Influence of Norepinephrine and Digitalis on Myocardial Oxygen Consumption in the Newborn Lamb
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ABSTRACT
The relationships between cardiac performance and oxygen usage were explored in 19 newborn lambs (5 hours to 8 days old), and the influence of inotropic stimulation was studied. A preparation was developed to measure coronary sinus flow and myocardial oxygen consumption (MVo2) under controlled hemodynamic conditions. Using a gelatin injection technique, we determined that more than 90% of the measured sinus flow originated from left heart tissue; less than 10% was derived from right ventricular myocardium. Changes in contractility were produced by intravenous infusion of norepinephrine or acetylstrophanthinid. Norepinephrine (1–2.4 μg/min kg−1) or acetylstrophanthinid (5 μg/min kg−1) produced increases in the maximal rate of rise of left ventricular pressure and large reductions in left ventricular end-diastolic pressure, but the changes in MVo2 in lambs with constant aortic blood pressure, cardiac output, and heart rate were minimal. Ventricular function curves demonstrated a significant relationship between end-diastolic pressure and MVo2 (P < 0.01). For a given left ventricular end-diastolic pressure, MVo2 was approximately 3 ml/min 100 g−1 left ventricle greater during the infusion of norepinephrine. Therefore, net changes in MVo2 with inotropic stimulation represented a balance between reciprocal changes in diastolic pressure (and volume) and contractility. The enhanced oxygen cost of increased contractility might be masked by a reduction in heart size (reduced wall stress). Ventricular function was reduced below initial control values following the infusion of norepinephrine in 3 lambs. This reduction correlated with a concomitant reduction in MVo2 and percent extraction but not with a reduction in flow. Lambs that did not show mechanical depression demonstrated no reduction in MVo2. These findings suggest a metabolic basis for catechol dependence which might have special importance for the newborn lamb with its incompletely developed catechol enzyme systems.

KEY WORDS coronary flow oxygen extraction injection mass acetylstrophanthinid ventricular function catechol dependence gelatin

Abundant evidence now supports the view that important structural, biochemical, and functional differences are present in newborn myocardium compared with adult myocardium; these differences are likely to be of major importance in circulatory adaptation following birth. The glycogen content is much greater in the newborn's myocardium (1), and left ventricular protein synthesis is markedly accelerated shortly after birth (2). Sympathetic innervation may be incomplete (3), and concentrations of myocardial norepinephrine and enzymes related to catecholamine metabolism are reduced (4). The latter observations are presumably responsible for a supersensitivity of fetal and newborn myocardium to catechol stimulation. Morphologic evidence suggests delayed development of the transverse tubular system (5) and continuing developmental changes in the conduction system (6) in those species which have been studied. More recent investigations have demonstrated differences in the muscle compliance of young animals compared with that of mature animals (4). Moreover, there probably are important differences in responsiveness to cardioactive drugs, notably norepinephrine and digitalis (7).

Therefore, this study was undertaken to determine patterns of coronary flow and myocardial oxygen metabolism in the newborn lamb; special attention was given to the interrelationships of functional and metabolic changes produced by the administration of norepinephrine and acetylstrophanthinid. The lamb provided a useful model for...
these studies because of the abundant information on the circulation of this species and the convenient anatomic arrangement that permitted the cannulation of the coronary sinus through the hemiazygos vein (8). Also, it was possible to identify with reasonable certainty those portions of myocardium which contributed to the metabolic measurements and to conclude that they were largely the same segments as those which contributed to the measurements of cardiac performance.

Methods

Nineteen newborn Dorset lambs of both sexes (5 hours to 8 days old) weighing 3.2-6.0 kg were used in this study. Each lamb was anesthetized with sodium pentobarbital (20 mg/kg, iv). The trachea was exposed and intubated; the chest was opened by a midline incision and ventilation was maintained with a Harvard constant-volume, positive-pressure pump. The ductus arteriosus was ligated, the descending thoracic aorta was cannulated (Fig. 1), and the systemic blood flow was measured with a Statham extracorporeal flow transducer (6.0 mm, o.d.) and a Medicon K2000 electromagnetic flowmeter. The flow transducer was calibrated in vitro with saline. Aortic flow was then passed through a heat exchanger and returned to the descending aorta. Blood temperature was maintained at 38 ± 1°C with the heat exchanger system and was continuously measured with a Yellow-Springs probe and telethermometer. Cephalic blood flow was abolished by ligating the brachiocephalic artery, and systemic blood flow and aortic blood pressure were controlled by a Sarns roller pump and an adjustable constant-pressure reservoir, respectively, in the extracorporeal circuit. The heat exchanger, reservoir, and tubing were primed with freshly drawn heparinized blood (5 mg/100 ml) from the maternal ewe. Arterial pH, Po2, and PCO2 were continuously monitored with a Jewett flow-through electrode assembly with three Beckman 160 physiological gas analyzers and were frequently checked with a blood gas analyzer and pH system (Instrumentation Laboratories). During the experiment, arterial Po2 and pH were kept within normal physiological limits (arterial Po2 90-120 mm Hg and pH 7.35-7.44) by the administration of oxygen or sodium bicarbonate if necessary.

