Major Alterations in Relaxation During Cardiac Hypertrophy Induced by Aortic Stenosis in Guinea Pig

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Left ventricular hypertrophy (LVH) was produced in guinea pigs after aortic stenosis (AS). The percentage of LVH in AS was determined by normalizing left ventricular (LV) weight by the mean LV weight of sham-operated controls ($n = 12$). After 3 weeks of cardiac overload, a mild LVH (30 ± 3%) was induced in 17 animals and a relatively severe LVH (56 ± 3%) was induced in 7 animals. LV papillary muscles were rapidly excised for mechanical studies. No significant differences were observed between control and mild hypertrophy groups. In contrast, a marked decrease in myocardial performance was seen in the more severe cardiac hypertrophy group and was expressed as a percentage of sham-operated levels (Vmax, 22%; active isometric force/mm², 23%; +dF/dt max/mm², 26%). Relaxation in this group was still more impaired than contraction (peak lengthening velocity, 14%; −dF/dt max/mm², 19%). Moreover, the load sensitivity of relaxation was present in both sham-operated controls and mild hypertrophy but almost disappeared in more severe hypertrophy. Isometric relaxation was delayed in the latter group, as shown by the 15% increase of the half-time of the decline of isometric relaxation ($t_{1/2}$). On the other hand, acute hypoxia (95% N_2–5% CO_2 for 20 minutes) also induced a fall in contractility and the disappearance of the load sensitivity of relaxation but with a 67% decrease of $t_{1/2}$. Thus, the mechanical analysis of relaxation allows the effects of chronic overload in relatively severe cardiac hypertrophy to be separated from those of acute hypoxia. Moreover, in severe cardiac hypertrophy, the impairment of the load sensitivity of relaxation with increased $t_{1/2}$ strongly suggests alterations of the sarcoplasmic reticulum, especially since the moderate decrease in the myofibrillar ATPase activity, which has been observed previously in guinea pig pressure overload, cannot account completely for the marked fall in myocardial performance. (*Circulation Research* 1987;61:107–116)

Chronic cardiac overload appears to modify the myocyte homeostasis in a complex manner, as shown by numerous biochemical, metabolic, or mechanical alterations described in the literature. These changes observed during cardiac hypertrophy are, in part, species specific. Thus, the rat myocardium adapts to chronic overload by changing the fast V_1 isomyosin to the slow V_3,[1] and this isomyosin shift is related linearly to alterations in contractility [2,3] Also, it has been shown that relaxation in the hypertrophied rat myocardium remains sensitive to the loading conditions, a mechanical property that generally seems to be present when the sarcoplasmic reticulum is normally functional and that, in the rat, is never impaired, whatever the degree or type of cardiac overload.[4,5] Moreover, acute hypoxia reversibly abolishes the load sensitivity of relaxation in both normal[6] and hypertrophied[5] rat cardiac muscle Under such conditions, relaxation characteristics are similar to those observed in normal frog myocardium,[7,8] where the sarcoplasmic reticulum is poorly developed.[9]

On the other hand, normal guinea pig heart muscle appears to be closer to that of human than to that of rat, considering 2 major regulatory mechanisms of contractility: 1) the isomyosin pattern, which is predominantly V_3[10], and 2) the calcium-induced calcium release from the sarcoplasmic reticulum, which is poorly developed[11] (A Fabiato, a personal communication). Normal guinea pigs were submitted to chronic cardiac overload induced by an abdominal aortic stenosis. Our study focused on the mechanical coupling between contraction and relaxation. The disappearance of the load sensitivity of relaxation suggests that, in guinea pig, chronic cardiac overload induces important abnormalities in Ca^{2+}-reaccumulation by the sequestering systems, especially by the sarcoplasmic reticulum, which may partly explain impairment in myocardial performance This is corroborated by the slight alterations recently observed in the Ca^{2+}- and Mg^{2+}-activated myosin ATPase activity, which cannot account totally for the marked fall in contractility.[12] Aortic stenosis in the guinea pig may represent an interesting model of cardiac hypertrophy, considering several biochemical analogies between heart muscle of humans and guinea pigs.

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Materials and Methods

Preparation of Animals and Surgical Procedure

Cardiac hypertrophy was induced in 5-month-old tricolor female Charles River guinea pigs (Paris) by chronic overload after subtotal aortic stenosis (AS). Surgery was performed under combined carfentanyl and etomidate anesthesia (Jansen Pharmaceutica, Beerse, Belgium); the animals were ventilated during the procedure. The abdominal aorta was dissected, and a metal clip was placed around the aorta just cranial to the renal arteries, which constricted the aorta to about 90% of the original surface area Meticulous postoperative care was taken. After 3 weeks of cardiac overload, the guinea pigs were killed and the hearts excised A left ventricular papillary muscle was used for mechanical studies Sham-operated control animals were subjected to the same operation but without a clip around the aorta. About 30% of animals that underwent surgery died either just after surgery or during the overall period of cardiac hypertrophy

At the moment of killing, the operated animals were without any sign of congestive heart failure, such as pulmonary or hepatic congestion and subcutaneous edema Guinea pigs were subdivided into 4 groups: Group 1, control group, $n = 12$, animals were sham-operated; Group 2, (AS^+), $n = 17$, moderate cardiac hypertrophy; Group 3, (AS^{++}), $n = 7$, relatively severe cardiac hypertrophy; Group 4, $n = 8$, normal heart muscles submitted to acute hypoxia. In spite of a slight decrease in body weight in overloaded animals, variance analysis did not show significant differences with respect to body weight between controls (563 ± 45 g), AS^+ (485 ± 36 g), and AS^{++} (451 ± 34 g). Physical characteristics of papillary muscles are listed in Table 1

Mechanical Analysis and Mounting Procedure

Left ventricular papillary muscles were quickly removed and vertically disposed in a chamber containing a Krebs-Ringer solution of (in mM) NaCl 118, KCl 4 7, $MgSO_4 7H_2O$ 1.2, KH_2PO_4 1 1, $NaHCO_3$ 24, $CaCl_2 6H_2O$ 2.5, and glucose 4 5. The bathing solution was bubbled with 95% O_2–5% CO_2 and maintained at pH = 7.4 and 29° C The muscle specimens were stimulated by means of 2 platinum electrodes that delivered rectangular pulses of 5-msec duration at a voltage slightly above threshold. Optimal stimulation frequency was 30 beats/min. After a stabilization period of 1 hour, the preload corresponding to Lmax was determined and the rest of the experiment was carried out at Lmax (i e , the initial length at the apex of the length-active tension relation) Acute hypoxia was induced in normal guinea pig cardiac papillary muscles by bubbling the solution with 95% N_2–5% CO_2 for 20 minutes. Depressant effects of hypoxia on mechanical performance were reversible after bubbling the solution again with 95% O_2–5% CO_2.

