Age and hypertrophy related changes in contractile post-rest behavior and action potential properties in isolated rat myocytes

Jutta Weisser-Thomas · Quan Nguyen ·
Manuela Schuettel · Daniel Thomas ·
Ulrike Dreiner · Christian Grohé · Rainer Meyer

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Abstract “Physiological” aging as well as early and progressive cardiac hypertrophy may affect action potential (AP) pattern, contractile function, and Ca^{2+} handling. We hypothesize that contractile function is disturbed in hypertrophy from early stages and is differently affected in aged myocardium. In vivo function, cardiomyocyte contractile behavior and APs were compared in Wistar-Kyoto (WIS) rats and spontaneously hypertensive rats (SHR) at different ages and degrees of hypertrophy (3–4, 9–11, 20–24 months). Post-rest (PR) behavior was used to investigate the relative contribution of the sarcoplasmic reticulum (SR) and the Na/Ca exchanger (NCX) to cytosolic Ca^{2+} removal. APs were recorded by

whole-cell current-clamp and sarcomere shortening by video microscopy. Cyclopiazonic acid was used to suppress Ca^{2+} ATPase (SERCA) function. Heart weight/body weight ratio was increased in SHR versus WIS within all age groups. Myocyte steady state (SS) shortening amplitude was reduced in young SHR versus WIS. Aging led to a significant decay of SS contractile amplitude and relengthening velocity in WIS, but the PR potentiation was maintained. In contrast, aging in SHR led to a decrease of PR potentiation, while SS contraction and relengthening velocity increased. $\text{APD}_{50\%}$ was always prolonged in SHR versus WIS. With aging, $\text{APD}_{50\%}$ increased in both WIS and SHR, but was still shorter in WIS. However, in old WIS the late AP portion ($\text{APD}_{90\%}$) was prolonged. Ca^{2+} handling and AP properties are disturbed progressively with aging and with increasing hypertrophy. Decreased amplitude of shortening and velocity of relengthening in aged WIS may be attributed to reduced SERCA function. In SHR, an increase in SR leak and shift towards transmembraneous Ca handling via NCX may be responsible for the changes in contractile function. A prolonged $\text{APD}_{90\%}$ in aged WIS may be an adaptive mechanism to preserve basal contractility. Therefore, the effects on contractile parameters and AP are different in hypertrophy and aging.

J. Weisser-Thomas · Q. Nguyen · M. Schuettel ·
U. Dreiner · R. Meyer
Institute of Physiology II, Universitätsklinikum Bonn,
Bonn, Germany

J. Weisser-Thomas · C. Grohé
Medizinische Klinik und Poliklinik II,
Universitätsklinikum Bonn,
Bonn, Germany

D. Thomas
Radiologische Klinik, Universitätsklinikum Bonn,
Bonn, Germany

J. Weisser-Thomas (✉)
Medizinische Klinik II, Universitaet Bonn,
Sigmund-Freud-Str. 25,
53113 Bonn, Germany
e-mail: jweisser@ukb.uni-bonn.de

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Introduction

Physiological aging as well as development of cardiac hypertrophy seem to affect contractile function and action potential (AP) shape. Contractile properties of cardiomyocytes are largely controlled by the AP shape reflecting the sum of all ionic currents, by the sarcoplasmic reticulum (SR) Ca^{2+} release and reuptake as well as transmembraneous Ca^{2+} fluxes via the L-type Ca^{2+} channel and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) (Bers 2001).

Pressure overload followed by cardiac hypertrophy leads to heart failure (Lorell and Carabello 2000). In heart failure, AP duration is increased and accompanied by a slow decay of the Ca^{2+} transient (Beuckelmann et al. 1992). Spontaneously hypertensive rats (SHR) are an established model for pressure overload induced hypertrophy (Bing et al. 1995; Brooks et al. 1994). However, studies of changes in Ca^{2+} handling during development of hypertrophy remain controversial. While steady state contractile function (Perez et al. 1993), SR Ca^{2+} ATPase (SERCA), NCX and phospholamban protein or RNA levels have been shown to be unchanged in young SHR rats (Perez et al. 1993; Ohta et al. 1995), others have found significant differences especially at older ages with enhanced hypertrophy (Zhu et al. 1999; MacDonnell et al. 2005). NCX activity in hypertrophy in SHR rats has been found to be decreased (Anderson et al. 1999) or increased (MacDonnell et al. 2005; Mace et al. 2003; Hasenfuss 1998; Houser et al. 2000).

Dynamic changes in Ca^{2+} handling during progression of hypertrophy and different ages seem to be the explanation for these controversial findings. With aging, Ca^{2+} transients show a reduced amplitude and/or a slower decay (Janczewski et al. 2002; Orchard and Lakatta 1985) which may result from a reduced SERCA function (Froehlich et al. 1978) and/or an increased NCX forward mode (Mace et al. 2003) or an increased Ca^{2+} leak through the SR Ca^{2+} release channel (ryanodine receptor) (Bers 2001; Song et al. 2006). In addition, SHR myocytes have been shown to display a change in structure of the T-tubular system leaving the ryanodine receptors back in a different cellular space resulting in a reduced ability to trigger SR Ca^{2+} release (Song et al. 2006). In aged rats, cardiomyocyte hypertrophy has been reported, combined with a deficiency in mitochondria, SR and the T-tubules (Lushnikova et al. 2001). AP duration

is prolonged, possibly brought about by a slower inactivation of the L-type Ca^{2+} current ($I_{\text{Ca,L}}$) and reduced outward K currents (Walker et al. 1993; Lakatta and Sollott 2002; Josephson et al. 2002; Liu et al. 2000).

