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ORIGINAL ARTICLE

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Mechanical activity and force–frequency relationship of isolated mouse papillary muscle: effects of extracellular calcium concentration, temperature and contraction type

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Abstract Cardiac physiology of the mouse is becoming increasingly important because the mouse is the mammalian model animal of choice for genetic modifications. However, mouse cardiac muscle is still poorly characterized under physiological conditions and inconsistent results have been published in the literature regarding mechanical activity especially the force–frequency relationship in isolated mouse muscle preparations. In this study we investigated systematically several mechanical parameters of isolated mouse papillary muscle such as force–frequency relation, twitch force, time to peak tension, relaxation time and post-rest potentiation at different experimental conditions. Extracellular calcium concentration ($[Ca^{2+}]$) was varied between 1.0 and 5.0 mM, temperature between 27°C and 37°C and force measurements were performed under isometric as well as auxotonic conditions. The mechanical activity of muscle preparations was found to be strongly dependent on $[Ca^{2+}]$ and temperature and slightly on contraction type. At low temperature and low $[Ca^{2+}]$ the force–frequency relation was strongly positive whereas at high temperature and high calcium it turned negative. The results of this study demonstrate a flat force–frequency relation in mouse papillary muscle at physiological conditions (37°C, $[Ca^{2+}]$ of 1.5 mM) and provide a reliable experimental basis for comparative studies with genetically altered mice.

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Introduction

Cardiac physiology of the mouse is becoming increasingly important [7, 10, 11] because the mouse is the mammalian species of choice for genetic manipulations. Overexpression as well as deletion of certain genes provide unique tools to study the functional importance of the respective proteins. For investigating cardiac function, techniques are well developed in larger mammals. It has become challenging to modify these techniques to apply them to the mouse heart with its very small size and extremely high physiological heart rate (500–600 beats/min [25]). Mouse cardiac muscle is still poorly characterized under physiological conditions. Inconsistent results have been published in the literature concerning the force–frequency relation (FFR) in mice.

In most mammalian species myocardial muscle preparations show an increase in steady-state twitch force in response to an increase of frequency of stimulation, a phenomenon first observed by Bowditch et al. [5] in isolated frog heart. The mechanism of this positive FFR has been attributed to an increase in total intracellular activator Ca^{2+} regulated by Ca^{2+} influx [14, 23]. Enhanced intracellular $[Ca^{2+}]$ leads to an increase of Ca^{2+} uptake into the sarcoplasmic reticulum with subsequent increased Ca^{2+} release. Recently, phospholamban and the Ca^{2+} pump of the sarcoplasmic reticulum were shown to play a major role in the frequency response of the heart [4, 19]. Despite clarification of the mechanisms underlying the FFR, it remains unclear why contradictory results about the FFR in mouse heart have been published. Reports about FFRs in mouse are ranging from negative FFRs to strongly positive relations [4, 7, 19, 26]. These studies differ with respect to the chosen experimental conditions, which often do not resemble the physiological

situation such as an extracellular [Ca^{2+}] of 2.5 mM or performing experiments at room temperature. However, in other species it has been shown that FFR can be modified by extracellular [Ca^{2+}] [16, 21]. In this study we investigated systematically how the mechanical activity of isolated mouse papillary muscle preparations, especially the FFR, is affected by differences in extracellular [Ca^{2+}] or temperature.

In addition, force measurements of isolated myocardial preparations are usually performed under isometric conditions which are far away from the in vivo situation of the beating heart. When active shortening of cardiac muscle preparations was allowed the membrane potential was more positive and intracellular [Ca^{2+}] increased compared to isometric contractions (cat and ferret papillary muscle [9, 15]). These differences in intracellular Ca^{2+} homeostasis upon auxotonic muscle contractions may also influence the FFR.

Therefore, in addition to systematic investigation of the influence of extracellular [Ca^{2+}] and temperature on the FFR of mouse papillary muscle, we established an experimental setup which allowed us to measure FFR at auxotonic contractions and compared these to isometric measurements.