Table 1. Physical Characteristics of Papillary Muscles in Sham-Operated (C), AS^+, and AS^{++}

	Weight (mg)	Length at Lmax (mm)	Cross-sectional area (mm^2)
C	9 0±0 6	3 2±0 2	2 8±0 1
	*	*	NS
AS^+	11 2±0 8	3 7±0 2	3 0±0 2
	*	*NS	*
AS^{++}	14 4±3 2	3 6±0 5	3 8±0 5

Data expressed as mean ± SEM. After analysis of variance, unpaired Student's t test was done between controls (C) and both AS^+ and AS^{++} NS, nonsignificant, $*p < 0$ 05

In both control and hypertrophied heart muscles, inotropy was modified by altering the external calcium concentration $[Ca^{2+}]_o$ and the stimulation frequency (Fr). Thus, mechanical data were measured successively at 3 values of $[Ca^{2+}]_o$: 2 5, 5, and 7 5 mM For each $[Ca^{2+}]_o$, 2 stimulation frequencies were used in succession: 30 and 10 beats/min. For each stimulation frequency, mechanical measurements were carried out after a steady-state period of 5 minutes

The force transducer, the electromagnetic lever system, and the whole electronic device have been described previously [4] Briefly, the load that is imposed on the muscle was determined by a servo-controlled current through the coil of the electromagnet The transducer used to measure the displacement of the lever consists of a photoelectric system. The equivalent moving mass of the whole system was 155 mg and the compliance was 0.2 μm/mN. The linearity of the system ranges from 0–2.5 mm of muscle shortening.

Mechanical Parameters

All mechanical parameters were calculated from 4 twitches. The first twitch was isotonic and was loaded with only the preload at Lmax. The second twitch was abruptly clamped to zero-load just after the electrical stimulus, the maximum unloaded shortening velocity (Vmax) was determined from this twitch by the zero-load clamp technique The third twitch was isometric at Lmax The fourth twitch was isotonic and was afterloaded to half-value of the isometric active force at Lmax Detailed explanations and definitions of mechanical indexes are given in Figures 1 and 2 and corresponding legends and in the subsequent text

Mechanical parameters characterizing the contraction phase, the relaxation phase, the coupling between contraction and relaxation, and the load sensitivity of relaxation are defined as follows.

CONTRACTION PHASE (Figure 1). Vmax, maximum unloaded shortening velocity at Lmax by means of the zero-load clamp technique; maxVc, maximum shortening velocity of the twitch with preload only; AF/mm^2, active isometric force at Lmax normalized per cross-sectional area; +dF/dtmax/mm^2, positive peak of isometric force derivative per mm^2; TPS, time to peak shortening of the twitch with preload only, TPF, time to peak force of the isometric twitch.

RELAXATION PHASE (Figure 1) maxVr, maximum lengthening velocity of the twitch with preload only; −dF/dtmax/mm^2, negative peak of isometric force de-

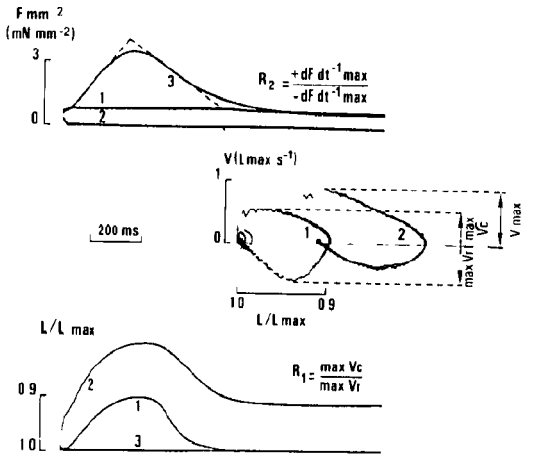

FIGURE 1 *Mechanical parameters of contraction and relaxation* Upper Trace: *Muscle force normalized per cross-sectional area (F/mm²) plotted vs time* Lower Trace. *Shortening length (L/Lmax) plotted vs time* Middle Trace *Shortening velocity-shortening length phase plane diagram Twitch 1 was loaded only with preload at Lmax Twitch 2 was loaded with same preload as that of twitch 1 and was abruptly clamped to zero load with critical damping just after stimulus Twitch 3, isometric twitch at Lmax, maxVc and maxVr, peak shortening velocity and peak lengthening velocity of Twitch 1, respectively,* R_1, *maxVc maxVr; Vmax, unloaded maximum shortening velocity of twitch 2 as determined by zero-load clamp technique,* $+dF/dtmax$ *and* $-dF/dtmax$, *positive and negative peaks of isometric force derivative at Lmax, respectively,* R_2, $+dF/dtmax$ $-dF/dtmax$

rivative at Lmax normalized per mm², $t_{1/2}$, half-time of the relaxation of the isometric twitch (Figure 2B)

COUPLING BETWEEN CONTRACTION AND RELAXATION (Figure 1). Coefficient R_1, maxVc/maxVr; coefficient R_2, $(+dF/dtmax/mm^2)/(-dF/dtmax/mm^2)$ Coefficients R_1 and R_2 test the coupling between contraction and relaxation under low and heavy loading conditions, respectively.

