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Mechanical parameters determined in dispersed ventricular heart cells

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Summary. A high-resolution laser diffraction system suitable for studying the basic mechanical properties of small contractile single cells has been developed. This method was used to establish the mechanical behavior of 95 ventricular cells isolated from adult guinea pig hearts. During contraction, the sarcomere length shortened from 1828 ± 43 nm (mean ± SD) to 1518 ± 99 nm. The maximal velocities were 1.98 ± 0.64 μm/s for shortening and 1.93 ± 0.54 μm/s for re-lengthening. The twitch duration from 20% shortening to 80% re-lengthening was 622 ± 120 ms.
Key words. Myocardial contraction; myocytes; sarcomere length; laser diffraction.

Introduction

Single cells isolated enzymatically from adult hearts represent a useful model for electrophysiological studies. They offer several advantages over multicellular preparations[5]. They have been used successfully to investigate the electrical behavior of the cell membrane as well as the function of individual ionic channels[4]. However, to date little is known about the mechanical properties of unloaded isolated myocytes[6, 9, 12]. This situation appears to be mainly due to technical limitations. Since no reliable and easy method for studying the mechanical properties was readily available, an opto-electronic system based on the principles of laser diffraction was developed. This system enabled the measurement of sarcomere length at rest as well as during contraction. The aim of this study was to assess the basic mechanical properties of enzymatically isolated mammalian myocytes under control conditions.

Methods

Cell isolation and experimental conditions

Cells were isolated from adult guinea pig ventricles by means of an enzymatic procedure as described by Kao[7] and modified for guinea pigs[11]. Briefly, adult guinea pigs were anesthetized with ether, killed by cervical dislocation, and the hearts excised quickly. The aorta was mounted on a plastic cannula and the heart perfused in a retrograde fashion with a cardioplegic solution containing 50 μmol/l Ca^{2+}. After this period, the perfusate was replaced with a solution containing collagenase (100 mg/100 ml) and hyaluronidase (100 mg/100 ml). Following isolation, the cells were allowed to recover for an hour suspended in standard bathing solution (see below). To perform an experiment, a small aliquot containing about 100 cells was transferred to the experimental chamber (volume: 1 ml). Once the cells had settled down, superfusion was started (flow rate: 3 ml/min). The chamber consisted of a circular Perspex frame with a glued-on glass bottom, fixed to a revolving device, and mounted on the stage of an inverted phase-contrast microscope (Diavert, Leitz, Wetzlar, FRG). An air isolated table-top system (Technical Manufacturing Corp., Peabody, MA, USA) prevented interferences from ground vibrations.

The composition of the bathing solution was as follows (in mmol/l): Na^+, 137; K^+, 5.4; Ca^{2+}, 1.8; Mg^{2+}, 0.5; Cl^-, 147; Glucose, 5.5; HEPES, 5; pH 7.4 (adjusted with NaOH). Temperature: 22–23 °C.

Laser diffraction measurements

To measure the sarcomere length of isolated myocytes at rest and during contraction the principle of laser diffraction was used. In order to do so, an opto-electronic system was developed which was suitable for small cells. Technical details and performance of the system have been described elsewhere[13] and will only be summarized briefly here. The beam diameter of a polarized 5 mW He-Ne laser (Melles-Griot, Irvine, CA, USA) was reduced to 50 μm using the modified fluorescence adaptor of the inverted microscope. After intensity adjustment by means of a polarizing filter, the beam passed through the cell under investigation, which acted as a diffraction grating. The resulting diffraction pattern was projected on to a high resolution linear image sensor (CCD-Camera. Texas Instruments, Dallas, TX, USA). A custom built electronic circuit determined the position of the first order diffraction maximum every 2 ms and delivered an analog voltage signal proportional to the sarcomere length. This signal was digitized by means of a transient recorder (W.+W., Basel, Switzerland) and stored by microcomputer for later analysis. Calibration of the opto-electronic device was performed by means of two standardized diffraction gratings

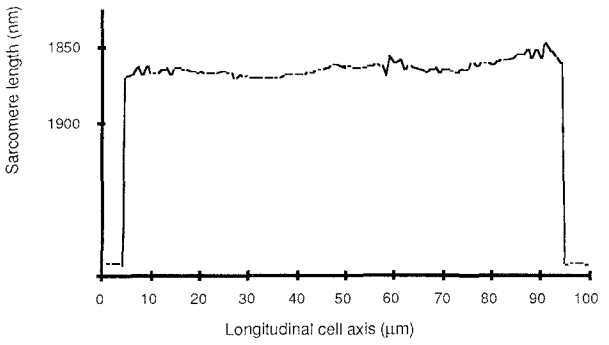

Figure 1. Scan along the longitudinal axis of a cell at rest showing the homogeneity of the mean sarcomere spacing. The beam diameter was 50 µm and the cell length approximately 100 µm. At both ends of the cell, the light intensity of the first diffraction maximum broke down below the threshold of detection, simulating a shorter cell length.