Post rest behavior is a tool to determine early abnormalities in Ca^{2+} removal from the cytosol (Bers 2001; Maier et al. 2000; Pieske et al. 1996), because it reflects the ability of the SR to store Ca^{2+} . During the rest, Ca^{2+} may leak out of the SR via ryanodine receptors. This Ca^{2+} may be transported out of the cell by the NCX or back into the SR by the SERCA. Post-rest shortenings are less sensitive to changes in AP shape and ionic currents than steady state shortenings (Bers 2001). Thus, the first beat after a rest period is a measure for the ability of the SR to store and release Ca^{2+} , as well as the NCX-mediated transmembraneous Ca^{2+} removal (Bers 2001; Maier et al. 2000).

Previous studies were undertaken to investigate age or hypertrophy related changes in cardiac function. However, direct comparisons of the influence of pressure induced hypertrophy and age at the same time still lack investigation. Our goal was to determine the influence of progressive hypertrophy and aging on steady state and post-rest contractile behavior as well as AP properties. The results show that with “physiological” aging the steady state contractile function is mainly affected and post-rest contractions are maintained. The late AP portion is prolonged which may help to restore the affected contractile function. In SHR cardiomyocytes during progression in hypertrophy and age, post-rest behavior becomes increasingly disturbed as a sign of impaired SR storage function shifting Ca^{2+} handling towards transmembraneous Ca^{2+} removal. These changes are accompanied by an early, general AP prolongation.

Materials and methods

Animals

All animal protocols have been approved by the local committee for animal care conformed to the NIH Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Wistar rats (WIS) and SHR (Charles River, Sulzfeld, Germany)

were housed at 24°C and 12 h dark-light cycle and fed with water and food ad libitum. For functional measurements, three age groups were established: young (3–4 months), middle (9–11 months) and old (20–24 months), see also Table 1.

Cardiac MRI measurements

MR-images were generated on a 4.7 T scanner (Varian INOVA, Palo Alto, Calif.). Rats were anesthetized using 1.5% isoflurane in room air applied over a nose cone. An ECG gated sequence (FLASH) was acquired in the short axis orientation, covering the entire LV with 9–11 slices. Imaging parameters were: flip angle 20–25°, TR 6 ms, TE 3 ms, FOV 5–6 cm², matrix size 128², zero-filled to 256², slice-thickness 1.5 mm, no gap, 11–12 cardiac phases/RR, 4 signal averages.

Global function The left ventricular (LV) epicardial and endocardial borders were manually traced on the end-diastolic image of each slice. LV mass was determined at end-diastole by summing the difference between the epi- and endocardial areas from the base to apex, then multiplying by the slice thickness and by the specific gravity of myocardium (1.05 g/cm³). Endocardial borders were drawn on end-systolic images so that end-diastolic (EDV) and end-systolic (ESV) volumes could be obtained. Stroke volume (SV) and ejection fraction (EF) were calculated as: SV=EDV–ESV and EF=SV/EDV. End-diastolic wall thickness was calculated from three measurements across the septum on a midventricular slice (largest EDV).

Myocyte isolation

Myocytes were isolated as previously described (Linz and Meyer 2000). Briefly, animals were weighed and hearts were removed and cardiopleged in EGTA-containing cold Tyrode solution containing NaCl 135 mM, KCl 4 mM, MgCl₂(H₂O)₆ 1 mM, HEPES 2 mM, EGTA 2.6 mM, pH 7.4. Heart weight and tibia length were measured and the heart was rapidly attached to a Langendorff apparatus and perfused with the Tyrode described above (37°C). Then, a high potassium solution was perfused (NaCl 4 mM, KCl 10 mM, MgCl₂ 1 mM, CaCl₂ 0.2 mM, K-Glutamate 130 mM). In a next step, this solution with the addition of 50 U/ml Trypsin Type I, (Sigma, St. Louis, Mo., USA), and finally with the addition of 0.6–1.0 mg/ml collagenase Type II (Sigma), was applied for digestion. The ventricles were cut down, chunked and allowed to settle in oxygenated experimental Tyrode containing NaCl 135 mM, KCl 4 mM, MgCl₂ 1 mM, HEPES 2 mM, CaCl₂ 1.8 mM, BSA 1 mM and Trypsin inhibitor. The solution was filtered through a 125-μm nylon mesh and centrifuged briefly. The cells were resuspended in fresh solution.

Functional measurements

Sarcomere length Cells were placed in a Laminin-coated, heated chamber on an inverted microscope (Zeiss IM 35, Germany) and continuously superfused with the experimental Tyrode described above at 36–37°C. Cells were field stimulated ~20% above threshold (~30 V, 0.1 ms) at 1 Hz by 2 gold electrodes

Table 1 Morphological analysis of animals' heart and cells at different ages expressed as mean±SD

