditions. Cardiac α₁-adrenoceptors may have clinical relevance in that sympathomimetic amines including nor-epinephrine possess higher affinity to α₁-adrenoceptors than β-adrenoceptors, and α₁-adrenoceptors play a compensatory role in patients with heart failure. Modulation of cardiac α₁-adrenoceptor–mediated signaling pathway could provide potential targets for the development of novel therapeutic strategy.

The role of cardiac α₁-adrenoceptors in regulation of myocardial contractility, including mechanism of action, signaling pathway, and pathophysiological and clinical significance, has been less clarified compared with the well-established β-adrenoceptor–mediated regulation. In this viewpoint, I will focus on the state-of-the-art of cardiac α₁-adrenoceptor–mediated basic mechanism and its pathophysiological relevance in relation to clinical implications.

**Regulation of Ca²⁺ Signaling**

The regulation of myocardial contractility by Ca²⁺ is achieved by either alteration of peak Ca²⁺ transients (CaT) that reflect the intracellular Ca²⁺ ([Ca²⁺]) mobilization during twitch contraction (upstream mechanism), myofilament Ca²⁺ sensitivity (central or downstream mechanism) or combination of both, in which the binding of Ca²⁺ to troponin C plays a central role (central mechanism) in cardiac EC coupling. Cardiac α₁-adrenoceptor stimulation elicits a moderate increase in peak CaT (20% of the maximum of β-adrenoceptor–mediated increase), whereas it induces the maximum positive inotropic effect (PIE) in an amount of 60% of the β-adrenoceptor–mediated maximum response in the rabbit ventricular myocardium.¹ The relation of peak CaT to contractile force is shifted to the left by α₁-adrenoceptor stimulation in a direction indicating an increase in myofilament Ca²⁺ sensitivity. The duration of contraction is prolonged because of retardation of relaxation in association with an abbreviation of CaT, likewise indicating an increase in myofilament Ca²⁺ sensitivity. These pieces of experimental evidence show that in contrast to β-adrenoceptor stimulation, the PIE of α₁-adrenoceptor activation is associated with an increase in myofilament Ca²⁺ sensitivity. The PIE of norepinephrine, epinephrine, and dopamine is mediated by both β-adrenoceptor and α₁-adrenoceptor activation. It is noteworthy that sympathomimetic amines, including norepinephrine, epinephrine, dopamine, and phenylephrine have higher affinity to cardiac α₁-adrenoceptors than β-adrenoceptors, indicating that the contractile regulation induced by sympathomimetic amines at lower concentrations is mediated predominantly by α₁-adrenoceptors, whereas at higher concentrations, the upstream mechanism activated by β-adrenoceptor stimulation is mobilized to induce a robust PIE required for more drastic adjustment of cardiac pump function.

**Mechanism of Myofilament Ca²⁺ Sensitization**

The signal transduction process responsible for the α₁-adrenoceptor–mediated myofilament Ca²⁺ sensitization has not yet been fully elucidated at the molecular level. This is largely because of characteristics of the cardiac α₁-adrenoceptor–mediated contractile regulation, including a prominent species-dependent variation of the signaling processes, diverse mechanisms involved in the regulation, and vulnerable coupling process that is readily affected by experimental and pathophysiological conditions. Cardiac α₁-adrenoceptors are coupled to various signaling pathways, the core process of which is stimulation of the phosphoinositide hydrolysis that results in subsequent generation of inositol 1,4,5-trisphosphate and diacylglycerol.² Although the role of 1,4,5-trisphosphate in the sustained PIE induced by α₁-adrenoceptor stimulation is not evident, diacylglycerol plays a crucial role in contractile regulation by activating protein kinase C (PKC) that catalyzes phosphorylation of sarcolemmal ion channels and ion transporters, and contractile proteins. It is shown in the rabbit perfused heart that the PIE of phenylephrine is associated with an increase in phosphorylation of a 15-kDa sarcolemmal protein and a 28-kDa cytosolic protein by PKC activation, but not with

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phosphorylation of phospholamban, troponin I, troponin T, and myosin-binding protein C.3

Potential candidates responsible for the cardiac α1-adrenoceptor–mediated myofilament Ca2+ sensitization may be intracellular alkalization (central mechanism) resulting from the PKC-dependent and PKC-independent activation of Na+–H+ exchanger,4 and myosin light chain 2 phosphorylation (downstream mechanism) induced by the activation of PKC.5,6 Although the importance of the former has been demonstrated definitely, experimental observations on the latter have currently been controversial in respect that the activation of myosin light chain kinase by PKC displays regional and species-dependent variations.

