Rested-State Contractions and Rest Potentiation in Spontaneously Hypertensive Rats

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To gain further insight into the excitation-contraction coupling mechanisms in hypertrophy, we studied rested-state contractions, rest decay curves, and rest potentiation under different experimental conditions using papillary muscles of spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar and Wistar-Kyoto (WKY) rats. Under constant stimulation at 1.1 Hz, contractility and relaxation were not significantly different in hypertensive when compared with normotensive animals. Rested-state contraction (the first beat after a rest interval of 15 minutes) increased to $159.2\pm23\%$ and $123.5\pm7.5\%$ of prerest values in Wistar and WKY rats, respectively, whereas in SHR it did not differ from prerest values $(92.8\pm9.8\%)$. Ryanodine, used to preferentially inhibit sarcoplasmic reticulum function, eliminated the differences in rested-state contractions observed between hypertensive and normotensive rats. Maximal rest potentiation (the first beat after a rest interval of 1 minute) was also significantly higher in Wistar and WKY rats than in SHR. These differences persisted at low extracellular Na^+, when Ca^{2+} efflux via the Na^+-Ca^{2+} exchanger was inhibited. Rest decay curves (the decay in contractility from maximal rest potentiation to rested-state contraction) showed a similar pattern in the three rat strains. The results suggest that the altered inotropic responses of the SHR arise from an alteration in calcium handling by the sarcoplasmic reticulum. Experiments on saponin-skinned trabeculae indicated that fractional calcium release induced by caffeine was significantly reduced in the SHR. We conclude that the altered inotropic response observed in SHR may reflect a diminished release of calcium from the sarcoplasmic reticulum. (*Hypertension.* 1993;22:306-314.)

KEY WORDS • hypertrophy • myocardial contraction • papillary muscles • rat, inbred SHR

Myocardial function in different models of hypertrophy and heart failure has been the subject of considerable study. The question of whether hypertrophy is associated with a depressed or normal inotropic state has been raised by numerous investigators and remains controversial.[1-10] It has been shown that the mechanical performance of the hypertrophied heart in spontaneously hypertensive rats (SHR) is preserved at 6 and even 12 months of age when conventionally evaluated; ie, no significant changes were observed in either maximum shortening velocity or peak developed tension.[9,10] By contrast, in vitro studies have shown several types of alterations at different stages of the excitation-contraction coupling mechanism, ie, the cellular membrane[11-13] — including the regulatory activity of both α- and β-adrenergic receptors[13] — the sarcoplasmic reticulum (SR),[14-16] and the myofibrils.[17] All these results were obtained in nonfailing hypertrophied hearts of SHR younger than 12 months.

In ventricular myocardium of most mammalian species, the force of contractions induced by rhythmic stimulation reflects the brief increase in cytosolic free calcium produced by calcium entry through the sarcolemma and

calcium release from intracellular stores. By contrast, contractions observed after rest intervals long enough to permit the development of a rested state (rested-state contractions, RSCs)[18] appear to depend primarily on calcium entry during membrane depolarization.[19] If rest intervals, longer than the basal interval but shorter than those required for the development of RSCs, are imposed, the first beat after the pause is potentiated (rest potentiation). As the rest interval is prolonged, the first beat after the pause increases up to a point (maximal rest potentiation) and then decreases, approaching the rested state asymptotically.[18] This rest-dependent decay of contractility is thought to reflect the gradual loss of calcium from intracellular stores.[19,20] In contrast to what has been described for most mammalian species, the RSC in rat ventricle is higher than steady-state contractions, suggesting that the rat ventricle maintains its store of releasable calcium more effectively over the rest period than other mammalian species.[21-23]

The study of rest potentiation, RSCs, and rest decay curves under different experimental conditions has provided a simple approach for obtaining important, albeit indirect, information on calcium movements and calcium stores of normal myocardium of different species, including the rat.[20,24,25] In the present investigation, we studied rest-dependent changes in contractile parameters to determine whether hypertrophy associated with the occurrence of spontaneous hypertension in the rat affects some of the mechanisms involved in the excitation-contraction coupling of the myocardium. We used interventions such as the addition of ryanodine and

reduction of extracellular sodium as experimental tools to "preferentially" inhibit either SR[26-28] or Ca^{2+} efflux.[20] Additional experiments with saponin-treated trabeculae allowed us to evaluate in situ SR function and myofilament Ca^{2+} responsiveness of the hypertrophied myocardium. Our results demonstrate that both RSC and rest potentiation are depressed in the SHR with respect to Wistar-Kyoto (WKY) and Wistar rats at a stage of evolution of hypertrophy in which basal contractility is still preserved. The data support the contention that this altered response of the SHR arises from an alteration in the mechanism of calcium release from intracellular calcium stores.

Methods

Male SHR and sex-matched normotensive Wistar and WKY rats were used in this study at 6 months of age. The SHR and WKY strains were originally derived from Charles River Breeding Farms, Wilmington, Mass. All animals were housed under identical conditions and had free access to a standard dry meal and water. Systolic blood pressure was measured weekly with the indirect tail-cuff technique. The rats were anesthetized with pentobarbital sodium (8 mg/100 g IP), with the hearts being rapidly removed and placed in an oxygenated Ringer's solution in which one or two papillary muscles from the left ventricle were dissected. Small left ventricular trabeculae, to be made hyperpermeable (see below), were also dissected from the SHR and WKY rat hearts.

Intact Papillary Muscles

The method used for mounting the preparations was essentially identical to that previously described.[8,29] Briefly, the papillary muscles were mounted vertically in chambers containing a Ringer's solution of the following composition (mmol/L): NaCl, 128.30; KCl, 4.70; $CaCl_2$, 1.35; $NaHCO_3$, 20.23; NaH_2PO_4, 0.35; $MgSO_4$, 1.05; and glucose, 11. The solution was equilibrated with a gas mixture of 5% CO_2–95% O_2, and the pH and temperature of the solution were kept constant at 7.40 and 37°C, respectively. Isometric mechanograms were recorded on a Hewlett-Packard 7404A oscillographic recording system equipped with a model 17403A carrier preamplifier and a model 17400C high gain preamplifier. The first derivative of developed tension (T) was obtained by an RC differentiator with a time constant of 2.5 milliseconds. The muscles were stimulated to contract at a frequency of 1.1 Hz by parallel platinum electrodes delivering 10-millisecond pulses at voltages 20% higher than the threshold of each preparation. After mounting, the papillary muscles were allowed to stabilize for 1 hour, during which the muscles were lengthened to the peak of their length-tension curve (L_{max}). After the stabilization period, different protocols were applied to different groups of muscles as described below.

