

A Review on Study of Various Inotropic Calcium Sensitizing Drugs in Congestive Heart Failure Treatment

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Abstract Acute Heart failure (AHF), clinical signs of low cardiac output, therapy with positive inotropic drug for cardiac care is mandatory. Three classes of inotropic drugs are currently available, like β -adrenergic agonists (especially dobutamine), phosphodiesterase (PDE) inhibitors (such as milrinone) and Ca^{2+} sensitizers (levosimendan). The mechanisms of action of these drugs, and important issues regarding their clinical indications and potential risks associated with their use are studied. Oral inotropes did not always improve mortality of the patients with heart failure (HF) partly because of possible direct toxic effects of these agents on myocytes, exacerbating arrhythmias, enhancing neurohormonal activity. Ca^{2+} sensitizers such as pimobendan and levosimendan were improve even mortality of the patients with HF through increasing cardiac contractility (CC) without a rise in (Ca^{2+})_i. However, these agents disappoint our expectations. We need the development of the agents which has more specific effect of Ca^{2+} sensitizing.

Keywords: calcium sensitizers, inotropic, heart failure

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1. Introduction

The treatment of acute heart failure (AHF) is based on the clinical presentation. New therapeutic options, such as natriuretic peptides, Ca^{2+} sensitizers and others in development, provide benefits beyond the usual drugs and can be used in scenarios where traditional agents would not be considered [1]. Ca^{2+} sensitizers are cardiotoxic agents that directly increase the Ca^{2+} sensitivity of cardiac myofilament and their effects on the Ca^{2+} -dependent force generation in cardiac muscle fibers. The propyl gallate, a strong antioxidant, increased the Ca^{2+} -sensitivity of cardiac myofilament in a dose-dependent and reversible manner. The propyl gallate is a Ca^{2+} -sensitizer with antioxidant activity, which might be beneficial for the treatment of CHF associated with oxidative stress than Ca^{2+} -sensitizers [2]. After open-chest cardiac surgery, ventricular function remains depressed (myocardial stunning). Catecholamines (epinephrine) improve ventricular function by increasing the intracellular [Ca^{2+}]_i concentration. In parallel, the oxygen consumption is increased. In the very insufficient ventricle, epinephrine can even become ineffective. Since Ca^{2+} -sensitizers provide another therapeutic way; the effects of epinephrine and levosimendan on postischemic hemodynamics were investigated. In contrast to epinephrine, levosimendan improves ventricular function without increasing oxygen demand, thereby considerably improving external efficiency. Even during epinephrine resistance in extremely dysfunctional hearts,

levosimendan successfully improves ventricular function [3]. A number of positive inotropic agents with diverse mechanisms of action have been discovered. Most of these cardiotoxic drugs exhibit characteristic electrophysiologic profiles. This prompted us to propose a classification scheme based on electrophysiologic principles, modifying the categories suggested. Class I actions designate positive inotropic mechanisms that enhance the transmembrane Ca^{2+} current by various means, such as beta-receptor stimulation (dobutamine), PDE inhibition (milrinone), direct stimulation of adenylate cyclase (forskolin), or direct modulation of Ca^{2+} channel gating (BAY K 8644). Class II action includes mechanisms that lead to elevation of intracellular sodium activity either by inhibiting the Na,K pump (digitalis) or by increasing transmembrane Na^{+} influx (DPI 201-106). Class III action involves a mechanism by which sensitivity of the myofilaments to Ca^{2+} increases (EMD 53998, levosimendan). This mechanism is not associated with apparent electrophysiologic manifestations. Positive inotropism is due to lengthening of the cardiac repolarization (almokalant) [4]. A key role in treating heart failure (HF) is played by inotropes and calcium (Ca^{2+}) sensitizer agents. The Ca^{2+} sensitizer agents give clinical and hemodynamic benefits in HF patients, after successful treatment with diuretics or ultrafiltration. These agents significant increase in cardiac cycle efficiency (CCE), cardiac output (CO), stroke volume (SV), and dP/dt(max) and a significant decrease in diastolic and diastolic arterial pressures and in systemic vascular resistance (SVR) have been observed at end of treatment. After the addition of

Ca²⁺ sensitizer levosimendan, a further reduction in signs and symptoms of HF observed. Few patients showed no response on several hemodynamic parameters without any significant improvement. The most HF patients treated with diuretics or ultrafiltration receive additional clinical and hemodynamic benefits [5,6]. The Ca²⁺ dependent interaction between troponin I (cTnI) and troponin C (cTnC) triggers contraction in heart muscle. HF is characterized by a decrease in CO, and compounds that increase the sensitivity of cardiac muscle to Ca²⁺ have therapeutic potential. The Ca²⁺-sensitizer, levosimendan, targets cTnC; however, its mechanism has been obscured by its instability. The levosimendan class of Ca²⁺-sensitizer works by binding to the regulatory domain of cTnC [7,8]. Diaphragm muscle weakness occurs in patients with HF and is associated with exercise intolerance and increased mortality. Reduced sensitivity of diaphragm fibres to Ca²⁺ contributes to diaphragm weakness in HF. The Ca²⁺ sensitizer levosimendan to restore the reduced Ca²⁺ sensitivity of diaphragm fibres from rats with HF. Diaphragm fibres were skinned and activated with solutions containing incremental Ca²⁺ concentrations and 10 µM levosimendan. Developed force was measured at each Ca²⁺ concentration, and force-Ca²⁺ concentration relationships were plotted. Ca²⁺ sensitivity of force generation was reduced in diaphragm muscle fibres from HF rats, compared with fibres from control rats. Maximal force generation was ~25% lower in HF diaphragm fibres than in control fibres. Levosimendan significantly increased Ca²⁺ sensitivity of force generation in diaphragm fibres from HF and control rats, without affecting maximal force generation. Levosimendan enhanced the force generating capacity of diaphragm fibres from HF rats by increasing the sensitivity of force generation to Ca²⁺ concentration. The result support effect of Ca²⁺ sensitizers on diaphragm muscle weakness in patients with HF [9]. The AHF is a major cause of hospitalizations. Severe dyspnea, pulmonary congestion and low CO with peripheral vasoconstriction and renal hypoperfusion is a main form of clinical presentation. Most patients with acute worsening have a pre-existing chronic HF, but AHF may also occur as a first manifestation of a previously unknown heart disease. Myocardial ischemia (MI), cardiac arrhythmias (CA), non-compliance with medication and infections are frequent precipitating factors. Management of AHF depends on the underlying HD and cause of decompensation. In patients with CHF vasodilators and iv diuretics are first-line drugs for rapid reduction of dyspnea and congestion. In patients with signs of low CO and oliguria, inotropic agents are also often administered to prevent further deterioration. Beta-adrenergic agents and PDE inhibitors correct the hemodynamic disturbance, but may also induce arrhythmias and worsen MI. Inotropic therapy therefore remains controversial. The Ca²⁺-sensitizers, represent a new therapeutic option. Levosimendan was shown to improve myocardial contractility without increasing oxygen requirements and to produce peripheral and coronary vasodilation. Study focused on pharmacology and therapeutic utility of levosimendan in patients with CHF [10].

Ca²⁺ sensitizers belong to a new class of cardiac enhancers that stimulate CC without causing intracellular Ca²⁺ overload or increasing myocardial oxygen demand.

Levosimendan, the most well studied Ca²⁺ sensitizer in the real clinical practice, produces greater hemodynamic and symptomatic improvement in patients with acute HF than traditional inotropes. Biological mechanisms are explaining the pleiotropic effects of levosimendan on the failing heart. Levosimendan has a unique dual mechanism of action by enhancing CC and causing peripheral vasodilatation. Immunomodulatory and antiapoptotic properties of levosimendan may be an additional biologic mechanism that prevents further cytotoxic and hemodynamic consequences of abnormal immune and neurohormonal responses in AHF, leads to cardioprotection, and beneficially intervenes in the progression of syndrome. Levosimendan exerts its cardioprotective effects through its antioxidant properties. Levosimendan does not increase markers of oxidative and nitrosative stress, in contrast to placebo treatment, in advanced CHF patients. Levosimendan has also been shown to activate mitoK(ATP) channels which are important mediators of ischemic preconditioning. Pharmacological modulation of K(ATP) channels may prove beneficial in patients at risk of MI, particularly those requiring inotropic support. Pleiotropic effects of levosimendan appear to have important clinical and prognostic implications in AHFS and ischemic heart disease (IHD) [11]. The role of Ca²⁺ in cardiac excitation-contraction (E-C) coupling has been established by simultaneous measurements of contractility and Ca²⁺ transients by means of aequorin in intact myocardium and Ca²⁺ sensitive fluorescent dyes in single myocytes. The E-C coupling process can be classified into 3 processes: upstream (Ca²⁺ mobilization), central (Ca²⁺ binding to TnC) and downstream mechanism. These mechanisms are regulated differentially by various inotropic interventions. Positive force-frequency relationship and effects of β-adrenoceptor stimulation, PDE-III inhibitors and digitalis are essentially exerted via upstream mechanism. Alpha-adrenoceptor stimulation, endothelin-1, angiotensin II, and clinically available Ca²⁺ sensitizers, such as levosimendan and pimobendan, act by a combination of the upstream and central/downstream mechanism. The effects of Ca²⁺ sensitizers such as EMD 57033 and Org 30029 are primarily induced via the central/downstream mechanism. Whereas the upstream and central mechanisms are markedly suppressed in failing myocytes and under acidotic conditions, Ca²⁺ sensitizers such as EMD 57033 and Org 30029 can induce cardiostimulatory effects under such conditions. Ca²⁺ sensitizers have high therapeutic potential for the treatment of contractile dysfunction in CHF and IHD, because they have energetic advantages and less risk of Ca²⁺ overload and can maintain effectiveness under pathological conditions [12]. Attempts to ameliorate cardiac contractile dysfunction by Ca²⁺ mobilizers, such as catecholamines, PDE-inhibitors and digitalis, play an important role in pharmacotherapy for CHF, but these agents possess disadvantages in causing Ca²⁺ overload resulting in arrhythmogenicity and damage to cardiomyocytes. Ca²⁺ sensitizers that act directly on contractile proteins are free from the risk of Ca²⁺ overload and they could improve haemodynamic parameters with minimum increase in energy expenditure even under pathological conditions, including acidosis and stunned myocardium. Potential of oral levosimendan in long-term treatment of chronic CHF in clinical settings is much more complex, the therapeutic relevance of Ca²⁺ sensitizers in

