Amrinone, Forerunner of Novel Cardiotonic Agents, Caused Paradigm Shift of Heart Failure Pharmacotherapy

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Amrinone has been a great forerunner of noncatechol and nonglycoside novel cardiotonic agents and given an extraordinary strong impact in the development of novel cardiotonic agents. Unexpectedly, amrinone failed to rescue the patients with chronic heart failure, thereby produced a prominent paradigm shift from inotropic to cardioprotective therapy, and contributed to advances in the current pharmacotherapy of chronic heart failure.

Mysterious Novel Cardiotonic Agent Acting Through Unknown Mechanism

In this study, Alousi et al showed that amrinone is a potent, long-acting positive inotropic agent when given orally or intravenously to either anesthetized or unanesthetized dogs. Amrinone was orally active with a rapid onset of action and duration of >5 hours and showed a wide separation between the positive inotropic and chronotropic effect. Amrinone increased contractile force and the rate of force development without changes in duration of the contractile cycle or time-to-peak tension, and in action potential or excitability even when it was given in relatively high concentrations. The toxicological studies attested to a low toxicity of amrinone ≤10 mg/kg, with less serious side effects such as lowering in blood pressure and an increase in heart rate compared with cardiac glycosides and catecholamines. The therapeutic index of amrinone was wide in contrast to that of cardiac glycosides that have a therapeutic index of ≈2 to 3, with life-threatening arrhythmias. Of greater interest was that amrinone did not increase cardiac output of the normal (nonfailing), but increased that of the failing heart model.

The mechanism of action of amrinone, however, was shown only by exclusion criteria. Amrinone did not seem to act via a catecholamine mechanism because its inotropic effect was not blocked by the β-receptor blocking agent propranolol or by depletion of cardiac norepinephrine with reserpine. It was

reported that there were no significant changes in the level of cardiac cAMP or phosphodiesterase (PDE) that could be responsible for the inotropic effect of amrinone. The original observations on this respect, however, were disproved soon in the subsequent studies as detailed in the following section. It was further shown that amrinone did not inhibit Na^+/K^+ ATPase activity.

The authors suggested that the positive inotropic with relatively weak chronotropic effect, the vasodilatory properties, the wide margin of safety, and the oral activity would make amrinone a prime candidate for clinical testing in patients with heart failure.

State-of-the-Art of Heart Failure Therapy in 1970s

In the late 1970s it was believed that the reverse of cardiac contractile dysfunction leading to improvement of the impaired cardiac pump function by means of positive inotropic agents is the mainstay of the pharmacotherapy of heart failure because the contractile dysfunction plays a key role in symptoms of heart failure directly by causing hemodynamic disorders and indirectly by driving compensatory mechanisms.

For this purpose, cardiac glycosides and catecholamines had long been administered to increase the cardiac contractile force to the patients with heart failure. However, cardiotonic agents belonging to these classes possess serious drawbacks, such as causing readily serious cardiac arrhythmias. In addition, cardiac glycosides have very narrow safety margin because of Ca^{2+} overload, and the inotropic effects of catecholamines are associated with an increase in heart rate and energetic disadvantages because of metabolic stimulation.

The development of orally available novel cardiotonic agents was a dream of both basic researchers and clinical cardiologists, and, therefore, an extensive effort had been accumulated to discover the novel agent to overcome these disadvantages by acting through novel mechanism of action, which could replace cardiac glycosides and catecholamines in the treatment of heart failure.

Consequently, the study of amrinone by Alousi et al caused a big sensation, driving several pharmaceutical companies to target on the development of novel cardiotonic agents. Furthermore, it was reported that amrinone induced a marked cardiotonic effect in patients already taking cardiac glycosides for severe heart failure without side effects of arrhythmias and altered arterial pressure.[1]

Amrinone with the bipyridine skeleton became the lead compound of subsequent development of novel cardiotonic agents, such as milrinone, enoximone, piroximone, and orprinone.

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## Mechanism of Action of Amrinone via PDE3 Inhibition

Meanwhile, the action mechanism of all of these novel cardiotonic agents became evident. In this context, it is noteworthy that in the subsequent issue of *Circulation Research*, Katz et al[2] sent a letter to the editor concerning amrinone. In the letter, they pointed out that the positive inotropic effect of amrinone shows a wide range of species-dependent variation among mammalian cardiac muscle. In the reply to the letter, Alousi and Farah[3] recognized that the positive inotropic effect of amrinone is markedly influenced by the species of animals and the stage of development. The order of sensitivity to amrinone is dog, cat, monkey, rabbit, and least in rat, and fetal cardiac tissue of the sheep heart does not respond to amrinone. These observations imply that amrinone elicits its positive inotropic effect by interacting with the intrinsic regulatory process of cardiac contraction.

