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ORIGINAL RESEARCH ARTICLE

Altered myocardial force generation in end-stage human heart failure

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Abstract

Aims This study aimed to elucidate the molecular background of increased Ca^{2+} sensitivity of force production in cardiomyocytes of end-stage human heart failure.

Methods and results Ca^{2+}-activated isometric force and the cross-bridge specific rate of force redevelopment (k_{tr}) were determined in Triton-skinned myocytes from end-stage failing and non-failing donor hearts. Measurements (control: pH 7.2, 0 mM inorganic phosphate (P_i)) were performed under test conditions that probed either the Ca^{2+}-regulatory function of the thin filaments (pH 6.5), the kinetics of the actin-myosin cross-bridge cycle (10 mM P_i), or both (pH 6.5, 10 mM P_i). The control maximal Ca^{2+}-activated force (F_o) and k_{trmax} did not differ between failing and non-failing myocytes. At submaximal [Ca^{2+}], however, both force and k_{tr} were higher in failing than in donor myocytes. The difference in the Ca^{2+} sensitivities of force production was preserved when the thin filament regulatory function was perturbed by acidosis (pH 6.5) but was abolished by cross-bridge modulation (i.e. by P_i) both at pH 7.2 and at pH 6.5. P_i induced a larger reduction in force but a smaller increase in k_{tr} in the failing myocytes than in the non-failing myocytes at submaximal [Ca^{2+}].

Conclusion The enhanced P_i sensitivity of the actin-myosin interaction suggests that the P_i release step of the actin-myosin cross-bridge cycle is modified during end-stage human heart failure. This might be of functional importance when P_i accumulates (e.g. during cardiac ischaemia). Moreover, this alteration can influence cardiac energetics and the clinical efficacy of sarcomere targeted agents in human heart failure.

Keywords Human heart failure; Cardiomyocyte; Myofilament; Actin-myosin cross-bridge cycle

Introduction

Alterations in myocardial proteins (molecular remodelling) contribute to the progressive diminution of cardiac performance during end-stage heart failure.[1,2] One of the factors involved in myocardial dysfunction is an increased myofibrillar Ca^{2+} sensitivity of force production, as a result of alterations in the expression,[3,4] and/or phosphorylation levels[5–7] of contractile proteins. Although interpretation of heart failure specific myofibrillar protein changes

is complicated by conflicting data of human hearts and of animal models,[8] it is generally accepted that down-regulation of β-adrenergic signalling leads to hypophosphorylation of contractile proteins and consequently to an increased Ca^{2+} sensitivity of force production during end-stage human heart failure.[9] Of note, phosphorylation statuses of contractile proteins are the function of a number of protein kinases and phosphatases, hence allowing fine tuning for contractile performance during health and disease.[8,10–12] In this regard, reduced levels of phosphorylation for constituents of the thin filament (e.g. troponin I) and of the thick filament (e.g. myosin binding protein C, myosin light chain 2) have been consistently documented for progressed stages of human heart failure in association with ischaemic and/or dilated cardiomyopathy.[7,13,14]

The Ca^{2+} sensitivity of force production is a result of the cooperative interplay between the thin and thick filaments in striated muscles.[15,16] Thus, it is controlled not only by the Ca^{2+} regulation of the thin filaments[17] but also by the kinetic properties of the actin-myosin cross-bridge cycle.[18] The aim of this study was to reveal putative alterations in the previous two processes in order to clarify the mechanistic background of the increased Ca^{2+} sensitivity of force production seen in association with end-stage human heart failure. We hypothesize that changes in the chemo-mechanical transduction of the actin-myosin cross-bridge cycle is an integral element within the pathogenesis of heart failure, where protein phosphorylation plays a central mechanism.

To characterize the relationships between the Ca^{2+} sensitivity and the kinetics of actin-myosin cross-bridges, the $[Ca^{2+}]$ dependencies of isometric force production and of k_{tr}, i.e. the cross-bridge specific rate constant of tension redevelopment after unloaded shortening and restretch, were measured simultaneously in Triton-permeabilized human left ventricular myocytes. Ca^{2+} regulation was modulated by acidification,[17] while inorganic phosphate (P_i) was added to probe the force-generating cross-bridge transitions.[19]

Our results indicate that kinetic alterations of the cross-bridge cycle have a direct impact on the Ca^{2+} sensitivity in the human myocardium and may also affect the conversion of chemical energy into force and shortening during end-stage human heart failure.

