Candidate mechanical stimuli for hypertrophy during volume overload

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Submitted 7 August 2003; accepted in final form 21 May 2004

Homes, Jeffrey W. Candidate mechanical stimuli for hypertrophy during volume overload. J Appl Physiol 97: 1453–1460, 2004. First published May 28, 2004; 10.1152/japplphysiol.00834.2003.—A myocyte system that senses and responds to mechanical inputs might be activated by any number of features of the time-varying length or force signals experienced by the myocytes. We therefore characterized left ventricular volume and wall stress signals during early volume overload with high spatial and temporal resolution. Left ventricular pressure and volume were measured in open-chest isoflurane-anesthetized male Sprague-Dawley rats 4 and 7 days after surgical creation of an infrarenal arteriovenous fistula or sham operation. Mean wall stresses were calculated by using a simple thick-walled ellipsoidal model. Consistent with previous reports, this surgical model produced a 66% increase in cardiac output and a 10% increase in left ventricular mass by day 7. A number of features of the time-varying volume signal (maximum, mean, amplitude, rates of rise and fall) were significantly altered during early volume overload, whereas many other proposed hypertrophic stimuli, including peak systolic wall stress and diastolic strain, were not. Treating hemodynamic variables more generally as time-varying signals allowed us to identify a wider range of candidate mechanical stimuli for hypertrophy (including some not previously proposed in the literature) than focusing on standard time points in the cardiac cycle. We conclude that features of the time-varying ventricular volume signal and related local deformations may drive hypertrophy during volume overload and propose that those features of the volume signal that also change during pressure overload might be the most interesting candidates for further exploration.

VENTRICULAR MYOCYTES CLEARLY have the ability to sense and respond to changes in their mechanical environment (21). These responses are believed to play an important role in normal cellular growth as well as in remodeling in a number of disease states. The classic experimental models of hypertrophy are pressure overload and volume overload. Both models produce an increase in ventricular mass, but the spatial pattern of protein assembly differs. In pressure overload, new sarcomeres are assembled primarily in parallel with existing sarcomeres, producing an increase in cell cross-sectional area (1, 2); at the ventricular level, this growth is reflected in an increased ratio of wall thickness to cavity radius (2, 12). During experimental volume overload, new sarcomere assembly has been reported to occur either preferentially in series with existing sarcomeres, producing relative lengthening of the cells and dilatation of the ventricular cavity (2, 11), or proportionately in series and parallel (15, 16, 18).

Because myocytes normally experience cyclic variations in both length and force, a cellular system that senses these quantities (or related variables such as strain and stress) might respond to minima, maxima, mean values, amplitudes of variation, frequency of variation, or rates of change. At the ventricular level, this suggests that minimum volume and wall stress, maximum volume and stress, mean volume and stress, amplitudes of volume and stress variation, heart rate, and rates of change of volume and stress should all be considered candidate mechanical stimuli for hypertrophy. Many of these possibilities or other related quantities have already been proposed in the literature to explain hypertrophy during hemodynamic overload (Table 1). However, previous studies of experimental volume overload in the rat have not quantified changes in all of these variables. In particular, most studies have measured only cardiac output and heart rate, allowing calculation of stroke volume but not measurement of the time-varying volume signal or any of its features.

In addition, most studies of volume overload have focused on time points several weeks after creation of the overload, when hypertrophy is established or when animals transition to heart failure. Because we are interested in the signals that trigger hypertrophy and because hypertrophy itself may alter those signals, we focused on very early time points, as close as possible to the onset of hypertrophy. Preliminary experiments suggested that the first 3–4 days after creation of the aortocaval fistula are dominated by postoperative recovery (reduced body and heart weights) in our experimental model, so we studied the period immediately after return to preoperative body weight, days 4 through 7.

The purpose of this study was therefore to completely characterize changes in the time-varying left ventricular volume and estimated wall stress signals during early volume overload with high spatial and temporal resolution. A number of features of the time-varying volume signal (maximum, mean, amplitude, rates of rise and fall) were significantly altered during early volume overload in this model, whereas many previously proposed candidate hypertrophic stimuli were not. Treating hemodynamic variables more generally as time-varying signals allowed us to identify a wider range of candidate mechanical stimuli for hypertrophy (including some not previously proposed in the literature) than focusing on standard time points in the cardiac cycle.