Actually, it became evident soon that these novel cardiotonic agents, including amrinone and milrinone, act by inhibition of the PDE3 activity and subsequent activation of cAMP/protein kinase A/Ca^{2+} pathway (Figure).

It was reported in the original article that cAMP was not involved in the mechanism of the positive inotropic action of amrinone, which was concluded from measurements of the global cAMP level of myocardial tissue. Because PDE3 inhibition induces a regional increase in cAMP in microdomain, mainly in the vicinity of sarcoplasmic reticulum membrane in myocardial cells (Figure),[4] it is extremely difficult to evaluate the role of cAMP in contractile regulation just based on the measurement of cAMP levels in myocardial tissue.

To overcome this difficulty, it was proposed that stimulation of muscarinic receptors is able to be used to differentiate the role of cAMP in mediating the positive inotropic effect of novel cardiotonic agents in mammalian ventricular muscle.[5] The muscarinic receptor agonist carbachol is able to inhibit selectively the cAMP-mediated positive inotropic effect of amrinone and milrinone.[5,6] In contrast, the positive inotropic effect of Ca^{2+} sensitizers, such as sulmazole[6] and Org 30019,[7] and the effect of cardiac α-adrenergic receptor agonist[8] are resistant to the inhibitory action of carbachol. The inhibition of the cAMP-mediated positive inotropic effect of amrinone is because of action at multiple sites, including lowering of cAMP via inhibition of adenylyl cyclase by activation of the inhibitory GTP-binding protein G_i (Figure), stimulation of PDE 2 by cyclic GMP, inhibition of protein kinase A, and activation of phosphatase. It should be noted that the increase in
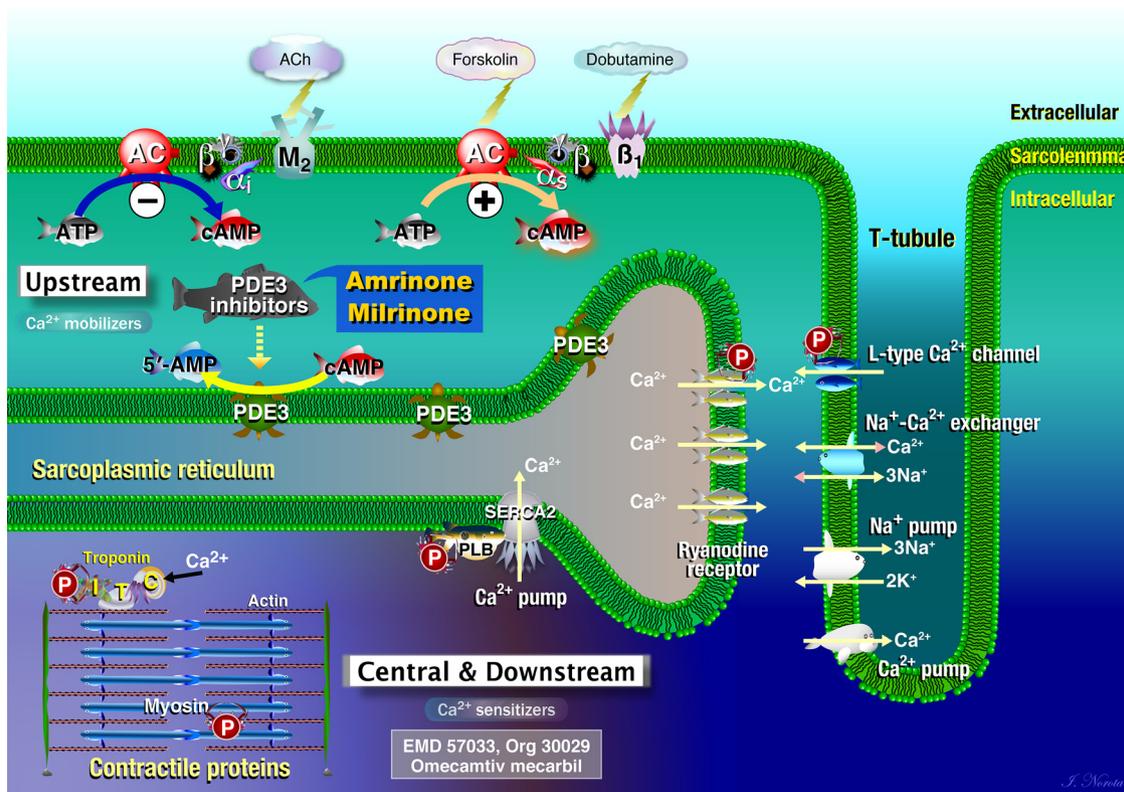
Figure. Tales of the Ocean: cardiac excitation–contraction coupling process focusing Ca^{2+} signaling and the site of action of inotropic agents. Although cardiotonic agents, including amrinone, milrinone, the β_1-receptor agonist dobutamine,[9] and forskolin, act via upstream mechanism, Ca^{2+} sensitizers, such as EMD 57033, Org 30029, and Omecamtiv mecarbil, act via central and downstream mechanism. M_2 receptor is coupled to activation of G_i protein to suppress the cAMP/protein kinase A (PKA) signaling. cAMP levels are finely tuned in the microdomain in the cardiomyocyte, phosphodiesterase 3 (PDE3) inhibitors increasing the cAMP level mainly in the vicinity of the sarcoplasmic reticulum membrane, whereas the β_1-receptor agonist elevating the level primarily near sarcolemma, but then all over the cardiomyocyte. Phosphorylation (P) of functional proteins involved in regulating Ca^{2+} signaling, such as L-type Ca^{2+} channels, ryanodine receptors, phospholamban (PLB), troponin I, and myosin binding protein C, catalyzed by PKA. Activation of muscarinic M_2 receptor by acetylcholine (ACh) (or carbachol) leads to lowering of cAMP partly through suppression of adenylyl cyclase (AC) via activation of Gi protein. SERCA2 indicates sarcoendoplasmic reticulum Ca^{2+}-ATPase.

