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Force-frequency-relation in human atrial and ventricular myocardium

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Abstract

In human heart failure, an increase in frequency of stimulation is followed by a reduced force of contraction *in vivo* and *in vitro*. The present study aimed to investigate whether a different origin of the myocardial sample or pretreatment with the cardioprotective agent 2,3-butanedione-monoxime (BDM) influences the force-frequency-relationship in electrically driven muscle strips taken from failing and nonfailing human myocardium. With as well as without pretreatment with BDM, the altered force-frequency-relationship in failing compared to nonfailing human ventricular myocardium can be observed. The effectiveness and the potency to increase force of contraction following an increase in frequency of stimulation was significantly higher in atrial than in ventricular myocardium in nonfailing and failing tissue. The different observations in atrial and ventricular myocardium provide evidence for functionally relevant differences in the electromechanical coupling between the human atrial and ventricular myocardium. (Mol Cell Biochem **119:** 73–78, 1993)

Key words: human myocardium, heart failure, force-frequency-relationship, inotropy, cardiac function

Introduction

In patients without clinical signs of heart failure, an increase in frequency of stimulation is followed by an increase in contractility *in vitro* [1–4]. In patients with normal left ventricular function, an increased contractility in response to rapid atrial pacing was demonstrated also *in vivo* [5]. In contrast, in patients with dilated cardiomyopathy only little or no enhancement in systolic and diastolic function during atrial pacing tachycardia was seen [5], thus, suggesting an important depression of inotropic and lusitropic function. *In vitro* studies using isolated electrically driven left ventricular papillary muscle strips taken from patients with severe heart failure have demonstrated that an increase in frequency of stimulation is accompanied by a reduced force of contraction [3]. Force of contraction increased in nonfailing myocardial tissue but it did not change significantly or it even decreased in failing human myocardium [3]. These studies, however, were performed after addition of 30 mmol/l 2,3-butanedione-monoxime (BDM) to the storage-solution to protect myocardial

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tissue from dissection injury. BDM, suggested to be a cardioprotective agent [6], exerts a variety of effects on myocytes, e.g. BDM decreases the sensitivity towards Ca^{2+} [7], BDM affects the slow inward Ca^{2+}-current [8, 9], and BDM influences cross-bridge-kinetics [10]. Altogether, BDM has been suggested to affect both Ca^{2+} availability and responsiveness of the myofilaments to Ca^{2+} [11]. Therefore, it is not unreasonable to speculate that pretreatment with BDM might influence myocyte properties and function. In order to isolate undamaged human left ventricular myocardium to study the influence of BDM-containing-solution on the force-frequency-relationship, we examined left ventricular human trabeculae. Left ventricular trabeculae can be used without causing dissection injury. To explore whether or not the different origin of the myocardial sample or the hemodynamic work load imposed on atria compared to ventricles influences the force-frequency-relationship, we studied the force-frequency-relationship in left ventricular and right auricular trabeculae from nonfailing myocardium.

Materials and methods

1. Myocardial tissue

Experiments were performed on isolated, electrically stimulated, human left ventricular and right atrial trabeculae. Tissue was obtained during cardiac transplantation (n = 12, 12 male; age: 47 years, range 19–62 yr; dilated cardiomyopathy). Patients suffered from heart failure clinically classified as NYHA IV on the basis of clinical symptoms and signs as judged by the attending cardiologist shortly before operation. Human atrial myocardium was also obtained from 9 nonfailing patients who underwent aortocoronary bypass operations. All patients gave written informed consent before surgery. Medical therapy consisted of diuretics, nitrates, ACE-inhibitors and cardiac glycosides. Patients receiving catecholamines, β-adrenoceptor- or Ca^{2+}-antagonists were withdrawn from the study. Drugs used for general anesthesia were flunitrazepam and pancuroniumbromide with isoflurane. Cardiac surgery was performed on cardiopulmonary bypass with cardioplegic arrest during hypothermia. Nonfailing human left ventricular myocardium was obtained from 4 donors who were brain dead as a result of traumatic injury. The cardioplegic solution (a modified Bretschneider solution) contained (in mmol/l): NaCl 15, KCl 10, $MgCl_2$ 4, histidine 180, tryptophan 2, mannitol 30 and potassium dihydrogen oxoglutarate 1.

