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Membrane Potentials and Contractile Events of Hypertrophied Rat Cardiac Muscle (41036)

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Abstract. Previous studies indicate that cardiac hypertrophy is associated with altered mechanical properties including prolonged contraction times and mechanical refractory periods and exaggerated aftercontractions in isolated rat papillary muscle preparations. The present study was designed to ascertain whether changes in transmembrane potentials accompany the altered mechanical properties. Responses of papillary muscles from hypertrophied hearts of deoxycorticosterone acetate-treated Wistar Kyoto (WKY) rats were compared to those of nonhypertrophied, sham-treated WKYs. Muscles connected to a force transducer in a bathing chamber containing a 27° oxygenated physiological salt solution were subjected to field stimulation and isometric tension measured. Intracellular microelectrodes were used to measure transmembrane potentials of single impaled cells. When compared to nonhypertrophied controls, the hypertrophied preparations had (1) longer action potentials and contraction times, (2) longer electrical and mechanical refractory periods, and (3) exaggerated transient depolarizations and aftercontractions following paired-pulse or high-frequency stimulation. Propranolol given to block the effect of endogenous catecholamine released by field stimulation decreased the electrical excitability and the tension output of all preparations but did not eliminate the differences between the hypertrophied and nonhypertrophied groups.

Cardiac hypertrophy has been shown to be associated with altered mechanical properties of isolated rat cardiac muscle preparations (1–5). These alterations include longer contraction times (1, 2), longer mechanical refractory periods (3, 4), and a tendency to develop exaggerated aftercontractions in response to paired-pulse stimulation (3–5). Because of the strong dependence of mechanical properties upon electrical events in cardiac muscle, it was of interest to determine what alterations of transmembrane potentials may accompany the altered mechanical properties. Therefore, in the present study, transmembrane potentials of single cells within isolated papillary muscle preparations of hypertrophied and nonhypertrophied rat hearts were measured. Simultaneous recording of isometric responses of the preparations allowed correlation of electrical and mechanical events.

The use of field stimulation to activate isolated papillary muscle preparations has been shown to release neural transmitters from nerve terminals which remain within the excised tissue (6, 7). Since catecholamines may promote the development of aftercontractions and their associated transient depolarizations in normal tissue (8, 9), and since alterations in cardiac catecholamine metabolism accompany rat cardiac hypertrophy (10, 11), additional studies were performed in the presence of propranolol, a β-blocking agent (12), to determine whether altered endogenous catecholamine effects were responsible for the differences seen between preparations of hypertrophied and nonhypertrophied cardiac muscle.

Materials and methods. *Animals.* Hypertension and cardiac hypertrophy were induced in unilaterally nephrectomized Wistar Kyoto (WKY) rats (39–42 weeks old) by treating animals with deoxycorticosterone acetate (DOCA) using a method previously described (4). Blood pressures of conscious, restrained warmed rats were monitored at biweekly intervals using an inflatable tail cuff and distally applied photocell. Adjustments were made in the DOCA injection (10 mg DOCA suspended in 0.2 ml sesame oil, s.c. biweekly) and drinking water (containing 1% NaCl) to maintain arterial systolic pressures at approximately 200 mm Hg for 12 weeks.

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Sham-operated, sesame oil-injected, age-matched littermate WKYs served as the control group, The degree of cardiac hypertrophy was assessed by comparing the wet ventricular weight and ventricular weight/body weight ratios of the DOCA-treated group (H-DT) with those of the sham-treated group (N-DS).

Papillary muscle preparation. After determining arterial pressure, the rat was anesthetized with ether, and the heart rapidly removed and placed in a well-oxygenated physiological salt solution (28–32°) containing (m*M*): NaCl 115, KCl 6.0, $MgCl_2$ 1.2, $CaCl_2$ 5.0, glucose 11.5, Tris–Cl 35.0, pH 7.4. This solution was used in previous studies from this laboratory and promotes the development of aftercontractions (4). The left ventricular posterior papillary muscle was dissected from the wall of the ventricle and tied at its base to a 5-mm Lucite post to be inserted into a muscle chamber. The valvular end of this muscle bundle was then separated from the ventricle and clamped into a 27-mm artery clamp which was attached to a Grass FT.03C force transducer. The muscle was quickly lowered into the muscle chamber containing the above salt solution (27 ± 1°) and the Lucite post attached rigidly to its moorings, placing the muscle in a horizontal position. Its length was adjusted to that which produced 1.0 g resting tension by moving the force transducer which was attached to a micrometer screw. Preoxygenated temperature-controlled solution was continuously circulated through the 3-ml chamber at a rate of approximately 20 ml/min. Supramaximal stimuli were generated by a Grass S88 stimulator via platinum plate electrodes positioned approximately 1 mm from each side of the muscle.

