Hypertrophied myocardium is more dependent on extracellular calcium than the normal cardiac muscle


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Summary

Background: The aim of this study was to analyze stable hypertrophied myocardial function and its response to inotropic maneuvers in rats submitted to renovascular hypertension for a 10-week period (RHT group, n=10).

Material/Methods: Myocardial performance was studied in isolated left ventricle papillary muscles in isometric contraction under the following conditions: at postrest contraction of 30 seconds (PRC), at extracellular calcium (ECa2+) chloride concentration of 1.25 and 5.20 mM, and after beta-adrenergic stimulation with 10–6 M isoproterenol (ISOP).

Results: The results were compared with normotensive Wistar controls rats (C group, n=10). In basal condition, resting tension, and contraction time (TPT) were greater, while relaxation time (RT50) tended to be longer in RHT than C group. PRC and ISOP promoted a similar change in muscle function response intensity (Δ) in both groups. ECa2+ shift did not change TPT in the C group and decreased TPT in the RHT animals; Δ was different between these groups. RT50 increased in C and decreased in RHT, both without statistical significance; however, Δ was different.

Conclusions: These results suggest that hypertrophied myocardial dysfunction may be attributed to changes in intracellular calcium cycling.

key words: renovascular hypertension • papillary muscle • myocardium function • calcium • isoproterenol

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**Background**

Myocardial hypertrophy is an adaptive response to ventricular overload [1]. Although there have been many investigations on myocardial function with pressure overload hypertrophy, the functional features of the myocardium remain controversial. In stable pressure overload left ventricular myocardial hypertrophy, mechanical activity has been reported as normal [2,3], enhanced [4,5], and depressed [6].

Inotropic maneuvers such as extracellular calcium (ECa\(^{2+}\)) concentration changes, \(\beta\)-adrenergic and postrest contraction (PRC) have been used to identify mechanical activity abnormalities in the hypertrophied myocardium, which cannot be observed at basal conditions. Furthermore, these maneuvers can help to understand the mechanisms implicated in myocardial dysfunction pathogenesis.

Studies of hypertrophied muscle with ECa\(^{2+}\) concentration changes and \(\beta\)-adrenergic stimulation showed that these maneuvers did not change the response [3–8] or made it worse [3] compared to normal rats. On the other hand, PRC intensified [9] or decreased [10] hypertrophied muscle function.

The aim of this study was to evaluate the mechanical performance of stable hypertrophied myocardium, its response to the cited inotropic maneuvers and to suggest which mechanisms are probably involved in these functional changes. There are no studies in literature using these maneuvers at the same time. Simultaneous use allows better evaluation of Ca\(^{2+}\) cycling and the \(\beta\)-adrenergic pathway in hypertrophied myocardium dysfunction.

**Material and Methods**

**Animal model and experimental protocol**

Eight-week-old male Wistar rats weighing 190 to 240 g were anesthetized \(ip\) with thiopental sodium (50 mg/kg). Renovascular hypertension (RHT) was induced by constricting the left renal artery to an outer diameter of 0.25 mm with the aid of a silver clip. The contralateral kidney was untouched (Goldblatt II hypertension model). All animals were housed in a temperature-controlled room (24°C) on a 12-h light/dark cycle, and food and water were supplied \(ad\) \(libitum\).

The rats were killed 10 weeks (RHT group, \(n=10\)) after surgery. Results were compared to sex-matched 18-week-old rats (control group, \(n=10\)). Systolic arterial pressure (SAP) was measured 1 week after surgery in RHT group and before killing in all animals, by tail cuff.

Left ventricular hypertrophy was evaluated by left ventricular weight to body weight ratio (LVW/BW) [11,12].

All experiments and procedures were performed in accordance with the US National Institute of Health’s Guide for the Care and Use of Laboratory Animals and were approved by Botucatu Medical School Animal Ethics Committee, São Paulo, Brazil.

