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Mechanical Properties of Cardiac Muscle from Spontaneously Hypertensive Rats: Accentuated Aftercontractions (39698)

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Several studies have indicated that a variety of indices of cardiac contractility and output may be elevated in the early phases of hypertension in both man and experimental animals (1-7). The hyperdynamic state may be a result of an expanded blood volume (1), a redistribution of blood resulting in an expanded central blood volume (2, 7), or an altered autonomic neural input to the heart (3, 6, 7). However, the possibility that alterations in intrinsic contractile properties of the muscle itself contribute to the hyperdynamic state has not yet been examined.

The spontaneously hypertensive rat (SHR) (8) has been shown to develop severe sustained hypertension which is initially associated with an elevation in cardiac output (5). In this study, isometric properties of papillary msucle preparations were examined to ascertain whether differences in mechanical properties between SHR and normotensive Wistar-Kyoto control rats (WKY) do exist.

Methods. Male albino spontaneously hypertensive rats (SHR) were compared to age-matched normotensive Wistar-Kyoto control animals (WKY).

Animals were anesthetized with sodium pentobarbitol, the carotid artery was cannulated, and blood pressure was measured using a Statham P23Db transducer connected to a Brush 440 recorder. The thorax was opened, and the heart was rapidly removed and placed in oxygenated physiological salt solution containing: NaCl, 115 m*M*; KCL, 6.0 m*M*; $MgCl_2$ and $CaCl_2$, 2.5 m*M*, glucose, 11.5 m*M*; Tris-Cl, 35.0 m*M*; pH 7.4. Following removal of a left ventricular papillary muscle, the heart was weighed. The muscle was mounted in a muscle bath equipped with (i) a water jacket for temperature control at 27 ± 0.5°, (ii) a gas portal for delivery of 100% O_2, and (iii) inflow and outflow portals for circulation of the solution. A 27-mm artery clamp was rigidly mounted in the bottom for attachment of one end of the muscle. An identical top clamp was attached to a silver chain connected to a moveable Statham isometric transducer (green cell). Changes in muscle length could be made and measured to a precision of ±0.01 mm. Output of the force transducer was amplified by a Gould transducer coupler (Type 114307 04) and was recorded on a Gould strip chart Model 440. The force signal was differentiated by a Gould differentiator coupler (Type 134214 01) and was also recorded.

The stimuli were provided by a Grass stimulator Model S88 and were applied through a pair of stainless steel electrodes positioned approximately 5 mm on either side of the muscle. Voltage and duration of the stimulus were adjusted to just elicit maximal mechanical responses from each preparation and, thus, were well below those levels required to release endogenous catacholamines (9).

After the muscle was mounted in the bath, it was stimulated at 0.2 Hz with a resting tension of 1-2 g for about 1 hr during which time the active tension reached a steady state.

(i) *Twitch characteristics at L_{max}.* The muscle was stretched in 0.5-mm increments to determine the length at which isometric tension development was maximal (L_{max}). At this length, resting and active tension, time-to-peak tension development (TPT), rate of tension development (dT/dt), and $^1/_2$ relaxation time ($^1/_2$ RT) were determined. Since this determination was made during the initial stretching sequence of length changes and muscle length-tension curves show considerable hysteresis (10), resting tensions are high. With a subsequent release of tension to 1.0 g, active tension did not decrease noticeably. At the end of the experiment, the muscle was removed and weighed. The cross-sectional area was esti-

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mated assuming a tissue density of 1.0 and a cylindrical muscle strip. All tension data were expressed per millimeter squared.

(ii) *Mechanical refractory period determined by paired pulses (MRP).* Following measurement of the twitch characteristics at L_{max}, muscle length was adjusted to that which gave a resting tension of 1.0 g. After equilibrating at 0.2 Hz at this new length, paired stimuli were presented. The pattern of presentation consisted of alternating four single stimuli with four paired stimuli with the overall frequency remaining at 0.2 Hz. The delay between the paired stimuli was progressively increased from 60 to 1500 msec in steps ranging from 25 to 500 msec. The paired pulse experiment was then repeated at a faster frequency of stimulation (0.5 Hz). The delay at which a response first appeared to the second stimulus of the pair was designated as the mechanical refractory period (MRP). As the delay was further increased, amplitudes of the individual responses to the two stimuli of the pair were measured. Aftercontractions (Fig. 1) were noted in many preparations following the paired pulse stimulation and were recorded as a percentage of the amplitude of the response to the first stimulus of the pair.