Transmembrane potentials were measured using 3 *M* KCl-filled glass microelectrodes. These electrodes were 5 mm in length and were attached to the Ag/AgCl electrode holder by a short segment of 3 *M* KCl-filled Silastic tubing which allowed for lateral movement. Tip resistance ranged from 10 to 20 *M*Ω Output was amplified by a WPI M701 microprobe system, displayed on an oscilloscope screen, and recorded on a Brush/Gould Model 440 recorder. Also recorded were the amplified tension signal and a stimulus marker.

Protocol. Muscles were stimulated at a frequency of 0.2 Hz and allowed to equilibrate for 30–60 min during which time active tension development reached a steady state. Muscle length was then adjusted to that at which tension development was maximal (L_{max}). (Resting tension at L_{max} ranged between 0.8 to 1.2 g.) Active tension development, time-to-peak tension, and time from peak to one-half relaxation were measured.

Single cardiac cells were impaled with microelectrodes and resting potential and action potential amplitudes were measured and corrected for tip potential obtained when the electrode was removed from the cell (average ± 5 mV). Times from the stimulus artifact to 50 and 80% repolarization were also measured. Data from at least five separate intracellular penetrations obtained at different locations in each whole muscle preparation were averaged for each testing condition to determine the mean response of the individual muscle.

Criteria applied to the selection of action potentials for data analysis included: (a) abrupt change of potential to between −60 and −100 mV upon penetration; (b) return to same resting potential after the action potential; (c) rapid rise time of the action potential upon stimulation; and (d) no obvious movement artifact. (Action potentials with discontinuities or late-developing slow waves correlated with the twitch were disregarded.)

Electrical and mechanical refractory periods were determined by applying extra stimuli at varying intervals following a control beat and identifying the minimum intervals at which electrical and mechanical responses could be evoked by the second stimulus. Electrical refractory periods were determined from at least five single cells from each preparation that held their penetration throughout the entire sequence of stimuli.

Aftercontractions and transient depolarizations occurred after paired-pulse stimulation in most preparations and their magnitude was assessed at a pairing interval of

300 msec at which, under the testing conditions used, they are most prominent. These events were also evoked by a short-duration, high-frequency stimulation (10 sec at 2.0 Hz) and their magnitudes were determined.

The β-blocker, propranolol (10^{-5} *M*), was added to the bathing solution to block effects of endogenous catecholamine release on the cardiac muscle (6, 7, 12) and the preceding protocol was repeated. Because of the catecholamine-independent membrane stabilizing effect of propranolol (13), all muscles became less excitable and stimulus parameters (duration and voltage) had to be increased to maintain maximal activation.

Data analysis. The average values of tension, transmembrane potential characteristics, and times for these events were determined for each preparation and then combined to determine the overall group means ± SE. Student's *t* test for unpaired data was applied to make statistical comparisons between preparations from the hypertrophied and nonhypertrophied hearts. The effect of propranolol on individual preparations was assessed by comparing responses before and after application of the drug using a paired *t* test.

Results. Information about the animals used in this study is given in Table I. The DOCA-treated rats (H-DT) had significantly higher arterial pressure and larger hearts than did the sham-treated rats (N-DS). This cardiac hypertrophy is also reflected in the higher heart weight/body weight ratio although this elevated ratio also reflects the smaller body weight of the DOCA-treated rats. The lengths of muscle between the clamp and the tie ranged from 5 to 9 mm and diameters between 0.5 and 1.0 mm. Muscles were selected so that cross-sectional areas were somewhat smaller than those used in previous studies (0.8–1.0 mm^2) (3–5) because microelectrode impalement of single cells was found to be easier to maintain in the thinner muscles with lower tension output.

