Hydralazine: Effect on Contraction Mechanics of WKY and SHR Rat Heart Muscle

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SUMMARY Chronic hydralazine treatment (2 weeks) in 22-week-old normotensive (WKY) and spontaneously hypertensive rats (SHR) significantly lowered the systolic blood pressure in both groups. Left ventricular papillary muscles from nontreated and treated WKY and SHR were placed in an isometric myograph, and contractile indices monitored. Nontreated WKY and SHR were not statistically different comparing: tension, maximum contraction and relaxation rates, time to maximum tension, total contraction time, or passive and active length-tension curves. Hydralazine-treated WKY and SHR had significantly reduced tension and maximum rates of tension development and relaxation; passive length-tension characteristics were not altered.

Stressing the papillary muscles with increased frequency of electrical stimulation (0.1–2 Hz) did not differentiate the various groups. Significant (p < 0.05) alteration with isoproterenol (10^-10 M) occurred with the hydralazine-treated WKY, which responded with a greater increase in relaxation rate than the hydralazine-treated SHR. It is suggested that the clinically very useful drug, hydralazine, causes a distinct contractile state alteration in rat myocardium after treatment sufficient to lower SHR blood pressure to a normal range. (Hypertension 3: 356-361, 1981)

KEY WORDS • hydralazine • normotensive rat • spontaneously hypertensive rat • papillary muscle • contraction mechanics • isoproterenol • frequency-force curves

Hydralazine antihypertensive therapy has proven clinically useful in chronically dilating arteriolar smooth muscle.1,3 The drug has a special affinity for arterial smooth muscle, but also binds to uterus, heart, kidney, liver, spleen, and brain;4 this binding to the effector sites may account for hydralazine's long duration of action in the face of a short plasma half-life. Although negative inotropic effects on the heart have been reported for certain cardiovascular drugs such as verapamil,6 no study has investigated the conceivable cardiac effects that hydralazine may have during chronic therapy.

Other investigators have demonstrated that hydralazine treatment does not reverse the hypertrophy of ventricular5 or aortic tissue6 from spontaneously hypertensive rats; however, it does reduce the force-generating ability of the aorta.7 The present study was designed to determine if hydralazine treatment produced a similar change in the mechanical characteristics of ventricular tissue. The contractile response of left ventricular papillary muscle from treated and nontreated rats to increasing frequency of stimulation and concentration of isoproterenol were determined as well as the passive length-tension characteristics.

Materials and Method

Animals and Treatment

Male normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR, Okamoto and Aoki strain) were purchased from Taconic Farms at age 15–16 wks. During the subsequent 5–6 weeks, the animals were divided into four groups: 1) normal WKY; 2) treated WKY; 3) normal SHR; and 4) treated SHR. Systolic blood pressure (BP) was monitored by the indirect tail cuff method. Each treated animal received hydralazine (Sigma) at 80 mg/liter of drinking water ad libitum for 2 weeks prior to papillary muscle experiments at age 21–22 weeks.

Papillary Muscle Preparations

Animals were killed by cranial concussion, and the left ventricular papillary muscles quickly excised from the hearts and placed in an isometric myograph8 with
bathing solution containing (in mM concentrations): NaCl 117.4, NaHCO₃ 25, Na₂HPO₄ 1.2, MgSO₄ 1.2, KCl 2.6, CaCl₂ 2.5, and glucose 11.1, which was saturated with 95% O₂-5% CO₂ at 26°C. Field stimulation by flat platinum-plate electrodes arranged on both sides of the muscle delivered square wave pulses of 5 msec duration at approximately 20% above threshold voltage with a stimulation rate of 0.1 Hz.

Protocol

Muscles were equilibrated for a minimum of 2 hours during which the preload was adjusted to the load at Lmax. Next, the muscles were driven at increasing frequencies from 0.1 to 2 Hz to define the frequency-force curve. The frequency was returned to 0.1 Hz, and, after a stable contractile response was obtained, isoproterenol was added to the bath in concentrations ranging from 10⁻⁷ to 10⁻⁵ M. The final manipulation involved chelation of Ca²⁺ and Mg²⁺ by changing the bathing medium to one containing 5 mM EGTA, 5 mM EDTA. Within 5 minutes, no contractions to electrical stimulation were recorded, and then the muscles were stretched 5%, 10%, 15%, and 20% above Lmax to achieve a passive length-tension curve.