	WIS			SHR		
	Young	Middle	Old	Young	Middle	Old
Animals (n)	11	6	5	8	5	6
BW (g)	441±92	472±142	657±105 ^a	197±41	270±63	181±23
HW (g)	1.57±0.29	1.33±0.31	2.6±0.4 ^a	1.22±0.17	1.49±0.34	1.43±0.18
HW/BW (g/kg)	3.97±0.51	3.02±0.41	3.70±0.22	6.31±1.33 ^a	5.43±0.06 ^b	7.92±1.44 ^b
TL (cm)	4.31±0.23	4.18±0.17	4.79±1.6	3.54±0.22	3.85±0.19	3.68±0.09
HW/TL	0.36±0.06	0.32±0.06	0.54±0.08 ^a	0.34±0.03	0.38±0.07	0.38±0.06 ^b
SL (μm)	1.911±0.029	1.883±0.033	1.880±0.023	1.898±0.026	1.898±0.034	1.898±0.024

WIS Wistar rats, SHR spontaneously hypertensive rats. Young 3–4 months, Middle 9–11 months, Old 20–24 months), BW body weight, HW heart weight, TL tibia length, SL resting sarcomere length

^a $p < 0.05$ versus young rats, ^b $p < 0.05$ versus WIS

placed in the bath. Signals were obtained using a video-based sarcomere length detection system (ION OPTIX, Milton, Mass., USA), digitized and stored on a PC for analysis.

After a run-in stimulation period to establish steady state contractions, the steady state contractions were interrupted and post-rest contractions after increasing periods of rest (2, 5, 10, 30, 60, 120 and 240 s) were recorded. Figure 1 shows an example for an original registration of a post-rest experiment. The change of shortening amplitude, shortening velocity and relengthening velocity were calculated by using the ION OPTIX IonWizard software. Pre-rest steady state values were compared to the first post-rest beat.

Action potential recordings Whole cell patch clamp experiments were performed at 36°C as reported previously (Weisser-Thomas et al. 2003). Briefly, borosilicate glass pipettes (6–7 mΩ) filled with a physiological internal solution containing NaCl 10 mM, K-Aspartate 120 mM, K-ATP 5 mM, MgCl₂ 1 mM, HEPES 10 mM, GTP 0.1 mM, were used. Cells were superfused with the experimental Tyrode, except BSA. Stimulation of cells and recording of APs were performed using an EPC7 (List, Darmstadt, Germany) amplifier. Electrical stimulation was used ~20% above threshold. Data were collected using AXON pclamp 6 software (Clampex, Axon Instruments, Foster City, Calif., USA), transformed on an AXON Digidata and analyzed later using AXON pclamp 8 software (Clampfit). The same post-rest protocol as described above was applied to obtain steady state and post-rest AP duration (APD_{50%} and APD_{90%}).

Fig. 1 Original registration of a post-rest experiment. After a run-in period, the steady state beating was interrupted by a 2-s rest period followed by the post-rest beating. After achieving a new steady state the 5-, 10-, 30-, 60-, 120- and 240-s pauses followed. It is visible that the first post-rest beat is larger than the last steady state beat (= post-rest potentiation of contraction)

Statistics

Statistics were performed either using the ANOVA model described below or a paired student's *t*-test. The dependent variables were treated as continuous variables for all analyses. The experimental unit was each individual cell. The experiment used a repeated measures design with each cell evaluated at up to six rest intervals. The null hypothesis was that there would be no difference between CONTROL and SHR or between age groups. Prior to analysis, all data were tested for normality using the Shapiro–Wilk test. The data were significantly non-normal for all variables. In order to apply ANOVA methods, a ‘normalized-rank’ transformation was applied. The rank-transformed data was analyzed using a mixed-model ANOVA for repeated measures using the rest interval as a covariate followed by multiple comparisons to detect significant mean differences between groups and ages. Multiple pairwise comparisons used the Bonferroni adjustment to maintain an experiment-wise type I error of 0.05 or less. Differences between means (rejection of the null hypothesis) were considered significant if the probability of chance occurrence was < 0.05 using two-tailed tests.

Results

In vivo cardiac function and morphological analyses

Body weight was higher in WIS animals in all age groups (Table 1), but heart weight/body weight ratio was always significantly higher in SHR. This is consistent with previous findings (Kokubo et al. 2005). In contrast, heart weight/tibia length ratio was increased in old WIS. These results show that in WIS overall weight and size increased with age, while in SHR both body weight and heart weight changed only a little thus developing an increase in heart weight/body weight ratio. Resting sarcomere length did not differ between SHR and control myocytes and did not change with age (Table 1). We did not observe any deaths of any animal either in the WIS control group or in the SHR group prior to the experiment.

Figure 2 shows original end-diastolic (left) and end-systolic (right) MR-images from young WIS (upper panel) and young SHR (lower panel) rats. A marked increase in left ventricular (LV) mass and

septum thickness (IVS) accompanied by an increase in ejection fraction (EF) was observed in the 3-month-old young SHR rat as a sign of compensated hypertrophy (Fig. 2; Table 2).

Steady state contractile function in young and aged myocytes

In total, 172 cardiomyocytes were investigated for functional measurements. In myocytes from young hearts, SS amplitude of shortening was reduced in SHR as compared to WIS ($0.081 \pm 0.006 \mu\text{m}$ vs $0.099 \pm 0.01 \mu\text{m}$; $p < 0.05$), but the velocity of relengthening was only slightly reduced (2.713 ± 0.228 vs $3.033 \pm 0.397 \mu\text{m/s}$; n.s.). The velocity of cell shortening and time to 90% decay of the twitch were not different ($3.874 \pm 0.334 \mu\text{m/s}$ vs $3.905 \pm 0.309 \mu\text{m/s}$ and 0.112 ± 0.002 s vs 0.113 ± 0.003 s, respectively; Fig. 3).

