Myocardial efficiency in stunned myocardium. Comparison of Ca\textsuperscript{2+}-sensitization and PDE III-inhibition on energy consumption

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Abstract

Objective: In stunned myocardium oxygen consumption is relatively high compared with the reduced ventricular function. On the other hand, inotropic stimulation is frequently required to improve postischemic ventricular dysfunction. However, inotropic agents which act via intracellular increased calcium result in a higher oxygen demand. Therefore Ca\textsuperscript{2+}-sensitization might be a favorable alternative. Methods: The effects of a novel Ca\textsuperscript{2+}-sensitizer (EMD 60263, 10 \textmu M, group 1) were compared with a phosphodiesterase (PDE) III-inhibitor (enoximon, 20 \textmu M, group 2) on 14 isolated, blood-perfused rabbit hearts during reperfusion after a global ischemia of 20 min. Ventricular function, the pressure–volume area (PVA, a measure of total mechanical work), and total myocardial oxygen consumption (MVO\textsubscript{2}) were assessed. Contractile efficiency (EF\textsubscript{cont}), derived from the reciprocal of the slope of the MVO\textsubscript{2}–PVA relation, and external efficiency (EF\textsubscript{ex}, stroke work/MVO\textsubscript{2}), were calculated. Results: At matched heart rate (group 1: 141 ± 10 min\textsuperscript{-1}, group 2: 151 ± 28 min\textsuperscript{-1}) and end-diastolic volume (1.3 ± 0.2 ml) systolic variables were significantly decreased in stunned myocardium: LVP\textsubscript{max} to 57 ± 13% of control value in group 1 and to 76 ± 7% in group 2, aortic flow to 20 ± 4 vs. 25 ± 8%. PVA was decreased to 57 ± 13 and 67 ± 11%, MVO\textsubscript{2} was non-significantly decreased to 73 ± 22 and 88 ± 14%. After administration of either inotropic agent LVP\textsubscript{max} was significantly improved to 96 ± 12 vs. 90 ± 8% compared with preischemic levels, aortic flow to 103 ± 24 vs. 88 ± 9%, and PVA 99 ± 11 vs. 89 ± 16%, respectively. EMD 60263 increased MVO\textsubscript{2} to control levels (107 ± 9%), and enoximon raised MVO\textsubscript{2} even more significantly above control (139 ± 13%). Both myocardial efficiency indices were significantly diminished during reperfusion: EF\textsubscript{ex} to 14 ± 9 vs. 23 ± 7% and EF\textsubscript{cont} to 71 ± 7 vs. 65 ± 9% compared with preischemic levels. EF\textsubscript{ex} (109 ± 21%) was significantly, but EF\textsubscript{cont} only slightly (84 ± 11%) increased after administration of EMD 60263, whereas EF\textsubscript{ex} (57 ± 13%) and EF\textsubscript{cont} (71 ± 12%) remained depressed after enoximon. Conclusions: In stunned myocardium, the decreased efficiency indices indicate that energy utilization is disturbed. Both agents recruited an inotropic reserve, whereas Ca\textsuperscript{2+}-sensitization seemed to be more favorable in terms of myocardial efficiency indices. These results indicate that alteration of myocardial calcium sensitivity contributes a major part to postischemic dysfunction. Therefore, Ca\textsuperscript{2+}-sensitization may potentially be a superior method for inotropic support in the postischemic heart. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

After brief episodes of ischemia, myocardial function remains depressed for hours or even days. The phenomenon of myocardial stunning is characterized by impairment of contractile function, despite almost complete integrity of the myocardium and restoration of normal or near normal coronary blood flow, i.e. oxygen supply [1]. Myocardial oxygen consumption is reported to be relatively high compared with postischemic function, supporting evidence that energy utilization is disturbed [2,3].

However, while baseline ventricular function is depressed, inotropic stimulation is often required in the clinical setting. Stunned myocardium retains the capacity to respond to various inotropic interventions [6], Via activation of β-adrenoceptors or selective inhibition of phosphodiesterase isoenzymes, inotropic stimulation can be achieved by increasing the amount of intracellular calcium, cycled with each beat [4,7]. Thus, this pathway is accompanied with higher energy demand, whereas the energy supply is often limited in stunned myocardium. Newer inotropic agents which act by altering myofilament calcium sensitivity without increasing intracellular calcium levels might achieve an inotropic action with smaller increases...
of energy demand [5]. The purpose of this study was to evaluate the consequences of inotropic stimulation on myocardial energetics in stunned myocardium. We investigated the effects of a phosphodiesterase inhibitor (enoximone) and a novel calcium sensitizer (EMD 60263), a thiaziazinone derivative, on myocardial efficiency in isolated rabbit hearts.

2. Materials and methods

2.1. Experimental preparation

The experiments were performed on a total of 14 male New Zealand White rabbits with an average age of 6 months and an average weight of 2200 ± 200 g; the rabbits were handled according to the animal welfare regulations of the German federal authorities. After rapid excision, the hearts were connected to a modified Langendorff setting and perfused with a modified, erythrocytes enriched Krebs–Henseleit solution containing (in mM): NaCl 119, NaHCO₃ 25, KCl 4.7, CaCl₂ 1.8, MgCl₂ 1.2, EDTA 0.5 and glucose 11. The buffer was equilibrated with 72% N₂, 22% O₂, 6% CO₂ at 37°C, giving a pH of 7.4. Bovine erythrocytes were added to achieve a hemoglobin concentration of 10 g/100 ml. The solution contained 4 g/100 ml albumin, and the Ca²⁺ concentration was adjusted to 2.5 mM.

A water-filled latex balloon was inserted into the left ventricular cavity via the left atrium. The balloon was connected to a ‘systemic’ circuit that contained two artificial valves and a windkessel. The aortic flow was measured via an ultrasonic flowprobe (T 206, Transonic Systems), aortic pressure (=afterload) with a pressure transducer (P 23 II, Statham). The circuit permitted changes in afterload and in preload conditions without alteration of coronary perfusion pressure. A 3-F microtip manometer (SPR-249, Millar), inserted into the balloon, was used to measure left ventricular pressure. For measurement of left ventricular dimensions, sonomicrometry was employed (system 6, Triton) using two ultrasonic crystals, glued to either side of the balloon. Different balloon sizes were used depending on the heart size.

Total coronary blood flow (CBF) was drained and measured with a second ultrasonic flow-probe. The difference in arteriovenous O₂ content (avD-O₂) was continuously measured using absorption spectrophotometry (AVOX systems). Arterial and venous samples of the perfusion medium were measured by a Lex-O₂-Con analyzer to control arterial equilibration and coronary venous measurements.

2.2. Experimental protocol

Control conditions were recorded after stabilization of left ventricular function, i.e. 20 min after the end of the instrumentation. The hearts were pseudo-randomized into two groups. The hearts of each group (n = 7) were subjected to a period of 20 min normothermic, no-flow ischemia. After 30 min reperfusion, data were recorded again, and finally the inotropic agents were administered. In group 1, the calcium-sensitizer EMD 60263 with a concentration of 10 μM was added. In group 2, the phosphodiesterase inhibitor III enoximone at a concentration of 20 μM was used.

During control and reperfusion, coronary arterial pressure was held constant at 80 ± 2 mmHg. All variables were measured at a constant temperature of 37.0 ± 0.5°C maintained by immersing the hearts in a temperature-controlled chamber. For baseline measurements left ventricular preload, estimated via the intraventricular diameter, was maintained constant as well. The hearts were weighed at the end of the protocol.

In order to assess the end-systolic pressure–volume relationship (Fig. 1), five to seven increasing preload conditions were chosen by stepwise lifting the preload reservoir. Data were recorded after stabilization at each particular preload, and the data were acquired during control, after 30 min reperfusion and after administration of the inotropic agents.

Fig. 1. The end-systolic pressure–volume relationship (ESPVR) was constructed by linear fitting of five to seven pairs of end-systolic pressures and volumes that were obtained by preload variation (panel A). The pressure–volume area (PVA) was assessed from the area enclosed by the systolic portion of the pressure–volume (PV) loop, the ESPVR and the end-diastolic pressure volume relation (EDPVR). Five to seven MVO₂ and PVA pairs were linearly fitted for construction of the MVO₂–PVA relationship (panel B). The MVO₂-intercept of this relationship is identical to the MVO₂ of the unloaded contraction.
2.3. Data acquisition

The following variables were continuously registered on a forced-ink chart recorder (Type 481, Brush): aortic flow, coronary flow, left ventricular (LV) pressure and inner diameter. Heart rate and LV \( \frac{dP}{dt} \) were derived from the pressure signal. At steady-state conditions after preload alteration, the variables were simultaneously stored digitally for later analysis at a sampling rate of 300 Hz.