2. Contraction experiments

Immediately after excision, the left ventricular muscles as well as the atrial tissue were placed in ice-cold preparated 2,3-butanedione-monoxime-containing Tyrode's solution (composition in mM: NaCl 119,8; KCl 5.4; $MgCl_2$ 1.05; $CaCl_2$ 1.8; Na HCO_3 22.6; NaH_2PO_4 0.42; glucose 5.05; ascorbic acid 0.28; Na_2EDTA 0.05, BDM 30) at room temperature or in Tyrode's solution without BDM and oxygenated by bubbling with 95% $O_2$5% CO_2 and delivered to the laboratory within 10 min. The experiments were performed on isolated, electrically driven muscle preparations. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75 ml glass tissue chambers for recording of isometric contractions. The bathing solution used was a modified Tyrode's solution, maintained at 37° C; its pH was 7.4. Isometric force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, FRG) attached to a Hellige Helco Scriptor (Hellige, Freiburg, FRG) or Gould recorder (Gould Inc, Cleveland, Ohio, USA). Each muscle was stretched and resting load was kept constant throughout the experiments. This was imperative, as both, relaxation and force generation are load dependent. The preparations were electrically paced at 1 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD 9), the voltage was 20% above threshold. All preparations were allowed to equilibrate at least 90 min in a drug-free bathing solution until complete mechanical stabilization. After 45 min the solution was changed. The force-frequency-relationship was studied starting with a rate of 0.5 Hz. Control strips performed in Tyrode's solution with identical composition as original experiments revealed maximally 20% reduction of baseline isometric tension over the period necessary to complete testing. The experiments were performed as described previously in detail [4].

Materials

2,3-Butanedione-monoxime was from SERVA (Heidelberg, FRG). All other chemicals were of analytical grade or the best grade commercially available. Applied agents did not change the pH of the medium.

Statistics

The data shown are means ± SEM. The drug concentration producing 50% of the maximum effect (EC_{50}) was graphically determined in each individual experiment. The EC_{50}-values are given with 95% confidence limits. Statistical significance was analysed using the Student's t-test for unpaired or paired observations (SPSS PC plus); $p < 0.05$ was considered significant [12].

Results

Figure 1 shows the frequency-dependent change in force of contraction in electrically driven left ventricular trabeculae from terminally failing myocardium after pretreatment with or without BDM-containing Tyrode's solution. Following an increase of frequency of stimulation, force of contraction was reduced in left ventricular trabeculae of terminally failing human hearts (dilated cardiomyopathy). BDM-pretreatment did not influence the force-frequency-relationship in the examined failing (see Fig. 1) and nonfailing (data not shown) human myocardium. In myocardium from patients without heart failure a positive force-frequency-relationship was observed ($p < 0.05$). Data are given in Table 1.

Figure 2 demonstrates the effect of increasing frequencies of stimulation in nonfailing and terminally failing ventricular and atrial trabeculae. In muscle strips from nonfailing human hearts, an increase in frequency of stimulation was followed by an increase in force of contraction. This holds true for atrial and ventricular myocardium. However, the effectiveness (△ mN atrial 2.4 ± 0.3 mN, ventricular 1.4 ± 0.12 mN) and the potency (mean EC_{50}: atrial 0.7 Hz, ventricular 1.3 Hz) to

