Differential Effects of Digoxin at Comparable Concentrations in Tissues of Fetal and Adult Sheep

William Berman, Jr., Peter J. Ravenscroft, Lewis B. Sheiner, Michael A. Heymann, Kenneth L. Melmon, and Abraham M. Rudolph

SUMMARY Electrocardiogram (ECG) electrodes and carotid arterial and superior vena caval (SVC) catheters were placed in eight nonpregnant ewes and 11 fetuses (109–129 days gestation) to measure heart rate, arterial pressure, P-R interval, left ventricular pre-ejection period (PEP), and left ventricular ejection time (LVET), before and after digoxin infusion into the SVC. After the ewes were killed, the steady state concentration of digoxin in plasma was related to the concentration in midbrain and left ventricular free wall. Although concentrations of digoxin in tissue differed between fetuses and ewes, tissue-plasma ratios were similar; the myocardial-plasma ratio was 87 for fetuses and 90 for ewes and the midbrain-plasma ratios were 6.4 and 5.3, respectively. In spite of these similarities, physiological and toxic effects differed at comparable plasma concentrations. Reduction in PEP/LVET ratio was greater in ewes than fetuses, and P-R interval prolongation was linearly related to digoxin concentration in fetuses but uncommon at plasma concentrations below 2 ng/ml in ewes. Arrhythmias occurred in six ewes, but in only one fetus, even though the mean steady state concentration of digoxin in plasma was 4.5 ng/ml in the fetuses and 2.3 ng/ml in the ewes. Atropine had little effect on digoxin-induced P-R interval prolongation, and isoproterenol produced no tachyarrhythmias in the fetuses. Age-related differences in inotropic and arrhythmogenic effects of digoxin exist and are related to differences in drug response rather than drug kinetics; this provides experimental support for the different dosage responses.

THE CLINICAL impression that newborns require and tolerate higher doses of digoxin per kilogram body weight than do adults had led to development of dosage schedules for infants and children that differ from those generally used for adults.1–4 Although the basis for this age-related difference is unclear, possible explanations include a difference in penetration of digoxin into target tissues and a difference in the target tissue response to comparable concentrations of digoxin. A single study on the relationship of age to the effects of digoxin on contractility of isolated rabbit papillary muscle6 suggests that digoxin has a less marked inotropic effect on newborn than adult myocardium at comparable concentrations producing submaximal responses. This finding agrees with clinical experience7 but has not been explored in studies that relate tissue or serum concentrations of digoxin to cardiac performance in vivo.

The ratio of myocardial-serum concentrations of digoxin has been used as an indicator of the capacity of the myocardium to bind digoxin.8–9 Kim et al.10 studied seven premature infants, four full term neonates, and four 12–15 years of age. However, the ratios of myocardial to serum concentrations of digoxin were similar and unrelated to age in the nine cases in which it was possible to calculate them. These results suggest that the higher myocardial concentrations of digoxin in the younger patients were attributable to higher plasma concentrations of digoxin rather than to an unusual property of the myocardium of the infant to bind digoxin. It also has been suggested that the newborn's myocardium is less sensitive to the tachyarrhythmic effects of digoxin than that of the adult.6,5 Because the pathogenesis of digitalis-induced arrhythmias is not clear, several hypotheses have been used to explain the resistance of neonates to the adult pattern of digitalis toxicity. Gillis et al.11 proposed that digoxin caused arrhythmias by stimulating the central autonomic nervous system. They showed that digitalis-induced arrhythmias in dogs were preceded by a synchronous discharge in the peripheral sympathetic, parasympathetic, and phrenic nerves. If the ability of digoxin to cause tachyarrhythmias is mediated through central stimulation of cardiac sympathetic nerves, then the inability of digoxin to reach the central nervous system of newborns, the underdevelopment of either central autonomic pathways, or cardiac sympathetic innervation12–14 could underlie age-related differences in the pattern of digoxin-induced arrhythmias. We studied the pharmacokinetics of digoxin in plasma, heart, and brain in fetal and adult sheep that had been given continuous infusions of digoxin to the point of steady state concentrations in tissue. We related changes
in myocardial contractility, atrioventricular conduction, and impulse formation to these kinetics. Our results suggest that age-related variations in response to digoxin and in the development of toxicity are due to differences in sensitivity of target organs to the drug rather than to differences in pharmacokinetics.

