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PROPERTIES OF VENTRICULAR MYOCYTES ISOLATED FROM THE HYPERTROPHIED AND FAILING HEARTS OF SPONTANEOUSLY HYPERTENSIVE RATS

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Objective: To investigate how the morphological and physiological properties of single myocytes isolated from the hypertrophied, failing left ventricles (LV) differ from those of normal or hypertrophied not failing ventricles. **Method**: Single myocytes were isolated separately from right (RV) and left ventricles (LV) of male spontaneously hypertensive rats (SHR) or Wistar-Kyoto (WKY) rats at the age of 6 and 12 months and of SHRs which developed or not developed heart failure at the age of 20—24 months. We measured cells dimensions, range and kinetics of electrically stimulated or initiated by caffeine contractions and Ca^{2+} transients, and investigated the response of cells to thapsigargin. **Results**: The transversal dimensions of the LV myocytes of 6 months old SHRs showed ~ 20% increase with respect to transversal dimensions of their RV myocytes and LV and RV myocytes of WKY rats. The difference did not change with progressing age and in the heart failure. The LV myocytes of 6 or 12 months old SHRs showed slowed kinetics of the Ca^{2+} transients and of contraction and relaxation and decreased contractile response to 2 s superfusion with 15 mM caffeine preceded by 5 mM Ni^{2+} used as an index of the sarcoplasmic reticulum (SR) Ca^{2+} content. Despite of this the range of shortening and relative contribution of the SR to contraction (assessed by measuring of the residual contractile response to electrical stimulation in cells poisoned with thapsigargin) or relaxation (assessed by calculation of the ratio of rate constants of the electrically stimulated and stimulated by 30 s superfusion with caffeine Ca^{2+} transients) was not altered in the hypertrophied myocytes. Properties of the LV myocytes of the 20—24 old SHRs with or without heart failure did not differ from those of LV myocytes of younger SHRs. The contractile response to caffeine of their RV myocytes dropped to the level of that in the LV myocytes. **Conclusion.** Our results suggest that transition from the compensated hypertrophy to the heart failue in 20—24 months old SHRs did not result from the further changes in properties of the surviving myocytes. Data from literature suggest that myocyte apoptosis and remodeling of the extra-myocyte space is the more likely reason.

Key words: *cardiomyocytes, hypertrophy, heart failure, SHR.*

INTRODUCTION

Cardiac hypertrophy leading to heart failure is one of the most common causes of debility and death. It is initiated by increase and/or redistribution of the forces developed and faced by the cardiac myocytes caused by various diseases of cardiovascular system like arterial hypertension, myocardial infarction, valvular heart disease or disseminated diseases of the heart muscle. The biological processes resulting in hypertrophy and failure are extensively studied in animal models and in the human hearts and proved to be similar disregarding of the disease. They consist of inhibition or stimulation of expression of genes encoding various proteins of the two main cellular populations of the heart muscle: myocytes and fibroblasts. Alterations concern the proteins involved in Ca^{2+} cycling and contractile system of cardiomyocytes and in intercellular conduction as well as the proteins of extracellular matrix. Decrease in expression and protein content of the Ca^{2+}-ATPase of sarcoplasmic reticulum (SERCA 2) have been reported in the hypertrophied hearts (1—5) or upon transition from compensated hypertrophy to heart failue (6) in animal models and in the failing human hearts (7). Decrease in expression of SERCA 2 may be accompanied by depression of expression of phospholamban (2—6) calsequestrin (4) and decrease in the density of ryanodine receptors (1, 4, 5, 8, 9). Increased activity of Na^{2+}/Ca^{2+} exchangers have been reported in human end-stage heart failure providing a mechanism compensating impaired relaxing function of the failing SR. However, resulting increased influx of Na^{2+} may be associated with membrane depolarization and enhanced arrhythmogenesis (10).

In the hypertrophied hearts of those animals which express α-myosin heavy chain the *ratio* of the β/α chains is increased (2, 11—13). Together with the impaired expression of the SR proteins this may account for slowing of contraction in the hypertrophied and failing hearts.

One of the animal models used in order to study the biology of the hypertrophied and failing heart is the ageing, spontaneously hypertensive rat (14, 15). These animals develop left ventricular hypertrophy with transition to the heart failure at the age of 18—24 months. Although the myocardial function during compensated hypertrophy and heart failure has been carefully studied in this animal model in the *in situ* and isolated hearts (14) as well as in isolated papillary muscles (14, 16) the function of single myocytes isolated from the SHR rats has never been, to our knowledge, investigated. In this paper we report the results of investigation of the dimensions, contractile function and Ca^{2+} handling in the myocytes isolated separatedy from the left and right ventricles of ageing male SHR rats during development of cardiac hypertrophy at the age of 6 and 12 months and cardiac failure at the age of 20—24 months.

MATERIALS AND METHODS

Experimental animals. 30 male SHR rats and 30 male Wistar- Kyoto rats at the age of 3 months were obtained from the Animals Facilities of the Mother and Child Health Center in Lodz, Poland, and maintained at the animal facility of the Medical Center of Postgraduate

Education. Technically successful experiments were performed in 6 SHR and 6 WKY rats at the age of 6 months, 6 SHR rats and 6 WKY rats at the age of 12 months, in 4 SHR rats which did not develop heart failure, and in 7 SHR rats which developed heart failure between 20 and 24 month. Unfortunately, some of the WKY rats died at the age of 16 months and we were not able to obtain good myocytes from the remaining ones. More WKY rats of the appropriate age were not available at the time span of this study. This is why we could compare results obtained in SHR rats at the age of 6 and 12 months with those obtained in the WKY controls, but we could compare properties of myocytes isolated from the failing hearts of SHR rats only with those of not failing hearts of SHR rats at the age of 12 and ~ 20 months. Most of he SHR rats developed at the age of 20—24 months cardiac failure manifest mainly by elaborate breathig and the lung congestion and oedema, which was the ultimate cause of their death. It developed rapidly within few hours without preceding warning symptoms, so that some animals were lost without experimental examination. The ratio of the lung weight in mg to the animal weight in grams was 14.9 ± 2.1 (range 10.0 – 22.3) in the rats which developed the heart failure whereas it was 5.8 ± 0.8 (range 4.7 – 8.25) in the rats without symptoms of heart failure. The ratio of weight of liver to the animal's weight did not differ between these group of rats. Pleural and peritoneal exudations, atrial thrombi and right ventricular hypertrophy reported by other authors in this animal model of cardiac failure (17) were rarely seen.