MATERIALS AND METHODS

All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (14) and approved by Columbia University’s Institutional Animal Care and Use Committee. A total of 37 adult male Sprague-Dawley rats were used for these studies;
Table 1. Early changes in proposed hypertrophic stimuli (rodent models)

<table>
<thead>
<tr>
<th>Proposed Stimulus</th>
<th>Reported PO</th>
<th>Reported VO</th>
<th>Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak systolic wall stress (12)</td>
<td>+ (8, 20)</td>
<td>+ (4, 6)</td>
<td>NS</td>
</tr>
<tr>
<td>End-diastolic wall stress (12)</td>
<td>+ (8)</td>
<td>+ (6)</td>
<td>+</td>
</tr>
<tr>
<td>Systolic fiber strain (27)*</td>
<td>− (20)</td>
<td>+ (26)</td>
<td>+</td>
</tr>
<tr>
<td>Diastolic fiber strain (6)*</td>
<td>+ (6)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Diastolic cross-fiber strain (22)*</td>
<td>NS (6)</td>
<td>NS (6)</td>
<td></td>
</tr>
<tr>
<td>Contractility (3)</td>
<td>NS (17, 25)</td>
<td>NS (4, 15, 28)</td>
<td>NS</td>
</tr>
<tr>
<td>Ventricular work (19)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ventricular efficiency (19)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean volume§</td>
<td>(7, 20)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Maximum volume§</td>
<td>(7)</td>
<td>+ (26)</td>
<td>+</td>
</tr>
<tr>
<td>Volume amplitude§</td>
<td>− (7, 20)</td>
<td>+ (9)</td>
<td>+</td>
</tr>
<tr>
<td>−dV/dt (19)</td>
<td>− (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+dV/dt§</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of reported changes in proposed hypertrophic stimuli during the first week of pressure overload (PO) or volume overload (VO) in rodent models. +dV/dt and −dV/dt, maximum rates of volume rise (filling) and fall (ejection), respectively. Reference numbers indicate study that proposed the stimulus or reported the changes: +, increase; −, decrease; NS, no significant change. *Midwall circumferential strain was considered equivalent to fiber strain and midwall longitudinal strain to cross-fiber strain. ‡Ejection work (EW) increased, but the ratio of EW to pressure-volume area (PVA) did not change. §Only EW and volume amplitude correlated significantly with left ventricular weight-to-body weight ratio (LV/BW) in aortocaval fistula (AVF) animals. §To our knowledge, these have not been proposed as hypertrophic stimuli previously.

surgical procedures are described in detail below. Experimental groups consisted of animals studied 4 days (n = 7) and 7 days (n = 7) after creation of an aortocaval fistula (AVF) and animals studied 4 days (n = 7) and 7 days (n = 8) after sham operation.

Surgical procedures. Infrarenal AVFs were created by using the method of Garcia and Diebold with minor modifications (4, 10). Animals were anesthetized with ketamine HCl (80 mg/kg) and xylazine HCl (12 mg/kg) and placed on a warming pad (TPump, Gaymar, Orchard Park, NY) to maintain body temperature. The abdomen was opened in the midline by aseptic technique. The intestines were externalized onto a sterile 2 by 2 and covered with a second sterile 2 by 2 to limit drying. The abdominal aorta and vena cava were exposed between the renal arteries and the femoral arteries by blunt dissection. The vessels were manually occluded above and below the fistula site, and a fistula was created by introducing an 18-gauge short-bevel needle into the abdominal aorta and through the common wall into the vena cava. After withdrawal of the needle, the vessel and surrounding field were blotted dry and the hole in the aorta was sealed with cyanoacrylate glue (Krazy Glue gel, Elmer’s Products, Columbus, OH). Patency was easily confirmed by the observation of red streaming blood and pulsation in the vena cava rostral to the fistula site. Total occlusion time was <5 min. Approximately one-third of the surgeries required a second occlusion to seal leaks, whereas in two cases the glue failed and the animals died of rapid hemorrhage. The amount of flow through the fistula and amount of blood loss during the procedure were qualitatively scored. The intestines were replaced, the abdomen was irrigated with 5 ml of 0.9% saline containing 50,000 units/ml penicillin G potassium, and the muscle layer was closed with 3-0 chronic gut suture. The skin layer was closed with glue and staples (Autoclip 9 mm, Becton-Dickinson, Sparks, MD), and the animals were allowed to recover on a warming pad. Each animal received a single dose of 0.3 mg/kg buprenorphine HCl subcutaneously for postoperative analgesia. Animals were monitored for postoperative discomfort but in general did not require additional doses of buprenorphine. Sham animals underwent an identical operation including blunt dissection to expose the vessels and occlusion of the vessels for a similar length of time but no perforation of the aorta or vena cava.

