Relationship of simultaneous atrial and ventricular pressures in stage 16–27 chick embryos

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Hu, Norman, and Bradley B. Keller. Relationship of simultaneous atrial and ventricular pressures in stage 16–27 chick embryos. Am. J. Physiol. 269 (Heart Circ. Physiol. 38): H1359–H1362, 1995.—Ventricular filling is determined by a dynamic balance between atrial and ventricular load and function. The embryonic cardiovascular system undergoes simultaneous growth and morphogenesis at the cellular, tissue, and organ levels to match the embryo's geometrically increasing metabolic demands. As part of our long-term investigation of atrial/ventricular coupling during primary cardiac morphogenesis, we defined the relationship between simultaneous atrial and ventricular pressures in the stage 16–27 white Leghorn chick embryo. We measured atrial and ventricular blood pressures with servo-null micropressure systems and sampled analog waveforms digitally at 500 Hz. Peak atrial pressure increased geometrically from 0.38 ± 0.03 to 1.21 ± 0.17 mmHg, while ventricular end-diastolic pressure increased linearly from 0.18 ± 0.03 to 0.55 ± 0.04 mmHg. The passive and active mean pressure gradients increased from 0.23 ± 0.04 and 0.20 ± 0.03 mmHg at stage 16 to 0.52 ± 0.10 and 0.62 ± 0.11 mmHg at stage 27, respectively. The atrioventricular pressure gradients were similar for stages 16, 18, and 21, then increased to stage 27. This diastolic pressure gradient identifies the atrioventricular orifice and developing endocardial cushions as a site of flow resistance that may influence both ventricular filling and chamber morphogenesis.

The embryonic heart is the first functioning organ, and the embryonic circulation expands and develops while simultaneously matching geometrically increasing metabolic demand. At the earliest stages, the embryonic heart is a curved tube with a single layer of outer myocardium, a thick layer of cardiac jelly, and a single layer of endothelial cells. At regions between the developing common atrium and common ventricle and between the ventricle and outflow chamber, the cardiac jelly forms thicker endocardial cushions. The incompressible cardiac jelly acts to amplify rhythmic myocardial contractions by increasing the ejection of blood from within the cardiac chambers, and the endocardial cushions coapt during contraction to facilitate forward blood flow. Thus, despite the absence of many of the structures of the mature heart, each embryonic chamber has distinct diastolic and systolic phases, and the embryonic heart generates forward pulsatile blood flow by the sequential contraction of the embryonic atrium, ventricle, and conotruncus.

The embryonic atrium displays conduit, reservoir, pump, and pacemaker functions similar to the mature heart. Simultaneous video microscopic measures of atrial perimeter and pulsed-Doppler measures of atrioventricular (AV) blood velocity show that passive ventricular filling occurs coincident with atrial filling and that the Doppler AV profile contains characteristic passive and active waves. From stage 16 to 24, the embryonic atrium increases in size and function, based on increasing heart rate, contraction velocity, and ventricular filling volume. The embryonic pacemaker rate is sensitive to changes in environmental temperature and local thermal stimulation and can be overdriven electronically. Embryonic heart rate increases during cardiovascular morphogenesis, and the cardiovascular circuit functions optimally near the intrinsic heart rate of a specific stage. The pump function of the embryonic atrium can also now be defined using the pressure-volume framework.

The atrial endocardium remains smooth walled during primary cardiac morphogenesis, in contrast to the trabecular ventricular endocardium. The embryonic ventricle undergoes geometric growth, and the myocardial wall transforms from a smooth, relatively thick shell to a porous, distensible, and trabecular meshwork. The structural morphogenesis of the ventricular wall corresponds to a progressive increase in ventricular diastolic properties, determined by an increasing rate of relaxation, increasing filling volumes despite decreasing filling times, and a rightward shift of the ventricular end-diastolic pressure-volume curve with development.

The interdependence of atrial and ventricular structural and functional maturation complicates the functional assessment of the developing cardiovascular system. Despite considerable data on the function of individual developing chambers, there have been relatively few studies with simultaneous direct measures of atrial and ventricular function. Therefore, as part of our long-term investigation of atrial/ventricular coupling during primary cardiac morphogenesis, we defined the relationship between simultaneous atrial and ventricular pressures in the stage 16–27 white Leghorn chick embryo. We measured atrial and ventricular blood pressures with servo-null micropressure systems and found a positive AV pressure gradient throughout ventricular filling. The pressure gradient may be a point of blood flow resistance critical to normal cardiac morphogenesis.