Methods

SURGICAL PREPARATION

In eight nonpregnant ewes, local anesthesia was induced with 1% lidocaine, and three silver-tipped electrocardiographic (ECG) electrodes were sewn into the subcutaneous tissue over the left chest wall. Premade lengths of polyvinyl catheter [inside diameter (i.d.), 0.052 inch; outside diameter (o.d.), 0.090 inch] were inserted through the jugular vein into the superior vena cava (SVC) and through the left carotid artery (CA) into the ascending aorta just above the aortic valve. The mechanical delay times of the premeasured CA catheters were determined by recording rapid pressure changes in a chamber with both the polyvinyl catheters and a catheter tip pressure transducer. The SVC catheters were used for infusion of digoxin.

Eleven ewes with fetuses of 109–129 days gestation were fasted 24–48 hours, given epidural anesthesia with 1% tetracaine HCl, 2 ml (20 mg) and placed supine on an operating table. Polyvinyl catheters (0.052 inch i.d.; 0.090 inch o.d.) were inserted through the ewe’s hindlimb artery and vein and advanced into the descending aorta and inferior vena cava (IVC), respectively. The ewes were given an intravenous infusion of 1000 ml of 10% dextrose in 0.9% saline during the operative procedure. Sodium pentobarbital, 60–300 mg, was administered intravenously for additional sedation, as required.

The uterine horn was exposed through a midline abdominal incision. A small hysterotomy was made to expose fetal parts. Premade polyvinyl catheters (0.040 inch i.d.; 0.070 inch o.d.) were inserted into the fetal IVC through the hindlimb vein and into the fetal SVC through the jugular vein. A catheter was also inserted into the amniotic cavity.

In the first two fetuses studied, polyvinyl catheters (0.030 inch i.d.; 0.048 inch o.d.) also were inserted through the hindlimb artery into the descending abdominal aorta and through the carotid artery into the ascending aorta just above the aortic valve. In the fetus, nearly all of the blood from the SVC passes into the right ventricle, pulmonary trunk, and via the ductus arteriosus into the descending aorta, and blood from the IVC passes to both ventricles. Since these flow patterns could cause differences in concentrations of digoxin in plasma from femoral and carotid arteries in the fetus, we used the two arterial catheters to determine whether the site of sampling affected concentrations of digoxin during continuous intravenous infusion of the drug through the SVC catheter. In the remaining fetuses, only the CA catheter was inserted and used to sample blood and measure arterial blood pressure. In three fetuses, a 12 F polyvinyl catheter was inserted suprapubically into the urinary bladder. Three silver-tipped ECG electrodes were sewn into the subcutaneous tissue over the left chest in eight of the 11 fetuses. The time delay of the premeasured fetal CA catheter was determined as described for the maternal CA catheter.

All catheters and wires were led to the ewe’s flank where they were protected in a cloth pouch sewn to the ewe’s skin. The surgical incisions were closed and the ewe was allowed to recover for 24–48 hours. Procaine penicillin (400,000 units) and dihydrostreptomycin (0.5 g) (2 ml Distycin) were administered intramuscularly to the ewe on the day of surgery. Kanamycin (400 mg) and potassium penicillin (1 million units) were administered into both the amniotic cavity and IVC of pregnant ewes on each of the first 5 postoperative days.

PHYSIOLOGICAL MEASUREMENTS

Pressures were measured with Statham P23Dc transducers and heart rate with a Beckman cardiograph. Pressures, ECG, and heart rate were monitored daily on a Beckman type RM direct writing recorder and recorded intermittently at a paper speed of 250 mm/sec. Fetal arterial pressures were corrected to amniotic fluid pressure as a zero reference level. The pre-ejection period (PEP) was measured as the interval in milliseconds from the onset of the QRS complex to upstroke of the carotid arterial pulse tracing, minus the catheter delay time. The left ventricular ejection time (LVET) was measured as the interval in milliseconds from the upstroke to the dicrotic notch of the carotid arterial pulse tracing. The measurements were made on four consecutive complexes with equal R-R intervals and the results were averaged. Arterial pH, Pco₂, Po₂, and hematocrit were measured on each of the study days.

