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EDITORIAL

Basic and Clinical Characteristics of PDE 3 Inhibitors as Cardiotonic Agents

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In this issue of *Journal of Cardiovascular Drugs and Therapy* Oleg E. Osadchii presents a comprehensive review on the recent advances in PDE research, namely, research on the well-established family of PDE isozymes, which are encoded by different genes, and the characteristics of contractile regulation produced by cardiac PDE inhibition [1].

The family of phosphodiesterase (PDE) isozymes that catalyze the conversion of the intracellular second messenger cyclic adenosine 3′,5′-monophosphate (cAMP) and cyclic guanosine 3′,5′-monophosphate (cGMP) to inactive 5′-AMP and 5′-GMP, respectively, plays an important role in the regulation of cardiac function by physiological and pharmacological interventions. Inhibition of cardiac PDEs is capable of enhancing the chronotropic, inotropic and dromotropic effects of sympathetic nerve stimulation due to increased cAMP accumulation and subsequent activation of cAMP-dependent protein kinase (PKA), mediated by activation of β-adrenoceptors. In addition, in the absence of β-adrenoceptor stimulation, inhibition of cardiac PDEs is able to produce a positive inotropic effect (PIE), which indicates that cAMP is generated by constitutive adenylyl cyclase activity in intact myocardial cells. Activation of PKA results in the phosphorylation of regulatory proteins involved in cardiac Ca^{2+} signaling and contractility, including the sarcoplasmic reticulum (SR) SERCA2a regulator phospholamban, Ca^{2+} release channels (ryanodine receptors), L-type Ca^{2+} channels (DHP receptors), Na^{+}/Ca^{2+} exchanger, troponin I, myosin-binding protein C (MBP-C) and myosin light chain 2 (MLC2), as shown in Fig. 1, leading to a pronounced characteristic PIE in association with a prominent increase in Ca^{2+} transients, acceleration of relaxation and abbreviation of contraction.

Selective inhibitors of PDE 3, such as amrinone, milrinone and olprinone, have been launched for the pharmacotherapy of cardiac contractile dysfunction in acute congestive heart failure (CHF) and in the aggravating phase of chronic CHF [2].

The PIE of the novel cardiotonic agents, pimobendan and levosimendan, is elicited by the combination of an increase in intracellular Ca^{2+} mobilization (upstream mechanism) due to PDE 3 inhibition and a Ca^{2+}-sensitizing action (central and/or downstream mechanism) on myofilaments [3]. Pimobendan is clinically available for oral administration in Japan, and the effectiveness of i.v. levosimendan in treating acute heart CHF has been demonstrated in middle-scale clinical trials in European countries [2].

Species-dependent expression of cardiac PDE isozymes

In cardiac muscle of various mammalian species, four PDE isozymes with different biochemical characteristics, PDE 1, PDE 2, PDE 3 and PDE 4, are expressed. PDE 1 is activated by the Ca^{2+}-calmodulin complex, PDE 2 is stimulated by cGMP, while PDE 3 and PDE 4 are isozymes with low K_m with high V_{max} values for cAMP and the former is inhibitable with cGMP. It has been shown that PDE 3 and PDE 4 play an important role in the regulation of cardiac function, and some clinically available cardiotonic agents, such as amrinone and milrinone, elicit a PIE through the inhibition of PDE 3 in most mammalian cardiac muscle. It should be noted, however, that there is a wide range of species-dependent variations in the expression of PDE