the treatment of chronic CHF [13]. Classic inotropic agents provide short-term haemodynamic improvement in patients with HF, but their use has been associated with poor prognosis. Inotropic agents, the Ca^{2+} -sensitizers, may provide an alternative longer lasting solution. Levosimendan is a relatively new Ca^{2+} sensitizer which offers haemodynamic and symptomatic improvement by combining a positive inotropic action via Ca^{2+} -sensitization and a vasodilatory effect via ATP-sensitive K^+ ($\text{K}(\text{ATP})$), Ca^{2+} -activated K^+ ($\text{K}(\text{Ca}^{2+})$) and voltage-dependent K^+ ($\text{K}(\text{V})$) channels activation. Levosimendan also seems to induce a prolonged haemodynamic improvement in patients with HF as a result of the long half-life of its active metabolite, OR-1896. Furthermore, levosimendan may have additional antiinflammatory and antiapoptotic properties, affecting important pathways in the pathophysiology of HF. Despite the initial reports for a clear benefit of levosimendan on short- and long-term mortality in patients with severe HF, the results from the recent clinical trials are rather disappointing, and it is still unclear whether it is superior to dobutamine in affecting survival of patients with severe HF. In conclusion, levosimendan is a promising agent for the treatment of decompensated HF. As further to its positive inotropic effect, it affects multiple pathways with key roles in the pathophysiology of HF. The effect of levosimendan on mortality in patients with HF will hopefully resolve the controversy as to whether levosimendan is superior to classic inotropic agents for the treatment of severe HF [14]. Ca^{2+} sensitizers also improve cardiac function by increasing the contraction of the myocardium without significantly increasing $(\text{Ca}^{2+})_i$ levels. Levosimendan also affects myocardial especially systolic waves of right ventricle and those of left ventricle [15]. PDE inhibitors as inodilators in HF are associated with promotion of arrhythmias. Ca^{2+} sensitizers have been proposed for the treatment of severe decompensated HF. The effect of levosimendan, a Ca^{2+} sensitizer, and milrinone, a PDE inhibitor, on ventricular arrhythmias was compared in a model of acute regional MI and reperfusion. Levosimendan, but not milrinone, significantly attenuated the pronounced increase in the number of ventricular premature beats (-63%), tachycardia (-50%), fibrillation (-70%), and inhomogeneity of ventricular electrical activation. Levosimendan significantly improved the overall survival rate. Levosimendan has a more beneficial profile than milrinone regarding the development of ventricular arrhythmias during and after regional MI [16]. The regulatory process of cardiac excitation-contraction coupling is classified into three categories; upstream (Ca^{2+} mobilization), central (Ca^{2+} binding to troponin C), and/or downstream (thin filament regulation of troponin C property or crossbridge cycling and crossbridge cycling activity itself) mechanisms. While a marked increase in contractile activity by the Frank-Starling mechanism is associated with only a small alteration in Ca^{2+} transients (downstream mechanism), the force-frequency relationship is primarily due to a frequency-dependent increase of Ca^{2+} transients (upstream mechanism) in mammalian ventricular myocardium. The characteristics of regulation induced by beta- and alpha-adrenoceptor stimulation are very different between the two mechanisms: the former is associated with a pronounced facilitation of an upstream mechanism, whereas the latter is primarily due to modulation of central

and/or downstream mechanisms. The α -Adrenoceptor-mediated contractile regulation is mimicked by endothelin ET(A)- and angiotensin II AT(1)-receptor stimulation. Acidosis markedly suppresses the regulation induced by Ca^{2+} mobilizers, but certain Ca^{2+} sensitizers are able to induce the positive inotropic effect with central and/or downstream mechanisms even under pathophysiological conditions [17].

2. Calcium Sensitizing Agents

The CHF is a long standing health issue. Traditionally, HF has been treated with a wide array of drugs such as diuretics, digitalis, catecholamine and non catecholamine inotropics, although treatment with these drugs bears adverse effects, such as the generation of arrhythmia and even death. A new class of drugs has recently exerted a positive impact on the treatment of patients with HF; these are the Ca^{2+} sensitizers that enhance myocardial contractility without increasing cytosolic Ca^{2+} . Levosimendan is a Ca^{2+} sensitizer that, besides increasing contractility, has a vasodilating effect due to the activation of $\text{K}(\text{ATP})$ channels, being both mechanisms responsible for an advantageous therapeutic option. Different studies have proven the efficiency and safety profile of the drug on various scenarios and populations; thereby considering levosimendan a real and safe alternative treatment for patients with acute or chronic ventricular failure that need intravenous pharmacological support [18]. The clinician's primary objective in treating a patient with decompensated HF is rapid and effective stabilization. This goal often is achieved through the use of inotropic support. Classic inotropic agents (β -adrenergic agonists and PDE-III inhibitors) can provide short-term hemodynamic benefits, but their long-term use has been correlated with poor survival rates. Ca^{2+} sensitizers comprise a new drug class that offers hemodynamic and symptomatic improvements without increasing cAMP and $(\text{Ca}^{2+})_i$ concentrations. These agents enhance contractility without a concurrent increase in the risk of cardiac events and thus represent a significant improvement over classic positive inotropic agents. Levosimendan is the most potent Ca^{2+} sensitizer to date, exhibiting a unique dual mechanism of action that combines a positive inotropic action mediated via Ca^{2+} sensitization and a vasodilator property via ATP-dependent K^+ channels. Data suggest that Ca^{2+} sensitizer agents represent a promising class of inotropic agents in a field that has seen few advances in recent decades [19]. Patients suffering from acute decompensated HF, immediately after infarction, or late in the progression of HF, need short-term, positive inotropic support in their therapy. The drugs acting through cAMP are used to increase the contractile force of the heart of such patients, although it is well-known that these kind of drugs may trigger arrhythmias and as a result may worsen the long-term prognosis of the patients. Levosimendan acting through Ca^{2+} sensitization of contractile proteins, has shed new light on inotropic therapy, and, importantly, has reduced mortality in AHF patients. The compounds which have been selected for detailed consideration are limited only to positive inotropic compounds that produce Ca^{2+} sensitization of contractile proteins. The difference between various Ca^{2+} sensitizing mechanisms mainly

focuses on the relaxation of cardiac muscle [20]. Levosimendan increases CC without increasing MVO_2 . This new molecule has no proarrhythmic effects and has anti-ischemic properties. Levosimendan hemodynamic effects, similar or superior to those of catecholamines, persist during one week. In a selected group of advanced HF patients levosimendan was associated with a mortality reduction in comparison with dobutamine. In spite of its cost, this inotropic agent appears very promising and it is expected that it will be widely used [21]. Cardiogenic shock is a condition associated with high mortality. The evidence base for choice of treatment is insufficient, but new therapeutic options and new understanding have led to some improvement in the prognosis. A class of HF medication is Ca^{2+} sensitizers. New options in the treatment of cardiogenic shock complicating acute myocardial infarction. Beta-adrenergic stimulation of the heart should, if possible, be avoided, because of increased myocardial oxygen requirement, Ca^{2+} overload of the cardiomyocytes, and increased mortality. Drug therapy using Ca^{2+} sensitizers is promising, but more controlled clinical trials are needed [22]. Along with the new methods moderating hyperactivated regulation mechanisms (e.g. renin or vasopeptidases inhibitors) promising is the field of the new inotropics active without increasing the supply of Ca^{2+} (Ca^{2+} sensitizers, the stimulators of sarcoplasmic Ca^{2+} ATPase). In the field of diuretics there may be expected the introduction of adiuretic blockers (akvaretics). The promising field is represented by the drugs intervening the metabolism of non-cellular matrix which are expected primarily to have a positive influence on the ventricle remodelling [23].

Ca^{2+} sensitizers are a new class of inotropes that share the in vitro properties of Ca^{2+} sensitization and PDE inhibition. Levosimendan is a distinct Ca^{2+} sensitizer, as it stabilizes the interaction between Ca^{2+} and troponin C by binding to troponin C in a Ca^{2+} -dependent manner, improving inotropy without adversely affecting lusitropy. It does not exhibit clinically relevant PDE inhibition at therapeutic concentrations. It also exerts vasodilatory effects, possibly through activation of several potassium channels and other less well characterized mechanisms. The pharmacokinetics of levosimendan are similar in healthy subjects and patients with HF and remain relatively unaltered by age, sex, and organ dysfunction. The levosimendan exerted potent dose-dependent positive inotropic and vasodilatory activity. Unlike conventional inotropes, levosimendan is not associated with significant increases in MVO_2 , proarrhythmia, or neurohormonal activation. The most common adverse effects are attributable to the vasodilation. The trials demonstrated favorable hemodynamic effects, improved tolerability, and a possible mortality benefit over dobutamine and placebo in patients who had acute symptoms of failure and required inotropic therapy. The long-term effect on patient outcomes is being confirmed in ongoing placebo- and inotrope-controlled trials. Levosimendan appears to be an effective inodilator devoid of the detrimental effects of conventional inotropes. Levosimendan may provide a promising alternative to conventional inotropes for patients with acutely decompensated HF [24]. The effects of the Ca^{2+} sensitizer levosimendan and that of its stereoisomer dextrosimendan on the cardiac contractile apparatus were studied using skinned fibers obtained from

guinea pig hearts. Levosimendan was found to be more effective than dextrosimendan in this model. The respective concentrations of levosimendan and dextrosimendan at EC_{50} were 0.3 and 3 μM . In order to explain the difference in efficacy as Ca^{2+} sensitizers, the binding of the two stereoisomers on cTn C was studied by nuclear magnetic resonance in the absence and presence of two peptides of cTnI. The two stereoisomers interacted with both domains of cTnC in the absence of cTnI. In the presence of cTnI-(32-79) and cTn I-(128-180), the binding of both levosimendan and dextrosimendan to the C-terminal domain of cTnC was blocked and only the binding to the N-terminal domain was observable. Differences in the overall binding behavior of the two isomers to cTnC were highlighted in order to discuss their structure to activity relation. The action of levosimendan as a Ca^{2+} sensitizer and positive inotrope relates to its stereoselective binding to Ca^{2+} -saturated cTnC [25].

Ca^{2+} affinity of cardiac troponin C (TnC) is regulated by the active cross-bridges (downstream-dependent mechanism). In the present study, we showed one of the methods to evaluate the downstream-dependent change in the Ca^{2+} affinity of TnC during contraction using the aequorin-injected ferret papillary muscle. For this purpose, the tension-dependent change in the extra- Ca^{2+} (a transient increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to a quick length reduction) was measured under various conditions. The regression line between the magnitude of tension reduction and the magnitude of the normalized extra- Ca^{2+} (the extra- Ca^{2+} was divided by $[Ca^{2+}]_i$ immediately before length change) (the normalized extra- Ca^{2+} -tension relation) in twitch contraction can be used for the estimation of the downstream-dependent change in the Ca^{2+} affinity of TnC. The normalized extra- Ca^{2+} -tension relation became shallow by EMD 57033 (EMD) (Ca^{2+} sensitizers) and by an increase in Ca^{2+} concentration in the solution ($[Ca^{2+}]_o$) in a concentration-dependent manner. However, 2,3-butanedione monoxime (BDM) (one of the desensitizers) antagonized the effects of EMD and higher $[Ca^{2+}]_o$ in a concentration-dependent manner. These effects of EMD and BDM were also observed in the normalized extra- Ca^{2+} -tension relation in tetanic contraction. The normalized extra- Ca^{2+} -tension relation became steep by shortening the initial muscle length before contraction in tetanic contraction. Length-tension relation in twitch contraction was significantly shifted upward by higher $[Ca^{2+}]_o$ and EMD, but BDM showed the opposite effects on them in a concentration-dependent manner. Thus, the downstream-dependent change in the Ca^{2+} affinity of TnC which physiologically functions in intact cardiac muscle can be evaluated using the normalized extra- Ca^{2+} -tension relation [26].