Materials and methods

Dissection of mouse papillary muscle

Adult mice (strain 129 Sv) of either sex (body weight 20–30 g, 12–20 weeks old) were killed by cervical dislocation. The hearts were immediately removed and transferred to a dissection chamber containing the following solution (mM): 135 NaCl; 4.7 KCl; 1.2 $MgCl_2$; 1.2 KH_2PO_4; 2.5 $CaCl_2$; 10 HEPES; 12 glucose; 5 sodium pyruvate; 20 mM BDM (2,3-butanedione monoxime). The solution was regularly exchanged with fresh solution warmed up to 32°C and gassed with 100% O_2. With the help of a stereoscopic microscope the anterior papillary muscle of the left ventricle was dissected together with a small portion of attached tissue of the ventricular wall. The papillary muscles had a diameter between 0.5 and 0.6 mm and were 2–3 mm long (measured under the microscope and calculated from length and weight); cross-sectional area was between 0.2 and 0.3 mm^2. The muscles were connected to small steel hooks and mounted in an organ bath.

Experimental setup

Muscle preparations were mounted horizontally in an organ bath which was thermostatically controlled and perfused at a rate of 5 ml/min (2 ml bath volume). The force of contraction was measured with a strain gauge transducer under isometric or auxotonic conditions. For auxotonic measurements a thin steel wire, which was flexible and acting as a spring (spring constant k=3 N/m), was attached to the lever of the transducer. Under these conditions a shortening of the preparation of 0.33 mm (10–15% of muscle length) would be achieved at a force of 1 mN (representative value of muscle preparations under these conditions). The preparations were field-stimulated by platinum electrodes. Under standard conditions pulses of 1 ms duration and a voltage 50% over threshold at a frequency of 1/s were used. The stimulation started immediately after transfer of the preparation to the organ bath.

The preparations were gradually stretched to a length where twitch force reached nearly its maximal value (90–95%). Muscles were then allowed to equilibrate for 45–60 min. The standard salt solution had the following composition (mM): 112.5 NaCl; 4.7 KCl; 1.2 $MgCl_2$; 1.2 KH_2PO_4; 25 $NaHCO_3$; 12 glucose; $CaCl_2$ was varied between 1.0 and 5.0. The solution was gassed with 95% O_2 and 5% CO_2 and had a pH of 7.4.

Force–frequency relation

After the equilibration period of 45–60 min (1.5 mM Ca^{2+}, stimulation frequency 1 Hz) postrest potentiation and the force–frequency relation were determined. Stimulation of muscles was interrupted for 1 min and the force of the first twitch thereafter was measured (postrest potentiation). Five minutes later muscles were stimulated with increasing frequencies (30–60 s each): 0.1, 0.2, 0.5, 1, 2, 3, 4 and 5 Hz and steady-state twitch forces for each frequency determined. To measure the effects of [Ca^{2+}] the same muscle preparation was perfused subsequently with [Ca^{2+}] of 1.0, 1.5, 2.5 and 5.0 mM. At each concentration muscles were allowed to equilibrate for 15–30 min at a stimulation frequency of 1 Hz before postrest potentiation and the force–frequency relation were performed as described above. [Ca^{2+}] was alternately varied in an ascending or descending protocol (n=4–6). Measurements directly performed after the first equilibration period (1.5 mM Ca^{2+}) were not used for evaluation.

This experimental protocol was performed under four different conditions: (1) 27°C, isometric contractions; (2) 27°C, auxotonic contractions; (3) 32°C, isometric contractions; (4) 32°C, auxotonic contractions. In addition, experiments were performed at 37°C (isometric and auxotonic) where measurements were restricted to [Ca^{2+}] of 1.5 mM.

Evaluation and statistics

Data are presented as means ±standard error of the mean and tested for statistical significance with the t-test (P<0.05).

Results

Example of an original recording of the force–frequency relation

We investigated the force–frequency relation of isolated mouse papillary muscle preparations under different experimental conditions. Figure 1 shows an original recording of twitch forces in the frequency range of 0.1 to 5 Hz at 1.5 mM Ca^{2+} and 27°C. The minimum of twitch force was found at a stimulation frequency of 1 Hz and force increased with higher frequencies up to 5 Hz. Force measurements at stimulation frequencies close to the physiological heart rate of mouse (550–620/min [11]) were limited by the appearance of incomplete relaxation due to the long twitch duration at 27°C. However, the frequency range investigated (1–5 Hz) can be estimated to include the corresponding basal heart rate of mouse at 37°C by assuming a temperature coefficient Q_{10} of 2.5. In that case a stimulation frequency of 4 Hz at 27°C will be equivalent to 10 Hz at 37°C. With a sudden increase of stimulation frequency the twitch amplitude decreased initially and increased with further stimulation to a new steady state. The increase of the contraction amplitude is attributed to the frequency-dependent increase of total intracellular activator calcium (see Introduction). At

