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

Masao Endoh

Cardiac α₁-adrenoceptor stimulation elicits a positive inotropic effect because of myofilament Ca²⁺ sensitization with a small increase in Ca²⁺ transients predominantly mediated by α₁₉-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 norepinephrine 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...
Mechanism of Intracellular Ca\(^{2+}\) Mobilization

The \(\alpha_1\)-adrenoceptor–mediated mobilization of [Ca\(^{2+}\)]_i like-wise involves diverse mechanisms. The increase in peak CaT is associated with a prolongation of action potential duration in the rabbit and rat because of the inhibition of sarcomemal K\(^+\) channels including I\(_{KATP}\) and I\(_{to}\), resulting in an increase in Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels and CaT-dependent PIE (upstream mechanism).\(^7\) Direct activation of L-type Ca\(^{2+}\) channels by the PKC-mediated phosphorylation may likewise contribute to the \(\alpha_1\)-adrenoceptor–mediated PIE by an increase in CaT in the rat.\(^7\) The PIE induced by \(\alpha_1A\)-adrenoceptor activation via upstream mechanism in transgenic mice with \(\alpha_1A\)-adrenoceptor overexpression involves RhoA/ROCK signaling pathway.\(^9\)

Species-Dependent Variation

The inotropic effect of \(\alpha_1\)-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 \(\alpha_1\)-adrenoceptor subtype-mediated contractile regulation exhibits a pronounced regional variation: activation of \(\alpha_1\)-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 \(\alpha_1\)-adrenoceptor stimulation in rats, guinea pigs, and mice (mediated by \(\alpha_1A\)-adrenoceptors in LV myocardium) is associated with a small increase in CaT because of the PKC-mediated activation of L-type Ca\(^{2+}\) channel and the inhibition of sarcomemal K\(^+\) channels,\(^7\) and an increase in myofilament Ca\(^{2+}\) sensitivity associated with the PKC-mediated myosin light chain 2 phosphorylation.\(^6\) Intracellular alkalinization because of stimulation of sarcomemal Na\(^{-}\)-H\(^{+}\) exchanger by \(\alpha_1A\)-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) pathway\(^4\) may contribute to an increase in myofilament Ca\(^{2+}\) sensitivity in rats.

The transient PIE observed in rats may be ascribed to the Ca\(^{2+}\) release from sarcoplasmic reticulum via 1,4,5-trisphosphate receptor activation through \(\alpha_1\)-adrenoceptor–induced phosphoinositide hydrolysis.\(^3\)

The sustained NIE of \(\alpha_1\)-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 Ca\(^{2+}\) 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 \(\alpha_1\)-adrenoceptor subtypes also play a key role in a wide range of species-dependent and regional variations of \(\alpha_1\)-adrenoceptor–mediated contractile regulation. The \(\alpha_1\)-adrenoceptors responsible for the PIE in the rabbit ventricular myocardium belong mostly to \(\alpha_{1B}\)-adrenoceptor.\(^7\) However, the activation of \(\alpha_{1A}\)-adrenoceptor induces an NIE in mice. In addition, \(\alpha_{1B}\)-adrenoceptor stimulation negatively modulates the \(\alpha_{1B}\)-adrenoceptor–mediated PIE in mice and rats and an increase in L-type Ca\(^{2+}\) channel activity induced by \(\alpha_{1A}\)-adrenoceptor activation through differential coupling to G proteins in rats.\(^8\) Although it is evident that \(\alpha_{1B}\)-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 iso-enzymes, the regulatory relevance and the detailed mechanism responsible for these intriguing observations remain to be elucidated in the future study.

The overexpression of \(\alpha_{1B}\) subtype in mice induces the LV contractile dysfunction, whereas overexpression of \(\alpha_{1A}\)-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, \(\beta_1\)-adrenoceptor and its subtypes in mediating the inotropic effect are in strong contrast to the \(\beta_1\)-adrenoceptor that consistently induces a PIE in the mammalian heart. Species-dependent variation of \(\alpha_1\)-adrenoceptor–mediated inotropy may be ascribed to the coupling to different types of G protein\(^8\) or activation of different types of PKC isoenzyme.