Data collection. Four or 7 days after the initial surgery, animals underwent a nonsurvival study to measure ventricular dimensions and pressures, which were then used to calculate other parameters of interest. Animals were anesthetized with isoflurane (3.5% induction, 2.5–3.0% maintenance) and ventilated at 60 breaths/min at a flow rate of 300–400 ml/min and 1:1 ratio of inspiration to expiration time to achieve a tidal volume of ~3.0 ml (SAR-830/P small animal ventilator, ITTC Life Science, Woodland Hills, CA). Body temperature was maintained with a warming pad. Great care was taken to minimize blood loss because of the potential effects on the data to be collected. The chest was entered by midline sternotomy between two straight hemostats placed from a small hole in the diaphragm to prevent blood loss. Bleeding was controlled by electrocautery. A retractor was placed, and four 1.0-mm sonomicrometers (Sonometrics, London, Ontario, Canada) were sewn to the epicardium with 6-0 silk suture in the following order and locations: 1) on the posterior wall two-thirds of the distance from apex to base and just lateral to the intraventricular groove (posterior); 2) on the anterior wall two-thirds of the distance from apex to base and just lateral to the intraventricular groove (anterior); 3) on the lateral wall under the left atrial appendage (base); and 4) at the left ventricular apex (apex). A precalibrated Millar SP-671 miniature pressure transducer (Millar Instruments, Houston, TX) was zeroed in warm distilled water and inserted into the left ventricle through a hole created with a 25-gauge needle. After instrumentation, the hemostats were removed, hemostasis was verified, and a needle ground electrode was placed into the muscle of the upper right hindlimb and connected to the Sonometrics system. The instrumentation period required 40–45 min from the start of induction.

A 5-s run of segment length and pressure data was recorded each 5 min for 1 h by using the commercial software SonoLab (Sonometrics) with an acquisition rate of 312 Hz. Anesthesia was maintained at a depth that just suppressed spontaneous respiration (typically 2.75%), and depth was monitored frequently by observation and by toe pinch. After 1 h of data acquisition, a tie was placed around the inferior vena cava and a single longer data run was acquired during inferior vena cava occlusion. The animal was then heparinized (100 units injected directly into each ventricle), and blood gases were measured in samples taken directly from the right and left ventricles. Blood-gas analysis was performed by use of an iStat portable blood-gas monitor (iStat, East Windsor, NJ) to verify the presence of a left-to-right shunt in fistula animals. Finally, hearts were arrested by retrograde perfusion with cold Krebs-Henseleit buffer containing 30 mM 2,3-butanedione monoxime and removed for analysis. The atria were trimmed away, and overall dimensions as well as the positions of the sonomicrometers were measured and recorded. The sonomicrometers were then removed, and the left and right ventricles were weighed separately and fixed in 3.7% formaldehyde for histological analysis.

Data analysis. With attention to signal quality during original acquisition, very little correction or filtering of the sonomicrometer signals was required. These signals were viewed by use of SonoView (Sonometrics), and the best quality base-apex and anterior-posterior signal pair was selected. Occasionally, single points fell well outside the length range of a given tracing and were corrected by using built-in software features; the number of corrections was never more than 10 in a data run of 1,500 points. Base-apex segment length, anterior-posterior segment length, and ventricular pressure (Fig. 1) were then exported to an ASCII file for analysis using custom routines written in Matlab (v. 5.0, The MathWorks, Natick, MA). In a few cases, a premature ventricular contraction occurred during data acquisition; in this case, the premature contraction and subsequent beat were excluded from the exported data.

Custom Matlab software was used to calculate the volume enclosed by the epicardium, with the assumption that the left ventricle can be

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modeled as a prolate spheroid truncated 50% of the distance from equator to base as described by Streeter and Hanna (23):

\[ V = \frac{\pi}{3}[(2 + 3(0.5) - (0.5)^3)](AP/2)^2(BA/1.5) \]

where \( AP \) is the measured anterior-posterior segment length and \( BA \) is the base-apex segment length. Cavity volume was calculated by subtracting wall volume on the basis of the measured left ventricular weight and a density of 1.06 g/cm\(^3\) for myocardium. End diastole was selected automatically as the point immediately preceding the rapid pressure upstroke and end systole as the point of maximal elastance, \( E_{max} \) = left ventricular pressure/left ventricular volume, assuming a zero volume intercept (Fig. 1). A graphical interface then presented each end diastole-end systole pair to the user for confirmation or rejection; in the case of rejection, the beat was excluded from further analysis. Less than 10% of all beats analyzed for this study were rejected.

Mean circumferential (\( \varepsilon_c \)) and longitudinal (\( \varepsilon_l \)) wall stresses were estimated using the formula suggested by Mirsky and recommended by Yin (29):

\[ \sigma_c = \frac{(Pbh)(1 - h^2/2a^2 - h/2b + h^2/8a^2)}{V} \]

\[ \sigma_l = \frac{(Pbh)(1 - h/2b)^2}{V} \]

where \( a \) is half the major axis length, \( b \) is half the minor axis length at the midwall, and \( h \) is the wall thickness. Wall thickness was calculated throughout the cardiac cycle from the epicardial segment lengths and known wall volume with the assumption that the endocardial and epicardial surfaces are confocal nested prolate spheroids and that wall volume does not change substantially during the cardiac cycle. Mean circumferential (\( \varepsilon_c \)) and longitudinal strain (\( \varepsilon_l \)) were estimated from midwall dimensions as

\[ \varepsilon_c = 0.5 \times [(b/b_0)^2 - 1] \]

\[ \varepsilon_l = 0.5 \times [(a/a_0)^2 - 1] \]

For end-diastolic strain, deformed long- \( (a) \) and short-axis \( (b) \) midwall diameters were taken at end diastole and reference dimensions \( a_0 \) and \( b_0 \) at the point of minimum pressure. For end-systolic strain, values of \( a \) and \( b \) at end systole were compared with \( a_0 \) and \( b_0 \) at end diastole.

Features of the time-varying volume, pressure, and stress curves were identified and averaged across all beats in a given data run as follows. Values of all parameters were calculated at end diastole and end systole. In addition, the minimum, maximum, mean, greatest rates of increase and decrease, and frequency of each signal were calculated for each beat. For each beat, ejection work (EW) was computed as the area inside the pressure-volume loop and pressure-volume area as EW plus the area bounded by the pressure-volume loop on the right, a line from the end-systolic point to the origin on the left, and the volume axis (24). This analysis was performed for data runs at 15, 30, and 45 min of the 1-h data collection period to confirm stability of the experimental preparation. Any parameter that varied >10% across these time points was flagged and reviewed. Typically this occurred when the absolute value of a parameter (e.g., minimum or diastolic pressure) was very small. To facilitate display and comparison, average interpolated volume, pressure, and stress curves were generated for each group as follows. Given heart rates near 360 beats/min and a data-acquisition rate of ~300 Hz, each heartbeat was represented in the data set as roughly 50 data points. All parameters were therefore interpolated for each beat at 50 evenly spaced points by linear local interpolation. These interpolated curves were averaged across all beats in a data run, then across three data runs (15, 30, and 45 min) for each animal, and finally across the animals in each group to create average curves.

Statistics. Statistical analysis was performed using InStat (GraphPad, San Diego, CA). Trends in body weight over the course of the study were analyzed by one-way repeated-measures ANOVA and then with a Dunnett’s multiple comparisons test to determine the time points at which body weight differed significantly from its preoperative value. For all other parameters, unpaired t-tests were used to determine whether values were significantly different between fistula and sham groups at each time point.

RESULTS

Twenty-one animals were randomized to fistula groups; three died during the surgery (two of acute hemorrhage, one during anesthesia), one died postoperatively of acute cardiac failure with massive pulmonary edema, and one was killed because of labored breathing indicative of acute failure, yielding a net survival rate of 76%. Of the 16 animals studied, two were excluded because right ventricular \( P_{O_2} \) fell within the 95% confidence interval of the sham mean, indicating minimal shunt flow. Of 16 animals that underwent sham surgery, there was one unexplained postoperative death.

