

Video Article

Contractility Measurements on Isolated Papillary Muscles for the Investigation of Cardiac Inotropy in Mice

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URL: <https://www.jove.com/video/53076>

DOI: [doi:10.3791/53076](https://doi.org/10.3791/53076)

Keywords: Medicine, Issue 103, Papillary muscle, contractility, mouse heart, β -adrenergic signaling, ischemia

Date Published: 9/17/2015

Citation: Uhl, S., Freichel, M., Mathar, I. Contractility Measurements on Isolated Papillary Muscles for the Investigation of Cardiac Inotropy in Mice. *J. Vis. Exp.* (103), e53076, doi:10.3791/53076 (2015).

Abstract

Papillary muscle isolated from adult mouse hearts can be used to study cardiac contractility during different physiological/pathological conditions. The contractile characteristics can be evaluated independently of external influences such as vascular tonus or neurohumoral status. It depicts a scientific approach between single cell measurements with isolated cardiac myocytes and *in vivo* studies like echocardiography. Thus, papillary muscle preparations serve as an excellent model to study cardiac physiology/pathophysiology and can be used for investigations like the modulation by pharmacological agents or the exploration of transgenic animal models. Here, we describe a method of isolating the murine left anterior papillary muscle to investigate cardiac contractility in an organ bath setup. In contrast to a muscle strip preparation isolated from the ventricular wall, the papillary muscle can be prepared *in toto* without damaging the muscle tissue severely. The organ bath setup consists of several temperature-controlled, gassed and electrode-equipped organ bath chambers. The isolated papillary muscle is fixed in the organ bath chamber and electrically stimulated. The evoked twitch force is recorded using a pressure transducer and parameters such as twitch force amplitude and twitch kinetics are analyzed. Different experimental protocols can be performed to investigate the calcium- and frequency-dependent contractility as well as dose-response curves of contractile agents such as catecholamines or other pharmaceuticals. Additionally, pathologic conditions like acute ischemia can be simulated.

Video Link

The video component of this article can be found at <https://www.jove.com/video/53076/>

Introduction

The investigation of proteins like ion channels referring their role for cardiac contractility is essential to discover different pathomechanisms and to establish new therapeutic strategies for cardiac diseases such as ischemia and heart failure.

Contractile function of mammalian cardiomyocytes is known to be modulated by various ion channels, transporters and other proteins. Action potential evoked activation of voltage dependent sarcolemmal L-type Ca^{2+} channels leads to Ca^{2+} influx from extracellular space and subsequently to Ca^{2+} -induced Ca^{2+} release (CICR)¹, which triggers cellular contraction². Ca^{2+} -signaling plays a central role in cardiac contractility and adaptation to physiological or pathological stress. Catecholamines activate cardiac β -adrenergic receptors, thus stimulating adenylyl cyclase (AC) which synthesizes cAMP. Being activated, protein kinase A (PKA) phosphorylates different intracellular and membrane associated proteins like L-type Ca^{2+} channels, phospholamban and ryanodine receptors resulting in modification of Ca^{2+} transients and cardiac contractility^{1,3,4}. cAMP is degraded by phosphodiesterase (PDE). Activation of Gs-coupled receptors other than β -adrenoceptors also leads to accumulation of cAMP.

The technique of contractility measurements in isolated ventricular muscle strips is well established for larger mammalian species⁵⁻⁸. Based on the possibility of gene targeting in mice it is important to establish methods to analyze murine cardiac physiology. However, existing data about the physiological properties of isolated muscle preparations in mice differ depending on experimental conditions⁹⁻¹².

The described method is used to analyze cardiac contractility of left ventricular papillary muscle preparations *in vitro*. Investigation of cardiac contractility is performed in the absence of influences modifying cardiac contractility *in vivo*, like blood pressure, neurohumoral stimulation and physical or metabolic stress. The beating rate of the contracting muscle preparation can be rigorously defined and changed arbitrarily. Twitch force can be analyzed in the context of specific stimuli such as calcium concentration, beating frequency or temperature. In addition, this method can be used to investigate different signaling pathway components and to compare cardiac performance of genetically modified mouse models by controlling experimental conditions mentioned above.

