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ORIGINAL PAPER

Autonomic boundary conditions for ventricular fibrillation and their implications for a novel defibrillation technique

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Abstract The sympathetic and parasympathetic divisions of the autonomic nervous system modulate cardiac rhythm and the probability of arrhythmia occurrence. Both increased sympathetic drive and hypoxia increase the likelihood for ventricular fibrillation (VF). Vagus nerve stimulation (VNS) can protect from fatal arrhythmias via cholinergic and nitrergic action. We sought to determine boundary conditions for VF and defibrillation by autonomic manipulations accompanied or not by hypoxic changes in urethane-anesthetized rats. VF was induced with (1) vagotomy, (2) systemic high-dose (>15 mg/kg) isoproterenol, and (3) hypoxemia. When VNS (50 Hz) produced cardiac standstill, it converted every VF episode (59/59). A nitric oxide synthase inhibitor did not reduce VNS efficacy (13/14 episodes converted), but addition of atropine reduced VNS efficacy (11/27 episodes converted). VF can be induced by autonomic derangements only under constrained conditions, including sympathetic over-activation, reduced parasympathetic input, and hypoxemia. VNS can provide an alternative method to defibrillate via its cholinergic action.

Keywords Acetylcholine · Arrhythmia · Sudden death · Vagus nerve · Nitric oxide

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Introduction

The autonomic nervous system (ANS) contributes to the electrophysiological stability of the heart. Both the sympathetic and parasympathetic divisions of the ANS modulate cardiac electrophysiology and thus potentially modulate the probability of occurrence of catastrophic arrhythmias such as ventricular fibrillation (VF).

Experimentally, various arrhythmias have been produced in dog hearts by direct [1] or indirect [2] sympathetic activation. Sympathomimetic amines and sympathetic stimulation also cause a decrease in the electrical stimulus current necessary to produce VF in dog hearts [3]. Conversely, blocking sympathetic innervation has been shown to be protective against ventricular tachyarrhythmias. In a coronary ligation model of myocardial infarction-related arrhythmias, dogs that underwent stellectomy required a greater current to produce VF than control animals [4]. Similar experiments have produced comparable results [5], and the concept is the basis for a large body of evidence which shows that blocking adrenergic input (specifically beta-adrenergic input) to the heart reduces sudden cardiac death after myocardial infarction (MI; [6]).

As early as 1860, vagus nerve stimulation (VNS) had been theorized to be protective against cardiac arrhythmias [7], but it did not receive much attention until the 1970s [8–10]. In one study using the canine coronary artery occlusion model with medium or high-intensity vagus nerve stimulation during occlusion, hearts took longer to develop VF during VNS compared to controls, the greatest benefits of which were seen during high-intensity stimulations [8]. Additional experiments with VNS or disruption of vagal activity in multiple species, including dogs [9, 11, 12], cats [13], and guinea pigs [14], have all supported the cardioprotective action of vagal activity. In human patients, vagal tone has been suggested as cardioprotective, as a loss of variability in heart rate (attributed to decreased vagal tone) is associated with increased mortality post-MI [15]. In addition, beta-adrenergic receptor blockers have been shown to reduce the current necessary for defibrillation [16], and they are more effective in preventing VF than drugs that increase parasympathetic tone [17–19].

The neurotransmitter basis for vagal protection of the heart includes acetylcholine (ACh) and nitric oxide (NO). In general, the vagus nerve innervates both atria and ventricles [20, 21] with a cardiotopic organization [22]. Muscarinic receptors are found mostly in the atria, but they are also found in the ventricles in a number of species [23], including rats [24]. Functional evidence of vagal control of the ventricles exists, as decreased cell contraction has been observed in the ventricles in guinea pigs when applying ACh [23]. However, it has also been shown that NO is released onto the left ventricle in response to electrical stimulation [25], and that NO may play an important role in protection against VF. In a Langendorff preparation in rabbits, the increase in threshold of current necessary for VF during VNS was blocked with the nitric oxide synthase inhibitor L-N^G-nitroarginine and restored with L-arginine, a NO precursor [26]. In later experiments with the same preparation, VNS was shown to cause this change in the VF threshold independent of muscarinic receptor activation [27].

The most common VF in humans occurs because of regional cardiac ischemia, as in MIs. However, hypoxemia has been implicated in some conditions to produce arrhythmias (e.g., obstructive apnea; [28]). While VF does not occur due to severe blood loss or asphyxia alone [29], hypoxemia may aid in VF induction. Conversely, greater oxygen availability can be cardioprotective against VF: following coronary ligation in dogs, placing the dogs in hyperbaric oxygen chambers increased survival and decreased VF occurrence [30].

The contribution of global cardiac hypoxia to VF is much less studied and may be of fundamental importance in diseases with autonomic/cardiac pathologies, as may be the case in sudden unexpected death in epilepsy (SUDEP). Given that activity on both divisions of the ANS is markedly increased during seizures [31–33], we asked whether changes in autonomic activity, accompanied or not by hypoxic changes to the heart, could precipitate ventricular fibrillation. In addition, we asked whether vagus nerve stimulation could convert this and other types of ventricular fibrillation.

Methods

All procedures were approved by an Animal Care and Use Committee and conform to National Institutes of Health (NIH) guidelines. Adult male Sprague–Dawley albino rats (3–9 months, 325–550 g; Harlan Sprague–Dawley, Chicago, IL, USA) were anesthetized with urethane (Sigma, St. Louis, MO, USA) at 1.5 g/kg ip and supplemented as necessary with 0.1 g/kg ip. The rats were subsequently moved to a warming pad to maintain body temperature and then prepared for surgery. The urethane preparation and many recording methods have been described previously [32–35]. A schematic representation of the typical experiments as described below is shown in Fig. 1.