**Functional study**

Ten animals from each group were killed by decapitation for functional study. The hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28°C. Trabecular carneae or papillary muscles were dissected from left ventricle (LV), mounted between 2 spring clips, and placed vertically in a chamber containing Krebs-Henseleit solution at 28°C and gassed with 95% O\(_2\) and 5% CO\(_2\). The composition of Krebs-Henseleit solution was as follows: 118.5 mM NaCl, 4.69 mM KCl, 1.25 mM CaCl\(_2\), 1.16 mM MgSO\(_4\), 1.18 KH\(_2\)PO\(_4\), 5.50 mM glucose, and 25.8 mM NaHCO\(_3\). The lower spring clip was attached to a Kyowa model 120T-20B force transducer by a thin steel wire (1/15,000 inch) which passed through a mercury seal at the bottom of the chamber. The upper spring clip was connected by a thin steel wire to a rigid lever arm above which a micrometer stop was mounted for the adjustment of muscle length. The lever arm was made of magnesium with a ball-bearing fulcrum and a lever arm ratio of 4:1. Preparations were stimulated 12 times/min with 5ms square wave pulses through parallel platinum electrodes, at voltages 10% greater than the minimum required to produce a maximal mechanical response.

The muscle were kept contracting isotonically with light loads for 60 minutes and then loaded to contract isometrically and stretched to the maximum of their length-tension curves.

After 5 minutes in after-loaded isotonic contractions, muscles were again placed under isometric conditions, and the peak of the length-tension curve (\(L_{\text{max}}\)) was carefully determined. A 15 minutes of stable isometric contraction was imposed before the experimental period and 1 isometric contraction was then recorded.

The following parameters were measured from isometric contractions: peak developed tension (DT, g/mm\(^2\)), resting tension (RT, g/mm\(^2\)), time to peak tension (TPT, ms), maximum rate of tension development (+dT/dt, g/mm\(^2\)/s), maximum rate of tension decline (–dT/dt, g/mm\(^2\)/s) and time from peak tension to 50% relaxation (RT\(_{50}\), ms).

The papillary muscle function was evaluated in the following conditions: PRC of 30 seconds, after increase the extracellular calcium concentration from 1.25 mM to 5.2 mM, and during \(\beta\)-adrenergic stimulation with isoprote- enol 10\(^{-6}\)M (ISOP).

At the end of each experiment, the muscle length at \(L_{\text{max}}\) was measured and the muscle between the 2 clips was blotted dry and weighed. Cross-sectional areas were calculated from the muscle weight and length by assuming cylindrical uniformity and a specific gravity of 1.0. All force data were normalized for the muscle cross-sectional area.

**Statistical analysis**

The data had normal distribution. Therefore, all data are reported as means ±SD. Morphologic and functional data in basal conditions were compared by \(t\) test for 2 independent samples. The study of functional parameters after each inotropic maneuver was done by \(t\) test for dependent samples. To compare the intensity of responses (\(\Delta\)) due to inotropic maneuvers, between RHT and control group, the independent \(t\) test was used. Differences between means which resulted in probability values (\(P\)) below 0.05 were considered
Data analysis was carried out with SigmaStat for Windows v2.03 (SPSS Inc, Chicago, IL).

### RESULTS

Mean values for morphometric parameters and SAP are summarized in Table 1. Renal artery clipping resulted in increased SAP (RHT: 179±22 mmHg vs. control: 133±6 mmHg, \( P<.001 \)) and LVW/BW (RHT: 2.92±0.41 vs Control: 1.96±0.10, \( P<.0001 \)). BW, RVW/BW, and CSA were similar in RHT and C groups.

Isometric data with inotropic maneuvers are summarized in Tables 2–4. Table 2 shows that in basal condition TPT and RT were higher in RHT than in C; other variables were similar in both groups. PRC increased DT, +dT/dt, and RT\(_{50}\) in RHT and C animals, but with the same intensity of response (\( \Delta \)). While TPT rose significantly only in the RHT.
group, RT and -dT/dt did not change with PRC; ∆ was also similar (Figure 1).

Table 3 shows that in basal conditions RT, TPT, and RT₅₀ were greater in RHT than in C rats. The ECa²⁺ change from 1.25 mM to 5.2 mM did not alter TPT in the C group, but decreased it significantly in the RHT group; ∆ was different between these groups. RT₅₀ increased in C and decreased in RHT animals, both without statistical significance; however ∆ was different between these groups. The maneuver promoted a significant and similar change in DT, +dT/dt, RT, and –dT/dt in RHT and C groups (Figure 2).

