Mechanisms and Pharmacological Classification of $\text{Ca}^{2+}$ Sensitizers

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Abstract: Since early 1980’s, an extensive effort has been accumulated for the development of novel cardiotonic agents to replace digitalis and catecholamines, the positive inotropic effect (PIE) of which are associated with serious adverse effects, including the induction of arrhythmias and cell injury due to the $\text{Ca}^{2+}$ overload. Several novel cardiotonic agents have been developed for the long-term treatment of the patients with chronic congestive heart failure (CHF), but currently none of the novel $\text{Ca}^{2+}$ mobilizers have succeeded in showing the definite improvement of the long-term prognosis in the chronic CHF patients. Certain novel agents with $\text{Ca}^{2+}$ sensitizing action have been shown to be more beneficial than $\text{Ca}^{2+}$ mobilizers in CHF models of experimental animals and small and/or middle scale clinical trials, which indicates that the development of novel $\text{Ca}^{2+}$ sensitizers for the treatment of the cardiac pump dysfunction could provide a breakthrough of the pharmacological therapy of the chronic CHF patients. In the early stage of the basic pharmacology for the development of novel $\text{Ca}^{2+}$ sensitizers, the classic muscarinic receptor agonist carbachol could be used as the pharmacological tool to differentiate the mode of action of novel $\text{Ca}^{2+}$ sensitizers having potential for the treatment of the chronic CHF patients.

Key words: $\text{Ca}^{2+}$ signaling $\text{Ca}^{2+}$ sensitizers $\text{Ca}^{2+}$ transients Carbachol Cardiotonic agents Congestive heart failure Levosimendan PDE3 inhibitors Pimobendan

1 Introduction
The pharmacological treatment of the patients with CHF has achieved a great advance in these three decades. However, the CHF remains still to be a serious cardiovascular disorder with an extremely high mortality. The cardiac pump failure due to the contractile dysfunction and arrhythmias are main causes of the death with the CHF. In early 1980’s, an extensive effort was started to develop novel cardiotonic agents to replace digitalis and catecholamines, both of which are associated with serious adverse effects. Afterwards, within a decade, several novel agents, such as amlinone, milrinone, olprinone, enoximone, piroximone, pimobendan, and levosimendan were developed and became available for the clinical use for the treatment of the CHF patients [1]. Although all of these novel cardiotonic agents improved the quality of life (QOL) of the patients with CHF, including the improvement of hemodynamic parameters and the exercise capability, most of these agents failed to show a beneficial effect on the prognosis of the chronic CHF patients, and clinical trials with some agents proved to aggravate the prognosis [2]. Based on the failure of these clinical trials with novel agents, a marked paradigm shift of the long-term pharmacological treatment of the chronic CHF patients occurred from the classic cardiac inotropic therapy to the cardiac protective therapy for reducing cardiac pre- and after-load, and suppressing remodeling. Nevertheless, the improvement of the cardiac contractile function by inotropic agents is of extreme importance under certain clinical situation, such as acute
CHF and aggravating phase of the chronic CHF [2].

In the cardiac excitation-contraction coupling, calcium ions (Ca$^{2+}$) play a key role in the regulation by binding to the regulatory protein troponin (Tn). The improvement of the cardiac contractility is achieved either by the increase in the mobilization of intracellular Ca$^{2+}$, the increase in the myofilament Ca$^{2+}$ sensitivity, and/or combination of both the mechanisms [3, 4].

2 Problem Formulation

By means of measuring simultaneously intracellular Ca$^{2+}$ transients (CaT) and the contractile activity in single cardiac myocytes loaded with Ca$^{2+}$ sensitive dyes such as fura-2 or indo-1 or in the ventricular myocardium loaded with the Ca$^{2+}$ sensitive bioluminescent protein aequorin, it is possible to differentiate the mode of action of cardiotonic agents from respect of the Ca$^{2+}$ signaling regulation [5]. One of advantages of these experimental procedures is that they are applicable to intact myocytes or myocardium isolated from a number of species from small rodents to large mammalians, the inotropic response of which shows a large species-dependent variation. Most experiments were carried out in right ventricular myocytes or ventricular myocardium isolated from rabbits and dogs.

