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Effect of Heart Rate Increase on Dorsal Aortic Flow before and after Volume Loading in the Stage 24 Chick Embryo

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ABSTRACT. In the stage 24 chick embryo, a paced increase in heart rate reduces stroke volume, presumably by rate-dependent decrease in passive filling. We hypothesized that rate-dependent stroke volume reduction could be abolished by volume loading. Dorsal aortic blood velocity was measured with a 20 mHz pulsed-Doppler meter from a 0.75-mm piezoelectric crystal (eight embryos), and atrioventricular velocity was simultaneously measured from the ventricular apex (six embryos). Sinus venosus pacing (stimuli of 1 ms duration and <4 mA) was performed at intrinsic rate (P:I) and at 150% of intrinsic rate (P:150%I). Volume loading was performed during P:150%I by intravenous injection of 7.5 μL of chick Ringer's solution. Using atrioventricular velocity profile, stroke volume was divided into the proportion due to passive (E-phase) and active (A-phase) filling. Stroke volume was compared during P:I, P:150%I, immediately (P:150%I′) and 30 s after (P:150%I″) volume loading. Data (mean ± SEM) were compared by ANOVA. During pacing, stroke volume (mm^2/cycle) decreased but increased after volume loading (I, 0.43 ± 0.03; P:I, 0.37 ± 0.03; P:150%I, 0.19 ± 0.03; P:150%I′, 0.24 ± 0.05; P:150%I″, 0.28 ± 0.04 ($p < 0.005$). During P:150%I, E-phase filling disappeared and was not restored by volume loading, whereas, A-phase filling diminished but was restored by volume loading. In stage 24 chick embryos, rate-dependent stroke volume decrease is reversed by volume loading that restores stroke volume due to an increase in active filling but not passive filling. Thus, even at rapid heart rate, the embryonic ventricle responds to volume loading, indicating that the Frank-Starling relationship functions during tachycardia in the embryonic heart. (*Pediatr Res* 26: 438–441, 1989)

Abbreviations

I, intrinsic heart rate
P:I, pacing at rate slightly faster than I
P:150%I, pacing at 150% of I
P:150% I′, P:150%I immediately after volume loading
P:150%I″, P:150%I 15–30s after volume loading
I″, P:150%I after cessation of pacing

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Heart rate change is integrally involved in cardiovascular development, but little is known regarding adaptation of the developing cardiovascular system to heart rate change. In the stage 24 chick embryo, a paced increase in heart rate resulted in decreased cardiac output and stroke volume, presumably by altering the passive phase of ventricular filling (1).

Inasmuch as the Frank-Starling relationship has been shown to function in the developing chick embryo (2), we hypothesized that volume loading would augment cardiac output and stroke volume during pacing-induced tachycardia. We found that in the stage 24 chick embryo, volume loading reversed the pacing-induced reduction in cardiac output and stroke volume.

MATERIALS AND METHODS

Fertile White Leghorn chicken eggs were incubated to Hamburger-Hamilton stage 24 (3). The embryo was exposed by opening the shell and its membranes. Blood flow velocity was measured with a 20 mHz pulsed-Doppler meter from a 0.75-mm piezoelectric crystal over the dorsal aorta (eight embryos) and simultaneously at the ventricular apex (six embryos). Dorsal aortic blood flow (mm^3/s) was computed as the product of mean velocity and aortic area (4). Dorsal aortic blood flow calculated in this way underestimates cardiac output because blood flow to the head and myocardium is not measured. For our purposes, we have assumed that blood flow to the head and myocardium is negligible. Heart rate was determined from the interval of cardiac cycles. Stroke volume (mm^3/cycle) was calculated as mean dorsal aortic blood flow/heart rate. Atrioventricular blood flow velocity profile was quantitated as passive (E-phase) and active (A-phase) filling, and the relative contribution of each phase to stroke volume was measured. These data were digitally sampled at 2-ms intervals (500 samples/s), stored on a Bernoulli disc and analyzed with the software package from RC Electronics, Inc. (Computerscope, Santa Barbara, CA).