Table 4 shows that in basal conditions RT and TPT were greater in RHT than in C animals. ISOP increased +dT/dt, –dT/dt, and decreased TPT and RT₅₀ in both groups, but with similar ∆. The ISOP raised DT only in group C and decreased RT in the RHT group; ∆ was also similar (Figure 3).

**DISCUSSION**

The aim of this investigation was to study the myocardial performance of stable hypertrophied cardiac muscle and its inotropic response to postrest contraction, ECa²⁺ changes, and beta-adrenergic stimulation. These maneuvers allow us to investigate the possible participation of Ca²⁺ cycling and the β-adrenergic pathway in hypertrophied myocardium dysfunction [5–7,9,10].

In this study, hypertrophied myocardium presented greater values in RT and TPT throughout the experiment. RT₅₀ was higher in RHT than C before ECa²⁺ increase. Although the 3 inotropic maneuvers provoked clear alteration in myocardial function, only increased ECa²⁺ concentration caused a marked difference in behavior between hypertrophied and normal muscles. ECa²⁺ increase did not change TPT in controls and reduced it significantly in the RHT animals; RT₅₀ increased in the C and decreased in the RHT animals, both without statistical significance; however, ∆ was different.

The result observed in basal situation, before first maneuvers (PRC), is in agreement with studies that have reported similar developed tension and increased contraction time [3,5,13], and in discordance with others that verified improvement of hypertrophied myocardium [9,10,14]. The TPT increase and the tendency toward longer RT₅₀, in basal condition, observed in RHT could be related to alterations in cardiac myosin composition and duration of the calcium transient. Several studies have shown that hypertrophied myocardium muscle induce a significant reduction in high-adenotriphosphate (ATPase) V₁ isomyosin expression in rats, which is offset by an increase in 2 lower ATPase, V₂ and V₃ isofoms [15]. The shift in the myosin isoenzyme towards the V₁ isofom may in part, explain the increased TPT observed in RHT.

Another possible mechanism involved in hypertrophied myocardium dysfunction is intracellular calcium transient
changes [16,17]; this could involve sarcomemal Na\(^+\)-Ca\(^{2+}\) exchange and L-type channel, sarcoplasmic reticulum’s (SR) cyclical release of Ca\(^{2+}\) through the Ca\(^{2+}\)-release ryanojine channel, sequestration of Ca\(^{2+}\) by the Ca-ATPase pump (SERCA), and the affinity of myofilament for Ca\(^{2+}\). PRC allows the study of SR participation in the cardiac muscle contraction-relaxation cycle. During the resting period, there is an increase in cytoplasmic calcium level. This mechanism is not completely clear; several studies suggest that during the rest period there is an increase of Ca\(^{2+}\) influx via the Na\(^+\)-Ca\(^{2+}\) exchange system [9,18], while others suggest a decrease Ca\(^{2+}\)-eflux via the same exchange system [19]. The increase in ECa\(^{2+}\) concentration alters the contraction and relaxation phases owing to an improvement in available myoplasmic Ca\(^{2+}\) concentration, by interfering with the operation of the sarcomemal Na\(^+\)/Ca\(^{2+}\) exchange, L-type Ca\(^{2+}\) channels and SR function [20]. The high cytosolic Ca\(^{2+}\) increased SR ryanodine channel opening and Ca\(^{2+}\) uptake by SR, due to phosphorylation of SR phospholamban, promoted by the activation of Ca\(^{2+}\)-calmodulin dependent protein kinase-II (CaMKII) [16,17].

The beta-adrenergic system, through receptor agonists, also influences the intracellular calcium transient. This system stimulation increases the rate of contraction and relaxation by formation of cyclic adenosine monophosphate (cAMP), which increases the activity of the cAMP-dependent protein kinase (PKA). This phosphorylates several proteins concerned with contraction and relaxation, and also troponin-I, which results in troponin C-Ca\(^{2+}\) sensitivity changes, increasing the rate of crossbridge detachment and relaxation [20,21].

PRC induced a similar change in all analyzed parameters, in both groups. This disagrees with other investigators who found improvement [9], or depression [10,13] of hypertrophied myocardium function. Our results suggest that Na\(^+\)-Ca\(^{2+}\) exchange and SERCA function may be preserved in RHT animals.