Mechanism of Intracellular Ca2+ Mobilization

The α1-adrenoceptor–mediated mobilization of [Ca2+]i likewise involves diverse mechanisms. The increase in peak CaT associated with a prolongation of action potential duration in the rabbit and rat because of the inhibition of sarcolemmal K+ channels including I_KATP and I_K, resulting in an increase in Ca2+ influx via L-type Ca2+ channels and CaT-dependent PIE (upstream mechanism).2 Direct activation of L-type Ca2+ channels by the PKC-mediated phosphorylation may likewise contribute to the α1-(namely α1A)-adrenoceptor–mediated PIE by an increase in CaT in the rat.4 The PIE induced by α1A-adrenoceptor activation via upstream mechanism in transgenic mice with α1A-adrenoceptor overexpression involves RhoA/ROCK signaling pathway.9

Species-Dependent Variation

The inotropic effect of α1-adrenoceptor stimulation shows a wide range of species-dependent variation in mammalian ventricular myocardium: mostly negative in mice, biphasic (or triphasic) in rats, and guinea pigs, positive being most pronounced in rabbits, moderate in humans and absent in dogs.7 In mice, the α1-adrenoceptor subtype-mediated contractile regulation exhibits a pronounced regional variation: activation of α1A-adrenoceptor subtype elicits a PIE in left ventricular (LV) myocardium whereas a negative inotropic effect (NIE) in right ventricular myocardium.6

The sustained PIE of α1-adrenoceptor stimulation in rats, guinea pigs, and mice (mediated by α1A-adrenoceptors in LV myocardium) is associated with a small increase in CaT because of the PKC-mediated activation of L-type Ca2+ channel and the inhibition of sarcolemmal K+ channels,3 and an increase in myofilament Ca2+ sensitivity associated with the PKC-mediated myosin light chain 2 phosphorylation.6 Intracellular alkalization because of stimulation of sarcolemmal Na+–H+ exchanger by α1A-adrenoceptor–mediated activation that requires both the extracellular signal regulated kinase (ERK)–dependent (p90(rsk) mediated) pathway of the mitogen-activated protein kinase (MAPK) cascade and ERK-independent (PKC mediated) pathway4 may contribute to an increase in myofilament Ca2+ sensitivity in rats.

The transient PIE observed in rats may be ascribed to the Ca2+ release from sarcoplasmic reticulum via 1,4,5-trisphosphate receptor activation through α1-adrenoceptor–induced phosphoinositide hydrolysis.3

The sustained NIE of α1-adrenoceptor stimulation in mice is associated with only a small decrease in peak CaT, suggesting that it is partly mediated by a decrease in myofilament Ca2+ sensitivity. The NIE may, in part, be because of a decrease in action potential duration in mice, rats, and guinea pigs and a suppressed cross-bridge cycling rate associated with troponin I phosphorylation induced by the activation of PKC. The NIE is gradually replaced to the sustained PIE in rats.4

Cardiac α1-adrenoceptor subtypes also play a key role in a wide range of species-dependent and regional variations of α1-adrenoceptor–mediated contractile regulation. The α1-adrenoceptors responsible for the PIE in the rabbit ventricular myocardium belong mostly to α1A-adrenoceptor.2,7 However, the activation of α1B-adrenoceptor induces an NIE in mice. In addition, α1B-adrenoceptor stimulation negatively modulates the α1B-adrenoceptor–mediated PIE in mice and rats and an increase in L-type Ca2+ channel activity induced by α1B-adrenoceptor activation through differential coupling to G proteins in rats.8 Although it is evident that α1B-adrenoceptor stimulation elicits an opposite action, that is, positive in rabbit and negative in mouse and rat, probably because of difference in the coupling process including G proteins and PKC isoenzymes, the regulatory relevance and the detailed mechanism responsible for these intriguing observations remain to be elucidated in the future study.