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Heart and body weights. Preliminary studies had suggested that both body weight and left ventricular weight decrease during the first 2–3 days after creation and sham surgery. We therefore focused on the first 3 days after return to preoperative body weight (postoperative days 4 to 7) as most clearly representing early hypertrophy. As expected on the basis of preliminary studies, body weight varied significantly with time in the 7-day fistula group (ANOVA P < 0.0001), being reduced relative to preoperative values at days 2 and 3 (P < 0.05) but not significantly different on days 4 through 7 (Fig. 2). Sham body weights were significantly reduced on postoperative day 2 and returned to preoperative values on day 3 (Fig. 2). There were no significant differences in body weight between the sham and fistula groups preoperatively, at 4 days, or at 7 days.

Both absolute and relative left and right ventricular hypertrophy were absent at day 4 but present by day 7 after creation of the fistula (Table 2). Left ventricular weight was 10% greater than sham (P = 0.001), and right ventricular weight was 27% greater than sham (P = 0.001) in the 7-day fistula group. Left ventricle-to-body weight ratio was 18% greater in the 7-day fistula group than in the 7-day shams (P = 0.003), whereas the ratio of right ventricular weight to body weight was 37% greater (P = 0.002).

Hemodynamics. Consistent with the presence of a large arteriovenous fistula, stroke volume was 98 and 92% greater in 4-day (P = 0.02) and 7-day (P = 0.0001) fistula groups, respectively, than in the corresponding sham groups, whereas cardiac output was 81% (P = 0.01) and 66% (P = 0.0004) greater (Table 2). Heart rate was lower in the fistula groups than in the sham groups, but this change was only significant at 7 days (P = 0.08 at 4 days, P = 0.003 at 7 days). At 7 days, the increase in stroke volume was due to a significant increase in end-diastolic volume (P = 0.01) without a change in end-systolic volume (Fig. 3). At 4 days, neither end-diastolic nor end-systolic volume was significantly different between the fistula and sham-operated groups. Diastolic and systolic pressures were not significantly different between fistula and sham-operated groups at either 4 days or 7 days.

Candidate hypertrophic stimuli. Table 1 lists a number of putative hypertrophic stimuli. Most of these signals showed little change 4 or 7 days after creation of a fistula (Table 3). End-diastolic mean wall stresses were higher in the fistula group at 7 days but not at 4 days. End-systolic mean circumferential strain was greater (more negative) in the fistula group at 4 and 7 days, and EW was increased in the fistula groups at both times (Table 3). Peak systolic wall stresses, end-diastolic strains, and contractility were not significantly different in the fistula groups. We also examined whether any of these stimuli were significantly correlated with the ratio of left ventricular weight to body weight (LV/BW) across the fistula groups and found that only EW (r² = 0.426, P = 0.011) and volume amplitude (r² = 0.390, P = 0.017) were significantly correlated with LV/BW. Changes in heart rate (HR), body weight (BW), and hemodynamic parameters measured in the present study (means ± SE). LV, left ventricle; RV, right ventricle; SV, stroke volume; CO, cardiac output; ESP, end-diastolic pressure; ESP, end-systolic pressure. *Statistically significant difference between sham and fistula groups.

Table 2. Heart weight, body weight, and hemodynamics during early volume overload

