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Vagal control of cardiac electrical activity and wall motion during ventricular fibrillation in large animals

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ABSTRACT

Vagal inputs control pacemaking and conduction systems in the heart. Anatomical evidence suggests a direct ventricular action, but functional evidence that separates direct and indirect (via the conduction system) vagal actions is less well established. We studied vagus nerve stimulation (VNS) during sinus rhythm and ventricular fibrillation (VF) in pigs and sheep to determine: 1) the range of unilateral and bilateral actions (inotropic and chronotropic) and 2) whether VNS alters left ventricular motion and/or electrical activity during VF, a model of abnormal electrical conduction of the left ventricle that excludes sinus and atrioventricular nodal function. Adult pigs (N = 8) and sheep (N = 10) were anesthetized with urethane and mechanically ventilated. VNS was performed in animals at 1, 2, 5, 10, 20, 50, and 100 Hz for 20 s. VF was induced with direct current to the ventricles or occlusion of the left anterior descending coronary artery. In 4 pigs and 3 sheep, left ventricular wall motion was assessed from endocardial excursion in epicardial echocardiography. In sheep and pigs, the best frequency among those tested for VNS during sinus rhythm to produce sustained electrical and mechanical ventricular standstill was 50 Hz for unilateral or bilateral stimulation. When applied during VF, bilateral VNS increased the variability of the dominant VF frequency, indicating a direct impact on the excitability of ventricular myocytes, and decreased endocardial excursion by more than 50% during VF. We conclude that the vagus nerve directly modulates left ventricular function independently from its effects on the conduction system.

1. Introduction

The vagus nerve can exert powerful cardioprotection for the heart. In addition to its well-established control of sinoatrial and atrioventricular nodal functions (Martin, 1977; Ardell and Randall, 1986; Neely and Urthaler, 1992), vagus nerve stimulation (VNS) can prevent ventricular fibrillation (VF) in myocardial ischemia (Myers et al., 1974) or during electrical stimulation (Kolman et al., 1975; Yoon et al., 1977; Brack et al., 2011), and vagotomy or atropine decreases the time to VF induced by myocardial ischemia (Corr and Gillis, 1974). VNS modulates VF severity by lowering defibrillation thresholds (Murakawa et al., 2003), exerting an effect on the electrical activity of the myocardium (Nazeri et al., 2011). It has even been shown to terminate VF in small animals, like rats (Naggar et al., 2012), though its control over ventricular arrhythmias appears weaker in large animals by comparison (Garrey, 1908).

These findings suggest meaningful vagal control of the ventricles (Brack et al., 2012). A growing number of anatomical and physiological studies show that the vagus nerve exhibits a direct effect on the left and right ventricles that is independent of its effect on the sinus and atrioventricular (AV) nodes. Histologic evidence in large animals such as cats (Johnson et al., 2004), dogs (Pauza et al., 2002), pigs (Crick et al., 1999a; Ulphani et al., 2010), sheep (Saburkina et al., 2010), and even humans (Pauza et al., 2000; Kawano et al., 2003) show diffuse vagal innervation of the ventricles. These studies largely use acetylcholinesterase staining techniques to demonstrate potential innervation, as acetylcholine is one of the known neurotransmitters involved in vagal action on the heart, the other being nitric oxide (Brack et al., 2009). In addition, vesicular acetylcholine transporter has been localized to areas including the ventricles in rats (Schafer et al., 1998). Muscarinic receptors have been localized to the ventricles of humans in studies searching for muscarinic cholinergic receptor mRNA (Hellgren et al., 2000; Wang et al., 2001; Zang et al., 2005a). Others have used immunohistochemical techniques for protein gene product 9.5 (PGP 9.5), a neuronal marker, to trace nerve fibers to the ventricles in humans (Crick et al., 1994) and pigs (Crick et al., 1999b).