LOAD SENSITIVITY OF RELAXATION. The concept of load sensitivity reflects the capacity of mammalian heart muscles to regulate the time course of relaxation according to loading conditions This phenomenon can be shown by applying abrupt load clamps during isotonic twitches (Figure 2A). In load-sensitive relaxation, the isotonic-isometric transition of relaxation is markedly changed in twitches abruptly load clamped to a given final level of load (Figure 2A) On the other hand, when relaxation is load insensitive, 1) the time courses of isometric relaxation of different afterloaded twitches almost coincide with the relaxation of the isometric twitch, and 2) abrupt load clamps fail to induce any difference in the occurrence of the isotonic-isometric transition of relaxation (Figure 2B). Such load-independent relaxation is typical of frog myocardium, in which the sarcoplasmic reticulum is poor and/or nonfunctional.[7,9] Quantification of the load sensitivity of relaxation has previously been achieved by means of 2 indexes [4,6] The first is the ratio of 2 areas, isotonic area·isometric area (isot. A:isom. A) and the second is the ratio of two times, t_1:t_2 (tRi). Both ratios are detailed in Figure 2C.

Statistical Analysis

The data were expressed as mean ± SEM. Analysis of variance and the Student's unpaired *t* test were used to compare the mean ± SEM in sham-operated and hypertrophied heart muscles for statistical significance. Linear regressions were performed by the least-squares method.

Results

Heart Weight and Cardiac Hypertrophy

After aortic stenosis, left ventricular (LV) weight (free wall and septum) significantly increased although body weight did not change significantly The percent of cardiac hypertrophy in overloaded animals was calculated from the left ventricular weight·body weight ratio normalized per the mean value of the same ratio determined in control animals. The degree of left ventricular cardiac hypertrophy was 30 ± 3% in AS^+ and almost double in AS^{++}, reaching 56 ± 3%.

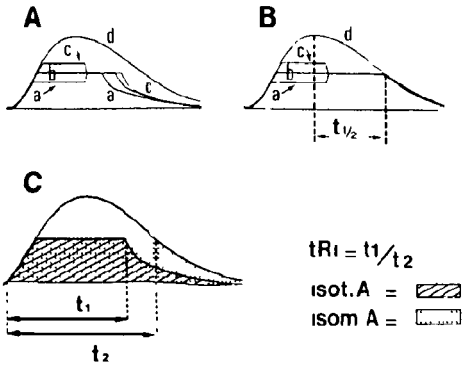

FIGURE 2 *Quantification of load sensitivity of relaxation Panels A, B, and C represent muscle force as a function of time.* Panels A and B: *Loading step (twitch a) and unloading step (twitch c) of same magnitude imposed at the same time Twitch b, isotonic level of load to which both twitches a and c were clamped, twitch d, totally isometric In Panel A, the transition between the isotonic and isometric phases of relaxation occurred at different times in twitches a, b, and c and always before relaxation of isometric twitch d, which characterizes the load-sensitive relaxation of mammals In Panel B, load clamps did not induce any significant differences in time course of relaxation of twitches a–d, which characterizes load-insensitive relaxation, as observed in frog myocardium* Panel C *Indexes used to quantify load sensitivity of relaxation Isot A (isotonic area) isom. A (isometric area) represented the ratio of 2 areas. The area "isot A" was limited by isotonic force vs time curve at 50% of active force at Lmax The area "isom A" was limited by isometric force time curve below same level of load Time to relaxation (tRi) represented the ratio of 2 times (t_1 and t_2). t_1, time to end of isotonic relaxation of twitch afterloaded at 50% of active force at Lmax: t_2, time at which isometric twitch was relaxed to same load level These two indexes tend toward 1 when the load sensitivity of relaxation disappears Time to half relaxation ($t_{1/2}$, Panel B), time from peak active isometric force at Lmax to ½ that value*

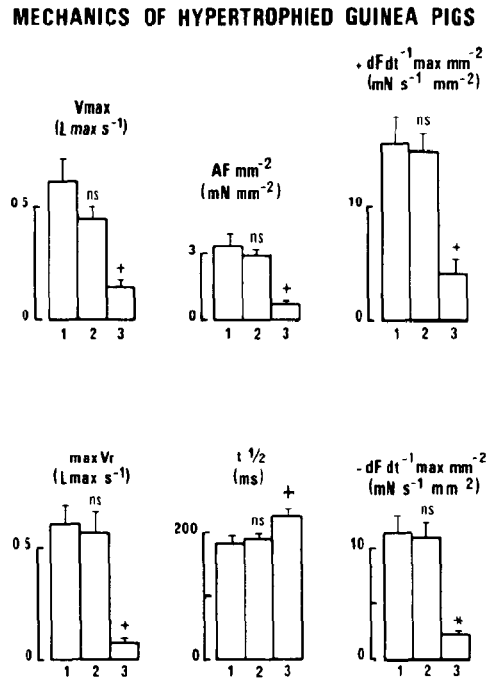

FIGURE 3 *Mechanical parameters of contraction (Vmax, AF/mm², and +dF/dtmax/mm²) and relaxation (maxVr, $t_{1/2}$, and −dF/dtmax/mm²) for control (C) (1), AS⁺ (2), and AS⁺⁺ (3) Abbreviations of parameters were defined in "Materials and Methods." After analysis of variance, Student's unpaired t test was carried out between C and both AS⁺ and AS⁺⁺ ns, nonsignificant, ⁺p<0 05, *p<0 001*

Mechanics

Mechanical parameters were calculated in C, AS⁺, and AS⁺⁺ groups, and mean ± SEM values of C were compared with those of AS⁺ and AS⁺⁺, respectively.

In the contraction phase (Figure 3), parameters characterizing the contraction phase (Vmax, AF/mm², and dF/dt max/mm²) decreased slightly in AS⁺ and significantly in AS⁺⁺. Similarly, TPS and TPF did not change after AS⁺ but were significantly diminished in AS⁺⁺ (Figure 4).