Surgery and electrophysiological recordings

A single superficial incision was made along the length of the rat's sternohyoid muscle, the trachea was exposed, and an endotracheal tube was inserted and tied in place with suture. Both vagus nerves were dissected, and a custom-made electrode [36] was placed along one of the nerves for stimulation of vagal efferents. The nerves were protected from drying with paraffin oil. Continuous blood pressure (BP) recordings were made from an arterial catheter (polyethylene tubing, cut square, 0.5 mm inside diameter, 0.8 mm outside diameter) in the femoral artery that was connected to a blood pressure transducer (CyQ; Columbus Instruments, Columbus, OH, USA). This catheter was also used for drug infusions. Copper bands coated with conductive gel were placed over the rat's wrists and an ankle for EKG recordings. All signals except the BP signal were amplified (A-M Systems model 1800; Sequim, WA, USA) and filtered to pass 1 Hz to 1 kHz. All signals were subsequently digitized at 2 kHz and stored on disk for analysis (InstruNet World; GW Instruments, Somerville, MA, USA).

A subset of animals had echocardiographic recordings taken during autonomic/hypoxemia-induced VF and during defibrillation to better understand changes in systolic function during these periods. Echocardiography was performed using the Phillips SONOS 5500 with a 15 MHz linear probe (Phillips, Andover, MA, USA). Animals were imaged in the optimal 2D-guided M-mode parasternal short

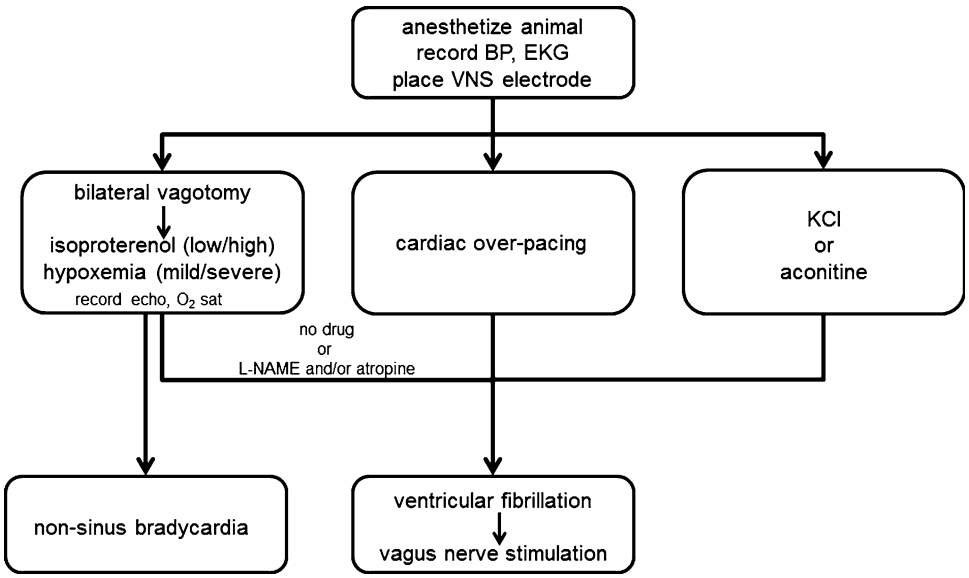

Fig. 1 Schematic representation of typical experiments. Rats were anesthetized with urethane, and a catheter was inserted in the femoral artery. EKG electrodes and VNS electrodes were placed. Autonomic/hypoxemia-induced VF and non-sinus bradycardia were studied. VF was induced in four ways, and attempts were made to convert VF with VNS in the presence of no drug or L-NAME and/or atropine

axis view with machine and gain settings adjusted for best image quality. Images were recorded and the parameters were measured for 3 cardiac cycles at a sweep speed of 100 mm/s. Left ventricular end systolic dimension (LVESD), left ventricular end diastolic dimension (LVEDD), septal wall thickness (SWT), and posterior wall thickness (PWT) were measured. The ejection fraction (EF) was calculated from the formula $EF = 100 \times [(LVEDD^2 - LVESD^2)/LVEDD^2]$ [37] assuming conical volumes.

VF induction and VNS defibrillation

Attempts to induce VF included a variety of methods. The first method used combinations of bilateral vagotomy (crushing nerve by tying suture knots), low-dose ($\leq$15 mg/kg intra-arterial) or high-dose (>15 mg/kg intra-arterial) systemic administration of isoproterenol (Research Biochemicals International, Natick, MA, USA), and periods of hypoxia using fixed-volume dead space attached to the endotracheal tube (1–6 ml). Oxygen rebreathing, as occurs with dead space application, is postulated as part of the mechanism of nocturnal sudden death, as in sudden death in epilepsy [38]. To avoid ischemic preconditioning, trials were kept as close together in time as possible. Trials of hypoxemia were repeated once an animal had recovered its baseline oxygen saturation. No difference was observed in the ability to produce VF between earlier and later trials of hypoxemia. Isoproterenol was used instead of stellate ganglion stimulation because stellate ganglion stimulation requires surgery that is frequently associated with pneumothorax, which prevents studies that include spontaneous respiration. In addition, isoproterenol can be given in doses with stable exposure to the drug. Isoproterenol was chosen instead of norepinephrine to mimic the immediate of the cardiac sympathetic nerve by preferentially activating beta adrenergic receptors. Doses of isoproterenol were administered in fixed volume bolus injections from 10 or 20 mg/ml solutions, and actual dosages per animal weight were calculated. Results were plotted after binning dosages by 5 mg/kg increments. A second method of VF induction was to over-pace the cardiac ventricles with electrical stimulation. These rats were mechanically ventilated with a rodent respirator (Harvard Apparatus, Holliston, MA, USA). A third method used infusion of potassium chloride (KCl; 100 mg/kg intra-arterial; Fisher Scientific, Fair Lawn, NJ, USA) or aconitine (50 μg/kg intra-arterial; Sigma, St. Louis, MO, USA; [39]) to elicit ventricular tachyarrhythmias or VF.

