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MECHANICAL RESTITUTION IN LEFT AND RIGHT GUINEA PIG VENTRICULAR PREPARATIONS

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A b s t r a c t

The present study was focused on identification of possible differences in the onset and time course of mechanical restitution in guinea pig multicellular heart preparations, i.e., papillary muscles from the right and left ventricles. Stimulated twitches of both preparations mounted in a horizontal perfusion bath were recorded simultaneously. Mechanical restitution curves were constructed at 25°C and 35°C and at stimulation rates of 0.5 Hz, 1 Hz and 2 Hz. No statistically significant differences in restitution curves of both preparations were found. It can be concluded that, in the assessment of mechanical behaviour of the heart, the guinea pig left ventricular papillary muscle appears to be a suitable substitute for the conventionally employed right ventricular papillary muscles.

K e y w o r d s

Mechanical restitution, Papillary muscle, Heart, Left and right ventricles, Guinea pig

INTRODUCTION

Regulations for handling animals in biomedical research urge the experimenter to economically utilise the sacrificed object. One of the most frequently employed preparations in studies of cardiac contractility is the papillary muscle or trabecula from the right ventricle. However, not in every heart can a suitable muscle be found. It is thus imperative to search for another tissue in the same heart instead of sacrificing another animal. The obvious candidate is the papillary muscle from the left ventricle. So far, this preparation has not been found suitable due to its larger diameter, putative insufficient oxygenation and, consequently, impaired function. The question arises whether these potential drawbacks really exist and, second, whether properties of this preparation differ from those of the right ventricular preparation in analogy to the well-known differences of the atrial muscle.

One of the possibilities of how to examine the quality of a multicellular preparation is to construct a mechanical restitution curve, i.e., the time course of contractility recovery between two beats. The relationship between the variable test interval and the magnitude of a contraction was first reported by *Bravený and Kruta* in 1958 as a mechanical restitution curve *(1)*. The course of this curve

reflects a number of variables like species, temperature, extra-cellular Ca^{2+} concentration, stimulation frequency, neurohumoral agents, ischaemia, cardioplegia, drugs used, etc. *(2).*

In our previous experiments on guinea pig hearts, we have noticed that, as a rule, at least one thin papillary muscle in the left ventricle may be found which is a candidate for contractility measurements. The aim of this study was to compare mechanical restitution in right and left papillary muscles isolated from the hearts of adult guinea pigs.

MATERIALS AND METHODS

In the study 19 left atria, 17 papillary muscles from the right ventricle and 12 papillary muscles from the left ventricle of guinea pigs were included. The body weight of animals varied from 230 to 380 (average, 330±50) grams; animals of both sexes were used.

The animals under deep ether anaesthesia were sacrificed by cervical dislocation. The chest was quickly opened and the heart immediately removed. This was placed in a preparation bowl with a cold (5°C) Krebs-Henseleit solution of the following composition: NaCl, 118 mM; $NaHCO_3$, 24 mM; KCl, 4.2 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.2 mM; glucose, 5.5 mM and Taurine, 10 mM. Both ventricles were opened and papillary muscles removed. Then, the left auricle strip was cut off and placed together with either the right or the left ventricle papillary muscle in the perfusion bath.

A horizontal bath for pharmacological studies (*Fig. 1*) was employed in our study. It was made from plastic, was double-walled and controlled by a thermostat. The temperature of a solution in the bath was kept constant during the experiment in the range of approximately 15°C to 37°C. There were two silver stimulation electrodes placed on the bottom, one for stimulation (below the clips that held the preparations) and one for grounding. The bath was filled with 10 ml Krebs-Henseleit solution that was aerated by means of a single bubbling stone placed at the edge of the bath. The stone was separated from the preparations by a barrier in order to prevent bubbles from causing artefacts in recording. It was possible to place two preparations in the bath at the same time and thus compare them under exactly the same conditions. Each muscle was fixed by a clip at one end and attached to a mechano-electric transducer (tensometer) by a thread bound to its opposite end. A simultaneous recording of the tension generated by the preparations was performed under isometric conditions, e.g., length of the preparation did not change during the experiment.

RESULTS

The experiments were carried out at 25°C and 35°C and fluctuations in temperature did not exceed 0.5°C. The preparations were stimulated with 1ms pulses of twice the current threshold. Stimulation frequencies were 0.5 Hz, 1 Hz and 2 Hz.