During HF, alterations occur in contractile protein expression and phosphorylation, which may influence the effects of Ca^{2+} -sensitizers. Two different Ca^{2+} -sensitizers were used: EMD 53998 (10 μM), which exerts its influence through the actin-myosin interaction, and OR-1896 (10 μM) (the active metabolite of levosimendan), which affects the Ca^{2+} -sensory function of the thin filaments. All of these effects were comparable in the donor and failing myocytes, but, in contrast with OR-1896, EMD 53998 considerably diminished the difference in the Ca^{2+} -sensitivities between the failing and non-failing myocytes. The action of Ca^{2+} -sensitizers under mimicked

ischemic conditions was impaired to a similar degree in the donor and the failing myocytes. Results indicate that the Ca^{2+} -activation of the myofibrillar system is altered in end-stage human HF. This modulates the effects of Ca^{2+} -sensitizers both under control and under mimicked ischemic conditions [27]. Ca^{2+} sensitizers exert positive inotropic effects without increasing intracellular Ca^{2+} . Thus, they avoid the undesired effects of Ca^{2+} overload such as arrhythmias and cell injury, but most of them may impair myocyte relaxation. However, MCI-154, also a Ca^{2+} sensitizer, has no impairment to cardiomyocyte relaxation. To clarify the underlying mechanisms, effects of MCI-154 on Ca^{2+} transient and cell contraction using ion imaging system, and its influence on L-type Ca^{2+} current and $\text{Na}^+/\text{Ca}^{2+}$ exchange current with patch clamp technique in rat ventricular myocytes as well. The results showed that MCI-154 had no effect on L-type Ca^{2+} current; MCI-154 concentration-dependently increased cell shortening, with a slight increase in Ca^{2+} transient amplitude and an abbreviation of Ca^{2+} transient restore kinetics; MCI-154 dose-dependently increased the electrogenic $\text{Na}^+/\text{Ca}^{2+}$ exchange current both in the inward and the outward directions in rat ventricular myocytes. These results indicate that MCI-154 exerted a positive inotropic action without impairing myocyte relaxation. The stimulation of inward $\text{Na}^+/\text{Ca}^{2+}$ exchange current may accelerate the Ca^{2+} efflux. The findings suggest that the improvement by MCI-154 of myocyte relaxation is attributed to the forward mode of $\text{Na}^+/\text{Ca}^{2+}$ exchange [28]. HF is characterized by sodium and fluid retention, sympathetic overactivation, parasympathetic withdrawal, vasoconstrictor activation and cytokine elevation. New therapies for HF attempt to control neurohormonal activation and limit progressive left ventricular dysfunction. Nesiritide (human B-type natriuretic peptide) is a new vasodilator that belongs to a new class of HF drugs known as natriuretic peptides. Nesiritide decreases pulmonary capillary wedge pressure, SVR, mean right atrial pressure and pulmonary artery pressure, while improving cardiac index, SV and HF symptoms. Many endothelin receptor antagonists are in various stages of development. Early clinical studies have demonstrated beneficial cardiovascular hemodynamic effects. Other new drugs for HF also include Ca^{2+} sensitizers, neutral endopeptidase and vasopeptidase inhibitors, aldosterone receptor antagonists, vasopressin antagonists and cytokine inhibitors. All are being actively investigated and many show significant promise as beneficial therapies in the treatment of HF [29].

The novel Ca^{2+} sensitizer levosimendan improves myocardial contractility without causing an increase in $(\text{Ca}^{2+})_i$ and cyclic AMP concentrations. It also has a vasodilator action due to an opening of the ATP-sensitive K^+ channels. In a double-blind clinical trial levosimendan was compared with dobutamine in 203 patients with severe low-output CHF. The pre-defined hemodynamic improvement was achieved in 28% of patients receiving levosimendan compared to only 15% with dobutamine. Levosimendan also reduced the 1- and 6-month mortality more than dobutamine. Levosimendan produced less MI and CA than dobutamine. Ca^{2+} sensitizers offer a therapeutic possibility in patients with decompensated low-output HF [30]. The finely-tuned increases and decreases in the $(\text{Ca}^{2+})_i$ levels in myocytes ultimately

regulate the contraction and relaxation of the heart. Therapeutic agents can improve or interfere with this delicate balance. Ca^{2+} sensitizers enhance CC by improving the use of Ca^{2+} that is available, rather than by inundating the cell with excessive Ca^{2+} , as is the case with traditional inotropes. With the sensitizing mechanism, the energy cost of contraction is maintained at a near-normal level, and the threat of arrhythmias and sudden death is low. Levosimendan is the first Ca^{2+} sensitizer to become available for the treatment of patients with AHF. In recent clinical studies, levosimendan increased CO and SV without significantly increasing oxygen demand. By its additional action as a vasodilator (via K^+ channel opening), levosimendan also corrects the hemodynamic decompensation, thus lowering the pulmonary capillary wedge pressure and SVR. Furthermore, levosimendan increases the coronary circulation thus leading to an improved function of the stunned myocardium and lessened ischemia. Taken together, levosimendan's primary Ca^{2+} -sensitizing action, along with its complementary vasodilator properties, make this new drug a highly promising agent for the treatment of patients with acute HF [31]. For increasing myocardial contractility in patients with HF, catecholamines, PDE-III inhibitors, and Ca^{2+} sensitizers are available. Improving myocardial performance with catecholamines and PDE inhibitors leads to increased $(\text{Ca}^{2+})_i$ concentration as an unavoidable side effect. An increase in intracellular Ca^{2+} can induce harmful arrhythmias and increases the energetic demands of the myocardium. Long-term trials with PDE inhibitors have raised concerns about the safety of positive inotropic treatment for HF. $(\text{Ca}^{2+})_i$ sensitizers are a new class of inotropic drugs. They improve myocardial performance by directly acting on contractile proteins without increasing $(\text{Ca}^{2+})_i$ load. Thus, they avoid the undesired effects of an increased intracellular Ca^{2+} load. Ca^{2+} sensitizers may enhance myocardial performance without increasing MVO_2 and without provoking fatal arrhythmias. Two Ca^{2+} sensitizers are available for the treatment of HF in men. Pimobendan is a drug with positive inotropic effects that additionally inhibits the production of proinflammatory cytokines. However, it exerts a significant inhibition of PDE at clinically relevant doses. Levosimendan is a Ca^{2+} sensitizer with no major inhibition of PDE at clinically relevant doses. It opens ATP-dependent K^+ channels and thus has vasodilating and cardioprotective effects. The most important studies of the long-term treatment of stable HF with pimobendan and on the short-term treatment of unstable HF with levosimendan are presented [32]. Regulation of CC by cardiotonic agents is achieved by an increase in $(\text{Ca}^{2+})_i$ mobilization (upstream mechanism), an increase in Ca^{2+} binding affinity to troponin C (central mechanism), or facilitation of the process subsequent to Ca^{2+} binding to troponin C (downstream mechanism). cAMP mediates the regulation induced by Ca^{2+} mobilizers such as beta-adrenoceptor agonists and selective PDE-III inhibitors acting through the upstream mechanism. These agents act likewise on the central mechanism to decrease Ca^{2+} sensitivity of troponin C in association with the cAMP-mediated phosphorylation of TnI. In addition to such a well-known action of cAMP, experimental findings have revealed that Ca^{2+} sensitizers, such as levosimendan, OR-1896, and UD-CG 212 Cl, require the cAMP-mediated signaling for induction of

Ca²⁺ sensitizing effect. These agents shift the [Ca²⁺]-force relationship to the left, but their positive inotropic effect (PIE) is inhibited by carbachol, which suppresses selectively the cAMP-mediated PIE. These findings imply that cAMP may play a crucial role in increasing the myofilament Ca²⁺ sensitivity by cross-talk with the action of individual cardiotoxic agents. No clinically available cardiotoxic agents act primarily via Ca²⁺ sensitization, but the PIE of pimobendan and levosimendan is partly mediated by an increase in myofilament Ca²⁺ sensitivity. Evidence is accumulating that cardiotoxic agents with Ca²⁺ sensitizing action are more effective than agents that act purely via the upstream mechanism in clinical settings. Further clinical trials are required to establish the effectiveness of Ca²⁺ sensitizers in long-term therapy for CHF patients [33].

HF is characterized by sodium and fluid retention, sympathetic overactivity, parasympathetic withdrawal, vasoconstrictor activation and cytokine elevation. New therapies for HF attempt to control neurohormonal activation and limit progressive left ventricular dysfunction. Nesiritide (human B-type natriuretic peptide) is a new vasodilator; it belongs to a new class of HF drugs known as natriuretic peptides. Nesiritide decreases pulmonary capillary wedge pressure, SVR, mean right atrial pressure and pulmonary artery pressure, while improving cardiac index, SV and HF symptoms. Many endothelin receptor antagonists are in various stages of development. Early clinical studies have demonstrated beneficial CV hemodynamic effects. Other new drugs for HF also include Ca²⁺ sensitizers, neutral endopeptidase and vasopeptidase inhibitors, aldosterone receptor antagonists, vasopressin antagonists and cytokine inhibitors. All are being actively investigated and many show significant promise as beneficial therapies in the treatment of HF [34]. Depression of myocardial contractility plays an important role in the development of HF and many inotropic agents were developed to improve the contractile function of the failing heart. Agents that increase cAMP, either by increasing its synthesis or reducing its degradation, exerted dramatic short-term hemodynamic benefits, but these acute effects were not extrapolated into long-term improvement of the clinical outcome of HF patients. Administration of these agents to an energy starved failing heart would be expected to increase myocardial energy use and could accelerate disease progression. The role of digitalis in the management of HF has been controversial, however, the recent large scale clinical trial has ironically proved that digoxin reduced the rate of hospitalization both overall and for worsening HF. More recently, attention was paid to other inotropic agents that have a complex and diversified mechanism. These agents have some PDE-inhibitory action but also possess additional effects, including cytokine inhibitors, immunomodulators, or Ca²⁺ sensitizers [35]. Levosimendan is one of the first agents of a new class of drugs known as Ca²⁺ sensitizers. These drugs are believed to increase CC by sensitizing cardiac myofibrils to Ca²⁺, and may therefore be of clinical benefit in the treatment of low-CO states, particularly CHF. In addition to sensitizing troponin to (Ca²⁺)_i, levosimendan has been shown to inhibit PDE-III, which may contribute to its positive inotropic effect, and open ATP-sensitive K⁺ channels (K(ATP)), which may produce vasodilation.

Unlike currently available intravenous inotropes, levosimendan does not increase myocardial oxygen utilization, has not been shown to be proarrhythmic, and has been used effectively in the presence of beta-blocking medications. Levosimendan also has not been shown to impair ventricular relaxation, which was an initial concern with this class of drugs. Clinical studies of levosimendan have demonstrated short-term hemodynamic benefits of levosimendan over both placebo and dobutamine. While large-scale, long-term morbidity and mortality data are scarce, the Levosimendan Infusion versus Dobutamine in severe low-output HF study suggested a mortality benefit of levosimendan over dobutamine up to 180 days after treatment. Clinical studies comparing levosimendan with other positive inotropes, namely milrinone, are lacking. Levosimendan treatment appears to be well-tolerated, with the primary adverse events being headache and hypotension. No clinically significant drug-drug interactions have been reported with levosimendan to date. The clinical future of levosimendan will depend on the results of larger, ongoing clinical trials [36].

The Ca²⁺ sensitizers like EMD 57033 (EMD) and CGP 48506 (CGP) may be advantageous for the treatment of human HF, as they increase force of contraction without increasing the (Ca²⁺)_i transients or energy consumption. However, whether or not Ca²⁺ sensitizers differ in their mode of action in human myocardium is not fully understood. The present study investigates the influence of EMD and CGP on force of contraction (FOC) and the (Ca²⁺)_i transient in left ventricular papillary muscle strips from left ventricular failing human myocardium as well as in right atrial trabeculae (RA) obtained from patients undergoing cardiac bypass surgery. Similar results were obtained in RA. Application of carbachol (100 μmol/l) had no effect on the positive inotropic effect of EMD or CGP. Both Ca²⁺ sensitizers significantly increased time to half peak relaxation as well as diastolic tension in DCM. EMD (10 μmol/l) and CGP (30 μmol/l) did not affect the Ca²⁺ transients in RA. The Ca²⁺ sensitizers EMD and CGP increase cAMP and Ca²⁺ independently from the FOC in the human myocardium. However, their therapeutic use in human HF may be limited as they impair relaxation [37]. Currently available therapies, which are based on three basic mechanisms of action (diuresis, exogenous vasodilators, and cAMP-dependent positive inotropes), have significant limitations that have encouraged the development of newer agents. The leading medications for this indication are representatives of three different therapeutic approaches, which include endogenous vasodilatory neurohormones (nesiritide), Ca²⁺ sensitizers (levosimendan), and neurohormonal antagonists (tezosentan). These three agents represent a new generation of therapeutics for this important medical problem and may provide the means not only to treat symptoms, but also to improve longer-term clinical outcomes [38]. Ca²⁺ sensitizers act on the central mechanism (Ca²⁺ binding affinity of TnC) and/or downstream mechanisms (thin filament regulation of actin and direct action on crossbridge cycling) of cardiac E-C coupling. Ca²⁺ sensitizers have mechanistic and energetic advantages over the agents that act through the upstream mechanism (Ca²⁺)_i mobilization). Ca²⁺ sensitizers and the agents that act through cAMP-mediated signaling process have been postulated to belong to different classes, however, recent

experimental findings revealed that certain Ca^{2+} sensitizers, such as levosimendan, OR 1896 and UD-CG 212 Cl, require cyclic AMP-mediated signaling for induction of the Ca^{2+} sensitizing effect. No clinically available agents act primarily via Ca^{2+} sensitization, but the positive inotropic effect of pimobendan and levosimendan is partly due to an increase in myofilament Ca^{2+} sensitivity. These agents are the hybrid of Ca^{2+} sensitizer and PDE III inhibitor. The extent of contribution of Ca^{2+} sensitizing effect of these agents to the clinical effectiveness to improve the hemodynamics in patients with HF is uncertain. These agents with Ca^{2+} sensitizing effect are clinically more effective than the agents that act purely via the upstream mechanism [39].

