EXPERIMENTAL STUDIES

Rapid Ventricular Pacing in Dogs With Right Ventricular Outflow Tract Obstruction: Insights Into a Mechanism of Sudden Death in Postoperative Tetralogy of Fallot

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Objectives. We explored the hypothesis that residual outflow tract obstruction and ventricular hyper trophy associated with rapid ventricular rhythm contribute to sudden death, in part because they result in humoral or hemodynamic changes that predispose to ventricular fibrillation, such as increased catecholamine release or decreased coronary flow, or both.

Background. Ventricular arrhythmia after surgical repair of tetralogy of Fallot has been associated with sudden death, particularly in patients with residual right ventricular hypertension. However, the mechanisms by which sudden death occurs remain unclear.

Methods. Seven awake, unanesthetized mature beagles with chronically elevated right ventricular pressure (high pressure group; right ventricular/left ventricular systolic pressure ratio >0.5) were compared with six beagles with low right ventricular pressure at rest and at the end of 5 min of ventricular pacing at 240 beats/min (low pressure group).

Results. In the high pressure group, cardiac output decreased during ventricular pacing (compared with sinus rhythm) from 304 ± 21 to 218 ± 21 ml/min per kg (p < 0.01) and plasma norepinephrine increased substantially from 673 ± 64 to 1,047 ± 92 pg/ml (p < 0.01). Comparable changes were not observed in the low pressure group. Plasma epinephrine levels were similar in both groups at rest and did not change with pacing. Postpacing norepinephrine levels from both groups correlated positively with both right ventricular systolic and diastolic pressure at rest and correlated negatively with the change in cardiac output from rest to pacing. Regional right ventricular myocardial blood flow increased with pacing in the low pressure group, whereas in the high pressure group it was increased at rest and did not increase further with pacing.

Conclusion. During ventricular pacing, dogs with right ventricular outflow tract obstruction and high right ventricular pressure had a decrease in cardiac output and an increase in plasma norepinephrine, coupled with a loss of right ventricular myocardial blood flow reserve. Similar changes may occur in postoperative patients with similar hemodynamics and tachyarrhythmia and could contribute to the occurrence of ventricular fibrillation and sudden death.

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Ventricular arrhythmia occurs in a high proportion of patients who have undergone surgical repair of tetralogy of Fallot (1-4), and in some of these patients has been associated with sudden death (5-9). Clinical evidence (10-12) suggests that the incidence of sudden death is increased in those patients who have residual abnormal right ventricular hemodynamics—specifically, elevated right ventricular systolic or end-diastolic pressure—but the reason for this increased incidence is unclear. Possible mechanisms of sudden death include humoral or hemodynamic factors favoring degeneration of ventricular tachycardia to ventricular fibrillation, such as increased catecholamine release or decreased coronary flow predisposing to ischemia, or both. Similar factors have been shown to predispose both experimental animal models (13) and patients with ischemic coronary artery disease (14-16) to ventricular fibrillation and sudden death.

Therefore, in this investigation we evaluated the humoral and hemodynamic consequences of a brief episode of rapid ventricular pacing in a canine model of chronic right ventricular pressure overload. Right and left ventricular hemodynamics, cardiac output, regional myocardial blood flow and plasma catecholamines were assessed at rest and at the end of rapid ventricular pacing in dogs 6 months after the placement of a pulmonary artery band, and results were compared with those obtained from age-matched control dogs.

Methods

Pulmonary artery band surgery. Ten 8-week old (7.5 to 4 kg) male or female purebred beagle puppies were used in
this procedure. A venous catheter was placed in the foreleg and anesthesia was induced with pentobarbital sodium (15 to 20 mg/kg intravenously) and maintained with additional pentobarbital as necessary. The dog was intubated and placed right side down on a heating pad to maintain rectal temperature at 37°C. Ventilation was maintained with a Harvard Apparatus small animal respirator. Blood gas determinations were performed intermittently and adjustments were made in ventilation to keep values within normal limits. Using sterile technique, a left thoracotomy was performed through the fourth intercostal space and the pericardium was opened to expose the base of the heart. The pulmonary artery trunk was dissected and umbilical tape was sutured in place around the main pulmonary artery. The band was made as tight as possible without decreasing systemic blood pressure as measured by an indwelling femoral artery catheter. The pericardium and chest were closed and the dog was placed in a heated cage to recover. Subsequently, all dogs were allowed to grow normally.

