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The Effects of Ouabain in Lambs with Depressed Myocardial Function

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Summary

The effects of ouabain infusion were tested in six lambs before and after depression of myocardial function by halothane anesthesia. Halothane reduced the left ventricular rate of pressure rise (dp/dt), stroke work, and stroke volume; the ratio of preejection period to left ventricular ejection time rose. Heart rate and systemic vascular resistance did not change. Before halothane, ouabain infusion did not alter the hemodynamic variables measured. After myocardial depression, ouabain infusion returned dp/dt, stroke work, stroke volume and the ratio of preejection period to left ventricular ejection time to control levels. Pacing studies showed a biphasic relationship between left ventricular dp/dt and heart rate. Maximal dp/dt occurred at a heart rate 42 beats/min higher than the resting rate.

These studies suggest resting myocardial performance in the healthy, newborn lamb is at near maximal level.

The tolerance to and physiologic effects of cardiac glycosides differ in newborn and adult sheep (1, 2). Studies have shown that age-related variations in the pharmacokinetics, metabolism, protein binding or tissue penetration of digoxin do not explain the biologic differences documented (3–7). The differences in drug effect may relate to developmental variation in the effects of cardiac glycosides on the myocardial sodium-potassium ATP-ase (Na-K ATP-ase) system. Recently, however, evidence has accumulated that the resting level of myocardial function in healthy newborn lambs is much higher than that in mature sheep (8–10). The high resting level of heart rate and myocardial function in the normal, nonfailing neonatal lamb heart may make inotropic stimulation with cardiac glycosides difficult to document.

We tested the effects of ouabain infusion on the hemodynamic function of newborn lambs. Drug effects were measured in undisturbed, chronically instrumented, awake animals; studies were repeated after myocardial depression by halothane anesthesia. We also studied the contribution of heart rate to resting myocardial function in newborn lambs by testing the effect of atrial pacing on the hemodynamic variables measured.

MATERIALS AND METHODS

ANIMAL PREPARATION

Studies were performed on six lambs ranging in age from 5–26 days and in weight from 3.8–12.5 kg. Animals were prepared surgically using 0.5% halothane anesthesia, administered by face mask, and pentobarbital sedation (5 mg/kg, IV). Polyvinyl catheters (0.050 inches ID; 0.090 inches OD) were inserted into the right atrium via the jugular vein and into the descending abdominal aorta via the femoral artery. A #5 French Millar catheter tip manometer was positioned in the left ventricle via the left carotid artery. Three electrocardiographic (ECG) electrodes were sewn to the chest wall. Catheters, wires and the Millar transducer cable were tunnelled subcutaneously to the left flank and encased in a mesh pouch, sewn to the skin. Animals were treated postoperatively with 50,000 units/kg/day of penicillin and 15 mg/kg/day of Kanamycin, given intramuscularly. Studies were performed 48 h or more after surgery.

RESTING STUDIES

Baseline measurements. Resting measurements of hemodynamic function were made while the lambs rested quietly, blindfolded in an open cage. Physiologic data were recorded on a Beckman R-611 direct writing recorder at paper speeds of 2.5, 10, 25, 100 and 200 mm/sec. Arterial pH, P_{CO_2}, P_{O_2} and hematocrit were measured on each study day. Aortic and right atrial pressures were recorded with Statham P23DB strain gauges. The ECG was recorded with an AC/DC coupler (Beckman 9806-A). Left ventricular pressure was recorded with the Millar catheter and used to generate its first derivative with respect to time (LV dp/dt) with a Beckman 9879 differentiating circuit, calibrated both internally and by application of an external triangular waveform. Heart rate was recorded from the left ventricular signal with a cardiotachometer (Beckman 9857). Systolic time intervals, preejection period (PEP) and left ventricular ejection time (LVET), were measured using the ECG, aortic and left ventricular tracings, as described previously (8). Cardiac output was measured using indocyanine green dye and an Electronics for Medicine DCCO-044 densitometer/output computer with internal standardization. Dye, 1.25mg, was injected into the right atrium; arterial blood was withdrawn sterilely at a rate of 38.2 ml/min for 20–30 sec to assure linearity of the downslope of the curve. Four output determinations were made on each study, the withdrawn blood reinfused after each determination and the results averaged. Systemic vascular resistance and stroke work were calculated from the appropriate measured variables.

Ouabain infusion. After baseline measurements had been made, lambs were given 50 μg/kg of ouabain, IV, and maintained on an infusion of 0.05 μg/kg/min of ouabain for 45 min. The loading and maintenance doses were modified from those used in adult dogs (11) in order to produce arrhythmias in the study animals. Subsequently, hemodynamic measurements were repeated.