2. Experimental procedure. The rats were weighed and anesthetized with the i.p. injection of chloralhydrate. Short piece of the stiff catheter was introduced into the right carotid artery and the blood pressure recorded with an electromanometer connected to the Siemens-Elema Mingograph 7 polygraph. Thereafter the chest was opened, the heart rapidly excised, washed in the cold Tyrode solution, blotted on the filter paper and weighed and myocytes isolated as described below. Lungs and liver were also excised, washed in cold Tyrode solution, blotted on filter paper and weighed. The ratio of the weight of the organs in mg to the weight of animal in grams was calculated.

3. Cells isolation. The aortic root was cannulated and the heart was perfused with the nominally Ca^{2+} free Tyrode solution at 37°C for 5 min. Thereafter perfusate was switched to the Tyrode solution containing collagenase (0.6 mg/ml, Boehringer, type I) and protease (0.06 mg/ml, Sigma) for 20—30 min. Isolation of cells from the hearts of the older animals (12 months and more) was difficult and required addition of 0.1 mg/ml of trypsin over the initial 5 min of perfusion with the enzymes containing solution. Thereafter the artia were cut away and the free wall of the right ventricle separated from the rest of the heart. Left and right ventricles were minced with scissors in the separate vessels containing Tyrode — enzymes solution, cells filtered through the nylon mesh and alloved to sediment. The supernatant was discarded and cells washed twice with the Tyrode solution containing Ca^{2+} at concentration rised gradually to 1.0 mM. This procedure yielded 70—85% of rod-shaped, viable cells in younger animals and ~ 60% in two older groups. However, in older groups attempts to isolate cells were often unsuccessful in that we did not obtain reasonable % of viable cells (at least ~ 40%) and/or they showed instability of the membranes manifest in frequent spontaneous contractions and poor adhesion to the glass bottoms of superfusion chambers. These cells were not used for experiment.

4. Cells superfusion, recording of contractions and intracellular Ca^{2+} concentration. The large drops of cells suspension in Tyrode solution were placed on the cover slip glued to the margins of a round hole cut in the bottom of plastic Petri dish mounted on the stage of an TV edge tracking system devised and built by J. Palmer, Cardiovascular Laboratories, School of Medicine, UCLA (for recording of contractions) or of an inverted microscope (Nicon, Diaphot) equipped for recording of cells fluorescence (for recording of cellular Ca^{2+} concentration). The inlet and outflow tubes of the rapid superfusion system modified from Rich *et al* (18) were immersed in the drops. After the cells had sedimented and stuck to the glass, the flow of the superfusing Tyrode solution was switched on. Thus the cells between the tubes were rapidly superfused with the stream of solution. This system enabled complete exchange of the solutions between the inflow and outflow tubes within ~ 300 msec. This was tested by recording of a change in resistance between two small

electrodes immersed in the flowing stream upon switching from distilled water to 100 mM KCl solution. Solutions from 4 separate containers were directed to the inflow tube by miniature magnetic valves. Two small platinum electrodes flanking the cells enabled their stimulation with the current pulses delivered by a programmed stimulator.

For measurement of fluorescence 15 µl of a solution containing 50 µl of 1.0 mM indo 1—AM dissolved in dry dimethylsulfide (DMSO), 2.5 µl of 25% (wt/wt) pluronic and 75 µl bovine calf serum was added to 500 µl of cells suspension. Cells were incubated for 20 min at room temperature, washed in Tyrode solution and stored for 30 min before use. A Nicon Mercury lamp was used as a source of epifluorescence. The exciting light was direced to the 100 × ultraviolet Fluoroglicerin-immersion objective (Nicon). A concentric diaphragm enabled illumination of a small fragment of a cell. Fluorescent light was collected from a microscopic field containing only one myocyte. The light was split by dichroic mirror into 405-nm and 395 nm wavelength beams and passed to two photomultipliers mounted in the holder attached to the side port of the microscope. The ratio of 405 to 495 fluorescence was obtained from the output of a Dual Channel Ratio Fluorometer (Biomedical Instrumentation Group, University of Pennsylvania, USA). No attempt to calibrate the signals in terms Ca^{2+} concentration was made. Cells were simultaneously illuminated with red (650—750) light through the bright-field optics of the microscope. A TV camera mounted in the place of one of the eye-pieces of the microscope enabled to see the cell image on the screen of the TV monitor.

5. Solutions. For cells isolation and throughout the experiments we used a Tyrode solution of the following composition (in mM): 144 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 0.43 NaH_2PO_4, 10.0 N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid (HEPES), 11.0 glucose, and 5.0 sodium pyruvate. The pH of the solution was adjusted with NaOH to 7.3 for cells isolation or to 7.4 for experiments. In the experiments $CaCl_2$ was added to concentration of 1.0 mM. All experiments were performed at stabilised room temperature (24°C).

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85—23, revised 1996).

6. Statistical evaluation. Dimensions of 30 randomly met myocytes of each ventricle were measured in each rat. For assessment of physiological properties of myocytes at least 6 cells of left and 6 cells of right ventricle were examined in each rat. The means of the data obtained in cells of a rat were used as the group means for further analysis. The data are shown as the means of group means ± SE. Student's t-test was used to compare the means of normaly distribution of continuous variable, and results were accepted as significant for $P < 0.05$. The X^2-test for normality was applied to check whether the cell dimensions or their functional parameters were normally distributed.

