Aortic Impedance and Hydraulic Power in the Chick Embryo From Stages 18 to 29

Kenneth G. Zahka, Norman Hu, Kenneth P. Brin, Frank C.P. Yin, and Edward B. Clark

Little is known about the hemodynamic properties of the rapidly expanding arterial bed during embryonic development. Using a servo-null pressure system and 20-MHz pulsed Doppler velocity meter, we recorded simultaneous dorsal aortic pressure and velocity waveforms. The waveforms were digitized at 3-msec intervals and subjected to Fourier analysis. We calculated hydraulic energy and the impedance spectrum to 10 Hz. From stages 18 to 29, heart rate (148±3 to 193±9 beats/min), systolic pressure (1.14±0.12 to 3.04±0.10 mm Hg), and mean dorsal aortic blood flow (21±2 to 214±19 mm3/min) increased. Peripheral vascular resistance (Zo: 30.4±4.8 to 6.4±0.7 dyne×sec/mm5), and the impedance moduli (Z1: 6.5±1.0 to 1.7±0.2 dyne×sec/mm5; Z2: 6.1±1.2 to 1.7±0.1 dyne×sec/mm5; Z3: 7.3±1.1 to 1.7±0.2 dyne×sec/mm5) decreased. Total hydraulic power increased from 48±7 to 2,606±96 nW, while the proportion of oscillatory energy increased from 29±2% to 65±4%. With development hydraulic load decreases, total external work increases and the dorsal aorta and embryonic vascular bed becomes more compliant. A greater proportion of total energy is expanded in pulsatile blood flow, suggesting that ventricular-arterial coupling is less efficient later in development.

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Pulsatile blood flow begins with the earliest cardiac contraction.1 In the mature animal, the vascular properties are an important determinant of cardiovascular function.2,3 Yet little is known about the rapidly expanding arterial bed during embryonic development. We hypothesized that the pulsatile characteristics of blood flow change with morphogenesis of the cardiovascular system.

Previous studies of the chick embryo have demonstrated a 10-fold increase in dorsal aortic blood flow and a tripling of systolic aortic pressure as the embryo grows from stage 18 to 29.4,5 The decrease in peripheral vascular resistance over this period has been attributed to the recruitment of new distal arterioles. These previous investigations, however, have only analyzed the steady-state components of blood flow.

Pulsatile blood flow requires an additional amount of work by the ventricle, compared with blood propelled at a constant rate. The magnitude of the increased work is in part determined by the mechanical properties of the vascular bed, including the distensibility and diameter of the large arteries.

We measured the developmental changes in dorsal aortic impedance and hydraulic load in the chick embryo from stages 18 to 29. Vascular impedance as well as resistance decreased, and the total hydraulic power increased during development.

Materials and Methods

Fertile white Leghorn eggs were incubated in a forced-draft, constant-humidity incubator and staged by the system of Hamburger and Hamilton.6 Each egg was placed on a dissecting microscope, blunted up, and the embryo was exposed by opening a window in the shell and removing the overlying membranes.

Phasic dorsal aortic pressure was measured with a servo-null pressure system4 with a 5-μm diameter tip micropipette inserted into the proximal dorsal aorta. The frequency response of this system was tested in vitro against a micromanometer using a sine wave pressure generator in a sealed chamber and was flat to 10 Hz.

Phasic aortic blood flow velocity was measured with a 20-MHz directional pulsed Doppler velocity meter.4 The 1-mm piezoelectric crystal was positioned at a 45° angle with a protractor jig over the dorsal aorta at the site of the insertion of the...
DORSAL AORTIC PRESSURE (mm Hg)

DORSAL AORTIC VELOCITY (mm/sec)

FIGURE 1. Simultaneous dorsal aortic pressure and velocity from a stage 24 chick embryo.

pressure and flow signals using the results of previous in vitro calibrations.

The analog records were digitized at a rate of 333 Hz and analyzed on a Data General MV8000 computer (Westboro, Massachusetts). The individual pressure and flow waveforms were displayed on the terminal screen. Only those pressure waveforms with a well-defined dicrotic notch and flow waveforms with no significant baseline drift or secondary rises in diastole were considered acceptable for analysis. Instantaneous aortic volume flow was calculated from the aortic flow velocity and the dorsal aortic diameter.