Protocol

NOTE: The basic steps of the isolation procedure are shown in **Figure 1**. All steps are described in detail in the following protocol. Papillary muscle isolation, mounting in organ bath chamber, acquisition and analysis is performed in a consecutive and compulsory timescale.

All animal experiments were performed in accordance with German legislation on protection of animals and were approved by the Ethics Review Board of University of Heidelberg.

1. Preparation of Instrumentation

1. For contractility measurements use a multichambered organ bath setup. Components of an *ex vivo* contractility organ bath setup are shown in **Figure 2**.

NOTE: To minimize the amount of time between the preparation of the papillary muscle and the beginning of the experimental protocol, set up equipment and prepare buffers prior to collecting experimental tissue. This provides the greatest amount of time that the tissue remains viable to conduct experiments.

2. Start up computer and data acquisition equipment. Turn on organ bath heat (preset to 32 °C) and allow units to preset temperature.
3. Prepare the acquisition equipment. Start recording data as mentioned in the software manual.
4. Check and conduct calibration (if necessary) on each channel and zero all channels to standardize the electric signal supplied from the respective force transducer (associated with that channel) to a 2-g force supplied by a certified weight.
5. Turn on gas supply (95% O₂/5% CO₂) to organ bath chambers. Continuously gas all chambers during the entire protocol measurements.

2. Preparation of Buffers and Physiological Solutions

1. Prepare a Krebs-Henseleit buffer solution to achieve final concentrations of 119 mM NaCl; 25 mM NaHCO₃; 4.6 mM KCl; 11mM glucose; 1 mM Na-pyruvate; 1.5 mM CaCl₂; 1.64 mM MgSO₄; 1.18 mM KH₂PO₄ and adjust pH to 7.4 (see **Table 1** Physiological solution).
2. Prepare separate Krebs-Henseleit buffer solution to use during isolation as described in step 2.1 with additional 30mM 2,3-Butanedione monoxime (BDM) and 50 IE Heparin/ml. Cool solution to 4°C (see Table 1 Preparation solution). For ischemia simulation, prepare ischemia-solution as described in Table 1.
3. For the protocol of the Ca²⁺-dependent contractility, add Ca²⁺ to the Krebs-Henseleit buffer solution to requested final concentration.

NOTE: prepare these solutions directly before its usage and gass with carbogen to avoid precipitation of Calciumcarbonate. Prewarm solution to 32°C before using.

3. Preparation of the Gassing Tube and Dissection Dish used during the Papillary Excision Procedure

1. Connect a soft gassing tube (external diameter 2-4 mm) to the gas connection (100% oxygen, carbogen alternatively) with expert working.
2. Create a closed ring of the gassing tube fitting inside the dissection dish. Puncture the gassing tube several times that a continuous bubbling is assured. This gassing tube can be used several times.
3. Prepare dissection dish with a silicon elastomer at the bottom (0.5 cm thick), so that pins can be stuck into it. This dish can be used several times.

4. Isolation of Left Anterior Papillary Muscle from Mice

NOTE: Before starting the isolation of the papillary muscle, check that the gas lines are clear of blockages.

1. Prepare organ bath setup as mentioned in the protocol section 1-3 before starting the preparation of the papillary muscles. Prepare all solutions at the day of the experiment.
2. Sacrifice the mouse (approximately 8-12 weeks old) by cervical dislocation according to expert working and institutional guidelines.
3. Fix the animal in dorsal position by fixing forepaws and hind paws on a dissection board, open the thorax lateral on both sides by cutting through the ribs with sharp bone scissors, then cut the diaphragm with a transversal cut. Remove the pericardium. Now the heart and the aorta are accessible.
4. Fix the heart with blunt forceps on the vascular truncus close to the heart and separate the heart quickly with scissors from the lungs and the surrounding tissue.
5. Transfer the beating heart to a Petri dish filled with cooled preparation solution gassed with oxygen (step 2.2). Allow the heart to beat and stimulate heart contractions softly via touching the cardiac apex with forceps.
6. As soon the heart is completely exsanguinous, transfer it to the dissection dish filled with cooled preparation solution (step 2.2) and gassed with O₂. From this step on use a stereomicroscope.
7. Fix the heart with a small pin through the right ventricle so that the heart is seen from dorsal (right ventricle is situated on the right side from the operator's view).
8. Using microsurgical scissors separate both atria on the atrioventricular level, cut connecting tissue from the ventricles and discard. Perform a cut through the ventricular wall from the AV-valve-level to the apex of the heart avoiding any mechanical pressure.
9. Open the ventricle and fix the left-sided free wall with forceps, thus having a look at both left ventricular papillary muscles.
10. Slightly cut away the ventricular tissue lateral on both sides of the left anterior papillary muscle preserving a part of the valvular sail on the papillary muscle preparation and avoid touching or stretching the papillary muscle as much as possible. Dissect the remaining ventricular wall tissue from the papillary muscle.

