Comparison of the responses of fetal and adult cardiac muscle to hypoxia

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Sir; Judy Y., and William F. Friedman. Comparison of the responses of fetal and adult cardiac muscle to hypoxia. Am. J. Physiol. 224(6): 1249-1253, 1973.—The responses of cardiac tissue isolated from fetal and adult sheep were compared after selective inhibition of aerobic and anaerobic metabolism. The frequency of contraction of adult right atria was significantly more depressed by oxygen deprivation or exposure to dinitrophenol than the fetal atria. Fetal right atria were more sensitive to inhibition of glycolysis by iodoacetate than the adult. No differences were observed in the ability of fetal and adult ventricular myocardium to generate tension with either oxygen or glucose deprivation. Both fetal and adult atrial and ventricular myocardium were more sensitive to the combination of oxygen deprivation and iodoacetate than either intervention alone. In the absence of glucose, oxygen deprivation depressed adult atrial and ventricular tissue to a greater extent than observed in the presence of glucose. In contrast, when fetal myocardium was exposed to the combination of oxygen and glucose deprivation, a secondary, positive chronotropic and inotropic response was observed that could be blocked by the beta-adrenergic blocking agent, propranolol ($10^{-6}$ M). The results suggest that the fetal heart is more dependent on glycolytic than aerobic metabolism as an energy source when compared to the adult heart. The secondary, positive chronotropic and inotropic response of fetal heart to the combination of glucose and oxygen deprivation is compatible with a preferential age-dependent release of cardiac catecholamines and a supersensitivity of fetal myocardium to norepinephrine when compared to the adult.

glycogen; sheep; isometric tension; frequency of contraction; oxidative phosphorylation; glycolysis, propranolol, iodoacetate, 2,4-dinitrophenol

Hypoxia represents a major hazard to both intrauterine and postnatal survival. Fortunately, the young of many species appear able to survive in the absence of oxygen for prolonged time periods when compared to adults (6, 10, 11, 15). In this regard, Dawes and associates (3) and Mott (13) have shown that a correlation exists between survival time and myocardial glycogen stores. While the association does not prove cause and effect, increased glycolysis has been suggested as an important metabolic compensation in cardiac hypoxia (2), and Opie (14) has attributed the enhanced ability of the fetus and newborn to withstand anoxia to their greater dependence on glycolytic metabolism. It has been shown that glycogen depletion by fasting decreases survival time, whereas reducing blood glucose by insulin administration without altering cardiac glycogen stores does not influence survival time (19). Of course, cardiac alterations in the absence of oxygen may occur both at enzymatic and structural levels. In this regard, past evidence suggests that one of the factors involved in the greater resistance of infant than of adult heart to anoxia may be age-related differences in intracellular proteolysis (4).

Since previous age-related survival studies have utilized whole animals, a specific comparison of the influence of profound hypoxia on the survival and performance of the fetal and adult heart has not been made. Accordingly, this study was designed to assess directly the effects of profound hypoxia and selected metabolic inhibitors on the spontaneous frequency of contraction of atria and the mechanics of contraction of ventricular myocardium isolated from fetal and adult sheep.

MATERIAL AND METHODS

Whole hearts were excised rapidly from 46 adult sheep (1-3 years old) after pentobarbital anesthesia (15 mg/kg iv). Pregnancies were confirmed radiographically in ewes with known breeding dates. The hearts of 48 fetal lambs (133 ± 4 days gestational age, term = 147 days) were removed rapidly after hysterotomy under spinal anesthesia (4 ml of 1% lidocaine). Spontaneously beating SA node-right atrial strips and right ventricular moderator bands or trabeculae carneae were isolated and suspended in a myograph containing oxygenated Krebs solution at 37°C as described previously (18). Whereas fetal atrial tissue was thinner than the comparable adult preparation, the atrial strips at both ages were less than 1.2 mm in thickness. The ventricular muscles were 4-12 mm in length and averaged 1.3 mm² (range 0.3-1.8) in cross-sectional area. The size and thickness of ventricular tissue from both age groups were comparable, since the cross-sectional areas of ventricular muscles isolated from both the fetus and adult were matched for the study of each intervention. Ventricular muscles and atria were held at one end by a spring-loaded clip forming the end of a rigid pin attached directly to a Statham (UC-3) force transducer. Isometric tension was measured at the apex of the length-active tension curve and for ventricular muscles was corrected for cross-sectional area and expressed in grams per square millimeter. Frequency of contraction was measured from spontaneously
were observed in either the magnitude or the time course of the response when fetus was compared to adult. In the first 10 min of profound hypoxia, and no differences in the presence of oxygen but continued in the fetus for 60-120 min. Spontaneous atrial contractions ceased in the adult beyond 10 min in the absence of oxygen. Active tension was measured at steady-state levels before and after any intervention. The results of this intervention only. Active tension was measured at steady-state levels before and after any intervention. The composition of the equilibrating Krebs solution was, in millimoles per liter: Na+, 146; K+, 3.6; Ca++, 2.5; Mg++, 1.2; Cl-, 126; H_2PO_4-, 1.2; SO_4-, 1.2; HCO_3-, 25; and glucose, 5.6. The solution was bubbled with 95% oxygen and 5% CO_2, which resulted in a pH of 7.4. The Po_2 of the oxygenated Krebs solution exceeded 600 mm Hg. When the myograph was bubbled with 95% nitrogen and 5% CO_2, the pH was maintained at 7.4, and Po_2 determined on the fluid bathing the muscle, never exceeded 10 mm Hg and averaged 4 mm Hg. The Po_2 was determined by a Beckman physiological gas analyzer (model 160). For both fetal and adult muscles the volume of the muscle bath and the rate of change of gas concentration were identical. Solutions of iodoacetate (IAA) and 2,4-dinitrophenol (DNP) were prepared freshly on the day of study and concentrations of 5 X 10^{-4} and 2.7 X 10^{-4} M, respectively, were maintained in the bathing medium in selected experiments. Changes in the frequency of contraction of spontaneously beating right atria and isometric tension of ventricular muscle were analyzed and the fetus and the adult were compared after: 1) substitution of oxygenated Krebs solution with nitrogenated Krebs solution, 2) addition of iodoacetate to the bath, 3) exposure to both IAA and nitrogenated Krebs solutions, 4) addition of DNP to the bath, 5) exposure to oxygenated Krebs solution prepared without glucose, 6) exposure to nitrogenated Krebs solution lacking glucose. The Student t test was used to analyze the statistical significance of differences between the two age groups.