The muscles were removed from the bath, and the muscle segment between the clamps was blotted on filter paper and weighed. The weight of the muscle, its length, and its density (1.06 g/cc) were used to calculate the cross-sectional area (based on that of a cylinder).

Data Analysis

All values are expressed as the mean ± SEM, and the difference between the various parameters was determined by the Student's t test; a p value of 0.05 or less taken as indicating significance using a two-tailed probability table.

Results

Contraction Mechanics (Nontreated)

A summary of the twitch contraction characteristics for WKY and SHR left ventricular papillary muscles appears in table 1. WKY and SHR had similar maximum force (F), maximum rate (dF/dt) of contraction and relaxation, time to maximum force (TMF), and total contraction times (TCT). There were no significant differences in cross-sectional areas between the treated and nontreated groups.

Contraction Mechanics (Hydralazine Treated)

Both hypertensive and normotensive rats responded to hydralazine treatment, with a significant (p < 0.05) decrease in systolic BP of 22 mm Hg in WKY and 70 mm Hg in SHR (fig. 1). The larger reduction in pressure of the SHR group shifted the values within normotensive limits (< 150 mm Hg); however, the treated SHR and WKY were still different (WKY lower). As illustrated in figure 1 and table 1, the papillary muscle contractile tension and rates of tension development and relaxation were significantly (p < 0.05) reduced in association with hydralazine treatment.

Active and Passive Length-Tension Dynamics

The active and passive length-tension curves for all groups are compared in fig. 2. It is evident that at 82% of Lmax and to Lmax a decrease in contractile tension

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**Table 1. Twitch Contraction Mechanics of Hydralazine-Treated and Nontreated WKY and SHR Papillary Muscles**

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>CSA (mm²)</th>
<th>Force/CSA (g/mm²)</th>
<th>dF/dt/CSA (g/s/mm²)</th>
<th>TMF (msec)</th>
<th>TCT (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>0.541</td>
<td>8.18</td>
<td>74.4</td>
<td>27.7</td>
<td>152</td>
</tr>
<tr>
<td>± 0.031</td>
<td>± 0.332</td>
<td>± 4.49</td>
<td>± 2.1</td>
<td>± 8.8</td>
<td>± 40.0</td>
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<tr>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>WKY + hydralazine</td>
<td>0.586</td>
<td>5.89*</td>
<td>57.2*</td>
<td>19*</td>
<td>150</td>
</tr>
<tr>
<td>± 0.018</td>
<td>± 0.522</td>
<td>± 5.44</td>
<td>± 1.62</td>
<td>± 7.3</td>
<td>± 52.7</td>
</tr>
<tr>
<td>n = 10</td>
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<tr>
<td>SHR</td>
<td>0.546</td>
<td>8.75</td>
<td>79.1</td>
<td>29.5</td>
<td>168</td>
</tr>
<tr>
<td>± 0.059</td>
<td>± 0.759</td>
<td>± 6.64</td>
<td>± 2.33</td>
<td>± 7.6</td>
<td>± 76.9</td>
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<td>n = 10</td>
</tr>
<tr>
<td>SHR + hydralazine</td>
<td>0.609</td>
<td>5.59*</td>
<td>49.8*</td>
<td>21.2*</td>
<td>161</td>
</tr>
<tr>
<td>± 0.041</td>
<td>± 0.533</td>
<td>± 4.58</td>
<td>± 2.95</td>
<td>± 5.5</td>
<td>± 30.7</td>
</tr>
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<td>n = 10</td>
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<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
</tbody>
</table>

*dF/dt is the electronically derived maximum rate of contraction (C) and relaxation (R); TMF is the time to maximum force development and TCT is the total contraction time; CSA is the cross-sectional area. All values are means ± standard error of means.