However, with increasing age and progression of hypertrophy, the changes in excitation contraction coupling develop differently in WIS as compared to SHR. There was a decrease of SS contractile amplitude in the aging control (WIS) animals, whereas an increase in SS contractile amplitude was observed in aging SHR myocytes (Fig. 3). Similarly, the velocity of relengthening which is a relative dimension of the

Table 2 In vivo functional parameters obtained by MRI

	WIS (young) $n=3$	SHR (young) $n=3$
EDV (cm^3)	0.369 ± 0.06	0.352 ± 0.03
ESV (cm^3)	0.566 ± 0.40	0.402 ± 0.33
EF (%)	73.3 ± 7.07	$86.5 \pm 7.82^*$
LV mass (mg)	448.2 ± 47.9	$722.4 \pm 104.8^*$
IVS (mm)	1.76 ± 0.12	$2.39 \pm 0.03^*$

Average data analysed from mri images above obtained in WIS and SHR rats

Left ventricular (LV) mass and ejection fraction (EF) is increased in SHR (Table 2)

EDV End-diastolic volume, ESV end-systolic volume, IVS intra-ventricular septum (mean $\pm$ SD)

* $p < 0.05$ versus WIS young

SERCA function decreased significantly to $2.384 \pm 0.204 \mu\text{m/s}$ in aged WIS (normal) myocytes, whereas an increase of relengthening velocity to $3.303 \pm 0.236 \mu\text{m/s}$ was measured in SHR myocytes (Fig. 3).

Post-rest shortenings in control and hypertrophied myocytes

PR behavior was used to study the relative contribution of SR Ca^{2+} uptake, SR Ca^{2+} release and NCX to Ca^{2+} removal from the cytosol. Figure 4a shows original registrations of PR shortenings in a young WIS myocyte compared to a young SHR myocyte. The first beat after the rest period was increased in both, WIS and SHR, but the increase of PR shortening was smaller in the SHR myocyte as compared to control. Figure 4b shows the age dependence of SS and PR contractions in WIS and SHR cells in absolute values. In young WIS, both PR and SS shortenings exhibited higher amplitudes of contraction than in older animals, while in SHR age dependant differences were less obvious. However, young WIS develop larger PR contractions as compared to SHR.

The extent of PR potentiation depends on a large amount on pre-rest SS SR loading conditions. Therefore, in order to emphasize the relation of age dependant PR shortening to the corresponding SS value their amplitude was standardized to the respective SS amplitude (Maier et al. 2000; Pieske et al. 1996; Fig. 4c).

The PR/SS shortening was greater in WIS myocytes with increasing rest periods as compared to

Fig. 2 Original cardiac MR-images from 3-month-old WIS (upper panel) and SHR (lower panel) rats. End-diastolic (right) and end-systolic (left) images show a clear hypertrophy with an increase in septum thickness of about 25% in the SHR rat

Fig. 3 Steady state average values are shown for myocyte peak shortening (*left*) and relengthening velocity (*right*) for all different age groups in WIS and SHR myocytes. While peak of contraction and relengthening velocity decays with physiological aging, there is an increase in steady state peak of shortening and relengthening velocity in progressive hypertrophy. $n=27$ cells for WIS young, $n=26$ cells for SHR young, $n=26$ cells for WIS middle, $n=11$ cells for SHR middle, $n=12$ cells for WIS old, $n=36$ cells for SHR old. Data are shown as mean $\pm$ SEM

SHR. In addition, the relative PR potentiation of shortening was maintained in older WIS, whereas there was a marked decrease in PR potentiation in the older SHR myocytes (Fig. 4c). This may reflect a SR Ca^{2+} leak or a change in SERCA/NCX activity in SHR. Accordingly, the PR/SS relengthening velocity was maintained in old WIS (Fig. 5a, left) whereas PR/SS relengthening velocity was reduced in old SHR (Fig. 5a, right).

PR contractions and relengthening velocity depend on the loading conditions of the SR, SERCA activity and the SR leak. To further account for the SR loading conditions, PR relengthening velocity was also standardized to the absolute magnitude of pre-pause SS shortening in μm (Fig. 5b). In young WIS and SHR, the PR relengthening velocity did not differ significantly. However, both ways of standardizing demonstrate a significant decreased relengthening velocity of old SHR as compared to old WIS as well as to young SHR at long rest periods.

Post-rest behavior is influenced by Ca^{2+} removal from the cytosol. Therefore, cyclopiazonic acid (CPA $3 \times 10^{-6}\text{M}$) was applied to partly block SERCA function in young WIS and SHR myocytes. SS shortening amplitude and relengthening kinetics were reduced by CPA in both WIS and SHR. However, in

the presence of CPA, the relative PR peak shortening and relengthening velocity became significantly smaller in SHR as compared to WIS (Fig. 6). This may be regarded as a sign for an increased SR leak in young SHR cardiomyocytes, which may become more obvious by pharmacological reduction of SERCA function during post-rest experiments.

