Intramyocardial pressure measurements in the stage 18 embryonic chick heart

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Chabert, Steren, and Larry A. Taber. Intramyocardial pressure measurements in the stage 18 embryonic chick heart. Am J Physiol Heart Circ Physiol 282: H1248–H1254, 2002.—Intramyocardial pressure (IMP) and ventricular pressure (VP) were measured in the trabeculating heart of the stage 18 chick embryo (3 days of incubation). Pressure was measured at several locations across the ventricle using a fluid-filled servo-null system. Maximum systolic and minimum diastolic IMP tended to be greater in the dorsal wall than in the ventral wall, but transmural distributions of peak active (maximum minus minimum) IMP were similar in both walls. Peak active IMP near midwall was similar to peak active VP, but peak active IMP in the subepicardial and subendocardial layers was four to five times larger. These results suggest that the passive stiffness of the dorsal wall is greater than that of the ventral wall and that during contraction the inner and outer layers of both walls generate more contractile force and/or become less permeable to flow than the middle part of the wall. Measured pressures likely correspond to regional variations in wall stress that may influence morphogenesis and function in the embryonic heart.

MECHANICAL FORCES affect form and function in the developing embryonic heart. Although this fact has been recognized for a long time, the nature and magnitude of these forces remain largely unexplored. One of these forces, blood pressure, has been measured in chick (3, 6, 9, 11, 14, 21) and zebrafish (7) embryos, but it is the ventricular wall stress due to this pressure that likely controls, in part, the processes of growth, remodeling, and morphogenesis (20).

Both solid and fluid components of the heart wall are subjected to stress. Currently, there is no direct way to reliably measure the stress in the solid part of the wall (8), and so, theoretical models are used to estimate solid stress (12, 25, 26). Intramyocardial pressure (IMP) in the fluid compartments, however, can be measured using a micropressure transducer. Although somewhat inconsistent, published measurements of IMP in the mature heart have yielded considerable insight into the mechanical behavior of the heart wall and have shown that IMP is affected by ventricular pressure (VP), perfusion pressure, and muscle contractility (5, 13, 15, 18, 23, 27).

The purpose of the present study was to measure IMP in the ventricle of the beating, stage 18 embryonic chick heart. This stage of development (3 days of incubation) coincides with the onset of myocardial trabeculation, a critical morphogenetic process (17). Results indicate that IMP varies regionally. In general, maximum systolic and minimum diastolic IMP were greater in the dorsal wall than in the ventral wall, but the peak active (maximum minus minimum) IMP distributions were similar in both walls. Peak active IMP at midwall was similar to peak active VP, but peak active IMP in the subepicardial and subendocardial wall layers was four to five times larger. We speculate that such regional variations in IMP may play a role in the growth and remodeling of trabeculae and may reflect material heterogeneity of the heart wall. In particular, results are consistent with the dorsal wall being stiffer than the ventral wall during diastole, whereas the inner and outer layers of both walls generate more stress and/or become less permeable to flow than the middle portion of the wall during systole.

METHODS

The small size of the stage 18 chick heart (<1 mm diameter) makes pressure measurements difficult. In this study, we used a servo-null micropressure system (model 5A, Instruments for Physiology and Medicine, San Diego, CA). Several previous studies have used this and similar systems to measure IMP in the mature heart (5, 13, 23, 27). The sensor probe (a micropipette) is small enough to minimize distortions due to its presence, and, when used properly, the system has good dynamic characteristics.

System setup and calibration. Heineman and Grayson (5) describe the operating principles of the servo-null system. A glass micropipette of 2- to 5-μm tip diameter was filled with filtered 2 M NaCl solution, sharpened, and connected to the system. To prevent degradation of the dynamic response, care was taken to ensure that the system was completely filled with fluid and free of any air bubbles. Pressures generated by the servo-null pump were measured using a fluid-filled transducer (model P23XL, Statham) with the signal recorded on a personal computer using data acquisition hardware and software (model MP100, Biopac Systems).