Vagus nerve stimulation and stimulation for cardiac over-pacing were performed using isolated pulse stimulators (A-M Systems model 2100). For VNS, biphasic current pulses (1–9.8 mA) that lasted for 1 ms were passed as 50-Hz trains for periods of 0.5–20 s. The currents used were supramaximal for efferent fiber activation so that stimulus trains (e.g., 50 Hz for several seconds) would optimally induce cardiac standstill [35]. For cardiac pacing, biphasic current pulses (2–10 mA) that lasted for 4–6.5 ms were passed as a 20- or 50-Hz train for a period of 4 s either horizontally or vertically across the left ventricle, or both ventricles. Recordings were reviewed using scripts written in Matlab (Mathworks, Natick, MA, USA). Cardiac standstill upon VNS was checked before VF induction. VNS defibrillation was successful when it converted VF to cardiac standstill with restoration of sinus rhythm after stimulus termination. It was considered to be unsuccessful if VF persisted after VNS. Spontaneous conversions of VF were not included in the count. They lasted 1–8 s, with a mean duration of 5.0 $\pm$ 2.2 s. They cannot be numerically compared to VNS conversions since the stimulation was only done for periods of VF which lasted longer.

Pharmacologic study of VNS defibrillation

Efficacy of VNS defibrillation was assessed when blocking the effects of the neurotransmitters NO and acetylcholine. A subset of rats that experienced VF was administered L-N^G-nitroarginine methyl ester (L-NAME; 27 mg/kg, intra-arterial; Tocris Bioscience, Ellisville, MO, USA) [40] alone or in combination with atropine (5 mg/kg intra-arterial; Phoenix Scientific, St. Joseph, MO, USA) during attempts to defibrillate with VNS. Atropine alone was administered in one animal, and its addition made VNS defibrillation ineffective for many attempts, something that had never been observed in other animals. Since the experiments involve loss of life, atropine was administered in other animals after using L-NAME.

Successes in VF induction and efficacy of VNS defibrillation are reported both as proportions of total successes to attempts (all conditions) and as average success rates across animals with standard deviations. Recordings were checked for ST elevation, where the J point was defined as, "the first point of inflection on the upstroke of the S wave" [41]. Statistical tests were done by performing one-way ANOVA, and post hoc tests are reported with Tukey's 95 % confidence interval (Minitab; Minitab, State College, PA, USA). The number of post-vagotomy premature ventricular contractions (PVCs) were compared to the number of post-vagotomy PVCs after addition of isoproterenol by paired Student's t test. In addition, this test was used to compare physiological measures taken from rats before VF or bradycardia to baseline measurements.

A total of 61 rats were used in these experiments. Twenty-three of 49 rats tested with the combinations of autonomic perturbations and hypoxia experienced an episode of VF. Eight rats were studied with cardiac overpacing and 20 rats, some of them from the autonomic/hypoxia group of animals, were tested with doses of KCl ($n = 17$) or aconitine ($n = 3$).

Results

Autonomic/hypoxia-induced ventricular fibrillation

A triad of conditions was found to be necessary to induce VF: (1) bilateral vagotomy, (2) systemic high-dose isoproterenol, and (3) severe hypoxemia (Fig. 2), where isoproterenol dose was defined as low ($\leq$15 mg/kg) or high (>15 mg/kg), and hypoxemia as mild (<7.5 ml/kg) or severe ($\geq$7.5 ml/kg). All other combinations of conditions (intact vagus/vagotomy/VNS, no/low-/high-dose isoproterenol, no/mild/severe hypoxemia) produced tachycardia, premature ventricular contractions, or non-sinus bradycardia (including bradycardia with irregular rhythm, or, more commonly, AV block of varying degrees). The conditions accounted for all stable VF inductions, except for one, which occurred in an animal with high-dose isoproterenol, mild hypoxemia, and bilateral vagotomy. VF could not be induced under other conditions, including high-dose isoproterenol and severe hypoxemia with intact vagi ($n = 3$). In comparing high- to low-dose isoproterenol, it was found that animals underwent fibrillation on average 32 % $\pm$ 31 of the time (39 of a total of 143 attempts in 29 rats) with high-dose isoproterenol at varying dead space doses, while no animal with low-dose isoproterenol underwent fibrillation (0 of 42 attempts; 13 rats; $p < 0.001$). Of note, severe hypoxemia was successful at producing VF over a range of isoproterenol doses 29 % $\pm$ 30 of the time (39 of a total of 143 attempts in 23 rats), while mild hypoxemia did not result in VF (0 of 42 attempts; 17 rats; $p < 0.001$). Those rats with severe hypoxemia that did not experience VF experienced bradyarrhythmias. A summary of VF induction successes can be found in Table 1.

We investigated EKG changes after administration of isoproterenol. Of 34 rats, only 1 experienced PVCs before addition of isoproterenol, while 12 experienced PVCs after the administration of isoproterenol. After vagotomy, the rats experienced 0.06 $\pm$ 0.3 versus 0.7 $\pm$ 1.2 PVCs/min after subsequent administration of isoproterenol ($p = 0.008$). It was additionally found that low-dose isoproterenol was sufficient to induce the maximal heart rate after vagotomy (Fig. 2a), and ST elevation was not observed in any (0/12) of the tested rats. ST elevation was observed in 56 % (10/18) of animals that experienced VF, but in none (0/24) of the rats with other arrhythmias.

