Influence of poststimulation potentiation and heart rate on the fetal lamb heart

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KIRKPATRICK, Stanley F., JAY NALIBOFF, PAUL T. PITLICK, AND WILLIAM F. FRIEDMAN. Influence of poststimulation potentiation and heart rate on the fetal lamb heart. Am. J. Physiol. 229(2): 318-323. 1975.—Isolated cardiac muscle techniques and studies of the chronically instrumented fetal lamb heart were employed to evaluate the ability of fetal myocardium to exhibit poststimulation potentiation. Isometric tension development and the response to paired electrical stimulation were significantly reduced in isolated fetal ventricular myocardium when compared to the adult (P < 0.001). As in the adult, increasing stimulation frequency raised fetal isometric tension via an increase in the rate of rise of tension development in the presence of reduction in time-to-peak tension. In seven fetal lambs the left ventricle was chronically instrumented with endocardial ultrasonic crystals and a high-fidelity micromanometer. After a 2-wk recovery period, heart rate was increased by atrial pacing from an average control level of 150 to 300 beats/min. Left ventricular dP/dt increased progressively and then fell beyond a heart rate of 270/min. When comparable pre- and immediate postpacing beats were analyzed, a step-wise increase in the velocity of left ventricular shortening and the mean rate of circumferential fiber shortening was observed in association with an increase in the extent of shortening. Thus, increases in the frequency of contraction exert a significant positive inotropic effect on the fetal heart.

atrial pacing; cardiac contractility; inotropic state; mean circumferential fiber shortening; chronic instrumentation; isolated cardiac muscle; left ventricular performance; left ventricular dimensions; paired electrical stimulation

IT HAS BEEN SUGGESTED that the potentiating effect of extra systoles on myocardial contractility may be poorly developed in the young mammalian heart (1). Moreover, questions have arisen concerning the influence on cardiac performance in the conscious animal of the relation between contraction frequency and contractility (14, 21). Accordingly, in the current study isolated heart muscle techniques have been utilized to compare directly the abilities of the fetal lamb and adult sheep heart to exhibit poststimulation potentiation. Furthermore, newly described methods appropriate to assess left ventricular function in situ in the chronically instrumented fetal lamb have been employed to evaluate the influence of heart rate on fetal cardiac function (10).

METHOD

Isolated cardiac muscle. After hysterotomy under spinal anesthesia (4 ml of 1% tetracaine hydrochloride), right ventricular moderator bands were removed rapidly from the hearts of 25 fetal lambs in the last 18 days of gestation (term = 147 days) and from 12 adult sheep after thiopental anesthesia (15 mg/kg). Muscles were suspended vertically in a myograph containing oxygenated Krebsbicarbonate solution at 30°C as described previously (20). The ventricular muscles were 5-14 mm in length and averaged 1.2 mm² (range 0.2-1.7) in cross-sectional area. The cross-sectional areas of ventricular muscles isolated from both the fetus and adult were matched so that the size and thickness of ventricular tissue from both age groups were comparable. Muscles were held at one end by a spring-loaded clip forming the end of a rigid pin attached directly to a force transducer (Statham UC-3) and stimulated through field electrodes at a frequency of 12 contractions/min with square-wave DC impulses of 4 msec duration and voltage less than 10% of threshold. Isometric tension was measured at the apex of the length-active tension curve and was corrected for cross-sectional area and expressed in grams per square millimeter. The stimulus artifact and active tension were recorded on a Clevite-Brush 260 recorder. In order to reach steady-state levels of tension before initiating any experiments, the muscles were equilibrated for 60 min. The composition of the Kreb’s solution was, in millimoles per liter: Na+, 146; K+, 3.6; Ca++, 2.5; Mg++, 1.2; Cl-, 126; H2PO4—, 1.2; SO4—, 1.2; HCO3—, 25; and glucose, 5.6. This solution was bubbled with 95% oxygen and 5% CO2 which resulted in a pH of 7.4. Active tension was measured at steady-state levels after altering the frequency of contraction between 12 and 90 contractions/min and during sustained postextrasystolic potentiation, using the shortest interval between the driving stimulus and effective premature stimulus. A Student t test was used to analyze the statistical significance of differences between fetus and adult.

