Acceleration of Ca^{2+} Repletion in the Junctional Sarcoplasmic Reticulum and Alternation of the Ca^{2+} -Induced Ca^{2+} -Release Mechanism in Hypertensive Rat (SHR) Cardiac Muscle

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Abstract: We estimated the time taken for a repletion of the junctional sarcoplasmic reticulum (JSR) Ca^{2+} stores from a family of mechanical restitution curves after twitches of various magnitudes in the cardiac muscle of hypertensive rats (SHR), using a method described previously (Tameyasu *et al.* *Jpn J Physiol.* 2004;54:209-19), to evaluate abnormality in Ca^{2+} handling by cardiac JSR in hypertension. We found no differences in contractility or in the time course of mechanical restitution between SHR and the controls (WKY) at 3 weeks of age. In comparison to WKY, 7- and 20-week-old SHR showed a greater rested state contraction (RST) and similar or smaller rapid cooling contraction, suggesting that their JSR contains a similar amount of Ca^{2+}

at saturation, but releases more Ca^{2+} upon stimulation. The adult SHR and WKY showed similar mechanical restitution time courses, but the adults had longer pretwitch latencies. The function $G(t)$ representing the time course of JSR Ca^{2+} store repletion in adult SHR exceeded the WKY value at $t \leq 0.5$ s, but the function $H(t)$ representing JSR $[\text{Ca}^{2+}]$ change corresponding to the mechanical restitution after RST was smaller in the adult SHR at $t \leq 0.5$ s, resulting in smaller $H(t)/G(t)$ in adult SHR at $t \leq 0.5$ s. Deviations of $G(t)$, $H(t)$, and $H(t)/G(t)$ from WKY were greater at 20 weeks than at 7. The results suggest an acceleration of JSR Ca^{2+} store repletion and an alternation of the Ca^{2+} -induced release of Ca^{2+} from the JSR in young adult SHR.

Key words: sarcoplasmic reticulum, Ca^{2+} -induced Ca^{2+} release, hypertrophied heart, hypertension, mechanical alternans.

In humans, systemic hypertension causes cardiac hypertrophy as a result of compensation, which, without adequate treatment, leads to cardiac failure and sudden death. Cardiac hypertrophy is associated with marked changes in myocardial contractility; the twitch contraction increases in magnitude and slows in both contraction and relaxation [1–5]. Such changes in myocardial contractility are attributed mainly to an alternation of the Ca^{2+} handling of the sarcoplasmic reticulum (SR). However, no consistent impairment has been found in the SR function of the compensated hearts of various animal models of hypertension. For example, the expression of both the SR Ca^{2+} ATPase and phospholamban increases in the hypertrophied heart of a mouse model [6], but all of the SR Ca^{2+} ATPase, lack of phospholamban and ryanodine receptor change in the spontaneously hypertensive rat (SHR) [7]. The increase, no change, and decrease of $\text{Na}^+/\text{Ca}^{2+}$ exchange activity have been reported in the hypertrophied hearts of canine, mouse, and SHR, respectively [6–8].

Measuring $[\text{Ca}^{2+}]$ change, directly or indirectly, in the lumen of the JSR in a beating heart improves our understanding of hypertrophy. Although Shannon *et al.* [9] directly measured $[\text{Ca}^{2+}]$ in the lumen of the junctional SR

(JSR) in beating myocytes isolated from the rabbit heart, its application has so far been quite limited [10]. It was also reported that in skeletal muscle, a lowering of measured $[\text{Ca}^{2+}]$ in the JSR lumen in response to an electrical stimulus occurred too late and continued to progress after closure of the Ca^{2+} release channels [11]. On the other hand, we studied the time course of mechanical restitution after twitch contractions of various magnitudes and found that mechanical restitution after a pretwitch latency period took a similar time course independently of the magnitude of the preceding twitch [12]. This finding led us to assume that a shorter latency after a smaller preceding twitch was due to a larger amount of Ca^{2+} remaining in the JSR Ca^{2+} stores after the preceding release and vice versa. We also presumed that Ca^{2+} is replenished in the JSR Ca^{2+} stores with a common JSR Ca^{2+} content versus time relationship after various degrees of JSR Ca^{2+} release. By analyzing mechanical restitution after twitches of various magnitudes with intact rat cardiac muscle based on these assumptions, and by assuming a simple function to convert twitch force to JSR $[\text{Ca}^{2+}]$ ($[\text{Ca}^{2+}]_{\text{JSR}}$), we estimated the time course of JSR Ca^{2+} store repletion and the relationship between the amount