To explore the VF induction, HR, BP, oxygen saturation, and heart dimensions were measured in rats that underwent hypoxemia. They were placed into three categories: non-sinus bradycardia, sinus bradycardia, and those that experienced VF (Fig. 3). In all animals, the heart rate showed a steady decline (Fig. 3a) beginning with the onset of increased airway dead space, and the HR right before VF or non-sinus bradycardia was found to be significantly different ($p = 0.003$). BP instability could be observed to occur in the middle of the time between onset of airway dead space and the onset of severe arrhythmia (Fig. 3b). The BP instability coincided with the plateau of oxygen saturation among these rats (Fig. 3c). The oxygen saturation for animals experiencing VF was significantly greater than that of the animals with non-sinus bradycardia ($p = 0.03$). As a measure of myocyte synchronicity, the amplitude of the R waves was not found to be significantly different among groups at arrhythmia onset.

Twelve animals underwent echocardiographic imaging throughout fibrillation/defibrillation trials (before, during, and after fibrillation and defibrillation) to assess the changes in LV function. Echocardiography showed no

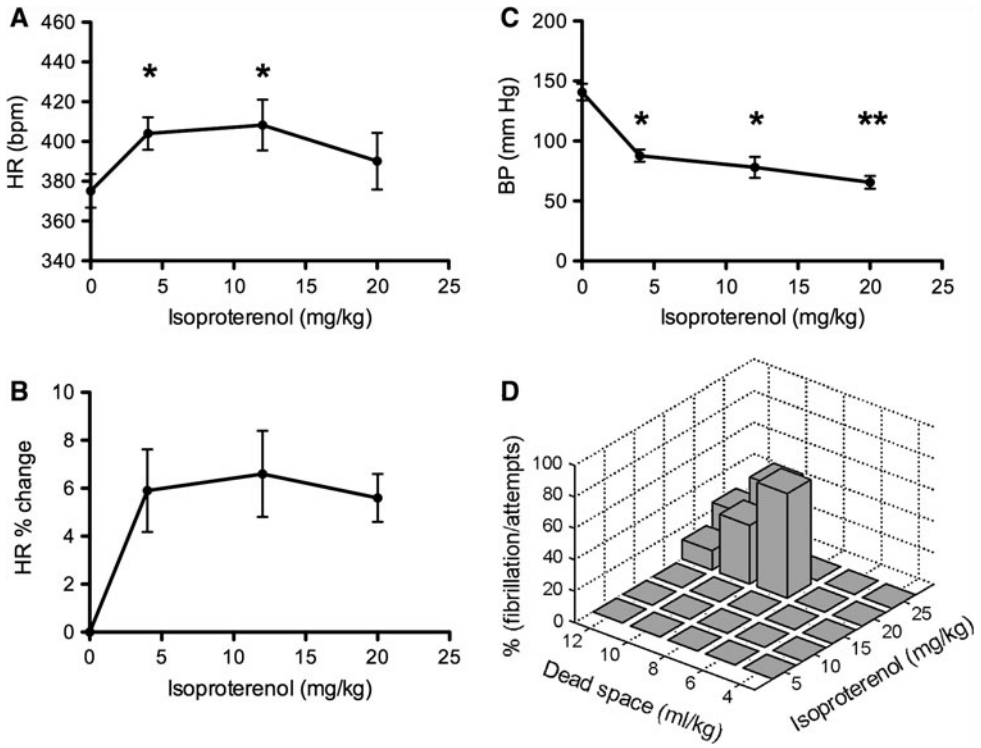

Fig. 2 Interaction of sympathetic activation with hypoxemia to precipitate VF. HR at increasing doses of isoproterenol after vagotomy is shown in (**a**). There were significant changes in HR over baseline (vagotomy, no isoproterenol; $p = 0.02$). Animals thus reached maximal HR at the lower doses. **b** shows the percent change in HR from baseline. The last three doses were found to yield changes similar to each other but distinct from baseline. The nonselective beta agonist also produced a decrease in mean BP (**c**, $p < 0.001$), and there were differences among the doses in post hoc tests (significantly different groups distinguished by *, **). In (**d**), a 3-dimensional graph of the success rates of achieving VF is shown. The graph shows an abrupt change in VF successes for both isoproterenol and dead space amounts

Table 1 VF induction rates post-vagotomy under varying isoproterenol doses and dead space volumes

	Low-dose iso	High-dose iso	Total	Average success % (SD)	*n* (rats)
Mild hypoxemia	0/20	0/22	0/42	0 (0)	17
Severe hypoxemia	0/22	39/121	39/143	29 (30)	23
Total	0/42	39/143			
Average success % (SD)	0 (0)	32 (31)			
n	13	29			

differences in LV measures between animals experiencing VF and those experiencing non-sinus bradycardia (Fig. 3d). However, sudden onset of LV systolic dysfunction was observed before VF, as can be seen by the sudden change in end systolic diameter midway through the time course up to the start of VF and a corresponding decrease in LV ejection fraction in the figure. Statistical tests on the cardiac measurements show significant changes in all cardiac dimensions for all groups at arrhythmia onset compared to baseline (see Fig. 3d). There was a trend towards a significant change in LV end diastolic dimension in rats with non-sinus bradycardia ($p = 0.06$).

Defibrillation via vagus nerve stimulation

In general, vagus nerve stimulation at 50 Hz with a sufficient current to activate all efferent fibers was able to cause cardiac standstill [29] (Figs. 4, 5, 6). Longer stimulus trains were accompanied by non-conducted P waves and ventricular escape beats later in the stimulus train. The cessation of the stimulus resulted in rebound tachycardia and sometimes in PVCs.