Cardiotonic agents that facilitate cardiac pump function by direct improvement of contractile dysfunction are indispensable for the treatment of hemodynamic disorders in AHF and CHF. Cardiotonic agents currently available for the treatment of hemodynamic crisis in CHF are catecholamines, selective PDE-III inhibitors and digitalis, all of which are Ca^{2+} mobilizers. Considering the number of serious adverse effects of these clinically available cardiotonic agents, development of agents that act via a novel mechanism of action may contribute to the progress of pharmacotherapy of CHF. Ca^{2+} sensitizers that act by increasing in myofilament Ca^{2+} sensitivity may be able to overcome the disadvantage of Ca^{2+} mobilizers. Ca^{2+} sensitizers do not increase activation energy, do not produce Ca^{2+} overload and may be effective even under pathophysiological states such as acidosis, myocardial stunning and HF. SCH00013 ((4,5-dihydro-6-[1-[2-hydroxy-2-(4-cyanophenyl)ethyl]-1,2,5,6-tetrahydro pyrido-4-yl]pyridazin-3(2H)-one)) is a novel Ca^{2+} sensitizer that elicits a moderate positive inotropic effect without significant alteration of Ca^{2+} transients. SCH00013 does not have a positive chronotropic effect and has a weak PDE III inhibitory action and class III antiarrhythmic action. SCH00013 prolonged the survival in a animal HF model with genetic cardiomyopathy. The oral bioavailability of SCH00013 is high and equivalent to that via intravenous administration. The pharmacological profiles of SCH00013 imply that this agent may be potentially beneficial for pharmacotherapy of contractile dysfunction in CHF [40]. Medical therapy for chronic HF has recently been extensively revised on the basis of a reduction of mortality in randomized trials. However, there are almost no available trials in children whose treatment for HF is adapted from what is known in adults. Digitalis play a role in HR variability but there is no evidence for an effect on mortality. Angiotensin-converting enzyme inhibitors lead to an improvement of left ventricular function and reduce mortality; in addition, they are well tolerated in children. Diuretics are useful in CHF but chronic administration may have deleterious effects. Beta-blocking agents reduce mortality and improve symptoms and the ejection fraction; however, they must be given cautiously and the up-titration phase must be very progressive. New promising drugs are emerging (Ca^{2+} sensitizers, PDE-inhibitors, angiotensin receptor [AT1] blockers) but at the present time their use in chronic HF has not proven to be efficient [41]. The effects of Ca^{2+} sensitizers are EMD 57033, MCI-154 and EGIS-9377 in cardiac preparations from streptozotocin-induced diabetic rats. In enzymatically dissociated

ventricular myocytes loaded with the Ca^{2+} probe indo 1, these Ca^{2+} sensitizers caused an increase in cell shortening without a significant effect on the $(\text{Ca}^{2+})_i$ transient. The contractile responses were substantially similar in myocytes from diabetic and age-matched control rats. In contrast, the contractile and $[\text{Ca}^{2+}]_i$ responses to pimobendan and isoproterenol were significantly less in diabetic myocytes. The Ca^{2+} sensitivity of tension in beta-escin-skinned trabeculae from diabetic hearts was not significantly different from that of controls. The effect of EMD 57033 on myofilament responsiveness to Ca^{2+} was identical in control and diabetic preparations. The slower time course of relaxation observed in diabetic papillary muscles was further prolonged in the presence of EMD 57033. However, the extent of the increase in relaxation produced by EMD 57033 did not differ between control and diabetic muscles, and the detrimental effect on resting tension was less pronounced in the two groups. In anesthetized rats, echocardiography showed that intraduodenal administration of EMD 57033 increased left ventricular systolic function without affecting variables of diastolic filling in both groups. Taken together, the present results suggest that Ca^{2+} sensitizers, unlike conventional inotropic agents, have the potential to increase in force of contraction to the same extent in nondiabetic and diabetic myocardium, possibly without exaggerating extremely the impairment of diastolic function in diabetes [42]. The sodium channel modulator DPI 201-106 has been described to possess Ca^{2+} -sensitizing properties. Therefore, the present study investigated the inotropic effect of the Na^{+} -channel modulator BDF 9148 (1 μM), a congener of DPI 201-106, in comparison with the Ca^{2+} -sensitizers CGP 48506 (1-50 $\mu\text{mol/l}$) and EMD 57033 (1-30 $\mu\text{mol/l}$) in electrically driven left ventricular cardiomyocytes isolated from guinea pigs. The changes of the contraction amplitude in comparison to the basal cell shortening (cell shortening in micron and %) were continuously recorded. BDF 9148, CGP 48506, and EMD 57033 exerted a significant increase in the contraction amplitude. The maximal positive inotropic effects of CGP 48506 (50 $\mu\text{mol/l}$) and EMD 57033 (30 $\mu\text{mol/l}$), respectively. However, only the Ca^{2+} -sensitizers CGP 48506 and EMD 57033, but not BDF 9148, prolonged the contractile twitch. Especially in patients with an already enhanced intracellular myocardial Ca^{2+} -concentration, Ca^{2+} -sensitizers, which impair relaxation, may be disadvantageous for therapeutical use despite their positive inotropic effect [43]. The Ca^{2+} binding to cTnC triggers contraction in heart muscle. In HF, myofilaments response to Ca^{2+} are often altered and compounds that sensitize the myofilaments to Ca^{2+} possess therapeutic value in this syndrome. One of the most potent and selective Ca^{2+} sensitizers is the thiadiazinone derivative EMD 57033, which increases myocardial contractile function both in vivo and in vitro and interacts with cTnC in vitro. Favorable hydrophobic interactions between the drug and the protein position EMD 57033 in the hydrophobic cleft of the protein. The drug molecule is orientated such that the chiral group of EMD 57033 fits deep in the hydrophobic pocket and makes several key contacts with the protein. This stereospecific interaction explains why the (-)-enantiomer of EMD 57033 is inactive. EMD 57033 complex with two regions of cTnI (cTnI and cTnI) reveal that the drug does not share a common

binding epitope with cTnI but is completely displaced by cTnI. These results have important implications for elucidating the mechanism of the Ca^{2+} sensitizing effect of EMD 57033 in cardiac muscle contraction [44].

The Ca^{2+} sensitizers increase myocardial contractile function without affecting Ca^{2+} homeostasis, which might be beneficial in the treatment of patients with HF. However, it remains uncertain whether Ca^{2+} sensitizers induce quantitatively similar inotropic responses in control and failing hearts. To compare their effects in normal versus failing hearts at the cellular level, shortening mechanics and ($[\text{Ca}^{2+}]_i$) transient were simultaneously measured in the left ventricular myocytes isolated from normal dogs and dogs with rapid pacing-induced HF. CGP 48506 and EMD 57033 exerted a positive inotropic effect in a dose (0.1-3 μM)-dependent manner in both normal and HF myocytes. The percent increase of cell shortening magnitude was comparable between the two groups. CGP 48506 and EMD 57033 did not affect the diastolic cell length and resting $[\text{Ca}^{2+}]_i$ level. They did not affect the duration of $[\text{Ca}^{2+}]_i$ transient dynamics. Thus Ca^{2+} sensitizers exerted comparable positive inotropic effects without affecting the rest cell length and rest $[\text{Ca}^{2+}]_i$ in normal and HF myocytes [45]. New therapeutic strategies as well as the development of drugs with more specific targets have been fueled by disappointments in the treatment of adult HF. Ca^{2+} sensitizers, vesnarinone and angiotensin channel blockers will be addressed in this manuscript. The physiologic and pharmacologic principles that justify their use in the management of HF are reviewed. Ca^{2+} sensitizers increase myocardial contractility and in part they bypass the adenylyl cyclase cascade, which gives them a more favorable energy profile. Vesnarinone is a quinolinone derivative with ion channel modulation properties, which result in a positive inotropic effect and prolongation of the action potential. In addition vesnarinone has immunomodulatory properties. Angiotensin-converting enzyme inhibitors are the cornerstones for the treatment of HF. The discovery of some putative drawbacks to ACE inhibition has challenged this supremacy. Angiotensin receptor blockers have been developed hoping to overcome these deficiencies. Myocardial developmental differences highlight the shortcomings of attempting to extrapolate data on drugs and cellular physiology in adults to children. Studies are needed addressing standards of care, quality of life, morbidity and mortality, neurohumoral activation, its modulation and the consequences of these therapies in pediatric HF [46]. The management of HF, pediatric pharmacology, the use of HF therapies including digoxin, ACE inhibitors, beta-adrenergic blockers, inotropic agents, diuretics, vasodilators, Ca^{2+} sensitizers, angiotensin and aldosterone receptor blockers, growth hormone, and future gene therapy. The etiology and course of ventricular dysfunction in children is poorly characterized. Furthermore, many changing developmental properties of the pediatric myocardium and differences in the etiologies of ventricular dysfunction in children compared with adults, invalidating the concept that children can safely be considered small adults for the purpose of understanding HF pathophysiology and treatment. However, strikingly little research in children with ventricular dysfunction exists in terms of well-designed large-scale studies of the epidemiology or

multicenter controlled clinical therapeutic trials. A future research agenda is proposed to improve understanding etiologies, course and treatment of ventricular dysfunction in children that is based on organized and funded cooperative groups since no one pediatric cardiac center treats enough children with a particular etiology of ventricular dysfunction. In conclusion, significant understanding of basic mechanisms of pediatric ventricular dysfunction and effective therapies for adults with ventricular dysfunction exist. A multicenter pediatric cardiac ventricular dysfunction network would allow improved understanding of diseases and treatments, and result in evidence-based medicine for pediatric patients with ventricular dysfunction [47].

The Ca^{2+} sensitizers may be advantageous for treatment in human HF by increasing cardiac force without increasing the Ca^{2+} transient or energy consumption. To study the mode of action of the Ca^{2+} sensitizers EMD 57033 (EMD) and CGP 48506 (CGP), their influence on butanedione monoxime (BDM)-mediated depression of cross-bridge cycling was analyzed in human myocardium (explanted hearts, dilated cardiomyopathy). The Ca^{2+} -sensitizing effect of CGP was accompanied by an increased Ca^{2+} sensitivity of myosin-ATPase activity, an increased slope of the Ca^{2+} force and Ca^{2+} ATPase curve, as well as a reduced maximal myosin ATPase activity. CGP and EMD reduced tension cost. In conclusion, EMD and CGP antagonize the BDM-mediated relaxation in troponin I-depleted cardiac muscle fibers. The Ca^{2+} -sensitizing effect of CGP seems to be dependent on an improvement of the myofilament cooperativity, whereas EMD seems to operate by increasing the force per cross-bridge [48].