In the relaxation phase (Figure 3), the 2 parameters, maxVr and −dF/dtmax/mm², were markedly decreased in AS⁺⁺ On the other hand, time to half relaxation ($t_{1/2}$) appeared to be significantly prolonged in AS⁺⁺.

Coupling between contraction and relaxation is shown in Figure 6. In AS⁺, maxVc and maxVr slightly decreased in similar proportion so that the coefficient R_1 did not change. In AS⁺⁺, however, maxVc decreased fourfold and maxVr sevenfold, so that R_1 increased significantly (Figure 6). Similarly, the coefficient R_2 did not change in AS⁺, although it increased significantly in AS⁺⁺ because the negative peak dF/dt/mm² was more depressed than the positive peak dF/dt/mm². Thus, in AS⁺, coefficients R_1 and R_2 testing the coupling between contraction and relaxation were not altered, even in the presence of 30% LV cardiac hypertrophy In contrast, in AS⁺⁺ in which 55% LV cardiac hypertrophy was observed, both R_1 and R_2 were markedly enhanced, which indicates that mechanical abnormalities of relaxation largely predominated over those of contraction.

The mechanical property of load sensitivity of relaxation can be shown in a series of afterloaded twitches and/or by applying load clamps during isotonic twitches (Figures 2 and 5). In normal guinea pig cardiac

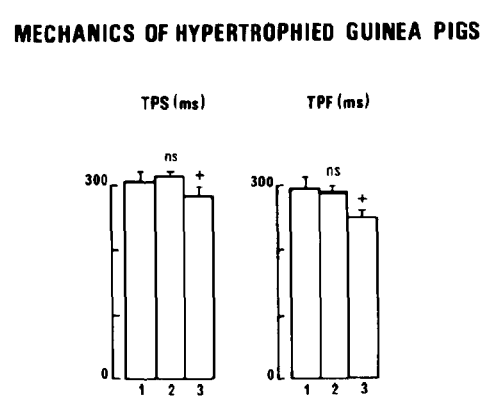

FIGURE 4 *Time to peak shortening (TPS) and time to peak force (TPF) in C (1), AS⁺ (2), and AS⁺⁺ (3). Unpaired t test was done between C and both AS⁺ and AS⁺⁺ ns, nonsignificant, ⁺p<0.05*

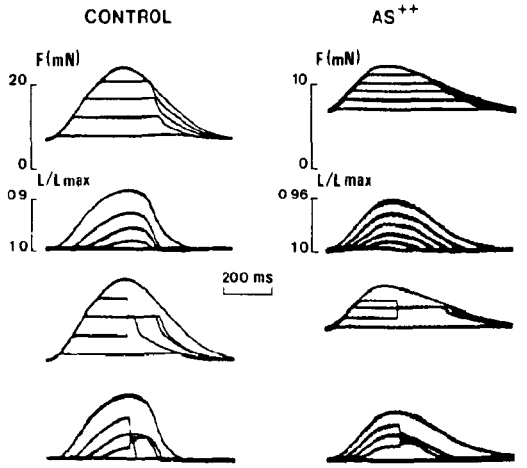

FIGURE 5 *Force (F) and shortening length (L/Lmax) plotted against time* Left panel *Typical sham papillary muscle.* Right panel *Typical AS⁺⁺ papillary muscle In upper part of each panel, a series of superimposed afterloaded twitches at different load level from preload only up to isometric twitch are presented In C, isotonic twitches were always over before isometric twitch In AS⁺⁺, very little differences were seen in time courses of relaxation of afterloaded twitches whose isometric relaxation almost coincided with that of totally isometric twitch In lower part of each panel were imposed a loading step and an unloading step of the same magnitude that were triggered at same time during contraction. In C muscle, load clamps induced marked differences in both onset and time course of isometric relaxation of afterloaded twitches Conversely, in AS⁺⁺ papillary muscle, load-clamps did not induce any significant differences in both onset and time course of isometric relaxation, which characterizes a load-insensitive relaxation In AS⁺⁺ papillary muscle, time to half relaxation was longer than in C*

papillary muscle, the isometric relaxation phases of the isotonic afterloaded twitches were distinct from each other and occurred before relaxation of the isometric twitch (Figure 5). Moreover, when load-clamp steps to a given final level of load were abruptly applied at time to peak shortening of the isotonic twitch, the isotonic-isometric transition of relaxation occurred at different times. This mechanical behavior characterized a load-sensitive relaxation (Figure 2A). On the other hand, in cardiac papillary muscle of animals with severe hypertrophy, very few differences were seen in the time course of relaxation of different afterloaded contractions up to the isometric state. Moreover, loading and unloading steps did not induce any differences in time courses of the isometric relaxation phases This mechanical behavior characterizes a load-insensitive relaxation (Figures 2B and 5). The degree of load dependence of relaxation was quantified by means of the 2 indexes, isot A · isom A and tRi (Figure 2C). No differences in load sensitivity of relaxation were observed between controls and AS^+ (Figure 6). On the contrary, this mechanical property was significantly decreased in AS^{++}, since both isot A · isom A and tRi approached 1 (Figure 6).

Effects of Acute Hypoxia in Normal Guinea Pig Papillary Muscle

After 20 minutes of hypoxia (Group 4), cardiac contractility was markedly depressed and the load sensitivity of relaxation disappeared (Figures 7 and 8).