Rested-state contractions. RSCs are defined as the contractions preceded by an interval of rest long enough that their force is not changed by further prolongation of the rest period. Preliminary experiments and experiments from other laboratories under similar experimental conditions[21] have shown that rest periods of 10 to 15 minutes were sufficient. In a first group of muscles, a period of 15 minutes was therefore applied to achieve

the rested state. Recovery of developed tension (DT) and maximal rate of rise of tension (+T) after stimulation was resumed was monitored until a steady state was reached. The last contraction before and the first contraction after the rest period were recorded at a high paper speed and considered to represent the steady state and RSCs, respectively. The general experimental protocol used in these studies is illustrated in the inset to Fig 1. In a second group of experiments, the same experimental protocol was followed in the presence of 3×10^{-8} mol/L ryanodine.

The effects of increasing ryanodine concentration in 10-fold steps from 10^{-10} mol/L on steady-state contractions (ie, while the muscles were paced at 1.1 Hz) were studied in a separate group of experiments. The ryanodine doses were cumulative and were allowed to equilibrate for 45 minutes before concentration was tested and changed.

Rest potentiation and rest decay curves. In a different series of experiments, the inotropic response of papillary muscles from the three rat strains was studied at different rest intervals, from 30 seconds to 15 minutes. The first beat after this latter rest interval is the RSC. After each test interval, the control stimulation rate was maintained long enough to ensure the disappearance of all the effects of the alteration of the stimulation interval. Any stimulation sequence thus included the perturbation of a single stimulation interval. This protocol was performed for each muscle in control Ringer's solution and in a solution with low extracellular [Na$^+$] (70 mmol/L). In an attempt to minimize the positive inotropic action of lowering extracellular Na$^+$, extracellular [Ca^{2+}] was decreased to maintain a constant [Ca^{2+}]-[Na$^+$] relation.

At the end of the experiment, the cross-sectional area was calculated from muscle length (at L_{max}) and weight, assuming a uniform cross section and a specific gravity of 1.0.

Chemically Skinned Trabeculae

Loops of fine silk were tied around the ends of selected left ventricular trabeculae (approximately 200 μm wide, 1 mm long). The preparations were excised and mounted horizontally in an acrylic chamber between an adjustable stainless-steel rod and a fixed-tension transducer (AE801 strain gauge, Aksjeelskapet Micro-Electronikk, Horten, Norway) and stretched approximately 30%. Experimental solutions at 22°C were pumped through the muscle bath at 0.35 mL/min and were removed by a vacuum line.[8]

Force-pCa relation. The trabeculae were treated with saponin (100 μg/mL) for 20 minutes[30] in a relaxing solution containing (mmol/L) KCl, 140; $MgCl_2$, 7; ATP, 5; phosphocreatine, 15; EGTA, 3; and imidazole, 25, as well as 0.1 mg/mL creatine kinase, pH 7.0. The calculated ionic strength of the relaxing solution was 0.180 mol/L. After treatment with saponin, the preparations were sequentially exposed to activating solutions containing selected concentrations of Ca^{2+}. The activating solutions were made by adding $CaCl_2$ to the relaxing solution. The concentration of $CaCl_2$ was adjusted to obtain a desired concentration of free Ca^{2+}. The free ionic concentrations in relaxing and activating solutions were calculated with a computer program provided by Fabiato and Fabiato.[31] After a stabilization period of 20 minutes, the relaxing

TABLE 1.   General Characteristics of Normotensive and Hypertensive Rats

| | Rat strain | | |
Characteristic	Wistar (n=32)	WKY (n=35)	SHR (n=37)
Systolic blood pressure (mm Hg)	126.25±2.28	129.43±1.8	194.19±3.69*†
Body weight (g)	332.19±10.53	292.79±6.33‡	268.91±3.76*†
Heart weight (g)	0.78±0.02	0.81±0.02	0.89±0.01*†
Heart/body wt (mg/g)	2.38±0.04	2.75±0.12‡	3.33±0.06*†

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. Values are mean±SEM.
*$P<.05$, SHR vs Wistar.
†$P<.05$, SHR vs WKY.
‡$P<.05$, WKY vs Wistar.

solution was replaced by activating solutions. The force induced by different calcium concentrations was recorded until a plateau was reached. For the characterization of the force-calcium curves, the data from each experiment were fitted individually to the Hill equation using a nonlinear least-squares method, where T (relative tension)=$[Ca^{2+}]^{nH}/(K+[Ca^{2+}]^{nH})$. The slope coefficient (nH) and the pCa ($-$log $[Ca^{2+}]$) that produced definite percentages of the maximal effect (from 10% to 90%) were computed for each curve.

Sarcoplasmic reticulum Ca^{2+} uptake and release. The experimental protocol used to study SR Ca^{2+} uptake was a modification of the method described by Endo.[32] Trabeculae were treated with a lower saponin concentration (50 μg/mL) to ensure preservation of the SR function.[33,34] After the treatment with saponin, Ca^{2+} in the SR was depleted by exposure to 25 mmol/L caffeine.[32,33] An increase in caffeine concentration to 30 mmol/L does not appear to release additional Ca^{2+} (see "Results," Fig 6A). The fibers were then immersed sequentially in (1) a Ca^{2+}-loading solution (pCa 6) for two different periods: 2 and 7 minutes, (2) a low-EGTA solution (Ca^{2+}-free relaxing solution containing 0.1 mmol/L EGTA), and (3) a caffeine solution (low-EGTA relaxing solution containing 25 mmol/L caffeine). Preliminary experiments indicated that loading periods longer than 5 to 7 minutes did not modify the caffeine-induced transient response. A maximum loading period of 7 minutes was therefore used in the present experiments. Because the concentration of EGTA in the caffeine solution is very low (weak buffer for calcium), a small amount of calcium released from the SR by caffeine can effectively raise the Ca^{2+} concentration around the myofilaments and produce measurable force responses. Ca^{2+} ion quickly diffuses out to a relatively large volume of the solution, bathing the trabeculae, and is chelated by free EGTA. Thus, the caffeine response is transient.[32] The protocol of this experimental series is illustrated in Fig 6A.

In a second series of experiments, the amount of Ca^{2+} released from the SR was examined by exposure to caffeine at various concentrations. The preparations were first loaded with calcium as described above for a fixed period of 7 minutes and then exposed to 1, 5, 10, 25, and 30 mmol/L caffeine. The loading and release procedure was repeated every 7 minutes. In both types of experiments, the amount of Ca^{2+} released from the SR was estimated by measuring both the amplitude of the caffeine-induced contraction[35,36] and the area under the caffeine-induced contraction.[33,37] Although this latter procedure was initially used in skinned fibers from

skeletal muscle[32] and subsequently adopted in skinned cardiac trabeculae,[33,37] the amplitude of the caffeine-induced contraction has been found to be more reproducible than the area under the curve of this contraction.[35] We have therefore used and then compared both methods.

Ryanodine was obtained from Calbiochem Corp, San Diego, Calif. Caffeine and saponin were obtained from Sigma Chemical Co, St Louis, Mo. All other chemicals used were of analytical grade.