METHODS

Fertile white Leghorn chicken eggs were incubated blunt end up in a forced-draft 38.5°C incubator. The study group included Hamburger-Hamilton stage 16 (2.5 days, n = 5),
stage 18 (3 days, n = 7), stage 21 (3.5 days, n = 4), stage 24 (4
days, n = 16), and stage 27 (5 days, n = 7) of a 46-stage
(21-day) incubation period (9). We opened the shell at the 
blunt end over the air sac and then incised the outer shell
membrane with microdissecting forceps to expose the embryo.
We measured simultaneously right atrial pressure and ventricu-
lar pressure with 10-μm tip glass cannulas connected to two
servo-null micropressure systems (model 900; World Precision
Instruments, Sarasota, FL) (11). Analog waveforms were
digitally sampled at 500 Hz (R. C. Electronics) and stored on 5
1/4-in. Bernoulli disk cartridges (Iomega, Roy, UT). We moni-
tored embryo temperature with a 3.0-mm flat disk thermistor
placed on the surface of the egg white adjacent to the embryo
and the vitelline vascular bed and maintained constant embryo
temperature at 37°C using a warming lamp.
We measured right atrial pressure in one-half of the stage
24 embryos (n = 8) and left atrial pressures in the remaining
embryos (n = 8). To measure the left atrial pressure, the
embryo was gently repositioned left side up, exposing the
atrium (3). Heart rate was calculated before and after embryo
repositioning. If the heart rate changed more than 10 beats/
min, the embryo was excluded from further analysis.
Heart rate was calculated from individual cardiac cycles. We
defined the period of passive ventricular filling from the point
of lowest ventricular pressure to the onset of active atrial
contraction (Fig. 1). The active phase began with the upstroke
of the atrial pressure curve and extended to the upstroke of the
ventricular pressure curve at end diastole. The atrial and
ventricular pressure waveforms were analyzed for peak and
mean pressures during passive and active ventricular filling
(end of isometric relaxation to onset of the A wave, and A wave
to end diastole, respectively) and for peak systolic pressures.
Ventricular dP/dt was derived mathematically using the Data-
pac waveform analysis software (RUN Technologies, Laguna
Niguel, CA). The areas under the curve of atrial and ventricu-
lar pressure during passive and active ventricular filling were
used to determine mean pressure gradients.
We analyzed at least 15 consecutive cycles for each embryo.
Data were analyzed by Student’s t-test and regression analy-
isis, with statistically significant differences defined as a P
value of < 5%. Values are presented as means ± SE.

RESULTS

Cycle lengths. Cycle length decreased from 492.2 ±
20.6 ms at stage 16 to 415.5 ± 16.4 ms at stage 27 (Fig.
2). The mean change in cycle length after embryo
repositioning from right lateral up to left lateral up at
stage 24 was −5.2 ± 0.2% (P < 0.05).

Analog pressure waveforms. The time series of simul-
taneously recorded atrial and ventricular pressures and
digital ventricular dP/dt shows that atrial pressure is
higher than ventricular pressure throughout ventricu-
lar filling (Fig. 1). Atrial pressure is lowest at the onset
of rapid atrial filling, which occurs coincident with the
onset of ventricular systole. The combined atrial and
ventricular waveforms are highlighted as follows: 1) atrial
peak pressure, 2) onset of ventricular ejection, 3) ventricu-
lar peak pressure, 4) ventricular end systole, 5) ventricu-
lar diastolic AV pressure crossover, 6) ventricular end-diastolic pressure, and 7) ventricular systolic AV pressure
crossover. In Fig. 1, point a represents the onset of passive ventricular filling, and point b represents
the onset of active ventricular filling.

Atrial and ventricular pressures. Peak atrial pressure
increased geometrically vs. embryo stage (r = 0.7266,
P < 0.05), while ventricular end-diastolic pressure
increased linearly (r = 0.9975, P < 0.05, Table 1).
Ventricular peak pressure increased geometrically with
stage (r = 0.9000, P < 0.05). All three pressures were
linearly related to stage-specific values for embryo weight
(r ≥ 0.9638, P < 0.05 for each, Fig. 2). In the subset of
stage 24 embryos where we measured either right or left
atrial pressure, atrial peak systolic pressures (0.60 ±
0.08 vs. 0.67 ± 0.09 mmHg, P > 0.05) and cycle lengths
(414.0 ± 12.9 vs. 443.0 ± 19.0 ms, P > 0.05) were
similar.

AV pressure gradients. Mean passive and active AV
pressure gradients were similar at each stage (P > 0.05
for all; Fig. 3). The passive and active mean AV pressure

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cycle Length, ms</th>
<th>Peak Atrial Systole, mmHg</th>
<th>Peak Ventricular Systole, mmHg</th>
<th>Ventricular End Diastole, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>492.2 ± 40.6</td>
<td>0.38 ± 0.03</td>
<td>1.16 ± 0.06</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>18</td>
<td>441.7 ± 10.1</td>
<td>0.48 ± 0.06</td>
<td>1.51 ± 0.06</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>21</td>
<td>418.8 ± 16.0</td>
<td>0.54 ± 0.11</td>
<td>1.68 ± 0.09</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>24</td>
<td>437.3 ± 25.1</td>
<td>0.80 ± 0.10</td>
<td>2.21 ± 0.18</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>27</td>
<td>415.5 ± 16.4</td>
<td>1.21 ± 0.17</td>
<td>3.14 ± 0.12</td>
<td>0.54 ± 0.04</td>
</tr>
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</table>

Values are means ± SE.
DISCUSSION

The embryonic cardiovascular system provides blood flow to the developing embryo and extraembryonic vascular bed during simultaneous structural and functional maturation. The onset of cardiac contraction in the avian heart begins at 29–33 h of incubation, shortly after the fusion of the paired heart tubes (19). Resting parameters of heart rate, ventricular and arterial blood pressure, and blood flow are available for the chick and rat embryo during primary cardiac morphogenesis. While the major focus of research on the developing circulation has been on ventricular pump function, the embryonic ventricle is extremely sensitive to preload and heart rate, highlighting the significance of AV coupling and atrial pacemaker, conduit, and pump functions.