Left ventricular pressure was measured with a 15-gauge needle passed through the apex into the left ventricular cavity. Aortic blood pressure and left ventricular pressure measurements were made with Sanborn transducers (267 series); the midlevel of the heart was used as the zero reference. The maximal rate of rise of left ventricular pressure (dP/dt max) was obtained using a resistance-capacitance differentiating circuit with a time constant of 0.268 msec. Heart rate was controlled by electrically pacing the left atrium with a Grass SD5 stimulator. The pressures, aortic blood flow, heart rate, lead II electrocardiogram, and left ventricular dP/dt measurements were recorded simultaneously on a multichannel oscillograph (Sanborn model 358) at chart speeds of 0.5-100 mm/sec.
Coronary flow was measured by the cannulation of the coronary sinus through the hemiazygos vein (8). The sinus was closed by stitch ligation 1–2 mm from its orifice in the right atrium, using direct vision. Flow from the sinus catheter (PE 240) was then diverted through Tygon tubing (1/8 inch, i.d.) to the external jugular vein. A T-connector was placed in the tubing for sampling and for timed collections of coronary sinus blood.

Arterial and venous blood samples were withdrawn simultaneously from the aorta and the coronary sinus for determinations of oxygen (9), lactate (10), and pyruvate (11) contents and for measurements of pH, PO₂, PCO₂, and hematocrit. Myocardial oxygen consumption (MV o₂), percent oxygen extraction, and lactate and pyruvate utilization were calculated (12).

Cardiac output was obtained by summing systemic blood flow and coronary sinus flow.

Norepinephrine (1.0–2.4 μg/min kg⁻¹) was infused into a femoral vein until a consistently elevated first derivative of left ventricular pressure (dP/dt max) was obtained. The protocol for acetylcholinesterase infusion (5 μg/min kg⁻¹) was similar except that a loading dose of 20 μg/kg was given over a 3-minute period. Measurements were made before the appearance of toxicity, evidenced by supraventricular arrhythmias or sustained ventricular tachycardia. Mean aortic blood pressure, systemic blood flow, and heart rate were held constant during the infusion of norepinephrine and acetylcholinesterase in 12 lambs. In 7 lambs ventricular function curves were performed under control conditions and during the administration of these agents.

All numerical data in this study were processed and analyzed by standard statistical methods (13), and values are given as means ± SE. Differences were considered significant when P < 0.05.

Distribution of Coronary Sinus Drainage in the Lamb Preparation.—Four hearts from lambs 2–4 days old were studied by retrograde injection of a pigmented gelatin mass containing Barosperse (14) through the sinus catheter at a constant pressure of 40 mm Hg. Using the methods employed in the present study this mass has been shown to fill venules and a few capillaries (14), and this finding was confirmed by histologic control. After the mass had set (30–45 minutes) the heart was dissected, and the various segments were weighed. Portions containing the pigmented mass were identified, further dissected, and weighed. As indicated in Figure 2, approximately 85% (range 82 to 90%) of the injected cardiac mass contributed to the left ventricular free wall and septum; approximately 8% (range 6 to 10%) was left atrial tissue. The right ventricular free wall contributed only 7%. Figure 2 also indicates the proportion of each cardiac component that was injected. Thus, 51% of the total heart weight was left ventricular free wall and septum; 24% of the total heart weight, but only 18% of that tissue contained injected vessels. Approximately 60% of the septal and the left atrial tissues were injected. None of the right atria was injected.