Data are expressed as mean±SEM. A multiple comparison test for statistical analysis of differences among group means (Peritz' F test) was used when more than two groups were studied.[38] When data from only two groups were compared, differences were analyzed by applying the Student's t test for nonpaired samples. A value of $P<.05$ was considered significant.

Results

General Characteristics of Rats and of Isometric Contractile Variables

None of the groups of animals studied showed evidence to suggest the presence of clinical cardiac failure. The general characteristics of the hypertensive and age-matched control rats at the time of the experiment are given in Table 1. Mean data for isometric contraction parameters are presented in Table 2. There were no significant differences in any of the mechanical parameters between SHR and either WKY or Wistar rats.

Rested-State Contractions

Fig 1 shows the overall changes in DT and +T after a 15-minute rest interval and after the RSC in Wistar and WKY rats and SHR. In both groups of normotensive animals, RSC was significantly higher than steady-state contraction values. The magnitude of the RSC in SHR was not significantly different from steady-state values and was significantly lower than the RSC in Wistar and WKY rats. In agreement with a previous study,[24] the recovery pattern in Wistar rats consisted of a monotonic decrease to steady state. A similar pattern was observed in WKY rats, although from a lower RSC, whereas there was no obvious difference between RSC and the following beats in SHR.

Effect of Ryanodine on Rested-State Contractions

In another set of experiments, RSCs were studied in the presence of ryanodine. The rationale for this group of experiments was to preferentially inhibit SR function[26-28] without affecting other aspects of the excita-

TABLE 2. Isometric Contraction Data From Normotensive and Hypertensive Rats

Parameter	Rat strain		
	Wistar (n=32)	WKY (n=35)	SHR (n=37)
DT (mN/mm^2)	7.94±1.18	14.01±1.96	11.07±1.67
+Ṫ ([mN/mm^2]/s)	144.45±19.99	231.48±29.89	192.18±28.71
−Ṫ ([mN/mm^2]/s)	95.26±13.04	150.43±20.09	127.89±20.69
TTP (msec)	62.53±1.96	72.00±3.51	70.25±2.91
$t_{1/2}$ (msec)	38.57±1.3	45.75±2.49	46.08±3.05
Cross-sectional area (mm^2)	0.77±0.04	0.89±0.05	0.86±0.04

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; DT, developed tension; +Ṫ, maximal rate of rise of tension; −Ṫ, maximal velocity of relaxation; TTP, time to peak tension; $t_{1/2}$, half relaxation time. Values are mean±SEM.

tion-contraction coupling process.[26,39] With this approach, tension development would be expected to depend primarily on calcium entry during accompanying membrane depolarization.[19,20,24]

Fig 2 shows that the recovery pattern from RSC in the presence of 3×10^{-8} mol/L ryanodine was virtually identical in the three groups. Ryanodine suppressed the potentiated RSC of Wistar and WKY rats and reversed the recovery pattern from a monotonic decrease to a mono-

FIG 1. *Plots show recovery of tension after 15-minute rest interval in Wistar rats, Wistar-Kyoto (WKY) rats, and spontaneously hypertensive rats (SHR). Abscissa values are post-rest beat numbers (ie, beats 1, 2, 3, 5, 7, and 10). Ordinate values are percentage of prerest steady-state tension. Inset shows experimental protocol. *Significant difference between SHR and Wistar rats; +significant difference between SHR and WKY rats. DT, developed tension; +Ṫ, maximal rate of rise of tension.*

tonic increase toward steady state. In SHR, ryanodine produced a depression of RSC similar to that in normotensive rats and also converted the flat pattern of recovery to a monotonic increase to steady state. The inset to Fig 2 shows that ryanodine produced essentially the same decrease relative to steady-state contraction in the three rat groups when muscles were paced at 1.1 Hz. Similar results were obtained when +Ṫ was analyzed. These experiments suggest that calcium influx accompanying membrane depolarization was not altered in the SHR.[19,20,24] They further suggest that an altered function of cardiac SR of SHR is involved in the difference in RSC observed between normotensive and hypertensive rats.

Postrest Potentiation and Rested-State Contraction: Influence of Low Sodium

The inotropic response of the three rat groups was studied at different rest intervals, from 30 seconds to 15 minutes. Because the decay in the magnitude of the first postrest contraction reflects the time-dependent loss of calcium from intracellular stores,[19,20] the main goal of this experimental series was to establish whether the diminished RSC observed in SHR could be explained by an increased time-dependent calcium loss from intracellular stores during the rest interval. The experiments were performed in control Ringer's solution and in a solution with low [Na$^+$] (70 mmol/L) and with Ca^{2+} also reduced by 50%. The latter was used to modify the transmembrane distribution of sodium and calcium in an attempt to inhibit the possible contribution of the Na$^+$-Ca^{2+} exchange process to the removal of calcium from intracellular stores.[20]