Atropine sulfate (0.4 mg into fetal IVC, 0.5 mg into ewe’s IVC) was administered to five fetuses and three ewes that had P-R interval prolongation but remained in sinus rhythm after infusion of digoxin, to test its effect on prolongation of the P-R interval associated with the administration of digoxin.

Next, dl-isoproterenol (Winthrop Laboratories) (0.5 μg/kg into the fetal IVC; 5 μg into the ewe’s IVC) was administered as a single bolus during digoxin infusion to four fetuses and eight ewes to test its effect on tacharythmia production. Fetuses and ewes were killed with pentobarbital.

DIGOXIN INFUSIONS

To obtain the maximum amount of data from each fetus and ewe, a series of plasma digoxin concentrations and physiological measurements were obtained in each. To relate the plasma concentrations to simultaneous myocardial concentrations of digoxin, steady state experiments were used since, at any point during steady state, the plasma to myocardial concentration ratio will be the same because of the linearity of the system and the definition of steady state. The value of this ratio for each animal can be obtained from the measured myocardial and plasma concentrations of digoxin at the final steady state. Accordingly, preliminary experiments were performed in both ewe and fetus to determine how long an infusion would have to be maintained in order to assure
steady state. The “elimination” or β half-life of a drug governs this process. Advantage could be taken of the placental clearance of the drug from the fetus to the ewe to achieve rapidly a pseudo steady state in the fetus.

Following an intravenous bolus injection of 5 mg of digoxin in the ewe, plasma concentrations of digoxin were measured and fitted to a biexponential decay curve. The average α half-life for three ewes was 0.5 hour and the average β half-life was 24 hours, with the highest 26 hours. Using this terminal half-life and the principle that after infusion for four half-lives at least 90% of the ultimate steady state concentration will have been reached, we gave infusions to eight ewes for 96 hours before taking measurements.

Following an infusion of digoxin into four fetuses for 6 hours at infusion rates of between 4 and 8 μg/kg estimated fetal weight per hour, the postinfusion decay curve for digoxin indicated that, in the fetuses, the average α half-life for digoxin was 0.5 hour, the average β half-life was 5 hours, and the average terminal γ half-life was 22 hours. The longest β half-life was 6 hours. Figure 1 shows a representative example of a postinfusion decay curve for digoxin from fetal plasma.

The concentration of digoxin in fetal plasma after continuous intravenous infusion into the fetus for 24 hours was approximately 8 times that in the maternal plasma. Thus placental clearance is the rate-limiting step for drug elimination in the fetus, and the long terminal (γ) half-life will only govern fetal plasma concentrations if the plasma digoxin concentration in the ewe exceeds that of the fetus. Under these conditions, the terminal half-life of digoxin in both animals must be the same, since the fetus ultimately depends on the ewe’s routes of elimination. The fetuses were therefore infused for just over four β half-lives (24 hours) before we assumed steady state had been reached, and successive steady states in any fetus were always at higher infusion rates than previous ones to assure that fetal and maternal plasma concentrations were never equal.

We measured the plasma concentration of digoxin 2 hours apart in the fetuses and 4 hours apart in the ewes at anticipated steady states to verify that steady state had been achieved. We estimated fetal weights in order to calculate fetal infusion rates on a per kilogram body weight basis. For each animal, we increased the infusion rate from one to three times at intervals of 24 hours for the fetuses and 96 hours for the ewes, attempting to achieve a maximum steady state concentration of digoxin that did not produce toxicity. At the final steady state concentration, the animals were killed by overdosage with 2 g of pentobarbital, iv. Left ventricular myocardium and midbrain samples were collected for an assay of digoxin.

The total clearance of digoxin at steady state was calculated using the expression: infusion rate (μg/kg/hour) = (total drug clearance (liters/kg/hour) × steady state concentration of digoxin in plasma (ng/ml)).

In three fetuses, two 1-hour urine collections were obtained after steady state had been achieved and the average renal clearance of digoxin was calculated. In one fetus, creatinine clearance and renal clearance of digoxin were measured simultaneously.

ANALYSIS OF TISSUES AND PLASMA

Concentrations of digoxin in plasma were measured by the assay described by Smith et al, modified by a sequential saturation technique. The coefficient of variation of the assay was 6% at 1 ng/ml and 13% at 3 ng/ml.