The pressure and flow waveforms were resolved into their Fourier components. Subsequent analysis was limited to those harmonics with a frequency of <10 Hz. Fourier analysis of the zero-flow baseline was used to determine the level of noise, and harmonics with moduli below the level of this baseline was excluded. The input impedance modulus and phase angle at each harmonic were calculated as the ratio of the respective pressure and flow moduli and differences of the phase angles.7,8 The potential and kinetic components of total external

<table>
<thead>
<tr>
<th>Stage</th>
<th>SBP* (mm Hg)</th>
<th>DBP* (mm Hg)</th>
<th>HR* (beats/min)</th>
<th>MDABF* (mm³/min)</th>
<th>PVR* (dyne x sec/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1.14±0.12</td>
<td>0.37±0.03</td>
<td>148±3</td>
<td>20.5±2.0</td>
<td>30.4±4.8</td>
</tr>
<tr>
<td>21</td>
<td>1.48±0.07</td>
<td>0.54±0.03</td>
<td>152±5</td>
<td>32.9±3.6</td>
<td>26.0±3.4</td>
</tr>
<tr>
<td>24</td>
<td>1.82±0.10</td>
<td>0.75±0.04</td>
<td>169±6</td>
<td>68.4±4.0</td>
<td>19.0±4.2</td>
</tr>
<tr>
<td>27</td>
<td>2.51±0.14</td>
<td>0.77±0.06</td>
<td>174±7</td>
<td>139.0±19.6</td>
<td>9.0±1.0</td>
</tr>
<tr>
<td>29</td>
<td>3.04±0.10</td>
<td>0.95±0.07</td>
<td>193±9</td>
<td>213.7±18.9</td>
<td>6.4±0.7</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MDABF, mean dorsal aortic blood flow; PVR, peripheral vascular resistance.

Values are mean±SEM.

*p<0.05.
were averaged, and then the data from each embryo were averaged. These data are reported as mean±SEM. We determined statistical significance by the Tukey’s studentized range test. Since we are describing variables that change continuously over development, for clarity we report only the statistical differences between stages 18 and 29. This does not imply lack of statistical difference between other stages.

Results

Dorsal aortic pressure and flow waveforms were similar to those recorded from mature animals despite the absence of semilunar valve apparatus (Figure 1). Heart rate, systolic pressure, diastolic pressure, and dorsal aortic blood flow increased (p<0.05) while peripheral resistance decreased (p<0.05) with development (Table 1).

The first (Z1), second (Z2), and third (Z3) impedance moduli decreased from stages 18 to 29 (p<0.05) (Figure 2). The phase angle of the first harmonic became less negative during development (p<0.05) (Figure 2). Total hydraulic power increased 50-fold from stages 18 to 29 (p<0.05) (Table 2). Steady-state power increased proportionately from stage 18 to 29 (p<0.05). However, the percentage of oscillatory power, that proportion of total hydraulic power expended in pulsatile blood flow, rose steeply from 28% in stage 18 to 53% in stage 27 and 65% in stage 29 (p<0.05).

Discussion

Little is known about the mechanisms that drive the expansion of the vascular bed during embryonic development. These studies describe the dynamic changes that we believe are integral to normal cardiovascular morphogenesis. They cover a range of cardiac development from the initial formation of the endocardial cushions and definition of the fourth aortic arches at stage 18 through the formation of the muscular septum, the conotruncal septum and the anlage of the semilunar valves at stage 29.

The mechanical properties of the aorta and the distal arterial bed may account for the progressive decrease in the impedance moduli observed in this study. Extrapolating from studies in mature animals, the lower impedance late in development suggests that the dorsal aorta is more distensible, is larger, or that there are less prominent wave reflections from the periphery. All three mechanisms likely play a role in the observed results.

Although it has not been possible to quantify arterial distensibility independently in the embryo, there is little apparent pulsation in the dorsal aorta itself in these stages. In contrast, the vitelline bed appears more pulsatile at stage 29 than at stage 18, suggesting that the mechanical properties of the vitelline bed contribute to the observed impedance spectrum. These apparent differences in properties of the embryonic and extraembryonic vasculature are consistent with their morphology. The morphology of the dorsal aortic wall is similar at stage 18.
and 29 and comprises a single endothelial cell layer and several mesenchymal cell layers that are surrounded by an amorphous extracellular matrix devoid of a muscularis or adventitial coat. The first elastic fibers are just beginning to form at stage 29. In contradistinction, the extraembryonic vitelline vessels have a well-developed muscular and adventitial layer.