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of JSR Ca^{2+} release and the stored Ca^{2+} in the JSR Ca^{2+} stores [12]. We applied this method to SHR cardiac muscle in the present study. The systemic blood pressure in SHR is 1.6 times higher than that in normotensive Wistar-Kyoto rats (WKY), used as controls, at an age as young as 4 weeks, and it increases rapidly until the age of about 10 weeks, then gradually at least until week 20, whereas it did not change appreciably after the age of 4 weeks in WKY [13, 14]. To evaluate which processes in the SR Ca^{2+} handling in SHR cardiac muscle are abnormal and how they proceed, especially in the early stage of cardiac hypertrophy, we therefore analyzed the short-term mechanical restitution of SHR cardiac muscle at three stages of hypertension: at the age of 3 weeks, before the development of severe hypertension, and at the ages of 7 weeks and 20 weeks, when SHR systemic hypertension was 2.0 and 2.4–2.7 times the WKY level, respectively [13, 14]. Since the right ventricular papillary muscle is much thinner and therefore more suitable for studying mechanical properties than the left ventricular papillary muscle, and since SHR developed pulmonary hypertension as well as systemic hypertension [15, 16], we chose to use isolated right ventricular papillary muscle in the present study on the assumption that the contractility of the right muscle changes in a way similar to that of the left one. The results showed no difference in any characteristic of the JSR Ca^{2+} handling between SHR and WKY at 3 weeks of age, but that an acceleration of the JSR Ca^{2+} store repletion and alternation of the JSR Ca^{2+} -induced Ca^{2+} -release mechanism occurred moderately in SHR at 7 weeks and more severely at 20 weeks of age.

MATERIALS AND METHODS

Preparation and experimental protocol. The hearts were isolated from SHR at the ages of 3, 7, and 20 weeks and from age-matched WKY. The SHR and their WKY were treated in accordance with the Japan Physiological Society's Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences. The experimental setup and protocol were similar to those described previously [12]. One end of a papillary muscle isolated from the right ventricle was pinned to the bottom of the experimental chamber, and the other was attached to a force transducer (FD pickup, Nihon Koden). Current pulses (5 ms duration) at a desirable frequency were applied through Pt wire electrodes as contractile stimuli to the muscle in standard Krebs solution (in mM; NaCl 118, KCl 2.5, CaCl_2 1.8, NaHCO_3 25, KH_2PO_4 1.2, MgSO_4 1.2, glucose 10, pH 7.2–7.4) equilibrated with a 95% O_2 and 5% CO_2 mixture at room temperature ($25 \pm 0.5^\circ\text{C}$). The resulting twitch force was recorded on an ink-writing oscillograph. Three serial stimuli were applied to the preparation at intervals of 60 s, producing an initial rested-state

Fig. 1. Short-term mechanical restitution after twitches of various magnitudes. **A:** An illustration showing the experimental protocol used to obtain mechanical restitution. RST is followed by a conditioning twitch contraction (P) with a given interstimulus interval. Subsequently, a test twitch contraction (T) was produced with an interval of t . The t varied with a given P, and f_t/f_0 was plotted against t . **B:** Short-term mechanical restitution after the RST was divided into three phases: (i) a pretwitch latency period, (ii) a phase of fast restitution of twitch contraction, and (iii) a subsequent phase of much slower restitution. **C** and **D:** Mechanical restitution curves obtained by varying the magnitude of a conditioning twitch contraction (P) in a papillary muscle preparation in WKY (**C**) and another in SHR (**D**). f_t/f_0 was plotted against t for curves I_a , I_b , I_c , and I_d . I_o represents mechanical restitution after RST and was obtained by plotting f_p/f_0 against the interval between RST and P. The magnitude of the conditioning twitch, f_p/f_0 , decreased in the order I_a , I_b , I_c , and I_d . Data points were fitted with a straight line.

contraction (RST) followed by a conditioning and a test twitch contraction (Fig. 1A). Mechanical restitution following a given conditioning twitch contraction was determined by varying the interval between the conditioning and the test twitch contractions. By varying the interval between the initial RST and the conditioning twitch, mechanical restitutions were examined after four conditioning twitch contractions with different magnitudes and after the RST. At the end of each experiment, a rapid cooling contracture was produced by rapidly introducing new cool (5°C) Krebs solution while draining off the old. Long and short diameters were measured at the base of the preparation under a binocular microscope to estimate a cross-sectional area, assuming that it was elliptical, and to compute force per unit of that area.

Estimation of time course of JSR Ca^{2+} store repletion.

The time course of JSR Ca^{2+} store repletion was estimated in a way similar to the one described previously [12]. Short-term mechanical restitution generally consists of three phases: (i) a latency period before a detectable twitch contraction is initiated, (ii) a rapid mechanical restitution, followed by (iii) a much slower one (Fig. 1). By observing that a lowering of the magnitude of a preceding twitch contraction shortens the latency without appreciably changing the rate of the phase (ii) restitution (Fig. 1, C and D), we hypothesized that the shorter latency is due to a larger amount of Ca^{2+} remaining in the JSR Ca^{2+} stores after the preceding release. Further, Ca^{2+} is replenished in the JSR Ca^{2+} stores with a common $[\text{Ca}^{2+}]_{\text{JSR}}$ versus time relationship after various degrees of JSR Ca^{2+} release.