For determinations of the concentration of digoxin in myocardium and brain, we selected samples from the left ventricular free wall and from the midbrain. Tissue samples weighed 200–850 mg (wet weight). The samples were frozen until assayed. Duplicate test and control samples were weighed and suspended in phosphate-saline buffer (pH 7.4). Fifty microliters of a solution of tritiated digoxin (0.15 ng/ml) were added to the test samples. Each sample was homogenized and sonicated for 20 seconds. Two extractions of the homogenate were performed, each with 10 ml of dichloromethane. The pooled dichloromethane phases were evaporated to dryness. The samples then were resuspended in 5 ml of a 5% bovine serum albumin-phosphate buffer-saline solution. The same amount of tritiated digoxin was added to the test samples and to the control samples. Liquid scintillation counting of 0.5 ml of each sample enabled the percent extraction to be calculated. An adjustment was made for incomplete extraction. The mean recovery of digoxin was 84% (range, 80–95%) from the samples of myocardium and 55% (range, 45–75%) from the samples of the midbrain. An aliquot of any sample was diluted when necessary. The results are expressed as nanograms of digoxin per gram of tissue (wet weight). The validity of the correction for extraction efficiency depends on the equality of extraction of tritiated and nontritiated digoxin from the tissue samples. Although not directly verified in this study, there are no theoretical grounds on which to suspect inequality of extraction.

STATISTICS

The relationship of the change in PEP/LVET to a logarithmic function of the steady state concentration of digoxin and to estimated gestational age was analyzed by
multiple regression. The percent change in P-R interval was regressed on the steady state concentration of digoxin. The significance of these relationships was tested by analysis of variance. A t-test for difference between means was used to compare the brain or myocardial tissue to plasma ratio of the concentrations of digoxin in the ewes vs. the fetuses.

**Results**

**BASELINE PHYSIOLOGICAL MEASUREMENTS**

Measurements of arterial blood gases and hematocrit levels in the fetus were normal for our laboratory, with the exception of high hematocrits in fetuses no. 9 and 11 (Table 1). The weights of these two was less than 80% of predicted weight; this finding suggests that they were runted.11 Arterial blood gases and hematocrits in all ewes were normal.

Heart rates prior to and during infusions of digoxin did not differ significantly by a paired t-test in either the fetuses or the ewes as long as there was not evidence of toxicity to digitalis. The ratio of PEP/LVET was the same for fetuses or the ewes as long as there was not evidence of toxicity to digitalis. The ratio of PEP/LVET was the same for fetuses and ewes prior to infusion of digoxin. Preinfusion values for heart rates, mean blood pressure, PEP, LVET, P-R interval, and PEP/LVET ratios are shown in Table 2.

**PHARMACOKINETICS**

The details of the infusion rates of digoxin and the values for clearance of digoxin are included in Tables 1 and 3. Because concentrations of digoxin measured from femoral and carotid arterial blood were identical in the first two fetuses, all subsequent blood samples for assay of digoxin were taken from catheters in the carotid artery.

The mean volume of distribution of digoxin during the β phase was 23 liters/kg in the ewes and 18 liters/kg in the fetuses. In the fetus, the placenta was the major route of elimination; renal clearance accounted for only 2% of total clearance of drug. In this regard, two of the lower values for total clearance were found in the polycythemic, runted fetuses (nos. 9 and 11, Table 1); it is possible that the umbilical blood flow was low in these two fetuses. Renal clearance of digoxin was 70% of the creatinine clearance in one fetus in which they were measured simultaneously.

Plasma sampled at 2-hour intervals in the fetuses or at 4-hour intervals in the ewes showed no significant difference in concentrations of digoxin at steady state. The mean steady state plasma concentration of digoxin at the final rate of infusion into the fetuses was 4.5 ng/ml compared with 2.3 ng/ml for the ewes (Table 4). The factor that prevented us from matching concentrations of digoxin in plasma at steady state for both groups was the high incidence of toxicity to digoxin in the ewes. Three ewes died from digoxin toxicity during higher rates of infusion. In contrast to the differences in steady state concentrations of digoxin in the two groups, the mean total clearance rates of digoxin per kilogram body weight for both groups were similar, 1.7 liters/kg per hour in the fetuses and 1.2 liters/kg per hour in the ewes.