The dorsal aortic diameter does increase from 0.29±0.003 mm in the stage 18 to 0.43±0.003 mm in the stage 29 embryo. Several authors have proposed normalization schemes including expression of the impedance spectrum in terms of velocity rather than volume flow to facilitate comparisons between vessels of different size. In the chick embryo, expression of the impedance spectrum in terms of velocity rather than volume would tend to diminish the differences in the impedance moduli between the stages evaluated in this investigation. We prefer to use flow volume in our calculations for several reasons. First, the units (nW) of the hydraulic energy are correct only if one uses flow volume. Second, from theoretical considerations, at a vessel bifurcation use of velocity rather than volume flow to calculate impedance would imply that velocity rather than volume was conserved. This clearly is incorrect.

Wave reflection may be important in early vascular hemodynamics. The qualitative analysis of wave reflections by inspection of the degree of oscillation of the impedance spectrum about the characteristic impedance at higher frequencies is not possible from our data due to the limited frequency response of the pressure and flow systems. We also cannot decompose the pressure and flow waves into their forward and backward components. Similarly, the crossing point of the phase angle cannot be evaluated due to the limited range of frequencies studied. Nevertheless, it is likely that wave reflections from the periphery probably decrease with development as the peripheral resistance falls with the recruitment of new arterioles and the sites of reflection become more distant as the aorta lengths. The effect of the latter would be modulated by any changes in wave velocity that may occur as the distensibility of the aorta changes.

TABLE 2. Mean, Oscillatory, and Total Hydraulic Power

<table>
<thead>
<tr>
<th>Stage</th>
<th>Mean* (nW)</th>
<th>Oscillatory* (nW)</th>
<th>Total* (nW)</th>
<th>% Oscillatory*</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>34±5</td>
<td>14±2</td>
<td>48±7</td>
<td>29±2</td>
</tr>
<tr>
<td>21</td>
<td>73±9</td>
<td>35±6</td>
<td>108±14</td>
<td>32±1</td>
</tr>
<tr>
<td>24</td>
<td>196±13</td>
<td>100±12</td>
<td>296±24</td>
<td>34±1</td>
</tr>
<tr>
<td>27</td>
<td>508±97</td>
<td>561±135</td>
<td>1,054±225</td>
<td>53±3</td>
</tr>
<tr>
<td>29</td>
<td>924±112</td>
<td>1,683±353</td>
<td>2,606±447</td>
<td>65±4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *p<0.05.

mizes the component of afterload due to the systemic venous pressure. Thus, our studies reflect total ventricular afterload including that imposed by venous pressure. The wall of the sinus venosus is fragile, and the reproducible measurement of simultaneous sinus venous, dorsal aortic blood pressure with dorsal aortic blood flow in the chick embryo, has not been possible due to excessive blood loss. It is not possible at this time to quantify the proportion of peripheral vascular resistance or impedance due to venous pressure at any given stage of chick embryonic development. The trends in total peripheral resistance and mean hydraulic power will be valid if the sinus venosus pressure either remains a constant percentage of the dorsal aortic pressure or increases in proportion to the dorsal aortic pressure during development. Sinus venosus pressure measured in embryos at comparable stages to those in this study (stages 21, 24, 27, and 29) indicates that sinus venosus pressure is lowest at stage 21 (0.04±0.01 mm Hg) and is greatest at stage 29 (0.47±0.26 mm Hg). While these were not measured simultaneously with dorsal aortic pressure or flow and while bleeding from the sinus venosus is a factor in the accuracy of the measurements, this does suggest that sinus venosus pressure increases in proportion to dorsal aortic pressure over the stages studied.

We found an increasing proportion of oscillatory power during development. In the mature animal, the proportion of total hydraulic power expended in pulsatile blood flow in the arterial circulation varies between 10% and 20%, At stage 18, the percent of oscillatory power was 28%, and it increased slowly until stages 27 and 29, when there is a striking and progressive increase in the percent of oscillatory power to 65%. This implies that at stages 27 and 29 there is less efficient transfer of cardiac energy into blood flow in the periphery. Why is there such a striking increase in oscillatory power? Our studies indicate that a significant portion of the energy is expended in producing pulsatility. Pulsatility, in turn, may be a factor that drives the development of the distal portion of the vascular system. Energy transferred to the vascular bed may be one link function and structure in vascular morphogenesis.

Embryologists have long speculated that there is a fundamental relation between function and form of the developing cardiovascular system. Our results...
emphasize that the embryonic circulation is distinctly different from that of the mature animal. These characteristics may in part permit pulsatile blood flow at a low pressure while driving the rapid expansion of the cardiovascular system.

References

KEY WORDS • embryonic aortic impedance • hydraulic power • vascular resistance • dorsal aortic blood flow
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