Chronic study of fetal cardiac performance. Surgical and technical modifications of sonocardiometry methods previously reported from this laboratory were utilized to study the intact fetal lamb heart (10). Seven pregnant ewes of mixed western breeds were selected for operation at known fetal gestational ages of 110-124 days. Pregnancies were timed from conception and radiographically confirmed. Figure 1 illustrates the general experimental situation. After delivery of the fetal head through the uterine incision, an arterial catheter was inserted into the fetal carotid artery. Through a separate neck incision a second catheter was placed into the fetal trachea to record intra-tracheal pressure variations and to provide a zero reference.
Fetal heart poststimulation potentiation

Left ventricular end-diastolic pressure was accurately measured to within 0.2 mmHg. The left ventricular diameter signal from the ultrasonic crystals was calibrated by means of an electronic circuit which provided 1-μs intervals. The accuracy, frequency response, and stability of the sonomicrometer have been reported previously (19). The error in measured left ventricular diameter induced by angular distortion up to 30° is 4% (10). The peak rate of rise of left ventricular pressure (dP/dt) and peak shortening (dD/dt) were obtained by electronic differentiation (Biotronex) of the high-fidelity pressure signal and the echo signal, respectively, with a frequency response linear to 100 Hz. Mean velocity of fiber shortening (VCF), in circumferences per second, was calculated from the ejection excursion of the diameter signal and normalized for end-diastolic diameter (9).

Recordings during the basal state and at paced heart rates per minute of 160, 210, 240, 270, and 300 were obtained during quiescent periods in the fetus when there were no respiratory movements. Pacing was maintained for 30 s at each level in all animals. Steady-state data were chosen for analysis at the end of each of the paced heart rates, and sufficient recovery time was allowed after each pacing period for heart rate and blood pressure to return to control levels. In order to evaluate changes in inotropic state induced by changes in contraction frequency, beats with similar R-R intervals (Fig. 6) were analyzed immediately prior to and after pacing that were matched with regard to systemic arterial pressure, left ventricular end-diastolic pressure, and left ventricular end-diastolic diameter (Fig. 2). The first or second beats after pacing were analyzed prior to any expected decline in a frequency-induced enhancement of contractility. Paired-t tests were used for statistical comparisons.

RESULTS

Isolated cardiac muscle. In all of the muscles isolated from both fetal and adult hearts, increasing the frequency of contraction over the range 12–90/min considerably augmented isometric tension development. Tension was increased in each of these muscles by relatively greater augmentation of the rate of tension development than beyond there is a slight, but significant, fall in peak systolic pressure. During the increase in heart rate induced by

for all other pressure data. The fetal head and neck were replaced and a left thoracotomy in the fourth intercostal space was performed. The pericardium was opened widely and bipolar pacing wires were sutured to the left atrial appendage. After placing a left atrial catheter through a purse-string suture, the cardiac apex was lifted and two 3.5-mm piezoelectric crystals were placed opposing one another on the endocardial surface of the left ventricle. Gentle traction to hold the crystals against the endocardial surface was maintained until hemostasis occurred. In order to obtain high-fidelity left ventricular pressure, a micromanometer pressure transducer (P12-Königsberg) was inserted into the apex of the left ventricle through a separate stab incision and secured with a purse-string suture. This pressure transducer was calibrated with left atrial and aortic pressure. The pericardium was left open and the wires and tubes passed through the fetal skin, uterus, and ewe’s flank and coiled in a cloth pouch sewn to the mother’s left flank. Postoperatively, ewes were housed in separate stalls and fed food, water, and alfalfa. Intramuscular injections of penicillin and streptomycin were administered daily for 10 days following surgery. Every other postoperative day each animal was brought to the laboratory and oriented to the area and personnel. Catheters were flushed and the fetal hematocrit was checked, if the value was <30%, the fetus was transfused with maternal blood to maintain a value above 30%.