HALOTHANE STUDY

Twenty-four or more h after resting studies, measurements were made again while animals were undisturbed in the open cage. Thereafter, halothane anesthesia (0.5–1%) was administered via face mask for 20 min before repeating hemodynamic measurements. Halothane anesthesia was continued during the administration of ouabain by the same technique used previously. Measurements were then repeated before discontinuation of halothane anesthesia and ouabain infusion.

PACING STUDIES

Twenty-four or more h after the halothane study, each of the six animals underwent tracheostomy and halothane anesthesia

Table 1. *Baseline hemodynamic data*

Animal (No.)	Age (days)	Weight (kg)	Hematocrit (%)	Potassium (meq·L^{-1})	Calcium (mg·dl^{-1})	Aortic mean pressure (mm Hg)	Right atrial mean pressure (mm Hg)	Heart rate (beats·min^{-1})	dp/dt (mm Hg·sec^{-1})	PEP/LVET	Cardiac output (ml·min^{-1}·kg^{-1})	Stroke volume (ml)	Stroke work (mm Hg·ml)	Systemic vascular resistance (mm Hg·min·kg·ml^{-1})
1	8	5.2	28	3.9	9.1	80	1	170	3000	0.312	387	11.8	944	0.204
2	10	4	31	4.0	8.9	79	2	135	4000	0.257	375	11.1	877	0.205
3	11	6.8	30	4.2	9.5	85	5	220	4400	0.282	444	13.7	1164	0.180
4	5	4.5	29	4.4	9.0	84	3	190	5400	0.355	406	9.6	806	0.200
5	26	13	34	4.0	9.6	85	4	185	3200	0.310	279	18.6	1666	0.290
6	18	9	32	4.1	9.1	88	5	195	3000	0.322	325	15	1320	0.255
$\bar{x}$	13	7.1	31	4.1	9.2	84	3	182	3833	0.306	369	13.3	1130	0.222
S.D.	7.7	3.4	2	0.2	0.3	3	2	28	958	0.034	59	3.2	325	0.041

Table 2. *Change in hemodynamic variables relative to baseline measurements after experimental intervention*

	Quabain infusion	Halothane anesthesia	Halothane and ouabain
dp/dt (mm Hg·sec^{-1})	3975 (3.8)[1]	2758 (−27.2)[2]	3817 (0.4)
Stroke volume (ml)	14.2 (5.4)	10.0(−25.6)[2]	13.6(1.0)
Stroke work (mm Hg·ml)	1181 (5.4)	766 (−30.1)[2]	1082 (−3.3)
PEP/LVET	0.280(−8.5)	0.411 (34.3)[2]	0.294(−3.9)
Heart rate (beats·min^{-1})	174(−4.4)	186 (1.6)	185 (1.6)
Systemic vascular resistance (mm Hg·min·kg·ml^{-1})	0.227 (3.3)	0.238 (7.5)	0.224 (0.9)

[1] Mean absolute value (mean % change from control).
[2] Differs from control, by analysis of variance, $P < 0.01$.

before right thoracotomy. Resting measurements were made before further manipulation. Next, pacing electrodes were sewn to the superior vena cava-right atrial junction and the right atrial lateral wall. The sinus node region was crushed with a hemostat to lower resting heart rate. The pacing electrodes were connected to a modified, electrically isolated pulse generator (Grass SD9) so that impulses of varying rate, 2 msec in duration, and 2–7 mA in amplitude could be delivered to the right atrium. Bipolar stimulation was delivered in such a way that the P wave morphology and vector on ECG resembled that during sinus rhythm. Hemodynamic variables were then measured before pacing at pacing rates from just above the spontaneous rate to 300 beats per min or when dp/dt and mean aortic pressure fell. Pacing was maintained for 5–10 sec at each rate, in increments of 10–20 beats·min^{-1} to the maximum rate, then in decrements of the same amount to the prepacing rate. For analysis, dp/dt values were normalized to the prepacing level and heart rates were normalized to the spontaneous, sinus rate before experimental intervention. Animals were killed by pentobarbital overdose at the conclusion of the study.

STATISTICS

The effects of ouabain infusion on resting myocardial function and on myocardial function depressed by halothane were tested by a two way analysis of variance (12). Identification of mean values differing from that of other groups was done by applying the standardized range test (13). The effects of pacing on the left ventricular dp/dt were examined by polynominal regression analysis.