*p < 0.05.
Effect of Hydralazine Treatment

Hydralazine was given ad libitum in the drinking water for 2 weeks. All values are means ± SEM, n = 8.

was associated with hydralazine treatment, but no change in the passive elastic properties. Preloads (g/mm²) at Lmax were: WKY 2.1 ± 0.35, SHR 2.6 ± 0.22, WKY with hydralazine 2.2 ± 0.39, SHR with hydralazine 2.2 ± 0.24. At the end of each experiment, the passive stress-strain curve at lengths greater than Lmax was determined (data not shown) after decreasing the calcium and magnesium to low levels, which prevented the response to electrical stimulation. There were no significant differences (p < 0.05) comparing all groups at 5%, 10%, and 15% above Lmax.

Frequency-Force Response

Increased frequency of stimulation from 0.1 to 2 Hz caused a typical (for rat) negative inotropic effect, with a decrease in force and maximum dF/dt of contraction (data not shown). No significant differences were observed comparing SHR and WKY decreases in maximum force and rate of force development. Maximum rate of relaxation changed from control as follows: WKY = -19 ± 6.7%, SHR = -15 ± 5.9%, WKY (hydralazine) = +2 ± 1.3%, SHR (hydralazine) = -21 ± 6.5%. At the highest frequency (2 Hz applied for 30 seconds), force decreased from control (0.1 Hz) as follows: WKY = -38 ± 3.2%, SHR = -42 ± 4.2%, WKY (hydralazine) = -45 ± 6.0%, SHR (hydralazine) = -55 ± 4.0%.

Effect of Isoproterenol

In contrast to the negative inotropic effect of increasing stimulation rate, stressing the papillary muscles with the beta-adrenergic agonist, isoproterenol, caused an increase in the maximum rate of contraction and relaxation in all groups (figs. 3 and 4), with the greatest increase occurring in the relaxation rates of treated WKY.

TMF and TCT were reduced similarly in all groups by isoproterenol. The maximum force in the presence of 5 x 10⁻⁶ M isoproterenol was as follows (100% = force at 0.1 Hz without isoproterenol): WKY = 93 ± 1.4% (p < 0.05), SHR = 91 ± 2.1% (p < 0.05), WKY (hydralazine) = 99 ± 2.5 (p > 0.05), and SHR (hydralazine) = 103 ± 1.6% (p > 0.05). These data indicate that isoproterenol significantly attenuated maximum force in nontreated muscles and that hydralazine treatment prevented this attenuation.

Discussion

Cardiac muscle mechanical properties of age-matched 22-week-old WKY and SHR rats were similar when compared at Lmax. These results support the studies of Heller et al. who used 7-, 12-, and 50-week-old WKY and SHR and emphasize that the mechanical properties of hypertrophied ventricles from SHR animals did not appear to undergo changes in muscle mechanics similar to those observed with experimentally induced pressure hypertrophy, i.e., decreased dF/dt, Vmax, and relaxation time.11-17 In our experiments, F, dF/dt, and contraction time of SHR ventricular muscles were not found to be different from those of WKY. Since our experiments were designed to study the effect of hydralazine on heart muscle, we did not experimentally examine the mechanisms that might interrelate to promote the proposed differences in ventricular muscle mechanics comparing pressure hypertrophy to genetic hypertension.

Our results further document the negative inotropic effect of increasing the frequency of stimulation of rat papillary muscles,14,16 and the lack of inotropic effect of isoproterenol on force at low stimulation rates.18 The most striking alterations with isoproterenol (10⁻⁴
to $5 \times 10^{-8}$ M) was the increase in WKY and SHR maximum relaxation rates to values 3–7 times the increase seen in maximum contraction rates. With paired pulse stimulation, Hamrell and Alpert reported that WKY had a greater maximum rate of tension development of the secondary twitch. Furthermore, addition of norepinephrine caused a shortened time to maximum relaxation and increased maximal rates of contraction in SHR and WKY; however, the control secondary contraction rate differences between the SHR and WKY persisted. It was suggested that there was possibly a decreased rate of Ca$^{2+}$ sequestration or a smaller pool of Ca$^{2+}$ available for release in SHR muscles, which might be due to an alteration in the cardiac cell membrane and/or the sarcoplasmic reticulum.

Relative to nontreated WKY and SHR, no significant differences were seen in the passive stress-strain curves of actively contracting muscles (stretched to $L_{\text{max}}$) or passive muscles where EGTA-EDTA was added to the bath. Hence, no differences in parallel elastic elements is suggested.