The above assumptions make it possible to determine the common $[\text{Ca}^{2+}]_{\text{JSR}}$ versus time relationship as follows. Let us consider an RST with a magnitude of f_o followed by an interval of ao by an RST of conditioning twitch contraction with a magnitude of fa , which is in turn followed by a test twitch contraction of the same magnitude (fa) with an interval of ap from the preceding twitch contraction, i.e., $y = fa$ at $x = ao$ on the mechanical restitution curve denoted by Io and $x = ap$ on the curve Ia in Fig. 2A. In this case, as shown in Fig. 2B, it is expected that after the JSR Ca^{2+} release to produce the RST, the $[\text{Ca}^{2+}]_{\text{JSR}}$ increases along a curve representing JSR Ca^{2+} store repletion versus time relationship, termed $G(t)$, and reaches the point Ao with a time lapse of ao , giving us the coordinates of $Ao \{ao, G(ao)\}$. JSR Ca^{2+} release upon stimulation at point Ao results in a reduction of $[\text{Ca}^{2+}]_{\text{JSR}}$ by $H(ao)$, which causes a conditioning twitch contraction with a magnitude of fa . After this Ca^{2+} release, $[\text{Ca}^{2+}]_{\text{JSR}}$ increases from point Ap along $G(t)$, the y-coordinate of which is given by subtracting $H(ao)$ from $G(ao)$, and again reaches the point Ao with a time lapse of ap , at which the JSR Ca^{2+} release upon stimulation produces a test twitch contraction with a magnitude of fa . The coordinates of point Ap are then $x = ao - ap$ and $y = G(ao) - H(ao)$. Thus the x- and y-coordinates of points Ao and Ap are determined. In a

Fig. 2. The procedure used to determine the time course of JSR Ca^{2+} store repletion from the short-term mechanical restitution. In **A**: Io represents phase (ii) in the mechanical restitution after RST, and Ia , Ib , Ic , and Id after twitches with magnitudes of fa , fb , fc , and fd , respectively. The twitch levels fa – fd are reached at times ao – do on line Io and at ap – dp on lines Ia – Id , respectively. **B**: Relationship between JSR Ca^{2+} store repletion and JSR Ca^{2+} release. Abscissa, time after onset of RST. Ordinate, change of $[\text{Ca}^{2+}]_{\text{JSR}}$ from the lowest level of $[\text{Ca}^{2+}]_{\text{JSR}}$ after JSR Ca^{2+} release producing RST. When RST is followed by a conditioning twitch contraction with a magnitude of fa and an interval ao and subsequently by a test twitch contraction with the same magnitude as fa with an interval of ap , the JSR releases Ca^{2+} at the point Ao in the amount of $H(ao)$, producing the conditioning twitch contraction. $[\text{Ca}^{2+}]_{\text{JSR}}$ then increases from point Ap and reaches the point Ao with a time expense of ap . This procedure determines the x- and y-coordinates of Ao and Ap . Similarly, the x- and y-coordinates of Bo – Do and Bp – Dp are determined using mechanical restitution curves I_o , I_b , I_c , and I_d . Connecting these points in chronological order gives a curve representing $G(t)$. For details, see text.

similar way, other pairs of points, $Bo\{bo, G(bo)\}$ and $Bp\{bo - bp, G(bo) - H(bo)\}$, $Co\{co, G(co)\}$ and $Cp\{co - cp, G(co) - H(co)\}$, and $Do\{do, G(do)\}$ and $Dp\{do - dp, G(do) - H(do)\}$, are determined by using paired mechanical restitution curves I_o – I_b , I_o – I_c , and I_o – I_d , respectively.

The values of the x-coordinates for points Ao , Bo , Co , Do and Ap , Bp , Cp , Dp were determined experimentally.

To determine the y -coordinates of these points, on the other hand, the magnitude of twitch was related to decrements of $[Ca^{2+}]_{JSR}$ in the following way. The magnitude of twitch was first related to a causal peak magnitude of cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_{cy}$) by using an empirical equation of $[Ca^{2+}]_{cy}$ (μM) = $1.6 f_i/f_o + 0.2$, which was determined by approximating the relation between the magnitudes of twitch contraction and peak $[Ca^{2+}]_{cy}$ reported by Backs *et al.* [17] with rat cardiac muscle and by assuming that $[Ca^{2+}]_{cy} = 0.1 \mu M$ at rest. The peak $[Ca^{2+}]_{cy}$ was then converted to a change of $[Ca^{2+}]_{JSR}$ by regarding the JSR volume as 1/1,000 of the cytoplasmic volume [18]. As we will describe later, the maximal twitch force was about 1.4 times greater in adult SHR than in the WKY, and assuming that the Ca^{2+} sensitivity of contractile elements in SHR preparation is similar to WKY, so that the equation $[Ca^{2+}]_{cy}$ (μM) = $2.24 f_i/f_o + 0.2$ was used to convert twitch force to a change of $[Ca^{2+}]_{cy}$ in SHR at the ages of 7 and 20 weeks.