7. Experimental protocols will be described for clarity in the Results section.

RESULTS

Morphological data

1. Cardiac hypertrophy index.

The ratio of the heart weight in mg/animal weight in grams was almost stable in the WKY rats till age of ~ 20 months. In the SHRs at 6 months the hypertrophy index was slightly, but not significantly higher than in WKYs. The index progressively increased in SHRs till the stage of heart failure and was significantly different from that of WKYs at the age of 12 and ~ 20 months

(WKY: 4.3±0.2, 3.5±0.15, 3.5±0.3, SHR: 3.9±0.4, 4.1±0.3, 4.9±0.2). The index in SHRs of 20—24 months was slightly, but not significantly lower than in the SHRs with the heart failure (4.7 ± 0.3).

2. Cells dimensions.

The length of myocytes did not differ neither between the left and right ventricles of SHRs nor between SHRs and WKY rats over the time span of this study. The width of the left and right ventricular myocytes of WKY rats and of the right ventricular myocytes of the SHRs did not differ significantly and was stable over the time span of this study. The width of the left ventricular myocytes of the SHR rats at the age of 6 months was by ~ 20% larger than that of other myocytes and id not increase during the further ageing and development of the heart failure (*Fig. 1*). The difference between the width of left ventricular myocytes of all SHRs and of right and left ventricular myocytes of WKYs and right ventricular myocytes of SHRs is significant statistically.

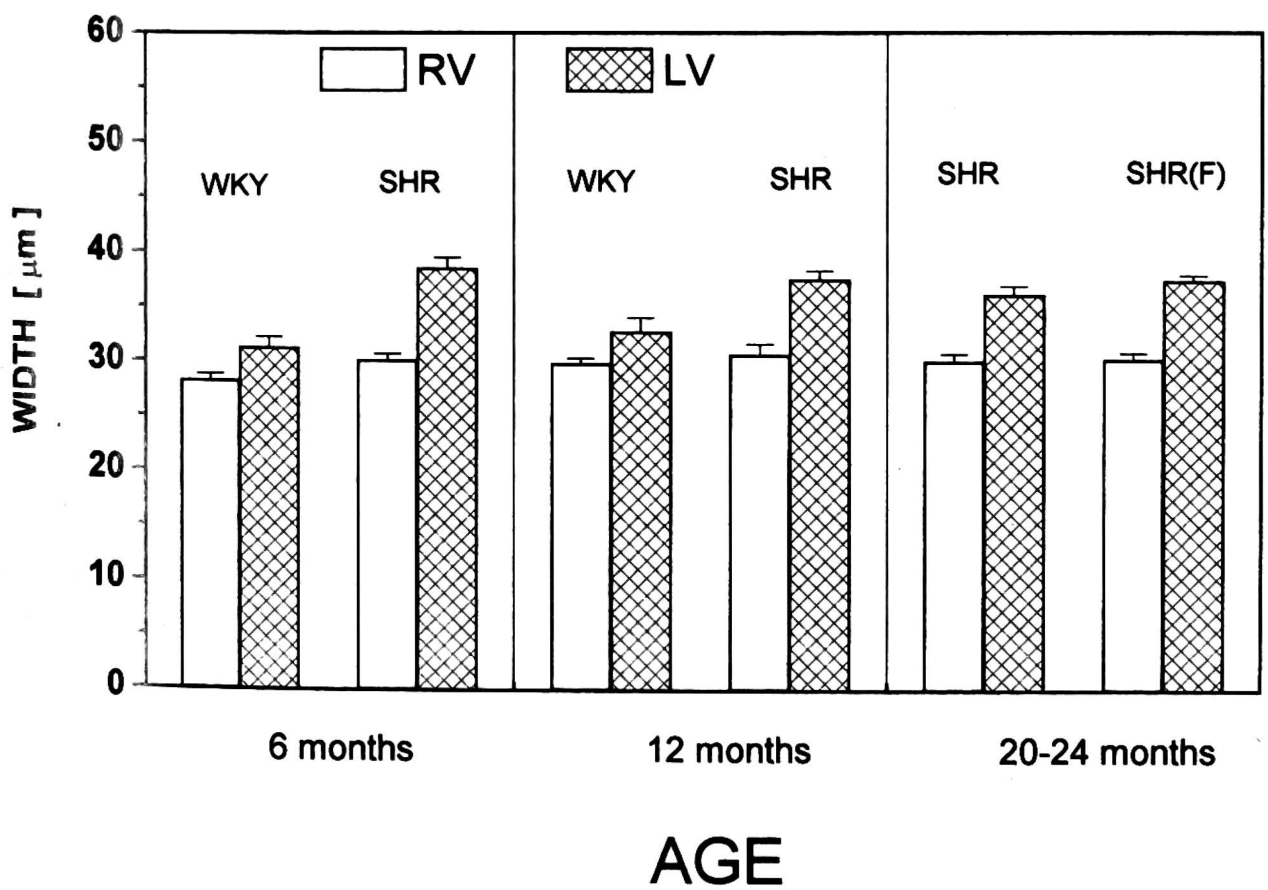

Fig. 1. Transversal dimensions of the right (RV) and left ventricular (LV) myocytes isolated from the hearts of Wistar-Kyoto (WKY) and spontaneously hypertensive rats without (SHR) and with the symptoms of heart failure (SHR(F). 30 cells of each ventricle of each rat were measured and the mean dimensions calculated. The bars represent the means of the group means ± SE. The differences between the RV and LV myocytes of the SHRs and between LV myocytes of SHRs and WKYs are highly significant statistically.