Data analysis. Data were averaged and reported as mean ± standard error of the mean. Differences between SHR and WKY groups were assessed by applying Student's *t*-test.

Results. SHRs had significantly higher arterial pressure when compared with age-matched normotensive WKYs as shown in Table I. Heart weights were somewhat

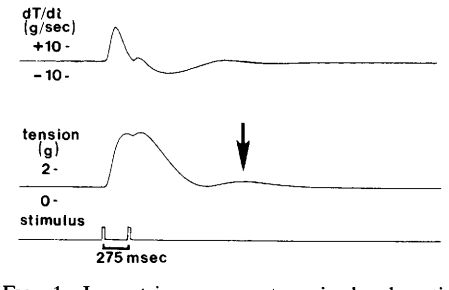

FIG. 1. Isometric response to paired pulse stimuli and subsequent aftercontraction (arrow) in a papillary muscle preparation from a spontaneously hypertensive rat.

TABLE I. INFORMATION ABOUT SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND NORMOTENSIVE WISTAR-KYOTO CONTROL RATS (WKY) USED IN THE EXPERIMENT

	SHR (N = 13)		WKY (N = 13)
Age (days)	84 ± 4[a]		81 ± 4
Body weight (g)	274 ± 22		290 ± 20
Heart weight (g)	0.93 ± 0.03		0.82 ± 0.07
H.W./B.W. × 100	0.33 ± 0.01	*	0.28 ± 0.01
Mean arterial pressure (mm Hg)	189 ± 6	*	120 ± 5

[a] Data reported as mean ± SEM.
* $P < 0.001$.

higher in SHRs than in WKYs as were heart weights expressed as percentage of body weight (H.W./B.W. × 100). However, cross-sectional areas of the SHR papillary muscle (0.94 ± 0.10 mm²) were not different from those of WKYs (0.88 ± 0.09 mm²).

(*A*) *Steady-state responses at L_{max}.* Comparison of isometric properties of the papillary muscles equilibrated at 0.2 Hz at L_{max} revealed no difference in resting tension (2.6 ± 0.3 g/mm² vs 2.6 ± 0.4 g/mm²), active tension (4.9 ± 0.5 g/mm² vs 5.4 ± 0.5 g/mm²), dT/dt (35 ± 4 g/mm²/sec vs 36 ± 4 g/mm²/sec), time-to-peak-tension development (166 ± 3 msec vs 167 ± 2 msec), or ½ relaxation time (176 + 7 msec vs 185 + 6 msec) between SHRs and WKYs, respectively.

(*B*) *Paired pulse responses. (i) Mechanical refractory period.* As shown in Table II, the MRP was found to be significantly longer in SHR preparations than in WKY preparations at both 0.2 and 0.5 Hz stimulation frequencies, even though increasing the frequency of stimulation decreased the MRP in both SHRs and WKYs.

As the delay between paired stimuli increased, the amplitude of the response to the second stimulus increased and, at the maximum delays investigated, approached the amplitude of the initial twitch. At delays below 500 msec, the second response was superimposed on the relaxation phase of the initial twitch, and precise quantitation of the amplitude of the isolated second response was not possible. No differences were detected between SHR and WKY preparations in the relationship between delay and

TABLE II. ISOMETRIC RESPONSE TO PAIRED STIMULI: MECHANICAL REFRACTORY PERIOD[a]

	SHR		WKY
At 0.2 hz (msec)	246 ± 4	← * →	229±6
	↑		↑
	**		**
	↓		↓
At 0.5 hz (msec)	221 ± 4	← * →	205 ± 5

[a] Minimal millisecond delay between paired stimuli that will elicit a second response.
* $P < 0.05$.
** $P < 0.01$.

the amplitude of the second response.

(ii) *Aftercontractions.* Aftercontractions were induced by paired pulse stimulation in many preparations. Table III summarizes the characteristics of these contractile events. The incidence and the amplitude of these events at both 0.2 and 0.5 Hz were greater in SHR than in WKY preparations. When the frequency of stimulation was increased from 0.2 to 0.5 Hz, the delay between paired stimuli that elicited the largest aftercontractions was decreased significantly ($P < 0.01$) in SHR preparations. When aftercontractions did appear in WKY preparations at 0.5 Hz, they were much smaller than at 0.2 Hz ($P < 0.001$).