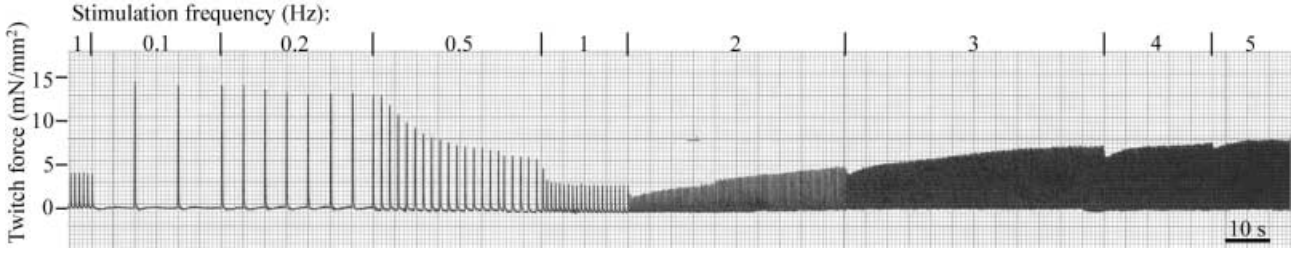

Fig. 1 Original recording of a typical force–frequency relationship (FFR) of isometrically contracting mouse papillary muscle measured at 27°C and an extracellular [Ca^{2+}] of 1.5 mM in the frequency range of 0.1–5 Hz. Note the positive inotropic effect in response to increasing stimulation frequency in the range of 1–5 Hz. Below 1 Hz an even stronger increase in twitch force was observed which can be attributed to very long intervals between the twitches

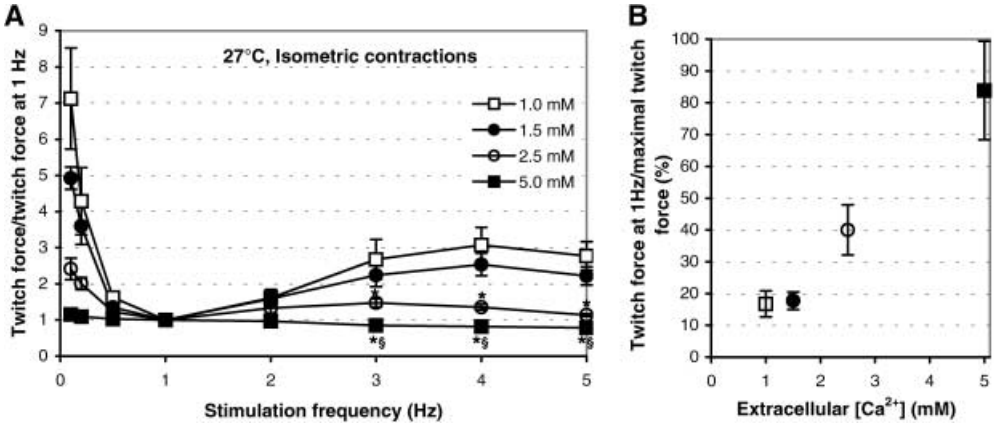

Fig. 2. **A** Effect of extracellular [Ca^{2+}] on the FFR of isometrically contracting mouse papillary muscles at 27°C. Twitch forces recorded at different stimulation frequencies were expressed in relation to twitch force at 1 Hz. In the range of 1–4 Hz FFR was clearly positive at 1.0 mM Ca^{2+}. With increasing [Ca^{2+}] FFR was attenuated resulting in a negative FFR at 5.0 mM Ca^{2+}. $^{*}P<0.05$ versus 1.0 and 1.5 mM Ca^{2+}, $^{\S}P<0.05$ versus 2.5 mM Ca^{2+}. **B** Ratio of twitch force at 1 Hz (F_1) to maximal twitch force (F_{max}) at different extracellular [Ca^{2+}] of the experiments of **A**. Note strong dependence of F_1/F_{max} on [Ca^{2+}]. High F_1/F_{max} may resemble high intracellular Ca^{2+} load at 1 Hz which cannot further enhanced by increasing stimulation frequency leading to a flat or negative FFR at [Ca^{2+}] of 2.5 and 5 mM

frequencies below 1 Hz twitch force was increased even more strongly, which is attributed to long intervals between the twitches comparable to the phenomenon of postrest potentiation (see Discussion).