The energy cost of contractility-enhancing drugs has been investigated in various experimental and clinical studies, and the Ca^{2+} sensitizers have been proven to be the most mechano energetically efficient. An "oxygen-wasting" effect in post-ischemic hearts was observed. The mechanism for this inefficiency has been thoroughly investigated, and seems to be caused by an inefficient excitation-contraction coupling and/or inefficiency in the contractile apparatus. The mechanoenergetic efficiency in HF needs further investigation [49]. Recent interest in so-called Ca^{2+} -sensitizing positive inotropic drugs has highlighted the potential problem of a positive effect on force development being offset, at least partially, by the negative effect that many of these drugs have on relaxation. The purpose of this study was to examine the interplay of contraction and relaxation in determining the overall cardiac effect of different positive inotropic drugs. Four drugs (calcium, dobutamine, EMD 57033, and CGP 48506) that were given at doses sufficient to increase similarly LVP-generating capability by approximately 20%. We show that, even though they produce equivalent changes in pressure-generating capability, these four agents produce dissimilar changes in relaxation capability, with dobutamine speeding relaxation, EMD 57033 slowing relaxation, and Ca^{2+} and CGP 48506 having little effect of relaxation. Similar relative effects were observed for drug-induced changes in the timing of pressure-generation events. These effects combine to produce different drug-induced changes in overall cardiac pump function judged by the relation between CO and HR. Dobutamine shifted the maximal CO to a higher HR. In

contrast, both Ca^{2+} sensitizers shifted the maximum in CO to a lower HR, whereas Ca^{2+} had no effect. Thus even though positive inotropic drugs may have similar effects on LVP generation, the overall benefit of such drugs on ventricular pump function will depend on how the drug also affects ventricular relaxation and ejection capabilities [50].

The Ca^{2+} sensitizers enhance systolic function, but may impair relaxation in vitro; these effects may differ in stunned and normal myocardium. We therefore studied the effect of EMD 57033 on systolic and diastolic function of normal and stunned porcine myocardium in vivo. 2. In conclusion, EMD 57033 restored systolic and diastolic function of stunned myocardium, and produced similar improvements in systolic and diastolic function in normal myocardium [51]. Thin filament regulation of contraction is thought to involve the binding of two activating ligands: Ca^{2+} and strongly bound cross-bridges. The specific cross-bridge states required to promote thin filament activation have not been identified. This study examines the relationship between cross-bridge cycling and thin filament activation by comparing the results of kinetic experiments using the Ca^{2+} sensitizers caffeine and bepridil. Bepridil produced a similar shift in the tension-pCa curves but had no effect on the kinetics. Thus bepridil increases the Ca^{2+} sensitivity through direct effects on TnC, whereas caffeine has significant effects on the cross-bridge interaction. Interestingly, caffeine also produced a significant increase in stiffness under relaxing conditions, indicating that caffeine induces some strongly bound cross-bridges, even in the absence of Ca^{2+} . The results are interpreted in terms of a model integrating cross-bridge cycling with a three-state thin-filament activation model. Significantly, strongly bound, non-tension-producing cross-bridges were essential to modeling of complete activation of the thin filament [52]. Compounds that sensitize cardiac muscle to Ca^{2+} by intervening at the level of regulatory thin filament proteins would have potential therapeutic benefit in the treatment of myocardial infarctions. Two putative Ca^{2+} sensitizers, EMD 57033 and levosimendan, are reported to bind to cTnC. The drug binding to [methyl-(13)C]methionine-labeled cTnC when free or when complexed with cTnI. In the absence of Ca^{2+} , neither drug interacted with cTnC. In the presence of Ca^{2+} , one molecule of EMD 57033 bound specifically to the C-terminal domain of free cTnC. NMR and equilibrium dialysis failed to demonstrate binding of levosimendan to free cTnC, and the presence of levosimendan had no apparent effect on the Ca^{2+} binding affinity of cTnC. Changes in the N-terminal methionine methyl chemical shifts in cTnC upon association with cTnI suggest that cTnI associates with the A-B helical interface and the N terminus of the central helix in cTnC. The binding of levosimendan to the cTnC.cTnI complex. However, levosimendan covalently bound to a small percentage of free cTnC after prolonged incubation with the protein. These findings suggest that levosimendan exerts its positive inotropic effect by mechanisms that do not involve binding to cTnC [53]. EMD 53998 (5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl]-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-one), the racemic mixture of (+)-enantiomer EMD 57033 and (-)-enantiomer EMD 57439, is a prototype of Ca^{2+} sensitizers that act via a central and/or down-stream mechanism in cardiac E-C

coupling. In rabbit ventricular cardiomyocytes loaded with indo-1/AM, EMD 53998 and EMD 57033 shifted the relationship between Ca^{2+} transients and cell shortening (systolic function) to the left to the same extent as compared with that of elevation of $[\text{Ca}^{2+}]_o$. EMD 57439 did not elicit a positive inotropic effect (PIE). The PIE of EMD 57033 was associated with a more pronounced decrease in the diastolic cell length than that of EMD 53998, whereas the systolic effects of these compounds were equivalent. These results indicate that weak PDE-III inhibition may exert a differential action on diastolic and systolic function. Thus, EMD 57439 antagonizes the Ca^{2+} -sensitizing effect of EMD 57033 on diastolic function with no effect on systolic function, which may lead to a decrease in diastolic cell length of a lesser extent with the racemate EMD 53998 compared with (+)-enantiomer EMD 57033 [54]. The Ca^{2+} sensitizers prolong myofibrillar force development in vitro and might therefore aggravate relaxation abnormalities of stunned myocardium. This is the first in vivo study of the effects of the thiadiazinone derivative EMD 60263 ((+)-5-(1-(alpha-ethylimino-3, 4-dimethoxy benzyl)-1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3, 6-dihydro-2H-1,3,4-thiadiazine-2-on), a Ca^{2+} -sensitizing agent with negligible PDE-III inhibitory activity, on diastolic function of regionally stunned myocardium. After producing stunning by two sequences of 10-min coronary artery occlusion and 30 min of reperfusion, anaesthetised pigs received either saline or 1.5 and 3.0 mg/kg of EMD 60263 or its enantiomer EMD 60264, which lacks the Ca^{2+} -sensitizing properties but shares the bradycardiac action via inhibition of the delayed inward rectifier K^+ current. In conclusion, both doses of EMD 60263 improved systolic as well as diastolic function of stunned myocardium. The high dose delayed relaxation of normal myocardium without adversely affecting systolic function [55]. Levosimendan is a new class of cardiac inotropic drugs, Ca^{2+} sensitizers. Levosimendan is a vasodilator both in vitro and in vivo, but its mechanism is not well understood. The cardiac target protein of levosimendan, troponin C, is a Ca^{2+} -binding EF-hand protein. This raises the possibility that levosimendan may also interact with smooth muscle EF-hand proteins, such as, calmodulin, the regulatory myosin light chains, or S100 proteins. The effects of levosimendan on $[\text{Ca}^{2+}]_i$, and force in porcine coronary arteries, with receptor-mediated (U46619) or KCl stimulation (Fig 1). At high levels of stimulation, levosimendan decreased force without changing or increasing $[\text{Ca}^{2+}]_i$, measured with the Ca^{2+} -sensitive fluorescent probe fura-2 in the intact artery. With lower levels of U46619, levosimendan (1 μM) lowered force by 70% and reduced $[\text{Ca}^{2+}]_i$ by 38%. The relationship between force and $[\text{Ca}^{2+}]_i$ for KCl stimulation are significantly rightward shifted, indicating Ca^{2+} desensitization by levosimendan. In contrast, the PDE-III inhibitor, milrinone, does not shift the force- Ca^{2+} relations but elicits relaxation via lowering $[\text{Ca}^{2+}]_i$. There was little change in pHi, indicating that the Ca^{2+} desensitization by levosimendan was not attributable to decreasing pHi. Levosimendan relaxes coronary arteries and lowers $[\text{Ca}^{2+}]_i$ by mechanisms different than milrinone. Results indicate a lowering of $[\text{Ca}^{2+}]_i$ by levosimendan consistent with opening of potassium channels and a relaxation that is independent of $[\text{Ca}^{2+}]_i$.

The mechanism might involve the direct effect of regulatory proteins themselves [56]. levosimendan on the smooth muscle contractile or

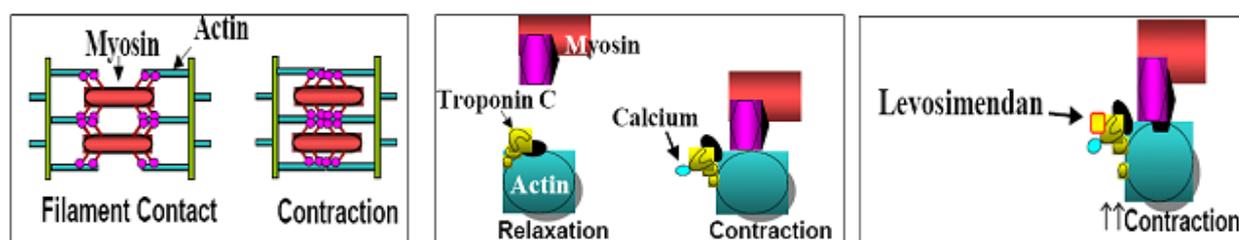


Figure 1. Calcium sensitisation of the contractile proteins in cardiac muscle by levosimendan

Due to shortage of donor hearts and increasing waiting-lists of patients with end-stage heart disease, new pharmacological principles for bridging therapies are necessary. The positive inotropic effects of cAMP-increasing drugs (catecholamines, PDE-inhibitors) are diminished in the failing myocardium. Hence, we investigated the usefulness and mechanism of the two Ca^{2+} sensitizers, levosimendan and CGP 48506 in preparations from end-stage failing human hearts since the exact mechanism of the positive inotropic effects is not yet clearly understood. CGP 48506 is an inotropic agent with Ca^{2+} -sensitizing properties in the human heart, that is devoid of inhibitory activity on human cardiac PDE isoenzymes. It offers, therefore, a new form of positive inotropic therapy that can be useful for the bridging treatment of HF before transplantation. On the other hand, levosimendan is a Ca^{2+} sensitizer showing less-effective inotropic effects accompanied by increased cAMP levels [57]. Newly developed Ca^{2+} sensitizers possess different mechanisms of action on contractile machinery. Increasing maximal Ca^{2+} -activated force in addition to enhancing Ca^{2+} sensitivity (MCI-154, EMD 53998, and EMD 57033) could exert pronounced positive inotropy and may provide a mechanoenergetic advantage over the classic Ca^{2+} mobilization in the chronically failing heart. EMD 53998 and EMD 57033 prolong crossbridge attachment time, resulting in negative lusitropy. In contrast, pimobendan, levosimendan, and MCI-154 accelerate left ventricular relaxation in HF, because Ca^{2+} sensitizing action of these agents may be prominent during the early phases of contraction. Therefore, Ca^{2+} sensitizers can avoid the legacy of problems associated with conventional inotropic interventions and may break through "reservation" to "preservation" in the treatment of chronic HF [58]. Physiological and pharmacological interventions are used to regulate cardiac contractile functions via modulation of Ca^{2+} signaling. The relevant regulatory mechanisms have recently been assessed in detail by use of novel experimental procedures, which include simultaneous measurements of intracellular levels of Ca^{2+} and contractile force in intact myocardial preparations loaded with the $(\text{Ca}^{2+})_i$ indicator aequorin and fluorescent dyes, namely, fura-2, indo-1 and fluo-3. Association with or dissociation from $(\text{Ca}^{2+})_i$ transients of contractile activity is taken as evidence that reflects the primary mechanism of action of individual inotropic interventions. In addition, motility assays of actin-myosin interactions *in vitro* have made it possible to define the site of action of Ca^{2+} sensitizers as troponin C and the interaction of the troponin-tropomyosin complex with actin or the actin-myosin interface at crossbridges. Frank-