The results of these experiments are presented in Fig 3A and 3B. For clarity, only the data obtained for WKY rats and SHR are shown, as the results for WKY and Wistar rats were similar. In control Ringer's solution, rest potentiation was maximal at rest intervals of 30 seconds and 1 minute in both WKY rats and SHR and then progressively decreased until RSC was reached. Although the decay pattern was similar in both rat groups, maximum rest potentiation was significantly greater in WKY rats than in SHR. Rest potentiation for both WKY rats and SHR in the presence of low sodium was of a lower magnitude than that observed with the control Ringer's solution, although peak values were similar to those obtained under control conditions. At low sodium, maximal rest potentiation was higher in WKY rats than in SHR, and in both groups the decline in contractility with longer pauses was abolished. Simi-
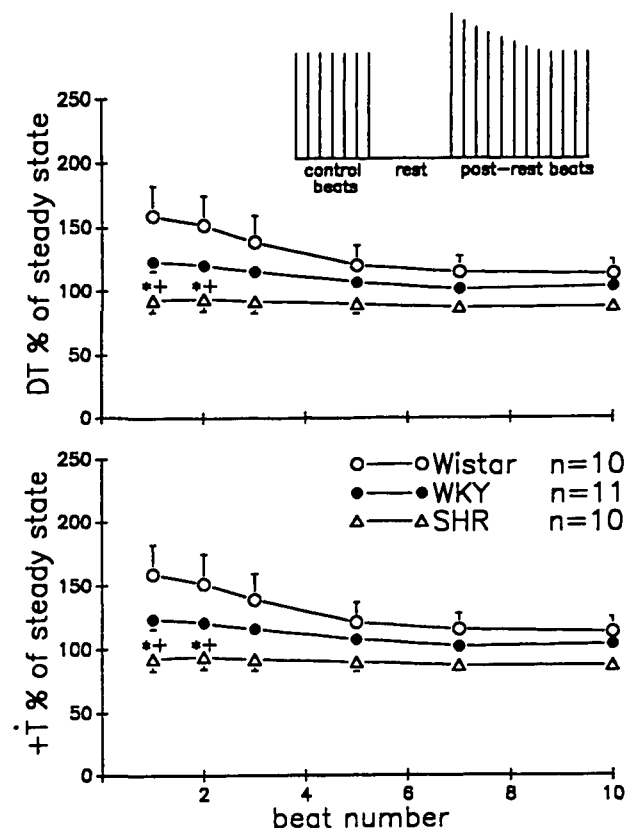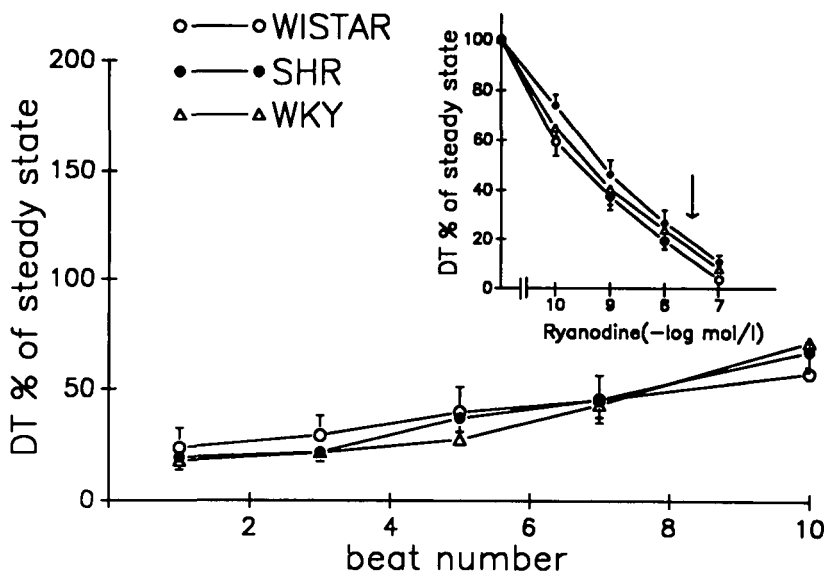
FIG 2. *Plots show postrest recovery of tension in the presence of 3×10^{-8} mol/L ryanodine as percentage of control tension (prerest steady-state tension in the presence of ryanodine) in Wistar rats (n=6), Wistar-Kyoto (WKY) rats (n=8), and spontaneously hypertensive rats (SHR, n=6). Abscissa values are as in Fig 1. Inset shows effects of increasing ryanodine concentration on steady-state contractions. All ryanodine concentrations produced essentially the same decrease in myocardial tension in the three rat groups. Arrow indicates ryanodine concentration used in postrest recovery experiments. Effects of different ryanodine concentrations were studied in 13, 11, and 15 papillary muscles from Wistar and WKY rats and SHR, respectively. DT, developed tension.*

lar results were obtained when DT instead of +T was analyzed. As shown in Fig 3A and 3B, the positive inotropic effect of lowering extracellular sodium was greater in WKY than in SHR papillary muscles ($166 \pm 11\%$ vs $133 \pm 14\%$ of control). This might explain

why maximal rest potentiation and RSC with low sodium were significantly higher in WKY rats than in SHR. However, the differences shown in Fig 3 were still present when the results obtained with low sodium were expressed as a percentage of their own control, ie, the twitch that preceded the pause with low sodium (data not shown).
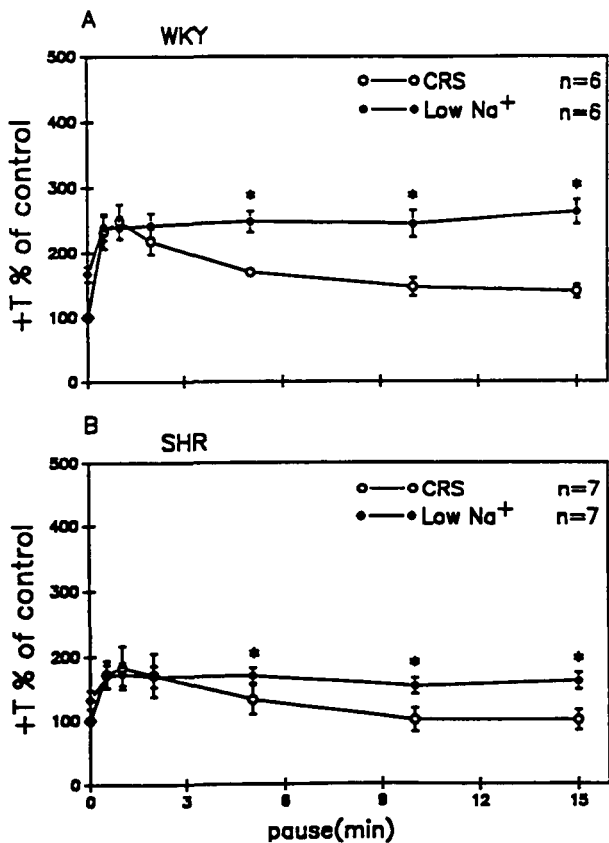

FIG 3. *Plots show first postrest contraction obtained after different time intervals or pauses (ie, 30 seconds and 1, 2, 5, 10, and 15 minutes) in control Ringer's solution (CRS) and low-sodium (70 mmol/L) Ringer's solution in Wistar-Kyoto (WKY) and spontaneously hypertensive rat (SHR) intact papillary muscles. Ordinate values are as in Figs 1 and 2. +T, maximal rate of rise of tension.*
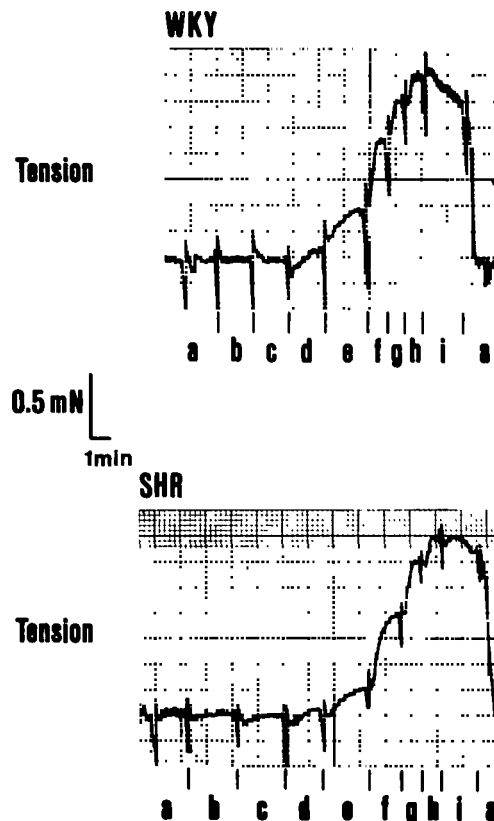