Instrumentation surgery. Six to 8 months after banding, the original 10 beagles, now weighing 8 to 11 kg; and 4 age- and weight-matched control dogs underwent surgery for instrumentation. Venous access was again obtained in the foreleg and anesthesia was induced with thiopental sodium (20 mg/kg intravenously). Each dog was intubated and mechanically ventilated. General anesthesia was maintained with 1% halothane. A left thoracotomy was performed and the pericardium opened. For long-term monitoring, the following were implanted. Polyvinyl catheters were placed in the left atrium, the aortic arch through the internal thoracic artery, the right atrium and the right ventricle. Two pairs of multistranded stainless steel wire (no. 5633 hookup wire, Cooner Wire Co.) with a loop formed at the end were sewn to the right ventricular epicardium with 6-0 synthetic nonabsorbable suture to be used for ventricular pacing. A 2.5F thermistor (Thermodilution Probe, American Edwards Laboratories) was placed in the main pulmonary artery to measure cardiac output. After instrumentation, all wires and cannulas were tunneled subcutaneously and exteriorized at the left lateral thorax. The pericardium remained open. The thoracotomy incision was closed in layers, and the dogs were allowed to recover as before. Meperidine hydrochloride (1 mg/kg per dose) was administered intramuscularly for analgesia, as necessary. Each animal received methicillin (500 mg), penicillin G (200,000 U) and streptomycin (250 mg) intramuscularly during the operation and once daily on each of the next 3 postoperative days. Beginning on the 1st postoperative day, each catheter was aspirated and injected with 0.4 ml of heparin solution (10,000 U/ml) to assure patency. The exteriorized wires and cannulas were protected by a nylon mesh vest. Animals were allowed to recover for 6 to 10 days before study.

Study protocol. Each animal, awake and unsedated, was placed in a nylon mesh animal support sling and allowed to acclimate to the surrounding conditions for 30 to 60 min. Polyvinyl catheters were attached to pressure transducers (Statham P23ID) with the zero set at midchest level. Adhesive electrocardiographic (ECG) leads were attached to each limb. Right and left atrial pressure, right ventricular pressure, aortic pressure and ECG lead II were recorded on a Gould no. 2800S physiologic recorder. Cardiac output was measured using right atrial injection of iced saline solution (0 to 3°C) and main pulmonary artery sampling. Injunctate temperature was measured directly. Cardiac output measurements were made with an American Edwards Laboratories cardiac output computer (model COM1). The initial value was discarded because of possible thermal loss to the catheter and the subsequent three cardiac output values were averaged, corrected for body weight and expressed in ml/kg per min. If any of the three measurements differed by >10% from their mean value, the process was repeated. Subsequently, regional myocardial blood flow was measured using radioactive microspheres. Finally, venous whole blood samples from the right atrium were collected into ethylene glycol tetraacetic acid (EGTA)-glutathione and heparin and immediately placed on ice for later determination of plasma catecholamine levels.

After values at rest were obtained, each dog underwent right ventricular pacing at 240 beats/min with ventricular capture confirmed on the surface ECG and blood pressure tracings; 240 beats/min was chosen as a rate approximately twice the sinus rate. Beginning precisely after a 5-min pacing interval but with continued pacing of the dogs, each measurement was repeated in the sequence just described. Finally, just before the termination of pacing, a second venous blood sample for plasma catecholamine levels was obtained and placed on ice. Both the rest and ventricular pacing venous blood samples were subsequently spun for 20 min at 4°C at 2,800 rpm to separate the cellular and plasma components. Plasma samples were stored at −80°C until analysis.

Calculation of regional myocardial blood flow. Regional myocardial blood flow was determined with radio-labeled microspheres (10- to 15-μm diameter, 3M), using the reference withdrawal method as previously described (17). Injections of microspheres labeled with either strontium-85, niobium-95 or scandium-46 were given in random order in each experiment at rest and again at the end of ventricular pacing. Reference arterial blood samples were withdrawn from the descending thoracic aorta at a constant rate with a withdrawal pump (Harvard Apparatus), beginning immediately before the injection of microspheres (given into the left atrium) and ending 90 s later. Each bottle of microspheres was placed in an ultrasonic bath for vortex agitation immediately before injection to ensure adequate dispersal of the microsphere suspension. Regional myocardial blood flow was calculated using the formula:

\[ Q_{\text{ref}} = Q_{\text{obs}} 	imes C_{\text{obs}}/C_{\text{ref}} \]

where \( Q_{\text{obs}} \) is blood flow to the myocardial tissue (ml/min), \( Q_{\text{ref}} \) is reference blood flow (ml/min), \( C_{\text{obs}} \) is counts/min in the tissue sample and \( C_{\text{ref}} \) is counts/min in the reference
blood sample (17). Blood flow to myocardial tissue (Q\text{ myocardium}) was divided by the appropriate sample weight and regional blood flow was expressed as ml/min per g. Data were obtained for both the left and right ventricles and were subdivided into subendocardial, midmyocardial and subepicardial regions.