Calcium dependence of the force–frequency relation

Extracellular [Ca^{2+}] was varied systematically between 1.0 and 5 mM and FFRs in a range of 0.1–5 Hz were recorded. The protocol was performed both for isometric and auxotonic conditions at 27°C.

Figure 2A shows FFR at [Ca^{2+}] of 1.0, 1.5, 2.5 and 5.0 mM for isometric muscle contractions. For better comparison steady-state twitch forces recorded at different stimulation frequencies were expressed in relation to twitch force at 1 Hz (F_1). At [Ca^{2+}] of 1.0 and 1.5 mM the FFR is clearly positive in the range between 1 and 4 Hz reaching relative values to F_1 of 3.1 (1.0 mM Ca^{2+}) and 2.5 (1.5 mM Ca^{2+}) at 4 Hz. As mentioned above this frequency range at 27°C can be estimated to include the physiological heart rate of mouse at 37°C. At 5 Hz twitch force declines, which may be attributed to insufficient energy supply of the muscle preparation in the organ bath. Increasing [Ca^{2+}] to 2.5 mM led to statistical significant flattening of the FFR with the maximum of twitch force at 3 Hz (1.5 in relation to F_1) and decreasing values at 4 and 5 Hz. Further increase of [Ca^{2+}] to 5 mM resulted in an overall negative relationship.

Thus, at a physiological [Ca^{2+}] of 1.5 mM mouse papillary muscle displayed at 27°C a positive FFR which turned negative at increasing [Ca^{2+}].

The absolute values of twitch forces at 1 Hz (F_1) were also strongly Ca^{2+} dependent whereas maximal twitch force of the muscle preparation (F_{max}, force of first twitch after a 1-min break of electrical stimulation=postrest potentiation) was independent of [Ca^{2+}]. Figure 2B illustrates F_1 in relation to F_{max} at different [Ca^{2+}]. Whereas at [Ca^{2+}] of 1.0 and 1.5 mM F_1 amounted below 20% of F_{max}, at [Ca^{2+}] of 5 mM F_1 was 85% of F_{max}. The enhancement of the ratio of F_1 to F_{max} at high [Ca^{2+}] may resemble higher intracellular Ca^{2+} load at 1 Hz at high [Ca^{2+}] compared to low [Ca^{2+}]. Thus, at high [Ca^{2+}], intracellular Ca^{2+} and twitch forces either cannot or can