FIG 4. *Typical records of experiments performed on saponin-skinned trabeculae from one Wistar-Kyoto (WKY) rat and one spontaneously hypertensive rat (SHR) show increase in tension as a function of pCa. From a to i, pCa=9 (relaxant solution), 7, 6.75, 6.5, 6.25, 6, 5.75, 5.5, and 4.5.*
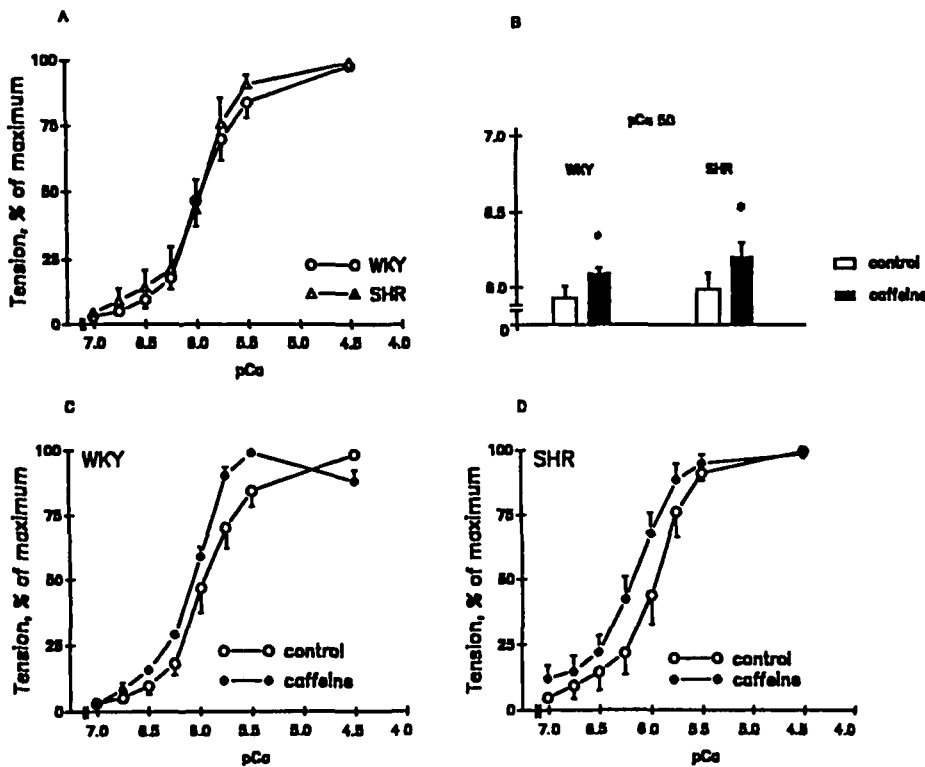
FIG 5. *Panel A: Plot shows tension-pCa relation in Wistar-Kyoto (WKY) and spontaneously hypertensive rat (SHR) saponin-skinned trabeculae. Tension is expressed as percentage of maximal tension. Mean Hill coefficients for control and hypertrophic trabeculae were 2.27 ± 0.3 and 2.23 ± 0.2, respectively. Panel B: Bar graph shows calcium concentration necessary to obtain 50% of maximal response (pCa 50) in absence and presence of 25 mmol/L caffeine. Panels C and D: Plots show effects of preincubation with 25 mmol/L caffeine on tension-pCa relation of saponin-skinned fibers from normal and hypertrophied hearts.*

These experiments provide evidence that the calcium loss from the cell during the rest interval is similar in SHR and WKY and Wistar rats. Rest potentiation is significantly lower in SHR than in WKY and Wistar strains, even when the calcium loss by the Na^+-Ca^{2+} exchanger is minimized.

Calcium Sensitivity of Myofilaments

Fig 4 shows sample recordings of calcium-induced contractions in skinned fibers obtained from WKY and SHR left ventricles. The summarized results of these experiments are presented in Fig 5A. No significant differences were found in either calcium sensitivity of myofilaments or maximal DT when SHR were compared with WKY rats.

Sarcoplasmic Reticulum Calcium Accumulation and Release

In the experiments described in this section, SR calcium uptake and release were estimated by the measurements of both the amplitude[35,36] and integral[33,37] of transient contraction induced by caffeine.

Because caffeine is known to affect calcium sensitivity of the myofilaments,[36] we conducted a pilot series of experiments to determine whether caffeine had a different effect on Ca^{2+} sensitivity of the myofilaments in SHR and WKY rats. Fig 5B through 5D show that, in the presence of 25 mmol/L caffeine, the pCa-tension curve shifted toward lower Ca^{2+} concentrations in both the WKY and SHR groups. The increase in myofilament calcium sensitivity was slightly greater in SHR than in WKY rats, but this difference was not significant.

In a second series of experiments, we examined whether the ability of the SR to accumulate calcium was altered in trabeculae from SHR hearts. The experimental protocol used in these experiments is depicted in Fig 6A. Fig 6B

shows the summarized results of experiments from six WKY and five SHR preparations. The data demonstrate that the time course of Ca^{2+} accumulation by the SR was similar in both rat groups. Typical examples of force transients from which the curves were constructed are shown in Fig 6C.

Fig 7 compares the calcium released from the SR by different caffeine concentrations after the same loading period of 7 minutes at pCa 6. As shown, the fractional release of calcium induced by submaximal caffeine concentrations was smaller in SHR than in WKY rats. This difference attained significant levels at a submaximal caffeine concentration of 10 mmol/L. Results similar to those shown in Figs 6 and 7 were obtained when the area rather than the amplitude of the caffeine-induced contractions was measured. After a calcium loading period of 2 minutes, the areas of caffeine-induced force transients were $68.9 \pm 6.4\%$ (WKY rats) and $66.4 \pm 3.2\%$ (SHR) relative to those obtained after 7 minutes of loading. The fractional release of calcium induced by 10 mmol/L caffeine was $110.9 \pm 18.4\%$ (WKY rats) and $64.8 \pm 9.9\%$ (SHR) when the areas were considered.

Discussion

A major finding of this investigation is that rest potentiation and RSC are depressed in SHR at a stage of evolution of myocardial hypertrophy when basal contractility is still preserved. The results further show that this may represent hypertrophic dependent changes of intracellular calcium handling at the SR level.