Response to single stimuli. Figure 1 is a diagrammatic representation of the mechanical and electrical responses of the preparations to a single stimulus applied at 0.2 Hz. The responses are reconstructed from the mean amplitude and duration data (± SE) obtained from the hypertrophied (H-DT) and nonhypertrophied groups (N-DS). Statistical comparison of the mean mechanical characteristics (top graph of Fig. 1) indicated that, although active tension development did not differ significantly between groups, times to peak tension and one-half relaxation were significantly longer in the hypertrophied group than the nonhypertrophied group. Comparison of the mean electrical characteristics of the two groups (bottom graph of Fig. 1) indicated that although resting potentials and action potential amplitudes did not differ significantly between groups, action potential durations (50 and 80% repolarization times) were significantly longer in the hypertrophied preparations than in the nonhypertrophied preparations.

Refractory periods. Assessment of the muscles' responses to extra stimuli applied at various times following a control beat (at 0.2 Hz) revealed that the refractory period for the electrical response was usually

TABLE I. ANIMALS USED IN THE STUDY

	N	Arterial pressure (mm Hg)	Heart weight (g)	Body weight	Heart weight/ body weight (mg/g)
DOCA-treated WKY (H-DT)	8	194 ± 5	1.27 ± 0.06	301 ± 18	4.25 ± 0.15
Sham-treated WKY (N-DS)	9	101 ± 5 **	0.89 ± 0.06 **	347 ± 18 **	2.60 ± 0.06 **

Note. Data represent mean ± SE.
** $P < 0.01$.

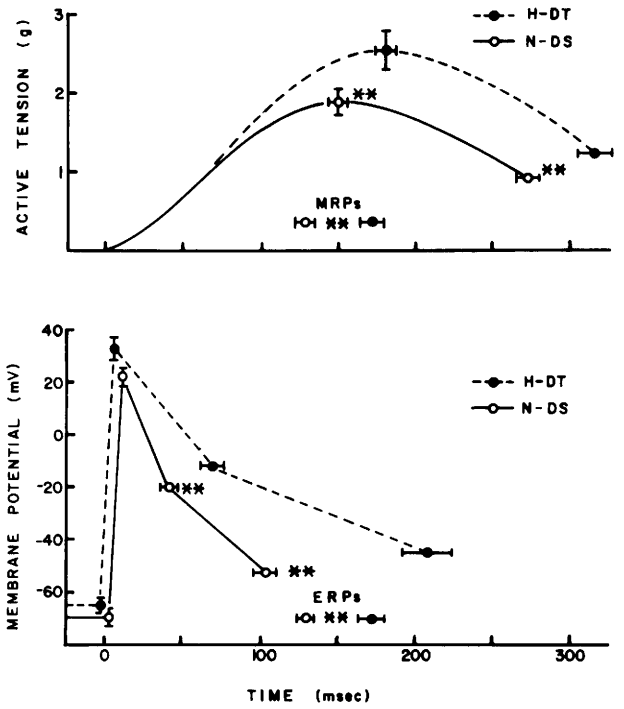

FIG. 1. Reconstructed tension (top) and membrane potential (bottom) responses of isolated cardiac muscle preparations to a single stimulus at 0.2 Hz. Data points represent mean ± SE of amplitude and duration variables obtained from H-DT and N-DS preparations. Mechanical refractory periods (MRP) and electrical refractory periods (ERP) for each group are indicated along the abscissa. ** indicates times to peak tension, to one-half relaxation, and to 50 and 80% repolarization of hypertrophied preparation were significantly different from those of nonhypertrophied preparations, $P < 0.01$.

identical to the refractory period for the mechanical response. These findings reflect the strong coupling of mechanical to electrical events. Furthermore, both the mechanical refractory period (MRP) and the electrical refractory period (ERP) were significantly longer in the hypertrophied than the nonhypertrophied preparations. Mean values (± SE) of these refractory periods are also indicated near the abscissas of the graphs in Fig. 1.

Aftercontractions and transient depolarizations. As has been previously reported (3, 5), these papillary muscle preparations develop aftercontractions in response to paired-pulse stimulation when tested in the specific bathing solution used in this study. Moreover, in the present study, high-frequency (2.0 Hz), short-duration (10 sec) stimulation also was found to produce large aftercontractions. The aftercontractions were always associated with small transient depolarizations of the individual cell membranes. Sample recordings of these events for an H-DT preparation are shown in Fig. 2. Figure 3 shows diagrammatic reconstructions of aftercontractions and transient depolarizations using mean data obtained from H-DT and N-DS groups. Comparison of the amplitudes of these events indicate that the hypertrophied preparations developed significantly larger aftercontractions and transient depolarizations than the nonhypertrophied preparations both following paired-pulse stimulation (300-msec pairing interval) and following a 10-sec, 2.0-Hz train of stimuli. In all cases, the peak of the transient depolarization either slightly preceded or occurred simultaneously with the peak of the aftercontractions.