Methods

Biopsies

Left ventricular biopsies were obtained during heart transplantation surgery from three explanted end-stage failing (New York Heart Association class IV) hearts (three males, age range 45–65 years) and from three non-failing donor hearts (two males, one female; age range 23–52 years). The heart failure resulted from ischaemic or dilated cardiomyopathy. The cardiac tissue was transported in cardioplegic solution, and upon arrival in the laboratory, the tissue was stored in liquid nitrogen. Phosphorylation levels of myofibrillar proteins of failing and non-failing myocardial tissue samples are given elsewhere.[7] The investigation conforms with the principles outlined in the Declaration of Helsinki.[20] All samples were obtained after informed consent had been received and with the approval of the local ethical committees, and the procedures followed were in accordance with institutional guidelines.

Myocyte isolation

Cardiomyocytes (8–9 per biopsy, 52 in total) were isolated mechanically and mounted in the experimental set-up as described previously.[21] 0.5% Triton X-100 was used to remove all the membranous structures of the myocytes. This allowed the study of myofibrillar contractile properties in the presence of a controlled intracellular environment without the presence of disturbing factors in the intact heart (i.e. hormonal factors, variable $[Ca^{2+}]$) or in multicellular preparations (myofibrillar disarray, diffusion limitations).

Experimental protocol

Isometric force measurements were performed at 15°C, and sarcomere length was adjusted to 2.1 μm in relaxing solution. The compositions of the relaxing and activating solutions used during force measurements were calculated as described by Fabiato (1981).[22] The pCa levels of the control (pH 7.2, 0 mM P_i) relaxing and activating solutions were 10 and 4.8, respectively. Solutions with other free $[Ca^{2+}]$ were obtained by mixing of the activating and relaxing solutions. After the first control activation (F_o) at saturating $[Ca^{2+}]$ (pCa = 4.8) *(Figure 1A)*, the resting sarcomere length was readjusted to 2.1 μm if necessary. The next four or five activations were carried out under various experimental conditions, followed by a bracketing control F_o determination. Measurements were continued until F_o had decreased below 80% of the value obtained at the beginning of the experiment. Force values at submaximal $[Ca^{2+}]$ were expressed relative to the nearest control F_o. To achieve maximal Ca^{2+} activation in the presence of 10 mM P_i and/or at pH 6.5, appropriate amounts of $CaCl_2$ were added to the activating solutions to cover a $[Ca^{2+}]$ range up to pCa 3.3.

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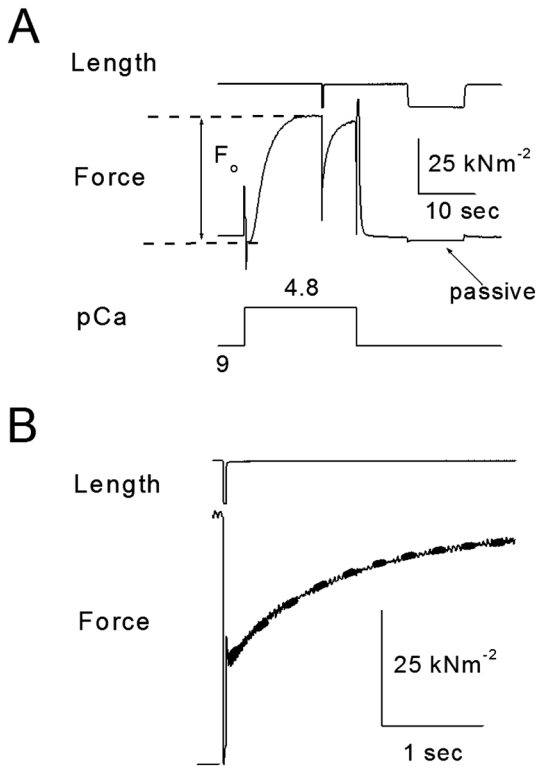

Figure 1 Panel A illustrates a force measurement on an isolated myocardial cell. Rapid length changes at the peak of the force development (at pCa 4.8; refer to also on the extended time scale in B) and in relaxing solution (pCa 10) served the determination of k_{tr} and the passive force component ('passive'). k_{tr} was defined as the time constant of the exponential fit (interrupted curve, panel B) to the force recovery following unloaded shortening (Δlength = 20% of the original segment length) and restretch ('release-restretch manoeuver').