2.4. Calculations and statistics

Hemodynamic data were computer-assisted analyzed with six to eight consecutive beats being averaged. The end-systolic pressure–volume relationship and the pressure–volume areas were calculated with the help of the computer program EASYDAT [8]. Coronary blood flow was normalized to 100 g wet weight. Myocardial oxygen consumption was calculated according to the Fick principle from normalized coronary blood flow and the difference in oxygen content of arterial and coronary venous perfusion medium.

The contractile efficiency (\( \text{EF}_{\text{cont}} \)) was determined as the inverse slope of the MVO\(_2\)-pressure–volume area relationship (Fig. 1), and the MVO\(_2\) for the unloaded contraction was assessed as the intercept of the MVO\(_2\)-pressure–volume area relationship with the MVO\(_2\) axis. As an index of external efficiency (\( \text{EF}_{\text{ex}} \)), the ratio of stroke work and myocardial oxygen consumption was determined at the same inner diameters, i.e. same preload. The stroke work in turn was calculated from the peak ventricular pressure and stroke volume.

Data are expressed as mean ± SD. Statistical analysis was performed with a statistical software package (Systat). Nonischemic and posts ischemic data were compared by paired \( t \)-testing. Analysis of variance (ANOVA) for repeated measurements was used to test differences in hemodynamic variables within any given group. When a significant overall effect was detected, a post hoc test with Bonferroni correction was performed to compare single mean values. A \( P \) value of less than 0.05 was considered indicative of a significant difference.

3. Results

Compared with control, all systolic global variables were significantly depressed in the posts ischemic reperfused hearts. Aortic flow was decreased in group 1 to 20 ± 4% and in group 2 to 25 ± 8% compared with preischemic levels, LVP\(_{\text{max}}\) to 57 ± 13 vs. 76 ± 7%, and \( \frac{dP}{dt}_{\text{max}} \) to 56 ± 12 (group 1) and 77 ± 9% (group 2) (Fig. 2).

Early relaxation, as measured in terms of \( \frac{dP}{dt}_{\text{min}} \), was impaired by 33 ± 9 and 29 ± 9%, whereas the end-diastolic left ventricular pressure remained essentially unchanged (8 ± 3 vs. 7 ± 3 mmHg in group 1 and 9 ± 2 vs. 7 ± 3 mmHg in group 2) (Fig. 2). Heart rate was similar in both groups during pre- and postischemic intervals (Fig. 3B). The

![Fig. 2. Effects of 20 min global no-flow ischemia and administration of inotropic agents ([□], EMD 60263; ■, enoximon) on aortic flow (AoF; A), peak left ventricular pressure (LVP\(_{\text{max}}\); B), \( \frac{dP}{dt}_{\text{max}} \) (C) and \( \frac{dP}{dt}_{\text{min}} \) (D). Note that all variables were significantly depressed during reperfusion (Rep) and were significantly increased after administration of the inotropic agent. The bars represent mean values ± SD, *control vs. reperfusion, *reperfusion vs. agent.](image)
decrease of coronary blood flow was not significant in both groups. Total oxygen consumption was slightly decreased by 27 ± 17% in group 1 and by 11 ± 8% in group 2 (Fig. 3C). In contrast, the pressure–volume area was significantly decreased by 43 ± 16 and 33 ± 22% (Fig. 3A). Oxygen consumption for the unloaded contraction was not significantly changed during reperfusion (by 12 ± 5% in group 1 and by 8 ± 6% in group 2) (Fig. 3D).