It has been shown that the cardiac pump function of the SHR is preserved at 1 year of age. At 18 months and older, changes in isolated muscle performance occur at a time when signs of cardiac failure are reported.[9] The
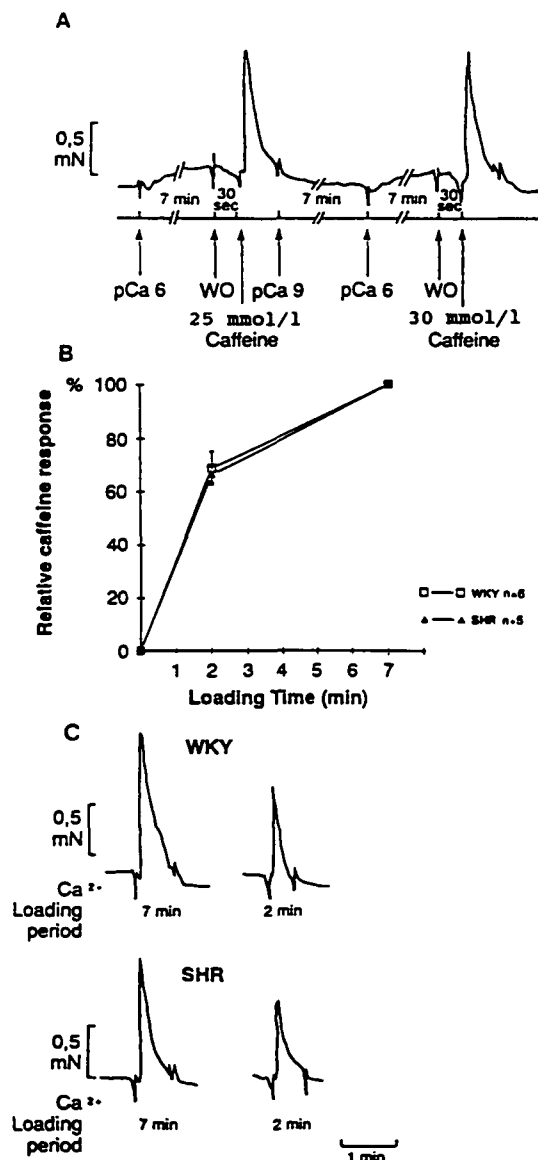
A

B

C

FIG 6. *Panel A: Experimental protocol. Caffeine concentrations higher than 25 mmol/L did not increase caffeine-induced tension transient. WO, washout with Ca^{2+}-free relaxing solution containing 0.1 mmol/L EGTA. Panel B: Plot shows Ca^{2+} accumulation in sarcoplasmic reticulum in Wistar-Kyoto (WKY) and spontaneously hypertensive rat (SHR) fibers. Amount of Ca^{2+} accumulated in sarcoplasmic reticulum was estimated from the relative amplitude of force transient induced by 25 mmol/L caffeine at the end of 10^{-6} mol/L Ca^{2+} loading period. Data are expressed as percentage of caffeine response after 7 minutes of Ca^{2+} loading. Panel C: Representative recordings of tension transients induced by 25 mmol/L caffeine in WKY and SHR skinned trabeculae after loading periods of 7 and 2 minutes with pCa 6.*

6-month-old hypertensive rats used in the current study did not show evidence of clinical cardiac failure, and basal contractility was preserved. The depression in RSC and postrest inotropy observed in this study therefore cannot be attributed to cardiac failure and might represent an early sign in the transition of the functional status of the hearts.
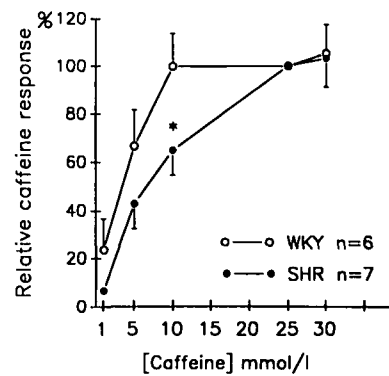

FIG 7. *Plot shows Ca^{2+} release by sarcoplasmic reticulum elicited by submaximal caffeine concentrations. Ca^{2+} release was estimated from the relative amplitude of force transient induced by different submaximal caffeine concentrations at the end of 10^{-6} mol/L Ca^{2+} loading period of 7 minutes. Data are expressed as percentage of the 25 mmol/L caffeine-induced response. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.*

In the present study, RSCs and rest potentiation were used as experimental tools to examine qualitative aspects of the dynamics of excitation-contraction coupling in the hypertrophic heart.[20,21,24] In the absence of changes in the intrinsic sensitivity to Ca^{2+} of the contractile element (Fig 5A), it seems reasonable to assume that changes in intracellular Ca^{2+} dynamics during the myocardial contraction-relaxation cycle may contribute substantially to the diminished postrest response of SHR. A decrease in calcium influx, an increase in the efflux of this cation, or a diminished release of calcium from intracellular stores on resumption of stimulation should be considered as possible intervening factors. The experiments with ryanodine point to the SR as the likely intracellular locus responsible for the depressed response of hypertrophic heart and suggest that calcium influx accompanying membrane depolarization was not altered in the SHR heart. The results obtained at different rest intervals in control and low-sodium solutions further indicate that calcium leak from cardiac cells was similar in SHR and normotensive animals. These findings are consistent with in vitro experiments on isolated SR membrane vesicles from hypertrophied ventricles of 5- to 6-month-old SHR,[16] as well as in hypertrophy induced by abdominal aortic constriction.[40] Taken together, the results obtained in intact papillary muscles leave us with the third of the alternatives suggested above; ie, an impairment of the calcium release mechanism might be involved in the diminished RSC and rest potentiation of SHR.

The availability of selective membrane-disruptive techniques using the detergent saponin[30,32-34,41] allows an in situ evaluation of SR function in hypertrophic myocardium. Although saponin treatment may destroy the transverse tubular network, SR function appears to be well preserved at the saponin concentration used in this study.[33,34,41] Calcium uptake by the SR was not altered in SHR compared with WKY preparations (Fig 6); however, the caffeine-induced calcium release mechanism was depressed (Fig 7). These findings correlate with the conclusions drawn from the current experiments on intact papillary muscles and suggest an alter-

ation of calcium release mechanisms in the hypertrophied heart of SHR. An alternative explanation for our results would be that cellular growth was not accompanied by a parallel development of the SR. Therefore, a given amount of Ca^{2+} released into a greater cytosolic volume would be expected to result in a lower tension via a simple dilution effect. Other experimental evidence indicates that myocyte growth was accompanied by a parallel or even greater increase in the development of sarcotubular membranes and myofibrillar proteins in hypertrophic hearts of SHR as well as in other models of hypertrophy.[16,42]

The calcium release mechanism has not been previously studied in SHR hearts. Experiments performed on saponin-skinned cardiac trabeculae from hypertrophic hearts of rats with abdominal aortic ligation failed to show significant changes in caffeine-induced calcium release.[41] The reason for the discrepancy between these results and ours is not clear and might be attributed either to differences in the inciting stimulus causing the hypertrophy or to a different stage of the hypertrophy. Moreover, more recent experiments from the same group suggested functional abnormalities of SR calcium release in the aortic ligation model of hypertrophy.[33] Experiments by Gwathmey and Morgan[43] suggest a decrease in the rate of both sequestration and release of calcium in hypertrophied ferret right ventricles. The prolongation of intracellular calcium transient observed in SHR failing hearts[44] is also consistent with the concept that hypertrophy impairs intracellular calcium handling.