Initially, the preparations were stimulated for 30 min at a frequency of 0.5 Hz at 25°C (control period) and contractions varying in prematurity were recorded according to the protocol (*Fig. 2*). The procedure was repeated at the basal frequencies of 1 Hz and 2 Hz. In this step, control periods lasted 15 min. Subsequently, temperature in the bath was increased up to 35°C and the stimulation protocol was repeated.

The recorded data were processed using a non-linear parameter estimation and the model developer MINSQ. The model introduced into the software presented the

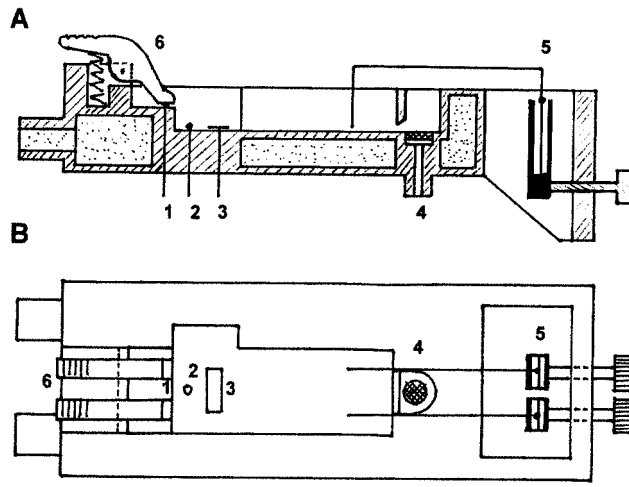

Fig. 1
Horizontal perfusion bath for heart multicellular preparations (A, side view; B, from above). 1, stimulation electrodes; 2, thermometer; 3, grounding electrode; 4, bubbling stone; 5, tensometers; 6, clips for holding preparations at distance.

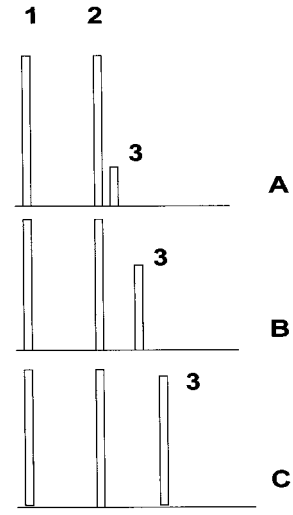

Fig. 2
Schematic diagram showing the progression of mechanical restitution of the cardiac muscle in relation to the prematurity of a stimulation pulse. 1, 2, regular stimulation pulses; 3, irregular stimulation pulse; A, B, C, degrees of prematurity ranging from very early intervals (A) to those close to the basic stimulation interval (C).

course of mechanical restitution as described earlier (*3*). It included one independent variable (X), one dependent variable (Y) and two parameters (TAU and TSH). TAU represents the time course of a mechanical restitution curve and TSH represents the time shift of the onset of the curve. The model is described by two equations:

$$A: = (1\text{-}EXP((-(TZ\text{-}TSH))/TAU)^{-1}$$

$$Y: = A*(1\text{-}EXP((-(X\text{-}TSH))/TAU))$$

where TZ is a stimulation interval in ms, and the limits for the fitting of TAU and TSH parameters are 10–2000 ms and 0–700 ms, respectively.

All restitution curves, constructed on the basis of the data recorded, were fitted with this model.

The well-known differences in mechanical restitution of atrial and right ventricular preparations (*2, 3*) were confirmed. This part of the study proved the validity of our experimental setup. The results obtained at the stimulation frequency of 0.5 Hz and temperatures of 25 and 35°C are shown in *Figs 3* and *4*.

Some differences were observed when the mechanical restitution curves of left and right papillary muscles were compared (*Figs 5,6*). They, however, were not significant when tested by the paired *t*-test.

To confirm this finding, we used the double essential Wilcoxon test. We compared TAU and TSH from all curves obtained in the experiments. No statistically significant difference between the mechanical restitution curve of the right and that of the left ventricular papillary muscle was found.