Response to profound hypoxia. The effects of profound hypoxia (95% N_2 - 5% Xe) on frequency of contraction and isometric tension are shown in Fig. 1, A and B. The spontaneous frequency of atrial contraction in the control state (oxygenated Krebs) is significantly higher in the fetus (135 ± 8 beats/min) when compared to the adult (79 ± 7 beats/min, P < 0.001). Frequency of contraction declined substantially in both groups. While the absolute reductions in spontaneous atrial rate were similar, a relatively greater reduction occurred in the adult (Fig. 1A). Spontaneous contractions ceased in the adult beyond 10 min in the absence of oxygen but continued in the fetus for 60-120 min at 60% of the control rate. The contractile force of ventricular muscle of both groups decreased rapidly within the first 10 min of profound hypoxia, and no differences were observed in either the magnitude or the time course of the response when fetus was compared to adult.

Response to iodoacetate. IAA (5 X 10^{-4} M) was added to oxygenated Krebs solution in order to block energy supply via glycolytic metabolism (1). The results of this intervention are shown in Fig. 2, A and B. Administration of IAA produced a significantly more marked decline in both the frequency of contraction of fetal atrial tissue and the tension generation of fetal ventricular myocardium when compared to the adult. An abrupt decline in the rate of fetal atrial contraction was observed at 35 min. Fetal right atria ceased beating at 50 min, whereas adult atria were unaffected as long as 120 min. Beyond 5 min, fetal contractile force declined steadily and the ventricular muscles could no longer be stimulated to contract after 40 min of IAA exposure. In contrast, a significant reduction in developed tension of adult muscles began only after 65 min, and 25% of control force existed at 120 min.

Response to combination of profound hypoxia and IAA. When tissue was exposed to both profound hypoxia and IAA, the responses were much more rapid than with either intervention alone. Adult atria ceased beating at 5 min and fetal atria at 8 min (Fig. 3A). Developed tension fell to zero within 8 min for both the fetus and adult. At any time beyond 4 min, fetal ventricular tissue was significantly more depressed than the adult (Fig. 3B).

Effect of 2,4-dinitrophenol. The effects of uncoupling oxidative phosphorylation by DNP are shown in Fig. 4, A and B. In oxygenated Krebs solution, there is a striking difference in the response to DNP (2.7 X 10^{-4} M) of fetal atria when compared to the adult. Adult atria were arrested within 5 min. After an initial rapid decline in frequency, fetal atria continued to contract at approximately 20% of control rates throughout the entire experiment (120 min).

The contractile force of fetal and adult right ventricular myocardium decreased rapidly within the first 5 min of DNP exposure. At both ages a low contractile force was then maintained.

Effect of glucose-free medium. During a 90-min study period, bathing fetal or adult muscles in oxygenated Krebs lacking glucose did not alter either contractile force or spontaneous frequency of contraction.

Effect of combination of glucose-free medium and profound hypoxia. Contracting fetal and adult cardiac tissue
FIG. 2. Effect of iodoacetate (IAA) on spontaneous frequency of right atria (A) and isometric tension of right ventricular myocardium (B). Number of experiments is in parentheses. Values and vertical bars are means ± SE.