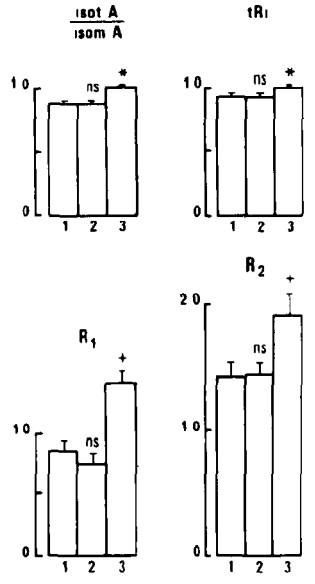

FIGURE 6. *Mechanical parameters of load sensitivity of relaxation (isot A · isom A and tRi) and of coupling between contraction and relaxation (R_1 and R_2). These parameters were defined in "Materials and Methods" and in Figures 1 and 2 C (1), AS^+ (2), and AS^{++} (3) The unpaired t test was done between C and both AS^+ and AS^{++}. ns, nonsignificant; $^+p<0.05$, $^*p<0.001$*

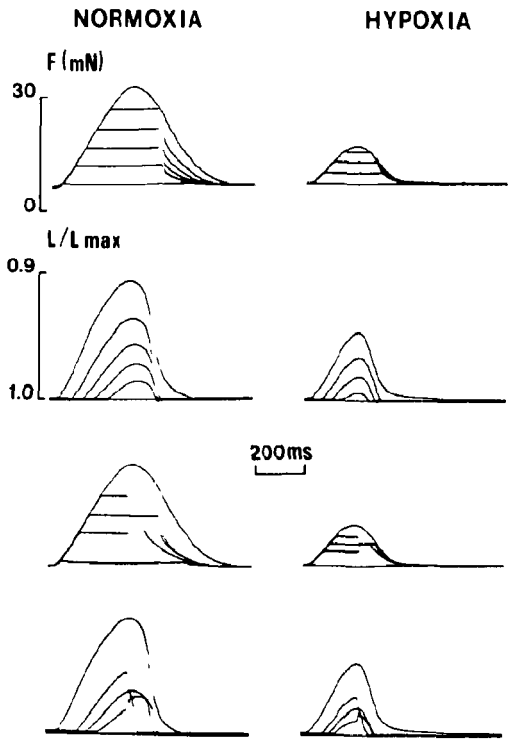

FIGURE 7 *Force (F) and shortening length (L/Lmax) plotted vs. time* Left panel. *Typical control papillary muscle in normoxic conditions* Right panel: *Same papillary muscle after 20-minute hypoxic period Upper and lower parts of each panel were presented respectively according to same protocol of loading modifications as seen in Figure 5. During hypoxia, load sensitivity of relaxation disappeared because no significant differences could be seen in time courses of isometric relaxation, both in a series of afterloaded twitches* (upper inset, right panel) *and of afterload-clamp steps* (lower inset, right panel) *During hypoxia, time to half relaxation ($t_{1/2}$) decreased*

However, contrary to what was observed in AS^{++}, $t_{1/2}$ and R_2 significantly decreased subsequent to hypoxia (Figures 3, 6, and 8). Thus, both cardiac hypertrophy and hypoxia abolished the sensitivity of relaxation to the loading conditions, but the time course of isometric relaxation was lengthened in the former case and shortened in the latter.

Effects of $[Ca^{2+}]_o$ on Active Force

At 30 beats/min and 2.5 mM $[Ca^{2+}]_o$, as compared with controls, AF/mm^2 did not appear to be significantly changed in AS^+ and conversely dropped markedly in AS^{++} (Figure 3). Similar results were observed at 10 beats/min and 2.5 mM $[Ca^{2+}]_o$, under these experimental conditions, AF/mm^2 (mN/mm^2) was equal to 2.1 ± 0.4 in controls, 2.4 ± 0.5 in AS^+, and 0.5 ± 0.1 in AS^{++}. In the 3 groups of animals and at 30 beats/min, AF/mm^2 increased when $[Ca^{2+}]_o$ augmented and was significantly higher at 7.5 than at 2 5 mM $[Ca^{2+}]_o$ (Table 2). Similar results were observed at 10 beats/min in controls but not in hypertrophied animals (Table 2). Interestingly, statistical analysis shows that, for a given $[Ca^{2-}]_o$ and for a given stimulation frequency, the normalized values of AF/mm^2, expressed as a

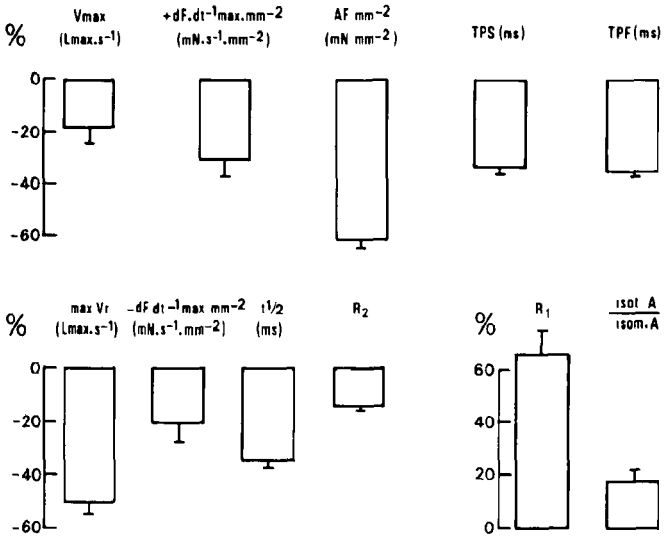

FIGURE 8 *Effects of hypoxia on mechanical parameters of contraction, relaxation, load sensitivity of relaxation, and coupling between contraction and relaxation in normal guinea pig papillary muscle. Data were expressed as a percent of respective control normoxic values. Abbreviations were defined in "Materials and Methods." Notice that after hypoxia, isot A·isom A increased and $t_{1/2}$ decreased*

percent of AF/mm² at 2.5 mM $[Ca^{2+}]_o$, did not differ significantly between controls, AS^+, and AS^{++} (Table 2).