11. Attach silk threads (7/0, metric 0.5) on both sides of the papillary muscle preparation, one on the valvular sail and one on the muscular part. Fix the preparation in the organ bath chamber filled with organ bath solution and gassed with carbogen, as temperature 32 °C is suggested.

5. Equilibration and Stimulation of the Papillary Muscle

1. Stimulate the papillary muscle preparation with rectangular pulses (duration of 2 ms and current of 100 mA) at stimulation frequency of 1 Hz immediately after the fixation in the organ bath.
2. Ensure a frequent change of the organ bath solution, either by continuous change or by frequent manual change (every 5 min) according to the setup of the organ bath type used.
3. Gradually increase the pretension until maximal twitch force is reached. Maximal twitch force is reached when the increase of the pretension is not followed by a further augmentation in the twitch force.
4. After 45-60 min of equilibration, start the experimental protocol.

6. Suggested Experimental Protocols for Contractility Measurements

NOTE: The experimental protocol outlined below comprises standard maneuvers to characterize cardiac contractility under physiological and pathophysiological conditions. In the representative section we describe these protocols in detail also showing representative results (see also Table 2).

1. Recording and analysis of the experimental protocols
 1. For recording, use a suitable software with adequate temporal resolution (acquisition rate ≥ 1 kHz).
 2. Analyze parameters including twitch force amplitude (height), time to peak tension (TTP) and half maximal relaxation time (R_{50}/TF_{fall} , respectively) as mentioned in the software program (as example Chart 5.5 of ADInstruments, see **Figure 4**).

Representative Results

The protocol of this manuscript for contractility measurements of isolated murine papillary muscle preparations is tuned to optimal conditions to achieve reproducible experimental results under physiological conditions. To define optimal experimental conditions we performed pilot experiments varying organ bath temperature and extracellular calcium concentration (see also¹²). The protocol described here was performed with an extracellular calcium concentration of 1.5 mM and a temperature of 32 °C.

Basal contractility properties

To characterize basal contractility properties, parameters for twitch force amplitude, twitch rate, time to peak (defined as the time required to reach maximal contraction force starting from 5% basal) and relaxation 50 time (half maximal relaxation time; defined as time required to reach half of relaxation force) are used in this protocol. A representative trace of twitches and the analysis of these parameters are illustrated in **Figure 3A-B**.

Calcium-dependent contractility

Extracellular calcium concentration crucially determines cardiac excitation-contraction coupling and by varying this parameter, the calcium sensitivity of the contractile apparatus in cardiomyocytes can be evaluated. As shown in **Figure 3C** the augmentation of the extracellular calcium concentration results in altered calcium transients and subsequently in altered twitch force generation. The calcium-concentration of the extracellular solution is increased step-by-step (*i.e.*, between 1.5 to 7mM). By applying this protocol, the calcium homeostasis regarding the calcium-dependent contractility of the contractile apparatus can be evaluated. In the organ bath setup used in this manuscript, the change of the extracellular calcium concentration was performed by changing the organ bath solution manually leading to a short interruption of the pacing and subsequently to an artificial postrest potentiation (see Force potentiation after non-pacing interval).