Ca^{2+} transients induced by amrinone, milrinone, or forskolin is abolished by the muscarinic receptor agonist.[6] It became evident later that the small mammalian species, such as rat and mouse, are less sensitive to amrinone because in these species PDE4, rather than PDE3, plays a main role in the hydrolysis of cAMP in contrast to that in larger mammalian species, including human and dog.

Hence, the article of Alousi et al created both excitement and controversy after its publication, acting as the catalyst for further research on the role of PDEs and the drug development related to PDEs in the treatment of heart failure. The article has been cited ≈340 times, which is rather modest compared with many other big impact *Circulation Research* articles (eg, of dobutamine).[9] There has been a resurgence of interest in PDEs in recent years, driven by the ability to better characterize the molecular localization and the effector targets of cAMP signaling. cAMP is complexly and locally regulated in subcellular functional compartments containing marcomolecular complexes, including adenylyl cyclase, β-adrenergic receptors, PDEs, and other enzymes and proteins.[10–12] The recent publications, however, have largely ignored the article of Alousi et al. Therefore, highlighting this article in a commentary could conceivably draw attention back to this early pioneering work.

Paradigm Shift of Pharmacotherapy of Heart Failure

During these 3 decades, extensive clinical trials have been performed to elucidate the clinical effectiveness of novel cardiotonic agents to prolong the life of patients with chronic heart failure. After accumulation of tremendous amount of clinical trials, it proved that novel cardiotonic agents acting through PDE3 inhibition effectively improved the quality of life of patients with heart failure, but surprisingly and disappointedly abbreviated the lifespan of the patients. Severe ventricular arrhythmias and cardiac sudden death were the main cause of the patient death, the mechanism of which could be ascribed to the intracellular Ca^{2+} overload mediated by cAMP in myocardial cells and excessive expenditure of energy and oxygen because of facilitation of cardiac contractility, heart rate, and metabolism. Among clinical trials, the outcome of the Prospective Randomized Milrinone Survival Evaluation study was one of the most influential, in which it was shown that, despite its beneficial hemodynamic actions, long-term therapy with oral milrinone (40 mg daily) increased the morbidity and mortality of patients with severe chronic heart failure.[13]

The failure of novel cardiotonic agents to rescue the patients with chronic heart failure caused a prominent paradigm shift in the treatment of chronic heart failure. The inotropic therapy had played a central role with diuretics, and control of neurohumoral factors and high blood pressure being ancillary therapy. The cardiac protection, unloading of the pump function and suppression of cardiac remodeling, namely cardiac hypertrophy and fibrosis, became the first choice, and the inotropic therapy became the secondary choice in the treatment of chronic heart failure.

Ironically, amrinone thus triggered the paradigm shift and contributed to establish the modern pharmacological therapy of chronic heart failure. Nowadays, primarily used are the cardioprotective and cardiac unloading agents that have been shown to prolong the life span of patients, such as angiotensin-converting enzyme inhibitors, angiotensin II AT_1 receptor blockers, β-adrenergic receptor blockers, and aldosterone antagonists, being followed by diuretic and inotropic agents. In this paradigm shift, especially impressive and of interest is the effectiveness of β-adrenergic blockers that had been previously contraindicated to the patients because of a potential risk for exacerbation of concealed heart failure syndrome. β-Blockers proved to effectively improve the mortality and morbidity of chronic heart failure patients, although the outcome of clinical trials was quite controversial depending on the type of β-adrenergic blockers used, the dosage regiment, and races in the early days.[14]