DISCUSSION

Most of the findings made in cardiovascular physiology have been obtained in different simple models, such as isolated perfused hearts, multicellular muscle strips and, recently, also in isolated cardiomyocytes and their parts. The quality of preparations is the main condition for obtaining valid scientific data. In isolated heart preparations, the crucial issue is proper oxygenation. These preparations are supplied with oxygen only by diffusion from the fluid in the bath. The conventionally used papillary muscle from the right ventricle with a diameter up to 1 mm is believed to be supplied by oxygen sufficiently. A similar preparation from the left ventricle, on the other hand, is suspected of developing ischaemia because its diameter is larger and the preparation is, therefore, usually discarded. Probably due to this fact there is not enough information on contractility parameters of the left ventricular multicellular preparations.

Mesaeli and *Juggi* (*4*) reported differences in mechanical restitution and post-extrasystolic potentiation curves between the right and left ventricles in isolated

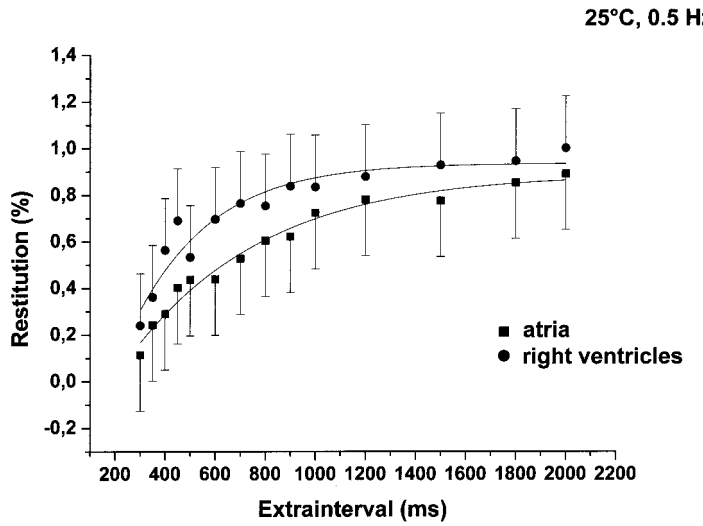

Fig. 3
Curve showing the mechanical restitution of left atrial muscles as compared to right ventricular papillary muscles. Extrainterval, period between a regular and a premature stimulation pulse. Temperature, 25°C; stimulation frequency, 0.5 Hz. Summary data from all experiments.

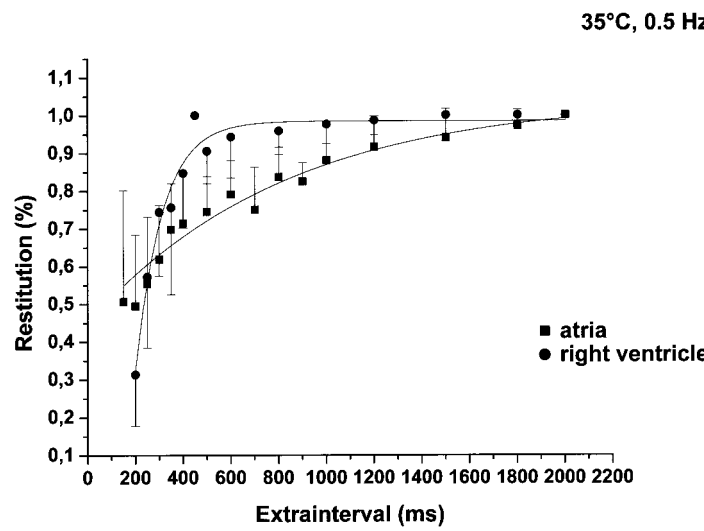

Fig. 4
Curve showing the mechanical restitution of left atrial muscles as compared to right ventricular papillary muscles. Extrainterval, period between a regular and a premature stimulation pulse. Temperature, 35°C; stimulation frequency, 0.5 Hz. Summary data from all experiments.