Force potentiation after non-pacing interval (PRP)

At the end of a contraction cycle calcium is sequestered into sarcoplasmic reticulum (SR) predominantly by an ATP-dependent calcium pump (SERCA, sarco-endoplasmic reticulum calcium-ATPase), thus lowering the cytosolic calcium concentration during the diastole. If the time interval between two contractions is enlarged, more calcium can be pumped back into the SR and subsequently more calcium can be released during the next excitation. The postrest-potentiation protocol (PRP) describes alterations of the twitch force amplitude after a previous stimulation with rest period of a defined duration. In human, rat as well as in murine cardiac tissue, an augmentation of the twitch force was shown^{10,12-14,16} (**Figure 3D**). It is known that a positive post rest potentiation is diminished or even adverse in failing cardiac tissue of human and rat^{13,14,16,17}. This protocol is used to evaluate the function of the diastolic calcium accumulation in intracellular stores^{4,15}.

Frequency-dependent contractility (FFR)

Force-frequency relation describes the correlation between beating rate and contractile twitch force (Bowditch effect)¹⁸. The force- frequency relation significantly differs between mammalian species and experimental conditions¹⁹. In mice, a specific correlation between beating rate and contraction strength was shown. At 32 °C, an initial negative FFR between stimulation rates of 0.1-1 Hz was shown, whereas the FFR was shown to be positive in the range of 1-4 Hz of stimulation⁹⁻¹² (**Figure 3E**).

In failing myocardium a negative correlation between beating rate and contractility is described^{20,21}. In this manuscript, the standard pacing-frequency (1 Hz) is reduced to 0.1 Hz and starting from there, the pacing-frequency is incrementally raised to 5 Hz (0.2; 0.5; 1; 2; 3; 4; 5 Hz).

β -adrenergic stimulation

Catecholamines like adrenaline and noradrenaline are hormones which are released during (patho-) physiological stress conditions modulating cardiac contractile force by activation of β -adrenoceptors. Iatrogenic application of catecholamines plays a prominent role in clinical treatment of patients with acute heart failure to enhance cardiac inotropy temporarily. This effect can also be seen and investigated in *in vitro* studies. As an agonist of β -adrenoceptors, Isoprenaline increases contractile twitch force. *In vivo*, an increase in beating frequency also occurs (positive inotropic effect), which additionally modulates the contractile response (Bowditch effect, see also FFR protocol described above). Using the method described here, the inotropic effect of Isoprenaline can be investigated at a fixed beating rate without that chronotropic effect (**Figure 3F**). The stimulation of β -adrenoceptors can also be achieved by other hormones such as histamine. In murine ventricular tissue, application of histamine results in a biphasic response which contains an initial positive inotropic effect, but with a transient decline in twitch force as shown in **Figure 3G**. For that reason an interpretation of data measured by application of histamine seems to be difficult; especially the underlying receptor-activation in mice is not entirely solved.

Ischemic conditions

Acute cardiac ischemia is defined as a critical limitation of blood flow leading to undersupply of the cardiac tissue with oxygen and nutrients resulting in loss of contractility, development of ischemic contracture and cell death. To simulate ischemia *in vitro*, muscles are exposed for 30 min to glucose- and pyruvate-free extracellular solution bubbled with 95 %N₂/5% CO₂. With that approach a depletion of ATP in the cardiomyocytes should be induced. Within few minutes after starting the ischemia-simulation, a decline of the twitch force occurs with appearance of an ischemic contracture pictured as a spontaneous increase in the preload tension (**Figure 3H**).

Component	Organ bath solution [mM]	Preparation solution [mM]	Ischemia solution [mM]
Sodium chloride	119	119	119
Sodium bicarbonate	25	25	25
Potassium chloride	4.6	4.6	4.6
Glucose	11	11	-
Sodium pyruvate	1.0	1.0	-
Calcium chloride dihydrate	1.5	1.5	1.5
Magnesium sulfate heptahydrate	1.64	1.64	1.64
Potassium phosphate monobasic	1.18	1.18	1.18
2,3-Butanedione-Monoxime	-	30	-
Temperature	32°C	4°C	32°C
Gassing	95% O ₂ / 5% CO ₂	100% O ₂	95% N ₂ / 5% CO ₂
pH	7.4	7.4	7.4

Table 1: Components and concentrations of the used buffer solutions.