FIG. 3. Effect of combination of profound hypoxia and iodoacetate (IAA) (5 × 10⁻⁴ M) on spontaneous frequency of right atria (A) and isometric tension of right ventricular myocardium (B). Number of experiments is in parentheses. Values and vertical bars are means ± SE.

FIG. 4. Effect of 2,4-dinitrophenol (DNP) (2.7 × 10⁻⁴ M) on spontaneous frequency of right atria (A) and isometric tension of right ventricular myocardium (B). Number of experiments is in parentheses. Values and vertical bars are means ± SE.

FIG. 5. Effect of combination of glucose deprivation and profound hypoxia on spontaneous frequency of right atria (A) and isometric tension of right ventricular myocardium (B) isolated from fetus and adult. Number of experiments is in parentheses. Values and vertical bars are means ± SE. Influence of propranolol (10⁻⁶ M) on fetal responses is shown in each panel.

Whole animals have been employed in past experiments designed to examine the relationship between survival during anoxia and the age of the animal (3, 13). It has not been possible to analyze the possible age dependency of the rate, but prolonged survival was still observed when compared to the adult (Fig. 5A).

Remarkable differences were also observed in the response of fetal and adult ventricular tissue to the combination of glucose and oxygen deprivation. An initial decrease in the contractile force of both fetal and adult tissue was observed. However, in contrast to the adult, a secondary, markedly positive inotropic response was observed in fetal myocardium which reached a peak at 60 min (Fig. 5B). When propranolol (10⁻⁶ M) was administered 30 min prior to the production of profound hypoxia the delayed, marked increase in the contractile force of fetal right ventricular myocardium was abolished (Fig. 5B).

DISCUSSION
resistance of the heart per se to oxygen deprivation in these studies, since multiple factors are operative in the ultimate demise of the anoxic animal. These factors include, in particular, body temperature, acid-base and electrolyte status, and the integrity of the central nervous system. Accordingly, the present investigation was designed to quantify and compare the responses of cardiac tissue isolated from fetal lambs and adult sheep to oxygen lack and selective inhibition of both anaerobic and aerobic metabolic pathways.

It must be recognized that the oxygenation of isolated cardiac tissue studied in a myograph is dependent on diffusion from the bathing solution and not on perfusion. To avoid problems in analysis that may have arisen because of oxygen diffusion limitations, the present investigation employed fetal and adult moderator bands that were matched for size and thickness for each experimental intervention. Moreover, when the responses were compared at either age of those ventricular muscles of small (0.3–1.1 mm²) and larger (1.1–1.8 mm²) cross-sectional areas, no differences were observed.

It has been demonstrated that fetal lambs of gestational ages comparable to those employed in this study have 3–4 times greater cardiac glycogen stores than the adult sheep (3). Moreover, a direct positive correlation exists between cardiac glycogen content and the duration of survival time during anoxia in a host of animal species (3, 17). Resistance to anoxia may be enhanced in the rat and rabbit by raising the cardiac glycogen by glucose infusion (9, 19) or by re-scription (16).

The results of this study demonstrate that the spontaneous frequency of atrial contraction in the fetus is maintained for substantially longer periods of time than the adult in the presence of profound oxygen deprivation or after uncoupling oxidative phosphorylation by DNP (12). In contrast, in the presence of oxygen when anaerobic glycolysis was blocked by IAA (1), the fetal, but not the adult, SA node – right atrial strips became quiescent. These findings are consistent with those observed in whole-animal experiments illustrating prolonged fetal survival under anoxic conditions, and support the proposal that the fetal heart has a much greater dependency on energy supplied via glycolysis than that of the adult (14). Oxygen deprivation and the uncoupling of oxidative phosphorylation by DNP produced comparable reductions in the ability of both fetal and adult ventricular myocardium to generate tension. In contrast, IAA administration resulted in a significantly greater negative inotropic response in fetal muscle. Thus, it would appear that the ability of the young heart to sustain contractile force as well as maintain heart rate is influenced profoundly by blockade of glycolysis.

A striking finding in the present study concerned the differences between fetus and adult heart when glucose deprivation was combined with oxygen lack. Under these conditions, fetal but not adult tissue exhibited a remarkable secondary rise in both frequency of contraction and isometric tension that was blocked by the beta-adrenergic antagonist, propranolol. Several factors may be responsible for this age-related contrast. Dhalla and associates (5) have demonstrated the importance of glucose for the maintenance of the heart’s catecholamine storage sites. In the latter studies glucose deprivation resulted in a shift of catecholamines from the granular to the soluble fraction, and a reduction in endogenous norepinephrine associated with leakage. Further, it appears that neural re-uptake of norepinephrine is dependent on the presence of glucose (20). Our present results suggest that hypoxia and glucose deprivation causes a greater release of the adrenergic neurotransmitter from fetal myocardium than adult. In this regard, past studies from this laboratory have shown that fetal lamb myocardium close to term is significantly more sensitive than adult tissue to the chronotropic and inotropic actions of norepinephrine (7, 8).

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REFERENCES