After twitch force was converted to changes of $[Ca^{2+}]_{JSR}$, points Ap , Bp , Cp , Dp , Do , Co , Bo , and Ao were connected to determine $G(t)$ in such a way that the resulting $G(t)$ increases monotonically and smoothly with time (Fig. 2B). The present analysis of mechanical restitution, however, does not provide a unique $G(t)$, but only the uppermost and lowermost $G(t)$ s. Then the lowermost $G(t)$ that increases with time at the lowest rate in the early phase and the highest rate in the later phase among possible $G(t)$ s was selected for the present purpose, since it seemed most realistic (for detail, see [12]). Neither the conversion function between twitch force and $[Ca^{2+}]_{JSR}$ change nor was the selection of $G(t)$ critical to the present conclusion, since they equally affected data from both SHR and WKY.

The function $H(t)$, which represents the amount of the reduction of $[Ca^{2+}]_{JSR}$ because of the JSR Ca^{2+} release to produce a twitch contraction upon stimulation, was simply determined by converting a twitch force to a change of $[Ca^{2+}]_{JSR}$. That is, $H(t)$ is the JSR representation of mechanical restitution after the largest twitch contraction.

Statistics. Data were expressed as mean $\pm$ SEM. Straight lines or curves were fitted to data using the least squares method unless otherwise stated. Statistically significant differences were calculated by Student's t -test and a two factor analysis of variance (ANOVA). A p value of less than 0.05 was considered significant.

RESULTS

1. Basic contractile parameters

Table 1 shows the basic contractile parameters in SHR and WKY together with their body (BW) and heart weights (HW). The HW/BW was greater in SHR than in WKY at the ages of 3, 7, and 20 weeks because of the smaller BWs and hypertrophied hearts in SHR compared with those of WKY, as reported earlier [13, 19]. The magnitudes of RST in SHR were equal to those in WKY at 3 weeks and increased to 1.31 and 1.46 times those of the latter at 7 and 20 weeks of age, respectively, together with a half-width increase of the RST, in agreement with earlier reports [3, 20]. The magnitude of rapid cooling contracture in SHR, which has commonly been used as an index of the SR Ca^{2+} content, was similar to that in WKY at 3 and 20 weeks and slightly smaller than that of WKY at 7 weeks of age. The results suggest that the SR function in SHR is normal at the age of 3 weeks and that the JSR saturated with Ca^{2+} releases more Ca^{2+} upon electrical stimulation in SHR than WKY at the ages of 7 and 20 weeks,

Table 1. Basic contractile parameters of cardiac papillary muscle and BW and HW in WKY and SHR.

		3 weeks	7 weeks	20 weeks
BW (g)	WKY	63.9 $\pm$ 0.9 (6)	303.6 $\pm$ 5.2 (7)	465.7 $\pm$ 14.9 (7)
	SHR	57.9 $\pm$ 1.9* (8)	257.6 $\pm$ 2.8*** (9)	405.0 $\pm$ 3.7** (9)
HW (g)	WKY	0.35 $\pm$ 0.02 (6)	0.97 $\pm$ 0.05 (7)	1.44 $\pm$ 0.05 (7)
	SHR	0.40 $\pm$ 0.01* (8)	1.35 $\pm$ 0.07*** (9)	2.26 $\pm$ 0.12*** (9)
HW/BW	WKY	5.4 $\pm$ 0.1 (6)	3.1 $\pm$ 0.1 (7)	3.1 $\pm$ 0.03 (7)
	SHR	6.8 $\pm$ 0.9** (8)	5.3 $\pm$ 0.6*** (9)	5.3 $\pm$ 0.2*** (9)
f_o (mN/mm ²)	WKY	17.9 $\pm$ 0.9 (5)	16.3 $\pm$ 0.8 (6)	16.0 $\pm$ 1.4 (5)
	SHR	17.6 $\pm$ 0.6 (6)	21.5 $\pm$ 0.8** (5)	23.4 $\pm$ 1.4** (6)
fr (mN/mm ²)	WKY	20.2 $\pm$ 1.3 (5)	20.0 $\pm$ 0.7 (5)	17.8 $\pm$ 0.6 (6)
	SHR	18.7 $\pm$ 0.5 (8)	16.3 $\pm$ 1.1*** (6)	16.6 $\pm$ 0.5 (6)
fw (ms)	WKY	242 $\pm$ 10 (6)	254 $\pm$ 7 (9)	287 $\pm$ 4 (6)
	SHR	260 $\pm$ 9 (8)	363 $\pm$ 13*** (9)	398 $\pm$ 17*** (6)

Sample number n is shown in parenthesis. f_o , magnitude of RST; fr , magnitude of rapid cooling contracture; fw , half width of RST. SHR vs. WKY; * p < 0.05, ** p < 0.01, *** p < 0.001.