Effect of propranolol. In both H-DT and N-DS preparations when propranolol was added to the bathing solution, active tension was depressed, mechanical and elec-

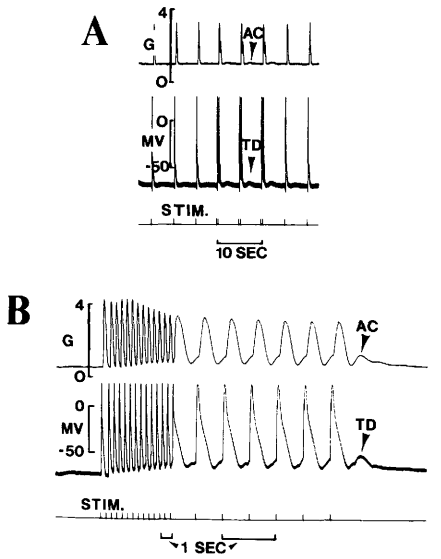

FIG. 2. Sample records of responses of DOCA-treated WKY preparation to repetitive stimuli. In each record, top trace is tension, middle trace is single cell membrane potential, and bottom trace is stimulus marker. (A) Response to single- and paired-pulse stimulation, pairing interval 300 msec. (B) Response to a 10-sec, 2.0-Hz stimulus train. Aftercontractions (AC) and transient depolarizations (TD) are indicated in each sample. Note time scale differences.

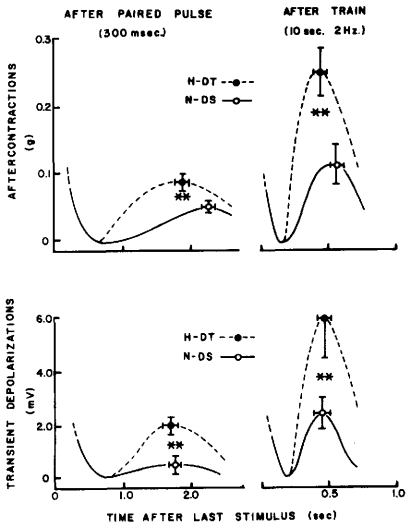

FIG. 3. Reconstructed aftercontractions (top) and transient depolarizations (bottom) occurring in isolated cardiac muscle preparations in response to paired-pulse (left) and high-frequency (right) stimulation. Data represents mean ± SE. ** indicates that the amplitude of the response of the hypertrophied preparations is significantly different from the response of nonhypertrophied preparations, $P < 0.01$.

trical refractory periods were prolonged, and the amplitude of aftercontractions and transient depolarizations was diminished. However, when the responses of the propranolol-treated H-DT papillary muscles were compared with those of propranolol-treated N-DS papillary muscles, it was found that H-DT preparations still had significantly longer times to peak tension, longer action potential durations, longer electrical and mechanical refractory periods, and larger aftercontractions and transient depolarizations than N-DS preparations. In addition, in the presence of propranolol, the action potential amplitude of the H-DT preparations was significantly greater than that of the N-DS preparations. These data are shown in Table II.

Discussion. The current studies indicate that alterations in certain characteristics of transmembrane potentials of rat cardiac muscle cells are correlated with changes in mechanical properties of isolated cardiac muscle preparations which accompany cardiac hypertrophy.

Prolonged action potentials of hypertrophied rat cardiac muscle have been reported by other investigators. Hayashi and Shibata (14) found that action potentials of hypertrophied ventricular muscle cells of SHRs were longer than those from nonhypertrophied hearts of WKYs. Aronson and Jeselsohn (15) also reported prolonged action potentials in hypertrophied heart muscle from renal-hypertensive rats. In addition, recent studies by Gulch *et al.* (16) indicate that in hypertrophied heart muscle from Goldblatt hypertensive rats the duration of the cardiac action potential is directly dependent upon the degree of hypertrophy. The present study confirms and extends these findings to the DOCA-treated rat model of cardiac hypertrophy. Furthermore, we have demonstrated that the prolonged action potentials of the hypertrophied cardiac muscle are associated with longer contraction times, longer electrical refractory periods, and