RESULTS

Baseline hemodynamic data are displayed in Table 1. Values concur with normal values reported previously (8). The animals ranged in age from 5–26 days at the time of surgery; 4 of 6 animals were less than 2 wk old. The variation in heart rate, dp/dt, stroke volume and stroke work between animals was moderate; values for mean aortic and right atrial pressure, PEP/LVET, cardiac output per kg and systemic vascular resistance were more consistent.

The effects of experimental intervention on six variables reflective of myocardial function are shown in Table 2. Because of the moderate variation in baseline values of dp/dt, stroke volume and stroke work between animals, changes after intervention were examined by a two way analysis of variance.

Ouabain infusion caused no significant change in left ventricular dp/dt, stroke volume, stroke work, heart rate, systemic vascular resistance or the PEP/LVET ratio from baseline values. Although 5 of 6 animals showed some arrhythmia during drug infusion (ventricular premature contractions in 3, junctional premature contractions in 2), physiologic measurements were made during periods of stable sinus rhythm.

Hemodynamic data measured prior to halothane anesthesia did not differ from measurements defined as baseline. Halothane anesthesia depressed myocardial function, as evidenced by significant reductions in left ventricular dp/dt, stroke volume and stroke work, and caused a significant elevation in the PEP/LVET ratio. Halothane anesthesia caused no consistent change in heart rate or systemic vascular resistance, as reported previously (14, 15). Ouabain administration during continued halothane anesthesia improved myocardial function. The values of left ventricular dp/dt, stroke volume, stroke work, and the PEP/LVET ratio are not different at rest, during ouabain infusion and during ouabain infusion with concommitant halothane anesthesia.

Heart rate affected left ventricular dp/dt. Before pacing, resting heart rate averaged 128 ± 9 beats·min^{-1} and dp/dt averaged 2705 ± 762 mm Hg·sec^{-1}. Figure 1 relates % changes in dp/dt to changes in heart rate induced by electrical pacing. The dp/dt value at the spontaneous resting heart rate prior to insertion of pacing electrodes was taken as the normalizing value. Sequential increments in heart rate raised dp/dt to a mean maximum value of 3377 ± 846 mm Hg·sec^{-1}, after which the dp/dt and aortic pressure fell. At the preintervention sinus rate of 180 beats·min^{-1}, dp/dt averaged 3109 ± 721 mm Hg·sec^{-1}. The peak left ventricular dp/dt occurred at pacing rates averaging 42 beats/min higher than the spontaneous sinus rate. Figure 2 depicts a sequential pacing study in wich the relationship of left ventricular dp/dt to heart rate can be seen well.

DISCUSSION

Immature subjects tolerate digoxin better than do mature ones. Several studies suggest the physiologic effects of digoxin also vary with age. Carefully controlled studies in fetal and newborn lambs, relating serum and myocardial concentrations of digoxin to its effect on myocardial function, have shown less inotropic effect in the younger animals than in ewes (1, 2).