3 Problem Solution

3.1 Mechanisms of Cardiotonic Agents

3.1.1 Ca$^{2+}$ Mobilizers

Certain cardiotonic agents exert the PIE purely by an increase in CaT with no alteration, as does the elevation of extracellular Ca$^{2+}$ concentration, or rather a decrease in the myofilament Ca$^{2+}$ sensitivity [6]. Ca$^{2+}$ mobilizers can be further classified into two subclasses based on the subcellular mechanism of action: cardiac glycosides that do not elevate myocardial cyclic AMP levels, and the agents such as β-adrenoceptor agonists and phosphodiesterase 3 (PDE3) inhibitors, the PIE of which is associated with an increase in myocardial cyclic AMP levels [7, 8]. Pieces of experimental evidence in animal CHF models and clinical trials have noted that the use of Ca$^{2+}$ mobilizers for the treatment of the chronic CHF patients is limited by serious adverse effects and disadvantages associated with their administration: 1) arrhythmias and the myocardial cell injury including apoptosis and necrosis due to Ca$^{2+}$ overload; 2) the energetic disadvantage to increase activation energy and/or metabolic energy by the agents such as catecholamines and PDE3 inhibitors; and 3) the loss of PIE under pathophysiological states, such as chronic CHF, acidosis and the stunned myocardium after ischemia.

3.1.2 Ca$^{2+}$ Sensitizers

Ca$^{2+}$ sensitizers overcome the disadvantages of Ca$^{2+}$ mobilizers in the following respects: 1) the energetic advantage that Ca$^{2+}$ sensitizers do not increase the activation energy and/or metabolic energy; 2) the PIE of Ca$^{2+}$ sensitizers is not associated with the potential risk of arrhythmias and cell injury due to the Ca$^{2+}$ overload; and 3) Ca$^{2+}$ sensitizers are capable of eliciting the PIE even under pathophysiological conditions, such as chronic CHF, acidosis and the stunned myocardium [4].

The relationship of the extent of PIE and the amplitude of CaT is shifted to the left of that with an elevation of the extracellular Ca$^{2+}$ concentration, by certain cardiotonic agents such as the α-adrenoceptor agonist phenylephrine, pimobendan and levosimendan, which indicates that these cardiotonic agents exert the PIE in association with an increase in the myofilament Ca$^{2+}$ sensitivity [9]. However, since these agents increase simultaneously the amplitude of CaT,
they act actually via at least two subcellular mechanisms, i.e., the modulation of regulatory proteins involved in the intracellular Ca\(^{2+}\) mobilization and myofibrillar proteins responsible for the Ca\(^{2+}\) sensitization. In contrast to Ca\(^{2+}\) mobilizers, namely PDE3 inhibitors and catecholamines, which are clinically available for the short-term application to avoid adverse effects or the loss of the effectiveness in the long-term administration, there are outcomes with the chronic CHF models in experimental animals and recent small and/or middle scale clinical trials showing that the agents with the Ca\(^{2+}\) sensitizing action exert a beneficial action in the long-term application. However, it is not yet confirmed in the large-scale clinical trial whether these agents are capable of prolonging the survival rate of the patients with chronic CHF [4].

3.2 Classification of Ca\(^{2+}\) Sensitizers Based on the Mechanism of Action

The site of action of Ca\(^{2+}\) sensitizers is not unity, and previous studies imply that at least following three regulatory sites or processes are responsible for increases in the myofilament Ca\(^{2+}\) sensitivity: 1) the increase in the Ca\(^{2+}\) binding affinity of TnC that is modulated by regulatory sites of TnI; 2) the Tn-tropomyosin complex to induce the feedback facilitation of the Ca\(^{2+}\) binding to TnC and/or to increase the regulatory effectiveness of the Tn-tropomyosin complex on the actin-myosin (AM) interaction (crossbridge cycling); and 3) the direct action on the myosin ATPase beyond the Ca\(^{2+}\) regulatory process [10, 11]. The information and pieces of the experimental evidence showing the exact site of action of Ca\(^{2+}\) sensitizers are still fragmentary and not sufficient, and await future study.