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The system was calibrated before each use and every time the pipette was changed. A water column was used first for static calibration; then the dynamic response was tested using a low-frequency acoustic speaker covering the end of a cylinder partially filled with normal saline. This device produced an approximate sinusoidal pressure waveform that was decomposed using Fourier analysis. Pressure was measured in the saline near the bottom of the cylinder simultaneously by the servo-null system and by another transducer (model SPC 350, Millar; Houston, TX) known to have excellent dynamic characteristics. Comparison of the dominant Fourier components of the two signals showed that the frequency response of the servo-null system was flat up to \( \sim 20 \) Hz. Thus because the heart rate at stage 18 is about 2 Hz, several harmonics of the pressure signal could be recorded reliably.

**Measurement procedure.** Fertile white Leghorn chicken eggs were incubated blunt end up at 38.5°C to Hamburger-Hamilton stage 18, as determined by external characteristics of the developing embryo (4). This stage corresponds roughly to 3 days of a 21-day incubation period. Cutting a window in the eggshell and removing the inner membrane exposed the embryo, which was kept warm by a lamp. During the experiments, images of the beating heart and the micropipette were recorded on videotape using a video recorder (model SVO-9500MD, Sony; New York, NY) and a camera (model 4915, Cohu; San Diego, CA) mounted on a dissecting microscope (model MZ8, Leica; Heerbragg, Switzerland).

A micromanipulator (model MMN-8, Narishige; Tokyo, Japan) was used to insert the micropipette into the ventral side of the ventricle at an angle approximately normal to the epicardial surface (see the line in Fig. 1B). The pipette was advanced through the heart in 50-µm increments, with the pressure being recorded for several seconds at each location. The reading on the manipulator micrometer provided the position of the pipette relative to the ground. The approximate position relative to the heart was determined by observation and by the shape of the pressure waveform, as described in **RESULTS**. Pressures were recorded at several locations across the ventral wall, the lumen, and the dorsal wall of the ventricle.

When the tip of the micropipette is immersed in fluid, changing the system gain does not affect the output signal (5). However, when the tip contacts a solid, such as a membrane, the tip is occluded and the signal changes with the gain (27). To be sure that the system was measuring a true fluid pressure, we altered the gain at each location. If the output signal changed as the gain changed, the data were discarded.

Pressure in the fluid just outside the heart was recorded for a reference, and then subtracted from the acquired pressures. Thus all reported pressures are relative to the external fluid pressure at the level of the heart.

**Data analysis and statistics.** Reported pressures were obtained by computing the mean values of five consecutive heartbeats. Measurements that were excessively noisy or sensitive to the system gain were discarded. In addition, embryos with heart rates >30% above or below the mean, peak ventricular pressures >30% above the mean, or negative end-diastolic pressures were excluded. Physiologically unrealistic pressure measurements can be caused by a partial blockage in the servo-null system. Reliable measurements were obtained for 7 of 12 embryos.

Measurements from the various locations were analyzed using the Friedman repeated-measures ANOVA on ranks with pairwise comparisons made using Dunn’s test (SigmaStat, SPSS; Chicago, IL). The statistical analysis was based on data from all seven hearts, although we were able to obtain acceptable data from all locations in only four hearts. The software handled missing data points automatically with a general linear model approach. Results are expressed as means ± SE, with statistical significance assumed for \( P < 0.05 \).

**RESULTS**

In the seven embryos included in this study, the mean heart rate was 98 ± 4 beats/min. End-diastolic and peak-systolic pressures in the lumen were 0.69 ± 0.46 and 1.60 ± 0.57 mmHg, respectively. These pressures and the recorded pressure waveforms (Fig. 2) are similar to those reported by other investigators for embryos of comparable ages (3, 6, 9, 11, 14, 21).

In general, the recorded IMP waveforms (Fig. 3) differed qualitatively from the VP waveforms (Fig. 2) and often exhibited biphasic behavior during diastole. In many embryos, for example, an initial fall in IMP was followed by a plateau and/or a “notch” and then a secondary pressure drop (Fig. 3). These characteristics, however, were not observed consistently. Hence, the IMP at end diastole could not be ascertained, and we...
report diastolic (minimum), systolic (maximum), and active (maximum minus minimum) pressure. Because the tip of the pipette was difficult to see under the microscope, the differences between the IMP and VP waveforms helped us determine whether the micropipette tip was located inside the wall or in the lumen.