Fig. 3. Relationships between the pretwitch latency and the magnitude of the conditioning twitch contraction (f_1/f_0) (A, B, C) and between the slope in the phase (ii) of mechanical restitution and f_1/f_0 (D, E, F). Ages 3 (A, D), 7 (B, E), and 20 weeks (C, F). Open circles, WKY; closed circles, SHR. $n = 5-8$. * $p < 0.05$, *** $p < 0.001$.

though the amount of Ca^{2+} in JSR at saturation in SHR is equal to or slightly less than that in WKY.

2. Mechanical restitution

Examples of mechanical restitution in SHR and WKY are shown in Fig. 1, C and D. An increase of the magnitude of a conditioning twitch contraction generally prolonged a pretwitch latency. The latencies of the SHR and WKY were similar at the age of 3 weeks, except that the former was greater than the latter at $f_1/f_0 = 1$. The latency increased with age in both groups, but at a greater rate in SHR; thus it was greater in SHR throughout the f_1/f_0 range at the age of 20 weeks (Fig. 3C).

The slope of phase (ii) in mechanical restitution was

constant and independent of the magnitude of the preceding twitch contraction in both SHR and WKY at all ages examined, except that the slope at $f_1/f_0 = 1$ in SHR at the ages of 7 and 20 weeks was smaller than the rest (Fig. 3, E and F).

3. $G(t)$ and $H(t)$

Neither $G(t)$ nor $H(t)$ showed significant age dependence in WKY. Both $G(t)$ and $H(t)$ in SHR at the age of 3 weeks were similar to WKY. At both 7 and 20 weeks of age, the SHR had a greater RST than the WKY, as stated previously. Because of the limited accuracy of estimation of the cross-sectional area of the preparation, it was tentatively assumed that the RST was 1.4 times greater in SHR

Fig. 4. $G(t)$ and $H(t)$ in WKY (A, B, C) and SHR (D, E, F). Ages 3 (A, D), 7 (B, E), and 20 weeks (C, F). Curves were fitted to data visually for $G(t)$ and by straight lines for $H(t)$ using the least squares method. Broken curves in E and F represent $G(t)$ and $H(t)$ in the age-matched WKY. Ordinate change of $[Ca^{2+}]_{JSR}$ after JSR Ca^{2+} release producing RST. Abscissa, time after the onset of RST before the conditioning twitch contraction. $n = 5-8$.

than in the WKY at these ages. Then a greater RST in SHR at the ages of 7 and 20 weeks resulted in a greater $G(t)$ than WKY. Similarly, a longer pretwitch latency in SHR at these ages shifted $H(t)$ rightward, compared to WKY (Fig. 4, E and F).

4. Efficiency of JSR Ca release

$H(t)/G(t)$, which represents the apparent, not the true, efficiency of JSR Ca^{2+} release, increased generally with increases of the value of $G(t)$ (Fig. 5), reaching unity eventually by definition. There was no age dependence in the plots of $H(t)/G(t)$ versus $G(t)$ in WKY at the ages of 3–20 weeks. Plots of $H(t)/G(t)$ versus $G(t)$ in SHR were similar to those in WKY at 3 weeks, shifted rightward at 7 weeks, and then rightward and downward at 20 weeks of age. This suggests that the apparent efficiency of the JSR Ca^{2+} release in SHR decreases with aging between 3 to 7 weeks of age and beyond, compared to WKY.

$G(t)$ was determined to be in the range of $t \leq 0.55$ s in the present study. By approximating $G(t)$ in this range of t with a logarithmic function and extrapolating it beyond $t = 0.55$ s, an overall time course of $G(t)$ was estimated. On the basis of the present result indicating that the Ca^{2+} content of the JSR at saturation in SHR is similar to that in the WKY, together with a previous report showing that the JSR loaded fully with Ca^{2+} releases half of its contents upon stimulation in normal rat and rabbit cardiac muscle [9, 13], the function $J(t)$ representing an absolute, not a change of, $[Ca^{2+}]_{JSR}$ as a function of time after maximal JSR Ca^{2+} release was considered to show the true, not the apparent, efficiency of the JSR Ca^{2+} release. That is, $J(t)$ is the sum of $G(t)$ and $[Ca^{2+}]_{JSR}$ immediately after the maximal release of JSR Ca^{2+} , whose concentration was 1.7 and 1.06 mM, respectively, for the WKY and the SHR aged 7 and 20 weeks. Since by definition $H(t)$ corresponds to mechanical restitution after the largest twitch contraction

(see MATERIALS AND METHODS), $H(t)/J(t)$ representing the true, instead of the apparent, efficiency of JSR Ca^{2+} re-

lease was estimated. Figure 6 shows the time courses of $J(t)$, $H(t)$, and $H(t)/J(t)$ thus obtained in the range of $t < 4$ s in SHR and WKY aged 20 weeks.