Action potentials in young, aged and hypertrophied aged myocytes

Figure 7a shows original tracings of steady state action potentials from young hypertrophied (SHR) and young control (WIS) myocytes (left) and from old SHR compared to old WIS myocytes (right); 86 cells were used for AP measurements. AP duration as measured as $\text{APD}_{50\%}$ and $\text{APD}_{90\%}$ were significantly prolonged in young SHR versus young WIS (Fig. 7a,b). There was an increase in $\text{APD}_{50\%}$ with age in both, WIS and SHR, (from 7.96 ± 0.39 ms to 10.99 ± 1.07 ms, and from 12.55 ± 0.62 ms to 17.80 ± 1.17 ms, respectively; $p < 0.05$), but the $\text{APD}_{50\%}$ remained significantly shorter in old WIS. In contrast, the late portion of the AP ($\text{APD}_{90\%}$) increased in old WIS to an extent that was comparable to the values of old SHR myocytes (Fig. 7b). These results show clear differences in AP

Fig. 4 a Original tracings of post-rest contractions are shown after a 30-s rest period for young WIS and SHR. The first post-rest beat is greater in WIS versus SHR while steady state shortenings are comparable. **b** The average magnitude of shortening in absolute values (μm) for PR and SS in age dependence for WIS and SHR. Young WIS exhibit a larger post-rest potentiation as compared to older WIS (* $p < 0.05$ vs old WIS, # $p < 0.05$ vs middle WIS, \$ $p < 0.05$ middle WIS vs old WIS). **c** To account for SR loading, the individual PR contractions were normalized to pre-rest SS values and shown here as mean $\pm$ SEM. The relative PR potentiation was maintained in old WIS. In SHR, the SS contractile amplitude did not change significantly with age. However, the relative PR contraction amplitude decreased with aging and increasing rest periods

kinetics between young myocytes, $\text{APD}_{50\%}$ and $\text{APD}_{90\%}$ are prolonged in SHR. During aging $\text{APD}_{50\%}$ increased in SHR myocytes while $\text{APD}_{90\%}$ remained unchanged. In WIS, both APD 's were prolonged. In young cardiomyocytes of both groups, PR $\text{APD}_{50\%}$ and $\text{APD}_{90\%}$ did not differ from SS values. In the old cardiomyocytes of both WIS and SHR, prolongation of the PR period was accompanied by a tendency to AP shortening, i.e., the longer the PR period the shorter the APD.

Discussion

This study focused on the changes in contractile function and AP shape during aging with or without development of cardiac hypertrophy. The main findings of the present study are that first, in early hypertrophy, the myocyte steady state contractile function is reduced and the AP duration is prolonged although in vivo contractile function is not altered. Second, in WIS myocytes, aging led to

Fig. 5 a,b Average values of PR relengthening velocity (*left* WIS, *right* SHR). In SHR cells, the post-rest potentiation and the relengthening velocity decay significantly with aging while they are maintained in the WIS control group. To account for pre-rest SR loading conditions PR relengthening velocity was also normalized to pre-rest magnitude of shortening (Fig. 5b). $n=27$ cells for WIS young, $n=26$ cells for SHR young, $n=12$ cells for WIS old, $n=36$ cells for SHR old. Data are shown as mean $\pm$ SEM

a decrease of steady state shortening amplitude as well as relengthening velocity. Post-rest potentiation of shortening and post-rest relengthening velocity are maintained during aging in normal myocytes while they are reduced progressively in SHR myocytes. Furthermore, an increase in $APD_{50\%}$ takes place in normal as well as in hypertrophied myocytes with age, but $APD_{50\%}$ is always shorter in normal aged myocytes versus age matched SHR. However, a separate increase in the late AP portion ($APD_{90\%}$) has been observed in normal aged cells which may reflect an adaptive mechanism to preserve contractile function.

Post-rest potentiation of contraction

Although cardiac function as measured by LV ejection fraction, systolic pressure or basal myocyte shortening magnitude may remain normal for a long time under hypertrophic or aging conditions, sub-clinical changes in contractile performance may already occur. Many of them are related to disturbed Ca^{2+} handling. Post-rest potentiation of contraction has been used to characterize Ca^{2+} handling in different species under physiological conditions (Bers 2001; Maier et al. 2000), and in heart failure (Pieske et al. 1996). Species/subjects with high SR storage capacity and/

Fig. 6 Cyclopiazonic acid (CPA) was applied to suppress SR Ca^{2+} uptake in WIS and SHR myocytes. Steady state shortening was depressed in both WIS and SHR. However, the PR/SS peak shortening and PR/SS relengthening velocity decayed significantly more in SHR. $n=26$ cells with CPA for WIS and $n=13$ cells for SHR. Data are shown as mean $\pm$ SEM

or high $[\text{Na}^+]_i$ therefore show a potentiation of the first contraction after a rest period (mice, rats, nonfailing humans), while myocytes with lower $[\text{Na}^+]_i$ or disturbed SR storage function “lose” Ca^{2+} during a rest period via the ryanodine receptor and therefore have a post-rest decay of contraction (ferret, cat, failing humans) (Bers 2001; Pieske et al. 1996). The rat myocytes studied here showed the physiological post-rest potentiation of shortening. However, with progressive age the rest potentiation decayed in SHR, but was maintained in aged WIS myocytes. The decreased post-rest potentiation in the aged SHR may be regarded as a sign for a change in SERCA/NCX ratio or an increased SR leak. Since basal velocity of relengthening as an indicator for SERCA Ca^{2+} reuptake did not decrease in aged SHR, an increased SR leak and/or NCX function may be most likely. Accordingly, after CPA application to reduce SR Ca^{2+} reuptake function, the post-rest potentiation and velocity of relengthening decayed significantly more in the SHR. Therefore, an increase in SR Ca^{2+} leak may be compensated by an adequate SERCA activity in young SHR myocytes as compared to young WIS myocytes, but can be unmasked by interventions like CPA treatment during post-rest contractions.