After administration of either agent, ventricular function was significantly improved. Aortic flow, LVP\textsubscript{max}, dP/dt\textsubscript{max} and dP/dt\textsubscript{min} were improved to almost control levels in both groups, whereas PVA was more improved by the calcium sensitizer (to 99 ± 17% of control) compared with enoximon (to 89 ± 13% of control value). Enoximon increased heart rate significantly by 30 ± 9% compared with control. In contrast, EMD 60263 reduced heart rate (13 ± 13%), without reaching significance. Analyzing the cardiac rhythm from the left ventricular pressure signal showed that application of either inotropic agent did not result in an increased occurrence of arrhythmias. Myocardial oxygen consumption was slightly increased above control with EMD 60263 (107 ± 9%), but enoximon raised MVO\textsubscript{2} significantly to 139 ± 13% compared with control levels.

Both efficiency indices were significantly diminished during reperfusion (Fig. 4). External efficiency was decreased to 14 ± 9% in group 1 and to 23 ± 7% in group 2. EMD 60263 improved the external efficiency index to control levels (109 ± 21%), enoximon to 57 ± 13% of control. The contractile efficiency – the reciprocal of the slope of the MVO\textsubscript{2}–PVA relation – was significantly depressed in both groups, to 71 ± 7% in the Ca\textsuperscript{2+}-sensitizer group and to 65 ± 9% in the enoximon group. After administration of EMD 60263, EF\textsubscript{cont} was non-significantly increased to 84 ± 11% of preischemic value and after enoximon, EF\textsubscript{cont} remained almost unchanged compared with reperfusion (71 ± 12% of control value). MVO\textsubscript{2} for the unloaded contraction increased non-significantly by 15 ± 6% in group 1 and 23 ± 8% in group 2.

4. Discussion

The relationship between ventricular function and myocardial oxygen consumption was investigated in stunned myocardium and after application of two different inotropic agents. The major findings were: (1) the calcium-sensitizer EMD 60263 and the phosphodiesterase (PDE) III inhibitor enoximon, recruited an inotropic reserve; (2) myocardial efficiency indices remained depressed after application of enoximon, whereas EMD 60263 improved both efficiency indices.

Depressed contractile function in viable myocardium at almost restored coronary flow, i.e. oxygen and energy...
supply, are major characteristics of stunned myocardium [1,2]. Possible causes underlying this phenomenon are free radical damaging of membranes and enzymes [9], calcium overload during ischemia and early reperfusion with activation of proteases and alteration of intracellular calcium-cycling [12]. In accordance with the literature, our data show a significant reduction of all functional parameters.

The relationship between ventricular function and myocardial oxygen consumption was investigated via the external efficiency. This term stresses the ratio between external work, i.e. stroke work (stroke volume times developed pressure), and the total energy demand, i.e. total myocardial oxygen consumption. In the present study, external efficiency was drastically reduced in both postischemic groups. Even after 30 min reperfusion, there was no substantial recovery. These results are in accordance with data from the literature; however, the decline in our data seems to be more pronounced, but the different types of ischemia (no-flow or low-flow ischemia), temperature and species might explain the difference with other studies.

Contractile efficiency was calculated by the relationship between MVO$_2$ and the pressure-volume area according to Suga and coworkers [13]. In contrast to the external efficiency, the MVO$_2$-PVA concept allows partitioning of the MVO$_2$ in one fraction that is needed for contraction and another that is non-work related, i.e. the unloaded contraction. This separation clearly showed a decrease of contractile efficiency in stunned myocardium and, in turn, an inefficient electromechanical coupling. The principal cause of myocardial stunning and loss of myocardial efficiency is not fully understood. Changes of energy requirements for calcium handling could increase myocardial oxygen consumption. However, there is evidence for a loss of Ca$^{2+}$-sensitivity of the myofilaments in stunned compared with normal myocardium as a major underlying mechanism. Based on experiments with isolated ferret hearts this mechanism was first proposed in 1987 by Kusuoka et al. [23]. Moreover, other groups found a decrease in the Ca$^{2+}$-sensitivity of isometric tension in skinned fibers from stunned pig myocardium [24] and in the intact ventricular muscle [12]. This alteration might implicate the increased energy demand of the myofilaments to generate force.