DISCUSSION

By observing the similarity of the time courses of mechanical restitution after twitches of various magnitudes, except for the pretwitch latency that was shorter after a smaller preceding twitch, we hypothesized that Ca^{2+} is replenished in the JSR Ca^{2+} stores with a common $[\text{Ca}^{2+}]_{\text{JSR}}$ versus time relationship after various degrees of JSR Ca^{2+} release and constructed $G(t)$ from a family of mechanical

Fig. 5. Relationship between $[\text{Ca}^{2+}]_{\text{JSR}}$ and the JSR Ca^{2+} release determined from $G(t)$ and $H(t)$. Ratio of the JSR Ca^{2+} release and the change of $[\text{Ca}^{2+}]_{\text{JSR}}$ after the JSR Ca^{2+} release producing RST was plotted against changes of $[\text{Ca}^{2+}]_{\text{JSR}}$. Open circles, WKY; closed circles, SHR. Ages 3 (A), 7 (B), and 20 weeks (C). Curves were fitted to data visually. The broken lines in C represent the $G(t)$ and $H(t)$ curves shown in B. $n = 6-8$.

Fig. 6. Estimated $J(t)$, $H(t)$, and $H(t)/J(t)$ up to $t = 4$ s in 20-week-old SHR and the WKY. It is assumed that $[\text{Ca}^{2+}]_{\text{JSR}} = 3.4$ mM at saturation in both WKY and SHR. In A: Curves for $J(t)$ in the WKY (solid) and SHR (broken) were obtained by fitting data in Fig. 4, C and F, respectively, with logarithmic functions and then adding the $[\text{Ca}^{2+}]_{\text{JSR}}$ remaining immediately after the JSR Ca^{2+} release, producing RST; $y = 3.4 - 1.98 \exp(-6.07 t)$ in the WKY, $y = 3.4 - 2.2 \exp(-15.2 t) - 0.83 \exp(-5.71 t)$ in SHR. Curves for $H(t)$ were obtained by fitting data from mechanical restitution in the WKY ($n = 5$) and SHR ($n = 5$) with logarithmic functions $y = 0.91 \ln(t) + 1.56$ in WKY, $y = 2 - 5.3 \exp(-4.1 t) - 0.9 \exp(-2.2 t)$ in SHR. B: $H(t)/J(t)$ curve in the WKY (solid) and SHR (broken). Ordinate, $[\text{Ca}^{2+}]_{\text{JSR}}$. Abscissa, time after onset of RST before the conditioning twitch contraction.

restitution curves after twitches of various magnitudes. In rat cardiac muscle, the action potential lengthens in a preceding interval < 0.25 s, causing a slight prolongation of Ca^{2+} influx with a reduced peak amplitude [21, 22]. Such a lengthening of action potential has little effect on the determination of the time course of mechanical restitution, since a sudden lengthening of the action potential does not affect the following JSR Ca^{2+} release to a significant extent, but increases the JSR Ca^{2+} content for the next release [22].

The curvature of $G(t)$ is sensitive to the manner in which twitch force is converted to a change of $[\text{Ca}^{2+}]_{\text{JSR}}$. A steady-state S-shaped relation between the contractile force and $[\text{Ca}^{2+}]$ reported in intact rat cardiac muscle [17], instead of the linear relationship used in the present study, leads theoretically to a $G(t)$ that increases with time initially at a decreasing rate, but later at an increasing rate. Such a $G(t)$ is unlikely. Actually, $[\text{Ca}^{2+}]_{\text{cy}}$ does not reach a steady state in a twitch contraction [23]. So the empirical linear relationship used in the present study may not be the best, but it is better than the S-shaped relationship.

Aging from 3 to 20 weeks had little effect on either $G(t)$ or $H(t)$ and therefore not on $H(t)/G(t)$, the apparent efficiency of the JSR Ca^{2+} release, in normal cardiac muscle in the present study. Although cardiac hypertrophy has already appeared in SHR at the age of 3 weeks, there were no differences in $G(t)$, $H(t)$, and $H(t)/G(t)$, or in the magnitude of rapid cooling contracture between SHR and WKY, indicating that the function of cardiac SR is normal in SHR at this age. In SHR at the age of 7 weeks, $G(t)$ started to increase with some delay but at a greater rate initially with a rightward shift of $H(t)$, compared to the WKY, resulting in a rightward shift of a plot of $H(t)/G(t)$ versus $G(t)$ (Fig. 5, B and C). All of the changes in $G(t)$, $H(t)$, and a plot of $H(t)/G(t)$ versus $G(t)$ became more prominent in SHR during aging from 7 to 20 weeks. That is, the adult SHR exhibits an increased rate of JSR Ca^{2+} store repletion after a maximal Ca^{2+} release, together with a decrease of the apparent efficiency of the JSR Ca^{2+} release. Such alternations of the SR function in SHR progress with aging. During aging from 7 to 20 weeks, the systemic blood pressure in SHR increases from 2.0 to 2.4–2.7 times that in the WKY [13, 14]. Since SHR can be a model of pulmonary as well as systemic hypertension [15, 16], it is expected that pulmonary blood pressure increases with aging in a way similar to that of the systemic blood pressure in SHR. It is then quite likely that the SR function in the right ventricular papillary muscle of SHR alters in response to an increase of pulmonary blood pressure. The delay in the JSR Ca^{2+} stores repletion in the adult SHR compared to the WKY may not be due to a prolongation of the SR Ca^{2+} uptake, since there is little difference in the time course of $[\text{Ca}^{2+}]_{\text{cy}}$ change in response to stimulation between young adult SHR and the WKY as long as the magnitude of their twitch force is comparable [7]. One possibility

that may account for the delay in the onset of $G(t)$ in the adult SHR is that the transportation of Ca^{2+} from the Ca^{2+} uptake sites in central SR to JSR may be abnormally slow in the adult SHR.