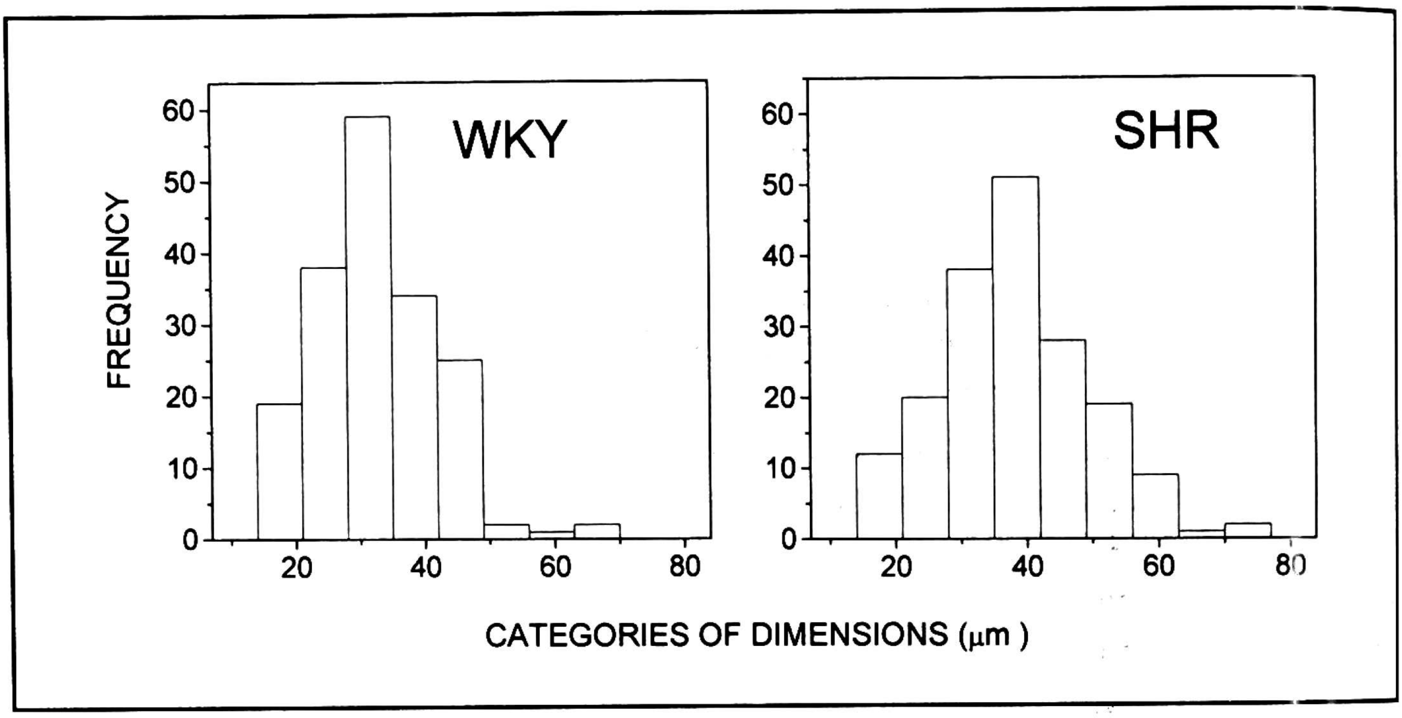

Fig. 2. Number of left ventricular myocytes isolated from the hearts of 6 spontaneously hypertensive (SHR) and 6 Wistar-Kyoto rats at the age of 6 months within the categories of transversal dimensions. Dimensions of 30 cells of each ventricle of each rat were measured (total 360 cells). Lower limit of dimensions did not change in SHRs as compared with WKYs.

3. *Fig. 2* shows the distribution of frequency within the categories of transversal dimensions of LV myocytes of the SHR and WKY rats at the age of 12 months.

It is clear that there is a decrease in the frequency in the categories of small dimensions and an increase in categories of larger dimensions with the increase of their upper limit. However, the lower limit did not change which suggests that hypertrophy was not uniform i.e. that not all myocytes increased their dimensions. This result conforms with that reported by Emanuel *el al.* (19) in the transgenic TGR (mREN2) 27 rats and suggests that we never know whether we deal with the hypertrophied cell or a cell, the dimensions of which did not change. This may decrease the differences between the mean parameters measured in cells isolated from the hypetrophied and not hypertrophied myocardium.

Range and kinetics of electrically stimulated contractions

At the steady state stimulation at the rat of 30/min cells shortened by 11.0%—13.5% of their resting length. The difference between the investigated groups of myocytes was not significant statistically. It is of particular interest that there was no difference between the range of shortening of the left ventricular myocytes isolated from the failing and not failing hearts (*Table 1*).

Measuring the time to peak shortening and time from peak shortening to 90% relaxation (*Table 1*) assesed kinetics of contraction. Time to peak shortening of the left ventricular myocytes of the 6 months old SHRs and time to peak shortening and relaxation time of these myocytes in 12 months old

Table 1. Range (% of resting length) and kinetics of shortening during elecrically stinulated contractions of single right ventricular (RV) and left ventricular (LV) myocytes isolated from the hearts of WKY and SHR rats. At least 6 cells of each ventricle were investigated in one animal and group means calculated. The data are the means of the group means ± SE.

Age (months)	No. of rats	WKY						SHR					
		RV			LV			RV			LV		
		range	TTPS	RT	range	TTPS	RT	range	TTPS	RT	range	TTPS	RT
6	6	11.8 ± 0.6	346 ± 24	411 ± 40	12.4 ± 0.6	347 ± 24	465 ± 29	12.4 ± 0.5	313 ± 90	352 ± 17	11.7 ± 0.4	402* ± 8	514 ± 44
12	4	11.0 ± 1.0	347 ± 28	391 ± 24	11.1 ± 0.3	356 ± 21	422 ± 30	11.6 ± 0.5	375** ± 21	460** ± 32	12.8 ± 1.0	418 ± 17	566* ± 15
20–24 (NF)	4							11.8 ± 0.9	333** ± 18	386** ± 19	13.5 ± 1.1	409 ± 31	541 ± 28
20–24 (F)	8 8							12.0 ± 0.4	376** ± 19	410** ± 25	11.8 ± 0.2	428 ± 17	513 ± 28

* significantly different from the respective WKY control
** significantly different from the left ventricular myocytes
TTPS time to peak shortening (ms)
RT time to 90% relaxation (ms)
NF myocytes isolated from not failing hearts
F myocytes isolated from the failing hearts

SHRs were significantly longer than respective times in right ventricular myocytes of SHRs and right and left ventricular myocytes of WKY controls. There was no further prolongation of these times in the left ventricular myocytes of SHRs at 20—24 months with the not failing or failing hearts. The difference in kinetics of Ca^{2+} transients in hypertrophied left ventricular myocytes of the SHR rats at the age of 12 months and that of respective WKYs is consistent with the changes in kinetics of contractions. We noticed significant increase of the time from the onset of the transient to 80% of its total amplitude, and increase of duration of the transient at 60% and 10% of its total amplitude. Surprisingly, the kinetics of Ca^{2+} transients in the myocytes of failing hearts ware more rapid again and did not differ from that in younger WKYs (*Table 2*).