Steady state contractile function and action potential properties at young age

In humans with mild hypertension, development of hypertrophy is a critical determinant for compensated cardiac function. Although LV ejection performance can be adequately increased by wall thickening in early hypertrophy the actual LV contractility is already depressed (Palatini et al. 2001). Due to the lack of human myocardial tissue at this early stage of hypertrophy, physiological data on the myocyte level are rarely available. On the molecular level, SERCA expression has been shown to be reduced by about 30% in human hypertrophic obstructive cardiomyopathy or aortic valve stenosis, whereas the expression of phospholamban and the NCX were unchanged (Schotten et al. 1999). Spontaneously hypertensive rats develop a stable hypertension of about 190 mmHg by the age of 3 months (Mill et al. 1998), and after 14 weeks the left ventricle is hypertrophied (Nand et al. 1997). An increased contractility has been found as response to pressure overload at this age (Mill et al. 1998) as well as reduced (Diaz et al. 2004) or unchanged contractile function (Perez et al. 1993). Accordingly here, in vivo ejection fraction and wall

Fig. 7 **a** Original tracings of action potential recordings of young myocytes (*left*) and old myocytes (*right*). **b** Average values. $n=36$ cardiomyocytes from young WIS and 30 cells from young SHR, $n=11$ for old WIS, and $n=9$ for old SHR. APs are prolonged in SHR versus WIS at young age. $APD_{50\%}$ is also longer in SHR at older age. However, with age WIS myocytes develop a clear prolongation of the late AP phase ($APD_{90\%}$)

thickness at 3 months were increased in SHR. However, basal myocyte contractility was slightly reduced and post-rest contractions were smaller in young SHR at this early state of hypertrophy. This shows that SR function was altered in early stages of hypertrophy and the alterations can be unmasked by interventions like post-rest contractions or drug application to suppress SERCA function. An increase in SR leak as well as a shift from intracellular (SR) Ca^{2+} cycling towards transmembraneous Ca^{2+} cycling via the NCX could be an explanation for this finding.

Along these lines here, a significant prolongation of the AP duration ($APD_{50\%}$ and 90%) has been observed in young SHR versus young WIS. A prolonged APD can increase Ca^{2+} influx via the $I_{Ca,L}$ and delay transmembraneous Ca^{2+} transport via NCX. This can increase SR Ca^{2+} load. Thus, young SHR cardiomyo-

cytes depend to a higher degree on external Ca^{2+} than WIS control myocytes.

Steady state contractile function and action potential properties change with aging

Left ventricular hypertrophy and development of heart failure increase with age. Impaired LV ejection reserve capacity manifest by a reduced ejection fraction, diastolic LV dilation and altered diastolic filling have been described (Lakatta and Sollott 2002). Besides others, a decreased SERCA function, a decreased response to beta-receptor stimulation with aging may be responsible for these changes (Lakatta 2003). However, sub-cellular findings are not always consistent, so that NCX function has been reported to be either increased (Mace et al. 2003), decreased

(Anderson et al. 1999) or unchanged (Abete et al. 1996) in aged myocardium.

Our results show that steady state shortening amplitude is reduced and relengthening velocity is prolonged during aging in WIS rats as a sign for disturbed SERCA function. Functional studies have been undertaken in normal aged rats showing reduced peak contraction (Cerbai et al. 1994), prolonged Ca^{2+} transients, slowed relaxation and a negative force–frequency relationship (Orchard and Lakatta 1985) which have been attributed to reduced SERCA uptake function with aging (Lakatta 2003). This is in good agreement with our finding of reduced shortening amplitude and a slower relengthening velocity in old versus young WIS cardiomyocytes. However, the post-rest potentiation of cell shortening was maintained in aged normal myocytes but decayed in the aged SHR myocytes. Since post-rest potentiation was maintained in aged WIS, the SR Ca^{2+} storage function seems to be maintained and thus the SR leak does not seem to increase during physiological aging.

In contrast to WIS, steady state shortening amplitude in SHR increases with increasing age. Also, relengthening velocity is fastest in oldest SHR cardiomyocytes. These findings indicate that the hypertensive rats investigated here did not (yet) go into heart failure, but rather they show a hyperactive state of contraction. In fact, not all SHR rats observed in other studies at this advanced age showed heart failure. Bing et al. (1995) and Brooks et al. (1994) reported that heart failure in SHR can occur between 18 and 24 months. However, they also described a group with stable hypertrophy at this age. According with our cellular findings they showed that base line peak systolic pressure was higher in the age matched old nonfailing SHR versus failing SHR or the control animals studied. However, Ca^{2+} transients were prolonged and force frequency response was negative in both failing and nonfailing SHR as compared to the control (Brooks et al. 1994). The increase in steady state shortening is accompanied by an $\text{APD}_{50\%}$ lengthening during aging in SHR cells. $\text{APD}_{50\%}$ in old SHR cardiomyocytes is significantly longer than in age matched WIS cells.

Although steady state contractile amplitude was increased in old as compared to young SHR, these cells were not able to preserve the physiological post-rest potentiation. Therefore, SR storage function seems altered in old SHR. The SR leak and/or the

NCX activity may be increased so that during rest a net Ca^{2+} efflux takes place. An explanation for these findings has been reported recently by Song et al. (2006). They found a spatial dispersion of the ryanodine receptor from the T-tubular system which goes along with desynchronized Ca^{2+} sparks. This in consequence led to a disturbed Ca^{2+} handling in SHR myocytes and may be related to arrhythmias in diverse models of heart failure.