Functional studies of vagal input to the heart have yielded additional evidence. Vagus nerve stimulation prolongs the effective refractory

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period of ventricular myocytes in intact dog hearts (Kolman et al., 1976) and in dog hearts after the sinus node was crushed (Martins and Zipes, 1980). In one study on guinea-pig ventricles, it was shown that acetylcholine decreased action potential duration and force of contraction in dissociated myocytes (Zang et al., 2005b). Left ventricular contractility, furthermore, is decreased in pigs and humans upon left VNS in vivo as measured by ventricular pressures (Lewis et al., 2001). Growing interest in the use of VNS in the treatment of heart failure (e.g., Klein and Ferrari, 2010) and prevention of arrhythmias (e.g., Zhang and Mazgalev, 2011) has been informed by these studies; however, sidedness and stimulation frequency remain unexplored.

We sought to study the effects of vagus nerve stimulation on ventricular function in large animals when the cardiac conduction system (including the conductive nodes and Purkinje fibers) was disrupted by testing vagal activity during VF. We aimed to find 1) the range of unilateral and bilateral vagal actions on the heart, 2) the vagal impact on individual ventricular walls, and 3) the effects of VNS during ventricular fibrillation.

2. Materials and methods

2.1. Surgery

All procedures were approved by an Institutional Animal Care and Use Committee and conform to National Institutes of Health (NIH) guidelines. A total of 8 pigs (4 male, 4 female; 25.0–30.0 kg) and 10 sheep (5 male, 5 female; 26.1–38.4 kg) were studied in these experiments. Animals were fasted the day prior to surgery, except for water that was given ad libitum. Pigs were initially anesthetized with a mixture of tiletamine/zolazepam (3 mg/kg of each; Fort Dodge Animal Health, Fort Dodge, IA) intramuscularly (IM). Sheep were anesthetized with a mixture of ketamine 1 mg/kg (Vedco, Saint Joseph, MO) and xylazine 2 mg/kg (Lloyd Laboratories, Shenandoah, IA) IM. For both pigs and sheep, the trachea was dissected, an incision was made between the tracheal rings, and an endotracheal tube was placed. The animal was subsequently mechanically ventilated on oxygen. An external jugular vein was dissected, and a venous catheter was placed. Urethane 1 g/kg (20% solution in water; Sigma, St. Louis, MO), was infused intravenously (IV) for surgical anesthesia. A total of 150–200 ml were given at a rate of 30 ml every 10 min. The choice of urethane was based on its long, stable plane of anesthesia and its preservation of vagal function (Hotta et al., 2009). Urethane is suitable for cardiovascular studies when given at a dose $\leq$1.5 g/kg, but at higher doses it has been reported to have negative inotropic effects on the heart (Maggi and Meli, 1986; Koblin, 2002).

Carotid arterial lines made of polyethylene tubing (2.0 mm outer diameter, 1.8 mm inner diameter) were placed in 4 pigs and 2 sheep. In all animals, both vagus nerves were carefully dissected adjacent to the common carotid artery and freed from connective tissue for a length of $\geq$4 cm. Platinum strip electrodes, insulated with nail polish over their outside surfaces, were wrapped around each nerve. Nerves were insulated and protected from drying with paraffin film. The magnitude of the bradycardic effect of VNS confirmed that the nerves were undamaged.

A constant current isolated pulse stimulator (A-M Systems model 2100, Sequim, WA) was used to stimulate either vagus nerve unilaterally, and both nerves could be stimulated with the same current together when connected in series. Stimulation current was titrated until the maximal effect on HR was observed (1–10 mA). VNS was performed as 1-millsecond square wave pulses on either or both nerves at 1, 2, 5, 10, 20, 50, and 100 Hz for 20 s.