Fetuses were studied after a full 2-wk recovery period. All signals were recorded on a Clevite-Brush oscillographic recorder and on magnetic tape. Zero reference was obtained by positioning the pressure gauges so that fetal intratracheal pressure at rest equalled atmospheric pressure.
FIG. 2. Fetus 138, 147 days' gestation, 14 days postoperative. A representative pacing study at 210 beats/min. Square, triangle, and circle mark beats used to obtain control, pacing, and postpacing data, respectively. Note similarity in left ventricular (LV) pressure and left ventricular end-diastolic diameter (LVEDD) pre- and postpacing. Heart rate is slightly slower following pacing. On first beat after pacing with LV pressure and LVEDD the same, there is a significant increase in rate of LV pressure rise (dP/dt). Postpacing beat was comparable to control with respect to LV systolic pressure and end-diastolic diameter but showed a marked augmentation in rate of rise of LV pressure (dP/dt) and in velocity (dD/dt) and extent (ΔD) of LV shortening. LA = left atrium.

Pacing, there were no significant changes observed either in the directly measured velocity of shortening, dD/dt, or in the calculated rate of mean circumferential fiber shortening, VCF. In contrast, dP/dt increased significantly and progressively up to a heart rate of 270/min and returned to near basal values at 300 beats/min.

An evaluation of the control and test beats that were chosen for analysis is shown in Fig. 6. No differences existed in left ventricular peak systolic or end-diastolic pressures or in left ventricular end-diastolic diameter. The slightly longer R–R intervals in test beats following pacing at 180, 210, and 240 beats/min would be expected to reduce the magnitude of the velocity parameters employed to determine the degree of postpacing potentiation. In comparing control beats and the first or second beats immediately postpacing, there is a striking and step-wise increase in both dD/dt and mean VCF (Fig. 7). Thus, at a heart rate of 300/min, dD/dt increased 47%, and mean VCF increased 39%. Blocking doses of propranolol (1 mg/kg) did not attenuate these indices of the contractile response to increased heart rate. The analysis in Fig. 6 (bottom panel) provides further evidence of an increase in contractile state related to the increase in paced heart rates, since the extent of shortening of the left ventricle increased progressively with increasing heart rate.

FIG. 3. A representative experiment showing relations between frequency of contraction and tension. In presence of a reduction in time-to-peak tension (middle panel), increase in isometric tension (top panel) associated with a marked rise in dP/dt (bottom panel).

FIG. 4. A comparison of isometric tension and response to paired electrical stimulation (PES) between fetus and adult. Each point and vertical bar represents mean ± SE. Numbers in parentheses refer to number of muscles studied.
FETAL HEART POSTSTIMULATION POTENTIATION

FIG 5. Measurements of peak systemic pressure, internal transverse left ventricular dimension (EDD, end-diastolic diameter; ESD, end-systolic diameter; ΔD, EDD - ESD), maximum rate of left ventricular diameter change (dD/dt), mean circumferential fiber shortening (Vcf), maximum rate of rise of left ventricular pressure (dP/dt) at basal heart rate (150 ± 15 beats/min) and at each of indicated paced heart rates. Asterisk denotes statistically significant differences from basal values (P < 0.05).

DISCUSSION

The current investigation was designed to assess the ability of the fetal heart to exhibit poststimulation potentiation and to evaluate the influence of heart rate on fetal left ventricular performance. Standard isolated muscle techniques were employed to analyze the former question while the latter issue was examined in the chronically instrumented fetus, since it is now quite clear the direct measurements of fetal myocardial performance derived from exteriorized animal experiments may be misleading (8, 10, 17).