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4-day Sham</th>
<th>4-day Fistula</th>
<th>7-day Sham</th>
<th>7-day Fistula</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV weight, mg</td>
<td>611±16</td>
<td>688±40</td>
<td>676±12</td>
<td>745±10*</td>
</tr>
<tr>
<td>RV weight, mg</td>
<td>165±5</td>
<td>177±12</td>
<td>173±7</td>
<td>221±9*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>246±6</td>
<td>241±8</td>
<td>277±5</td>
<td>261±11</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>2.49±0.09</td>
<td>2.87±0.20</td>
<td>2.44±0.04</td>
<td>2.88±0.12*</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.67±0.03</td>
<td>0.74±0.06</td>
<td>0.63±0.02</td>
<td>0.86±0.06*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>373±9</td>
<td>346±11</td>
<td>385±6</td>
<td>333±14*</td>
</tr>
<tr>
<td>SV, μl</td>
<td>173±16</td>
<td>326±49*</td>
<td>187±17</td>
<td>360±27*</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>65±6</td>
<td>111±14*</td>
<td>72±6</td>
<td>119±9*</td>
</tr>
<tr>
<td>RV PO2, Torr</td>
<td>66±3</td>
<td>81±4*</td>
<td>63±2</td>
<td>84±5*</td>
</tr>
<tr>
<td>EDV, μl</td>
<td>445±75</td>
<td>570±95</td>
<td>319±45</td>
<td>535±57*</td>
</tr>
<tr>
<td>ESV, μl</td>
<td>271±63</td>
<td>244±60</td>
<td>131±29</td>
<td>174±36</td>
</tr>
<tr>
<td>EDP, cmH₂O</td>
<td>6.1±0.7</td>
<td>9.1±1.5</td>
<td>5.7±0.5</td>
<td>7.8±1.2</td>
</tr>
<tr>
<td>ESP, cmH₂O</td>
<td>120±11</td>
<td>130±12</td>
<td>122±6</td>
<td>106±8</td>
</tr>
</tbody>
</table>

Changes in heart weight (HR), body weight (BW), and hemodynamic parameters measured in the present study (means ± SE). LV, left ventricle; RV, right ventricle; SV, stroke volume; CO, cardiac output; ESP, end-diastolic pressure; ESP, end-systolic pressure. *Statistically significant difference between sham and fistula groups.

Volume and stress signal features. Average interpolated volume and stress curves are shown in Fig. 4. Most features of the time-varying volume signal were significantly different between fistula and sham groups at 7 days. Maximum volume (P = 0.01), the amplitude of volume variation (P = 0.0002), mean volume (P = 0.04), and maximum rates of volume rise (+dV/dt, P = 0.01) and fall (−dV/dt, P = 0.0002) were all significantly greater in the fistula animals, whereas minimum volume was not significantly different (Table 4). Of these signals, only amplitude of volume variation (P = 0.03) and −dV/dt (P = 0.05) were significantly greater in 4-day fistula animals than in the 4-day shams. None of the features of the time-varying circumferential or longitudinal wall stress signals (maximum, minimum, amplitude, mean, or maximum rates of rise or fall) were significantly different between fistula and sham groups at 4 or 7 days.

Fig. 2. Trends in body weight (means ± SE) in sham-operated animals (gray circles, n = 8) and in animals with abdominal aortocaval fistulae (AVF; black circles, n = 7). Preoperative and postoperative body weights are plotted at 0 and 0.2 days for clarity. AVF body weight was reduced on days 2 and 3 and returned to preoperative value on day 4; sham body weight was reduced on day 2 and returned to preoperative value on day 3. *Body weight significantly different from its preoperative value by Dunnett’s multiple-comparison post-test (ANOVA P < 0.0001 for both groups). AVF and sham body weights were not different preoperatively, on day 4, or on day 7.
suggested approaches to prioritizing these candidate signals for subsequent in vitro studies.

Candidate stimuli: narrowing the field. Because so many mechanical and chemical signals change simultaneously during experimental volume or pressure overload, most investigators have focused on signals that both change during overload and meet other a priori conditions. For example, some investigators have postulated that a hypertrophic stimulus should be normalized by the hypertrophy it induces, forming a negative feedback loop (6, 12). Other possible strategies include focusing on stimuli that show the largest relative changes, stimuli that change before the onset of hypertrophy, stimuli that correlate quantitatively with the degree of hypertrophy in a given model, or stimuli that explain the responses observed in multiple different experimental models.

About half of the candidate mechanical signals considered in this study showed no significant difference between the sham and the fistula groups (Tables 1, 3, 4). Among the remainder, features of the time-varying volume signal showed the largest relative increases, typically 50% at 7 days. Two of these volume signals, the amplitude of volume variation and $-dV/dt$, were also among only four candidate signals significantly increased by 4 days, closer to the onset of hypertrophy (Table 4); the others were end-systolic circumferential strain and EW, both related to the amplitude of volume variation (Table 3). Interestingly, EW and volume amplitude were also the only two candidate signals from Table 1 that were significantly correlated with LV/BW across all animals in this study. Taken together, these results suggest that volume amplitude, $-dV/dt$, and related signals are the most fruitful candidates for further investigation.

Given what is already known about myocyte mechanotransduction, it seems unlikely that features of the time-varying cavity volume signal are transduced directly. Local deformations associated with the cavity volume signals reported here are more likely candidates for in vitro study. It is therefore perhaps surprising at first glance that the estimated strains were not more prominently altered by volume overload. Because stroke volume increased in fistula animals entirely by an increase in end-diastolic volume, measures normalized by stroke volume increased in fistula animals entirely by an increase in end-diastolic volume, whereas at 4 days both related to the amplitude of volume variation (Table 3).

The overall goal of our work is to identify candidate mechanical stimuli for hypertrophy and directly test them one at a time in vitro. Because biological systems typically have nonlinear responses, we consider it critical to test candidate stimuli at physiologically relevant levels and therefore chose to first carefully quantify changes in candidate stimuli in vivo during experimental volume overload. We characterized the time-varying left ventricular volume and estimated wall stress signals during early volume overload with high spatial and temporal resolution. On the basis of these data, we identified several candidate mechanical stimuli for hypertrophy, including some that have not been previously recognized or proposed. Below is a discussion of these candidate stimuli and

DISCUSSION

The overall goal of our work is to identify candidate mechanical stimuli for hypertrophy and directly test them one at a time in vitro. Because biological systems typically have nonlinear responses, we consider it critical to test candidate stimuli at physiologically relevant levels and therefore chose to first carefully quantify changes in candidate stimuli in vivo during experimental volume overload. We characterized the time-varying left ventricular volume and estimated wall stress signals during early volume overload with high spatial and temporal resolution. On the basis of these data, we identified several candidate mechanical stimuli for hypertrophy, including some that have not been previously recognized or proposed. Below is a discussion of these candidate stimuli and
end-diastolic quantities, such as ejection fraction, fractional shortening, or end-systolic strain may minimize apparent changes during volume overload.

One additional strategy suggested above was to search for signals that explain observed responses in multiple different experimental models, for example during both pressure and volume overload. Among the signals considered here, volume amplitude, \(-dV/dt\), and systolic fiber strain all increase in early volume overload and decrease in early pressure overload (Table 1); these are intriguing candidates for explaining differential aspects of hypertrophy in the two models, such as relative cell lengthening in volume overload vs. thickening in pressure overload. By contrast, end-diastolic wall stress, mean volume, and maximum volume increase in both early volume and early pressure overload (Table 1) and therefore might merit exploration as stimuli for features of hypertrophy common to these two models, such as net protein accumulation.

**Comparison to previous reports.** Consistent with past reports, we found that cardiac output is increased entirely because of an increase in stroke volume with unchanged or slightly depressed heart rate during the first week after creation of an arteriovenous fistula in the rat (9, 13, 15, 28). We observed smaller elevations in end-diastolic pressure (EDP) and less depression of end-systolic pressure and contractility than reported by some other investigators using rat arteriovenous fistula models; this is largely a function of fistula size. A review of reports of hemodynamics during the first week after arteriovenous fistula in rats suggests that shunts larger than 75% of control cardiac output are associated with acute depression of systolic pressure generation, whereas smaller shunts are not (4, 9, 13, 15, 28). In most cases, larger fistulae are also more likely to cause an elevation of EDP; however, at least two studies have reported a significant increase in EDP with a shunt size similar to ours (9, 28).

The cardiac output and volume values reported here compare favorably to prior studies using different measurement methods. Dividing our measurements of cardiac output by body weight produced cardiac index values of 237 ± 31 and 256 ± 22 ml·min\(^{-1}\)·kg\(^{-1}\) for the 4-day and 7-day sham groups, respectively, which fall between Flaim et al.’s sham group average of 200 ml·min\(^{-1}\)·kg\(^{-1}\) in 500-g rats (9) and Huang et al.’s sham group average of 300 ml·min\(^{-1}\)·kg\(^{-1}\) in