Name	Protocol	Analysed parameter	Read out/ possible conclusion
Postrest potentiation (PRP)	Pacing-pause from 10-60sec	First twitch / basal	SR calcium load
Force-frequency relation (FFR)	Stimulation rate from 0.1 to 5 Hz	Twitch force	Frequency dependent contractility
concentration-response curve	Increase agent concentration	Twitch force	Agent-mediated contractility (EC ₅₀ , IC ₅₀)
Dependency on extracellular calcium	Vary [Ca ²⁺] _{ex} from 1.5 to 7mM	Twitch force	Calcium-dependent contractility
Ischemic conditions	95% N ₂ / 5% CO ₂ , depletion of glucose & pyruvate	Twitch force, hypoxia-dependent contracture	Cardiac contractility under ischemic conditions

Table 2: List of suggested experimental protocols to investigate cardiac contractility in isolated papillary muscle preparations.

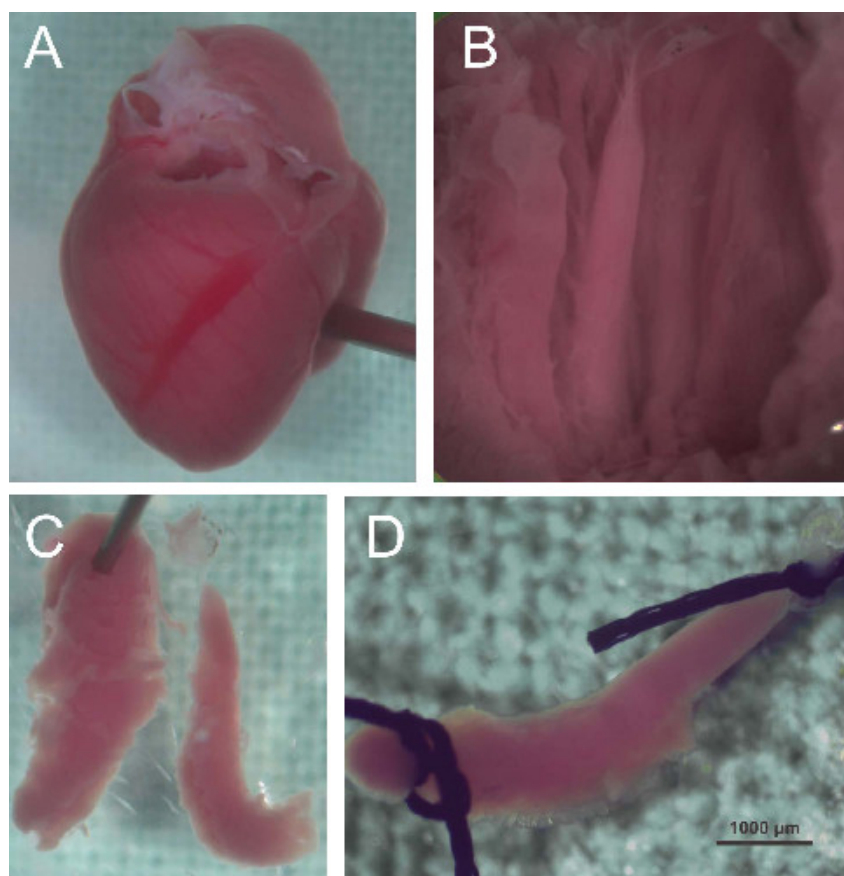


Figure 1. Key steps of the papillary muscle preparation. (A) Dissection of both atria. (B) View on the left ventricular anterior papillary muscle. (C) Dissection of the papillary muscle from the ventricular wall. (D) Attachment of two silk threads before the fixation in the organ bath chamber. [Please click here to view a larger version of this figure.](#)