The species-dependent differences of the positive inotropic effect of amrinone noted in the letter of Katz et al[2] and in the reply by Alousi and Falah[3] are currently being elucidated at the level of intracellular compartmentalization of cAMP and PDEs with novel molecular, biochemical, and imaging tools.[10–12]

Amrinone, milrinone, and some other novel PDE3 inhibitors are currently used to treat acute heart failure associated with acute cardiac contractile dysfunction that constitutes cardiovascular emergency to result in acute hemodynamic failure in the absence of immediate improvement of cardiac contractile function. Thus, with selective targeting of cAMP pools, the original dream of Alousi et al may have finally reached fruition with heart failure patients being treated with immediate elevation of cardiac contractility.

Potential Cardiotonic Agents Acting Through Novel Mechanism

The effort for the development of ideal cardiotonic agents, initiated by amrinone, has currently been further continuing. In this context, it is noteworthy that the cardiac Ca^{2+} signaling in contractile regulation can be classified into 3 processes: Ca^{2+} binding to troponin C (central mechanism), regulation of Ca^{2+} transients (upstream mechanism), and the process subsequent to Ca^{2+} binding to troponin C (downstream mechanism)[15,16] (Figure). Although several clinically available cardiotonic agents act via upstream mechanism, potential promising candidates in the future are Ca^{2+} sensitizers[15,16] that act through central and downstream mechanism. They have energetic advantages acting by an increase in myofilament Ca^{2+} sensitivity without expenditure of the activation energy and free from Ca^{2+} overload. Pimobendan and levosimendan were developed and are clinically available in some countries. These agents actually increase the myofilament Ca^{2+} sensitivity but are also associated with an increase in Ca^{2+} transients mediated by cAMP because of PDE3 inhibition. The positive inotropic effect of levosimendan is abolished, whereas the effect of pimobendan is partially inhibited by the muscarinic receptor agonist. Levosimendan may increase Ca^{2+} transients because of PDE3 inhibition and enhance the Ca^{2+} signal facilitated by cAMP at the level of myofilaments.[17] Acceleration of relaxation by accumulation of a small amount of cAMP induced by PDE3 inhibition may be favorable for the contractile regulation by these agents with respect to avoiding potential diastolic disturbance. Although several cardiotonic agents, such as theophylline, caffeine, sulmazole,[15] Org 30029,[7] levosimendan,[17] and

pimobendan, have both PDE inhibitory and Ca^{2+}-sensitizing actions with different extents, these actions are not always associated with each other and can be separated in other pure agents. PDE3 inhibitors, including amrinone, milrinone, and EMD 57439 [(−)-enantiomer of the thiadiazine derivative],[18] possess no Ca^{2+}-sensitizing action, whereas Ca^{2+} sensitizers, such as EMD 57033 [(+)-enantiomer of the thiadiazine derivative],[18] CGP 48506,[16] and α-adrenergic agonist,[8] have no PDE inhibitory action.

It is shown in middle-scale clinical trials that these agents are capable of improving the quality of life of patients with chronic heart failure, and do not seem to increase morbidity and mortality of the patients. However, the situation that the development of ideal cardiotonic agents is earnestly desired for the therapy of heart failure has currently not been altered since amrinone appeared as a novel cardiotonic agent. Ca^{2+} sensitizers act likewise through various mechanisms at contractile proteins.[15,16,18] Whereas Ca^{2+} sensitizers could theoretically induce diastolic dysfunction, the agents that act through Ca^{2+}-sensitizing mechanism, such as Org 30019,[7] EMD 57033,[18] and α-adrenergic receptor agonist,[8] prolong the duration of contraction with a decrease in the rate of early phase of relaxation, but rather accelerate the late phase of relaxation.[7,8,18] These observations indicate that diastolic dysfunction may not readily occur with these Ca^{2+} sensitizers, provided that the patients are not experiencing extensive tachycardia. This issue, however, requires careful investigations under various clinical situations in the future. A novel agent acting through unique mechanism, such as the selective activation of myofilament ATPase, is still under the phase of early clinical trial.[19] Further effort is necessary to achieve the goal to establishing clinical effectiveness.

Pharmacotherapy by means of cardiotonic agents requires the presence of intact myocardial cells. However, especially in heart failure developing subsequent to acute coronary syndrome, the lack of sufficient number of myocytes limits the effectiveness of pharmacotherapy. Therefore, the future non-pharmacological options to increase myocardial cells or to reverse the impaired Ca^{2+} regulatory apparatus by gene and stem cell–based approaches to improving cardiac pump function and increasing the effectiveness of cardiotonic agents are currently under extensive development.[20–22]

Disclosures

None.

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