We are well aware that the differences described between hypertrophic and control hearts may arise from factors not directly studied in our experiments, such as changes in the morphometry or energetics of the myocytes. For the present experiments, papillary muscles from SHR hearts having a cross-sectional area similar to those from Wistar and WKY hearts were chosen. Thus, the influence of different geometric characteristics systematically affecting the response of the papillary muscles of SHR is improbable. For the same reason, the diminished rest potentiation and the RSC of SHR can hardly be associated with a higher degree of hypoxia in the core of these preparations, unless the oxygen consumption of SHR was higher than in normotensive rats. A normal or decreased oxygen consumption with an improved economy of force production has been described in pressure-hypertrophied hearts.[45-48] This improvement in the economy of force production is likely to contribute to the ability of these preparations to generate nearly normal isometric twitch tension, even though the preparations may have extremely large cross-sectional areas.[48] Another possibility to be considered is that both groups of muscles have a similar degree of hypoxia, the hypertrophic myocardium being affected by hypoxia more than the normal myocardium. This possibility cannot be ruled out in our experiments but seems improbable, because no mechanical signs of hypoxia were detected in any of the preparations. The preparations were stable at least during the last 20 minutes of the stabilization period, and no decrease in DT nor any increase in resting tension[49] occurred in any of the preparations used throughout the experiments. Moreover, a similar biochemical and mechanical re-

sponse to hypoxia has been described in papillary muscles of 6-month-old SHR and WKY rats.[49]

In summary, our experiments suggest that the depressed RSC and rest potentiation observed in SHR are determined by modifications in the cellular handling of activator calcium. The experiments further suggest that the intracellular locus of this defective behavior is the SR, possibly through an alteration of the calcium release mechanism. At the stage of the hypertrophy studied here, this defective calcium release would appear only under conditions that require an elevated cardiac inotropism. In connection with this, it is worth emphasizing that in the diminished inotropic response to β-adrenergic stimulation of SHR[50] — a finding generally attributed to an alteration of the β-adrenergic system[50-52] — postreceptor mechanisms like the one described here may also play a role.

Acknowledgments

References
1. Bing O, Matsushita S, Fanburg B, Levine H. Mechanical properties of rat cardiac muscle during experimental hypertrophy. *Circ Res.* 1971;28:234-245.
2. Hamrell B, Alpert N. The mechanical characteristics of hypertrophied rabbit cardiac muscle in the absence of congestive heart failure: the contractile and series elastic elements. *Circ Res.* 1977; 40:20-25.
3. Kissling G, Gassenmaier T, Wendt-Gallitelli MF, Jacob R. Pressure-volume relations, elastic modulus and contractile behavior of the hypertrophied left ventricle of rats with Goldblatt II hypertension. *Pflugers Arch.* 1977;369:213-221.
4. Newman WH, Webb J. Adaptation of left ventricle to chronic pressure overload: response to inotropic drugs. *Am J Physiol.* 1980; 238(*Heart Circ Physiol* 7):H134-H143.
5. Saragoça M, Tarazi R. Left ventricular hypertrophy in rats with renovascular hypertension: alterations in cardiac function and adrenergic responses. *Hypertension.* 1981;3(suppl II):II-171-II-176.
6. Cappasso JM, Strobeck JE, Malhotra A, Scheuer J, Sonnenblick E. Contractile behavior of rat myocardium after reversal of hypertensive hypertrophy. *Am J Physiol.* 1982;242(*Heart Circ Physiol* 11):H882-H889.
7. Gende OA, Mattiazzi A, Camilión MC, Pedroni P, Taquini C, Gomez Llambí H, Cingolani HE. Renal hypertension impairs inotropic isoproterenol effect without β-receptor changes. *Am J Physiol.* 1985;249(*Heart Circ Physiol* 18):H814-H819.
8. Pedroni P, Pérez G, Mattiazzi A. Calcium sensitivity of isometric tension in intact papillary muscles and chemically skinned trabeculae in different models of hypertensive hypertrophy. *Cardiovasc Res.* 1990;24:584-590.
9. Bing OHL, Wiegner AW, Brooks WW, Fishbein MC, Pfeffer JM. Papillary muscle structure-function relations in the aging spontaneously hypertensive rat. *Clin Exp Hypertens A.* 1988;10:37-58.
10. Conrad CH, Brooks WW, Robinson KG, Bing OHL. Impaired myocardial function in spontaneously hypertensive rats with heart failure. *Am J Physiol.* 1991;260:H136-H145.
11. Lee SW, Schwartz A, Adam RJ, Yamori Y, Whitmer K, Lane L, Wallick E. Decrease in Na^+,K^+-ATPase and [^{3}H]ouabain binding sites in sarcolemma prepared from hearts of spontaneously hypertensive rats. *Hypertension.* 1983;5:682-688.
12. Kawamura M, Watari S, Nagati T, Kimura T, Ohkubo T, Kawanishi M, Nagano M. Calcium binding and Na^+-Ca^{2+} exchange in cardiac sarcolemma in experimental hypertension. In: Jacob R, Gulch RW, Kissling G, eds. *Cardiac Adaptation to Hemodynamic*

Overload, Training and Stress. Darmstadt, FRG: Steinkopff; 1983: 182-188.

13. Sharma RV, Butters CA, Bhalla RC. Alterations in plasma membrane properties of the myocardium of spontaneously hypertensive rats. *Hypertension.* 1986;8:583-591.

14. Aoki K, Ikeda N, Yamashita K, Hotta K. ATPase activity and Ca^{2+} interaction of myofibrils and sarcoplasmic reticulum isolated from the hearts of spontaneously hypertensive rats. *Jpn Heart J.* 1974; 15:475-484.

15. Limas C, Cohn JN. Defective calcium transport by cardiac sarcoplasmic reticulum in spontaneously hypertensive rats. *Circ Res.* 1977;40(suppl I):I-62-I-69.

16. Cuneo ME, Grassi de Gende AO. Cardiac sarcoplasmic reticulum characteristics in hypertrophic hearts from spontaneously hypertensive rats. *Basic Res Cardiol.* 1988;83:286-295.

17. Morano I, Lengsfeld M, Ganten U, Ganten D, Rüegg JC. Chronic hypertension changes myosin isoenzyme pattern and decreases myosin phosphorylation in the rat heart. *J Mol Cell Cardiol.* 1988; 20:875-886.

18. Koch-Weser J, Blinks RJ. The influence of interval between beats on myocardial contractility. *Pharmacol Rev.* 1963;15:601-651.

19. Reiter M, Vierling W, Seibel K. Excitation-contraction coupling in rested-state contraction of guinea-pig ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol.* 1984;325:159-169.

20. Sutko JL, Bers D, Reeves JP. Postrest inotropy in rabbit ventricle: Na^+-Ca^{2+} exchange determines sarcoplasmic reticulum Ca^{2+} content. *Am J Physiol.* 1986;250:H654-H661.