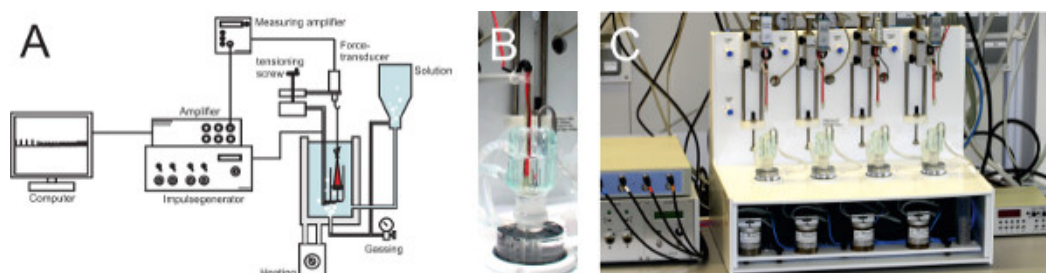


Figure 2. Organ bath setup. (A) Schematic setting of the organ bath setup. Water jacket organ bath chambers equipped with aeration glass frits provide stable temperature control. To generate square-wave pulses field electrodes are attached to the tissue support. A force transducer senses the contraction of the muscle thereby amplifying the generated signal. This signal is filtered and transferred back to the computer by an A/D converter. The signal is then digitized and can be saved for later analyses. (B) Picture of a single papillary muscle preparation fixed in the organ bath chamber from the organ bath setup (C). [Please click here to view a larger version of this figure.](#)