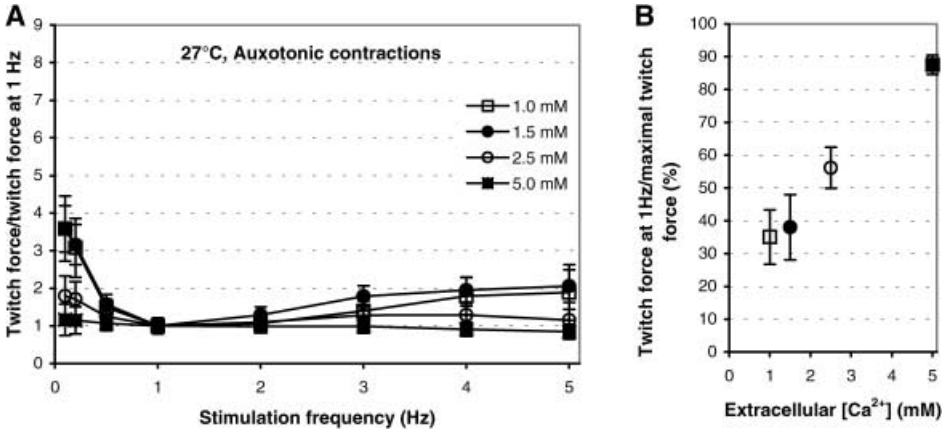

Fig. 3. **A** Effect of extracellular [Ca^{2+}] on the FFR of auxotonically contracting mouse papillary muscles at 27°C. Twitch forces recorded at different stimulation frequencies were expressed in relation to twitch force at 1 Hz. In the range of 1–4 Hz FFR was less positive with increasing [Ca^{2+}] but the Ca^{2+} dependence was not as strong as observed for isometric conditions (see Fig. 2A). **B** Ratio of twitch force at 1 Hz (F_1) to maximal twitch force (F_{max}) was also dependent on [Ca^{2+}]. F_1/F_{max} at [Ca^{2+}] of 1.0 and 1.5 mM was higher under auxotonic compared to under isometric conditions (see Fig. 2B), which indicates differences in Ca^{2+} homeostasis of both contraction types and may result in different FFR

only slightly be further enhanced by increasing stimulation frequencies leading to a flat FFR.

At frequencies below 1 Hz twitch force was increased, which is attributed to the very long intervals between the twitches, a phenomenon comparable to postrest potentiation. In fact, twitch forces recorded at 0.1 Hz were only slightly below that of postrest potentiation. Since at 1.0 mM [Ca^{2+}] F_1 was very low in relation to F_{max}, as mentioned above, twitch force was increased up to seven times at 0.1 Hz in relation to F_1 whereas at 5.0 mM Ca^{2+} twitch force at 0.1 Hz was only 1.2 in relation to F_1.

Auxotonic contractions

The same experimental protocol was performed allowing auxotonic muscle contractions (shortening fraction of about 10%).

Figure 3A illustrates that the effect of [Ca^{2+}] on the FFR under auxotonic conditions was qualitatively similar as under isometric conditions but less pronounced. FFR was positive at low [Ca^{2+}] and turned negative at high [Ca^{2+}]. The relation at 1.0 mM Ca^{2+} is below that of 1.5 mM Ca^{2+}; however, these values were not statistically significant from each other.

The differences between the corresponding relations of auxotonic compared to isometric conditions were statistically significant and most prominent at low [Ca^{2+}]. 1.0 mM Ca^{2+}: increase of twitch force to 1.7 in relation to F_1 at auxotonic compared to 3.0 at isometric conditions. 1.5 mM Ca^{2+}: 2.0 compared to 2.5. 2.5 mM and 5.0 mM Ca^{2+}: no differences. In addition, the ratio of F_1 to F_{max} (Fig. 3B) differed under auxotonic compared to isometric conditions at corresponding [Ca^{2+}]. At 1.0 and 1.5 mM Ca^{2+} F_1 was about 30% of F_{max} under auxotonic conditions whereas it amounted to 15–20% at isometric conditions. This indicates that calcium homeostasis in muscles stimulated at 1 Hz varies between auxotonic and isometric contractions and the higher ratio of F_1 to F_{max} under auxotonic conditions may resemble higher intracellular calcium load at 1 Hz and lead to a flatter FFR.

Influence of temperature

To investigate the effect of temperature FFRs were additionally determined at 32°C (from 1.0 to 5.0 mM Ca^{2+}) and 37°C (1.5 mM Ca^{2+}) from isometrically as well as auxotonically contracting muscles. Increasing temperature from 27°C to 32°C and 37°C led to a flattening of the FFR especially at low [Ca^{2+}], whereas at high [Ca^{2+}] the FFR remained unchanged (slightly negative). To compare the FFRs at the various temperatures the ratio of twitch force at 4 Hz (F_4) to twitch force at 1 Hz (F_1) is shown in Fig. 4A for the physiological [Ca^{2+}] of 1.5 mM. As clearly visible, increasing temperature from 27°C to 37°C led to a flat or slightly negative relation under both isometric and auxotonic conditions. The flattening of the FFR induced by increasing temperature was accompanied by an increase in F_1 reflected by a higher ratio of F_1/F_{max} (Fig. 4B) similar to observations following a rise in [Ca^{2+}] (Figs. 2, 3B).

Single twitch records

Time to peak tension (Ttp) and time to 50% relaxation (R_{50}) of single twitches at 1 Hz at the various experimental conditions are summarized in Table 1. Whereas Ttp and R_{50} were only slightly affected by [Ca^{2+}] both parameters were reduced to about 50% by an increase in temperature of 10°C. No significant differences were observed between the corresponding data of isometrically and auxotonically contracting muscles.