In echocardiographic studies to assess LV function throughout fibrillation/defibrillation trials, the segments of the LV were found to be hypokinetic and to move asynchronously during fibrillation, as compared to that of normal beats (Fig. 4b). VNS defibrillation caused LV segments to become akinetic during stimulation until escape beats appeared.

A total of 23 rats experienced autonomic activation/hypoxia-induced VF. Trains $\geq$1.5 s were necessary to

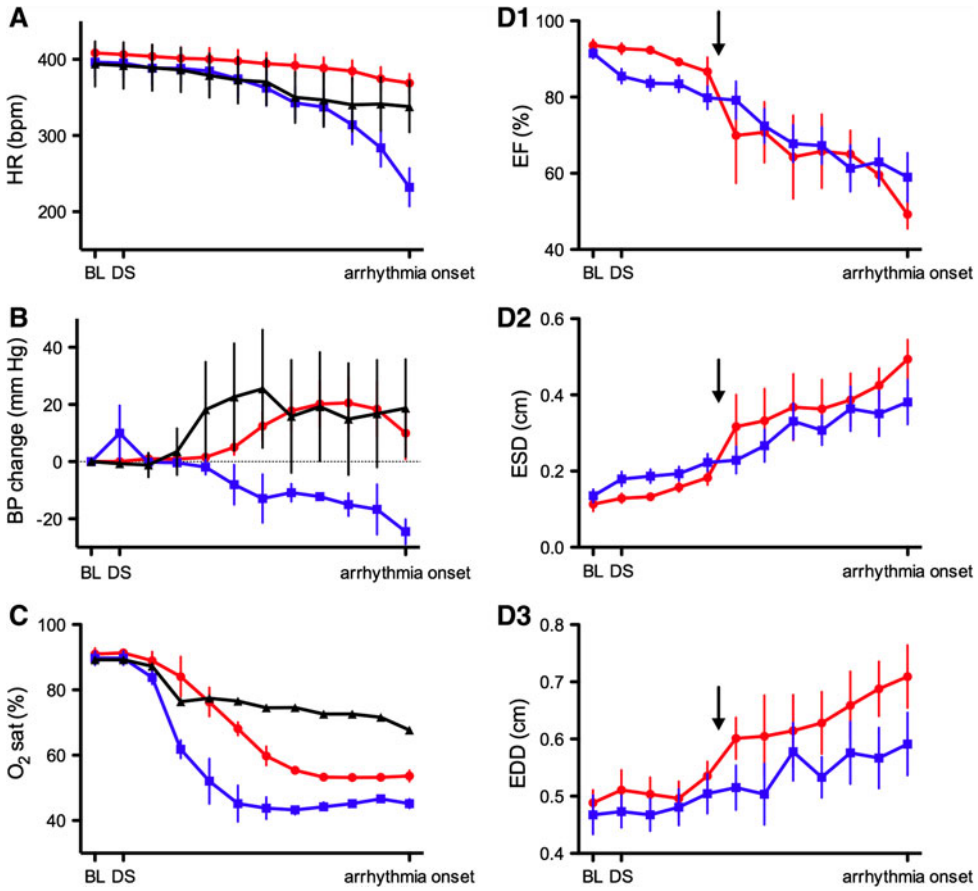

Fig. 3 Changes in systemic and cardiac variables in the time before arrhythmia onset. Changes in HR over time while dead space (*DS*) had been in place until arrhythmia onset are shown in (**a**). This was preceded immediately by a baseline (*BL*) measurement. HR was measured for overlapping groups of animals entering VF (*red*/*circle*), non-sinus bradycardia (*blue*/*square*), and sinus bradycardia (*black*/*triangle*). Measurements were made over normalized time, i.e., at time intervals relative to the dead space placement and arrhythmia onset (VF, sinus/non-sinus bradycardia). These were generally about 1–3 min in duration. **b** shows the change in BP over the same time scale, where the BP in the non-sinus bradycardia group decreases significantly compared to the other two groups ($p = 0.004$ at last time point). **c** shows oxygen saturation at this time scale, and a measurement from one animal with sinus bradycardia is shown for reference. **d1**–**d3** show echocardiographic measurements of end systolic diameter (*ESD*), end diastolic diameter (*EDD*), and ejection fraction (*EF*) along the same time scale as (**a**–**c**). No appreciable differences could be seen between VF and non-sinus bradycardia, except for a shift in EF in the VF group (*arrow*). This was observed as a change in systolic function, as can be seen in the drastic shift in ESD (**d2**). While the shifts are suggestive, the data do not show statistical significance (color figure online)

defibrillate. These rats experienced VF 1–4 times per animal. It was found that 85.7 % of all VF episodes (30 of a total of 35 episodes in 23 rats) were defibrillated successfully with VNS in these animals, the other episodes ending spontaneously. The conditions of stimulation ranged in current from 1 to 4.5 mA at 50 Hz and lengths of trains from 0.5 to 20 s. Across all conditions, a total of 1.1 ± 0.3 VNS attempts were required to defibrillate successfully. Among animals that experienced any period of cardiac standstill during VNS, although some ventricular escape beats could occur, all 30/30 VF episodes were defibrillated (Fig. 4a).