3.3 Application of the Muscarinic Cholinergic Stimulation for Differentiation of the Mechanism of Action of Ca\(^{2+}\) Sensitizers

Previously we proposed that the muscarinic cholinergic agonist carbachol could be used for differentiating the cyclic AMP-mediated and cyclic AMP-independent Ca\(^{2+}\) mobilization in the dog ventricular myocardium [12]. The use of carbachol for this purpose was effective especially to clarify whether the cyclic AMP generation contributes to the PIE of novel cardiotonic agents such as amlinone, milrinone, olprinone and other PDE3 inhibitors, namely because the increase in myocardial cyclic AMP levels induced by these agents was relatively small and not detectable up to the concentrations of these agents that induce ca. 80% of the maximal response [7].

It is evident that the muscarinic cholinergic receptor stimulation is also useful to differentiate the mode of action of Ca\(^{2+}\) sensitizers when cyclic AMP is involved in the PIE in combination with the myofilament Ca\(^{2+}\) sensitizing mechanism. The PIE of Ca\(^{2+}\) sensitizers such as Org 30029 [13], EMD 57033 [14], SCH00013 [15] and CGP 48506 that possess little or only weak PDE3 inhibitory action is resistant to the inhibitory action of carbachol, and the PIE of pimobendan with relatively stronger PDE inhibitory action is more susceptible to carbachol. The PIE of these agents in the presence of carbachol was induced in association with no increase in CaT [4]. In contrast, the PIE of levosimendan is abolished by carbachol [16, 17], the PIE being induced during and after washout of carbachol and levosimendan, since carbachol is washed out faster than levosimendan [17].

These observations imply that there are at least two classes of Ca\(^{2+}\) sensitizers: 1) the myofilament Ca\(^{2+}\) sensitivity is increased without alteration of Ca\(^{2+}\) mobilization; 2) the Ca\(^{2+}\) mobilization is increased first by the accumulation of cyclic AMP due to the PDE3 inhibition that leads to a prominent PIE via an amplification process at the level of myofilaments. Since the currently developed Ca\(^{2+}\) sensitizers are mostly associated with the
PDE3 inhibitory action, the simple pharmacological procedure to differentiate these two classes of Ca\(^{2+}\) sensitizers is of extreme usefulness in the course of the development of novel Ca\(^{2+}\) sensitizers.

While the detailed subcellular mechanism of the carbachol-induced inhibitory action is out of scope of this article, the inhibition of the cyclic AMP-mediated signaling process at different levels is responsible for the functional inhibition: 1) the decrease in cyclic AMP generation due to inhibition of adenyl cyclase via activation of the GTP binding coupling protein G\(_i\); 2) the activation of phosphatase leading to dephosphorylation of the regulatory proteins phosphorylated previously via cyclic AMP-mediated process; and 3) the inhibition at the level of the activation of protein kinase A [7, 8, 12].

It is noteworthy that there is another class of agents, such as \(\alpha\)-adrenoceptor agonists, endothelin and angiotensin II that elicit the PIE by combination of an increase in Ca\(^{2+}\) mobilization with myofilament Ca\(^{2+}\) sensitization [4, 8]. The PIE of the agents belonging to this class is not susceptible to the inhibitory action of carbachol, because the PIE of these agents is induced through the cyclic AMP-independent signaling process [12]. These agents play a crucial role as endogenous functional and genetic regulators, but are not used as cardiotonic agents, since they are more active as vasoconstrictors and stimulate cardiac remodeling.

### 4 Conclusion

Based on these pieces of experimental evidence, it is evident that carbachol is able to differentiate two classes of Ca\(^{2+}\) sensitizers: the former agents induce the PIE in the presence of carbachol at the baseline level of CaT purely by increasing the myofilament Ca\(^{2+}\) sensitivity, while the agent such as levosimendan exerts the Ca\(^{2+}\) sensitizing action by enhancing the Ca\(^{2+}\) responsiveness in combination with the increased Ca\(^{2+}\) level through the cyclic AMP-mediated process by the PDE3 inhibition induced by the compound. The pharmacological characteristics of Ca\(^{2+}\) sensitizers preferable for the treatment of the chronic CHF patients are not known at the moment, but it is necessary and useful to clarify the mode of action of these agents for the development of novel Ca\(^{2+}\) sensitizing agents most beneficial for the patients with chronic CHF. The classic pharmacological tool carbachol could be effectively applied to differentiate the mode of action of Ca\(^{2+}\) sensitizers in the early stage of basic pharmacological research for development of novel cardiotonic agents having potential to be developed in the future for the improvement of cardiac pump function and prognosis in the patients with chronic CHF.

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