The magnitude of rapid cooling contracture in SHR, which is commonly used as an index of the SR Ca^{2+} content, was equal to or even smaller than that in the WKY in the present study (Table 1), consistent with that in earlier histochemical studies [24, 25], but it contradicted the findings of another study with voltage-clamp and $[\text{Ca}^{2+}]_{\text{cy}}$ measurement, showing that SHR myocytes have an increased SR Ca^{2+} content [4]. This discrepancy may have resulted from different kinetics in the SR Ca^{2+} handling between the tissues and isolated single myocyte preparations, though a further sophisticated study is required to settle this question. In the present study, the true efficiency of JSR Ca^{2+} release, i.e., $H(t)/J(t)$, was derived on the basis of an assumption that JSR Ca^{2+} content saturated with Ca^{2+} in SHR is similar to that in the WKY. If this is really so, the true efficiency is smaller in adult SHR than in the WKY in the early phase of JSR Ca^{2+} store repletion, but increases after the $H(t)$ value; therefore the magnitude of twitch contraction in SHR exceeds those in the WKY (Fig. 6B). The present study accounts for the higher $[\text{Ca}^{2+}]_{\text{cy}}$ transient and therefore for the greater magnitude of twitch contraction in adult SHR than in the WKY at a low stimulus frequency [1–5] because of increased JSR Ca^{2+} release resulting from an increase in the true efficiency of the JSR Ca^{2+} release, not from an increased SR Ca^{2+} content as was claimed previously [4].

Heart rate in SHR and the WKY at ages of around 20 weeks is about 370 beats per min (bpm) *in vivo* [14]. This rate may be about 120 bpm or have an interval between beats of 0.5 s under the present condition (25°C) with a presumed temperature coefficient of about 3. A steady-state twitch force at 120 bpm was 0.52 and 0.50 fo, or $H(t) = 0.93$ and 1.22 mM $[\text{Ca}^{2+}]_{\text{JSR}}$, in WKY and SHR, respectively (unpublished data). These values of $H(t)$ predict steady-state twitches with intervals of 0.34 s and 0.45 s in WKY and SHR, respectively, when computed using the graph shown in Fig. 4, C and F. This means a discrepancy between the steady-state twitch force *in vivo* and that predicted from a set of $G(t)$ and $H(t)$ obtained in the present study. This discrepancy remains to be elucidated.

Consequently, the present study suggests an acceleration of the JSR Ca^{2+} stores repletion and an alternation of the mechanism of Ca^{2+} -induced release of Ca^{2+} from the JSR in young adult SHR. The true efficiency of the JSR Ca^{2+} release in young adult SHR may be lower during beating at a high frequency, but greater at a low frequency. Such abnormalities in the Ca^{2+} handling of the SR probably advance with aging.