Active IMP varied regionally across the heart wall (Fig. 4). In some embryos, we were able to measure pressure for two or three passes of the micropipette in different directions through the heart (c.f. Fig. 4, A and B), but in other embryos, only one pass produced reliable data (c.f. Fig. 4 C).

The abscissa in Fig. 4 represents the location of the micropipette tip relative to the ground. Because the heart also moved due to the pushing and pulling force of the pipette, as well as due to the heartbeat, we did not know precisely the location of the tip relative to the heart. The data, however, show a fairly consistent trend, with active IMP being much higher in the inner and outer layers than in the middle part of the wall. Thus we divided the IMP data into six regional groups (c.f. Fig. 1 C, n = 5 for each region): inner dorsal (ID), middle dorsal (MD), outer dorsal (OD), inner ventral (IV), middle ventral (MV), and outer ventral (OV) walls. In addition, we measured the VP in the lumen of each heart (L, n = 7).

Active pressure was consistently higher in the inner and outer regions of both walls than at midwall or in the lumen. To analyze this trend, we selected from each passage the maximum pressures in regions ID, OD, IV, and OV and the minimum pressures in regions MD, MV, and L. These values were then averaged over all passages for each heart to obtain the data in Fig. 5 C. Finally, the means of these data computed over all hearts provided regional pressures (see Fig. 6). Regional diastolic and systolic pressures were computed similarly (Fig. 5, A and B).

The trends in the data suggest that diastolic and systolic IMP were greater in the dorsal wall than in the ventral wall, with the largest values occurring in the subepicardial layers of the dorsal wall (Fig. 6, A and B). In both walls, systolic IMP tended to be higher in the inner and outer layers than at midwall (Fig. 6 B). In addition, IMP in all parts of the dorsal wall tended to be greater than the lumen pressure (VP) during both diastole and systole. Although many of these differences were not statistically significant, the systolic pressures in regions OD and IV were significantly larger than the systolic pressures in regions MV and L.

In contrast, active IMP distributions were qualitatively similar in the dorsal and ventral walls, with the IMP in the inner and outer regions of both walls being about four to five times as large as the active lumen pressure.

Fig. 4. Pressure distributions measured in 3 hearts (represented by different symbols) are plotted as a function of position of the micropipette tip relative to the ground. Solid symbols represent passages of the tip from the ventral to the dorsal side of the heart; open symbols are measurements taken in the opposite direction. The inserted abbreviations correspond to the regions defined in Fig. 1C.
pressure, whereas midwall IMP was similar to that in the lumen (Fig. 6C). This trend also was observed in each individual heart (Fig. 5C). Active pressures in the inner and outer layers of the wall (regions ID, OD, IV, and OV) were not significantly different from each other, with the same being true of the active midwall and lumen pressures (regions MD, MV, and L). However, the active pressures in regions ID and OD were larger than those in regions MD and L, whereas the pressures in regions IV and OV were larger than those in regions MV and L.

**DISCUSSION**

The main finding of this study is that the stage 18 embryonic chick heart contains significant regional variations in fluid pressure. In particular, our data suggest that 1) diastolic and systolic IMP are greater in the dorsal wall than at corresponding locations in the ventral wall; 2) active IMP in the subendocardial and subepicardial layers of the dorsal and ventral walls is four to five times larger than the pressures at midwall and in the lumen; and 3) characteristic IMP and VP waveforms differ markedly. As discussed below, we speculate that these pressure patterns reflect regional variations in material properties of the embryonic heart.

**Morphological and engineering background.** At stage 18, the chick heart is a curved tube consisting primarily of the primitive atrium, the ventricle, and the conotruncus (Fig. 1A). (The morphology of the embryonic human heart is similar at a comparable developmental stage.) Although there are not yet formed...
valves, the stage 18 heart pumps in a pulsatile fashion (9). In addition, the heart is in the initial phases of the process of myocardial trabeculation, as the originally smooth ventricular wall transforms into a meshwork of sponge-like trabeculae (17) (Fig. 7). Later, the trabeculae compact to form the highly ordered fiber architecture of the mature heart (10, 19).