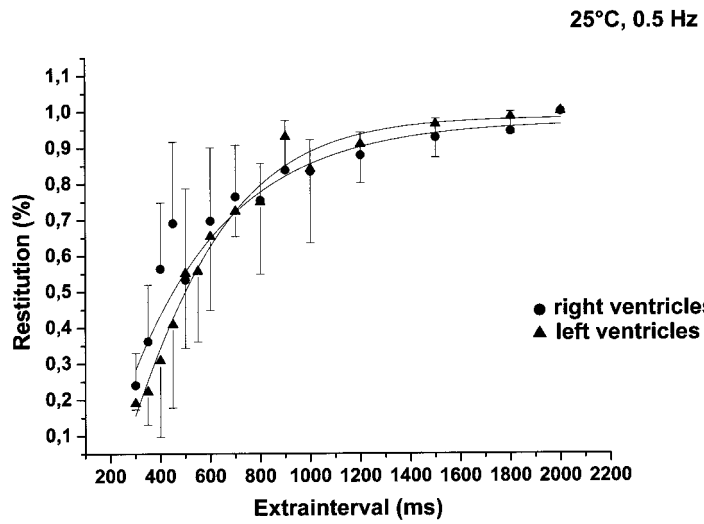

Fig. 5
Curve showing the mechanical restitution of right as compared to left ventricular papillary muscles. Extrainterval, period between a regular and a premature stimulation pulse. Temperature, 25°C; stimulation frequency, 0.5 Hz. Summary data from all experiments.

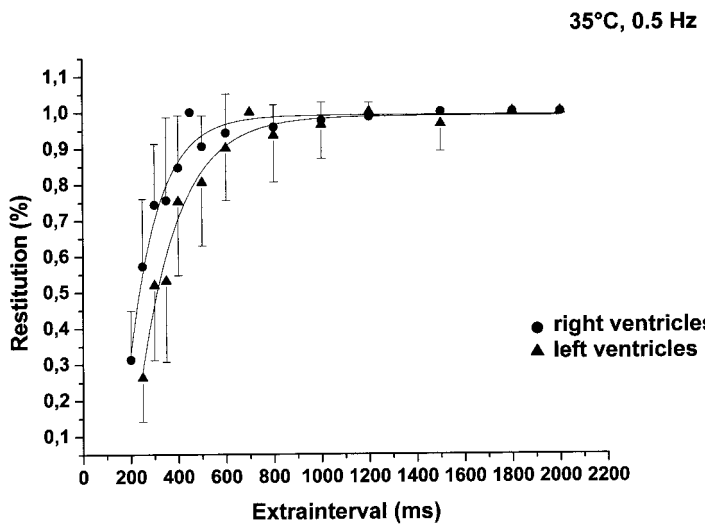

Fig. 6
Curve showing the mechanical restitution of right as compared to left ventricular papillary muscles. Extrainterval, period between a regular and a premature stimulation pulse. Temperature, 35°C; stimulation frequency, 0.5 Hz. Summary data from all experiments.

rat perfused hearts. They concluded that the interval-force relationship of a rat heart (exemplified, for instance, by time constants of restitution curves) provided a useful tool for quantifying and comparing right and left ventricular function. However, the authors admitted that this animal model showed distinct differences from other species. For this reason, our findings were not regarded as contradictory to their results, because the regulation of contractility processes in the guinea pig cardiac muscle, our experimental model, is known to be different from that in the rat heart.

In conclusion, there was no significant difference in the characteristic parameters of mechanical restitution, i.e., its onset and time course, between the papillary muscles of right and left ventricles in the guinea pig. These papillary muscles may, therefore, be considered equally suitable for assessing contractile behaviour of the guinea pig heart.

A c k n o w l e d g e m e n t s

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MECHANICKÁ RESTITUCE U PREPARÁTŮ IZOLOVANÝCH Z PRAVÉ A LEVÉ KOMORY SRDCE MORČETE

S o u h r n

Cílem této studie bylo zjistit případné rozdíly v začátku a časovém průběhu mechanické restituce u multicelulárních preparátů izolovaných ze srdcí laboratorních morčat – papilárních svalů z pravé a levé komory srdeční. V horizontální perfuzní lázničce byly současně registrovány stimulované stahy obou preparátů. Průběh mechanické restituce byl zkoumán při teplotách 25°C a 35°C a při stimulačních frekvencích 0.5 Hz, 1 Hz a 2 Hz. Nenalezli jsme signifikantní rozdíl v mechanické restituci obou preparátů. Pro hodnocení mechanických vlastností srdce se zdá být papilární sval z levé srdeční komory laboratorního morčete vhodnou náhražkou běžně užívaného papilárního svalu z komory pravé.

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