(1754 nm and 1666 nm. Leybold Heraeus, Huerth, FRG) before and after each experiment.

Experimental protocol

The cells used for an experiment were selected on the basis of morphological criteria. Quiescent, rod-shaped cells with clearly visible striation patterns and without blebs were chosen. They were focussed in the field of vision and rotated and aligned appropriately to the direction of the line sensor and the position of the laser beam. During an experiment, the proper alignment of the cell was controlled by visual inspection through the ocular of the microscope. Cells were stimulated with platinum electrodes using stimuli of twice threshold amplitude and 0.5-ms duration delivered by a Stimulator (Grass SD-9, Quincy, MA, USA). A digital timer (Devices 3290, Digitimer Ltd. Welwyn Garden, GB) triggered the stimulator and the digital recording device at a frequency of 0.5 Hz. After onset of stimulation, a cell was allowed to equilibrate for 5 min before collection of data began.

Results

Sarcomere length at rest and during contraction

To determine the sarcomere length of myocytes it was also important to investigate the uniformity of the sarcomeres within an isolated cell. To do this, a resting myocyte was scanned along its longitudinal axis by continuously shifting the microscope stage. During these measurements, the laser beam always spread over a muscle area equivalent to approximately 25 sarcomeres. Figure 1 illustrates the result of such a longitudinal scan experiment. Quantitative analysis yielded a mean sarcomere length of 1865 nm and an inhomogeneity of ± 3 nm (SD). This shows that the sarcomere length was rather uniform over the entire cell length. Therefore, it seems justifiable to ascribe a unique sarcomere length to each cell. After establishing this point, the sarcomere behavior of stimulated myocytes was investigated. Figure 2 illustrates the result of an experiment in which the diffraction pattern was determined during a single twitch. The lower part of the figure shows a plot of the measured sarcomere length versus time, and the upper part depicts its first derivative, i.e. the rate of length change per sarcomere versus time. The incidence of electrical stimulation (0.5 Hz) is indicated by the dotted vertical line. Within 32 ms after stimulation, the sarcomeres started to shorten. The peak shortening was reached at 292 ms after stimulation, and re-lengthening was nearly complete (80% relaxation) at 505 ms after stimulation. During the twitch the sarcomere length changed from 1831 nm to 1580 nm at the peak, i.e. by 14%. The maximal rate of shortening of 1.7 µm/s was observed at 102 ms and at a sarcomere length of 1742 nm, while the maximal rate of re-lengthening of 2.1 µm/s took place at 446 ms and at a sarcomere length of 1671 nm.

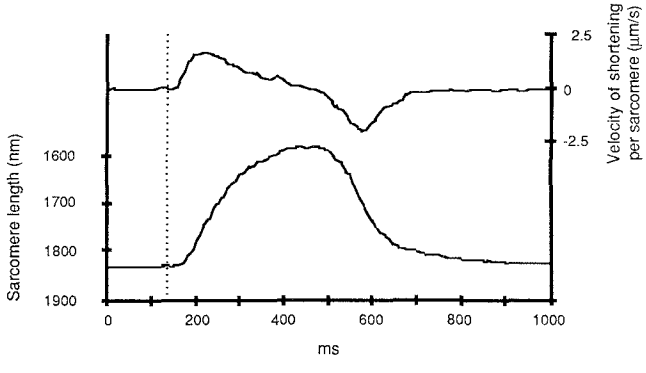

Figure 2. Sarcomere length (left scale) and rate of shortening and re-lengthening (right scale) during a twitch of a single cardiac myocyte (····· = stimulus at 125 ms).

The table summarizes the data collected from 95 myocytes (7 hearts) at rest and during a twitch. The first column shows the overall mean values obtained. During a contraction, sarcomere length decreased from 1828 ± 43 nm (SL diast.; mean ± SD) to 1518 ± 99 nm (SL syst.), i.e. by 17%. The maximal rates of sarcomere shortening (ΔSL syst.) and re-lengthening (ΔSL diast.) turned out to be comparable, i.e. 1.98 ± 0.64 µm/s and 1.93 ± 0.54 µm/s respectively. On the average, the twitch lasted 622 ms (time interval between 20% shortening and 80% re-lengthening).

In order to get an idea about the mechanical stability of the cells, 14 consecutive twitches were analyzed with regard to the parameters discussed above. The coefficients of variation (standard deviation/mean value) calculated for the various mechanical parameters are listed in the second column of the table. The coefficients of variation in the third column were obtained by comparing the mechanical properties of cells from the same population. Therefore, the figures listed provide an estimate of the homogeneity of isolated cells from the same heart. The figures in the fourth column were gained by comparing sarcomere parameters of cells from different cell populations. Thus, they allow an estimate of the homogeneity of cell populations from different hearts.