**Determination of plasma norepinephrine and epinephrine levels.** These levels were determined by high performance liquid chromatography with electrochemical detection as previously described for human plasma (18).

**Statistical methods.** The data were tabulated and expressed as the mean ± SEM. Within-group comparisons (two-sample) were made using the Student paired t test and between-group comparisons were made using nonpaired t tests. Linear regression analysis was used to compare individual values of postpacing plasma norepinephrine levels with right ventricular systolic and diastolic pressures and with the change in cardiac output from rest to pacing.

**Approval.** This protocol was reviewed and approved by the Baylor College of Medicine and the Texas Heart Institute Animal Care and Use Committees. These studies conformed to the "Position of the American Heart Association on Research Animal Use" adopted by the Association on November 11, 1984.

**Results**

**Hemodynamic data (Table 1).** Dogs were placed in the group with high right ventricular pressure if the right ventricular systolic pressure at rest was ≥50% of the left ventricular systolic pressure. Seven dogs from the banded pulmonary artery group met this criterion (high pressure group). The control (low pressure) group consisted of six dogs (the four nonbanded control dogs and two additional dogs with ineffective bands). One dog from the banded pulmonary artery group was excluded at the time of study because of fever, lethargy and peripheral edema suggestive of systemic infection.

Heart rates at rest were similar for dogs with low or high right ventricular pressure (Table 1). Ventricular pacing was performed in the same fashion for all animals in both groups. In the low pressure group, right ventricular systolic pressure was 57 ± 5 mm Hg and did not change significantly from rest to ventricular pacing; in the high pressure group, it was 92 ± 9 mm Hg at rest and decreased significantly to 65 ± 2 mm Hg during ventricular pacing. In fact, in all animals in the experimental group, the pressure tracing during ventricular pacing demonstrated an alternans pattern, and the pressure depicted in Table 1 represents an average pressure over 10 beats (Fig. 1). Right ventricular end-diastolic pressure at rest was also higher in the high pressure group than in the control group (10.7 ± 1.3 vs. 5 ± 0.6 mm Hg, p < 0.01). During ventricular pacing, right ventricular end-diastolic pressure decreased in the low pressure group but tended to increase in the high pressure group. Although the changes from rest to pacing were not significant in either group, a marked differ-

Figure 1. Representative tracings from a single dog in the low right ventricular pressure (Control) group and from a single animal in the group with high right ventricular pressure (PA Band) at rest and at the end of 5 min of ventricular (V) pacing. Ao = aortic pressure; ECG = electrocardiographic lead II; LV = left ventricular pressure; RV = right ventricular pressure.
ence in right ventricular end-diastolic pressure between groups was appreciated during ventricular pacing (14 ± 2.2 vs. 3.5 ± 1.5 mm Hg, p < 0.01). Aortic systolic pressure, aortic mean pressure and left atrial mean pressure did not vary significantly within either group or between the groups at rest or at the end of ventricular pacing. In the high pressure group, however, a small transient decrease in aortic pressure was noted with the initiation of pacing, but recovered by the end of the pacing interval when our measurements were made.

Cardiac output. Cardiac output at rest was similar in both groups (304 ± 21 and 312 ± 23 ml/min per kg, respectively, in dogs with high or low right ventricular pressure) and cardiac output in the control group did not vary significantly from rest to ventricular pacing. However, in the high pressure group, cardiac output was decreased at the end of ventricular pacing by an average of 28% (from 304 ± 21 to 218 ± 21 ml/min per kg).

Plasma norepinephrine levels. Figure 2 demonstrates plasma norepinephrine and epinephrine concentrations measured at rest and after ventricular pacing in both the control dogs and the dogs with high right ventricular pressure. Plasma norepinephrine levels in the dogs with high right ventricular pressure (Fig. 2A), increased significantly during ventricular pacing compared with their paired values at rest. Furthermore, this change in norepinephrine from rest to pacing in the high right ventricular pressure group was significantly different from that in the control group (that is, the ventricular pacing norepinephrine level in the experimental group was significantly higher than that in the control group). Rest levels, however, were not significantly different.