Effects of $[Ca^{2+}]_o$ on maxVr and $-dF/dtmax/mm^2$

At 30 beats/min, in controls, AS^+, and AS^{++}, maxVr and $-dF/dtmax/mm^2$ increased when $[Ca^{2+}]_o$ was increased from 2.5 mM to 5 and 7.5 mM (Figures 9 and 10). In controls and AS^+, significant differences were observed between the values of both maxVr and $-dF/dtmax/mm^2$ at 2 5 and 7 5 mM $[Ca^{2+}]_o$, respectively. On the other hand, in AS^{++}, the influence of $[Ca^{2+}]_o$ variations was more moderate than in control and AS^+ (Figures 9 and 10). For a given $[Ca^{2+}]_o$, there were no significant differences between controls and AS^+ regarding the mean values of both maxVr and $-dF/dtmax/mm^2$, respectively. Conversely, for each $[Ca^{2+}]_o$, mean values of maxVr and $-dF/dtmax/mm^2$ were significantly lower in AS^{++} than in controls and AS^+

At 10 beats/min, the increase in $[Ca^{2+}]_o$ slightly increased the mean values of both maxVr and $-dF/dt$ max/mm² (Figures 9 and 10). However, in a given group, these 2 parameters did not show significant differences at 2 5 and 7.5 mM $[Ca^{2+}]_o$, respectively. For a given $[Ca^{2+}]_o$, there was no significant difference in maxVr between C, AS^+, and AS^{++} (Figures 9 and 10)

Effects of Stimulation Frequency on maxVr and $-dF/dtmax/mm^2$

In controls and AS^+, and for each $[Ca^{2+}]_o$, augmentation of Fr increased both maxVr and $-dF/dt$ max/mm². Conversely, in AS^{++} and for each $[Ca^{2+}]_o$, increased Fr did not significantly change maxVr and $-dF/dtmax/mm^2$ (Figures 9 and 10).

Effects of $[Ca^{2+}]_o$ and Stimulation Frequency on the Load Sensitivity of Relaxation

In controls and AS^+, for a given Fr, the increase in $[Ca^{2+}]_o$ did not change isot. A:isom. A significantly (Figure 11), and for a given $[Ca^{2+}]_o$, the increase in Fr slightly decreased isot. A:isom. A (Figure 11). Moreover, for any $[Ca^{2+}]_o$ or Fr, relaxation was totally load-insensitive in AS^{++} (Figures 5 and 11).

Table 2. Effects of $[Ca^{2+}]_o$ and Stimulation Frequency on Isometric Active Force

	30 beats/min			10 beats/min		
Groups	$[Ca^{2+}]_o$ 2 5 mM	$[Ca^{2+}]_o$ 5 mM	$[Ca^{2+}]_o$ 7 5 mM	$[Ca^{2+}]_o$ 2 5 mM	$[Ca^{2+}]_o$ 5 mM	$[Ca^{2+}]_o$ 7.5 mM
C	100%	145±12%	196±15%	100%	116±19%	158±40
AS^+	100%	176±16%	189±20%	100%	152±19%	119±29%
AS^{++}	100%	113±11%	150±19%	100%	98±11%	119±15%
F test		$F^2_{24}=3\ 93$	$F^2_{19}=0\ 08$		$F^2_{20}=2\ 35$	$F^2_{16}=0\ 96$
		<0 05	NS		NS	NS
t test		NS				

Peak active force was successively calculated in sham-operated (C), AS^+, and AS^{++} at 10 and 30 beats/min and at 3 different $[Ca^{2+}]_o$, 2.5, 5, and 7 5 mM For each stimulation frequency (Fr) and at 5 and 7 5 mM $[Ca^{2+}]_o$, data were expressed as a percent of respective values at 2 5 mM $[Ca^{2+}]_o$. The F test was done in the 3 groups for each $[Ca^{2+}]_o$ and each Fr At 10 beats/min, the F test did not show significant differences and the *t* test was not done At 30 beats/min and 7 5 mM $[Ca^{2+}]_o$, similar results were observed At 30 beats/min and 5 mM $[Ca^{2+}]_o$, the value of the F test permitted execution of the *t* test, in this case, the *t* test showed no significant differences between C, AS^+, and AS^{++} Thus, in all cases, the percent changes in active force did not significantly differ in C, AS^+, and AS^{++}, although the absolute magnitudes of active force were significantly lesser in AS^{++} than in C (see Figure 3 and text)

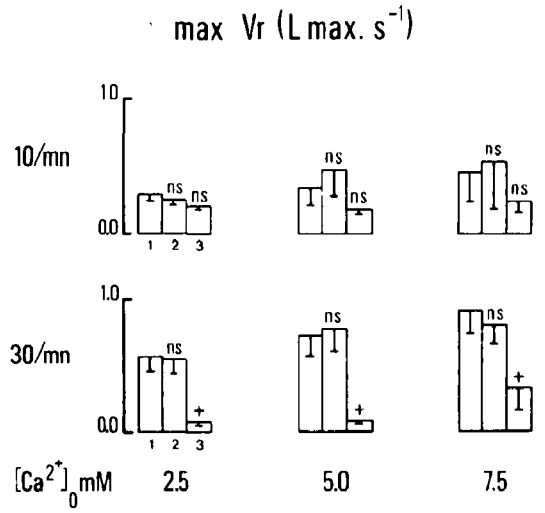

FIGURE 9. *Maximum lengthening velocity at Lmax (maxVr) in C (1), AS⁺ (2), and AS⁺⁺ (3) maxVr calculated at 2 stimulation frequencies (10 and 30 beats/minute) and at 3 different $[Ca^{2+}]_o$ (2 5, 5, and 7 5 mM). Student's unpaired t test was done between sham and both AS⁺ and AS⁺⁺ ns, nonsignificant; $^{+}p<0\ 05$*