Staircase phenomenon

The measurements presented so far have been determined under steady-state conditions, either at rest or stimulated at a frequency of 0.5 Hz. In the absence of electrical stimuli, all myocytes investigated were quiescent mechanically. Utilizing such cells, the mechanical behavior upon onset of stimulation has also been examined. The result of a representative experiment is illustrated in figure 3. It shows the continuous recording of consecutive diffraction signals, expressed in absolute sarcomere length and plotted versus time. Stimulation at 1 Hz was started at t = 1.4 s. The figure demonstrates that the peak sarcomere length altered in a beat-to-beat fashion, thus giving rise to a positive staircase. A steady-state was reached after 6 beats. By then the change in sarcomere length during a twitch had increased from 135 nm to 192 nm, i.e. by 42%. The staircase phenomenon is associated with an increase in rate of sarcomere shortening as indicated by the progressive steepening of the optical signal. There was no sign of incomplete sarcomere re-lengthening during diastole, which would be indicative of a sustained tension increment. This suggests that the cell was in perfect physiological condition.

Summary of the sarcomere length measurements. SL = sarcomere length. ΔSL = maximal velocity of sarcomere shortening (syst.) and re-lengthening (diast). Twitch duration (t) was measured from 20% contraction to 80% relaxation.

	Mean	Coefficient of variation		
		Multiple twitches in one cell	Multiple cells in a population	Multiple populations
SL diast.	1828 nm	0.09%	1.5%	1.8%
SL syst.	1518 nm	0.14%	5.3%	5.7%
Δ SL syst.	1.98 µm/s	9%	30%	21%
Δ SL diast.	1.93 µm/s	2.7%	27%	10%
t(20–80)	622 ms	2.2%	18%	14%
n =	95 cells	14 twitches	7 popul.	7 popul.

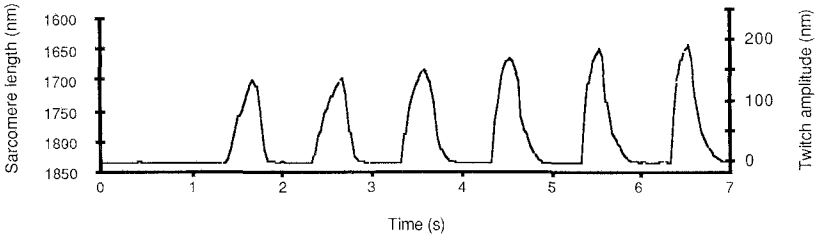

Figure 3. Positive staircase after onset of stimulation. A train of pulses was applied at 1 Hz starting at t = 1.4 s. The plot shows the sarcomere spacing in absolute values (left hand scale, in reverse order) and twitch amplitude (right hand scale) versus time. Twitch amplitude is represented as sarcomere shortening in nm.

Effect of $[Ca^{2+}]_o$ on the sarcomere length

In another set of experiments, the relationship between the $[Ca^{2+}]_o$ and the mechanical behavior was studied. To carry out these measurements, $[Ca^{2+}]$ in the bathing fluid was altered every 7 min changing from 3.6 mmol/l to 1.8 mmol/l (control), 0.9 mmol/l, 0.45 mmol/l, and 3.6 mmol/l. Bracketing was used to correct for the occasional mechanical rundown of a cell during the course of an experiment (duration up to 35 min). Before each solution change, the diffraction signals at rest and during the twitch were measured. Figure 4 depicts the analyzed data of such an experiment. It shows a plot of the twitch amplitude as a function of $[Ca^{2+}]_o$. The twitch amplitude represents the sarcomere shortening during a twitch normalized to the shortening in the standard solution ($[Ca^{2+}]_o$ = 1.8 mmol/l). As indicated by the figure, there was a positive correlation between the peak sarcomere length changes and the $[Ca^{2+}]_o$. Over the range of $[Ca^{2+}]_o$ tested, there was no significant change in sarcomere length detectable during diastole.

