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 (M\text{Vo}_2) 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 acetylstrophanthidin. Norepinephrine (1-2.4 \mu g/min kg\(^{-1}\)) or acetylstrophanthidin (5 \mu g/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 M\text{Vo}_2 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 M\text{Vo}_2 (P < 0.01). For a given left ventricular end-diastolic pressure, M\text{Vo}_2 was approximately 3 ml/min 100 g\(^{-1}\) left ventricle greater during the infusion of norepinephrine. Therefore, net changes in M\text{Vo}_2 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 M\text{Vo}_2 and percent extraction but not with a reduction in flow. Lambs that did not show mechanical depression demonstrated no reduction in M\text{Vo}_2. 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, acetylstrophanthidin, 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 cathechol 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 acetylstrophanthidin. The lamb provided a useful model for...
these studies because of the abundant information on the circulation of this species and the convenient anatomical 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 flow 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 oscillogram (Sanborn model 358) at chart speeds of 0.5–100 mm/sec.

Right: Preparation for control and measurement of left ventricular performance and measurement of coronary sinus blood flow. P.A. = pulmonary artery, P.V. = pulmonary vein, L.A. = left atrium, L.V. = left ventricle, R.V. = right ventricle, R.A. = right atrium, I.V.C. = inferior vena cava, S.V.C. = superior vena cava, and B.C.A. = brachiocephalic artery. Left: Detailed posterior view of the lamb heart showing coronary sinus cannulation through the hemiazygos vein. Sinus is closed by stitch ligation near entry to the right atrium.

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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 (¼ 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, P_O2, P_C02, 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 acetylstrophanthidin 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 acetylstrophanthidin 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 tissue was 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 88% of this tissue by weight contained injected vessels. By contrast the right ventricular free wall accounted for 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 Acetylstrophanthidin 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, and EXT. = extraction.

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Figure 5

Relationships of stroke volume and myocardial oxygen consumption to left ventricular end-diastolic pressure. Both curves shift to the left with the administration of either inotropic agent. The lamb used in these experiments was 1 day old and weighed 3.6 kg. Heart rate and aortic blood pressure were held constant.

Figure 6

Calculated regression lines showing the relationship between myocardial oxygen consumption ($\text{MVO}_2$) and left ventricular end-diastolic pressure (LVEDP) before (control) and during norepinephrine (NE) infusion in seven lambs.

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 $\text{MVO}_2$ 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, $\text{MVO}_2$ 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 $\text{MVO}_2$ 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
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 ($\text{MVO}_2$) is plotted at points which correspond to those on the left. $\text{MVO}_2$ is markedly reduced in the second control state (open triangles). Heart rate = 200/min and aortic blood pressure = 75 mm Hg.

In Figure 8 (bottom) in which $\text{MVO}_2$ increased somewhat from $9.4 \pm 1.0$ to $12.3 \pm 1.0$ ml/min 100 g$^{-1}$ left ventricle and remained at that level during the second control state.

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 M\(\text{VO}_2\). These findings were unrelated to arterial Po\(_2\), 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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