**TABLE 2 Hemodynamic Variables Measured Prior to Digoxin Infusions**

<table>
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<tr>
<th>Animal no.</th>
<th>Gestational age (days)</th>
<th>Weight (kg)</th>
<th>Infusion rate of digoxin (µg/kg/hr)</th>
<th>Steady state concentration of digoxin in plasma (ng/ml)</th>
<th>Total clearance of digoxin (liters/kg/hr)</th>
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<tr>
<th>Animal no.</th>
<th>Weight (kg)</th>
<th>Infusion rate of digoxin (µg/kg/hr)</th>
<th>Steady state concentration of digoxin in plasma (ng/ml)</th>
<th>Total clearance of digoxin (liters/kg/hr)</th>
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<td>52</td>
<td>2.4</td>
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* Mean ± SD.
The data are described by relating a logarithmic

time (PEP/LVET) ratio for fetuses and ewes.

The relationship of the concentration of digoxin in

plasma to change in pre-ejection period-left ventricular ejection
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R interval is linearly related to the concentration of digoxin in fetuses but not in ewes.

Atropine did not decrease the digoxin-induced P-R interval prolongation in ewes. Atropine decreased the digoxin-induced P-R interval prolongation by small amounts in three of five fetuses, but never to preinfusion levels.

Arrhythmias occurred after digoxin infusion in one of eight fetuses and in six of eight ewes. The one fetus that developed toxicity to digoxin showed atrioventricular (AV) dissociation when the concentration of digoxin was 9.0 ng/ml. Administration of atropine did not reverse this arrhythmia. Five ewes developed arrhythmias during infusions of digoxin alone and a sixth developed junctional tachycardia when the concentration of digoxin in its plasma was 1.2 ng/ml and isoproterenol was administered concurrently. The arrhythmias in the ewes included three episodes of junctional tachycardia when concentrations of digoxin were of 2.1-3.0 ng/ml, third-degree AV block when the concentrations of digoxin was 1.3 ng/ml, and sinus pause with junctional escape when the concentration of digoxin was 3.6 ng/ml. No ventricular extrasystoles were observed.

The average heart rate increase after isoproterenol administration was 101 beats/min in the ewes and 106 beats/min in the fetuses. Isoproterenol caused moderate reductions in P-R interval associated with drug-induced sinus tachycardias, but produced no tachyarrhythmias in the fetuses whose steady state concentrations of digoxin in plasma were 2.7-4.2 ng/ml.

**Discussion**

These studies confirm that the physiological and toxic effects of digoxin vary with age. Our observations were made at steady state concentrations of digoxin and thereby assure equilibrium of digoxin between plasma and tissue.

The β half-life controlled the time required to reach steady state in the fetus. Because the digoxin crossing the placenta from fetus to ewe was distributed in a large volume, maternal concentrations of digoxin in plasma remained well below those of the fetus over our infusion times and did not contribute to the steady state concentrations of the drug reached in the plasma of the fetus. If the ewe's concentration of digoxin had been equal to or greater than that of the fetus, the fetal elimination half-life would have coincided with the ewe's half-life, and the fetal concentration of digoxin in plasma would not have approached 95% of steady state level in 20 hours. The compartmental model accounts for the delay in distribution of digoxin between the plasma and the peripheral tissues. This delay can account for the poor correlation of concentration of drug in plasma with an effect observed in a target organ. Unless the drug has been administered long enough for equilibrium to be achieved, it is unreasonable to expect that the concentration of drug in plasma will be predictive of drug effect in other tissues. The results of previous studies relating concentrations of digoxin in plasma to effect or to toxicity in infants and children are difficult to interpret because steady state concentrations of drug in plasma or tissues may not have been achieved.10, 22, 23

We used systolic time intervals to quantify changes in myocardial contractility after digoxin administration. Whereas no single index of myocardial contractility reflects myocardial muscle performance independently of other variables of the circulatory system,24 the PEP normalized for LVET (PEP/LVET) seemed fitting for this study for the following reasons: (1) the PEP/LVET ratio is independent of variations in age and heart rate,25, 26 allowing direct comparisons between fetal and adult responses without requiring analysis of percent change; (2) PEP and LVET were measured after placement of ECG electrodes and a carotid arterial catheter, not requiring instrumentation of the left ventricle with catheter tip transducers; (3) previous studies in both adults and children confirm dose-dependent changes in systolic time intervals caused by digitalis.27, 28