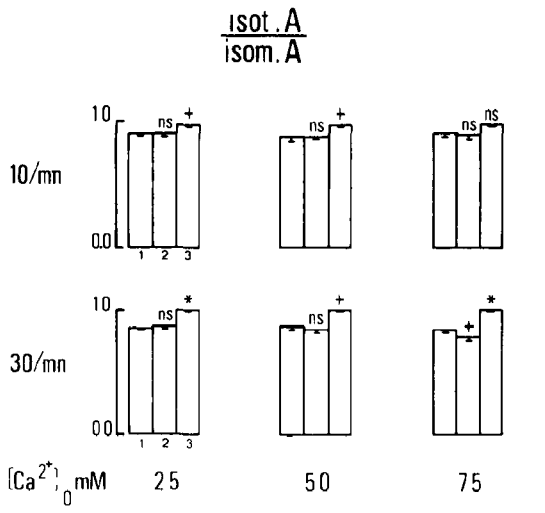

FIGURE 11. *Load sensitivity of relaxation was quantified by isot A isom A (see Figure 2) C (1), AS⁺ (2), and AS⁺⁺ (3) Isot A isom A was calculated successively at 10 and 30 beats/min and at 2 5, 5, and 7 5 mM $[Ca^{2+}]_o$ The unpaired t test was done between C and both AS⁺ and AS⁺⁺. ns, nonsignificant. $^{+}p<0\ 05$; $*p<0\ 001$.*

Discussion

Mechanics of papillary muscles from normal and hypertrophied guinea pigs were studied at two different degrees of cardiac hypertrophy. As long as the cardiac hypertrophy was moderate (30 ± 3% in Group 2, AS⁺), i.e., during the compensatory phase of the hypertrophy, intracellular mechanisms that regulate myocardial performance were not altered since none of the mechanical parameters of contraction and relaxation differed significantly from those of controls and

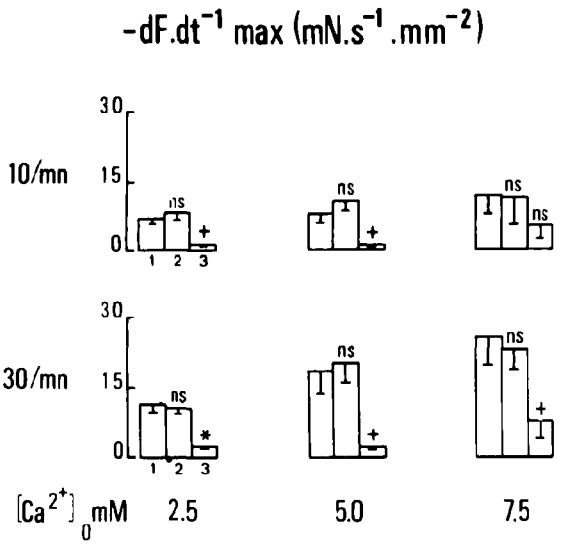

FIGURE 10 *Negative peak of isometric force derivative at Lmax ($-dF/dtmax/mm^2$) in C (1), AS⁺ (2), and AS⁺⁺ (3) $-dF/dtmax/mm^2$ was successively calculated at 10 and 30 beats/min and at 2.5, 5, and 7 5 mM $[Ca^{2+}]_o$ The unpaired t test was done between C and both AS⁺ and AS⁺⁺ ns, nonsignificant, $^{+}p<0.05$, $*p<0.001$.*

since the responsiveness to changes in $[Ca^{2+}]_o$ and Fr was very similar to that obtained in controls. But in severe cardiac hypertrophy (56 ± 5% in Group 3, AS⁺⁺), marked impairment of mechanics was observed as shown by the fall in contraction and relaxation parameters, by alterations in the responsiveness to changes in $[Ca^{2+}]_o$ and Fr, and by the disappearance of the load sensitivity of relaxation.

At least two important mechanisms that regulate the homeostasis of inotropy in normal guinea pig heart muscle differ strikingly from what has been described in rat cardiac muscle and appear to be relatively close to what has been observed in human heart muscle. First, cardiac myosin ATPase activity in guinea pigs and in humans is not very different [13] Second, the magnitude of the calcium-induced calcium release (CICR) from the sarcoplasmic reticulum of skinned adult ventricular cells of guinea pig is between the CICR of human ventricle and that of rabbit ventricle and, consequently, is much lower than that of the rat.[11] (A. Fabiato, personal communication). These two factors may partly explain the low myocardial performance of normal guinea pig left ventricular papillary muscle as compared with that of normal rat.[5,14,15] Moreover, the maximum speed of shortening length has previously been found to be linearly related to the myosin ATPase activity in both skeletal and cardiac muscle of different species.[13,16,17]

In cardiac hypertrophy, a similar linear relation has been obtained between the maximum unloaded shortening velocity and either the myosin ATPase activity or the V_1 and V_3 isomyosins [2,3] However, for a comparable degree of cardiac hypertrophy and for a comparable drop in maximum speed of shortening, the fall in myosin ATPase activity has definitively been shown to be decreased markedly during chronic cardiac over-

load induced in rat and is either normal or only slightly affected by the process in guinea pigs or in humans [12,18–21] These latter modifications in ATPase activity cannot explain totally the major drop in myocardial performance observed in the AS^{++} group of the present study

Other processes, such as modifications of Troponin C (TnC) affinity for Ca^{2+}, might be involved to account for the fall in contractility observed in AS^{++} Of interest is the fact that the absolute magnitude of the active force was significantly lower in AS^{++} than in controls, although the percent changes were not significantly different (Table 2 and Figure 3). The differences in absolute values of the active force between controls and AS^{++} reflect a lesser ability of hypertrophied heart muscles to generate force at any given $[Ca^{2+}]_0$ However, the nonsignificant shift of percent values suggests that the sensitivity of the myofilaments to Ca^{2+} did not differ in controls and AS^{++}. Similar results have been shown recently in control and hypertrophied ferrets.[22]