The wall of the stage 18 chick ventricle is composed primarily of a compact outer layer of myocardium (epimyocardium) with circumferential-radially oriented porous muscular ridges protruding into the lumen (Fig. 7, a and b). These ridges, which are connected by struts, represent the first trabeculations. Because no coronary arteries are present at this stage, the metabolic requirements of the heart muscle are met by direct blood flow through the trabecular spaces with each beat. Therefore, many fluid-filled pockets are available for taking pressure measurements.

The microstructure of the ventricle suggests that the intramyocardial fluid is relatively mobile, and so the wall can be treated, to a first approximation, as a poroelastic material, i.e., a fluid-filled porous elastic solid. According to a poroelastic model for the embryonic heart (25), the pore fluid pressure (IMP) falls below VP during diastole drawing blood into the wall and rises above VP during systole forcing blood out. Moreover, poroelasticity theory predicts that the IMP magnitude depends on VP (a boundary condition), the deformation rate (heart rate), and the material properties of the solid part of the wall. In particular, for a given VP and heart rate, the theory predicts that IMP increases with increasing tissue modulus (stiffness), increasing contractility, and decreasing hydraulic permeability (1, 24, 25).

Hypothetical mechanisms. With this background, we now propose mechanisms to explain our results. First, the relatively large values of diastolic IMP in the dorsal wall (Fig. 6A) suggest that the passive stiffness (modulus) is higher or the permeability is lower in the dorsal wall than in the ventral wall. Morphological evidence suggests that both may be true because the trabeculae appear to be denser in the dorsal wall (Fig. 7c).

Second, the active IMP distributions (Fig. 6C) indicate that changes in stiffness, contractile force, or hydraulic permeability from diastole to systole are similar in both walls. Thus relatively high values of the total systolic IMP in the dorsal wall (Fig. 6B) probably are due mainly to greater diastolic IMP in that wall (Fig. 6A). Furthermore, geometric symmetry in the active IMP measurements suggests that systolic changes in mechanical properties of each wall are greater in the inner and outer regions than near midwall (Fig. 6C).

Third, the reasons for the shape of the IMP waveforms may relate to the contraction pattern in the stage 18 embryonic heart. Consider, for example, the biphasic fall in IMP after the peak (Fig. 3). During systole, a contractile wave travels from atrium to conotruncus (see Fig. 1A) generating the pressure build-up that eventually ejects the blood. After the wave passes the venous end of heart, that section begins to relax and local IMP falls, even as the arterial end is still contracting with VP remaining high throughout the ventricle. The large VP then forces rapid elastic expansion of the relaxing section, the pores enlarge, and pore pressure (IMP) falls further at a faster rate.

Finally, it is important to note that, whereas the measured diastolic IMP was generally lower than diastolic VP in the ventral wall, even to the point of slight suction in regions MV and OV, such was not the case for the dorsal wall (Fig. 6A). If VP always exceeds IMP in the trabecular spaces, then blood cannot flow into the wall to provide metabolic support. It may be that some of our measurements in the relatively dense dorsal wall may have been recorded inside trabeculae where, due to lower hydraulic permeability, IMP likely is larger than in the pores. Such intratrabecular pressure would not affect blood flow directly. These measurements also may have contributed to the relatively large variability in our data (Fig. 5).

Verifying these conjectures will require direct measurements of the regional poroelastic properties of the ventricular wall, a difficult task, that to our knowledge, has not yet been attempted. In addition, more realistic models that include heterogeneous poroelastic properties of the wall may help shed more light on the underlying mechanisms.