Of the 8 rats that underwent rapid cardiac pacing to induce VF, VF was induced 1–9 times per animal. No difference was observed between different stimulation sites used for over-pacing. An EKG feature of the VF in these animals was that it was more than twice the amplitude of the VF experienced by rats that underwent the autonomic activation/hypoxia protocol. Of the total number of VF episodes, 78.4 % (29/37) were defibrillated by VNS. The conditions of the VNS stimulations ranged from 2.75 to 9.8 mA with 20- or 50-Hz trains that lasted 3 s in duration. On average, 2.8 ± 2.7 attempts were necessary to defibrillate. When limiting the count to only animals that were stimulated with 50-Hz trains, VNS defibrillation had an 88.9 % (16/18) success rate with only 1.5 ± 0.8 attempts. All other episodes of VF spontaneously converted. Successful defibrillation elicited a brief period of cardiac

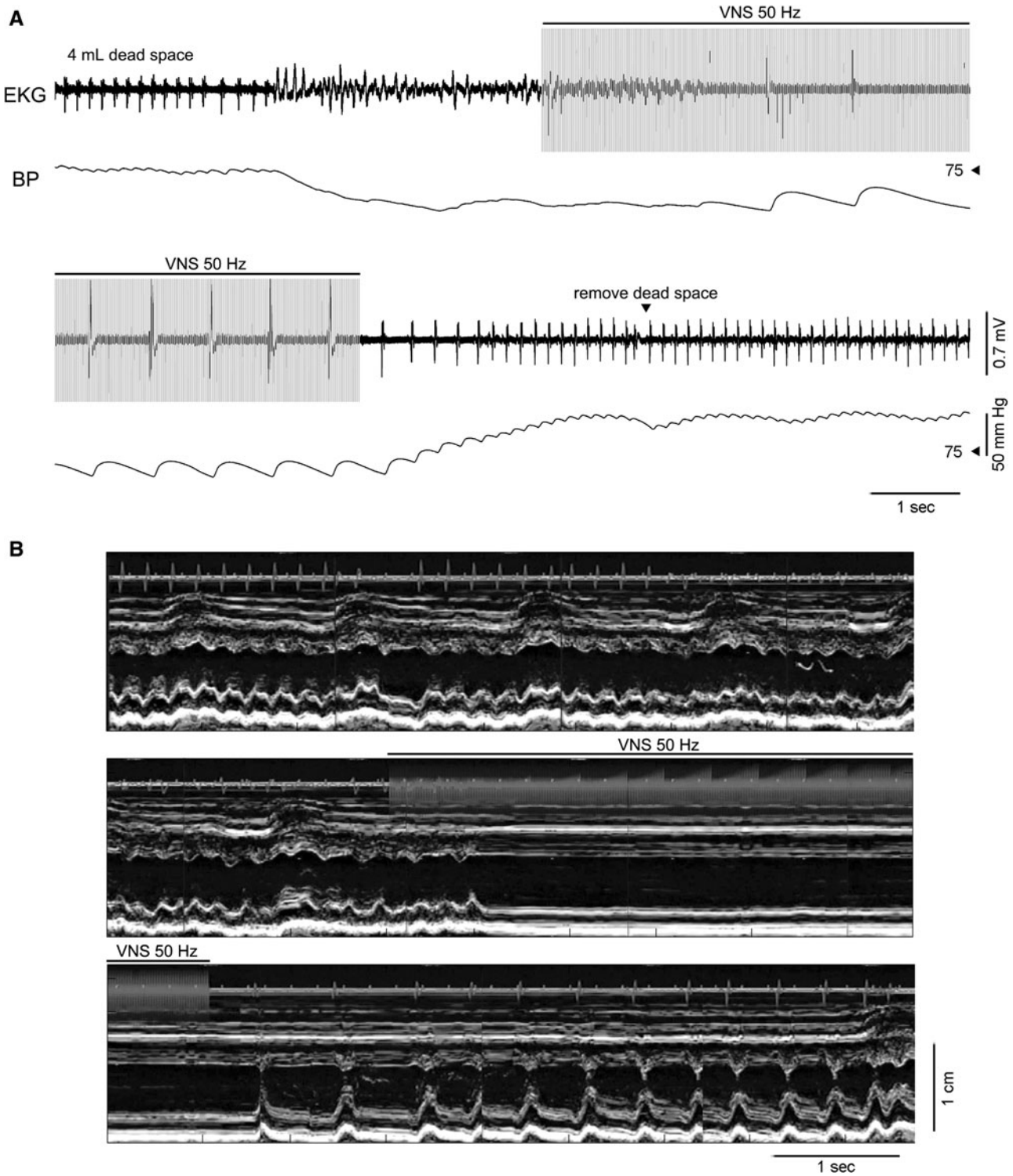

Fig. 4 Spontaneous entry into VF and conversion with VNS. EKG and BP tracings for a rat that underwent autonomic activation/hypoxia induced VF and VNS defibrillation are shown in (**a**). The rat was infused with isoproterenol 26 mg/kg. Four milliliters of dead space tubing were placed over the endotracheal tube for 38 s before fibrillation. In (**b**), the M-mode image of a different rat undergoing the same type of fibrillation is shown. The rat was infused with isoproterenol 30 mg/kg and had a 6-ml dead space placed over the endotracheal tube for 65 s before fibrillation. The dead space was removed after the period shown in the image. In both (**a**) and (**b**), the right vagus was stimulated with 3.5-mA, 1-ms pulses at 50 Hz to defibrillate