The major reason for undertaking this study was to investigate the ventricular myocardial contractile state after hydralazine treatment. Sen et al. found that hydralazine treatment of SHR rats at an age similar to that used in this study reversed the hypertensive state but, interestingly, did not reduce ventricular weight. Hydralazine allowed persistence of cardiac hypertrophy, while arterial pressure was lowered. A similar persistence of hypertrophy has been observed in aortae from hydralazine-treated SHR rats; how-
ever, such treatment was associated with a decrease in contractile function. For this reason, we examined the contractile function of cardiac muscle.

As with arterial tissue, the present study indicates that hydralazine also impairs the force-generating capacity of cardiac muscle (fig. 1 and table 1) from both WKY and SHR rats. This is not due to a change in the active length-tension relationship or accompanied by a change in the passive length-tension characteristics (fig. 2). In addition, hydralazine treatment is also associated with a significant decrease in the maximum rates of both contraction and relaxation (table 1).

Not only does hydralazine treatment alter the 0.1 Hz contraction characteristics of papillary muscles, but it also alters the response to increasing frequency of stimulation and increasing concentration of isoproterenol. As indicated in the Results section, the response of rat papillary muscles to an increase in frequency of stimulation is a reduction in maximum force development and in the maximum rate of contraction and relaxation; whereas the response to isoproterenol is a decrease in maximum force development, and an increase in the maximum rates of contraction and relaxation. Hydralazine treatment prevents the reduction in maximum force development seen in both SHR and WKY rats with increasing isoproterenol concentration but not with increasing frequency of stimulation. In addition, in WKY rats, it prevents the reduction in maximum rate of relaxation associated with increased stimulus frequency and further enhances the increase in maximum rate of relaxation seen with isoproterenol stimulation (fig. 3).

The mechanism by which hydralazine treatment contributes to the observed changes in contractile characteristics of papillary muscles from normotensive and hypertensive rats is unknown. The decrease in maximum contractile response of papillary muscles stimulated at 0.1 Hz may be the result of an alteration in the passive or active length-tension characteristics, a decrease in calcium availability, a decrease in the energy supply through metabolism, and/or a reduction in the contractile protein content. As shown in table 1 and figure 2, there are no changes in tissue cross-sectional area or the length-tension characteristics after hydralazine treatment. This suggests that the decrease in maximum contractile response is not due to a change in the physical properties of the tissue. It is generally assumed that the rate of contraction and relaxation reflect fluctuations in the intracellular ionic calcium concentration due to the activity of various cellular controllers of intracellular calcium concentration. Therefore, the observation that hydralazine treatment results in a reduction in the maximum rates of relaxation and contraction implies that hydralazine may also affect the intracellular regulation of calcium concentration.

It might be suggested that the reduction in maximum force development associated with increasing isoproterenol concentration is due to the saturation of the contractile system by calcium. The attenuation of this effect of isoproterenol by hydralazine treatment further suggests an effect of hydralazine on cellular calcium regulation. Finally, the observation that the changes in maximum relaxation rate due to stimulus frequency and isoproterenol are altered only in papillary muscles from WKY rats suggests that the action of hydralazine may be different in normotensive and hypertensive animals.

These effects of hydralazine could be due to hydralazine bound to the papillary muscle and, therefore, present in the in vitro muscle bath. In addition, the effects could result from the long-term effect of hydralazine or one of its metabolites on cellular processes during the 2-week treatment period, or a result of the decrease in afterload produced by hydralazine treatment. The present study does not permit a differentiation between these various mechanisms of action. However, data from arterial tissue suggest that the reduction in maximum force development is not due to the binding of hydralazine to arterial tissue. In addition, in two experiments where hydralazine was added directly to the muscle bath, only an increase in the threshold for electrical stimulation occurred without a change in maximum force development of papillary muscles.

In summary, these data suggest that 2 weeks of hydralazine treatment result in a significant reduction in the maximum force generating capacity and rates of relaxation and contraction of papillary muscles from both normotensive and spontaneously hypertensive rats. In addition, such treatment alters various aspects of the response of papillary muscles to increasing stimulus frequencies and isoproterenol concentration.

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References

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