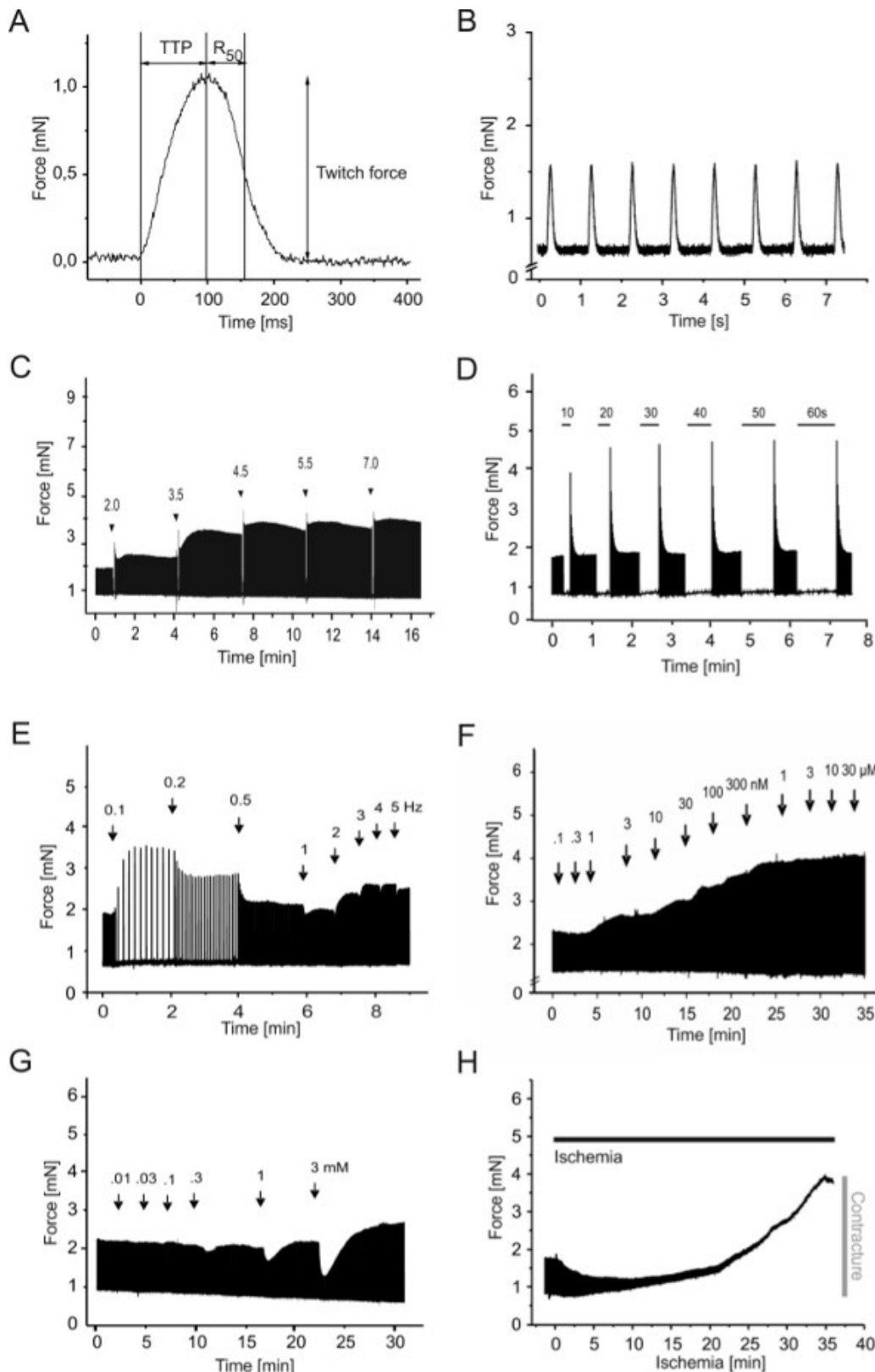


Figure 3. Representative results of contractility measurements of murine left anterior papillary muscles. (A) Representative analysis of twitch force amplitude, Time to peak tension (TTP) and half maximal relaxation time (R_{50}) of a single twitch. Representative recordings of stimulated twitches of murine papillary muscle preparations under basal stimulation with a beating rate of 1 Hz (B), during increasing extracellular calcium concentrations (C), during defined resting intervals (post rest potentiation, PRP) (D), or different stimulation rates (force-frequency relation, FFR) (E) and after application of increasing concentrations of Isoprenaline- (F) or Histamine-application (G). Representative recording of stimulated twitches and preload during ischemia-stimulation (H). [Please click here to view a larger version of this figure.](#)

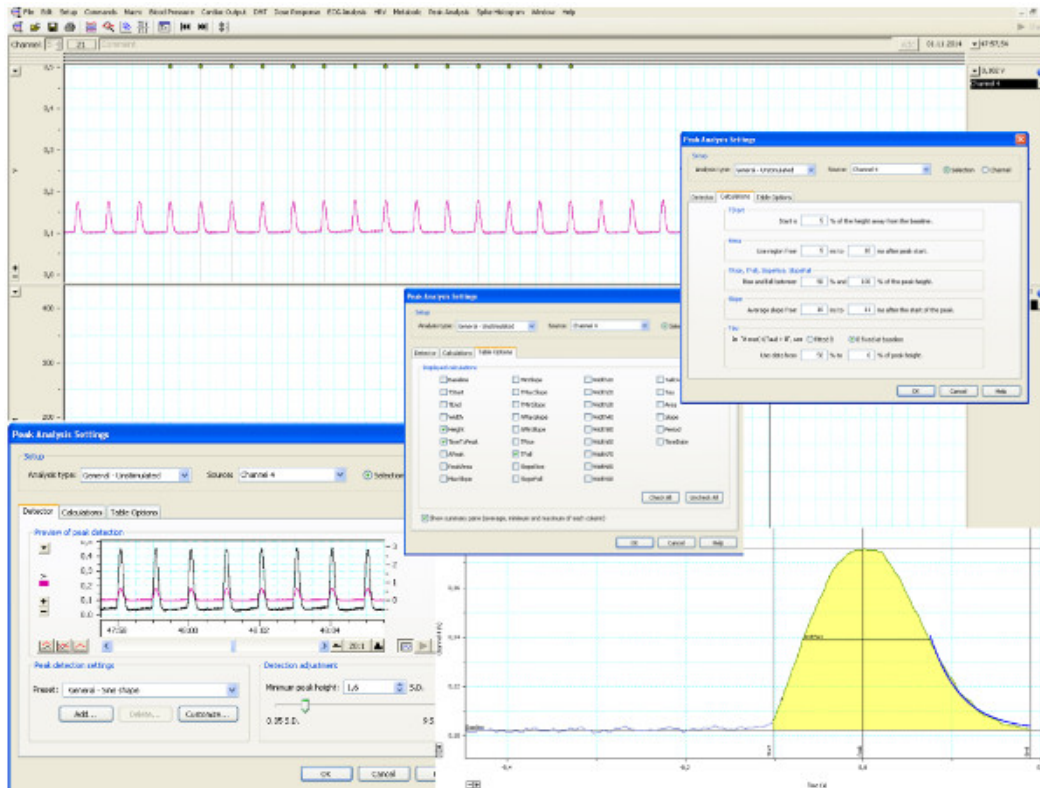


Figure 4. Analysis of contractile parameters. To characterize contractile function, parameters including twitch force amplitude (height), time to peak tension (TTP) and half maximal relaxation time (R_{50}/T_{Fall} , respectively) are analyzed in this protocol using the software program Chart 5.5 (ADInstruments). [Please click here to view a larger version of this figure.](#)

Discussion

In this manuscript we describe a method to investigate contractility of murine papillary muscle *in vitro* which can be used to answer several scientific questions related to heart physiology and pathology in mice as well as to support the analysis of transgenic lines and the discovery of new pharmaceutical approaches to treat heart dysfunctions. We illustrate the use of this method to assess physiological, pathological and pharmacological properties of cardiac muscle contractility (see Fig 3). Additional applications not illustrated here include evaluation of intracellular pathways using different pharmacological agents (see also ¹²).