In three lambs the right and left coronary arteries were cannulated post-mortem and independently perfused with blood from a reservoir with a mean pressure of approximately 75 mm Hg. In all hearts repeated determinations indicated that a constant fraction (65–70%) returned from the coronary sinus cannula in each heart. None of the right coronary flow entered the sinus but was drained through other venous channels.

These observations provide evidence that the coronary sinus drainage in these preparations was derived principally from the left ventricle and septum, with a small contribution from the left atrium; probably less than 10% came from right heart tissue. Presumably, all of the flow which entered the coronary sinus catheter originated from left coronary artery inflow.

Results

Effects of Norepinephrine and Acetylcholinesterase on Left Ventricular Contractility and Myocardial Oxygen Usage.—Individual data from six lambs, 5 hours to 2 days old, are presented in Figure 3. Values were obtained immediately before and during norepinephrine infusion (1–2 μg/min kg⁻¹); aortic blood pressure, cardiac output, and heart rate were maintained constant. All lambs demonstrated a positive inotropic response mani-
Responses of six newborn lambs to norepinephrine (NE) infusion. LV dp/dt MAX = maximal rate of rise of left ventricular pressure, LVEDP = left ventricular end-diastolic pressure, CF = coronary flow, C = control, MVO₂ = myocardial oxygen consumption, BW = body weight, AP = aortic pressure, CO = cardiac output, HR = heart rate, EXT. = extraction.

Tests by an increase in left ventricular dp/dt max and a fall in end-diastolic pressure. MVO₂ increased minimally in five lambs and decreased in one. On the average, MVO₂ increased approximately 12% during the infusion of norepinephrine, a change which was barely significant (P < 0.05) and resulted from a small increase in both oxygen extraction (42-47%) and coronary flow (25-27 ml/min).

These observations may be compared with data obtained from six lambs before and during the administration of acetylstrophanthidin (Fig. 4). The mean toxic dose was 104 ± 10 /µg/kg. Reported measurements were made before the appearance of toxicity. All lambs showed striking positive inotropic responses. The mean left ventricular dp/dt max fell 4 cm H₂O. Only one lamb demonstrated an important increase in MVO₂, but the slight change in the mean was not significant. Oxygen extraction increased in five of the six lambs, but the coronary flow did not increase in any.

Lactate and Pyruvate Uptake.—The above findings indicate that both norepinephrine and acetylstrophanthidin produced pronounced augmentation of left ventricular contractility in the newborn lamb. However, under controlled hemodynamic conditions, the inotropic changes were associated with minimal augmentation of MVO₂. Moreover, the mean uptake of lactate increased with norepinephrine infusion from 1.93 ± 1.44 to 6.73 ± 1.43 mg/min 100 g⁻¹ left ventricle and with acetylstrophanthidin infusion from 0.72 ± 3.61 to 4.14 ± 1.39 mg/min 100 g⁻¹ left ventricle; pyruvate uptake was unchanged during the infusion of either agent.

Relationship of Changes in Ventricular Performance to Oxygen Consumption.—In the experiments cited above the administration of either norepinephrine or acetylstrophanthidin led to a substantial reduction in end-diastolic pressure (Figs. 3 and 4). This reduction reflected a decrease in cardiac size and implied a reduction in wall tension as well as preloading. To determine if the minimal changes in MVO₂ represented a balance between reciprocal changes in diastolic size and contractility, ventricular function curves were determined in seven lambs by sequentially augmenting aortic blood flow in steps. Samples were obtained at each measured point and MVO₂ was computed. A representative study from one of these lambs illustrates the relationship between stroke volume and left ventricular end-diastolic pressure (Fig. 5, left) and the relationship between MVO₂ and left ventricular end-diastolic pressure (Fig. 5, right). Each increment in left ventricular end-diastolic pressure was associated with an increase in MVO₂ as well as stroke volume. Moreover, when contractility was increased by norepinephrine (top) or acetylstrophanthidin (bottom) both curves shifted to the left. For any given end-diastolic pressure both stroke volume and MVO₂ were substantially greater. However, it is apparent from these relationships that, if there was little change in stroke volume but a reduction in left ventricular end-diastolic pressure with the infusion of an inotropic agent as in the earlier experiments, a substantially smaller increase or even a decrease in MVO₂ would be recorded, as in Figures 3 and 4.