Table 2. Kinetics of electrically stimulated Ca^{2+} transients of the left ventricular myocytes isolated from SHR snd WKY rats.

Age (months)	No. of rats	WKY			SHR		
		time to 80% amplitude (ms)	duration at 60% amplitude	duration at 10% amplitude	time to 80% amplitude (ms)	duration at 60% amplitude	duration at 10% amplitude
6	6+6	34 ± 0.6	312 ± 9	512 ± 13	37 ± 3.2	348 ± 15	575 ± 14*
12	6+6	28 ± 2.7	251 ± 15	428 ± 41	38 ± 2.4*	382 ± 11*	651 ± 29*
20–24	6 (F)	—	—	—	42 ± 4.5	351 ± 15	492 ± 4

* significantly different from the WKY rats
(F) heart failure

Contribution of sarcoplasmic reticulum to activation of contraction and to relaxation

1. The SR Ca^{2+} *content.*

The SR Ca^{2+} content was assessed by measuring the contractile response of cells to superfusion of 15 mM caffeine. Caffeine was superfused for 2 sec., i.e. up to the summit of cell shortening. Since the range of cell shortening under the effect of caffeine depends on the amount and rate of release of Ca^{2+} from the SR and on the rate of Ca^{2+} transport out of the cell mostly by Na/Ca exchange, the latter was eliminated by superfusion of 5.0 mM Ni^{2+} 5 sec prior to and during caffeine superfusion. *Fig. 3* shows the responses of a cell to electrical

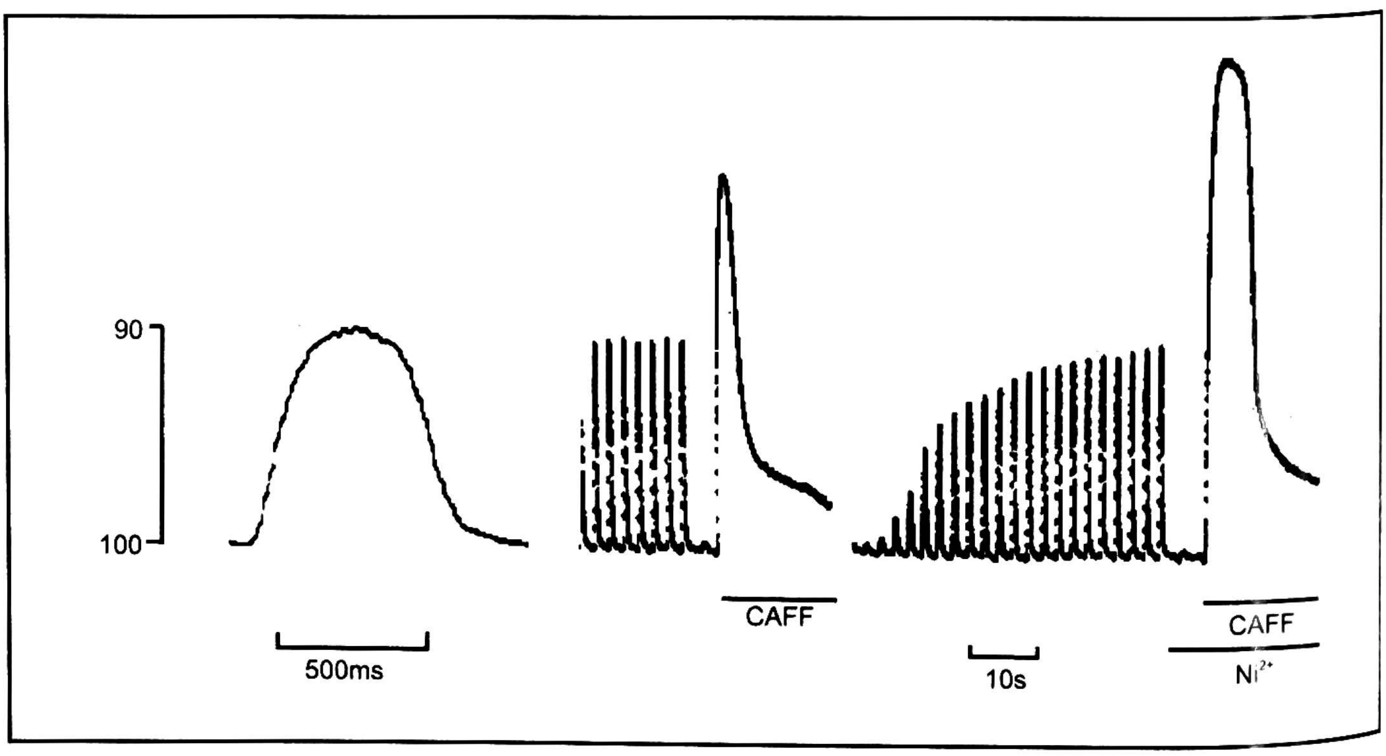

Fig. 3. The shortening of the single myocyte isolated from the left ventricle of the WKY rat at the age od 6 months initiated by electrical stimulation at the rate of 30/min, superfusion of 15 mM caffeine or 15 mM caffeine preceded by 5 mM Ni^{2+}. Left scale: cell shortening in % of resting length.