21. Janczewski AM, Lewartowski B. The effect of prolonged rest on calcium exchange and contractions in rat and guinea-pig ventricular myocardium. *J Mol Cell Cardiol.* 1986;18:1233-1242.

22. Shattock MJ, Bers DM. Rat vs. rabbit ventricle: Ca flux and intracellular Na assessed by ion-selective microelectrodes. *Am J Physiol.* 1989;256:C813-C822.

23. Lewartowski B, Zdanowski K. Net Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} uptake in resting single myocytes of the rat heart: comparison with guinea-pig. *J Mol Cell Cardiol.* 1990;22:1221-1229.

24. Bers D. Calcium influx and sarcoplasmic reticulum Ca release in cardiac muscle activation during postrest recovery. *Am J Physiol.* 1985;248:H366-H381.

25. DuBell WH, Houser SR. Rest decay of calcium transients and contractility in feline ventricular myocytes. *Am J Physiol.* 1990;259: H395-H402.

26. Sutko JL, Kenyon JL. Ryanodine: a modifier of sarcoplasmic reticulum calcium release in striated muscle. *Fed Proc.* 1985;44: 2984-2988.

27. Marban E, Wier WG. Ryanodine as a tool to determine the contributions of calcium entry and calcium release to the calcium transient and contraction of cardiac Purkinje fibers. *Circ Res.* 1985; 56:133-138.

28. Rousseau E, Smith JS, Meissner G. Ryanodine modifies conductance and gating behavior of single Ca^{2+} release channel of sarcoplasmic reticulum. *J Biol Chem.* 1986;261:6300-6306.

29. Mattiazzi AR, Cingolani HE, de Castuma ES. Relationship between calcium and hydrogen ions in heart muscle. *Am J Physiol.* 1979;237:H497-H503.

30. Thomas G, Groß R, Pfitzer G, Rüegg JC. The positive inotropic dihydropyridine BAY K 8644 does not affect calcium sensitivity or calcium release of skinned cardiac fibres. *Naunyn Schmiedebergs Arch Pharmacol.* 1985;328:378-381.

31. Fabiato A, Fabiato F. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J Physiol (Paris).* 1979;75:463-505.

32. Endo M. Calcium release from the sarcoplasmic reticulum. *Physiol Rev.* 1977;57:71-108.

33. Furukawa N, Bassett A, Furukawa T, Myerburg RJ, Kimura S. Hypertrophy alters the effect of $Ins(1,4,5)P_3$ on Ca^{2+} release in skinned rat heart muscle. *Am J Physiol.* 1991;260:H1612-H1618.

34. Herland JS, Julian FJ, Stephenson DG. Halothane increases Ca^{2+} efflux via Ca^{2+} channels of sarcoplasmic reticulum in chemically skinned rat myocardium. *J Physiol.* 1990;426:1-18.

35. Fabiato A, Baumgarten CM. Methods for detecting calcium release from sarcoplasmic reticulum of skinned cardiac cells and the relationships between calculated transarcolemmal calcium movements and calcium release. In: Sperelakis N, ed. *Physiology and Pathophysiology of the Heart.* Boston, Mass: Martinus Nijhoff; 1984:215-254.

36. Wendt LR, Stephenson DG. Effects of caffeine on Ca-activated force production in skinned cardiac and skeletal muscle fibers of the rat. *Pflugers Arch.* 1983;398:210-216.

37. Su JY, Kerrick WG. Effects of halothane on caffeine-induced tension transients in functionally skinned myocardial fibers. *Pflugers Arch.* 1979;380:29-34.

38. Harper JF. Peritz' F test: basic program of a robust multiple comparison test for statistical analysis of all differences among group means. *Comput Biol Med.* 1984;14:437-445.

39. Fabiato A. Effects of ryanodine in skinned cardiac cells. *Fed Proc.* 1985;44:2970-2976.

40. de la Bastie D, Levitsky D, Rappaport L, Mercadier JJ, Marotte F, Wisnewsky C, Brovkovich V, Schwartz K, Lompré AM. Function of the sarcoplasmic reticulum and expression of its Ca^{2+}-ATPase gene in pressure overload-induced cardiac hypertrophy in the rat. *Circ Res.* 1990;66:554-564.

41. Kimura S, Bassett A, Saida K, Shimizu M, Myerburg J. Sarcoplasmic reticulum function in skinned fibers of hypertrophied rat ventricle. *Am J Physiol.* 1989;256:H1006-H1011.

42. Page E, McCallister J, Burian W. Quantitative electron microscopic description of heart muscle cells: application to normal, hypertrophied and thyroxin-stimulated hearts. *Am J Cardiol.* 1973; 31:172-181.

43. Gwathmey JK, Morgan JP. Altered calcium handling in experimental pressure overload hypertrophy in the ferret. *Circ Res.* 1985; 57:836-843.

44. Bing OHL, Brooks WW, Conrad CH, Subha S, Perreault CL, Morgan JP. Intracellular calcium transients in myocardium from spontaneously hypertensive rats during the transition to heart failure. *Circ Res.* 1991;68:1390-1400.

45. Gamble WJ, Phornphutkul C, Jumar AE, Sanders GL, Manasek FJ, Monroe RG. Ventricular performance, coronary flow and MV_{o2} in aortic coarctation hypertrophy. *Am J Physiol.* 1973;224: 877-883.

46. Kissling G, Rupp H. The relationship between oxygen consumption and isoenzyme pattern of myosin in chronically pressure-loaded myocardium of the rat. *Pflugers Arch.* 1981;391(suppl):R5. Abstract.

47. Kissling G, Rupp H, Mallot L, Jacob R. Alterations in cardiac oxygen consumption under chronic pressure overload: significance of the isoenzyme pattern of myosin. *Basic Res Cardiol.* 1982;77: 255-269.

48. Alpert NR, Mulieri LA. Increased myothermal economy of isometric force generation in compensated cardiac hypertrophy induced by pulmonary artery constriction in the rabbit: a characterization of heat liberation in normal and hypertrophied right ventricular papillary muscles. *Circ Res.* 1982;50:491-500.

49. Brooks WW, Ingwall JS, Conrad CH, Holubarsch C, Bing OHL. Tolerance to hypoxia of myocardium from adult and aged spontaneously hypertensive rats. *Am J Physiol.* 1987;252:H1096-H1114.

50. Saragoça M, Tarazi R. Impaired cardiac contractile response to isoproterenol in the spontaneously hypertensive rat. *Hypertension.* 1981;3:380-385.

51. Prashad N. Reduced levels of cAMP-dependent protein kinase in spontaneously hypertensive rat. *J Biochem.* 1985;260:10125-10131.

52. Kumano K, Upsher M, Khairallah PA. Beta-adrenergic receptor response coupling in hypertrophied hearts. *Hypertension.* 1983; 5(suppl I):I-175-I-183.