The presence of a positive inotropic effect with increasing frequency of contraction and paired electrical stimulation has been documented amply on isolated cardiac tissue preparations (2, 11, 18, 23). The positive inotropic effect of increased contraction frequency has also been demonstrated in the whole mammalian heart, both in isolated preparations and anesthetized open-chested animals (4, 15). Some past studies of the conscious, normal animal have raised questions concerning the importance of the relation between contraction frequency and cardiac performance (14, 21), although recent investigations in conscious dogs have shown considerable enhancement of inotropic state during increased heart rate (12). The current study further analyzes the relation between contraction frequency and contractility in the conscious animal by analyzing the transients in left ventricular dimensions and pressure during and immediately after pacing. The fetal heart was chosen for study, since the suggestion had been made that the young mammalian heart may not be capable of poststimulation or postextrasystolic potentiation (1).

The results of the isolated ventricular muscle experiments show clearly that a mature frequency-force relationship exists in isolated fetal cardiac muscle and that substantial increases in force generation result from postextrasystolic potentiation. The finding of significantly lower levels of isometric tension at \( L_{\text{max}} \) in the fetus than in the adult agrees with past observations from our laboratory in which reductions in force generation and the extent and velocity of shortening appeared to be related to a lesser amount of fetal contractile mass per unit weight of myocardium than the adult (7). Although paired electrical stimulation produced a profound increase in force development in fetal muscle, a significantly greater increase was observed in the adult. Whether or not this finding...
may be explained by age-related differences in excitation-contraction coupling remains conjectural.

It is important to appreciate some of the difficulties that arise in assessing a change in contractility at high contraction frequencies in the chronically instrumented animal. As heart rate was increased by pacing, there was a marked reduction in resting fiber length, as indicated by the fall in left ventricular end-diastolic diameter. One would anticipate that this change in length would produce a decrease in the velocity of shortening (3, 16, 18, 22 and in peak left ventricular dP/dt (5, 6, 13). In addition, slightly lower left ventricular peak systolic pressures existed during pacing at progressively smaller left ventricular cavity sizes so that one would also anticipate a fall in systolic wall tension (16). Thus, the alterations in preload and afterload would appear to be directionally opposite with respect to viewing a change in left ventricular contractile state. Because of these considerations, the relationship between inotropic state and contraction frequency in these young, in situ hearts was analyzed by comparing control beats with beats in the immediate postpacing period with similar R-R intervals which are matched with regard to systolic pressure and end-diastolic dimensions and pressures (Fig. 6). Since the test beats are the first or second postpacing, they occur prior to any expected decline in a frequency-induced enhancement of contractility. Under these matched loading conditions, an increase in a velocity parameter (Fig. 7) or an increase in the extent of shortening (Fig. 6, bottom panel) may be considered a positive inotropic effect.

In the present study no significant increases were observed either in directly measured left ventricular velocity or in the calculated rate of mean circumferential fiber shortening during pacing. In contrast, the rate of rise of left ventricular pressure increased significantly up to a heart rate of 270 beats/min with a return to basal values at 300 beats/min (Fig. 5). The increase in dP/dt, despite a fall in left ventricular end-diastolic diameter and systolic wall tension, suggested that this isovolumetric index more sensitively reflects a frequency-induced augmentation in contractility than the ejection parameters that were measured during the course of atrial pacing. It is likely that the ultimate decline in dP/dt was a reflection of incomplete relaxation of the ventricle, a markedly shortened fiber length that resulted from inadequate time for ventricular filling, and perhaps, diminished coronary blood flow.

The results of the present study demonstrate that increased frequency of contraction exerts a significant, positive inotropic effect on the normal fetal heart. A direct relation existed between the magnitude of inotropic potentiation and the rise in heart rate. The augmentation in contractility was observed best after fiber length increased immediately after pacing, but was masked during pacing by the rate-induced reduction in fiber length. These results are quite comparable to findings in the chronically instrumented adult dog (12). As in the latter study, it did not appear in the present investigation that the increase in contractility was related to beta-adrenergic stimulation, since no differences in the responses to pacing were observed after pretreatment with the beta-adrenergic blocking agent, propranolol.

In the past there have been questions raised about the presence of contraction frequency-related changes in contractile state in the newborn (1), as well as in the conscious animal (14, 21). The results of the present investigation provide support for the presence and importance of poststimulation potentiation in the intact, undisturbed animal in the perinatal period.

REFERENCES