These arguments indicate that other regulatory mechanisms of contractility might be altered in guinea pig chronic cardiac overload Ultrastructural damages at the level of myofilaments, Z-lines, or connective tissue might explain in part the lower force generated by hypertrophied guinea pig heart muscles. Histological changes induced by chronic cardiac overload have not been studied extensively in guinea pig contrary to other mammal species However, the disappearance of the load sensitivity of relaxation in AS^{++} argues in favor of an impairment of the Ca^{2+}-accumulating systems, particularly the sarcoplasmic reticulum.[4 7 8] The load sensitivity of relaxation is not present under various experimental conditions in which the sarcoplasmic reticulum is poorly developed, nonfunctional, or inhibited. These experimental arguments are indirect, so the links between the SR function and the load sensitivity of cardiac relaxation remain speculative. Moreover, under parallel conditions, the CICR from the sarcoplasmic reticulum is absent. For example, ventricular myocardium of frog and of newborn rat, in which the CICR is absent,[11] presents a load-insensitive relaxation[7 23]; caffeine, which partially inhibits the sarcoplasmic reticulum,[24] suppresses the spontaneous beats of skinned cardiac cells[11] and abolishes the sensitivity of relaxation to the loading conditions[4,25]; a decrease of the external calcium concentration diminishes both the CICR from the sarcoplasmic reticulum[11] and the load sensitivity of relaxation in rat[23], and sarcomere length modulates the CICR from the sarcoplasmic reticulum,[26] and the decrease in initial muscle length depresses the load sensitivity of relaxation [23]

Moreover, the magnitude of the load sensitivity of relaxation in normal guinea pig myocardium is between that of normal rat and frog myocardium,[4 6] and is close to that obtained in adult rabbit myocardium.[27] The degree of the load sensitivity of relaxation, which is obtained by comparing myocardium of various species, parallels that of the CICR from the sarcoplasmic reticulum described by Fabiato and Fabiato [11] This corroborates that variations of the rate and amount of Ca^{2+}-accumulation by the sarcoplasmic reticulum are always closely related to changes in the CICR from the sarcoplasmic reticulum [11]

The load sensitivity of relaxation disappeared in AS^{++}. This disappearance suggests an impairment of the Ca^{2+}-reaccumulation by the sarcoplasmic reticulum in AS^{++} as has been described previously during cardiac hypertrophy and failure induced in mammals,[28–34] and particularly in human heart muscle.[35–37] In the present study, the force decay was slowed and delayed as shown by the increase of time to half relaxation ($t_{1/2}$) and of the coefficient R_2. In ferret cardiac hypertrophy, such prolonged time courses of isometric relaxation ($t_{1/2}$) and of calcium transient measured with aequorin may be due to a decrease in the rate of calcium uptake by the sarcoplasmic reticulum [22] Regulation of the load sensitivity of relaxation probably involves mechanisms other than those involving the sarcoplasmic reticulum and, in particular, TnC-actomyosin interactions. It has recently been shown by Sys et al[38] that a diminution in load sensitivity of relaxation might be due to an accelerated disappearance of actomyosin interactions, particularly during hypoxia.[5 6] In this case, relaxation of the isometric twitch is abbreviated, and the time to half relaxation decreases. We also found similar results during hypoxia induced in normal guinea pig (Figures 7 and 8). This shows that impairment of regulatory mechanisms governing the sensitivity of relaxation to the loading conditions in guinea pig appears to differ during cardiac hypertrophy and during hypoxia and that papillary muscles in AS^{++} are not hypoxic because both $t_{1/2}$ and R_2 vary in opposite directions during chronic overload (AS^{++}) and hypoxia, respectively.

This mechanical behavior observed during cardiac hypertrophy in the guinea pig differs from that previously described in the rat. It has been shown that cardiac pressure and/or volume overload in rat never induces significant alterations of the load dependence of relaxation, whatever the type or degree of chronic overloading.[4] The differences in load sensitivity of relaxation between the rat and the guinea pig during cardiac hypertrophy might be due in part to a greater efficiency of Ca^{2+} transport through the sarcoplasmic reticulum membrane in the former species than in the latter one, considering both Ca^{2+}-release[11] and Ca^{2+}-reaccumulation.[39] Thus, during cardiac overload, alterations of Ca^{2+} movements through the sarcoplasmic reticulum membrane may appear to be more marked in the guinea pig than in the rat.

Generally, in chronic cardiac overload, TPF and TPS have been shown to be prolonged.[5,14,18,22,40,41] This constitutes a compensatory mechanism, providing more time for adequate force to be developed. In contrast, TPF and TPS in cardiac overload in the present study were significantly decreased in AS^{++} as compared with controls. It has been shown that, in cardiac overload, increased TPF is coordinated with myothermal changes so that there is a longer period of time for force development.[40] In contrast, these authors have

found that, in case of combined pressure overload and thyrotoxic stresses (PT_2, rabbit model), the decrease in time-independent heat in conjunction with the decrease in TPF provides an inadequate quantity of free Ca^{2+} for activation although the slight decrease in actin-activated myosin ATPase activity cannot account for the marked fall in contractility. From a mechanical and biochemical point of view, the PT_2 rabbit model of cardiac hypertrophy presents several similarities with that of AS^{++} in guinea pig.[12,18,20,40]

In conclusion, cardiac hypertrophy induced by chronic aortic stenosis in guinea pig involved major alterations in myocardial performance. Relaxation was strongly modified, particularly in AS^{++}, as is witnessed by the disappearance of the sensitivity of relaxation to the loading conditions. Such alterations in relaxation have not been observed previously in similar models of rat cardiac hypertrophy, so the mechanical behavior, which follows a chronic overload, and, especially relaxation-contraction coupling, appear to be markedly species-dependent. The fall in myocardial performance in hypertrophied guinea pig cannot be totally accounted for by the relatively slight changes in myosin ATPase activity,[12] and the specific perturbations of relaxation (alteration of load sensitivity and increase in $t_{1/2}$) strongly suggest an impairment of the sarcoplasmic reticulum. In terms of myosin ATPase activity and of calcium movement through the sarcoplasmic reticulum, guinea pig heart muscle appears to be relatively close to that of human and far from that of rat. This is of interest considering the mechanical differences observed during a similar chronic pressure overload induced in guinea pig and in rat, particularly when the relaxation phase is analyzed.[42]

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KEY WORDS • cardiac hypertrophy • sarcoplasmic reticulum • isomyosins