The rate of force redevelopment (k_{tr}) was determined by using release-restretch manoeuvers, as illustrated in Figure *1*B.[23] At low [Ca^{2+}] ($P < 25\%$ of the control F_o), the low signal-to-noise ratio prevented k_{tr} determination.

Data analysis

Ca^{2+}-force relations were fitted to a modified Hill equation:

$$F = F_o\left[Ca^{2+}\right]^{nHill} / \left(Ca_{50}{}^{nHill} + \left[Ca^{2+}\right]^{nHill}\right)$$

where F is the steady-state force at a given [Ca^{2+}], while F_o, nHill, and Ca_{50} (or pCa_{50}) denote the maximal Ca^{2+}-activated force at saturating [Ca^{2+}], the slope, and the midpoint of the sigmoidal relationship, respectively.

All values in the text and figures are given as means ± S.E.M. Mean values were compared through unpaired Student's *t*-tests. Differences were considered significant when $P < 0.05$.

Results

The isometric force (F_o) and the rate constant of tension redevelopment (k_{trmax}) under control conditions (pH 7.2; 0 mM P_i) for failing ($38 \pm 4\,kN\,m^{-2}$ and $1.00 \pm 0.06\,s^{-1}$, respectively) and the non-failing ($36 \pm 3\,kN\,m^{-2}$ and $0.93 \pm 0.02\,s^{-1}$) myocytes did not differ significantly at saturating [Ca^{2+}]. In accordance with the findings in previous studies[3,4,6] we observed an increased Ca^{2+} responsiveness of the contractile apparatus in end-stage failing human myocardium *(Figure 2A;* $\Delta pCa_{50} = 0.15$, $P < 0.05$). Our experiments also showed that not only the isometric force but also k_{tr} (Figure *2*B) in failing myocytes was markedly higher at submaximal [Ca^{2+}] ($\Delta k_{tr} = 0.16\,s^{-1}$ at pCa 6.0) than in non-failing myocytes.

Modulation of the cross-bridge function by adding 10 mM P_i decreased force at saturating [Ca^{2+}] to a similar

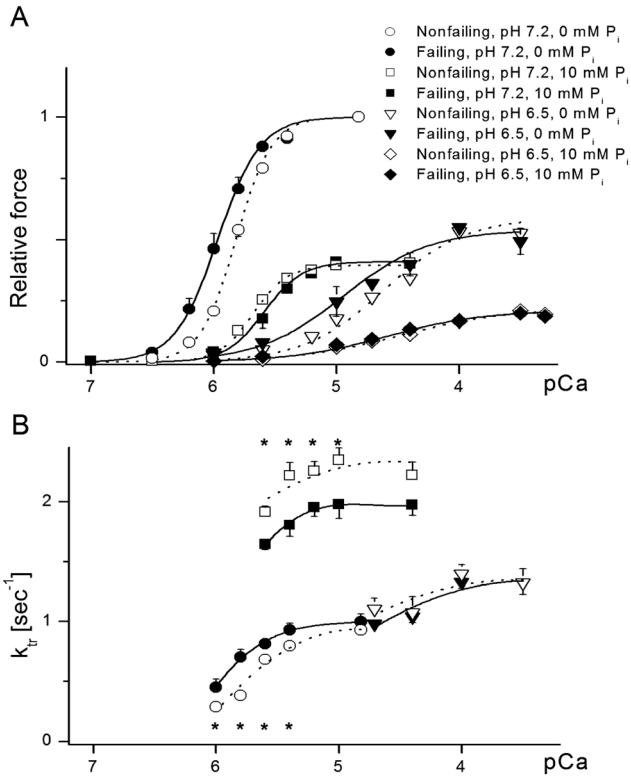

Figure 2 The Ca^{2+} dependence of isometric force production (A) and the rate constant of tension redevelopment (k_{tr}) (B) in the absence or presence of 10 mM inorganic phosphate (P_i) at pH 7.2 and pH 6.5. Closed symbols with continuous lines and open symbols with dashed lines depict results for failing and non-failing myocytes, respectively. Corresponding force and k_{tr} values are indicated by identical symbols for all metabolic conditions. The lines are the results of Hill fits to the relative force values in A. Error bars denote the S.E.M. when this is larger than the size of the symbols, and asterisks (*) denote significant differences between means of k_{tr} values for failing and non-failing myocytes.