Starling mechanism operates at the level of the binding of Ca^{2+} ions to troponin C and subsequent regulatory processes, while the force-frequency relationship is mainly ascribed to an alteration in the intracellular mobilization of Ca^{2+} ions. Cardiotonic agents can be classified as follows: 1) agents that act via a cAMP-dependent or a cAMP-independent mechanism; and 2) agents that facilitate the mobilization ($\text{Ca}^{2+})_i$ or increase in myofibrillar sensitivity to Ca^{2+} ions. Regulatory mechanisms mediated via the phosphorylation of functional proteins induced by cAMP, which is responsible for the actions of novel cardiotonic agents, beta 1-adrenoceptor partial agonist and selective inhibitors of PDE-III, have currently been clarified in more detail. Ca^{2+} sensitizers are of extreme therapeutic interest because of their ability to increase myocardial contractility without an increase in activation energy; they are devoid of risks of arrhythmogenicity and myocardial cell death from $(\text{Ca}^{2+})_i$ overload; and they effectively reverse contractile dysfunction under pathophysiological situations, such as acidosis or myocardial stunning [59]. Current medical treatment of chronic HF makes use of a combination of diuretics, cardiac glycosides and ACE inhibitors. The latter have improved the chances of survival of patients with chronic cardiac insufficiency. The combination of hydralazine hydrochloride and isosorbide dinitrate also improves survival, but direct comparison of both regimens provided evidence for a less favourable effect than that of the ACE inhibitors. Inhibition of neuroendocrine activation has been demonstrated only for ACE inhibitors and cardiac glycosides. The use of beta blockers represents a new therapeutic strategy that over the long term improves cardiomyocyte function, CO at rest, and physical performance. For this indication, however, beta blockers should be used with extreme caution and at very low initial doses. Ca^{2+} sensitizers, modulators of intracellular Ca^{2+} and/or sodium homeostasis, imidazolin receptor antagonists with an action on the central nervous system and AT1 receptor antagonists [60].

During HF, force production by the heart decreases. This may be overcome by Ca^{2+} -sensitizing drugs, which increase myofibril Ca^{2+} sensitivity without necessarily altering intracellular Ca^{2+} concentration. However, Ca^{2+} sensitizers slow the relaxation of intact cardiac muscle. We used diazo-2, a caged chelator of Ca^{2+} , to study the effects of the Ca^{2+} sensitizers caffeine and CGP 48506 on the intrinsic relaxation rate of cardiac myofibrils. Relaxation was fitted by a double-exponential decay, and the rate constants were found to be independent of force and preflash Ca^{2+} concentration. Thus, myofibril relaxation need not be slowed by Ca^{2+} -sensitizing agents

but can even be accelerated. Despite similarities in their effects on myofibril Ca^{2+} sensitivity, caffeine and CGP 48506 affect the myofibrils at least partly via different mechanisms (Palmer and Kentish, 1997). The effect of cardiotonic drugs with a Ca^{2+} -sensitizing effect on cardiac mechanoenergetics in the failing heart is not fully understood. Accordingly, we measured left ventricular (LV) contractility (E_{max}) and the relation between MVO_2 (VO_2) and pressure-volume area (PVA; a measure of LV total mechanical energy) before and during enhancement of contractility by infusion of dobutamine (DOB) or pimobendan (PIMO) in six cross-circulated hearts isolated from pacing-induced HF (FL) dogs, and compared the results with those reported in normal hearts (NL). Although the baseline E_{max} was much lower in FL dogs than in NL dogs, DOB and PIMO comparably enhanced E_{max} in the FL dogs. The O_2 cost of contractility, defined as the increase in unloaded VO_2 at zero PVA divided by the increase in E_{max} , obtained during an enhancement of contractility with DOB, was significantly higher in FL dogs than in NL dogs, suggesting that a larger amount of O_2 is consumed during Ca^{2+} cycling with DOB in FL dogs. In contrast, the O_2 cost of contractility with PIMO was similar between FL and NL dogs. Furthermore, the VO_2 per minute was significantly higher with DOB than with PIMO partly because of an excessive positive chronotropic effect of DOB. The (1) PIMO exerts a positive inotropic effect comparable to that of DOB in both NL and FL dogs; (2) the O_2 cost of contractility with DOB is higher in FL dogs than in NL dogs; and (3) PIMO has a relative O_2 -saving effect compared with DOB in FL dogs [61]. Positive inotropic compounds may be harmful in the long-term treatment of chronic CHF because they may induce a Ca^{2+} overload, unwanted changes in cross-bridge kinetics and an acceleration in HR. Therefore, the effects of a variety of cardiotonic agents on the heat released from small guinea pig papillary muscles contracting isometrically using rapid antimony-bismuth thermopiles. The economy of muscle contraction was lowest with PDE inhibitors and highest with Ca^{2+} sensitizers. Compared with an increase in extracellular Ca^{2+} concentration, β_1 -adrenoceptor stimulators and PDE inhibitors profoundly decrease the economy of myocardial contraction, and Ca^{2+} -sensitizers (pimobendan and EMD-53998) slightly increase myocardial economy, whereas ouabain and the Ca^{2+} channel agonist BAY K 8644 have no effect on this parameter. In addition, the evidence that acceleration of HR may be harmful not only from an energetic point of view: an increase in HR may also decrease the contractility of the failing human myocardium. An positive inotropic compound should have no, or even negative, chronotropic effects, should not be mediated by increases in Ca^{2+} transients, and should decelerate, rather than accelerate, cross-bridge kinetics [62].

Depression of myocardial contractility plays an important role in the development of HF; therefore, intensive interest and passion have been generated to develop cardiotonic agents to improve the contractile function of the failing heart. Inotropic agents that increase cAMP, either by increasing its synthesis or reducing its degradation, exert dramatic short-term hemodynamic benefits, but these acute effects cannot be extrapolated into long-term improvement of the clinical outcome in patients with advanced HF. Administration of these agents

to an energy-starved failing heart would be expected to increase myocardial energy use and could accelerate disease progression. The role of digitalis in the management of HF has been controversial, but ironically the drug has now been proved to favorably affect the neurohormonal disorders and its reevaluation is now being intensively investigated. Recently, attention has been focused on other inotropic agents that have a complex and diversified mechanism. Recent clinical studies have demonstrated that they are potentially useful in the long-term treatment of HF patients. These agents have some PDE-inhibitory action but also possess additional effects, including acting as cytokine inhibitors, immunomodulators, or Ca^{2+} sensitizers. However, their therapeutic ratio is narrow and further studies are warranted to establish their optimal doses and their eventual status in the treatment of HF [63]. Most patients with chronic CHF are subjected to symptomatic treatment, predominantly with drugs. It has become clear that treatment with unloading drugs is probably more beneficial than treatment with inotropic agents. It has been widely recognized that the neuroendocrine compensatory changes associated with CHF afford an important target for drug treatment. This may also hold for some of the changes in receptor density, such as the down regulation of cardiac β -adrenoceptors. The clearly changing insights into the backgrounds of drugs for the treatment of CHF are critically discussed. Apart from the changing views and appreciation of the currently used drugs (diuretics, ACE inhibitors, digoxin, β -adrenoceptor agonists), the following new approaches are discussed: β -blockers, angiotensin II receptor antagonists, ibopamine, Ca^{2+} antagonists, inhibitors of ANP degradation, vasopressin antagonist, vesnarinone, and Ca^{2+} sensitizers (van Zwieten, 1997). The effect of two Ca^{2+} sensitizers, EMD 57033 (without significant PDE inhibition) and ORG 30029 (with PDE inhibition) in human hearts, EMD had no effect on the peak of the $[\text{Ca}^{2+}]_i$ transient; it prolonged the time course of the $[\text{Ca}^{2+}]_i$ transient in both nonfailing and failing myocardial fibers. ORG increased the peak of the Ca^{2+} transient and prolonged the time course in preparations from both nonfailing and failing hearts. Both EMD and ORG shifted the $[\text{Ca}^{2+}]_i$ -force relationship toward lower $[\text{Ca}^{2+}]_i$ (EMD > ORG). The Ca^{2+} sensitizers EMD 57033 and ORG 30029 increased active force development in nonfailing and failing human myocardium, but both impaired relaxation in failing myocardium to a greater extent than in nonfailing human myocardium in a concentration-dependent fashion [64]. The effect of cardiotonic drugs with Ca^{2+} -sensitizing effect (Ca^{2+} sensitizers) on cardiac mechanoenergetics is not fully understood. Accordingly, the effects of milrinone (a PDE inhibitor) and sulmazole (Ca^{2+} sensitizer with a PDE-inhibiting effect) on left ventricular mechanics and energetics were studied. These results suggest that the two positive inotropic drugs exhibit similar mechanoenergetic effects in the normal canine heart despite the different mechanisms of action [65]. The effect of EMD 53998 (EMD) (0.1-100 $\mu\text{mol/l}$), chemically a racemic thiadiazinone derivative, suggested to be a potent Ca^{2+} -sensitizer, was studied in human failing and nonfailing left ventricular myocardium. For comparison, the effects of the pimobendan (0.1-300 $\mu\text{mol/l}$), isoprenaline (Iso) (0.001-3 $\mu\text{mol/l}$) as well as CaCl_2 (1.8-15 mmol/l Ca^{2+})

were investigated. The positive inotropic responses were examined in electrically driven human left ventricular papillary muscle strips from terminally failing hearts and nonfailing donor hearts. The effect of EMD on the Ca^{2+} -sensitivity of skinned fiber preparations from the very same human failing hearts were studied as well. EMD and pimobendan increased force of contraction (FOC) in a concentration-dependent manner. As judged from the EC50-values, EMD increased FOC more potently than pimobendan. EMD was significantly more effective than pimobendan to increase FOC in papillary muscle strips as well as from nonfailing hearts. Only in terminally failing myocardium, EMD increased FOC as effectively as Iso. After inotropic stimulation with EMD, pimobendan, or Iso, carbachol (1000 $\mu\text{mol/l}$) reduced FOC in left ventricular papillary muscle strips, indicating a cAMP-dependent mode of action. In skinned fiber experiments, EMD increased Ca^{2+} -sensitivity significantly more than pimobendan. EMD increases FOC in human myocardium via sensitizing of the contractile proteins towards Ca^{2+} and by inhibition of PDE-III-isoenzymes. EMD is a potent Ca^{2+} sensitizing agent in human myocardium. Thiadiazinone derivatives could be one step in the evolution to more potent and selective Ca^{2+} -sensitizers [66].