stimulation, caffeine superfusion, and caffeine superfusion during inhibition of Na/Ca exchange by 5.0 mM Ni^{2+}. Most cells relaxed upon reperfusion of pure Tyrode solution, however, some of them remained more or less contracted and few died. The mean shortening of right ventricular myocytes of 6 and 12 months old SHRs and of right and left ventricular myocytes of WKY rats of respective age due to caffeine + Ni^{2+} superfusion ranged from $36.1 \pm 3.7\%$ to $39.0 \pm 1.0\%$ of the cell resting length. Small differences between these groups of cells were not significant statistically. The range of shortening of the left ventricular myocytes of the SHRs at the age of 6 and 12 months was $29.2 \pm 0.8\%$ and $29.8 \pm 0.7\%$, respectively, and was fignificantly lower than that of the right ventricular myocytes of SHRs and right and left ventricular myocytes of WKY rats. The range of shortening of the left ventricular myocytes of the failing hearts dropped to $27.4 \pm 2.1\%$, however, the difference between them and left ventricular myocytes of not failing hearts and of SHR rats at the age of 12 months was not significant. The range of shortening stimulated by caffeine in the right ventricular myocytes of failing and not failing hearts of SHRs at the age of ~ 20 months also decreased and id not differ from that of the left ventricular myocytes. These differences illustrated in *Fig. 4.* (top panel) suggest that the Ca^{2+} content of the SR of the hypertrophied left ventricular myocytes of SHRs was less than that of their right ventricular myocytes and of all myocytes of WKY rats. Again it is important that there was no significant difference between myocytes of failing and not failing hearts of the SHRs.

2. Contribution of the SR to activation of contraction.

The contribution of the SR to activation of contraction was investigated in the left ventricular myocytes of SHRs and WKY rats using a selective blocker of the Ca^{2+}-ATPase of SR, thapsigargin (TG). Cells were stimulated at the rate 30/min and after steady state has been obtained, superfusing solution was switched to that containing 10^{-6} M TG. Every 3 min stimulation was stopped and contractile response to 10 sec superfusion of 15 mM caffeine tested. The initial effect of TG consisted of a marked prolongation of relaxation and slight slowing of shortening of the electrically stimulated cell. Later both shortening and relaxation became very slow and amplitude of shortening decreased. These changes were accompanied by decrease in contractile response to caffeine until it completely disappeared showing that there was no releaseable Ca^{2+} in the SR. Electrically stimulated contractions remaining after disappearance of the responses to caffeine were regarded as activated by Ca^{2+} diffusing to sarcoplasm from the sources other than the SR. Difference between the control shortening and this remaining after TG was regarded as % of contraction activated by Ca^{2+} normally released from the SR. It ranged from $67 \pm 4\%$ to $72 \pm \%$ and did not differ significantly between the myocytes of the SHR and WKY rats nor between the myocytes of failing and not failing hearts of SHR rats.

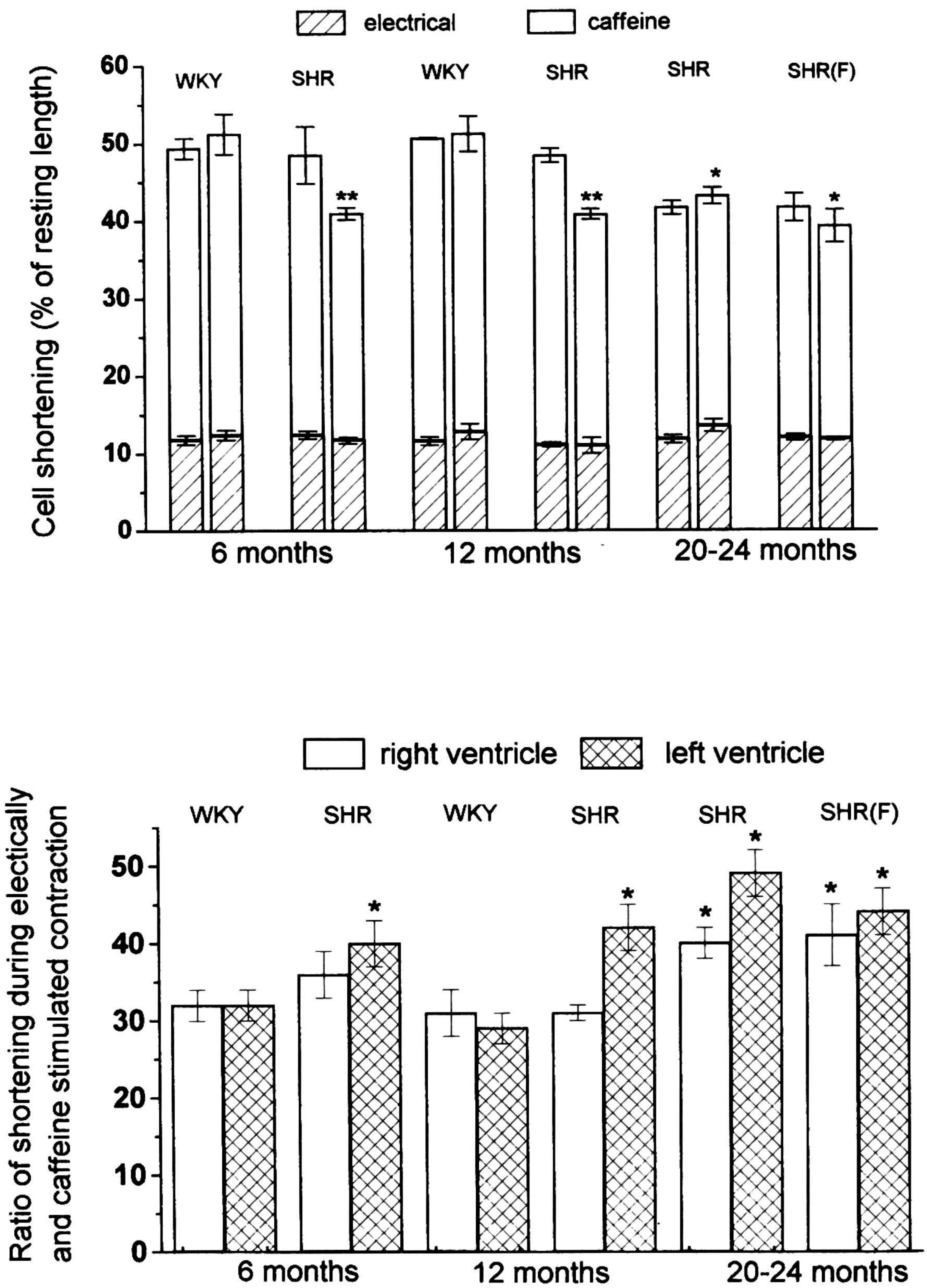

Fig. 4. Top: Range of shortening of single myocytes isolated from the right (RV) and left ventricles (LV) of WKY and SHR rats initiated by electrical stimulation or caffeine. SHR(F): rats which showed symptoms of the heart failure. At least 6 cells of each ventricle of each animal were investigated and the group means calculated. The bars represent the group means ± SE. n = 6—7.