The overexpression of α1B subtype in mice induces the LV contractile dysfunction, whereas overexpression of α1A-adrenoceptor displays a marked enhancement of cardiac contractility, but induces ventricular fibrosis, pathological cardiac remodeling, and premature death.10 In respect to a prominent species-dependent variation, α1-adrenoceptor and its subtypes in mediating the inotropic effect are in strong contrast to the β-adrenoceptor that consistently induces a PIE in the mammalian heart. Species-dependent variation of α1-adrenoceptor–mediated inotropy may be ascribed to the coupling to different types of G protein5 or activation of different types of PKC isoenzyme.

Similarity of Inotropic Responses to α1-Adrenoceptor Agonists, Endothelin-1, and Angiotensin II

α1-Adrenoceptor agonists, endothelin isopeptides, and angiotensin II, which share the signal transduction pathway in activating the phosphoinositide hydrolysis, elicit identical inotropic effects in respect to a moderate increase in peak CaT, and a shift of the relation of contractile force to peak CaT to the left and an identical species-dependent characteristic.11 These pieces of evidence imply strongly the role of phosphoinositide hydrolysis in inducing the myofilament Ca2+ sensitization by these receptor agonists. Pieces of current
experimental evidence, however, fail to fully support the role of phosphoinositide hydrolysis in myofilament Ca$^{2+}$ sensitization induced by stimulation of these different types of receptor. For example, the phorbol ester phorbol 12,13-dibutyrate that substitutes for diacylglycerol in activating PKC does not enhance, but inhibits the PIE of $\alpha_1$-adrenoceptor agonists and endothelin-1 (ET-1), which may be ascribed to the inhibition of receptor coupling to phospholipase C activation in the rabbit.\(^7\) In rat and human ventricular myocardium, activation of PKC by phorbol esters elicits an NIE associated with a decrease in CaT and without or with a decrease in myofilament Ca$^{2+}$ sensitivity.\(^8\) PKC inhibitors, however, can suppress only partially the PIE of $\alpha_1$-adrenoceptor agonists in the rabbit papillary muscle.\(^7\) The coupling of $\alpha_1$-adrenoceptor stimulation to different types of PKC isoenzyme, activation of certain types of which is inhibitory and may contribute to diverse actions of PKC activators and inhibitors.

### Contractile Regulation by Crosstalk of ET-1 With Norepinephrine

In cardiovascular disorders, such as congestive heart failure and ischemic heart diseases, endogenous regulators, including norepinephrine, ET-1, angiotensin II, and other cytokines, are released from various types of cell. It seems likely that the cardiac contraction might be regulated by crosstalk among these endogenous regulators because plasma levels of these regulators are elevated. In dog ventricular myocardium, ET-1 alone does not affect the contractile function, but in the presence of subthreshold concentration of norepinephrine at 0.1 to 1 nmol/L that produces no inotropic effect by itself, ET-1 elicits a PIE. The PIE of ET-1 is associated with the activation of PKC\(\varepsilon\), a small increase in CaT and an increase in myofilament Ca$^{2+}$ sensitivity.\(^9\) Pharmacological analysis indicates that the PIE induced by crosstalk of ET-1 with norepinephrine requires the simultaneous activation of protein kinase A and PKC. In the presence of higher concentrations of norepinephrine at 0.1 to 1 \(\mu\)mol/L that induces a PIE via $\beta$-adrenoceptor activation by itself, ET-1 produces a pronounced NIE in association with a decrease in CaT, which is mediated by an activation of pertussis toxin-sensitive Gi proteins, and protein kinase G and phosphatases (similar to the accentuated antagonism observed with the muscarinic receptor agonist in mammalian ventricular myocardium).\(^1\) Therefore, the cardiac contractility is regulated either positively or negatively by crosstalk of ET-1 with norepinephrine through the activation of different signaling pathways, which are dependent on the concentration of norepinephrine released from sympathetic nerve endings under various pathophysiological conditions.