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Table 1 Parameters of Ca^{2+}-force relations

Conditions	Failing pCa_{50}	Non-failing pCa_{50}	Failing nHill	Non-failing nHill	Failing F_o	Non-failing F_o
pH 7.2, 0 mM P_i	5.97 ± 0.04*	5.82 ± 0.01	2.49 ± 0.17*	2.99 ± 0.13	1	1
pH 7.2, 10 mM P_i	5.59 ± 0.07†	5.68 ± 0.02†	2.78 ± 0.44	3.10 ± 0.23	0.41 ± 0.02†	0.40 ± 0.01†
pH 6.5, 0 mM P_i	4.94 ± 0.08*†	4.61 ± 0.09†	1.25 ± 0.27†	1.31 ± 0.22†	0.54 ± 0.04†	0.59 ± 0.03†
pH 6.5, 10 mM P_i	4.59 ± 0.19†	4.47 ± 0.08†	1.10 ± 0.15†	1.00 ± 0.07†	0.21 ± 0.02†	0.22 ± 0.01†

*$P < 0.05$ vs non-failing myocytes.
†$P < 0.05$ vs control conditions (pH 7.2, 0 mM P_i). Values were obtained on a total of 52 myocytes.

extent (to 41 ± 2 and 40 ± 1% respectively) in the failing and non-failing myocytes. P_i induced a rightward shift in the Ca^{2+}-force relationship, which was, however, significantly larger for the failing myocytes than for the non-failing myocytes. This completely eliminated the initial difference in Ca^{2+} sensitivity between the failing and non-failing myocytes (Figure *2*A).

Modulation of the thin filament Ca^{2+}-sensory function by reducing the pH of the solutions from 7.2 to 6.5 caused a similar decline in F_o (to 54 ± 4 and 59 ± 3%, respectively) in the failing and non-failing myocytes and also shifted the Ca^{2+} sensitivity relations of the failing and non-failing myocytes in an almost identical manner. However, also at pH 6.5, the addition of 10 mM P_i abolished the difference in the Ca^{2+}-force relations of the failing and non-failing myocytes, (Figure *2*A). An overview of the parameters describing the Ca^{2+}-force relations is given in Table *1*.

P_i caused a marked increase in k_{trmax} both in the failing and in the non-failing myocytes (from 1.00 ± 0.06 and 0.93 ± 0.02 s^{-1} to 1.97 ± 0.09 and 2.22 ± 0.11 s^{-1}, respectively; $P < 0.05$) (Figure *2*B). However, there was one conspicuous difference: the P_i-induced increase in k_{tr} for the failing myocytes at submaximal [Ca^{2+}] was significantly less than that for the non-failing myocytes. Acidosis increased k_{trmax} by ~30% in both groups (to 1.28 ± 0.08 and 1.32 ± 0.12 s^{-1} in the failing and non-failing myocytes, respectively). Determination of k_{tr} at pH 6.5 was feasible only between pCa 3.5 and pCa 4.71 and in the absence of P_i. In these cases, no significant difference in k_{tr} was observed between the failing and non-failing myocytes.

Discussion

This study clearly shows the following: (1) the difference in the Ca^{2+} sensitivities of force production between failing and non-failing myocardium is eliminated by 10 mM P_i. (2) P_i causes a smaller acceleration of the cross-bridge kinetics in the failing than in the non-failing myocytes, whereas modulation of the Ca^{2+}-regulatory function of the thin filament affects force generation similarly in failing and non-failing hearts.

In our previous studies, we have shown that the increased Ca^{2+} responsiveness in failing human hearts most likely results from deceased phosphorylation of myosin light chain 2 and troponin I, in particular bisphosphorylation of serines 23 and 24.[23] However, we did not observe a difference in myosin light chain 1 and troponin T phosphorylation between donor and failing hearts.[7,13]

In this study, our primary aim was to explore the molecular background of the altered cardiac pump function during end-stage human heart failure. To obtain a more detailed picture, the determination of Ca^{2+}-force relation was paralleled by k_{tr} measurements in the absence or presence of ischaemic metabolites.