We also found that sarcoplasmic Ca\(^{2+}\) elevation elicited a better relaxation and contraction time in RHT than in C rats. This is not in agreement with literature. While some works showed similar improvement in mechanical activity for both groups [7], Baudet and associates [9] found less response in hypertrophied muscle. The decrease in RT\(_{50}\) with ECa\(^{2+}\) elevation suggests that, perhaps, SERCA activation, owing to phosphorylation of SR phospholamban, promoted by CaMKII, is depressed in the RHT group. The TPT rise with ECa\(^{2+}\) change suggests that, the SR ryanodine channels are less sensitive to Ca\(^{2+}\) in hypertrophied cardiac muscle. Other investigators previously observed the ryanodine

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**Table 4. Isoproterenol effect on isometric data.**

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>ISOP</th>
<th>Δ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DT (g/mm²)</strong></td>
<td>Control</td>
<td>6.32±1.69</td>
<td>6.85±1.56</td>
<td>0.53±0.51</td>
</tr>
<tr>
<td></td>
<td>RHT</td>
<td>6.79±2.39</td>
<td>7.13±2.28</td>
<td>0.34±0.65</td>
</tr>
<tr>
<td><strong>RHT (g/mm²)</strong></td>
<td>Control</td>
<td>0.75±0.29</td>
<td>0.68±0.30</td>
<td>-0.07±0.09</td>
</tr>
<tr>
<td></td>
<td>RHT</td>
<td>1.05±0.34</td>
<td>0.97±0.26</td>
<td>-0.08±0.12</td>
</tr>
<tr>
<td><strong>TPT (ms)</strong></td>
<td>Control</td>
<td>144.00±15.98</td>
<td>107.89±17.51</td>
<td>-36.11±13.06</td>
</tr>
<tr>
<td></td>
<td>RHT</td>
<td>171.67±32.88</td>
<td>129.44±25.67</td>
<td>-42.33±10.63</td>
</tr>
<tr>
<td><strong>+dT/dt (g/mm²/s)</strong></td>
<td>Control</td>
<td>67.67±15.87</td>
<td>97.11±22.22</td>
<td>29.44±13.54</td>
</tr>
<tr>
<td></td>
<td>RHT</td>
<td>64.22±19.61</td>
<td>83.67±24.41</td>
<td>19.44±12.72</td>
</tr>
<tr>
<td><strong>–dT/dt (g/mm²/s)</strong></td>
<td>Control</td>
<td>23.44±4.53</td>
<td>47.11±8.64</td>
<td>23.67±8.28</td>
</tr>
<tr>
<td></td>
<td>RHT</td>
<td>27.78±11.44</td>
<td>47.22±12.71</td>
<td>19.44±7.80</td>
</tr>
<tr>
<td><strong>RT(_{50}) (ms)</strong></td>
<td>Control</td>
<td>178.44±41.13</td>
<td>97.22±21.34</td>
<td>-81.22±24.04</td>
</tr>
<tr>
<td></td>
<td>RHT</td>
<td>187.00±62.12</td>
<td>109.56±26.33</td>
<td>-77.44±37.50</td>
</tr>
</tbody>
</table>

Values are mean ±SD; RHT – renovascular hypertension; base – baseline values; ISOP – isoproterenol 10\(^{-6}\) M; DT – peak developed tension; RT – resting tension; TPT – time to peak tension; +dT/dt – maximum rate of tension development; –dT/dt – maximum rate of tension decline; RT\(_{50}\) – time from peak tension to 50% relaxation; Δ – intensity of treatment response; NS – not significant (t test).
channels and CaMKII decrease in stable myocardial hypertrophy [20,22,23].

Beta-adrenergic stimulation promoted similar intensity of response (Δ) in both groups. This is in agreement with some authors [8] and in discordance with others who found inotropic response depression in the hypertrophied muscle [2,3,5]. Our data suggest that the beta-adrenergic pathway, L-type Ca\(^{2+}\) channel, SR function to uptake Ca\(^{2+}\) via PKA, and troponin C-Ca\(^{2+}\) sensitivity were preserved in the hypertrophied muscles.

## Conclusions

Our investigation shows that there is a change in the relaxation and contraction phases of the hypertrophied muscle after 10 weeks of RHT and that these alterations may be related to sarcoplasmic reticulum calcium transient via CaMKII and ryanodine channels.

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## References:


