Ventricular-vascular uncoupling by acute conotruncal occlusion in the stage 21 chick embryo

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Keller, Bradley B., Masaaki Yoshigi, and J oseph P. Tinney. Ventricular-vascular uncoupling by acute conotruncal occlusion in the stage 21 chick embryo. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2861–H2866, 1997.—Embryonic ventricular diastolic and systolic function was evaluated during normal ejection (coupled) and during acute ventricular outflow tract occlusion (uncoupled) in the stage 21 chick embryo. We simultaneously measured ventricular pressure with a servo-null system and ventricular dimensions using video microscopy. Experimental protocols included 1) baseline recording followed by acute conotruncal (CT) ligation (n = 15) and 2) baseline recording, preload increase using Krebs-Henseleit buffer (3 µl), preload reduction via venous hemorrhage, and then CT occlusion (n = 20). Ventricular epicardial cross-sectional area was converted to internal volume using wall volume measures and assuming an ellipsoid geometry to produce pressure-volume loops. We calculated the time constant of ventricular pressure decline using a monoeponential decay function with a pressure asymptote. As previously noted, heart rate was unaffected by acutely altered preload or afterload. CT ligation increased end-systolic pressure, maximal +dP/dt, and the time constant of ventricular pressure decline and decreased stroke volume indexed for end-diastolic volume. Thus the embryonic ventricle has significant contractile reserve masked in vivo by the dynamic coupling between the ventricle and arterial circulation.

This study shows that the embryonic ventricle generated a significantly higher systolic pressure when ejection into the arterial tree was prevented by acute conotruncal (CT) occlusion, resulting in a more linear end-systolic PV relation. However, the increased end-systolic pressure generated after acute CT occlusion was associated with a prolonged rate of ventricular relaxation (r), suggesting prolonged calcium reuptake by immature myocytes. As expected, the increase in ventricular afterload associated with CT occlusion was associated with decreased SV for matched EDVs. Thus embryonic myocardial contractile reserve is masked in the ejecting state by rapid changes in arterial impedance that occur in response to altered systemic blood flow.

METHODS

Embryo preparation and developmental staging. Fertilized white Leghorn chicken eggs were incubated blunt end up in a forced draft incubator to Hamburger-Hamilton stage 21 (3.5 days) of a 46-stage (21-day) incubation period (12). This embryonic stage corresponds to 11.5 embryonic days in mice (23) and to Streeter Horizon XIV in humans (25). The egg was positioned on a photomicroscope stage, and then access to the embryo was gained by opening the shell and removing a small region of extraembryonic membranes. Ambient temperature between 37 and 38°C was maintained using ambient heat lamps and a double-walled glass chamber (custom design; Radnoti Glass Technologies, Monrovia, CA) perfused at 15 l/min with 40°C water using a circulating water bath (model EX221; Neslab Instruments, Portsmouth, NH). All studies were performed in ovo.

Hemodynamic preparation. We measured ventricular pressure and dimensions using an integrated physiology and morphometry workstation that has been previously described in detail (19). Briefly, video images were acquired using a photomicroscope (model M400; Wild Leitz, Rockleigh, NJ), video camera (model 70-newvicon tube; Dage-MTI, Michigan City, IN), a video recorder (model VR9670; Magnavox), and a time-date generator (model VTG-33; FOR.A, West Newton, MA). A 50-µm division scribed glass standard was recorded in the plane of each embryo after imaging for planimetry software calibration.

We simultaneously measured intraventricular pressure with a servo-null system (model 900A; World Precision Instruments, Sarasota, FL). A fluid-filled glass capillary pipette was positioned with the use of a micromanipulator (Leitz, Wetzlar, Germany) to puncture the ventricle. The servo-null system is linear (y = 0.995x – 0.23, r = 0.99, SE = 0.11 mmHg) to a standing water column over the range of 0–10 mmHg (6). The frequency response of the servo-null pressure system was determined by pop test, and the actual pressure decay of the system can be approximated as a second-order
system (33). Intraventricular pressure was calculated as the difference between measured pressure and the pressure recorded when the tip was placed in extraembryonic fluid adjacent to the ventricle.

Preload alteration. Acute volume alterations were produced using a 10-µl graduated syringe (Hamilton, Reno, NV) connected by plastic tubing and a three-way stopcock to a reservoir of warmed, oxygenated Krebs-Henseleit buffer (KHB) and a polished, 5-µm-tip diameter glass pipette. We inserted the glass pipette into the sinus venosus to alter ventricular preload. After the recording of baseline pressure and video data, an event marker was triggered, and ventricular preload was increased by a single 3-µl injection of buffer. Ventricular preload was acutely decreased by severing a fourth-order vitelline vein, which results in a gradual reduction in ventricular preload over 180 s or by the acute withdrawal of 3 µl of venous blood (19, 32).

CT occlusion. Acute occlusion of the embryonic ventricular outflow tract, CT, was produced using a single strand of 10–0 nylon placed around the CT using microforceps and then loosely tied in an overhand knot (7). The loose CT suture was tied tightly to occlude the outflow tract, preventing ventricular ejection.

Experimental protocols were performed as follows: group I, pressure data recorded at baseline and then after isolated acute CT occlusion (n = 15); and group II, pressure and image data recorded at baseline and then after increased ventricular preload (KHB infusion), reduced preload (venous hemorrhage or blood withdrawal), and acute CT occlusion (n = 20). After data was recorded for each embryo, ventricular tetany was induced by the topical application of 50 µl of 2 M NaCl to the outer surface of the ventricle. Ventricular pressure and dimension data with or without acute alterations in ventricular preload, the CT knot was then tied tightly to occlude the outflow tract, preventing ventricular ejection.

Data acquisition and signal processing. In the first experimental protocol, we continuously recorded analog pressure waveforms at a sampling rate of 500 Hz with an analog-to-digital board (AT-MIO16; National Instruments, Austin, TX). We used custom analysis software (Labview; National Instruments, Austin, TX) and simultaneously view the recorded pressure waveforms in sequential 5-s windows. Individual waveforms were reviewed for signal stability, signal noise, and waveform reproducibility. Three consecutive cardiac cycles were then chosen for each window. These data files were used to calculate time derivative of pressure (dP/dt) and τ. In the second experimental protocol, a separate analog device sampled the waveform at 15.75 kHz and superimposed the analog pressure waveform onto the video image in real time (model PONV; Ogden Scientific, Spencerport, NY; see Ref. 19). The device also placed zero, full-scale, and pressure baseline markers onto video fields for pressure scale calibration. Composite video fields were recorded on VHS tape. These data files were used for all calculations of PV loop data.

Video image processing. Individual video fields were analyzed at workstations that included a minicomputer, frame-grabbing board, and image analysis software (19). Intra- and interobserver error of area measurement by planimetry is not significant (P > 0.29 and P > 0.96, respectively; see Ref. 19). The video measurement protocol for each embryo included 1) calibration of measurement software for length (mm) and area (mm²) from the recorded standard; 2) measurement of the absolute horizontal position of zero, full-scale, and instantaneous pressure markers on vertical lines 50 or 51; 3) conversion of positional values into millimeters mercury; 4) tracing of sequential video fields for epicardial ventricular cross-sectional area (mm²); 5) planimetry of epicardial ventricular area of the maximally contracted ventricle; 6) ventricular volume derived from area using a simplified ellipsoid geometric model V = 0.65·A³/² where V is volume and A is area; and 7) cavity volume calculated as total volume minus wall volume calculated from the tetanized ventricle using the same model equation (19). The ellipsoid equation was derived from equations for the cross-sectional area of an ellipsoid (A = πab), where a is the semimajor axis and b is the semiminor axis, and the volume of an ellipsoid of revolution [V = (4π/3)ab²] and assumed a fixed aspect ratio (a/b = 4/3). End diastole was defined as the onset of ventricular contraction, and end systole was defined at the time of maximum pressure-to-volume ratio.

Basic hemodynamic parameters. Individual pressure waveforms were analyzed for cycle length, maximal +dP/dt (+dP/dtmax), and maximal − dP/dt (−dP/dtmax). dP/dt was calculated using numerical differentiation. We calculated τ, the rate of pressure decay.

Fig. 1. Representative ventricular pressure and differentiated pressure (dP/dt) waveforms of a stage 21 chick embryo plotted on a time scale (top) in seconds (s). The expanded pressure decay and duration scale (bottom) displays ventricular pressure from the onset of diastole at P0 (pressure at the onset of relaxation), at 20, 30, 40 ms, and at Pmin (minimum pressure).
ventricular pressure decline as suggested by Braunstein et al. (4, 31), to include a pressure asymptote as follows: \( P(t) = a \cdot e^{-t/\tau} + b \) where \( P \) is pressure, and \( t \) is time.

Ventricular pressure at the onset of relaxation, \( P_0 \), was defined at \(-dP/dt_{\text{max}}\) for each cardiac cycle. Ventricular pressure data were then partitioned starting at \( P_0 \) for the subsequent 20, 30, and 40 ms and to \( P_{\text{min}} \) (Fig. 1). We calculated \( \tau \) by fitting raw pressure data using nonlinear least-squares regression using Sigmaplot (Jandel Scientific, Corte Madera, CA). Curve-fitting initial conditions were \( a = 2.5 \), \( \tau = 10 \), and \( b = 0 \).

We calculated end-diastolic and end-systolic pressures and volumes and SV from each PV loop. We calculated the EDV versus SV relationship from PV loops generated during alterations in ventricular preload and from PV loops generated during acute CT occlusion. We attempted to analyze end-systolic PV data using linear and second-order regression equations but were unable to adequately fit the data due to the limited range of end-systolic volumes (15).

Statistical analysis. All data were summarized as means \pm SE. We used one-way repeated-measures analysis of variance to determine statistical significance for repeat measures within groups, e.g., EDV at baseline and after buffer infusion, hemorrhage, and CT clamp. Linear regression analysis was used to determine the relationship between EDV and SV during ventricular ejection versus during acute CT occlusion. Statistical significance was defined by a probability value of \( P < 0.05 \).

RESULTS

Baseline embryonic heart rate was 133.9 \pm 2.3 beats/min, and heart rate was unchanged by alterations in ventricular preload (\( P = 0.88 \)) or by acute CT occlusion (\( P = 0.88 \)). Representative ventricular pressure and volume tracings at baseline and after buffer infusion, venous hemorrhage, and acute CT occlusion are shown in Fig. 2, and corresponding representative PV loops are shown in Fig. 3. During volume infusion, EDV increased dramatically with little increase in developed pressure.

As expected, EDV increased from 0.27 \pm 0.02 to 0.39 \pm 0.05 mm\(^3\) after volume infusion (\( P < 0.05 \)), decreased to 0.19 \pm 0.02 mm\(^3\) after preload reduction (\( P < 0.05 \)), and then increased to 0.35 \pm 0.03 mm\(^3\) after acute CT occlusion (\( P < 0.05 \), Fig. 4). EDV after CT occlusion was greater than baseline (\( P < 0.05 \)) but was similar to volume infusion (Fig. 4).

End-systolic pressure calculated from PV loops was 2.32 \pm 0.07 mmHg at baseline, 2.47 \pm 0.12 mmHg after volume infusion (\( P > 0.05 \)), and 2.12 \pm 0.08 mmHg (\( P > 0.05 \)) after venous hemorrhage. However, after acute CT occlusion, end-systolic pressure increased (3.60 \pm 0.08 mmHg, \( P < 0.05 \), Fig. 4). Consistent with the increase in end-systolic pressure, \(-dP/dt_{\text{max}}\) increased from 44.2 \pm 2.2 to 72.4 \pm 6.8 mmHg/s after CT clamp (\( P < 0.001 \)). As previously noted, SV varied linearly with EDV both during alterations in ventricular preload and after acute CT occlusion (Fig. 5).

After acute CT occlusion, there was no change in \(-dP/dt_{\text{max}}\) from baseline (\(-47.1 \pm 2.8 \text{ vs.} -45.3 \pm 1.4 \text{ mmHg/s}, P = 0.37 \)). The \( \tau \) calculated from each cardiac cycle was significantly influenced by the duration of pressure decline analyzed (Table 1). After acute CT occlusion, \( \tau \) was prolonged as calculated for each duration of pressure decay (Table 1).
DISCUSSION

Ventricular contractile reserve. At each developmental stage, ventricular pump performance and ventricular-vascular coupling in the embryo are similar to fetal (9), neonatal (26, 29), and adult hearts (15, 24). Scanning electron micrographs of developing embryonic myocytes reveal myofibrils in varying states of maturation with a significantly smaller volume fraction of aligned sarcomeres than the mature cardiomyocyte (7). Despite myocyte immaturity and geometric simplicity, the embryonic heart alters SV linearly in response to changes in EDV, resulting in a moderate range of functional adaptation to altered circulating blood volume (19, 32). However, the current study was performed to evaluate the embryonic contractile reserve that may be masked by dynamic changes in arterial load during altered SV. As expected, acute CT clamping significantly increased systolic pressure and end-systolic diameter likely produce increased end-systolic wall stress in the embryo (9, 20). In addition, the increased proximal resistance to ejection produced by CT clamping likely prolongs the time each myocyte generates force in an isometric mode before ejection and muscle shortening. This delay in “shortening deactivation” results in an increase in developed force toward a maximal “isometric” contraction (16). Thus we speculate that an acute increase in wall stress and force generation after CT clamping may be associated with an increase in intracellular calcium concentration, and further studies are needed to measure intracellular calcium concentration during acute alterations in ventricular preload and afterload and to correlate embryonic contractile reserve with myocyte excitation-contraction coupling.

Ventricular relaxation. In contrast to the increase in contractile function after acute CT clamping, diastolic function was affected adversely by acutely increased afterload. Our values for \( \tau \) before CT clamp were similar to previously published values for the chick embryo (4). Acute CT occlusion prolonged \( \tau \) in the embryonic heart, as has been noted for the mature left ventricle (13). Numerous studies have detailed the increased systolic pressure and end-systolic diameter likely produce increased end-systolic wall stress in the embryo (9, 20). In addition, the increased proximal resistance to ejection produced by CT clamping likely prolongs the time each myocyte generates force in an isometric mode before ejection and muscle shortening. This delay in “shortening deactivation” results in an increase in developed force toward a maximal “isometric” contraction (16). Thus we speculate that an acute increase in wall stress and force generation after CT clamping may be associated with an increase in intracellular calcium concentration, and further studies are needed to measure intracellular calcium concentration during acute alterations in ventricular preload and afterload and to correlate embryonic contractile reserve with myocyte excitation-contraction coupling.

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Table 1. Time constant of ventricular relaxation at baseline and after acute clamp

<table>
<thead>
<tr>
<th>Duration</th>
<th>Baseline</th>
<th>CT Clamp</th>
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<tbody>
<tr>
<td>20 ms</td>
<td>38.9 ± 3.6</td>
<td>69.2 ± 13.7*</td>
</tr>
<tr>
<td>30 ms</td>
<td>36.8 ± 5.8</td>
<td>88.2 ± 38.5*</td>
</tr>
<tr>
<td>40 ms</td>
<td>26.5 ± 2.4</td>
<td>58.2 ± 14.6*</td>
</tr>
<tr>
<td>( P_{\min} )</td>
<td>24.6 ± 1.6</td>
<td>35.8 ± 2.7*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. CT, conotruncal. Analysis of ventricular pressure decay using increasing durations of pressure decline starting at the onset of ventricular relaxation \( (P_0) \) and increasing from 20 ms after \( P_0 \) to the time of minimum pressure \( (P_{\min}) \). * \( P < 0.05 \) vs. baseline.
functional immaturity of the sarcoplasmic reticulum in developing embryonic and fetal rabbit, rat, mouse, and chick cardiomyocytes, highlighting the dependence of the embryonic myocyte on transsarcolemmal calcium via Na\(^+\)-Ca\(^2+\) exchange and L-type calcium channels (1, 2, 10, 14, 30). After acute CT clamp, the prolonged time course of pressure decay is consistent with prolonged calcium reuptake, likely due to the combination of increased intracellular calcium contraction after CT clamping and rate-limited reuptake. Of interest, chronic CT banding increases systolic pressure versus control embryos without prolonging \( \tau \) (13). When embryonic myocardial load is chronically altered, the embryonic myocardium compensates by altering the rate of myocyte division, e.g., resulting in myocyte hyperplasia after chronic CT banding (7) or hypoplasy after chronic calcium channel blockade (8) or left atrial ligation (28). Further studies of in vivo and in vitro embryonic muscle function are required to determine how altered myocardial growth rate influences the rate of maturation of the embryonic contractile apparatus.

Cycle length response. It is also worth briefly mentioning that acutely increased afterload was not associated with a compensatory change in cycle length. Previous studies of acute alterations in loading conditions in the embryo have shown a lack of chronotropic response to compensate for altered ventricular performance (3, 19, 31). However, the embryonic heart rate is linearly related to environmental temperature, likely due to direct effects on intracellular metabolic rates (5). The probable explanation for the lack of chronotropic responsiveness to altered ventricular load in the early embryo is the absence of a functional autonomic nervous system during primary cardiovascular development (22).

End-systolic PV relations. The basic model of a time-varying elastance assumes that arterial load is constant (24, 27). In contrast to the mature circulation, the embryonic CV system rapidly alters systemic vascular resistance in response to altered blood flow (31). Consequently, ventricular PV loops generated during alterations in ventricular preload display smaller changes in developed pressure than in SV, resulting in an extremely curvilinear end-systolic PV relationship (19). This rapid vasoactive response to altered blood flow in the embryo confounded our initial attempts to calculate a “maximum ventricular elastance.” End-systolic PV points from PV loops generated for the ejecting and outflow tract occluded ventricle during alterations in preload show that acute “uncoupling” of the ventricle from the vascular system reduced the curvilinearity of the end-systolic PV relationship.

Adaptive mechanisms. With recognition of the importance of the acute functional response to increased ventricular afterload, the broader question is the mechanism by which the developing CV system (heart and vasculature) adapts to chronic alterations in hemodynamic loading conditions. After chronic CT banding, the embryonic chick heart accelerates myocyte division, resulting in a larger heart with an increased number of normal-sized myocytes (7). Ventricular pressure is increased after CT banding, but, interestingly, the rate of ventricular pressure decay is unchanged after myocardial adaptation to chronically increased afterload (13). Improved relaxation in the setting of accelerated growth is likely crucial for the embryo, because ventricular filling is very sensitive to changes in ventricular diastolic function in this relatively low-pressure system (18). Insights into the unique adaptive mechanisms present in the developing cardiovascular system may aid our understanding of cardiovascular adaptation in the neonatal and mature heart.

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