### Cardiac $\alpha_1$-Adrenoceptors in Heart Failure and Pathophysiological Relevance

In mouse ventricular myocardium, $\alpha_{1A}$-adrenoceptor stimulation induces an NIE in the right ventricular myocardium, which is switched to a PIE in heart failure, whereas $\alpha_{1A}$-adrenoceptor activation elicits consistently a PIE in LV muscles, which is unaffected by heart failure.\(^6\) These observations indicate that $\alpha_1$-adrenoceptors are coupled to different signaling processes in the right ventricular and LV myocardium to result in contractile regulation differentially influenced by heart failure. In rat congestive heart failure, the $\alpha_1$-adrenoceptor–mediated PIE is preserved or becomes dominating when compared with the $\beta$-adrenoceptor–mediated PIE, although it is impaired in cardiac hypertrophy compared with healthy animals. In human whole heart, stimulation of $\alpha_1$-adrenoceptors induces a PIE, the magnitude of which is attenuated in patients with heart failure compared with healthy subjects. Nevertheless, it functions in a compensatory fashion together with $\beta_2$-adrenoceptors to maintain cardiac inotropy.\(^1\) Actually, norepinephrine evokes an $\alpha_1$-adrenoceptor–mediated PIE comparable to that induced via $\beta$-adrenoceptors in failing human ventricular muscle. Overall, the PIE mediated by activation of $\alpha_1$-adrenoceptor and crosstalk may play a significant compensatory role in the maintenance of cardiac pump function, the inhibition of which by the antagonists of $\alpha_1$-adrenoceptor directly or receptors involved in the crosstalk could cause unpredictable or yet unrecognized cardiovascular responses in patients with heart failure.

### Perspectives and Clinical Implications

It is noteworthy that the cardiac $\alpha_1$-adrenoceptor–mediated inotropic response shows peculiar characteristics that it is readily altered or diminished under experimental or pathophysiological conditions, which is in strong contrast to a stable $\beta$-adrenoceptor–mediated PIE. The $\alpha_1$-adrenoceptor–mediated activation of phospholipase C, the ERK, p38-MAPK, or stimulation of hypertrophy that occurs in intact mouse heart is lost in cultured cardiomyocytes.\(^1\) In addition, a pronounced regional difference in $\alpha_1$-adrenoceptor–mediated signal transduction processes exist among mammalian species. Therefore, although small rodents, especially mice and rats, are most frequently used for the study pursuing the pathophysiological basis of cardiac disorders, they are not able to provide a suitable experimental model in respect to the role of $\alpha_1$-adrenoceptor and its signaling processes that contribute to the pathophysiology in patients. Nevertheless, the knockout of $\alpha_{1C}/\alpha_{1D}$-adrenoceptor in mice exacerbates the pressure overload–induced heart failure, which supports the large-scale clinical trial ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) that the $\alpha_1$-adrenoceptor antagonist doxazosin increased heart failure in patients with hypertension.\(^1\) The study with human atrial and ventricular myocardium implies the existence of regional difference in $\alpha_1$-adrenoceptor–mediated coupling processes in myosin light chain 2 phosphorylation likewise in the human heart.\(^1\)

When the sympathetic activity is increased, an elevation of blood pressure due to the activation of predominant vascular $\alpha_1$-adrenoceptors makes it extremely difficult to detect the contribution of cardiac $\alpha_1$-adrenoceptors to a fine contractile adjustment. However, it is evident that the activation of cardiac $\alpha_1$-adrenoceptors mediates the PIE of sympathomimetic amines including norepinephrine at concentrations lower than those activating $\beta$-adrenoceptors likewise in human. The experimental observations in the dog\(^1\) imply that cardiac $\alpha_1$-adrenoceptors stimulated by norepinephrine in combination with other endogenous regulators could play an important role in contractile regulation under pathophysiological conditions. It may be energetically advantageous compared with the
β-adrenergocortland effect--mediated effect in expenditure of the excessive activation and metabolic energy by the latter. In addition to inotropic regulation, cardiac α₁-adrenoceptors are involved in regulation of the development of physiological cardiac hyper trophy, ischemic preconditioning, and cardiac cell survival signaling pathway. Thus, the signaling pathway activated by cardiac α₁-adrenoceptors may provide a potential target for improving cardiac contractile function in various heart diseases.

In the end, I apologize that I had to skip several important original papers in the text because of the format of Viewpoints, which will be hopefully covered by the reference lists of the cited papers.

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**References**


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