The inefficiency of the myocardium has also been attributed to ischemia-induced injury such as uncoupling of oxidative phosphorylation [14]. However, no data exist confirming mitochondrial uncoupling [15]. In the present study, MVO$_2$ for the unloaded contraction did not differ significantly between normal and stunned myocardium. MVO$_2$ for the unloaded contraction, which contains the fraction for basal metabolism and another for excitation–contraction coupling, increases with increasing contractile state and decreases with decreasing contractile state [16]. Therefore, our results suggest that the maintained MVO$_2$ for the unloaded contraction is inadequately high in relation to the decreased postischemic contractile state. In a previous study of our group, basal MVO$_2$ after KCl arrest was assessed in a similar protocol. Basal MVO$_2$ was not elevated in postischemic hearts, suggesting that the fraction of MVO$_2$ attributable to excitation–contraction is disproportionately high in stunned myocardium [17]. Therefore the excess oxygen utilization at a state of reduced contractile function cannot be explained by an increased metabolic need for postischemic repair mechanisms taking place during this condition [8] and indeed, calcium transport mechanisms of cell membranes and the sarcoplasmic reticulum are impaired in stunned myocardium [10,11].

Inotropic support is often necessary for the long-lasting myocardial dysfunction after brief periods of ischemia, i.e. perioperatively in cardiac surgery patients after ischemic arrest. Most of the clinically used inotropic drugs, such as α- and β-adrenergic agonists, and PDE III-inhibitors, act by increasing Ca$^{2+}$-loading of the cytosol and sarcoplasmic reticulum, leading to an increase of the Ca$^{2+}$ transients [4]. But this is associated with a disproportionate increase in myocardial oxygen requirement [18]. A number of new cardiotonic drugs do not act through increased cytosolic calcium transients, but affect also Ca$^{2+}$-sensitization (i.e. shift in the pCa–force relation) in both skinned and intact cardiac muscle preparations [5]. Thiadiazine derivatives

![Image](https://example.com/image.png)

**Fig. 4.** Effects of 20 min global no-flow ischemia and administration of inotropic agents (□, EMD 60263; ■, enoximon) on external efficiency (EF$_{ex}$; left panel) and contractile efficiency (EF$_{cont}$; right panel). The bars represent mean values ± SD, $P < 0.05$, *control vs. reperfusion, †reperfusion vs. agent.
vary widely in their ability to sensitize the myofilaments towards Ca\(^{2+}\) and to increase cellular cyclic AMP levels via their PDE III inhibitory action; the (+)-enantiomer EMD 60263 has been described as the first Ca\(^{2+}\)-sensitizer devoid of PDE III inhibitory activity [19]. The concentration (IC\(_{50}\)) of EMD 60263 required to reach half-maximal PDE III inhibition activity is reported to be >30 \(\mu\)M [20], a concentration clearly higher than the one used in our study.

The inotropic actions of the Ca\(^{2+}\)-sensitizer EMD 60263 and the PDE III inhibitor enoximon were tested in a constant afterload preparation to eliminate any afterload effects. In the isolated rabbit heart preparation, postischemic ventricular dysfunction was significantly improved with both agents, reaching almost preischemic control levels with the selected drug concentrations. These findings were not unexpected and are in accordance with the notion that ventricular function can be improved by recruiting an inotropic reserve [20]. An in vivo study in pigs demonstrated that EMD 60263 increased systolic segment shortening of stunned and nonstunned myocardium, the effect being more pronounced in stunned than in nonstunned myocardium [21]. According to the concept of loss of Ca\(^{2+}\)-responsiveness of the myofilaments as a major cause of stunning, these data and our results demonstrate that Ca\(^{2+}\)-sensitizers are very effective inotropic agents in this particular setting of stunned myocardium. Interestingly, only enoximon increased heart rate significantly, but no arrhythmogenic effects were found in the presence of either agent in the stunned myocardium.

The external efficiency was substantially improved to control levels with EMD 60263 (109%), whereas EF\(_{ex}\) remained depressed with enoximon (57%). After administration of both drugs, contractile efficiency was slightly increased with EMD 60263 and remained unchanged with enoximon. With respect to the better improvement of myocardial efficiency with the Ca\(^{2+}\)-sensitizer compared with the PDE III inhibitor, the present data also underline the reduced Ca\(^{2+}\)-responsiveness as a major cause for energy wastage in the stunned myocardium. Therefore, Ca\(^{2+}\)-sensitization also improves the energetic balance, whereas other inotropic agents do not. Another advantage of Ca\(^{2+}\)-sensitization compared with phosphodiesterase inhibition, with its known vasodilatory effects, might be that no systemic vasodilatory properties were seen. This was shown in an in vivo study in pigs [21]. However, despite the quite interesting perspectives of Ca\(^{2+}\)-sensitization in the management of postoperative heart failure, there are only a few clinical trials with the agent Levosimendan. In a clinical study, this Ca\(^{2+}\)-sensitizer, which also has phosphodiesterase-inhibiting properties, showed an enhancement of cardiac performance after cardiopulmonary bypass in humans [25].