Comparison with other studies. Although IMP has not been measured previously in the embryonic heart, several investigators have measured it in the mature heart (5, 13, 15, 18, 23, 27). In general, the reported results are somewhat inconsistent and appear to depend on measurement technique. As Westerhof (22) summarizes, some authors have found subendocardial IMP higher than VP, whereas others have found IMP to be lower than peak systolic VP (5). Most groups, however, agree that IMP decreases relatively smoothly from endocardium to epicardium, as suggested by some theoretical models (22). This distribution may be related to the greater stress and strain predicted by

![Fig. 7. Confocal images of stage 18 chick heart. Transverse sections near middle of ventricle (a) and near apex (b) illustrate circumferential-radial trabecular ridges (arrows) with large holes. Sagittal section (c) showing trabeculations that are denser in the dorsal wall than in the ventral wall of the ventricle. Scale bars, 250 μm.](http://ajpheart.org)
models for the subendocardial layers (26). In contrast, our data show relatively sharp IMP peaks in both the inner and outer layers, suggesting sharper demarcations in material stiffness or hydraulic permeability in the embryonic heart compared with those of the mature heart.

Implications for cardiac development. The measured IMP distributions likely accompany regional variations in wall stress (27) that may influence cardiac morphogenesis and function. On a functional level, the flow of blood within the trabecular wall depends, in part, on IMP distributions (25). The importance of mechanical stress for morphogenesis is illustrated, for example, by studies showing that increased ventricular pressure in the chick embryo leads to accelerated growth and to thicker and denser trabeculations (2, 16). Our data suggest that systolic wall stress is elevated in the inner and outer parts of the wall. Stress concentrations in the epimyocardium may induce growth of the trabeculae, whereas high stress near the lumen may stimulate global growth of the ventricle to accommodate the increasing blood volume during development. Currently, however, we can only speculate on the possible role that mechanical stress plays in heart development. Future studies are needed, for instance, to explore the relationship between wall stress and gene expression in the embryonic heart.

Limitations. Several limiting factors must be kept in mind when interpreting our results. First, as in the mature heart, IMP measurements in the embryonic heart may be affected by the introduction of a probe into the wall that disturbs the surrounding tissue and, thereby, alters the stress field. This may be especially problematic here, given the relatively small pressures measured at some locations. Previous studies have shown that among the currently available techniques servo-null systems minimize this problem due to the relatively small size of the micropipette tips (23). In addition, as discussed in the Methods section, we confirmed that, by checking the response to changes in the system gain, the tip was surrounded by fluid.

Second, as the micropipette tip penetrates the endocardium or epicardium, it may stretch the membranes, locally increasing stress and IMP. Sharpening the tips minimized this possibility. Moreover, we observed little membrane stretching once the tip punctured the wall, and repeated passes through the heart yielded similar values for the IMP, regardless of the direction of pipette travel (Fig. 4). Thus we do not feel that locally high values of IMP are artifact.

Third, mean heart rate of our embryos (98 beats/min) was significantly lower than normal rate for stage 18 chick embryos (about 140 beats/min). This discrepancy was likely due to the inability of our egg warming system to maintain a constant temperature during the recording period (approximately a half hour or more). In chick embryos, heart rate and temperature are directly related. However, Yoshigi and Keller (28) have shown that peak ventricular pressure in the stage 24 chick embryo changed by <10% when heart rate varied between ~100 and 200 beats/min. Our own tests with stage 18 hearts have shown a similar insensitivity to heart rate (data not shown). Thus the influence of heart rate on our results is likely not significant.

Fourth, we were unable to find a way to determine the precise location of the pipette tip relative to the heart. With experience, it became somewhat easier to judge whether the pipette was located within the wall, in the lumen, or somewhere else, and the different characteristic waveforms of the VP and IMP (Figs. 2 and 3) helped in this regard. In most cases, however, we could not see the tip, and the motion of the heart due to its beating may have changed the location of the tip in the wall even when the tip remained stationary relative to the ground. In addition, the morphology of the heart at this stage made it impossible to know whether the micropipette passed through a trabecula or the space between trabeculae (c.f. Fig. 7).

Despite these problems, the consistency of the data leads us to believe that we are reporting the correct trends, namely that subepicardial and subendocardial active IMP in the stage 18 chick ventricle is significantly higher than the active pressures at midwall and in the lumen. Nevertheless, the door is open for more conclusive and expansive studies. For instance, it would be interesting to measure IMP in isolated beating and arrested hearts, as well as in hearts at different developmental stages. Such information would help determine the importance of wall stress during cardiac morphogenesis.

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