In our experiments, the AP duration was prolonged with both, aging and hypertrophy. However, in old WIS the $\text{APD}_{50\%}$ was still significantly shorter than in the old SHR myocytes. Interestingly, the late portion of the AP ($\text{APD}_{90\%}$) was markedly prolonged in the old WIS myocytes. An AP prolongation has been found in normotensive aged rat ventricular myocytes (Janczewski et al. 2002; Walker et al. 1993; Josephson et al. 2002; Cerbai et al. 1994; Jullien et al. 1989) and SHR myocytes (Diaz et al. 2004; Cerbai et al. 1994). The underlying mechanisms have been shown to involve a slower inactivation of the $\text{I}_{\text{Ca,L}}$ with age (Janczewski et al. 2002; Walker et al. 1993; Josephson et al. 2002) or a reduction of the I_{to} current density (Lakatta 2003; Tomita et al. 1994) which was directly related to the duration of hypertension and aging (Lakatta 2003). Liu et al. (2000) reported a greater I_{to} with an increased rate of activation in aged Fisher rats, while the I_{to} inactivation kinetics and steady-state inactivation were unaltered. They also found a separate increase in $\text{APD}_{90\%}$, which was comparable to our findings. It may be concluded from these findings that the slow inactivation of $\text{I}_{\text{Ca,L}}$ and the increased NCX transport are involved in the late $\text{APD}_{90\%}$ changes with aging. In addition, DuBell et al. (1991) have shown previously that the APD in rats is proportional to the amplitude of the Ca^{2+} transient. By inhibition of the NCX with lithium they were able to show that the magnitude of the late portion of the AP plateau phase depends partly on the NCX. In contrast, the SHR myocytes investigated here showed an increased AP duration ($\text{APD}_{50\%, 90\%}$) from early hypertrophy and age on, although their shortening amplitude was smaller than that of age matched WIS. Therefore, the AP prolongation with aging may be due to a different mechanism than during early hypertrophy. AP prolongation is a mechanism to increase Ca^{2+} inward flux and thus is able to compensate reduced SR storage of any reason. This was demonstrated nicely by Janczewski et al. (2002), who stimulated old rat

cardiomyocytes with their natural long AP's and with (shorter) AP's of young rats using AP clamp. With short AP's, old cardiomyocytes exhibited smaller Ca^{2+} transients, a slower rate of rise and decay of Ca^{2+} and a reduced SR load. Old myocytes seem to use the AP prolongation to preserve a better SR loading (Janczewski et al. 2002).

The question has been raised whether or not hypertrophy and aging might have similar effects on the heart or not (Lakatta 1987). Physiological aging and aging in combination with hypertension both lead to changes in cardiomyocyte function and AP prolongation. However, SHR cardiomyocytes exhibit signs of an increased SR leak, whereas aged matched WIS cardiomyocytes develop reduced shortening amplitude and prolonged relaxation, which points to a decreased SERCA function.