Figure 6 shows the calculated regression lines relating MVO₂ to left ventricular end-diastolic pressure obtained from seven lambs during the control state and during the administration of
norepinephrine. The slopes are precisely parallel, and, at a given left ventricular end-diastolic pressure, the \( M_{\text{VO}_2} \) was approximately 3 ml/min 100 g\(^{-1}\) left ventricle higher during the infusion of norepinephrine. Therefore, a fall in left ventricular end-diastolic pressure from 8 to 4 cm H\(_2\)O would result in no net change in \( M_{\text{VO}_2} \) consequent to the infusion of norepinephrine. This observation is consistent with the data obtained from six different lambs (Fig. 3) in which a comparable fall in left ventricular end-diastolic pressure occurred and the slight average change in \( M_{\text{VO}_2} \) was of marginal significance.

Relationship of Postnorepinephrine Depression to Cardiac Oxygen Metabolism.—The relationships between ventricular function and \( M_{\text{VO}_2} \) were studied in five lambs following the infusion of norepinephrine (1.1–2.4 \( \mu \)g/min kg\(^{-1}\) ). Ventricular performance was reduced below the levels that existed before infusion in three lambs, and this reduction was uniformly associated with a reduction in \( M_{\text{VO}_2} \). A representative study is shown in Figure 7 from a 1-day-old lamb. Infusion of norepinephrine (2 \( \mu \)g/min kg\(^{-1}\) ) caused an increase in both stroke volume and \( M_{\text{VO}_2} \) for a given left ventricular end-diastolic pressure under conditions of constant heart rate and aortic blood pressure. The control curve obtained following cessation of norepinephrine infusion was substantially lower than the initial control curve. Similarly, \( M_{\text{VO}_2} \) at any given end-diastolic pressure was uniformly lower. The mean data from these lambs are shown in Figure 8 (top). Left ventricular dP/dt max increased from 3640 ± 170 to 5480 ± 300 mm Hg/sec with norepinephrine infusion but fell to 1960 ± 220 mm Hg/sec after the norepinephrine infusion was stopped. The left ventricular end-diastolic pressure was also much higher (12.1 ± 2.1 cm H\(_2\)O) than it was in the initial control state (5.0 ± 0.5 cm H\(_2\)O). Mean \( M_{\text{VO}_2} \) increased from 11.2 ± 1.1 to 14.1 ± 0.9 ml/min 100 g\(^{-1}\) left ventricle but fell to 8.9 ± 1.2 ml/min 100 g\(^{-1}\) left ventricle during the second control state and in the face of higher left ventricular end-diastolic pressure. These values may be contrasted with the values in Figure

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Representative lamb (1 day old, 3.64 kg) showing marked reduction in left ventricular performance (triangles, left) following the administration of norepinephrine (NE) compared with the control curve (open circles) obtained before the administration of norepinephrine. On the right, myocardial oxygen consumption ($MVO_2$) is plotted at points which correspond to those on the left. $MVO_2$ is markedly reduced in the second control state (open triangles). Heart rate = 200/min and aortic blood pressure = 75 mm Hg.

Values for coronary flow and percent oxygen extraction are shown in Figure 9. Flow increased in both groups and remained elevated. In contrast, oxygen extraction fell markedly from $65.3 \pm 2.3\%$ to $31.1 \pm 5.3\%$ in those lambs showing reduced contractility following the administration of norepinephrine. Extraction was unchanged (approximately 60%) in those which did not demonstrate postnorepinephrine mechanical depression (Fig. 9, bottom). There was no correlation between the appearance of postnorepinephrine cardiac depression and the rate of infusion, the duration of infusion (17–28 minutes), or the total amount of norepinephrine infused (25–57 $\mu$g/kg).

**Discussion**

Studies investigating the interrelationships between myocardial function and metabolism in the newborn must consider a number of unique features which represent important differences from the adult. For example, because of the relative prominence of the right ventricle in the early postnatal period, assessment of the metabolic cost of changes in left ventricular performance should be based on samples obtained from blood which has perfused the left ventricular myocardium.
Admixture of venous blood from the right ventricular myocardium would tend to obscure or invalidate the results. The preparation described in this study has the advantage of providing samples of mixed venous blood which have originated primarily from the left heart vascular territories with minimal contribution (less than 10%) from the right heart (Fig. 2). Thus, a high degree of accuracy might be expected in assessing proportional changes in cardiac metabolism which are consequent to alterations in left heart performance.