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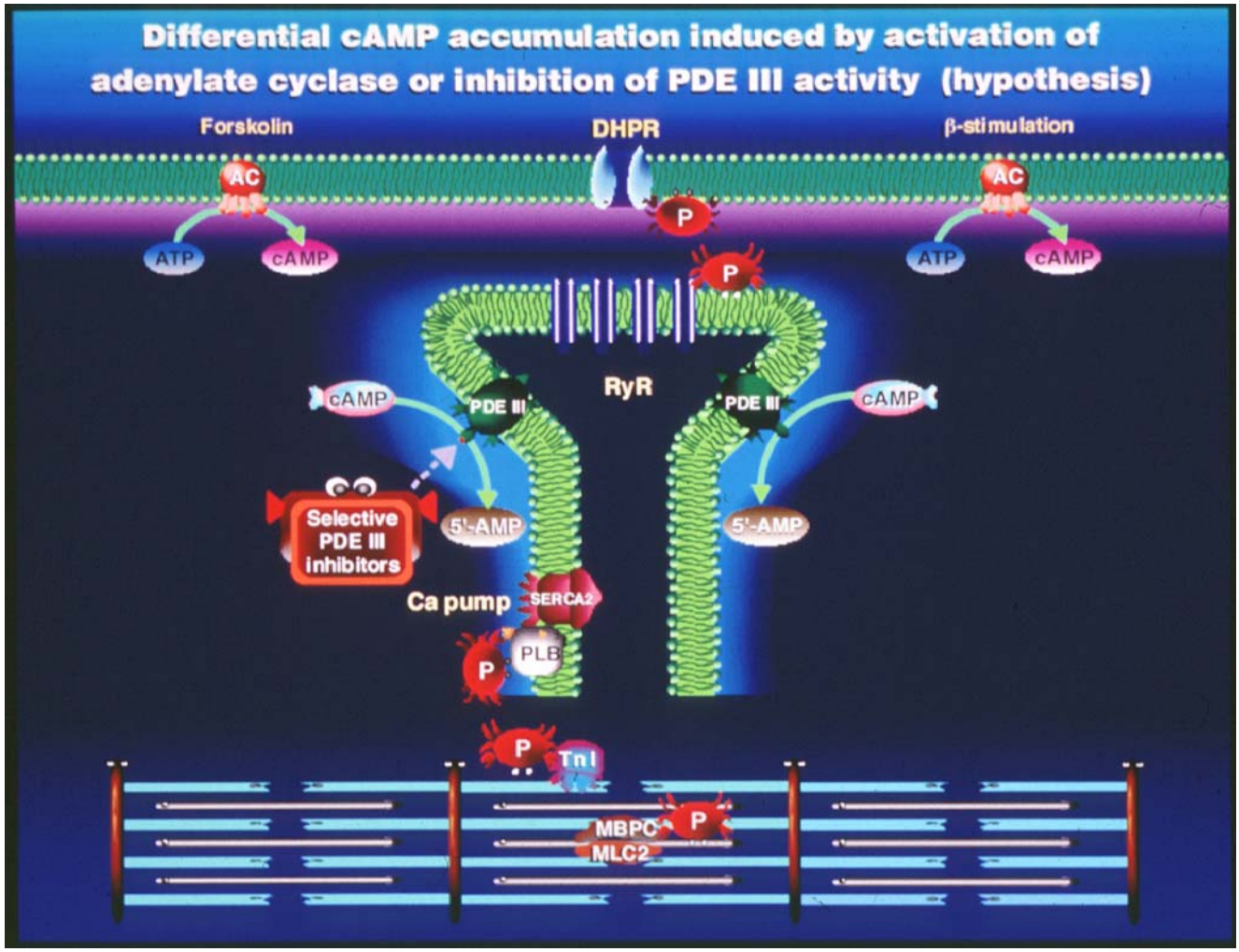

Fig. 1 Regulatory proteins phosphorylated by PKA activation, and the potential regional difference in cAMP accumulation induced by PDE 3 inhibition and β-adrenoceptor- (or forskolin-) induced adenylyl cyclase activation

isozymes among mammalian species. The inhibition of PDE 4 appears to play a key role in the hydrolysis of cAMP in rat ventricular myocytes, but appears to play different roles in larger mammalian species [4]. This species dependence of the expression of PDE isozymes has to be taken into consideration in the interpretation of experimental data obtained from smaller mammalian species, including mice, especially as mice are employed ordinarily as cardiovascular disease models due to the ease of gene manipulation in this species. Indeed, it has been shown that the inhibition of PDE 4 elicits more pronounced effects on cardiac contractility than PDE 3 inhibition in rodents [5].

Accumulation of cAMP and PIE induced by PDE inhibitors

Selective PDE 3 inhibitors induce accumulation of cAMP to a lesser extent than non-selective PDE inhibitors, such as theophylline and 3-isobutyl 1-methylxanthine (IBMX), and catecholamines, in intact cardiac muscle [6]. Indeed, amrinone had been postulated to elicit a PIE via an unknown mechanism, because it did not produce a detectable increase in cAMP levels when contractile force was increased in dog ventricular myocardium [7], while it was clearly shown that the PIE of amrinone is due to the accumulation of cAMP induced by selective PDE 3 inhibition [8]. The reason for the lesser extent of cAMP accumulation induced by PDE 3 inhibitors may be ascribed to an inhomogenous elevation of regional cAMP levels induced by different stimulatory interventions in intact myocardial cells. Cardiac PDE 3 is densely localized on the SR membrane, which may lead to cAMP accumulation mainly around the SR membrane to preferentially affect SR Ca^{2+} uptake and release mechanisms [9]. While it is supposed that chronotropic/inotropic selectivity may be different between catecholamines that act on adenylyl cyclase located in the sarcolemma and PDE 3 inhibitors acting on the SR membrane (Fig. 1), there is no systematic study that addresses this issue. There are some studies indicating that the pharmacological features of

amrinone and milrinone are not the same, the former acting preferentially on vascular dilating, while the cardiac selectivity being higher with the latter.