Levosimendan, a Ca^{2+} sensitizer acting through TnC, accelerated the proportional association rate and decelerated the dissociation rate of crossbridges. The effect of levosimendan on crossbridge kinetics occurred before the peak twitch tension was achieved. Thus, the compound did not change the actual relaxation phase of twitch tension. Since the effect on the association was more pronounced than on the dissociation of crossbridges, levosimendan shifted the entire twitch tension curve to the left. Based on the dissociation rate analysis levosimendan seems to act preferentially as a Ca^{2+} sensitizer at low concentrations. At high concentrations the PDE-III inhibitory properties of levosimendan modulated its effect on the early relaxation processes. In contrast, PDE III inhibition is probably the primary mechanism of action for MCI-154. Pimobendan, and EMD 53998 at low concentrations, whereas their direct effects on crossbridge kinetics contributed to the positive inotropic action at high concentrations. The Ca^{2+} sensitizing mechanisms of these compounds seemed to be based almost exclusively on the decelerating effect on dissociation of crossbridges [67]. Ca^{2+} sensitizers are enhancing contractility with modest effects on energy utilization. EMD 57033 enhances contractility and prolongs relaxation. Its effects are modulated by HR, $[\text{Ca}^{2+}]_o$, and contraction mode, with positive inotropic effects being more prominent for ejecting beats [68]. Inhibitors of PDE-III enhance cardiac contractile force by elevating the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ by impairing cAMP degradation thus increasing cAMP levels. The drugs are more effective in healthy than in failing hearts since basal cAMP production is diminished in the latter. However, long term treatment with PDE-III inhibitors does not appear to be beneficial due to increased risk of potentially lethal arrhythmias caused by augmentation of $[\text{Ca}^{2+}]_i$ [1]. This risk should be absent in Ca^{2+} sensitizers. Thiadiazinone derivatives have been synthesized in which the potency for Ca^{2+} sensitization is many-fold larger than the potency for PDE-III inhibition. The Ca^{2+} -sensitizing action resides in the [+] enantiomers, while the [-] enantiomers show weak

PDE-III inhibition. In the enantiomer pair [+] EMD 60263 and [-] EMD 60264, only the former concentration-dependently increased force of contraction in isolated cardiac preparations and myocytes. In the Langendorff-perfused guinea-pig heart, force was reversibly increased, whereas [-] EMD 60264 even produced a negative inotropic response despite of its PDE inhibitory activity. Heart rate (HR), however, was reduced by both enantiomers. Perfusion pressure remained unaffected. The effects were fully reversible upon wash-out of the enantiomers. [+] EMD 60263 also enhanced cell shortening of human myocytes from both normal and failing hearts. In contrast to the opposite effects on contractility, both enantiomers prolong the action potential duration by blocking the rapidly activating component of the delayed rectifier K^+ current. The therapeutic potential of these agents has yet to be assessed in clinical studies [69]. The role of cTnC as a target protein for the Ca^{2+} sensitization by levosimendan, pimobendan, MCI-154 and EMD 53998 was evaluated using purified recombinant human cTnC. For determination of Ca^{2+} - and magnesium-dependent binding of the compounds to cTnC a new type of cTnC-HPLAC column was used. Furthermore, dansylated cTnC was utilized to study the effect of the Ca^{2+} sensitizing compounds on Ca^{2+} -induced conformation of cTnC. Only levosimendan showed Ca^{2+} -dependent and to a lesser extent magnesium-dependent retention in the cTnC column. The findings indicate that levosimendan binds both to the N-terminal and C-terminal domains of cTnC. In agreement with this, only levosimendan shifted the Ca^{2+} -induced fluorescence curve of dansylated cTnC to the left. Levosimendan at 3 μM decreased the value of Ca_{50} to 1.19 μM . In conclusion, it is suggested that the mechanism of Ca^{2+} sensitizing effect of levosimendan, unlike that of the other Ca^{2+} sensitizers, is based on Ca^{2+} -dependent binding to the N-terminal domain of cTnC. This is proposed to amplify the trigger of contraction induced by cTnC in the cardiac muscle [70]. Levosimendan is a novel positive inotropic drug targeted to increase contraction force of the heart through its Ca^{2+} -dependent binding to cTnC. The Ca^{2+} -sensitizing effect of levosimendan on contractile proteins as well as its positive inotropic and lusitropic effects in paced guinea pig papillary muscle. The Ca^{2+} sensitization induced by levosimendan in fibers skinned with saponin was dependent on the perforation velocity of cell membranes. Levosimendan was almost ineffective in slowly perforated fibers, but was the most potent Ca^{2+} sensitizer in fibers with rapidly perforated cells. The perforation-dependent Ca^{2+} sensitization was probably due to changes in phosphorylation state of contractile proteins during the slow dissection of fibers. It is noteworthy that the Ca^{2+} -sensitizing effect of levosimendan was not affected by acidic pH. Levosimendan at therapeutically relevant concentrations markedly increased Ca^{2+} sensitivity, being more potent than EMD 53998, pimobendan, and MCI-154. The lack of effect of levosimendan on maximum tension supports the hypothesis that levosimendan increases Ca^{2+} sensitivity through its action on cTnC. Unlike EMD 53998, levosimendan did not increase myosin ATPase activity, indicating that it did not increase the cycling rate of myosinactin crossbridges. In paced papillary muscles, levosimendan induced positive inotropic effect without

changing relaxation time. Thus, levosimendan was devoid of the main negative factors described for Ca^{2+} sensitizers [71].

Some of the problems with inotropic agents and describes the new concept of increasing cardiac myofilament sensitivity to Ca^{2+} . Presently used inotropic agents act by increasing the intracellular concentration of Ca^{2+} in cardiac myocytes by either cAMP-dependent or cAMP-independent mechanisms. There is concern that elevation of cAMP and/or cytosolic Ca^{2+} might be proarrhythmic and increase mortality in patients with CHF. Ca^{2+} sensitization represents a new approach to the treatment of CHF. Drugs that sensitize the contractile proteins to Ca^{2+} enhance myocardial contractility without changes in the cytosolic Ca^{2+} concentration. Ca^{2+} sensitization can be achieved by an increased affinity of troponin-C for Ca^{2+} (pimobendan), by stabilization of the Ca^{2+} -induced conformational change of troponin-C (levosimendan) or by direct interference with the myosin-actin interaction (MCI-154, EMD 53998, and EMD 57033). Ca^{2+} sensitization reduces the risk for Ca^{2+} overload and has a favorable effect on MVO_2 . Inhibition of cardiac relaxation is a possible adverse effect of Ca^{2+} sensitizers owing to an expected higher level of contractile tension during diastole. However, most of the reported Ca^{2+} sensitizers have additional PDE-III inhibitory activity, which is associated with a positive lusitropic effect, but from the standpoint of mortality PDE inhibition might not be beneficial in the long run. Most Ca^{2+} sensitizers have a hemodynamic profile characteristic of inodilators. Clinical data on Ca^{2+} sensitizers are yet very sparse but ongoing clinical trials are awaited [72]. The adverse drug events (ADEs) during levosimendan treatment cannot be predicted in detail. The tolerability of levosimendan in human, ADEs, values before and after treatment. The most common ADE seen in healthy volunteers is headache, reported by some 40% of subjects in oral dosing but only 10% in i.v. dosing. The incidence of headache does not correlate well with the total daily dose of the drug. However, the controlled release formulations tested appear to cause vasodilatory symptoms more frequently than i.v. or rapid release oral formulations. The other typical vasodilatory ADEs seen in healthy volunteers are nausea, palpitation, and dizziness. Symptomatic hypotension is rarely encountered. It appears that HF patients tolerate the vasodilatory actions of the drug better than healthy volunteers. Only individual cases of headache, vertigo, and flushing have been reported, and injection site irritation has been the most commonly reported ADE. Even though some increase in HR is seen with high doses of the drug, there are thus far no signs of an increased incidence of ventricular tachyarrhythmias, nor have there been any noteworthy changes in the clinical laboratory safety tests. However, that at least in i.v. dosing the drug is devoid of ADEs with significant medical seriousness [73]. The Ca^{2+} sensitizers may influence myocardial energetics by their action on Ca^{2+} turnover and on crossbridge behavior. Using a myothermal method, the effects of the Ca^{2+} sensitizer EMD-53998 on Ca^{2+} cycling, crossbridge behavior, and myocardial energy turnover were compared with the effects of an increase in $(\text{Ca}^{2+})_o$ from 1.25 to 7.5 mM and with the effects of the catecholamine isoproterenol. All three inotropic interventions increased isometric force development in

right ventricular rabbit papillary muscles. Relaxation time was decreased with isoproterenol, unchanged with high Ca^{2+} , and increased with EMD 53998. Ca^{2+} cycling-related energy consumption, as measured by tension-independent heat, increased by 234% with high Ca^{2+} , by 439% with isoproterenol, and by 77% with EMD 53998. In contrast to high Ca^{2+} and isoproterenol, EMD 53998 increased economy of crossbridge cycling by increasing the force-time integral of the individual crossbridge cycle. The EMD 53998 acts by PDE inhibition and myofilament Ca^{2+} sensitization. The latter effect is in part mediated by alteration of crossbridge behavior. Because of its effects on Ca^{2+} cycling and crossbridge function myocardial energy turnover was reduced significantly with EMD 53998, whereas energy turnover was unchanged with high Ca^{2+} and was increased with isoproterenol. The Ca^{2+} sensitizer levosimendan was investigated in isolated failing human myocardium. Levosimendan dose-dependently increased isometric tension. The inotropic effect was associated with increased rate of relaxation and reduced relaxation time. Measurements of $(\text{Ca}^{2+})_i$ using the photoprotein aequorin suggest that levosimendan acts by increasing myofilament Ca^{2+} sensitivity and by increasing cAMP due to PDE inhibition. However, the contribution of the cAMP system to the action of levosimendan appears to be rather small. Therefore, the finding of a positive lusitropic effect of levosimendan may be consistent with the notion that levosimendan binds to troponin-C and increases Ca^{2+} sensitivity only at high (systolic) $(\text{Ca}^{2+})_i$ concentrations [74]. The mechanisms of action of the Ca^{2+} -sensitizing agents like levosimendan, pimobendan, MCI-154, and EMD 53998. By using purified human recombinant troponin-C (cTnC), the role of cTnC as a target protein for these compounds was investigated. Accordingly, the Ca^{2+} -dependent binding to cTnC and the stabilizing effects of the compounds on the Ca^{2+} -induced conformation of dansylated cTnC were studied. Only levosimendan showed Ca^{2+} -dependent action on cTnC. Of the studied compounds, levosimendan was the most potent Ca^{2+} sensitizer in skinned fiber experiments. Furthermore, EMD 53998 and MCI-154, but not levosimendan and pimobendan, increased myosin ATPase activity, indicating that they may enhance the cycling rate of myosin-actin crossbridges. Levosimendan probably enhances the association rate but decreases the dissociation rate of myosin-actin crossbridges. Therefore, levosimendan does not seem to affect the actual relaxation phase. The other Ca^{2+} sensitizers, however, appear to act mainly by decreasing the dissociation rate of crossbridges. The weak Ca^{2+} -sensitizing effect of pimobendan may be based on indirectly mediated increase in affinity of cTnC for Ca^{2+} . MCI-154 might act in a similar way but, like EMD 53998, MCI-154 also acts on myosin-actin crossbridges. The levosimendan binds in a Ca^{2+} -dependent manner to the N-terminal domain of cTnC, which magnifies the extent of the contraction produced by cTnC when it is Ca^{2+} -activated [75].