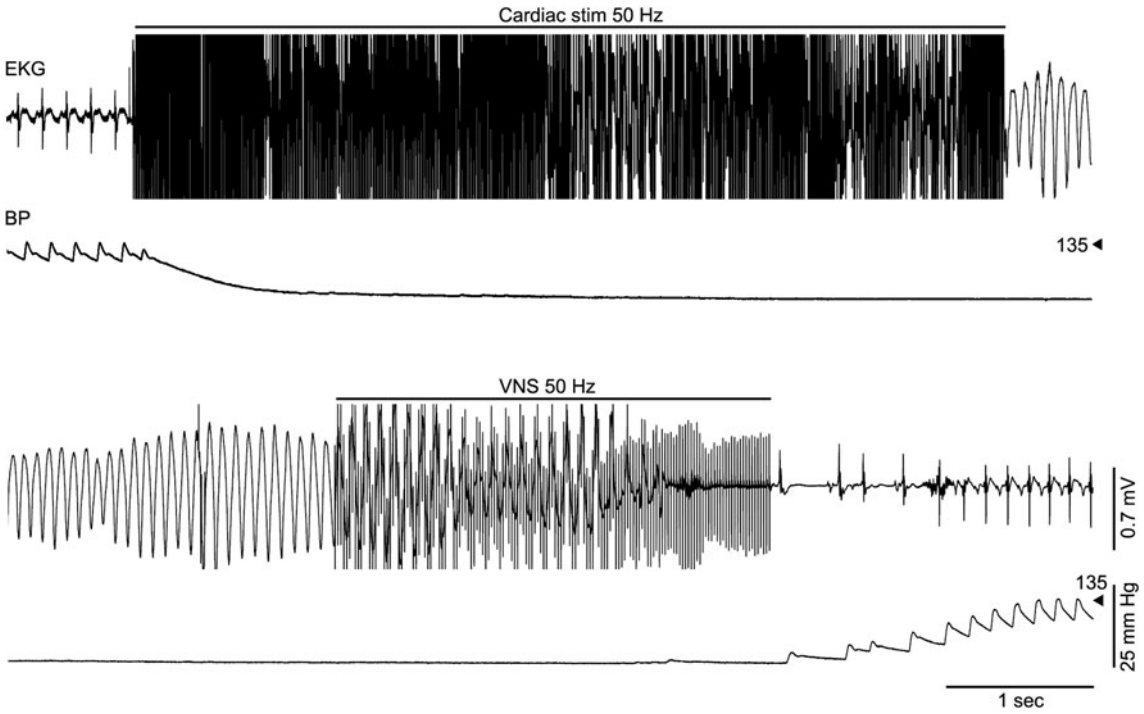

Fig. 5 VF from over-pacing converted with VNS. EKG and BP tracings of a rat that underwent VF and VNS defibrillation after cardiac electrical over-pacing stimulation are shown. Cardiac stimulation was performed by stimulating the ventricles with 4-mA, 4-ms pulses at 50 Hz. Defibrillation was accomplished by stimulating the left vagus with 4-mA, 1-ms pulses at 50 Hz

standstill before returning to sinus rhythm (Fig 5). Of the animals that experienced any period of cardiac standstill during VNS, all (29/29) VF episodes were defibrillated.

In pooling the above sets of VF episodes from all rats, left versus right vagus nerve stimulation efficacy was compared. A total of 25 rats had left VNS and 5 rats had right VNS, for a total of 30 animals. It was found that, among those with left VNS, 68.9 % (71/103) of VF episodes were defibrillated successfully, compared to 88.9 % (8/9) with right VNS. Cardiac standstill could be achieved with either right or left VNS.

None of the rats infused with KCl had their ventricular tachyarrhythmias stopped or converted by VNS. A range of stimulation intensities (1–4.5 mA) and train durations (3–20 s) were attempted, but KCl rendered VNS ineffective for cardioversion. The VF induced in the 3 rats by aconitine infusion could not be brought to cardiac standstill or successfully cardioverted by either a range of currents (1–4.5 mA) or a range of train durations (3–20 s). Table 2 summarizes VNS defibrillation among all of the VF induction methods tested.

Neurotransmitter basis of defibrillation by VNS

Four rats that underwent rapid cardiac pacing and 3 rats that underwent autonomic activation/hypoxia-induced fibrillation were also infused with either L-NAME alone or with L-NAME and atropine and were tested with VNS. The results from these animals were pooled.

VNS was applied using a variety of currents (3–9.8 mA) and train durations (3–5 s) at 20 or 50 Hz. Among the rats treated with L-NAME, 94 % ± 14 (13 of 14 attempts in 6 rats) of VF episodes were successfully defibrillated (Fig. 6a), and among the rats treated with L-NAME and atropine, 31 % ± 29 (11 of 27 attempts in 5 rats) were defibrillated (Fig. 6b). Rats without these drugs had been converted successfully at a rate of 81 % ± 32 (59 of 72 attempts in 31 rats). These data are summarized in Table 3. A statistical difference was found among the groups ($p = 0.004$), and post hoc tests showed no difference between control animals and animals with L-NAME, but a difference between these two groups and those animals with L-NAME and atropine.

Discussion

Combinations of vagotomy, systemic isoproterenol, and hypoxemia were studied to define conditions necessary to evoke VF. VF required high sympathetic drive accompanied by little or no vagal activity within a specific "window" of oxygen saturation. ST segment elevation is often a precursor to VF even in the absence of coronary occlusion. Other combinations of autonomic action and