In the 10 dogs from both groups for which cardiac output data were available, linear regression analysis was performed, comparing individual plasma norepinephrine levels obtained during ventricular pacing with the percent change in cardiac output from rest to ventricular pacing. As Figure 3 demonstrates, the greater the decline in cardiac output during ventricular pacing, the higher the plasma norepinephrine value (r = −0.73, p < 0.02).

Linear regression analysis was also performed comparing ventricular pacing norepinephrine levels with right ventricular systolic pressure at rest (r = 0.63, p = 0.02) and with right ventricular end-diastolic pressure at rest (r = 0.79, p < 0.01) (Fig. 4).

Plasma epinephrine levels. Plasma epinephrine did not vary significantly from rest to ventricular pacing within either the control group or the dogs with high right ventricular pressure (Fig. 2B). In the high pressure group, both rest and pacing levels of plasma epinephrine tended to be higher than in the control group, but this difference was not statistically significant.

Regional myocardial blood flow. Regional myocardial blood flow was measured in the right and left ventricles in tissue segments divided transmurally to include the subendocardial, midmyocardial and subepicardial regions. As a result of injection or sampling difficulties, data were available from four of six control dogs and five of seven dogs from the group with high right ventricular pressure. In the control animals, right ventricular subendocardial blood flow at rest averaged 1.18 ± 0.03 ml/min per g. Comparable values were obtained in the midmyocardial and subepicardial regions. Blood flow subsequently increased in all three regions during ventricular pacing. In the high right ventricular pressure group, however, myocardial blood flow at rest was elevated compared with that in control dogs (subendocardial flow 1.66 ± 0.28 ml/min per g) and did not increase with ventricular pacing. The percent change in myocardial blood flow from rest to ventricular pacing was comparable for each of the transmural regions in the control group (Fig. 5). In addition, the failure to increase blood flow with pacing was evident in all three regions of the myocardium in the dogs with high right ventricular pressure. However, because of a
greater degree of variability in the midmyocardial and subepicardial regions, the difference between the two groups for the change in blood flow from rest to pacing was statistically significant in the subendocardial region only. In contrast, myocardial blood flow in the left ventricle at rest was similar in both groups and blood flow increased with ventricular pacing across all regions of the ventricular wall in both groups (data not shown).

**Discussion**

The data from this study clearly demonstrate that dogs with abnormal right ventricular hemodynamics (a chronically elevated right ventricular systolic and end-diastolic pressure) respond differently to rapid ventricular pacing than do control dogs. During rapid ventricular pacing, the dogs with high right ventricular pressure demonstrated a decrease in cardiac output, no increase in right ventricular myocardial blood flow and a significant elevation in circulating plasma norepinephrine levels. Furthermore, plasma norepinephrine levels correlated closely with the change in cardiac output and the right ventricular systolic and end-diastolic pressures.

**Decreased cardiac output.** There are several possible explanations for the decrease in cardiac output during pacing in the dogs with high right ventricular pressure. The tendency for an already elevated right ventricular end-diastolic pressure to increase and for left ventricular end-diastolic pressure to decrease implies impaired right ventricular emptying as one potential mechanism. This impaired emptying could simply be caused by the mechanical obstruction to increased flow through a small fixed orifice in the pulmonary artery. Alternatively, right ventricular hypertrophy is likely to have caused a decrease in right ventricular compliance, which may have resulted in poor diastolic filling associated with the rapid heart rate. Even right ventricular systolic dysfunction might serve as an additional explanation in this setting. Previous studies from other laboratories (19,20) have associated right ventricular pressure overload with decreased intrinsic contractile function of right ventricular myocardium.

Other potential mechanisms that could contribute to the reduction in cardiac output include a change in left ventricular compliance due to septal hypertrophy (as a consequence of right ventricular hypertrophy) (21,22), as well as the development of right ventricular ischemia (23).

**Decreased myocardial blood flow reserve.** Previous studies (24) have shown that myocardial oxygen consumption increases with tachycardia or pacing, and in normal hearts a concomitant increase in regional myocardial blood flow occurs to meet the increased oxygen demand (25-27). In the present study, both in the control dogs with low right ventricular pressure and in the dogs with high right ventricular pressure, left ventricular myocardial blood flow in-
creased with pacing. Similarly, right ventricular regional myocardial blood flow increased by 40% to 55% during pacing in the control group, but in the high-pressure group, it was increased at rest and did not increase further with pacing. The observation that rest right ventricular myocardial blood flow per gram was increased in this group is consistent with previous reports (28–32) of both chronic left and chronic right ventricular hypertrophy.