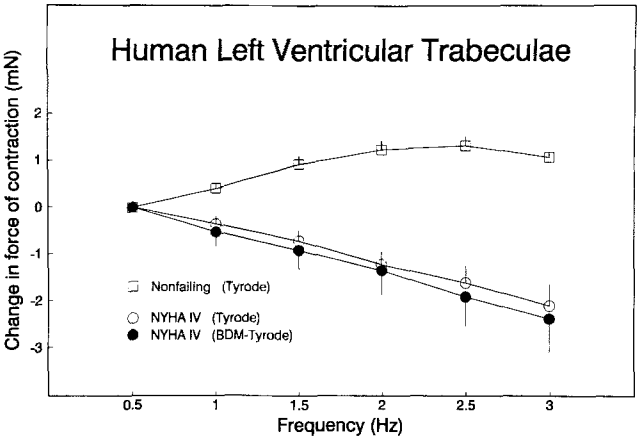

Fig. 1. Change in isometric force of contraction (ordinate, mN) plotted as a function of frequency of stimulation (0.5 Hz to 3 Hz) (abscissa) in electrically driven left ventricular trabeculae from terminally failing myocardium due to dilated cardiomyopathy and nonfailing control tissue (n = 5; basal force of contraction: 2.5 ± 0.3 mN). Muscle strips of terminally failing myocardium were either stored in BDM-containing Tyrode's solution (basal force of contraction: 3.9 ± 0.7 mN, n = 8) or in Tyrode's solution (basal force of contraction: 4.3 ± 0.7 mN, n = 6). Results are presented as mean ± SEM.

Table 1. Force-frequency-relationship in ventricular and atrial trabeculae from nonfailing and failing myocardium

	n	basal FOC (mN)	1 Hz: delta mN (% basal)	2 Hz: delta mN (% basal)
NONFAILING				
AUT	13	4.1 ± 0.5*+	2.4 ± 0.3*+	2.2 ± 0.3*+
EC_{50}		0.7 (0.5–0.8)*+	(163 ± 8)*+	(159 ± 9)*
PAP	13	1.8 ± 0.3+	0.4 ± 0.1+	1.4 ± 0.1+
EC_{50}		1.3 (1.2–1.5)	(123 ± 4)+	(190 ± 12)+
FAILING				
AUT	4	1.2 ± 0.2*	0.2 ± 0.1*	1.0 ± 0.2*
EC_{50}		1.6 (1.0–2.4)	(117 ± 6)*	(180 ± 10)*
PAP	48	2.5 ± 0.2	−0.3 ± 0.1	−0.6 ± 0.1
EC_{50}		1.7 (1.5–1.9)	(92 ± 2)	(82 ± 4)

*$p < 0.05$ vs PAP
+$p < 0.05$ vs Failing
AUT = Right auricular trabeculae
PAP = Left ventricular trabeculae
FOC = Force of contraction
(% basal) = Change in force of contraction 1 Hz (2 Hz) vs. 0.5 Hz stimulation frequency in mN.