Discussion

The laser diffraction method introduced enables the determination of the sarcomere length of small contractile cells with great precision. However, structural irregularities, such as local differences in sarcomere spacing or oblique lattice planes, may affect the fine structure of the diffraction pattern and thus lead to imperfect measurements[1]. Appropriate measurements demonstrated that such problems were small when investigating healthy myocytes. Screening experiments revealed a high degree of homogeneity for the arrangement of sarcomeres along the cellular axis (SD ± 3 nm). The measurements carried out on myocytes isolated from adult guinea pig ventricles yielded a value of 1828 ± 43 nm (mean ± SD) for the sarcomere length at rest. This value is in good agreement with previous reports on dispersed heart cells from adult guinea pig ventricle, where a direct measurement by means of a filar micrometer yielded a mean resting sarcomere length of 1870 ± 30 nm in a sample where cells with sarcomere lengths shorter than 1800 nm were discarded[10]. In contrast, laser diffraction measurements performed on intact cardiac muscle have revealed an unloaded sarcomere length of 2010 ± 80 nm[8]. In intact muscle the connective tissue exerts a considerable preload on the individual cardiac cells even under resting conditions; a phenomenon which is completely absent in isolated myocytes. In other words, the resting sarcomere length in unloaded single myocytes and preloaded cardiac tissue cannot be compared. With regard to the sarcomere length prevailing during the twitch, the situation is different. In isolated myocytes the laser diffraction technique revealed a minimal sarcomere length of 1518 ± 99 nm during peak shortening. This value is in good agreement with the 1.6 µm reported in the literature for intact heart muscle[3, 14]. This conformity indicates that in the unloaded condition, shortening is limited mainly by the passive viscoelastic forces developed by the contractile cells themselves.

The mechanical stability of the isolated myocytes may be assessed by the twitch amplitude. In a given cell it remained relatively constant over time. It varied only a little among cells from the same or even different populations. In contrast, the time course of the contractile signal, i.e. its duration, and the velocity of sarcomere shortening and re-lengthening showed considerable variations not only among different cells but also in a particular cell. By and large, laser diffraction yielded velocities of sarcomere shortening and re-lengthening which deviate from those found both in isolated myocytes (3.4 µm/s)[6] and intact cardiac tissue (4 µm/s at a sarcomere length of 1.8 µm)[14]. However, if one takes into account the differences in experimental conditions, such as species, temperature and $[Ca^{2+}]_o$, the agreement is reasonably good.

In the present study, an attempt was made to perform all measurements only on Ca^{2+}-tolerant myocytes. This was achieved by selecting the cells by means of morphological criteria. Investigating such cells, a positive correlation could be established between $[Ca^{2+}]_o$ and amplitude of the contractile signal or the velocity of sarcomere shortening and re-lengthening. Upon onset of electrical stimulation, the cells underwent a physiological staircase phenomenon similar to the one observed in intact tissue of most species[2]. The cells showed no signs of Ca^{2+}-overload, such as progressive sarco-

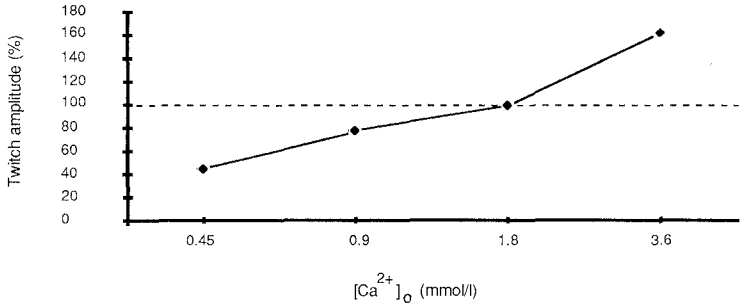

Figure 4. Twitch amplitude (represented as sarcomere shortening) of a single cardiac myocyte exposed to different concentrations of extracellular calcium. The values have been normalized with respect to that at the standard calcium concentration (1.8 mmol/l, dotted line). Stimulation frequency was 0.4 Hz.

mere shortening with time, a large amplitude of the first twitch after a rest period, or aftercontractions. In cases of severe Ca^{2+} overload the diastolic sarcomere length was found to be considerably shorter, though. Subsequent measurements showed a gradual development of irreversible contractures.

In conclusion, the contractile parameters investigated demonstrate that isolated adult myocytes exert a mechanical behavior similar to that of intact cardiac muscle. They represent a useful preparation for studying mechanical properties alone or in conjunction with electrical measurements.

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Currents through ionic channels in multicellular cardiac tissue and single heart cells

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Summary. Ionic channels are elementary excitable elements in the cell membranes of heart and other tissues. They produce and transduce electrical signals. After decades of trouble with quantitative interpretation of voltage-clamp data from multicellular heart tissue, due to its morphological complexness and methodological limitations, cardiac electrophysiologists have developed new techniques for better control of membrane potential and of the ionic and metabolic environment on both sides of the plasma membrane, by the use of single heart cells. Direct recordings of the behavior of single ionic channels have become possible by using the patch-clamp technique, which was developed simultaneously. Biochemists have made excellent progress in purifying and characterizing ionic channel proteins, and there has been initial success in reconstituting some partially purified channels into lipid bilayers, where their function can be studied.
Key words. Cardiac tissue; single heart cell; voltage clamp; patch clamp; ionic channel current.