* significantly different from RV of younger SHR rats and RV and LV of younger WKY rats.

** significantly different from RV of SHRs and RV and LV of WKY.

Bottom: relative index of fractional Ca^{2+} release from the SR.

* significantly different from the right ventricle or respective ventricle of WKY.

The amplitude of cells shortening and contribution of Ca^{2+} released from the SR activation of contraction did not differ between the hypertrophied and normal myocytes. However, the SR of hypertrophied myocytes contained less Ca^{2+} than that of other cells. This suggests that in the hypertrophied myocytes larger % of Ca^{2+} stored in the SR was released to activvate contraction than in the normal cells. This was assesed by calculating the ratio of cells shortening during the electrically stimulated contractions to the shortening elicited by superfusion of caffeine $+Ni^{2+}$ (*Fig. 4*, bottom panel). In right ventricular myocytes of SHR rats and in myocytes of the WKY rats it ranged from 0.29 ± 0.02 to 0.36 ± 0.03 and did not differ significantly between the groups. In the left ventricular myocytes of SHR rats it ranged from 0.40 ± 0.02 in the not failing hearts of SHR rats at the age of ~ 20 months which is close to fractional SR Ca^{2+} release measured in the hypertrophied LV myocytes of the aortic banded rats by Delbridge *et al.* (20). The differences between the left ventricular myocytes of SHR and WKY rats are significant. In the right ventricular myocytes of the SHR rats at the age of 20—24 months with failing and not failing hearts the *ratio* was also increased.

3. Relative contribution of carcoplasmic reticulum to relaxation.

This was assessed in left ventricular myocytes by comparing the rate constant of relaxation from the electrically stimulated Ca^{2+} transients and transients stimulated by 30 sec superfusion of caffeine according to protocols published by Bassani *et al* (21) and Negretti et al (22) (*Fig. 5*). As shown by these authors relaxation from the electrically stimulated transient results from Ca^{2+} reuptake by the SR, outward Ca^{2+} transport by Na/Ca exchange and sarcolemmal Ca^{2+}-ATPase, and mitochondrial Ca^{2+} uptake. Caffeine releases Ca^{2+} from the SR and blocks its reuptake by the SR. Therefore relaxation from caffeine stimulated Ca^{2+} transient depends on the relaxing factors other then the SR,

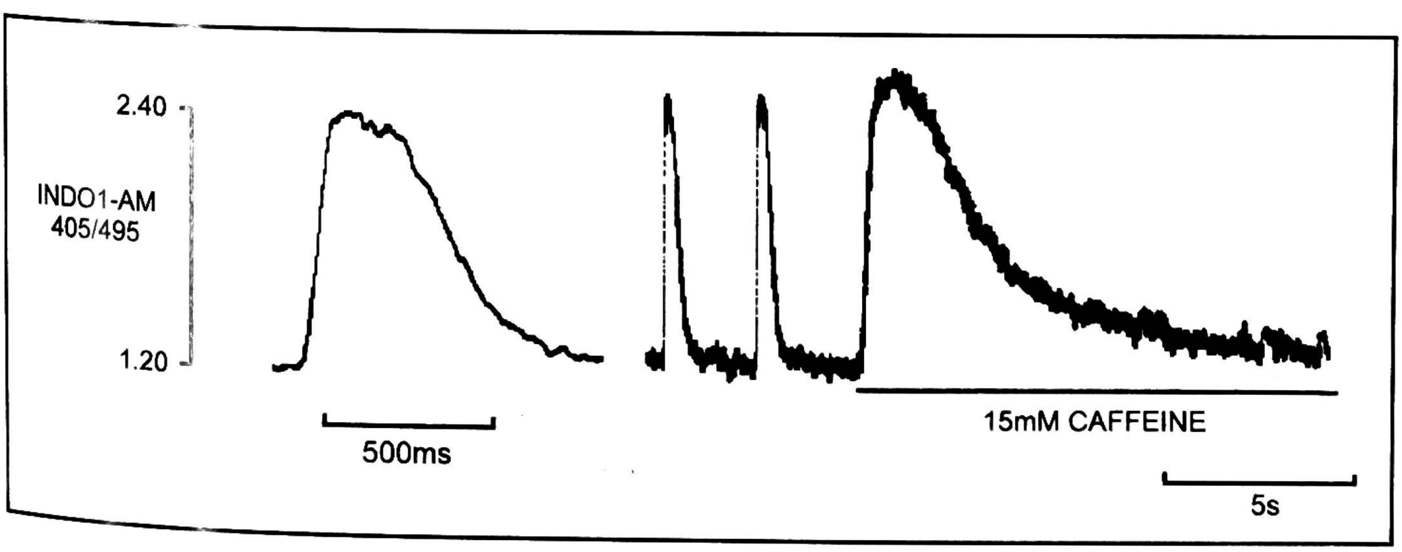

Fig. 5. The transients of Indo 1 fluorenscence initiated in the single myocyte of the LV of the WKY rat at the age of 6 months, initiated by electrical stimulation at the rate of 30/min or by caffeine. Left scale: Indo 1 fluorescence in arbitrary units.

the Na/Ca exchange being most effective. So the rate constant of relaxation from the electrically stimulated transient divided by the rate constant of relaxation from the caffeine stimulated transient times 100 reflects the % constribution of the SR to relaxation from the former. It ranged from 90 ± 2.6 to 94 ± 0.5 and did not differ significantly between the myocytes isolated from the hypertrophied or failing hearts of SHR and from the hearts of WKY rats.