In a series of experiments to study the relationship between cAMP accumulation and the PIEs induced by various cardiotonic agents, it has been found that the PIE and the increase in Ca^{2+} transients mediated by cAMP are readily inhibited by muscarinic cholinergic receptor activation by carbachol or ACh in association with a lowering of cAMP levels elevated beforehand by PDE inhibition, or by β-adrenoceptor or histamine receptor stimulation, in canine ventricular myocardium [10]. In guinea-pig ventricular myocardium, ACh suppresses the PIE induced by β-adrenoceptor stimulation, but without lowering the cAMP level [11]. It has become evident that muscarinic receptor stimulation antagonizes the PIE mediated by cAMP via different signal transduction processes, including: 1) suppression of adenylyl cyclase via inhibitory G proteins (G_i proteins); 2) activation of phosphatases leading to dephosphorylation of phosphorylated regulatory proteins; 3) suppression of PKA via an unknown mechanism; and 4) potential stimulation of PDE 2 by cGMP accumulated following muscarinic receptor stimulation. No matter what subcellular mechanism is involved, muscarinic receptor stimulation is generally accepted and employed to differentiate the role of cAMP in induction of the PIE by novel cardiotonic agents.

Effectiveness of PDE inhibitors in the treatment of CHF

Since the early 1980s, when the selective PDE 3 inhibitor amrinone was first introduced as a novel cardiotonic agent for the treatment of CHF patients, a number of PDE 3 inhibitors, including milrinone, olprinone, enoximone, piroximone and vesnarinone, have been developed. Their clinical effectiveness has been examined to clarify whether these agents could replace digitalis and catecholamines in the pharmacotherapy of acute as well as chronic CHF patients. In the treatment of chronic CHF patients, it became evident that selective PDE 3 inhibitors are capable of improving the quality of life (QOL) of NYHA class II and III patients, but exacerbate the prognosis by elevating the mortality rate of these patients. Namely, oral milrinone was shown to increase the rate of lethal ventricular arrhythmias, even though it effectively improved the QOL of these patients [12]. In a middle-scale clinical trial, vesnarinone, which possesses an inhibitory action on PDE 3, in association with K^+ channels, ameliorated the mortality rate; however, subsequent large-scale clinical trials have shown that it shortens the survival of patients with chronic CHF [2]. Since then, the development of selective PDE 3 inhibitors for the treatment of chronic CHF patients has been completely abandoned, but these agents are used to improve contractile dysfunction in acute CHF and in the aggravating phase of chronic CHF, as injectable preparations, to overcome the hemodynamic emergency [2].

Characteristics of the PIE of levosimendan

Levosimendan has been shown to be effective in improving the hemodynamics and symptoms in CHF patients. Levosimendan can act through at least three signalling pathways to elicit beneficial effects on CHF patients: 1) it increases myofilament Ca^{2+} sensitivity by stabilizing the conformation of the Ca^{2+}-troponin C complex; 2) it inhibits PDE 3 with a high affinity; and 3) it activates K^+_{ATP} channels in vascular smooth muscle cells and mitochondrial membranes [13]. As a Ca^{2+} sensitizer, levosimendan has peculiar characteristics in that the PIE is associated with an increase in Ca^{2+} transients, and the PIE is susceptible to the inhibitory action of the muscarinic receptor agonist carbachol [14, 15]. Although the PIE of certain Ca^{2+} sensitizers, such as EMD 57033 and Org 30029, is not associated with an increase in Ca^{2+} transients [16], detailed examination of the $[Ca^{2+}]_i$-force relationship in the intact ventricular myocardium revealed that the PIE of levosimendan is associated with an increase in Ca^{2+} transients over a wide range of concentrations [14, 15]. Furthermore, the PIE of levosimendan is abolished in the presence of carbachol, which has been shown to selectively inhibit a cAMP-mediated pathway. Acidosis partially suppresses the PIE of levosimendan by decreasing the amplitude of Ca^{2+} transients, leaving its Ca^{2+} sensitizing action unaffected [15]. The increase in Ca^{2+} transients may be ascribed to a moderate accumulation of cAMP, and it is postulated that the PIE of levosimendan is elicited by a combination of a