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**Table 4. Volume signal features during early volume overload**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4-day Sham</th>
<th>4-day Fistula</th>
<th>7-day Sham</th>
<th>7-day Fistula</th>
</tr>
</thead>
<tbody>
<tr>
<td>min V, (\mu l)</td>
<td>251 ± 60</td>
<td>226 ± 50</td>
<td>121 ± 30</td>
<td>170 ± 35</td>
</tr>
<tr>
<td>max V, (\mu l)</td>
<td>479 ± 76</td>
<td>593 ± 96</td>
<td>352 ± 42</td>
<td>556 ± 54*</td>
</tr>
<tr>
<td>amp V, (\mu l)</td>
<td>229 ± 19</td>
<td>367 ± 54*</td>
<td>231 ± 18</td>
<td>386 ± 24*</td>
</tr>
<tr>
<td>mean V, (\mu l)</td>
<td>359 ± 68</td>
<td>411 ± 72</td>
<td>230 ± 36</td>
<td>366 ± 48*</td>
</tr>
<tr>
<td>(-dV/dt), ml/s</td>
<td>-6.1 ± 0.6</td>
<td>-8.3 ± 0.8*</td>
<td>-6.7 ± 0.3</td>
<td>-9.2 ± 0.4*</td>
</tr>
<tr>
<td>(+dV/dt), ml/s</td>
<td>7.5 ± 0.7</td>
<td>10.0 ± 1.5</td>
<td>7.2 ± 0.6</td>
<td>11.3 ± 1.4*</td>
</tr>
</tbody>
</table>

Changes in features of the time-varying volume signal in the present study (means ± SE). Min V, minimum volume; max V, maximum volume; amp V, amplitude of volume variation; mean V, mean volume. *Statistically significant difference between sham and fistula groups.
Heart and body weight changes during early volume overload. Some studies have reported significant increases in ventricular-to-body weight ratios as early as 3 days after creation of volume overload (5). However, the significant early drop in body weight we observed on days 2 and 3 suggests that these increased ratios may reflect the postoperative drop in body weight rather than a true ventricular hypertrophy. We observed very similar body weight trends in the AVF and sham groups in this study. We tested the possibility that the drop we observed was due to the use of a relatively long-acting anesthetic (ketamine/xylazine) for the initial procedure by anesthetizing a subset of observed was due to the use of a relatively long-acting anesthetic, very similar body weight trends in the AVF and sham groups, similar to Brower et al.’s (4) mean balloon volume of 327 $\mu$L at a pressure of 12.5 mmHg in isolated left ventricles from control rats weighing between 400 and 484 g.

The primary limitation of this study is that hemodynamics were measured in anesthetized, open-chest animals. It would be preferable to chronically instrument animals and track the early changes in hemodynamics after creation of volume overload. However, given the concerns raised by the present study about postoperative stress confounding the study of early overload, subjecting animals to chronic implantation of instrumentation seemed as likely to compromise as to improve data quality. Good agreement with cardiac output and pressure measurements made by other investigators in both conscious and anesthetized closed-chest rats (4) suggests that these errors were relatively small. Another potential source of error was drift or offset in the Millar miniature pressure transducer. The calibration slope of the transducer was remarkably stable across all studies. However, the offset varied considerably from study to study. This offset was removed by using the assumption that minimum left ventricular pressure in an open-chest rat should be approximately zero. The calculated offsets varied considerably but were not significantly different from zero in any group, confirming that noise rather than signal was removed with this correction.

Summary. This study characterized standard hemodynamic measures as well as the time-varying volume and stress signals present at the onset of hypertrophy in a rat volume overload model. A number of features of the time-varying volume signal (maximum, mean, amplitude, rates of rise and fall) were significantly altered during early volume overload in this model, whereas many previously proposed hypertrophic stimuli were not. Treating hemodynamic variables more generally as time-varying signals allowed us to identify a wider range of candidate mechanical stimuli for hypertrophy (including some not previously proposed in the literature) than focusing on standard time points in the cardiac cycle. We conclude that several features of the time-varying ventricular volume signal and related local deformations may drive hypertrophy during volume overload and propose that those features of the volume signal that also change during pressure overload might be the most interesting candidates for further exploration.

ACKNOWLEDGMENTS

The author thanks Dr. Gregory Brower at Auburn University for help in learning the surgical volume overload technique and Bozena Malyszko for help with data analysis for this study.

GRANTS

This work was funded by a Biomedical Engineering Research Grant (RG-00-0062) from the Whitaker Foundation.

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