Similarity of Inotropic Responses to \(\alpha_1\)-Adrenoceptor Agonists, Endothelin-1, and Angiotensin II

\(\alpha_1\)-Adrenoceptor agonists, endothelin iso-peptides, 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 Ca\(^{2+}\) sensitization by these receptor agonists. Pieces of current
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, a small increase in CaT and an increase in myofilament Ca$^{2+}$ sensitivity. PKC inhibitors, however, can suppress only partially the PIE of α₁-adrenoceptor agonists in the rabbit papillary muscle. The coupling of α₁-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.

Cardiac α₁-Adrenoceptors in Heart Failure and Pathophysiological Relevance

In mouse ventricular myocardium, α₁A-adrenoceptor stimulation induces an NIE in the right ventricular myocardium, which is switched to a PIE in heart failure, whereas α₁B-adrenoceptor activation elicits consistently a PIE in LV muscles, which is unaffected by heart failure. These observations indicate that α₁-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 α₁-adrenoceptor–mediated PIE is preserved or becomes dominating when compared with the β-adrenoceptor–mediated PIE, although it is impaired in cardiac hypertrophy compared with healthy animals. In human whole heart, stimulation of α₁-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 β₂-adrenoceptors to maintain cardiac inotropy. Actually, norepinephrine evokes an α₁-adrenoceptor–mediated PIE comparable to that induced via β-adrenoceptors in failing human ventricular muscle. Overall, the PIE mediated by activation of α₁-adrenoceptor and crosstalk may play a significant compensatory role in the maintenance of cardiac pump function, the inhibition of which by the antagonists of α₁-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 α₁-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 β-adrenoceptor–mediated PIE. The α₁-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. In addition, a pronounced regional difference in α₁-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 α₁-adrenoceptor and its signaling processes that contribute to the pathophysiology in patients. Nevertheless, the knockout of α₁A/α₁B-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 α₁-adrenoceptor antagonist doxazosin increased heart failure in patients with hypertension. The study with human atrial and ventricular myocardium implies the existence of regional difference in α₁-adrenoceptor–mediated coupling processes in myosin light chain 2 phosphorylation likewise in the human heart. When the sympathetic activity is increased, an elevation of blood pressure due to the activation of predominant vascular α₁-adrenoceptors makes it extremely difficult to detect the contribution of cardiac α₁-adrenoceptors to a fine contractile adjustment. However, it is evident that the activation of cardiac α₁-adrenoceptors mediates the PIE of sympathomimetic amines including norepinephrine at concentrations lower than those activating β-adrenoceptors likewise in human. The experimental observations in the dog imply that cardiac α₁-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-adrenoceptor–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 hypertrophy, ischemic preconditioning,17 and cardiac cell survival signaling pathway.18 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.

Sources of Funding
This study was supported, in part, by a Grant-in-Aid for Scientific Research on Priority Areas and Scientific Research (B) from the Ministry of Education, Science, Sports and Culture, Japan.

Disclosures
None.

Acknowledgments
We thank Dr Kuniaki Ishii, Yamagata University School of Medicine, and Dr Thomas Eschenhagen, University Medical Center Hamburg-Eppendorf, for reading the article and having provided valuable advices.

References

Key Words: dopamine ■ myosin light chain ■ potassium channels ■ protein kinase C ■ troponin C
Cardiac α₁-Adrenoceptors and Inotropy: Myofilament Ca²⁺ Sensitivity, Intracellular Ca²⁺ Mobilization, Signaling Pathway, and Pathophysiological Relevance
Masao Endoh

doi: 10.1161/CIRCRESAHA.116.309502

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/119/5/587

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/