Table 1 Increases in myofilament Ca^{2+} sensitivity and Ca^{2+} transients induced by novel agents. −: no increase; ±: slight or moderate increase; +: marked increase

Ca^{2+} Sensitizers		
Association with increases in Ca^{2+} transients		
	Ca^{2+} sensitizing action	Increases in Ca^{2+} transients
EMD57033	+	−
Org 30029	+	±
Levosimendan	+	±
SCH00013	+	±
Pimobendan	+	+
MCI-154	+	+

small increase in Ca^{2+} transients and its enhancement induced by troponin C-mediated Ca^{2+} sensitization. In patients with chronic CHF, it is likely that the cAMP-mediated pathway targeted by PDE 3 inhibition has been disrupted; therefore, the Ca^{2+} sensitizing action becomes dominant [17]. The long-term effectiveness of levosimendan in chronic CHF patients is under examination in large-scale clinical trials.

Inotropic therapy and development of novel cardiotonic agents

The mainstay of the current pharmacotherapy for chronic CHF is cardioprotective therapy, representing a shift from the inotropic therapy that was the first choice pharmacotherapy 30 years ago. Since the early 1980s, extensive efforts have been made to develop novel cardiotonic agents to replace digitalis and catecholamines, because these agents have serious disadvantages such as arrythmogenicity and unfavourable pharmacokinetic characteristics. During this time, it was believed that the improvement in QOL, namely short-term amelioration of hemodynamic parameters and exercise tolerance, would lead to favourable prognosis, including a decrease in the morbidity and mortality of patients. However, it has become evident that novel agents, such as amrinone and milrinone, exert unfavourable effects on the prognosis, even though these agents effectively improve the QOL of CHF patients. This realisation caused a large paradigm shift in pharmacotherapy from cardiotonic to cardioprotective therapy [18].

Nevertheless, cardiotonic agents with fewer adverse effects are inevitable for the treatment of cardiac contractile dysfunction in certain serious cardiogenic hemodynamic disorders. While selective PDE 3 inhibitors have limited indication in the long-term treatment of chronic CHF, they play an important role in the maintenance of cardiac pump function in cardiogenic emergency situations due to contractile dysfunction, such as acute CHF or the aggravating phase of chronic CHF. For the long-term treatment of chronic CHF patients, Ca^{2+} sensitizers that induce the PIE with no or low increase in Ca^{2+} transients by enhancing the Ca^{2+} binding signals at the level of contractile proteins, thin and thick filaments. These agents may have the advantages that: 1) they do not produce a Ca^{2+} overload leading to cardiac arrhythmias and myocardial cell injury; 2) they do not use activation energy required for intracellular/transcellular Ca^{2+} flux; and 3) their cardiotonic effects may be maintained even under pathophysiological conditions, such as acidosis, stunned myocardium and CHF, when Ca^{2+} mobilizers lose their effectiveness [16, 19]. Although there are currently no clinically-available Ca^{2+} sensitizers that act purely via a Ca^{2+} sensitizing mechanism, pimobendan (oral preparation) and levosimendan (i.v. injectable preparation), which are clinically available in Japan and European countries, act by a combination of PDE 3 inhibition and myofilament Ca^{2+} sensitization.

It is noteworthy that a number of drugs, including classical agents like caffeine and theophylline, and the novel agents presented in Table 1, share PDE inhibitory and Ca^{2+} sensitizing action [2]. These agents increase Ca^{2+} transients by accumulation of cAMP. Accumulation of cAMP to a small extent may be preferable to prevent a risk of diastolic dysfunction that could result from an increase in Ca^{2+} sensitivity to a diastolic level of $[Ca^{2+}]_i$. While there is evidence indicating that these agents elicit more favorable effects than pure Ca^{2+} mobilizers, such as sympathomimetic amines and PDE 3 inhibitors, it remains to be clarified which mechanisms contribute more significantly to their clinical effectiveness as therapeutic agents to improve cardiac contractile dysfunction in CHF patients [2]. The dream to develop novel cardiotonic agents that act via a unique mechanism of action to replace digitalis and catecholamines in the treatment of CHF originated 30 years ago, and has been achieved only partially by the successful development of PDE 3 inhibitors such as amrinone and milrinone for acute CHF. CHF remains a serious cardiovascular disorder with a high rate of mortality; the development of novel agents for the long-term treatment of chronic CHF, by future studies, is awaited.

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