Heart rate was increased by pacing using square wave stimuli of 1 ms duration at twice diastolic threshold (<4 mA). Pacing the right sinus venosus from bipolar Teflon-coated silver electrodes (12 μm diameter) increased the heart rate, presumably without altering the sequence of cardiac excitation and contraction. Mean dorsal aortic blood flow was measured sequentially: during I, P:I, P:150%I, P:150%I′, P:150%I″, and I″ (Figure 1). Volume loading was performed during pacing by intravenous injection of 7.5 μL of chick Ringer's solution (120 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L $CaCl_2$) over a 5-s interval. A complete study was performed in less than 2 min. The data (mean ± SEM) were analyzed by analysis of variance and $p \leq 0.05$ reported as statistically significant.

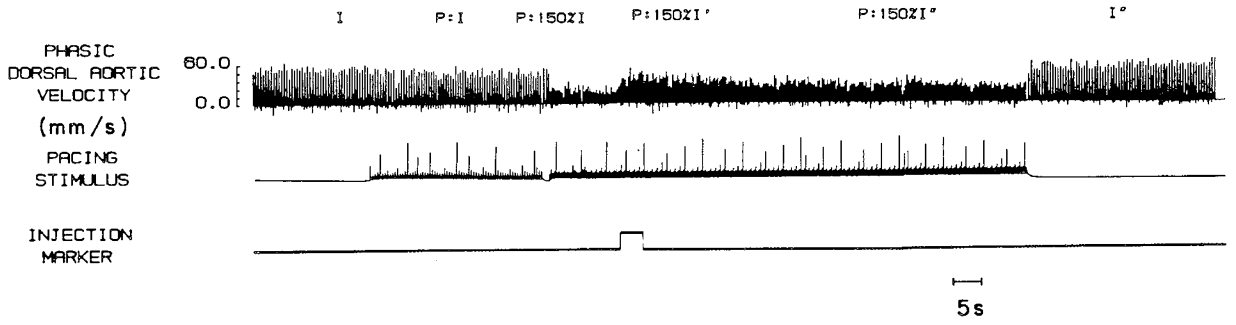

Fig. 1. Volume loading protocol in stage 24 embryo. Dorsal aortic velocity is recorded during I, P:I, P:150%I, P:150%I' and P:150%I" and I". Volume loading (injection marker) occurred over ≤5-s interval.

RESULTS

Average baseline cycle length (I) was 400 ms (heart rate = 150 bpm). After volume loading, the baseline cycle length (I") was 454 ms (heart rate = 132 bpm). Dorsal aortic blood flow significantly decreased during pacing at 150% of intrinsic heart rate (Fig. 2); after volume loading the aortic blood flow decrease was reversed. Stroke volume decreased during pacing (Figs. 1 and 3) but then increased during and after volume loading. Stroke volume increased at pacing termination (I"), presumably due to Frank-Starling effect of volume loading.

During I, P:I, and I", the phasic ventricular filling velocity showed a two component waveform with an E-phase and A-phase portion (Fig. 4). While pacing the A-phase was present, but the E-phase disappeared and did not reappear until pacing was terminated, despite volume loading (Figs. 4 and 5).

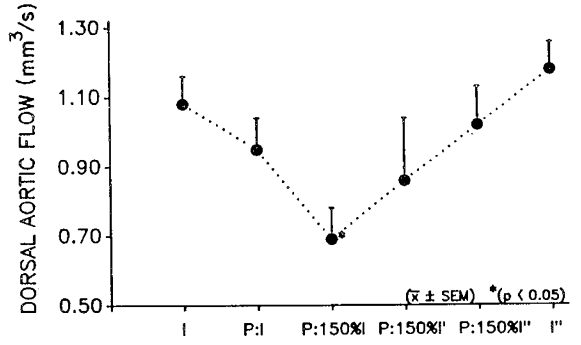

Fig. 2. Effect of interventions on dorsal aortic blood flow (mm/s). During P:150%I, average aortic blood flow (n = 8) is significantly reduced. During volume loading (P:150%I), there is a significant increase that persists during P:150%I". During I", dorsal aortic blood flow is increased as a result of volume loading.

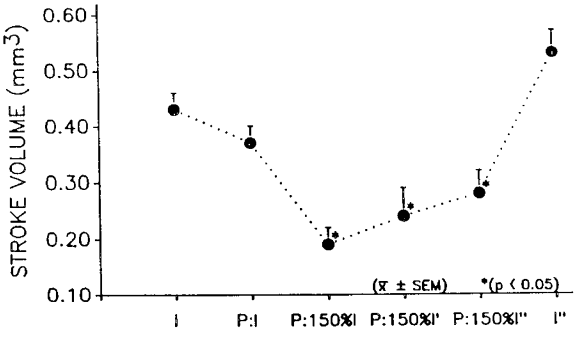

Fig. 3. Effect of interventions on stroke volume. During P:150%I, average stroke volume (n = 8) is significantly reduced. During volume loading, there is a slight increase that becomes more obvious during P:150%I". During I", stroke volume is significantly increased due to the effect of volume loading.