Cardiac diseases such as ischemic heart disease and heart failure display an eminent clinical problem and remain the leading cause for mortality and morbidity worldwide²². For many proteins expressed in the heart, their role for cardiac contractility is still poorly understood or not studied until now. The contractility measurement on isolated papillary muscles demonstrates a meaningful and valid approach to study cardiac contractility *in vitro* in mouse models.

Although the method is technically feasible and shows a good reproducibility, there are several critical steps necessary to ensure its success: First, after isolating the heart, ensure a blood free solution during the preparation procedure to be able to work properly (visualization). Another critical step is that tissue preparation is performed carefully to ensure viability by avoiding any touching or stretching of the papillary muscle. As described in the method section try to keep the valvular sail connected to the papillary muscle to ensure a trouble-free attachment of the silk thread. After fixation of the preparation in the organ bath chamber, change the organ bath solution several times during the first minutes to flush out the BDM. Increase the pretension of the preparation slightly and gradually until no more increase of twitch force occurs. There are some exclusion criteria for the evaluation of the single protocols such as arrhythmic beats and spontaneous increase of the pretension as a sign for ischemic conditions. Several experimental protocols such as postrest potentiation and force-frequency relation can be performed with the same preparation sample, whereas a new preparation sample should be used for the analysis of drug-mediated effects on contractility. The preparation protocol is optimized to isolate the left anterior papillary muscle. Adapting the protocol, also the posterior muscle can be used for this method of contractility measurements.

This method of isolated papillary muscle measurement provides various information about the calcium-, temperature- and frequency-dependent contractility as well as about the contractile response after applying pharmacological agents or the simulation of pathological conditions. However, the interpretation and extrapolation of these results needs to be handled with scientific care. The described method is an *in vitro* model of cardiac muscle, disconnected from its normal environment and neural innervation. Therefore, the experimental conditions are not physiological and the results received in this protocol cannot be transferred without in-depth interpretation to the situation *in vivo*. For example, the method does not take into account changes in blood pressure, hormones or extrinsic neural control. The supply of oxygen and metabolic supplements for the preparation in the organ bath is limited by diffusion. An insufficient supply of oxygen and metabolic supplements would lead to ischemic conditions and subsequently invalid experimental results. To avoid this, the experimental conditions should be optimal, especially concerning

oxygenation, concentration of calcium and temperature of the organ bath solution. With the experimental conditions of the protocol described here, valid results can be afforded.

In this manuscript a method of simulating ischemia-like conditions is described. Additionally to the simulation of hypoxia, all energetic supplements of the organ bath solution were depleted to mimic the ischemic state *in vivo* as good as possible. In contrast to hypoxia, it was shown that the simultaneous depletion of oxygen and energy supply leads to changes in calcium transients comparable to alterations seen *in vivo*²³.

The procedure described for the isolation of mouse papillary muscle can be adapted to other species, e.g., rat. However the optimal conditions of the organ bath setup could vary between different animal models and may be adapted. Parameters that may need adaptation are the temperature and the calcium concentration of the physiological solution.

In summary, this contractility method provides a very powerful approach to assess heart physiology, pathophysiology and pharmacology. When used properly, it provides the ability to study cardiac contractility in an isolated but well controlled environment.

Disclosures

The authors declare that they have no competing financial interests.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (KFO 196 "Signaltransduktion bei adaptativen und maladaptiven kardialen Remodelling-Prozessen", FR 1638/1-2) and by the DZHK (German Centre for Cardiovascular Research, a part of the German Centres of Health Research, which is a BMBF (German Ministry of Education and Research) initiative).

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