Discussion. Hypertension in SHRs develops between 4 and 8 weeks of age and is firmly established by the time the animal is 8–10 weeks old (11). Cardiac hypertrophy, however, is evident at even earlier ages (12), suggesting that increased afterload may not be the only factor stimulating abnormal growth of the heart. The animals used in this study were approximately 12 weeks of age, and both hypertension and cardiac hypertrophy were evident.

Since isometric properties of papillary muscles from SHRs equilibrated at L_{max} are not different from WKYs, the tissue's maximum tension-generating capabilities are apparently not influenced at this time by the hypertension or hypertrophy. These findings imply that the slow-developing cardiac hypertrophy seen in SHRs may have more of the characteristics of volume-induced hypertrophy, which does not depress contractile properties of isolated muscle (13), than of pressure-induced hypertrophy, which does depress these properties (14, 15). This suggestion is supported by studies of Pfeffer

TABLE III. ISOMETRIC RESPONSE TO PAIRED PULSE STIMULATION: CHARACTERISTICS OF AFTERCONTRACTIONS[a]

	SHR		WKY
At 0.2 Hz			
Incidence	13/13		10/13
Optimal delay (msec)[b]	280 ± 8	*	265 ± 8
Amplitude (%)[c]	4.2 ± 0.5	**	2.6 ± 0.5
At 0.5 Hz			
Incidence	13/13		6/13
Optimal delay (msec)[b]	217 ± 8	*	248 ± 8
Amplitude (%)[c]	4.9 ± 0.4	***	0.4 ± 0.4

[a] Data reported as mean ± SEM.
[b] The delay between stimuli of a paired pulse presentation that elicits the largest amplitude aftercontraction.
[c] Percentage of control, control being the amplitude of the response to the first stimulus of the pair at the optimal delay.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

et al. (16) who report that the hypertrophy that accompanies the hypertension in SHRs up to 24 weeks of age is associated with the "stable peak pumping ability" of the heart when expressed per gram of myocardium.

While isometric characteristics of single contractions do not differ between SHRs and WKYs, the way in which two mechanical responses interact is different. The prolonged mechanical refractory period (MRP) seen in the SHRs reflects an altered time course of recovery events associated with a twitch. One component of this interval is the electrical refractory period (ERP) of the membrane. The longer MRP of SHRs might reflect a prolonged ERP. This possibility is supported by a report of a prolonged action potential plateau phase in ventricular cells from SHR preparations (12).

The MRP may also depend upon the time course of replenishment of the "activator Ca^{++} stores" of the sarcoplasmic reticulum. This is the major source of Ca^{++} for coupling excitation to contraction although a significant amount of extracellular Ca^{++} does move into the cell during the action potential (18). If the net rate of filling of this store is depressed, the MRP may be prolonged. Recent studies (19) indicate that Ca^{++}-uptake and -binding by sarcoplasmic reticulum from SHR cardiac muscle is de-

pressed. Yet, with paired pulses in this study, as the delay was increased, no differences were detected between SHR and WKY muscles. Since the amplitudes of mechanical events observed in this period are most likely to reflect the amount of Ca^{++} available for contraction, the results of this aspect of the study alone do not point to obvious differences in the rate of Ca^{++} recycling between SHR and WKY muscle.

An intriguing finding of this study was the accentuated frequencies of occurrence and strength of aftercontractions in the SHR preparations. The mechanism(s) responsible for aftercontractions is presently obscure. These mechanical events have been described by others to be exaggerated by high [Ca^{++}], epinephrine, and low temperature, yet are independent of propagated membrane voltage changes (20, 21). The observation that SHRs are more prone to develop aftercontractions leads to the suspicion that events associated with Ca^{++} cycling are altered in SHR heart muscle.

Summary. Isometric properties of the isolated cardiac muscle of spontaneously hypertensive rats (SHR) were compared to those from age-matched normotensive Wistar–Kyoto (WKY) preparations to assess the contribution of alterations in intrinsic contractile properties of cardiac muscle to the well-documented increase in cardiac output seen during the early phases of hypertension.

At L_{max}, there were no detectible differences in resting tension, active tension, rate of tension development, or rate of relaxation between SHR and WKY preparations, indicating that neither the hypertension nor the accompanying cardiac hypertrophy influences these particular contractile characteristics. However, interactions between individual contractions of cardiac muscle were different. With paired pulse stimulation, SHR preparations had longer mechanical refractory periods and larger and more frequent aftercontractions. Therefore, during the SHR's high cardiac output phase, certain, but not all, of the intrinsic mechanical properties of heart muscle are different from age-matched WKYs.

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