DISCUSSION

This study was designed to test the hypothesis that volume loading would augment cardiac output and stroke volume during pacing-induced heart rate increase. We found the stroke volume decrease resulting from a paced increase in heart rate is reversed by volume loading, which restores stroke volume due to an increase in active (A-phase) ventricular filling. Results of this study confirm that even at rapid heart rate, the embryonic ventricle responds to volume loading, presumably by the Frank-Starling mechanism, which is known to be operative at this developmental stage (2).

Pacing the sinus venosus maintained a normal sequence of atrioventricular excitation and contraction. In support of this assumption is the observation that during pacing near the intrinsic rate, cardiac output and stroke volume do not change significantly. Both E-phase and A-phase ventricular filling occur when pacing near the intrinsic rate. We assume that the decrease in stroke volume and cardiac output observed during pacing is a result of the effect of increased heart rate on ventricular filling rather than an alteration in ventricular excitation sequence.

Varying conclusions regarding the importance of heart rate on control of cardiac output have been made from previous studies in the developing animal. There are important methodologic differences in these studies. Most studies have been performed in the late gestational fetal lamb (5–7). The study of Anderson *et al.* (7) evaluated a number of variables and accounted for many of the differences reported in previous studies. Importantly, they noted that experimentally induced heart rate variations produced changes in end-diastolic volume and contractility which substantially influence stroke volume. Further, when left ventricular end diastolic volume was controlled, the rate-related changes in stroke volume were eliminated. Anderson *et al.* (7) concluded that there was no developmental change in the relationship between heart rate and left ventricular output from late gestation through adulthood.

The effect of paced increase in heart rate differs from the effects of hyperthermia-induced heart rate increase. Hyperthermia results in an increase in cardiac output while stroke volume is unaffected (8). The differing effects may be due to a difference in the extent of heart rate increase. For example, in the stage 24 chick embryo, a temperature increase from 37 to 40°C results in a heart rate increase of 117% as opposed to the pacing-induced 150% increase used in our study. However, the extent to which other factors, hemodynamic or metabolic, influence the different response to heart rate increase is not known.

In a previous study (1), we concluded that the decrease in cardiac output and stroke volume that occurred during pacing-induced heart rate increase in the immature chick embryo, is related in part to loss of the passive phase of ventricular filling.

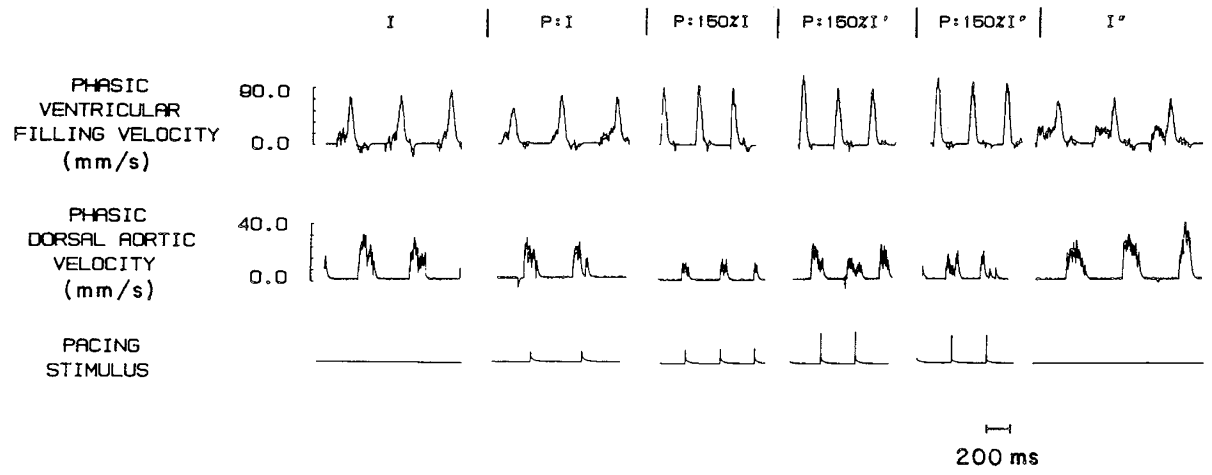

Fig. 4. Phasic velocities at different conditions. During I, atrioventricular velocity (phasic ventricular filling) has A-phase and E-phase component. E-phase is absent during P:150%I, but not during P:I. Administration of fluid bolus results in an increase in aortic velocity during both P:150%I′ and P:150%I″, but E-phase filling is not restored. During I″, E-phase filling returns, and dorsal aortic velocity increases presumably due to volume loading.