In other studies of animals with chronic left ventricular hypertrophy, transmural flow to the myocardium during rapid pacing or during exercise has remained constant or, alternatively, has increased, depending on the study. Consistently, however, there has been a transmural redistribution of perfusion away from the subendocardial region. This decrease in the endocardial/epicardial flow ratio has been used as an indicator of diminished myocardial blood flow reserve under stress and to suggest that the subendocardial region is predisposed to ischemia under these conditions (27–30). Similarly, these criteria have been applied to studies of the hypotrophic right ventricle. Manohar (33) demonstrated a decrease in the endocardial/epicardial flow ratio in young swine with chronic right ventricular hypertrophy during ventricular pacing at 275 beats/min, and Murray and Vatner (32) found both a decreased vasodilator capacity and a decreased endocardial/epicardial flow ratio with adenosine administration in dogs with chronic right ventricular hypertrophy.

Similar results were not obtained in our study. In the dogs with high right ventricular pressure, we did not measure a redistribution of myocardial blood flow away from the subendocardium during pacing. Rather, transmural blood flow to the right ventricle did not increase in this group at a time of increased oxygen demand. Although the observed difference in blood flow from rest to pacing relative to the control group proved to be significant only in the subendocardial region, our data raise the possibility that with pacing, either subendocardial or transmural ischemia could develop in the right ventricle in the group with high right ventricular pressure.

It is not clear why our study found no redistribution of flow from the subendocardium to subepicardium with pacing in our study dogs with high right ventricular pressure. One possible explanation is that accurate measurement of subepicardial flow may not have been possible in this group because of the degree of epicardial fibrosis present, resulting in spuriously low values for subepicardial blood flow both at rest and with pacing.

Increased norepinephrine. Samples for plasma catecholamine determination were obtained from the right atrium and thus reflect changes in whole body catecholamine release rather than changes in catecholamine release across the myocardium. In this study, postpacing plasma norepinephrine levels correlated closely with the change in cardiac output from rest to pacing. It is likely that the observed increase in plasma norepinephrine measured at the end of ventricular pacing in the dogs with high right ventricular pressure was due to increased sympathetic tone in response to the decrease in cardiac output. The observation that systemic blood pressure transiently dipped downward with the initiation of pacing but recovered at 5 min of pacing when our hemodynamic measurements were made, even in the presence of decreased cardiac output, supports the view that systemic vascular resistance increased in response to an increase in alpha-adrenergic tone. The fact that norepinephrine increased and epinephrine did not is perplexing, unless one considers that a phased response of the two catecholamines may exist (that is, norepinephrine responds first to relatively mild changes in perfusion and epinephrine responds only when larger changes in pressure and perfusion are encountered). To date, however, such a phased response has not been reported by other investigators.

As for the potential consequences of increased norepinephrine release, previous electrophysiologic studies (34,35) suggest that norepinephrine may shorten the refractory period of ventricular muscle fibers and catecholamine-enhanced pacemaker activity may initiate diastolic depolarization in Purkinje fibers that do not normally show pacemaker activity. Nonuniform distribution of sympathetic activity that might occur in the right ventricle as a consequence of ischemia could result in a nonuniform alteration in refractoriness and thus predispose to ventricular fibrillation (35). Even in the absence of ischemia, catecholamine release has been associated with the progression of ventricular tachycardia to ventricular fibrillation (36).

Limitations of the canine model. The canine model employed in this study was limited in that it evaluated a single aspect of postoperative tetralogy of Fallot that might be associated with sudden death (namely, residual right ventricular hypertension). Not addressed by this model were other conditions that might contribute to the incidence of ventricular arrhythmia and fibrillation in patients after surgical treatment of tetralogy of Fallot, including scarring from infundibular resection and ventriculotomy (37), the effect of long-standing hypertension on scar composition and the effects of long-standing myocardial fibrosis (38). The absence of these other predisposing conditions in our animal model may explain why we did not see spontaneous ectopic beats at rest and did not induce ventricular tachycardia with our pacing protocol.

Conclusions. Nevertheless, our results do suggest mechanisms that might contribute to the development of a fatal arrhythmia in the postoperative patient with congenital heart disease. The dogs in our study with elevated right ventricular pressure demonstrated high circulating norepinephrine levels and the potential for right ventricular transmural ischemia associated with rapid ventricular pacing. Tachyarrhythmias in patients after surgical treatment of tetralogy of Fallot who have residual right ventricular hypertension, in addition to ventriculotomy, scarring and fibrosis, could create the physiologic substrate for a reduced ventricular fibrillation threshold by mechanisms similar to those de-
scribed in this study. Further studies are warranted to explore this possibility.

References