The effect of digoxin on myocardial contractility, as reflected by a reduction in the PEP/LVET ratio, was less marked in fetuses than in ewes. If our findings are confirmed by future studies in man, they would support the current practice of achieving concentrations of digoxin in the plasma of neonates which would carry a high probability of producing toxicity in older children and adults. We did not determine maximal changes in contractility which digoxin can produce in fetuses and ewes because the concentrations of drug in plasma we could achieve were limited by arrhythmic side effects. This study does not provide information about possible age-related differences in the half-life or renal clearance of digoxin, since a fetal rather than a newborn animal model was used. Accordingly, conclusions about age-related variations in digoxin clearance as a possible explanation for the smaller digoxin dosages required in premature as compared to full term infants cannot be drawn.

The mechanisms underlying the age-related differences in response to digoxin are undetermined. Our results show that the concentration of digoxin in myocardium has the same relationship to the concentration in plasma in both the fetal and the adult sheep. This dispels the speculation that the concentration of digoxin in the myocardium relative to plasma varies with age despite the higher concentrations in the myocardium than would be expected from the concentrations in the plasma we observed in one fetus and one ewe. Such variability has been noted in other studies16, 29 and remains unexplained. The possibility still exists that myocardial concentrations of digoxin at an active site, myocardial receptor affinity for digoxin, or myocardial receptor density might vary with age; these variables were not examined in this study.

The relationship between contractile response and concentration of digoxin in plasma differed. This age-dependent variability in the effects of digoxin on myocardial contractility correlated both with the clinical impression that newborns and infants not only tolerate but require higher per kilogram doses of digoxin than adults and with data obtained in vitro.6 No explanation of these age-related differences in contractility is provided by this study. Friedman26 has used electron micrographic findings to postulate that intrinsic differences in contractility between the developing and the mature heart may be related to variation in the ratio of myofibrils to noncontractile.
elements. In right ventricular moderator bands, 60% of the mass was myofibrils in adult sheep, but only 30% was myofibrils in the fetus. Since fetal myocardium has less contractile tissue per unit mass than adult myocardium, comparable concentrations of digoxin in the myocardium might not be expected to produce equivalent increases in contractile force in fetuses and adults, particularly if there is no preferential concentration of drug by contractile tissue.

As previously mentioned, the effects of digoxin on cardiac rhythm may be related to its central autonomic stimulatory effects. Part of the inotropic effect also may depend on the autonomic nervous system. Although Koch-Weser failed to show dependence of the inotropic effects of digoxin in isolated preparations on release of myocardial norepinephrine, the role of the central autonomic nervous system in intact animals remains undefined. Differences in the intramyocardial distribution of digoxin, in the effects of digoxin on calcium flux, or in binding of digoxin to membrane-localized sodium-potassium ATPase also might help to explain age-related differences in the inotropic effects of the drug.

The effects of digoxin on conduction and automaticity differed between fetuses and ewes. There was a graded, continuous relationship between prolongation of the P-R interval and the steady state concentrations of digoxin in the plasma of fetuses. Prolongation of the P-R interval in ewes occurred in a more "all-or-none" fashion. Commonly, after concentrations of digoxin in plasma reached or exceeded 2 ng/ml, the prolongation appeared. This age-related difference in the effect of digoxin on AV conduction agrees with results of previous studies. In both fetuses and ewes, administration of atropine had little effect on the prolongation of the P-R interval produced by digoxin.