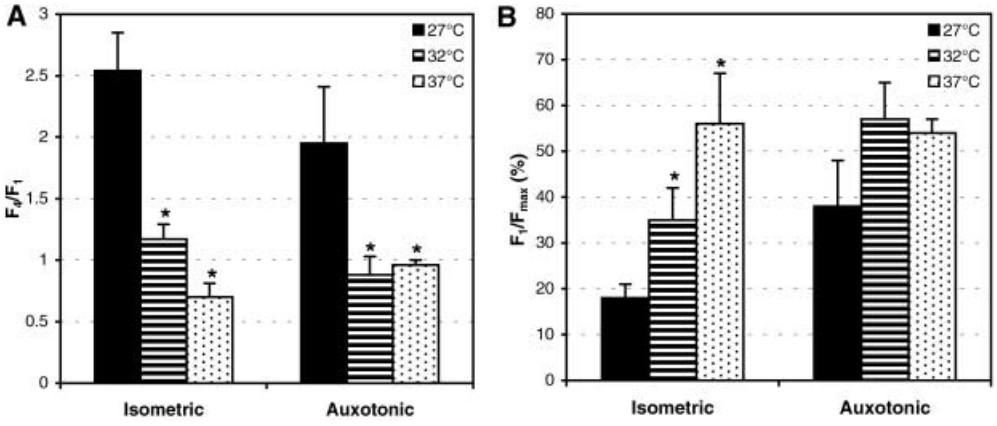

Fig. 4A, B Effect of temperature on FFR. **A** The ratio of twitch force at 4 Hz (F_4) to twitch force at 1 Hz (F_1) was used as a parameter to characterize the FFR at different temperatures (27°C, 32°C and 37°C) under isometric and auxotonic conditions (1.5 mM Ca^{2+}). Note the significant decrease of F_4/F_1 with increasing temperature. **B** The decrease of F_4/F_1 was accompanied by an increase in F_1/F_{max} similar to observations following a rise in [Ca^{2+}] (see Figs. 2, 3). *P<0.05 versus 27°C

Table 1 Time to peak tension (Ttp) and time to 50% relaxation (R_{50}) of single twitch recordings at 1 Hz under the various experimental conditions. Whereas Ttp and R_{50} were only slightly affected by [Ca^{2+}] both parameters were reduced to about 50% by an increase in temperature of 10°C.

Contraction type	Temperature (°C)	[Ca^{2+}] (mM)	Time to peak tension (Ttp, ms)	Time to 50% relaxation (R_{50}, ms)
Isometric	27	1.0	81.3±3.1	67.5±6.6
		1.5	90.0±6.8	62.5±6.3
		2.5	96.3±4.7*	62.5±7.5
		5.0	106.3±2.4*	72.5±6.0
	32	1.5	67.5±4.8	43.8±4.3§
	37	1.5	58.8±3.1§	32.5±2.5§
	27	1.0	92.0±2.6	64.0±1.9
Auxotonic		1.5	90.8±4.4	64.2±2.0
		2.5	92.5±3.6	59.2±2.7
		5.0	100.0±2.9*	67.5±4.0
	32	1.5	63.3±2.1§	41.7±4.0§
	37	1.5	47.0±1.2§	28.0±1.2§