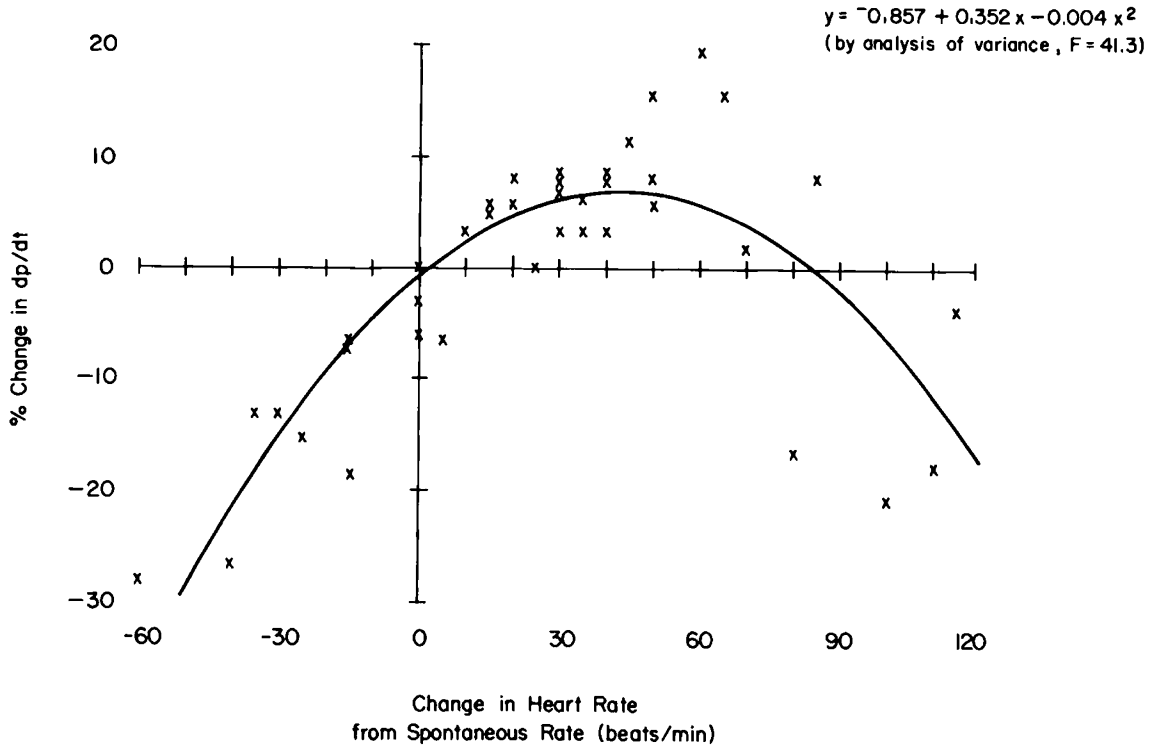

Fig. 1. The relationship of left ventricular dp/dt to the change in heart rate induced by sinus node destruction and subsequent atrial pacing.

Other studies in infants and children have examined the physiologic response to digoxin as assessed by systolic time intervals and echocardiographic determinations of cardiac chamber size and function.

Park *et al.* (16) studied the effects of digoxin on systolic time intervals in infants with congestive heart failure due to a variety of congenital cardiac anomalies. Four of 6 patients with ventricular septal defect, 0 of 3 patients with patent ductus arteriosus, and 2 of 3 patients with primary myocardial disease responded to therapy; response occurred only in patients with abnormal time intervals before therapy. Baylen, *et al.* (17) showed no consistent change in the left ventricular dimensions of sick, premature infants with patent ductus arteriosus who underwent medical management for congestive heart failure. Three studies have documented the effects of digoxin on echocardiographically determined systolic time intervals of infants undergoing digoxin therapy for congestive heart failure. Pinsky, *et al.* (18) studied two groups of premature infants with mean serum digoxin concentrations of 1.7 and 3.5 ng/ml, respectively. No difference in the extent of change of systolic time intervals was found. Each group showed a reduction of about 10% in left ventricular ejection time and 20% in preejection period after therapy. Although raw data were not quoted, the PEP/LVET ratio can be estimated to have changed from 0.333 pretherapy to 0.296 posttherapy. Sandor, *et al.* (19) assessed left ventricular function in 18 neonates with a variety of congenital cardiac anomalies; one group had a mean digoxin concentration of 2.0 ng/ml after therapy, the other a concentration of 3.6 ng/ml. Mean changes in systolic time intervals were small and indistinguishable between the two groups. The change in the PEP/LVET ratio was largest in those few infants with a higher than normal ratio before therapy. The assessment of digoxin effect was complicated by the concommitant administration of other decongestive therapy, especially furosemide. Hofstetter, *et al.* (20) studied left ventricular systolic time intervals, shortening fraction of the left ventricular minor axis (SF) and velocity of circumferential fiber shortening (Vcf) in 12 neonates and infants before and after full digitalizing doses. Mean SF and Vcf were normal before therapy in both neonates and infants; SF and Vcf increased approximately 15% in neonates (3–13 days of age) and 30% in infants (1–10 months of age) after therapy. The PEP/LVET ratio was 0.283 before therapy and 0.303 after therapy in the neonates; corresponding values were 0.332 and 0.306 in the infants.

These studies in infants and children show baseline estimates of contractile cardiac function, even in the presence of congestive symptoms, are often normal. The responses to digoxin are variable, small, and do not show a dose-response relationship. These findings are in contrast to several studies examining the response of left ventricular function to digoxin therapy in adults (21–23). Although differences in the physiologic response to digoxin may reflect developmental changes in the interaction of digoxin with its membrane receptor (24), there is little evidence to substantiate age-related differences in the binding to, dissociation from or inhibiting effect of digoxin on the myocardial Na-K ATP-ase system (25–29).

Several studies have shown substantial differences in both the preejection phase and ejection phase levels of cardiac function in young and old subjects. Studies in sheep have established substantially higher levels of cardiac output·kg^{-1} (8–10), dp/dt (8) and heart rate (8–10) in newborn lambs relative to ewes; PEP/LVET does not vary with age (1, 8). Assessments of myocardial function in normal lambs and in infants with structural cardiac anomalies and congestive heart failure, but normal left ventricular pump function, have led to speculation that the limited response to cardiac glycosides in some patients may reflect the high resting level of cardiac function in the subjects studied (30).