Fetuses attained higher concentrations of digoxin than ewes in both plasma and tissue but had fewer episodes of arrhythmia. No tachyarrhythmias were observed in the fetuses whose concentrations of digoxin in plasma ranged from 2.7 to 9.0 ng/ml. In contrast, three of six ewes developed tachyarrhythmias at concentrations of 2.1-3.0 ng/ml. Stimulation of the central autonomic nervous system has been implicated in the genesis of digoxin-induced tachyarrhythmias in mature animals. If this is so, then differences in conduction and in the incidence of tachyarrhythmias between fetuses and ewes could relate to one or more of the following differences: (1) the entry of digoxin into the central nervous system, (2) the central autonomic response to digoxin, (3) the transmission of centrally originated sympathetic stimuli to the myocardium, or (4) the myocardial response to sympathetic stimulation. Because we have found that entry of digoxin into the central nervous system is similar in fetuses and ewes (Table 4), differences in exposure of central autonomic centers to digoxin cannot explain possible differences in sympathetic discharge which might account for the age-related conduction and automaticity findings. Fetal and adult autonomic centers could, however, respond differently to identical concentrations of digoxin in critical areas of the brain. We infused isoproterenol to determine whether β-adrenergic stimulation in the fetus would produce an incidence of tachyarrhythmias comparable to that observed in the ewes. None of the fetuses developed arrhythmias after administration of isoproterenol in doses that increased heart rate. We conclude that age-related differences in the sensitivity of the myocardium to circulating catecholamines probably do not explain the age-related differences in the incidence of tachyarrhythmias we observed. Differences in either sympathetic innervation of the myocardium or its response to sympathetic stimulation could explain variations with age in the pattern of toxicity to digoxin. The latter postulate may reflect a developmental imbalance between parasympathetic and sympathetic cardiac innervation. Further study is needed to determine when these age-related differences in response to digoxin disappear and what non-neurological mechanisms may be involved.

Acknowledgments

We thank Roberto A. Marques, James J. Powers, Christine Roman, Louise Wong, Alan Nisen, and Les Williams for their expert technical assistance.

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THE MICROCIRCULATION is able to adapt the transport of solutes to the metabolic needs of the tissues. A primary vasomotor mechanism is a change in supply of metabolites by variation of total blood flow to the tissue. The control of this process resides at the arterioles. A second mechanism regulates the distribution of blood to the exchange area. This postarteriolar adaptation could result from (1) variation in the number of effectively perfused capillaries, (2) shift of blood between shunt (nonexchanging) and capillary (exchanging) circuits, or (3) a combination of 1 and 2. Previous work has not resolved the relative contributions of these postarteriolar mechanisms to the adaptive response to increased metabolic demands. Recently, Chien \cite{1} proposed a model of dual circulation that evaluates transcapillary exchange and the partition of microvascular flow into capillary and shunt flow. Chien's hypothesis was used in this study to assess the partition of microvascular flow in skeletal muscle and the adaptive microcirculatory response to muscle contraction and vasodilation.

Methods

PERFUSED MUSCLE PREPARATION

Experiments were performed on five mongrel dogs, anesthetized with sodium pentobarbital (30 mg/kg, supplemented as required). The right gracilis muscle was completely isolated vascularity by cannulating the gracilis artery and vein and tying off the collateral blood vessels located near the knee joint (present in only two dogs of this series). The muscle was left in situ and was perfused

### SUMMARY

The postarteriolar response of capillary transport and microvascular flow distribution to muscle contraction and to adenosine was measured by the indicator dilution technique in isolated dog gracilis muscles perfused with blood at controlled flows. A model of dual circulation was used to analyze the partition of microvascular flow. The extraction (E) of $^{125}$I-iodoantipyrine (IAp) served as an indicator of capillary flow whereas the capillary transport capacity coefficient ($PS C$) of $^{22}$Na was used to assess the changes in capillary surface area available for exchange. Muscle contraction produced by electrical stimulation of the motor nerve increased mean E-IAp from 0.94 ± 0.03 (SD) to 0.95 ± 0.01 and produced a 2.0- to 2.9-fold increase in $PS C$-Na. Intra-arterial adenosine produced results similar to those caused by muscle contraction. We conclude that (1) in resting muscle, most of the flow circulates through exchanging blood vessels and (2) in addition to the primary mechanisms of arteriolar vasodilatation, a substantial increase in the number of capillaries available for exchange of materials plays an important role in the adaptive response to increased metabolic demand.
Differential effects of digoxin at comparable concentrations in tissues of fetal and adult sheep.

W Berman, Jr, P J Ravenscroft, L B Sheiner, M A Heymann, K L Melmon and A M Rudolph

_Circ Res._ 1977;41:635-642
doi: 10.1161/01.RES.41.5.635

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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