*P<0.05 versus 1.0 mM Ca^{2+}, §P<0.05 versus 27°C

Discussion

Optimal conditions for studies with isolated mouse cardiac muscle

The left anterior papillary muscle is particularly suitable for studying contractility in an isolated mouse heart muscle preparation since it can be excised with minimal mechanical injury and it is similar in size in a homogeneous group of animals. Its diameter of about 0.6 mm, however, may be critical for sufficient substrate and oxygen supply. For rat ventricular preparations a critical diameter for sufficient oxygen supply of 0.2 mm was suggested [22]. Therefore, preparations of thin mouse ventricular trabeculae would be preferable. However, such studies are very rare and trabeculae suitable for preparation were found only in a minority of mouse hearts [7]. To reduce energy demand, studies with isolated myocardial muscle preparations are usually performed with reduced activation frequencies, often with 1 Hz instead of about 10 Hz which is the normal heart rate in mouse. The twitch forces measured in our experimental system under the various experimental conditions were usually in the range of 5–10 mN/mm^2 and maximal twitch forces (postrest potentiation) amounted to 20–25 mN/mm^2. These values were much lower than that reported for several other myocardial preparations but were in agreement with the studies of Bluhm et al. [4] and Meyer et al. [19] using isolated mouse papillary muscles. At [Ca^{2+}] below 2.5 mM twitch forces measured by us are also in the same range as those reported by Gao et al. [7] and Kögler et al. [12] for thin (diameter of 0.1–0.2 mm) mouse ventricular trabeculae. Thus, we think that the energy supply of the papillary muscle is usually sufficient at least at a reduced stimulation rate of 1 Hz. Further indirect evidence that our conditions guarantee sufficient energy supply is provided by measuring FFR in a frequency range up to 5 Hz. Since papillary muscles

exhibited nearly the same or higher twitch forces at 4 and 5 Hz (depending on experimental conditions), where the energy consumption can be estimated to be about 4–5 times higher, the energy supply will be certainly sufficient at 1 Hz. However, we cannot rule out that at high-frequency stimulation the energy supply will reach a critical level. At stimulation rates above 5 Hz a decline of twitch amplitude occurred after reaching the steady state, which probably can be interpreted as a sign of insufficient energy supply. An increase of $[Ca^{2+}]$ above the normal extracellular level of 1.5 mM increases contraction amplitude and metabolic rate and therefore the energy supply may reach a critical level earlier. An increase of $[Ca^{2+}]$ certainly influences other responses of the tissue as well. One should therefore be cautious with alterations of the normal $[Ca^{2+}]$. In several recent studies this aspect was not considered [4, 18, 19]. A reduction of temperature to 32°C may be recommendable in order to be safe that the energy supply is sufficient. However, a reduction of temperature may also effect contractility, for example by changing the myofibrillar Ca^{2+} sensitivity. Thus, one should try to optimize conditions as close as possible to the physiological in vivo situation in order to prevent changes in Ca^{2+} homeostasis, FFR or other undefined influences of contractility. Also one should consider using auxotonically instead of isometrically contracting myocardium since this is much closer to the in vivo beating heart. As will be discussed below differences between both contraction types have been observed.

Effects of $[Ca^{2+}]$ and temperature on the force–frequency relation

In this study the FFR of mouse papillary muscle was investigated at extracellular $[Ca^{2+}]$ between 1.0 and 5 mM, temperatures of 27°C to 37°C and under isometric compared to auxotonic muscle contractions. We were able to show that, dependent on experimental conditions, the FFR in mouse papillary muscle can be steeply positive or clearly negative. Studies on FFR of myocardium of large mammals have consistently shown a positive FFR [6, 21] whereas in rat and in mice the results vary substantially. In theory, species with short action potentials of cardiomyocytes such as the mouse are assumed to exhibit a negative FFR [2, 20]. Since we showed that FFR in mice is dependent on temperature and $[Ca^{2+}]$ different findings in the literature may be due to differences in experimental conditions. Reports of negative FFR in mouse were based on (unphysiological) high $[Ca^{2+}]$ of 2 or 2.5 mM [4, 19]. Our results show that a reduction of $[Ca^{2+}]$ to physiological levels can turn the FFR positive. Gao et al. [7] reported twitch force increasing sevenfold over a range of stimulation rates of 0.2–4 Hz working at room temperature and 1.0 mM Ca^{2+}. Our results suggest that this increase in force is predominantly due to the low temperature level. Our data are in agreement with the work of Layland and Kentish [16] who showed that a positive FFR in rat trabeculae can be converted to a negative FFR by elevating $[Ca^{2+}]$ (from 1.0 to 8.0 mM). The effects of temperature on FFR observed by this group also fit our results in the frequency range of 1–5 Hz. However, at frequencies below 1 Hz Layland and Kentish [16] report a further reduction of twitch force, whereas we observed in mouse papillary muscle a several-fold increase in twitch force at 0.1 Hz compared to 1 Hz. This increase in force at very low frequencies probably resembles the phenomenon of postrest potentiation described in the ventricular muscle of many species [1, 3].