The molecular interactions regulating myofilament activity in heart cells, focus is on the interaction between TnC, the Ca^{2+} -receptor and TnI, an inhibitory protein. It is this interaction that appears to form a molecular switch that turns on the thin filament. It will be seen that control of the actin-myosin reaction is not only through Ca^{2+} -binding to TnC, but also through steric, cooperative and

allosteric processes involving all of the main myofilament proteins-actin, myosin, TnM, TnT, TnC, and TnI. The process is modulated by covalent and non-covalent mechanisms. The process is altered in diverse myopathies and pathologies of the heart and is a target for pharmacological manipulation by a inotropic agents, "Ca²⁺-sensitizers" [76]. The signal transduction process mediated by cAMP that leads to the characteristic positive inotropic effect (PIE) in association with a positive lusitropic effect has been well established. Relationships between accumulation of cAMP, changes in (Ca²⁺)_i transients and the PIE differ, however, depending on the mechanism of particular drugs that affect different steps in the metabolism of cAMP. Selective partial agonists of beta 1-adrenoceptors and inhibitors of PDE-III cause the accumulation of less cAMP for a given PIE than does isoproterenol. In addition, in aequorin-microinjected canine ventricular muscle, selective inhibitors of PDE III, OPC 18790 and Org 9731, produced smaller decreases in the responsiveness of myofilaments to Ca²⁺ ions than isoproterenol, while a partial agonist of beta 1-adrenoceptors, denopamine, elicits a decrease in Ca²⁺ responsiveness of the same extent as does isoproterenol. Activation of myocardial alpha 1-adrenoceptors, as well as stimulation of receptors for endothelin and angiotensin II, which accelerates hydrolysis of phosphoinositide (PI) to result in production of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) are associated with very similar inotropic regulation: the dependence on the species of animals of induction of the PIE; an excellent correlation between the extent of acceleration of hydrolysis of PI and the PIE; isometric contraction curves associated with a negative lusitropic effect; the PIE associated with increases in myofibrillar responsiveness to Ca²⁺ ions; and the selective inhibition of the PIE by an activator of protein kinase C (PKC), phorbol 12,13-dibutyrate (PDBu), with little effect on the PIE of isoproterenol and Bay k 8644. A novel class of cardiotoxic agents, namely, Ca²⁺ sensitizers such as EMD 53998 and Org 30029, act on the Ca²⁺-binding site of TnC, increasing the affinity of these sites for Ca²⁺ ions, or at the actin-myosin interface to facilitate the cycling of cross-bridges. These agents produce a PIE with little change or decrease in Ca²⁺ transients and may bring about a significant breakthrough in the development of drugs for reversal of myocardial failure in the treatment of CHF [77,78]. Positive inotropic agents that increase the sensitivity of myofilaments to Ca²⁺, these drugs appear to augment contractility independently of cAMP or Ca²⁺, and thus may have fewer of the adverse side effects seen with other currently available agents. A significant negative lusitropic effect of the Ca²⁺ sensitizer EMD 53998 in ferret papillary muscle, although this effect was considered to be outweighed by powerful augmentation of contractility. The impairment of relaxation by Ca²⁺-sensitizers may be even more severe when myocardial Ca²⁺ is abnormally elevated, such as in hypoxia and end-stage HF. The effects of EMD 53998 and milrinone on contractility and Ca²⁺ flux in a cell culture model of myocardial hypoxia. The increased Ca²⁺ sensitivity results in marked impairment of relaxation under hypoxic conditions, possibly due to the impaired Ca²⁺ sequestration and increased Ca²⁺ availability exhibited by hypoxic myocytes. The effects of Ca²⁺ sensitizers can be strongly influenced by the prevailing

status of (Ca²⁺)_i handling, and may be deleterious in the diseased or ischemic myocardium [79]. The narrow margin of safety of cardiotoxic glycosides has led to novel cardiotoxic agents that are superior to the glycosides. Cardiotoxic drugs acting on β-adrenoceptors and inhibitors of cAMP PDE have been used for the treatment of HF. Ca sensitizers are of interest, mechanism of action may be beneficial for the failing heart. Cardiotoxic agents with a novel mechanism of action such as gingerol and xestoquinone have been isolated from natural sources. The 9-methyl-7-bromo-eudistomin D is a powerful radiolabeled Ca²⁺ releaser having caffeine-like properties, provide a promising tool for the molecular mechanism of the Ca²⁺ release process [80].

The imidazole-containing compounds carnosine and homocarnosine, endogenous to skeletal and cardiac muscle, have effect on the contractile behaviour of skinned skeletal and cardiac muscle. Carnosine, at millimolar concentrations which are near physiological for many skeletal fibres, and in a concentration-dependent fashion, shifts the curve relating Ca²⁺ to steady-state tension to lower Ca²⁺ in both skeletal (frog) and cardiac (rat) muscle preparations. Of other imidazoles endogenous to heart, homocarnosine is somewhat more effective, while N-acetyl L-histidine is much less so. The maximum level of Ca²⁺-activated force is increased significantly by homocarnosine in cardiac trabeculae. The cellular imidazoles related to carnosine are natural 'Ca²⁺ sensitizers' in striated muscle [81]. Inotropic agents alter MVO₂ by influencing HR, by influencing preload and afterload due to vasodilation, and by direct effects on the myocardium. The inotropic agents which act by increasing cAMP in the failing human myocardium increase myocardial energy turnover by their effects on excitation-contraction coupling, resulting in a considerable increase in the amount of Ca²⁺ cycling. Glycosides, which increase contractile force independent of cAMP, increase Ca²⁺ cycling moderately and do not influence myocardial energy turnover significantly. Ca²⁺-sensitizers, by increasing Ca²⁺-affinity of contractile proteins, may increase contractile force and decrease myocardial energy turnover. The energy-saving effect of reduced preload and afterload may counterbalance a direct myocardial energy-wasting effect of some inotropic agents. An increase in HR due to inotropic interventions is unfavorable since 1) oxygen consumption increases in proportion to HR, and 2) contractile force of the failing human myocardium decreases. The increasing HR increases contractile force and CO in nonfailing human myocardium, but decreases cardiac performance in the failing human heart. In light of the inverse force-frequency relation in failing human myocardium, negative chronotropic drugs may represent a class of "positive inotropic" agents. Agents reducing HR may be beneficial from an energetic point of view by reducing MVO₂ and by improving myocardial perfusion due to a prolongation of diastole. The role of Ca²⁺ in muscle activation is emphasis on interactions between the regulatory proteins TnC, TnI, actin and myosin. These interactions depend on Ca²⁺, but they also influence the Ca²⁺ binding to TnC. They may be affected by short "competitive" peptides whose sequences are similar to the amino-acid sequences on the protein-interaction sites. They may be also affected by certain Ca²⁺ sensitizers that influence the Ca²⁺ sensitivity of myofilaments. The Ca²⁺

sensitizers are drugs which increase force development in striated muscle by sensitizing myofilaments to Ca^{2+} . The increase Ca^{2+} affinity of the regulatory domain of Ca^{2+} binding protein TnC. The Ca^{2+} binding proteins are calmodulin and skeletal troponin C. The Ca^{2+} -sensitizing action of drugs on TnC. A model of human cTnC in three- Ca^{2+} state has been constructed. When Ca^{2+} is bound to Ca^{2+} site II of cTnC an open conformation of the protein results, which has a hydrophobic pocket surrounded by a

few polar side chains. Complexation of three drugs, trifluoperazine, bepridil, and pimobendan, to the hydrophobic pocket, cTnC an interaction occurs between Gln-50 and Asp-88, which has a long-range effect on Ca^{2+} binding. The binding modes of drugs, where a strong interaction with Asp-88 exists, can effectively prevent the interaction between Asp-88 and Gln-50 in the protein, and are proposed to be responsible for the Ca^{2+} -sensitizing properties of the drugs [82,83,84].

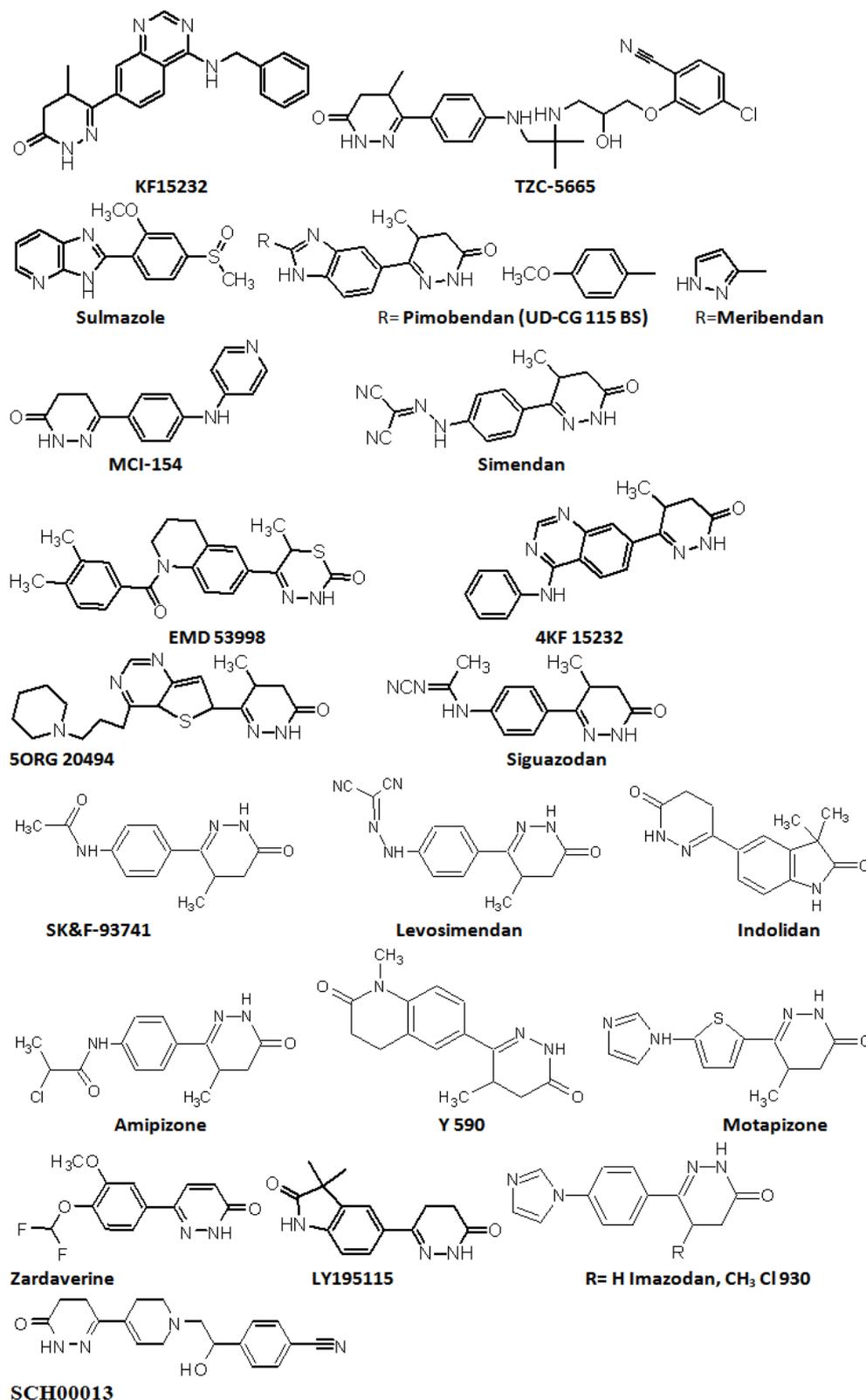


Figure 2. Structure of some effective calcium sensitizing agents

3. Conclusion

The Ca^{2+} ions are key regulators of skeletal muscle contraction. By binding to contractile proteins, they initiate a cascade of molecular events leading to cross-bridge formation and ultimately, muscle shortening and force production. The ability of contractile proteins to respond to Ca^{2+} attachment, also known as Ca^{2+} sensitivity, is often compromised in acquired and congenital skeletal muscle disorders. Undoubtedly, major physiological causes of weakness for patients. The strong molecular and cellular evidence that pharmacological modulators of some of the contractile proteins, also termed Ca^{2+} sensitizers, are efficient agents to improve Ca^{2+} sensitivity and function in diseased skeletal muscle cells. In fact, they compensate for the impaired contractile proteins response to Ca^{2+} binding. Such Ca^{2+} sensitizing compounds are successfully used for reducing problems in cardiac disorders (CD). Therefore, in future, these drugs may represent an emerging class of agents to enhance the quality of life of patients suffering from skeletal muscle weakness [85-87]. The development of Ca^{2+} -sensitizing agents has led to a new approach to the treatment of CHF. These novel inotropic agents act directly on the contractile protein systems and correct the reduced responsiveness of the myofilaments to Ca^{2+} . The inotropic activity of this class of cardiotoxic agents may represent an important pharmacologic approach to the future treatment of acute and chronic CHF. The cardiotoxic effects of Ca^{2+} sensitizers, with particular emphasis on the hemodynamic actions of these drugs in various in vitro and in vivo heart preparations. The therapeutic exploitation of these agents and delineate their possible role in the therapy of CHF.

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