REFERENCES

1. Conrad CH, Brooks WW, Robinson KG, Bing OHL. Impaired myocardial function in spontaneously hypertensive rats with heart failure. *Am J Physiol.* 1991; 260: H136-45.
2. Gwathmey JK, Warren SE, Briggs GM, Coelas L, Feldman MD, Phillips PJ, Callahan M Jr, Schoen FJ, Grossman W, Morgan JP. Diastolic dysfunction in hypertrophic cardiomyopathy: effect on active force generation during systole. *J Clin Invest.* 1991; 87: 1023-31.
3. Brooksby P, Levi AJ, Jones JV. Contractile properties of ventricular myocytes isolated from spontaneously hypertensive rat. *J Hypertens.* 1992; 10:521-7.
4. Brooksby P, Levi AJ, Jones JV. Investigation of the mechanisms underlying the increased contraction of hypertrophied ventricular myocytes isolated from the spontaneously hypertensive rat. *Cardiovasc Res.* 1993; 27: 1268-77.
5. Mill JG, Novaes MAS, Galon M, Nogueira JB, Vassallo DV. Comparison of the contractile performance of the hypertrophied myocardium from spontaneous hypertensive rats and normotensive infarcted rats. *Can J Physiol Pharmacol.* 1998; 76: 387-94.
6. Ito K, Yan X, Tajima M, Su Z, Barry WH, Lorell BH. Contractile reserve and intracellular calcium regulation in mouse myocytes from normal and hypertrophied failing hearts. *Circ Res.* 2000; 87: 588-95.
7. Shorofsky SR, Aggarwal R, Corretti M, Baffa JM, Strum JM, Al-Seikhan BA, Kobayashi YM, Jones LR, Wier WG, Balke CW. Cellular mechanisms of altered contractility in the hypertrophied heart. Big hearts, big sparks. *Circ Res.* 1999; 84: 424-34.
8. Sipido KR, Volders PGA, Marieke de Groot SH, Verdonck F, Van de Werf F, Wellens HJJ, Vos MA. Enhanced Ca²⁺ release and Na/Ca exchange activity in hypertrophied canine ventricular myocytes. Potential link between contractile adaptation and arrhythmogenesis. *Circulation.* 2000; 102: 2137-44.
9. Shannon TR, Guo T, Bers DM. Ca²⁺ scraps. Local depletions of free [Ca²⁺] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca²⁺ reserve. *Circ Res.* 2003; 93: 40-52.
10. Kubalova Z, Terentyev D, Viatchenko-Karpinski S, Nishijima Y, Gyrke I, Terentyeva R, da Cunha DNQ, Sridhar A, Feldman DS, Hamlin RL, Carnes CA, Gyrke S. Abnormal intrastore calcium signaling in chronic heart failure. *Proc Natl Acad Sci USA.* 2005; 102: 14104-9.
11. Bradley SL, Jingsong Z, Leandro R, Thomas RS, Gustavo B, Eduardo R. Depletion "scraps" and dynamic buffering inside the cellular calcium store *Proc Natl Acad Sci USA.* 2006; 103: 2982-7.
12. Tameyasu T, Tanaka M, Ogura S, Sato S. Characterization of junctional sarcoplasmic reticulum Ca²⁺ content and release by short-term mechanical restitution in cardiac muscle. *Jpn J Physiol.* 2004; 54: 209-19.
13. Fukuda S, Tsuchikura S, Iida H. Age-related changes in blood pressure, hematological values, concentrations of serum biochemical constituents and weights of organs in the SHR/lzm, SHRSP/lzm and WKY/lzm. *Exp Anim.* 2004; 53: 67-72.
14. Kokubo M, Uemura A, Matsubara T, Murohara T. Noninvasive evaluation of the time course of change in cardiac function in spontaneously hypertensive rats by echocardiography. *Hypertens Res.* 2005; 28: 601-9.
15. Janssens SP, Thompson BT, Spence CR, Hales CA. Functional and structural changes with hypoxia in pulmonary circulation of spontaneously hypertensive rats. *J Appl Physiol.* 1994; 77: 1101-7.
16. Aharinejad S, Schraufnagel DE, Bck P, MacKay CA, Larson EK, Mikovsky A, Marks Jr SC. Spontaneously hypertensive rats develop pulmonary hypertension and hypertrophy of pulmonary venous sphincters. *Am J Pathol.* 1996; 148: 281-90.
17. Backx PH, Gao W-D, Azan-Backx MD, Marban E. The relationship between contractile force and intracellular [Ca²⁺] in intact rat cardiac trabeculae. *J Gen Physiol.* 1995; 105: 1-19.
18. Moravec CS, Bond M. Calcium is released from the junctional sarcoplasmic reticulum during cardiac muscle contraction. *Am J Physiol.* 1991; 87: H989-97.
19. Brooksby P, Levi AJ, Jones JV. The electrophysiological characteristics of hypertrophied ventricular myocytes from the spontaneously hypertensive rat. *J Hypertens.* 1993; 11: 611-22.
20. Manso AM, Encabo A, Ferrer M, Balfagn G, Salaices M, Marin J. Changes of cardiac homeostasis in spontaneously hypertensive rats. *J Auton Pharmacol.* 1999; 18: 123-30.
21. Schouten VJA. The negative correlation between action potential duration and force of contraction during restitution in rat myocardium. *J Mol Cell Cardiol.* 1986; 18: 1033-45.
22. Bouchard RA, Clark RB, Giles WR. Effects of action potential duration on excitation-contraction coupling in rat ventricular myocytes. Action potential voltage-clamp measurements. *Circ Res.* 1995; 76: 790-801.
23. Isenberg G, Etter EF, Wendt-Gallitelli M-F, Schiefer A, Carrington WA, Tuft RA, Fay FS. Intrasarcomere [Ca²⁺] gradients in ventricular myocytes revealed by high speed digital imaging microscopy. *Proc Natl Acad Sci USA.* 1996; 93: 5413-8.
24. Moravec CS, Keller E, Bond M. Decreased inotropic response to beta-adrenergic stimulation and normal sarcoplasmic reticulum stores in the spontaneously hypertensive rat heart. *J Mol Cell Cardiol.* 1995; 27: 2101-9.
25. Keller E, Bond M, Moravec CS. Progression of left ventricular hypertrophy does not change the sarcoplasmic reticulum calcium store in the spontaneously hypertensive rat heart. *J Mol Cell Cardiol.* 1997; 29: 461-9.