These experiments address the effect of the resting level of cardiac function on the extent of physiologic response to cardiac glycosides. Ouabain infusion, at doses established to achieve therapeutic serum concentrations and high enough to precipitate cardiac arrhythmias, produced no measurable effect on hemodynamic function in healthy, resting lambs. Halothane, an established myocardial depressant which does not provoke consistent changes in heart rate or systemic vascular resistance (31–33), depressed hemodynamic performance significantly. When ouabain was administered to the lambs with depressed myocardial performance, functional variables improved significantly to levels not different from those at rest. These results are consistent with previous studies on the effects of cardiac glycosides in healthy newborn and fetal sheep (1, 2). Because of the high cardiac output demand after birth (8), intrinsic neural and hormonal inotropic

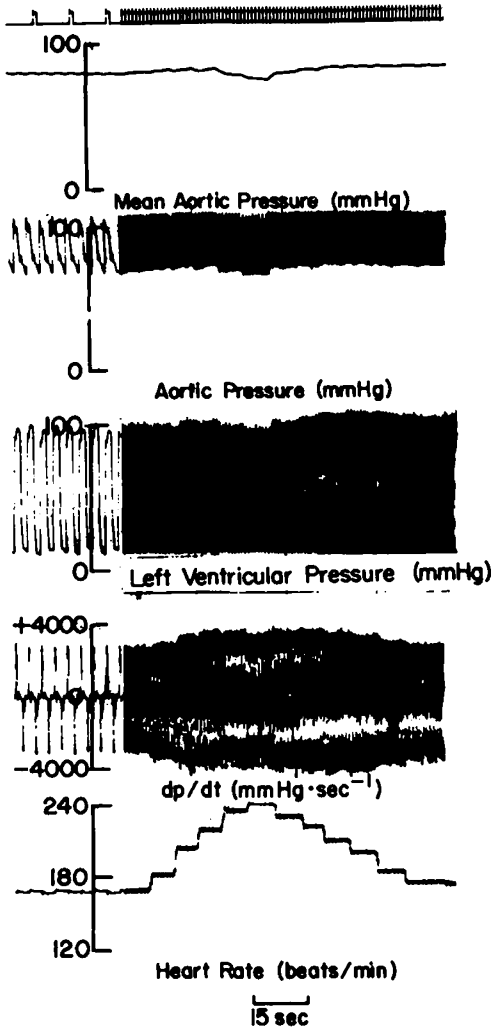

Fig. 2. Pacing study showing the effect of changing the paced atrial rate on aortic blood pressure, left ventricular pressure and left ventricular dp/dt.

mechanisms may stimulate myocardial function to the point where the effects of exogenously administered inotropic agents may be masked. This study was designed to test that possibility by depressing myocardial contractility before glycoside administration. Halothane was chosen because of its ability to depress contractility without affecting heart rate or loading conditions consistently. Although the inotropic effect of ouabain could not be shown in healthy lambs, contractility was improved by ouabain after myocardial depression by halothane. This pattern emphasizes the importance of considering the baseline contractile state in the evaluation of any inotropic intervention.

Studies designed to examine the relationship of dp/dt to heart rate were performed in anesthetized animals with open chests. Before pacing, spontaneous heart rate and dp/dt were lower than in awake animals. The reduction in left ventricular dp/dt was due, in part, to the reduced rate and, in part, to the experimental preparation. The maximum dp/dt achievable with pacing was less than the control dp/dt at sinus rates. The relationship of rate to dp/dt, therefore, was examined by normalizing the dp/dt value to the prepacing value and normalizing the heart rate to the spontaneous sinus rate in the control, intact animal. Left ventricular dp/dt varied biphasically with cardiac rate; dp/dt fell at heart rates below the spontaneous rate, rose to a peak at a heart rate averaging 42 beats/min more than the spontaneous rate and then fell at higher rates. Although these pacing studies in anesthetized animals with open chests may not be applicable to the intact subject, they suggest that left ventricular dp/dt in the newborn lamb at rest may be very close to its maximum rate-related level.

These studies do not pursue possible age-related variation in the mechanism of action of cardiac glycosides. They do show that a part of the apparent resistance of some subjects to the physiologic effects of glycosides results from the high baseline level of heart rate and myocardial function which exist after birth. Because a large percentage of neonates and infants with congestive cardiac failure due to anatomic deformity have normal left ventricular function, as assessed by echocardiography and angiography, the potential for benefit to these patients by inotropic stimulation with digoxin may be limited.

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