Atrial conduit function is important in the embryonic heart, since up to one-half of ventricular filling volume occurs before the onset of atrial systole (3). Venous inflow patterns in the embryonic right atrium display preferential streaming across either the right or left AV groove, depending on the origin of venous return. In addition, there are regional sources for venous return that result in preferential streaming through the embryonic heart and preferential arterial distribution (10). Thus alterations in venous return due to changes in peripheral tissue perfusion, vascular resistance, or capacitance could affect ventricular morphogenesis and function (14).

Atrial systole is also an important determinant of embryonic ventricular filling. Retrograde flow is seen in the sinus venosus and central veins during atrial systole, consistent with recordings of inferior vena caval flow acquired with transvaginal pulsed-Doppler ultrasound in the early human embryo (22). The AV cushion functions as a valve to prevent retrograde flow during systole (1). Atrial afterload relates to the combination of AV resistance to flow and ventricular diastolic properties. As further evidence of the pulsatile nature of the embryonic heart, rapid atrial filling occurred coincident with the onset of ventricular systole.

Atrial peak pressure increased geometrically with stage, as did ventricular peak pressure. This increase in systolic function is consistent with the increase in myocardial mass and myocyte maturation associated with chamber development. Our finding that the right and left atrial pressures were similar in the stage 24 chick embryo is consistent with anatomic data that the atrium is a common chamber until stage 29 (6 days) when atrial septation and fusion of the ventral and dorsal endocardial cushions are completed. The avian atrial septum remains fenestrated in the area of the foramen ovale following primary cardiac morphogenesis. Both venous return to the atrium (20) and distal ventricular outflow tract obstruction (6) can influence trans-atrial blood flow and atrial septal morphogenesis.

We found that atrial pressure exceeds ventricular pressure throughout ventricular filling. The pressure gradient is likely due to a resistance to flow across the developing AV cushions. The pressure drop across the AV cushions is actually the pressure difference during AV flow (occurring sometime after point a in Fig. 1) and may be estimated by the mean pressure difference during ventricular filling. This pressure drop should be similar during passive and active atrial filling unless atrial contraction resulted in a reduced AV orifice area due to boundary effects. The onset of passive ventricular filling (point a) begins after ventricular pressure falls below atrial pressure (point 3) minus the pressure drop across the AV cushions. The onset of active ventricular filling is likely coincident with atrial systole (point b), though a small additional volume of blood related to inertial effects of passive flow may fill the ventricle. To determine the actual onset and end of AV blood flow, simultaneous Doppler blood velocity and chamber pressures are required.

One intriguing finding that requires further investigation is the similar AV pressure gradients from stage 16 to 21 in contrast to the increasing gradients at stages 24 and 27. One explanation could be that the AV orifice enlarges proportionally to the increase in AV flow. The progressive rise in AV pressure gradient after stage 21 may be due to maturation of the right and left AV orifices with fusion of the primum septum to the AV cushions, resulting in two smaller AV orifices with higher resistance to flow than the preceding common orifice. The impact of changing AV resistance on ventricular filling is of particular importance to syndromes including AV valve stenosis or atresia that are associated with ventricular chamber hypoplasia (5). In addition, the analysis of blood pressure and flow across AV cushions is also relevant to defining the mechanisms responsible for partitioning the ventricle and aortic sac.

Obviously, measures of AV pressure and flow gradients are also dependent on the maturation of ventricular diastolic properties. Embryonic ventricular diastole is influenced by diastolic filling time (4, 12), ventricular geometry (18), and ventricular material properties (23). Similar AV pressure gradients from stage 16 to 21 in the context of increasing AV flow reflect a decrease in AV orifice resistance associated with enlarging heart size.
Thus a small but significant pressure difference exists between the developing atrial and ventricular chambers. Because embryonic cardiovascular function increases dramatically to support rapid embryo growth, subtle deviations in structural or functional maturation may have dire consequences on the mature system. Parameters of normal cardiovascular function are now available in the human embryo from 8 wk of gestation, emphasizing the importance of defining the normal pressure and flow gradients present in the developing heart.

This work was supported by National Heart, Lung, and Blood Institute Specialized Center of Research Award in Pediatric Cardiovascular Diseases PO5-HL-51498 (to N. Hu and B. B. Keller) and Physician Scientist Award III-02498 (to B. B. Keller).

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Received 31 January 1995; accepted in final form 22 May 1995.

REFERENCES