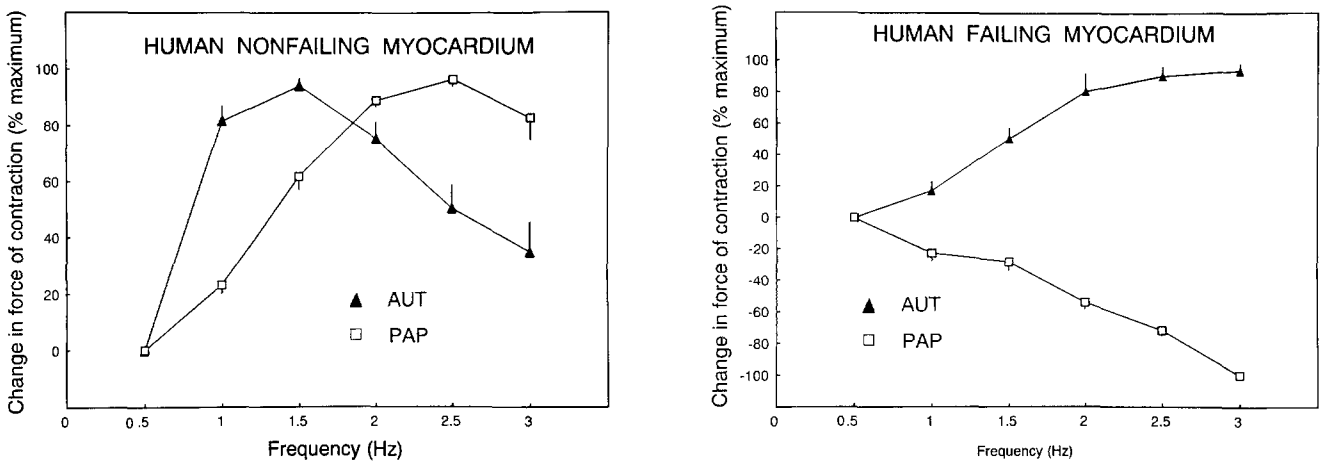

Fig. 2. Change in isometric force of contraction (ordinate, %) plotted as a function of frequency of stimulation (0.5 Hz to 3 Hz) (abscissa) in electrically driven left ventricular trabeculae from nonfailing myocardium (A) (PAP, n = 13; basal force of contraction 1.8 ± 0.2 mN; AUT, n = 9; basal force of contraction: 4.1 ± 0.5 mN) and from terminally failing myocardium (B) (PAP, n = 48; basal force of contraction 2.4 ± 0.2 mN; AUT, n = 4; basal force of contraction: 1.2 ± 0.2 mN). Results are presented as mean ± SEM.

increase force of contraction following an increase in frequency of stimulation was significantly lower in atrial than in ventricular nonfailing human myocardium. In human failing myocardium the force-frequency-relationship was found positive in right atrial tissue, but negative in ventricular trabeculae.

Discussion

The different force-frequency-relationship in nonfailing and failing human myocardium can be demonstrated in freshly isolated human myocardium with [3] as well as without the use of BDM [this study]. Consistently, we report that the use of BDM as 'cardioprotective solution' does not artifactually influence the force-frequency-relationship in human myocardium. However, the force-frequency-relationship is different in human atrial and ventricular myocardium.

In vivo, adrenergic reflex mechanisms may be activated to compensate reduced myocardial contractility in heart failure. These mechanisms may mask changes in contractility in response to different frequencies of stimulation. Therefore, isolated, electrically driven cardiac preparations may provide a useful tool to examine isometrically the effect of different stimulation frequencies on force of contraction. Under this experimental approach, additional influencing factors like changes of preload or afterload do not affect the developed myocardial force of contraction. In previous studies, it has been demonstrated that BDM protects the human myocardium from cutting injury [3], thus facilitating dissection and preservation of muscle strip preparations [3]. Addition of BDM resulted in a significantly higher force of contraction in electrically driven human papillary muscle strips after longterm storage (4° C, 10 h, 24 h) in BDM-containing tyrode's solution than in tyrode's solution [13]. BDM affects via a variety of mechanisms myocardial contractility [7–11] and exerts concentration-dependently negative inotropic activity in isolated muscle strip preparations from animals [10, 14] and humans [3, 15]. Experiments performed without the use of BDM showed a significant difference between the force-frequency-relationship (up to 3 Hz) in nonfailing and terminally failing human tissue [14]. Consistently in patients with normal left ventricular function, rapid atrial pacing initiated an increase in contractility, i.e. increase in peak + dP/dt and an increase in the peak-systolic pressure to end-diastolic volume rate [5]. In patients with dilated cardiomyopathy, however, these increases in contractility indices were absent or diminished [5]. *In vitro*, stimulation frequencies from 0.5 Hz up to 3 Hz were followed by a progressive decrease in force of contraction in muscle preparations from the failing myocardium [4]. Human papillary muscle strip preparations and left ventricular trabeculae from nonfailing hearts increased, demonstrating that the positive force-frequency-relationship can be detected in preparations with [3] as well as without pretreatment with BDM [4, this study]. Therefore, the demonstrated reversibility of BDM-mediated effects [15, this study] opens the possibility to use BDM as a cardioprotective agent for isolating preparations of animal and human myocardium. In addition, BDM

might be a beneficial supplement for cardioplegic solution for open heart surgery as well.