Acidification reduced pCa_{50} and the cooperativity (nHill) of force production both in the failing and in the non-failing myocytes, while the increase in k_{trmax} was small. These findings are consistent with the notion that the pH has a major impact on the Ca^{2+}-binding function of troponin C but has little direct effect on the transitions between the weak and strong cross-bridge states.[17] 10 mM P_i reduced F_o more than acidosis, but it induced a smaller rightward shift in the calcium-force relations and did not change the Hill coefficient either in the failing or in the non-failing myocytes. These alterations, together with the large increase seen in k_{tr}, are in agreement with the view that P_i, via reducing the overall free energy of MgATP hydrolysis, affects the cross-bridges directly[19,24,25] and has no direct effect on the Ca^{2+}-binding function of troponin C.[17] It is now generally accepted from animal studies that P_i reverses the P_i-release step of the cross-bridge cycle by mass action, thereby decreasing the proportion of cross-bridges in the high-force conformation.[19,24–26] Furthermore, the P_i-induced changes in pCa_{50} are considered to be a consequence of the altered distribution of actin-myosin states within the cross-bridge cycle.[17]

An elevation in the rate of the cross-bridge transition from the non-force-generating state to the force-generating state (f_{app}) increases pCa_{50}.[18] Under control conditions, the higher k_{tr} ($f_{app} + g_{app}$) values of the failing myocardial cells are thus logically related with the observed increase in pCa_{50}. Reduction of the pH from 7.2 to 6.5 evoked comparable reductions in the Ca^{2+} sensitivities of force production and—with respect to k_{tr}—it also did not discriminate between the failing and the non-

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failing myocytes. These observations suggest that the thin filament regulation during human heart failure is not intrinsically altered despite a probable reduction in the level of troponin I phosphorylation.[5,7]

In contrast, the P_i sensitivity of pCa_{50} and k_{tr} differed significantly between the failing and the non-failing myocytes. P_i decreased pCa_{50} more, whereas it increased the Ca^{2+} sensitivity of k_{tr} less in the failing myocytes than in the non-failing myocytes. It is concluded, therefore, that the molecular interplay between cross-bridge transitions and the Ca^{2+} sensitivity of force production is characteristically altered in the failing human heart. This most likely involves the chemo-mechanical transduction process per se through a reduction in the rate of the P_i-release step and/or enhancement of the reverse reaction. The elimination of the higher Ca^{2+} sensitivity of force production by 10 mM P_i in the failing myocytes occurred both at pH 7.2 and at pH 6.5, in perfect agreement with the notion that the P_i effect is independent from that of protons.[24,27]

Functional implications

The F_o and k_{trmax} values at saturating [Ca^{2+}] of failing and non-failing human myocytes did not differ significantly from each other under any metabolic conditions and thus corroborated previous findings of our group.[27] During physiological cardiac activity, the thin filaments are far from Ca^{2+} saturation. Differences in the mechanical parameters of failing and non-failing hearts at intermediate [Ca^{2+}] therefore bear important functional consequences for the cardiac performance. The increase in the apparent Ca^{2+} sensitivity of force production might be deleterious for the diastolic function, but it could potentially compensate for the reduced amplitude of the Ca^{2+} transient during systole. Here, we show that the disturbance in cross-bridge cycling is coupled to an enhanced P_i dependence of Ca^{2+} sensitivity in failing myocardium. Hence, P_i accumulation, e.g. during ischaemia, may eliminate the advantage of an increased Ca^{2+} reactivity. Moreover, slowing of the P_i-release step might explain the increased economy of contraction observed in failing hearts.[26] Nevertheless, it is to be acknowledged that end-stage heart failure is a complex syndrome, where in addition to alterations of sarcomeric proteins in cardiomyocytes, pump function may also depend on the largely unknown interplay of numerous cell types in the myocardium.

Recent pharmacological research resulted in the development of so-called direct myosin activator agents for the management of systolic heart failure.[28] These drugs bind to the myosin catalytic domain and operate by an allosteric mechanism through the modulation of P_i-dependent myosin function. In animal models of heart failure, cardiac myosin activation gave promising results, while in humans, convincing results are still awaited.[29] Our present results imply that the eventual success of new sarcomere targeted agents will depend not only on their pharmacokinetic binding characteristics to a well-defined target molecule but rather on their complex action on the actin-myosin cross-bridge cycle during human heart failure.

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Declaration of interest

None declared.

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