A thoracotomy was performed on all animals by incising the skin and subcutaneous tissue between two ribs on the left side over the heart. The ribs were kept retracted for access to the heart for epicardial ultrasound and/or induction of ventricular fibrillation (VF). VF was induced in all animals by placing large (1.5 cm rings), low impedance silver chloride electrodes over the ventricles and stimulating with direct current driven by 3–5 V for several seconds (Protek triple DC power supply model 3033B, Tempe, AZ). Epicardial ultrasound was started within 2 s of VF induction, and VNS was initiated 5–30 s after VF induction. We clamped the left anterior descending coronary artery (LAD) with a hemostat for several minutes in 5 pigs as an alternative method for inducing VF. Bilateral 50 Hz vagus nerve stimulation was performed during this period. After testing VNS, normal sinus rhythm was successfully restored by using an external defibrillator (Cardiac Science, Burdick model DC-190, Waukesha, WI). To verify the effect of VNS in the absence of sympathetic tone, the beta adrenergic receptor antagonist propranolol (1.5 mg/kg IV; Research Biochemicals, Inc., Natick, MA) was tested in 2 sheep. Animals were sacrificed either by injecting 10 ml intracardiac Euthasol (a mixture of pentobarbital 390 mg/ml and phenytoin 50 mg/ml; Virbac, Fort Worth, TX) or by not terminating VF.

2.2. Data acquisition

Pulse oximetry, electrocardiogram (EKG), blood pressure (BP), and echocardiographic images were recorded continuously. Pulse oximetry was recorded by placing a pulse oximeter clip over one of the ears (Med Associates, Georgia, VT). EKG was recorded from limb lead pin electrodes placed subcutaneously, and the signal was amplified and filtered to pass 1 Hz to 1 kHz (A-M Systems, model 1800, Sequim, WA). BP was recorded from the arterial catheter, which was connected to a BP transducer (CyQ, Columbus Instruments, Columbus, OH). Pulse oximetry, EKG, and BP were digitized at 2 kHz and stored on disk for analysis (Spike2; Cambridge Electronic Design, Cambridge, UK).

Epicardial echocardiography was performed with 4 pigs and 3 sheep using the Phillips SONOS 5500 with a 3 MHz linear probe (Phillips, Andover, MA) during sinus rhythm and during VF. The probe was placed over the LV for a short axis view, and data was recorded in both M-Mode and 2D video. Video tapes were digitized (Pinnacle Dazzle DVC 100; Corel, Mountain View, CA) and stored to disk using Matlab software (Image Acquisition Toolbox; Mathworks, Natick, MA).

2.3. Data analysis

Heart rate response to VNS was quantified by counting the number of ventricular beats in a 20-second period of VNS at a particular frequency using the EKG, BP, and/or pulse oximetry data. Electrical artifact from stimulation could be removed using Matlab (Mathworks, Natick, MA) with the Signal Processing Toolbox. To remove electrical artifact from the EKG while the animals were in sinus rhythm, the samples during each pulse were excluded and missing data was estimated using a least squares method. After removing frequencies below 5 Hz, a notch filter was applied to the signal at the frequency of the vagal train to remove sinusoidal artifact. The resulting waveform showed the EKG signal buried within the stimulation with resolution sufficient to visualize the p-waves in the vast majority of recordings. Sample PR interval lengths (from p-wave onset to QRS complex onset) were measured from the filtered signal as well. Brief transient electrical activity at the beginning and end of stimulation could sometimes be observed with this method, particularly with larger artifacts, such as occurred with bilateral VNS.

Alternatively, a simpler method was used to analyze the underlying EKG signal of VF during VNS because discrete events were not being counted. A Butterworth bandpass filter was used to filter the VF record between 4 and 40 Hz, a bandwidth containing the dominant VF frequencies (Carlisle et al., 1990; Indik et al., 2006). Color spectrograms were constructed using 2048 points for each frequency spectrum with 90% overlap and zero-padding to 8192 points. Dominant VF frequency at a particular time was found by locating the frequency with greatest amplitude in the frequency spectrum (Indik et al., 2006). To assess the extent of frequency heterogeneity during VF, spread of the dominant