Hearts of newborn mammals will demonstrate vigorous positive inotropic responses to norepinephrine (15), digitalis glycosides (7), or sympathetic nerve stimulation (16). Moreover, precise quantification of dose-response relationships has been achieved with isolated cardiac muscle from the fetus, newborn, and adult (4). The present study was designed to estimate the oxygen cost of inotropic changes in relation to the changes in performance engendered by norepinephrine or acetylstrophanthidin. With aortic blood pressure and stroke volume constant, both agents elicited pronounced positive inotropic changes evidenced by a fall in left ventricular end-diastolic pressure and a simultaneous rise in left ventricular dP/dt max (Figs. 3 and 4). Moreover, the responses to acetylstrophanthidin were no less than the responses to norepinephrine (1-2 μg/min kg⁻¹). The suggestion that neonatal lambs less than 3 days old are supersensitive to norepinephrine (4) is not supported by these findings. In most lambs the increase in contractility was accompanied by a very small augmentation of MVo₂. The average changes were of borderline significance, however. Correspondingly, changes in coronary flow and myocardial oxygen extraction were generally quite small.

It has been reported that with controlled hemodynamic conditions the adult dog manifests a uniform increase in MVo₂ with the administration of norepinephrine (17). However, Sarnoff and co-workers reported that MVo₂ did not show changes with doses of norepinephrine sufficient to produce large increments in contractility (18), but with very high doses of norepinephrine augmentation of MVo₂ was observed. Our observations are entirely consistent with those of Sarnoff et al. Moreover, our findings concerning the oxygen cost of acetylstrophanthidin administration under controlled hemodynamic conditions do not differ from earlier findings in the adult dog (19). Thus, under the conditions of these experiments, pronounced augmentation of contractility appeared but was associated with minimal changes in MVo₂. These findings are generally consistent with comparable findings for adult heart preparations.

When the contractility of isolated cardiac muscle strips is augmented with norepinephrine or acetylstrophanthidin, MVo₂ is uniformly increased (20, 21). It is also well established that tension development is an important determinant of MVo₂ (22, 23). Indeed, the oxygen cost of augmentation of contractility with norepinephrine may be similar in magnitude to the accompanying alterations in tension development (24). Thus it appears likely that the minimal net changes in MVo₂ recorded in the earlier studies of Sarnoff et al. (18) and in our own study (Figs. 3 and 4) represent a summation of the oxygen cost of increased contractility reflected by the left ventricular dP/dt max and decreased tension reflected by the fall in left ventricular end-diastolic pressure.

To support this concept, we measured MVo₂ at different levels of end-diastolic pressure produced by changing the cardiac output while the heart rate and the aortic blood pressure were held constant. Figure 5 demonstrates that MVo₂ increased with increasing left ventricular end-diastolic pressure. Moreover, for any given left ventricular end-diastolic pressure, MVo₂ was substantially greater during the infusion of norepinephrine or acetylstrophanthidin. In the absence of compliance changes (4) left ventricular end-diastolic pressure must reflect diastolic volume and directional changes in wall tension. Therefore, these relationships and the regression data (Fig. 6) show that, with inotropic stimulation, reduction of cardiac size, which is sufficient to counterbalance the oxygen cost of increased contractility, may occur and result in little or no net change, assuming constancy of other hemodynamic variables.

A progressive decline in cardiac function has been observed during sustained norepinephrine infusion (25, 26), and reduced contractility has been reported in isolated cardiac muscle preparations following relatively brief exposure to norepinephrine (27). Three of five lambs in the present study showed depression of contractility following the infusion of norepinephrine when compared with values obtained before the infusion. Myocardial oxygen extraction and MVo₂ were uniformly reduced in these lambs. Contrastingly, two lambs which failed to manifest mechanical evidence for
postnorepinephrine depression also showed no change in extraction or $\text{MVo}_2$. These findings were unrelated to arterial $\text{Po}_2$, $\text{pH}$, serum calcium levels, serum potassium levels, or blood glucose concentrations. There was no limitation of oxygen supply; coronary flow and oxygen content were normal. A potential explanation is suggested by the observation that in some tissues high concentrations of exogenous norepinephrine will markedly inhibit norepinephrine synthesis from tyrosine (28). It is not established whether this inhibition is also true for myocardium, nor is information available concerning the rate of recovery of synthetic processes in cardiac neural structures. These considerations may be of special importance in newborn myocardium where sympathetic innervation and development of norepinephrine enzyme systems may be incomplete (4).

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References


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