An important aspect of these studies is that the origin of the myocardial tissue greatly influences the force-frequency-relationship. In isolated trabeculae from nonfailing and failing myocardium, the frequency-dependent increase in force of contraction was more pronounced in atrial than in ventricular tissue. In atrial and ventricular myocardium the density of 1,4-dehydropyridine-binding sites is unchanged [16]. Thus, differences in the number of sarcolemmal Ca^{2+}-channels do not influence the different force-frequency-relationship. In addition, in human failing and nonfailing myocardium the binding to 1,4-dihydropyridine-binding sites was also unchanged [17]. The basic characteristics of L-type Ca^{2+}-currents of ventricular myocytes are qualitatively similar to those described in human atrial cells and other mammalia species [18, 19]. Thus, the significantly altered force-frequency-relationship in failing compared to nonfailing tissue and the differences observed in atrial and ventricular myocardium most likely are not due to differences in transsarcolemmal Ca^{2+}-currents, but may be due to differences in intracellular Ca^{2+}-handling. This is in accordance with measurements in isolated human myocytes. Beuckelmann et al. [20] in this respect observed significant differences in isolated human cells from myopathic and control hearts; e.g. resting Ca^{2+}-levels were higher, and the rate of diastolic Ca^{2+}-decay was slowed. Possible mechanisms may relate to a reduced Ca^{2+}-efflux through the Na^{+}/Ca^{2+}-exchange system, or may be due to a reduced re-uptake of Ca^{2+} into the sarcoplasmatic reticulum.

In simultaneous measurements with the Ca^{2+}-indicator protein aequorin, the peak amplitude of the Ca^{2+}-transient and the resting intracellular Ca^{2+}-concentration increased along with increasing stimulation frequency [21]. Therefore, the mechanical deterioration in the failing heart, e.g. during a frequency increase, is not due to a reduced availability of cystolic Ca^{2+} rather than due to a diminished Ca^{2+}-re-uptake from the cytosol. Hence, pronounced elevation of Ca^{2+} intracellularly will worsen the force-frequency-relationship [21]. Consistently, interventions that increase intracellular Ca^{2+} excessively such as high Ca^{2+} decreased augmentation of tension following an increase in stimulation frequencies in human muscle strip preparations [21].

The sensitivity of the myofibrils to Ca^{2+} might also influence the force-frequency-relationship in atrial and ventricular myocardium. Using skinned fiber preparations from human myocardial tissue, the sensitivity to Ca^{2+} was observed to be different in atrial and ventricular myocardium [22]. The different sensitivity to Ca^{2+} in atrial and ventricular myocardium may be related to differences in myosin isoenzyme expression [23–25]. For instance, the change in Ca^{2+}-sensitivity in atrial myocardium of patients with heart failure has been reported to be related to an elevation of right atrial pressure [22]. Differences in contraction coupling might also explain the differences observed in the frequency-dependent increase in force of contraction in atrial and ventricular tissue. The present study clearly demonstrates that experiments performed with atrial tissue may be not necessarily comparable to the situation in ventricular myocardium. This holds true for receptor-dependent inotropic mechanisms, e.g. β-adrenoreceptor-mediated increase in force of contraction [24], as well as for receptor-independent inotropic mechanisms, e.g. force-frequency-relationship (this study). On the other hand, the missing increase in $+ dp/dt_{max}$ or in end systolic pressure-volume ratio after atrial pacing tachycardia in patients with dilated cardiomyopathy might have their origin in the changes demonstrated in these experiments in isolated trabeculae.

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