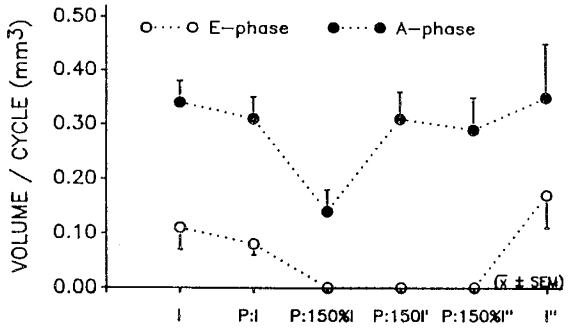

Fig. 5. Contribution of E-phase and A-phase velocity to ventricular filling. During pacing at 150% intrinsic rate, E-phase velocity disappears. A-phase velocity contribution diminishes during P:150%I, but is restored during P:150%I′ and P:150%″.

Why the heart appears to be critically rate dependent on passive filling at this stage of development is unclear. However, the heart rate-dependent decrease in stroke volume is diminished by volume loading that restores stroke volume apparently by augmenting active but not passive filling. The possibility that Ca^{++} in the infusate may have been responsible for the increase in stroke volume cannot be totally excluded. However, this seems unlikely as the study of Faber *et al.* (9) demonstrated stroke volume increase after intravascular injection of Ringer's solution and a greater increase after injection of dextran in isotonic saline.

In the embryo, the developmental heart rate changes are paradoxical. In the chick embryo, heart rate increase is parallel to body mass (10), although after hatching there is a reciprocal relationship as the animal matures (11). The cause of the gradual increase in embryonic heart rate has not been determined.

The stage 24 embryo is in the midpoint of primary cardiac morphogenesis associated with dramatic physiologic, electrophysiologic, and pharmacologic changes in the heart. For example, cardiac myocytes develop recognizable myofibrils between stages 9 and 10 (12). At stage 24, myocytes still have immature appearing ultrastructure with unorganized myofibrils, although, the heart is capable of coordinated contraction. Electrogenic Na-K pumping is present in the very young embryo (13). Fast Na^+ channels increase rapidly during early cardiac development, but during intermediate stages, functional fast Na^+ channels coexist with a high density of slow Na^+ channels as well as slow Ca^{++} channels (13). Available evidence suggests that although both muscarinic and β-adrenergic receptors are present in the stage 24 embryo, functional innervation has not occurred (13). During these dramatic myocardial changes, the embryo is undergoing rapid increase in body wt; *e.g.*, there is a 15-fold increase from stage 18 (17.7 mg) to stage 29 (267 mg), with a concomitant 9-fold increase in ventricular wt (0.35 to 3.18 mg) (10). In addition to changes at the myocyte level, gross cardiac morphology is also changing rapidly. For example, the embryonic ventricle is relatively smooth walled at stage 18 and rapidly develops marked trabeculae so that at stage 24, the chamber is sponge-like with a thin free wall. By stage 29, trabecular fusion has divided the primitive ventricle into left and right ventricles, and the ventricular septum is nearly complete (14). Diastolic ventricular function changes in association with these profound changes in ventricular structure. The end diastolic pressure increases from 0.33 mm Hg at stage 18 to 0.40 mm Hg at stage 24 and 0.82 mm Hg at stage 29 (10). The ratio of passive to active ventricular filling (E/A ratio) decreases from 1.7 at stage 18 to 0.2 at stage 24 (15). These changes are consistent with a stiffening or decreased compliance of the ventricular chambers. The developmental change in compliance may be one factor dictating the developmental change in heart rate.

All of these factors speak to a complex interrelationship of preload, afterload, myocardial contractility, and heart rate during cardiac development. Understanding this interrelationship is essential for defining normal development and the influence on morphogenesis.

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