TABLE II. MECHANICAL AND ELECTRICAL CHARACTERISTICS OF PROPRANOLOL-TREATED PAPILLARY MUSCLES

	H-DT		N-DS
Active tension (g)	2.2 ± 0.4		1.5 ± 0.2
Time to peak (msec)	176 ± 7	**	140 ± 6
½ Relaxation time (msec)	132 ± 7		124 ± 13
Mechanical refractory period (msec)	195 ± 11	*	155 ± 9
Aftercontractions, pairing[a] (g)	0.07 ± 0.02	*	0.02 ± 0.01
Aftercontractions, train[b] (g)	0.13 ± 0.03	*	0.03 ± 0.01
Resting potential (mV)	71 ± 1		68 ± 2
Action potential (mV)	95 ± 2	*	85 ± 3
50% Repolarization time (msec)	95 ± 8	**	43 ± 3
80% Repolarization time (msec)	231 ± 22	**	97 ± 8
Electrical refractory period (msec)	195 ± 11	*	157 ± 9
Transient depolarizations, pairing[a] (mV)	1.8 ± 0.3	*	0.3 ± 0.2
Transient depolarizations, train[b] (mV)	2.6 ± 0.6	*	1.6 ± 0.6

Note. Data represent mean ± SE.
[a] Amplitude of the response following paired-pulse stimulation, 300-msec pairing interval.
[b] Amplitude of the response following high-frequency (2.0 Hz), short-duration (10 sec) train of stimuli.
* $P < 0.05$.
** $P < 0.01$.

longer mechanical refractory periods. Taken together, these various studies suggest that cardiac hypertrophy is a phenomenon affecting the entire excitation–contraction sequence of cardiac muscle performance.

Transient depolarizations were found to accompany aftercontractions evoked by either paired-pulse or high-frequency short-duration stimulation. Furthermore, the amplitude of these electrical and mechanical events was exaggerated in hypertrophied preparations. Since catecholamines have been reported to promote the development of aftercontractions and transient depolarizations (10, 11) in normal cardiac tissue, it might be suspected that an increased effect of endogenous norepinephrine released from residual nerve terminals by the field stimulation might account for the exaggeration of these events in hypertrophied cardiac muscle. In order to eliminate any possible catecholamine effect, the β-blocker, propranolol, was used. The dose used of this drug was intentionally large in order to ensure complete blockade. At this dose, propranolol also has been found to have a pronounced membrane stabilizing effect (13). This influence rendered the cardiac muscle preparations of the present study much less excitable and depressed the contractile response. In spite of these effects, however, the hypertrophy-dependent differences in aftercontractions, transient depolarizations, refractory periods, and action potential durations were not eliminated by the addition of propranolol. Thus, these findings indicate that the differences between the hypertrophied and nonhypertrophied preparations were not a result of differences in acute release of, or sensitivity to, endoge-

nous catecholamines. This, of course, does not rule out a long-term, chronic effect of altered catecholamine influences on the heart.

Mechanisms responsible for the development of aftercontractions and transient depolarizations are not well understood. These electrical and mechanical events are reported to be promoted in normal cardiac muscle by paired-pulse or high-frequency stimulation, low temperatures, low Na^+ concentrations, high Ca^{2+} concentration, and digitalis intoxication (17–23). Voltage clamp studies of digitalis-intoxicated preparations suggest that transient depolarizations are associated with a slow inward sodium current and an oscillatory release of Ca^{2+} from intracellular stores (19, 20). Whether similar mechanisms account for the development of aftercontractions and transient depolarization in hypertrophied cardiac muscle has not been ascertained although there are suggestions that Ca^{2+} handling by the sarcoplasmic reticulum is altered by cardiac hypertrophy (24).

Summary. Characteristics of simultaneously recorded membrane potentials and isometric tension responses of isolated papillary muscles from hypertrophied and nonhypertrophied rat hearts were determined. When compared with nonhypertrophied preparations, hypertrophied preparations were found to have longer action potentials, longer contraction times, and longer electrical and mechanical refractory periods. When subjected to paired-pulse or high-frequency, short-duration stimulation, hypertrophied preparations developed larger aftercontractions and larger transient depolarizations than did nonhypertrophied preparations. Propranolol treatment of the preparations did not eliminate these differences between hypertrophied and nonhypertrophied preparations, suggesting that endogenous catecholamines were not responsible for the differences.

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