In an attempt to analyse the effects of temperature and $[Ca^{2+}]$ on FFR we calculated the ratio of twitch force to the maximal twitch force that can be generated after a break of stimulation. The lower the ratio of twitch force at 1 Hz to maximal twitch force (F_1/F_{max}) the more positive was the FFR. Thus, muscles showing a positive or negative FFR do not differ so much in the absolute twitch forces that can be achieved by high-frequency stimulation, but they do differ in the absolute minimal force values at 1 Hz. This suggests that muscles exhibiting a flat or negative FFR have a relatively high intracellular calcium load at 1 Hz which cannot further be enhanced by increasing stimulation frequency. In contrast, muscles with a positive FFR may have a relatively low intracellular calcium load at 1 Hz which can be further increased at higher frequencies.

Auxotonic contractions

To our knowledge this study is the first to investigate systematically the effect of auxotonic contraction on FFR in isolated myocardial muscle preparations. However, it has been reported that rat papillary muscles mounted at constant muscle length did not contract isometrically but displayed shortening of sarcomeres in the central region of the muscle preparation at the expense of the damaged ends [13]. Thus, the so-called isometric contractions in our study may also be auxotonic to some minor degree. Nevertheless, mounting the papillary muscle in a way that allows shortening of the whole preparation will certainly result in a higher degree of sarcomere shortening and be closer to the in vivo situation of the beating heart. At physiological conditions, i.e. 37°C and 1.5 mM Ca^{2+}, no significant differences of the FFR between so-called isometrically and auxotonically contracting muscles could be observed. However, at lower temperatures (27°C) the positive inotropic effect of increasing stimulation frequency observed during isometric twitches was weakened under auxotonic conditions. This effect may be due to differences in calcium cycling of both contraction types. Cat and ferret papillary muscles were shown to exhibit increased intracellular $[Ca^{2+}]$ as well as a more positive membrane potential and a longer action potential during auxotonic as compared to isometric twitches [9, 15]. An increased intracellular calcium load during auxotonic twitches at 1 Hz would explain our observation of a higher F_1/F_{max} ratio and could result in a flatter FFR. In addition, in the heart sarcomeres display a positive force–

length relationship. Therefore, auxotonic contractions accompanied by more shortening of the muscle preparation than isometrically contracting muscles display smaller twitch forces. Furthermore, under auxotonic conditions stronger contraction will not only result in a higher twitch force but will also result in more shortening. Thus, the degree of muscle shortening may also influence the FFR and may contribute to the flatter FFR observed under auxotonic compared to isometric conditions.

Implication of the FFR in vivo

The physiological importance of a positive inotropic effect of increased frequency is certainly not the same in different species. In small animals, such as the mouse, maximal heart rate is only 30% above heart rate at rest in contrast to larger mammalian species where heart rate can be increased two- to threefold [11, 25]. Therefore FFR may play a minor role in regulating cardiac performance in mice and rats. Thus, a flat or slightly negative FFR in isolated mouse papillary muscle does not necessarily indicate ineffective contractility but may be close to the physiological situation. A minimal force-frequency dependence in murine heart in vivo was recently shown by Georgakopoulos and Kass [8]. The most likely explanation for this minimal force-frequency dependence is a rapid calcium cycling and enhanced sarcoplasmic reticular calcium buffering. In mouse a calcium re-circulation fraction of about 90% was reported compared to 50% values in human heart [8, 17, 24]. Evidence for high calcium recycling and little calcium flux across the plasma membrane comes also from our observation of the independence of maximal twitch force, i.e. postrest potentiation, from extracellular calcium.

Thus, in mice in vivo cardiac performance might be mainly enhanced by other mechanisms than increasing heart rate, such as adrenergic stimulation or the Frank-Starling mechanism.

In conclusion, we found that the isolated mouse papillary muscle is a good experimental model for investigating various aspects of cardiac physiology. Basic contractile parameters and, in particular, the FFR were found to be strongly dependent on $[Ca^{2+}]$, temperature and the type of contraction. This may explain the different results reported in the literature about a positive inotropic effect caused by increased frequency in mouse heart. Under conditions that most closely resemble the physiological situation, mouse papillary muscle exhibits a flat FFR which supports the results of a recent in vivo study [8].

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