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References

- Abete P, Ferrara N, Cioppa A et al (1996) The role of aging on the control of contractile force by Na^+ - Ca^{2+} exchange in rat papillary muscle. *J Gerontol A Biol Sci Med Sci* 51: M251–M259
- Anderson SE, Gray S, Atherley R et al (1999) Na-dependent changes in intracellular Ca in spontaneously hypertensive rat hearts. *Comp Biochem Physiol A Mol Integr Physiol* 123:299–309
- Bers DM (2001) Excitation-contraction coupling and cardiac contractile force, 2nd edn. Kluwer, Dordrecht, The Netherlands
- Beuckelmann DJ, Nabauer M, Erdmann E (1992) Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circulation* 85:1046–1055
- Bing OH, Brooks WW, Robinson KG et al (1995) The spontaneously hypertensive rat as a model of the transition from compensated left ventricular hypertrophy to failure. *J Mol Cell Cardiol* 27:383–396
- Brooks WW, Bing O, Litwin SE, Conrad CH et al (1994) Effects of treppe and calcium on intracellular calcium and function in the failing heart from the spontaneously hypertensive rat. *Hypertension* 24:347–356
- Cerbai E, Barbieri M, Li Q, Mugelli A (1994) Ionic basis of action potential prolongation of hypertrophied cardiac myocytes isolated from hypertensive rats of different ages. *Cardiovasc Res* 28:1180–1187
- Diaz ME, Graham HK, Trafford AW (2004) Enhanced sarcolemmal Ca^{2+} efflux reduces sarcoplasmic reticulum Ca^{2+} content and systolic Ca^{2+} in cardiac hypertrophy. *Cardiovasc Res* 62:538–547
- duBell WH, Boyett MR, Spurgeon HA, Talo A, Stern MD, Lakatta EG (1991) The cytosolic calcium transient modulates the action potential of rat ventricular myocytes. *J Physiol* 436:347–369
- Froehlich JP, Lakatta EG, Beard E et al (1978) Studies of sarcoplasmic reticulum function and contraction duration in young adult and aged rat myocardium. *J Mol Cell Cardiol* 10:427–438
- Hasenfuss G (1998) Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovascular Res* 39:60–76
- Houser S, Piacentino V III, Weisser J (2000) Abnormalities of calcium cycling in the hypertrophied and failing heart. *J Mol Cell Cardiol* 32:1595–1607
- Janczewski AM, Spurgeon HA, Lakatta EG (2002) Action potential prolongation in cardiac myocytes of old rats is an adaptation to sustain youthful intracellular Ca^{2+} regulation. *J Mol Cell Cardiol* 34:641–648
- Josephson IR, Guia A, Stern MD et al (2002) Alterations in properties of L-type Ca channels in aging rat heart. *J Mol Cell Cardiol* 34:297–308
- Jullien T, Cand F, Fargier C et al (1989) Age-dependent differences in energetic status, electrical and mechanical performance of rat myocardium. *Mech Ageing Dev* 48:243–254
- Kokubo M, Uemura A, Matsubara T et al (2005) Noninvasive evaluation of the time course of change in cardiac function in spontaneously hypertensive rats by echocardiography. *Hypertens Res* 28:601–609
- Lakatta EG (1987) Do hypertension and aging have a similar effect on the myocardium? *Circulation* 75(1 Pt 2):169–177
- Lakatta EG (2003) Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part III: cellular and molecular clues to heart and arterial aging. *Circulation* 107:490–497
- Lakatta EG, Sollott SJ (2002) Perspectives on mammalian cardiovascular aging: humans to molecules. *Comp Biochem Physiol A Mol Integr Physiol* 132:699–721
- Linz KW, Meyer R (2000) Profile and kinetics of L-type calcium current during the cardiac ventricular action potential compared in guinea-pigs, rats and rabbits. *Pflugers Arch* 439:588–599
- Liu SJ, Wyeth RP, Melchert RB et al (2000) Aging-associated changes in whole cell K^+ and L-type Ca^{2+} currents in rat ventricular myocytes. *Am J Physiol* 279:H889–H900
- Lorell BH, Carabello BA (2000) Left ventricular hypertrophy: pathogenesis, detection, and prognosis. *Circulation* 102:470–479
- Lushnikova EL, Nepomnyashchikh LM, Klinnikova MG (2001) Morphological characteristics of myocardial remodeling during compensatory hypertrophy in aging Wistar rats. *Bull Exp Biol Med* 132:1201–1206
- MacDonnell SM, Kubo H, Crabbe DL et al (2005) Improved myocardial β -adrenergic responsiveness and signaling with exercise training in hypertension. *Circulation* 111:3420–3428
- Mace LC, Palmer BM, Brown DA et al (2003) Influence of age and run training on cardiac Na^+ / Ca^{2+} exchange. *J Appl Physiol* 95:1994–2003
- Maier LS, Bers DM, Pieske B (2000) Differences in Ca^{2+} -handling and sarcoplasmic reticulum Ca^{2+} -content in

- isolated rat and rabbit myocardium. *J Mol Cell Cardiol* 32:2249–2258
- Mill JG, Novaes MA, Galon M et al (1998) Comparison of the contractile performance of the hypertrophied myocardium from spontaneous hypertensive rats and normotensive infarcted rats. *Can J Physiol Pharmacol* 76:387–394
- Nand V, Doggrell SA, Barnett CW (1997) Effects of veratridine on the action potentials and contractility of right and left ventricles from normo- and hypertensive rats. *Clin Exp Pharmacol Physiol* 24:570–576
- Ohta K, Kim S, Hamaguchi A et al (1995) Expression of sarcoplasmic reticulum Ca^{2+} -ATPase mRNA in the hypertrophied heart of young spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol Suppl* 22:S228–S229
- Orchard CH, Lakatta EG (1985) Intracellular calcium transients and developed tensions in the rat heart muscle: a mechanism for the negative interval-strength relationship. *J Gen Physiol* 86:637–651
- Palatini P, Frigo G, Visentin P et al (2001) Left ventricular contractile performance in the early stage of hypertension in humans. *Eur J Appl Physiol* 85:118–124
- Perez GN, Petroff MV, Mattiazzi A (1993) Rested-state contractions and rest potentiation in spontaneously hypertensive rats. *Hypertension* 22:306–314
- Pieske B, Sutterlin M, Schmidt-Schweda S et al (1996) Diminished post-rest potentiation of contractile force in human dilated cardiomyopathy. Functional evidence for alterations in intracellular Ca^{2+} handling. *J Clin Invest* 98:764–776
- Schotten U, Koenigs B, Rueppel M et al (1999) Reduced myocardial sarcoplasmic reticulum Ca^{2+} -ATPase protein expression in compensated primary and secondary human cardiac hypertrophy. *J Mol Cell Cardiol* 31:1483–1494
- Song LS, Sobie EA, McCulle S et al (2006) Orphaned ryanodine receptors in the failing heart. *Proc Natl Acad USA* 103:4305–4310
- Tomita F, Bassett AL, Myerburg et al (1994) Diminished transient outward currents in rat hypertrophied ventricular myocytes. *Circ Res* 75:296–303
- Walker KE, Lakatta EG, Houser SR (1993) Age associated changes in membrane currents in rat ventricular myocytes. *Cardiovasc Res* 11:1968–1977
- Weisser-Thomas J, Piacentino V 3rd, Gaughan JP et al (2003) Calcium entry via Na/Ca exchange during the action potential directly contributes to contraction of failing human ventricular myocytes. *Cardiovasc Res* 57:974–985
- Zhu B, Zhang J, Liu N (1999) The sarcoplasmic reticulum Ca handling protein and left ventricular hypertrophy in SHR. *Zhonghua Yi Xue Za Zhi* 79:286–288