The MVO\(_2\)-PVA relationship has been shown to be more sensitive in the settings of ischemia [22] compared with other efficiency assessments. The absence of complete recovery of contractile efficiency, even after Ca\(^{2+}\)-sensitization, indicates that in stunned myocardium energy utilization is disturbed and can scarcely be improved.

5. Conclusion

In our model of myocardial stunning, the imbalance of oxygen consumption to decreased ventricular function, as demonstrated by the decline of efficiency indices, shows the energetic inefficiency of the myocardium. Ca\(^{2+}\)-sensitization and PDE III inhibition both can recruit an inotropic reserve. Because Ca\(^{2+}\)-sensitization seems to be more favorable in terms of myocardial efficiency, we conclude that Ca\(^{2+}\)-desensitization is an attribute of the phenomenon of myocardial stunning. The maintained MVO\(_2\) for the unloaded contraction in stunned myocardium and its moderate change after Ca\(^{2+}\)-sensitization also indicate that disturbances in excitation–contraction coupling contribute to the inefficiency. Taken together, concerning myocardial efficiency the Ca\(^{2+}\)-sensitizer seems to exert more beneficial effects in posts ischemic inotropic stimulation.

References

Appendix A. Conference discussion

Dr L. von Segesser (Lausanne, Switzerland): You are working in an isolated rabbit model, and you can therefore not appreciate what happens to the peripheral resistance when you modify the heart function.

Dr Sunderdiek: No, we can’t do it in this model.

Dr von Segesser: But your drugs would; they would also act on the muscles of the vessels?

Dr Sunderdiek: Yes, they certainly would, and there are a couple of studies using the calcium sensitizer in pigs by the group of Verdow from Rotterdam, and they showed that the systemic blood pressure was well maintained.

Dr J. Svennevig (Oslo, Norway): Would you just very briefly indicate possible clinical implications.

Dr Sunderdiek: A clinical implication would be, for instance, a state of low cardiac output postoperatively after an operation with myocardial ischemia, i.e. coronary bypass surgery. Then we will have the need for inotropic intervention, and the calcium sensitizer maybe be a good alternative to improve myocardial function postoperatively.

Dr von Segesser: But then you would increase afterload with this drug, whereas with enoximon you would decrease afterload. Am I right?

Dr Sunderdiek: The data do not show an influence of the calcium sensitizer EMD 60263 on systemic blood pressure. So probably with an increase of cardiac output we might have at least an indirect increase of afterload conditions. But so far, at the moment there are not so many in vivo studies using the calcium sensitizer in pigs by the group of Verdow from Rotterdam, and they showed that the systemic blood pressure was well maintained.

Dr von Segesser: Then we might have at least an indirect increase of afterload conditions. But so far, at the moment there are not so many in vivo measurements to show reliable data of afterload changes.

Dr C. Stamm (Boston, MA, USA): PDE III inhibitors, just like catecholamines, actually desensitize contractile proteins to calcium via PKA and troponin I. With a pure, 100% calcium-sensitizing agent one would expect an impairment of relaxation associated with the improvement of contractility. Did you observe this?

Dr Sunderdiek: No, we did some further studies, because the presented data showed only dP/dt max as a parameter of early relaxation with no real alterations. In further studies we also measured the relation of τ and the end-diastolic pressure–volume relation, and both parameters showed a decrease of diastolic function after administration of the calcium sensitizer.

Dr Stamm: Most of those calcium-sensitizing agents have some PDE III-inhibiting properties. Do you know to which extent this is true for the EMD that you used?

Dr Sunderdiek: We got some data from the group of Dr Ravens from Essen in Germany, and they said that in this dosage of 10 μM, the extent for phosphodiesterase inhibition of this agent would be very low. So, I can’t tell you any exact percentage, but I would guess that the agents property of phosphodiesterase inhibition would be lower than 10% of the total inotropic effect.