DISCUSSION

This study shows that hypertrophied left ventricular myocytes of SHR rats differ from their not hypertrophied right ventricular myocytes and from left and right ventricular myocytes of the age and sex matched WKY rats by the slower kinetics of contraction and relaxation and respective changes in the Ca^{2+} transients and by lower content of Ca^{2+} in the SR, albeit relative contribution of the SR to activation of contraction and relaxation is not changed. The important finding is that the morphological and physiological properties of hypertrophic left ventricular myocytes isolated from the failing hearts of SHR rats did not differ from those of left ventricular myocytes of SHR rats isolated from not failing hearts.

Slow kinetics of contraction and altered Ca^{2+} handling of the hypertrophied myocardium of not failing and failing hearts is a common finding in animal models (11, 17, 23) and in humans (7, 24). This is usually accompanied by a decrease in contractile force of the multicellular preparations (11, 17, 26) or single myocytes (25) from the failing hearts. However, in our experiments we did not find decrease in the range of electrically stimulated contractions of hypertrophied myocytes even when they were isolated from the failing hearts.

Slow kinetics of contraction may depend on the impaired Ca^{2+} handling, on metabolic disorders or on the changes in contractile proteins. We found that the contractile response of the myocytes isolated from the hypertrophied and failing left ventricles to caffeine superfusion is less than in the not hypertrophied myocytes. This may depend on several changes in the cell properties. One of them could be a decrease in the sensitivity of contractile proteins to Ca^{2+}. However, no change (27) or an increase, rather than decrease (28) in the sensitivity of the contractile system to Ca^{2+} have been reported in the compensated and decompensated cardiac hypertrophy in the SHRs, although in the aortic banded rats it seemed to be decreased (29). Thus it is likely that decrease of the contractile response to caffeine resulted from the lower amount of Ca^{2+} released from the SR by this compound. This could depend on decreased amount of Ca^{2+} stored in the SR or on poorer response of the Ca^{2+} release channels of the SR. One of the reasons of the former could be the decreased rate of Ca^{2+} uptake by the SR. However, in contrast to aortic banded rats (3), in SHR model of hypertrophy and failure expression and protein content of the SR Ca^{2+}-ATPase is not decreased (12). In our experiments the relative contribution of SR to relaxation, which depends on the rate of the Ca^{2+} uptake by the SR was not decreased in the hypertrophied myocytes. The other reason of reduced response of the myocytes to caffeine could be the decreased density of ryanodine receptors reported in the hypetrophied rat myocardium (8, 9) or their impaired function as reported in the hypertrophied and failing dog heart (30). This may lead to reduced ability of I_{Ca} to trigger Ca^{2+} release from the SR during electrically stimulated contractions as reported in single myocytes isolated from hypertrophied and failing hearts of hypertensive Dahl SS/Jr rats (31). However, in contrast to the hypertrophied myocytes of the aortic banded rats (20), the fractional Ca^{2+} release from the SR of electrically stimulated myocytes of hypertrophied and failing ventricles seemed to be increased in our experiments since despite lower response to caffeine the amplitude of shortening was not diminished. This result is not compatible with failure of the ryanodine receptors. The other reason of a decreased Ca^{2+} content of the SR of SHR rats could be the decreased expression and content of calsequestrin reported in pressure over-load induced cardiac hypertrophy in rabbit (4). This seems to be most likely in our experimental model. Despite the differences in the SR Ca^{2+} content between the hypertrophied and not hypertrophied myocytes we did not find any difference in the relative contribution of the SR to activation of contraction between the myocytes isolated from the normal WKY hearts and from the hypertrophied or failing hearts of SHRs. This was assessed by measuring of percent of contraction left after complete depletion of the SR Ca^{2+} by thapsigargin, a selective blocker of the Ca^{2+} -ATPase of the SR (32—35). Also relative contribution of the SR to relaxation did not differ between these groups of myocytes which is in contrast to the results of Beuckelmann *et al* (24) who found that contribution of the SR to relaxationn from the Ca^{2+} transients is diminished in the myocytes isolated from the failing human hearts. Thus it seems that slower kinetics of contraction of our hypertrophied myocytes depended on factors other than impaired Ca^{2+} handling. These might be the metabolic disorders, the changes in contractile proteins or both. It has been shown by Tian *et al* (36) that cumulating of ADP due to poisoning of the heart with iodoacetamide leads to impaired relaxation and increased left ventricular diastolic pressure and that the hypertrophied rat hearts are not able to maintain a low diastolic ADP concentration. Indeed, a linear relationship between increases in [ADP] and left ventricular diastolic pressure was found in rat left ventricular hypertrophy due to aortic banding (37). The other likely reason of slow kinetics of contraction may be the increase in β/α myosin heavy chain ratio reported in SHR by Bing et al (11) and Bolyut *et al* (12).

Bing *et al* (11) and Conrad *et al* (17) found that the developed force decreases and kinetics of contraction of isolated papillary muscles slow down upon transition from compensated hypertrophy to heart failure in SHRs. Thus the lack of differences in morphological and physiological properties between the hypertrophied myocytes from the failing and not failing hearts of the SHR rats in our experiments supports the notion that transition from the compensated hypertrophy to heart failure results in this animal model from decrease in number of viable myocytes and from the remodeling of extramyocyte space rather, than from the impairment of function of the surviving myocytes (17). It has been already well documented that the number of apoptotic myocytes increases largely upon transition to heart failure in SHR rats (38). Since the adult cardiac myocytes do not proliferate, this must lead to the decrease of the total number of viable, contracting cells. On the other hand it has been shown that fibrosis developing already during the compensated hypertrophy increases rapidly upon transition to heart failure (16, 39) due to four- to fivefold stimulation of expression of the genes encoding fibronectin and collagen I and III (12). This certainly worsens the working conditions of the surviving myocytes and increases the stiffness of the heart muscle impeding the diastolic fillling of the ventricles.

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