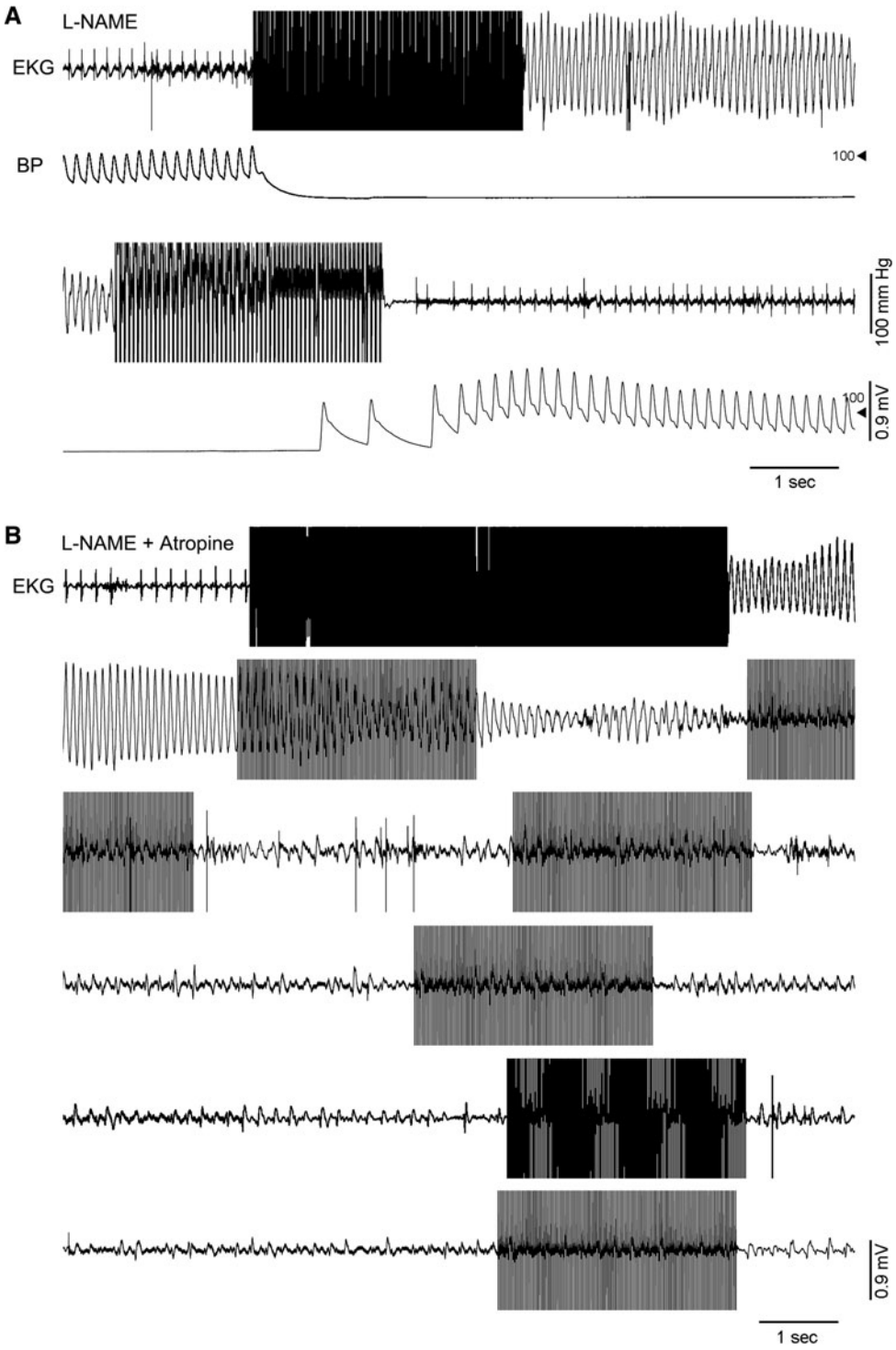

Fig. 6 Cholinergic and nitrergic contributions to cardioversion. EKG and BP tracings from a rat infused with L-NAME 27 mg/kg that underwent VF and VNS defibrillation after cardiac over-pacing are shown in (**a**). In a different rat infused with L-NAME 27 mg/kg and atropine 5 mg/kg, VNS defibrillation was ineffective (**b**). In both examples, cardiac over-pacing was performed by stimulating the ventricles with 4-mA, 4-ms pulses at 50 Hz. Defibrillation was accomplished by stimulating the left vagus with 4-mA, 1-ms pulses at 50 Hz

hypoxemia caused PVCs, tachycardia, or severe bradycardia. Saturations above the "window" did not result in VF, and those below it resulted in severe bradyarrhythmias and eventually death. We conclude that it is difficult to bring a normal heart to VF by autonomic over-activation. VNS converted VF by bringing about cardiac standstill under multiple conditions, and this was found to occur via the cholinergic action of the vagus nerve. We conclude that VNS may be a useful alternative to external defibrillation devices.

A mechanism for autonomic/hypoxia-induced ventricular fibrillation

One question that arises from the results is what effect does isoproterenol have at doses beyond the maximal heart rate?

Table 2 VNS defibrillation under a variety of conditions

VF induction method	VF episodes	Defibrillation (50 Hz)	Spontaneous conversions	*n* (rats)
Autonomic/ hypoxemia	35	30	5	23
Cardiac over-pacing	37	29	8	8
KCl	17	0	0	17
Aconitine	3	0	0	3

Table 3 Pharmacologic manipulation of VNS defibrillation success rates

	VF episodes	Defibrillation (50 Hz)	Average success % (SD)	*n* (rats)
Pre-drug	72	59	81 % (32)	31
L-NAME	14	13	94 % (14)	6
L-NAME and atropine	27	11	31 % (29)*	5

Use of atropine alone prevented VNS defibrillation in all attempts in one animal

* Indicates significantly lower than all other groups ($p = 0.004$)

Indeed, VF could not be induced when the isoproterenol dose was limited to that sufficient reach the maximal HR. Several lines of evidence suggest that isoproterenol-induced ventricular tachyarrhythmias may be mediated via myocardial ischemia. In general, high-dose isoproterenol in animals has been found to quickly produce local ischemic changes before finally causing necrosis [42–44]. It has been shown that isoproterenol produces infarct-like necrosis and that it is even more cardiotoxic than epinephrine or norepinephrine [44]. The cardiotoxicity is attributed to a number of causes, including: (1) myocardial Ca^{2+} buildup resulting in cell injury, (2) relative hypoxia of parts of the myocardium, (3) increased membrane permeability, (4) myofilament overstimulation, and (5) ATP depletion [44]. Thus, in addition to the effects of sympathetic over-excitation, it is possible that VF was induced with isoproterenol by causing myocardial ischemia. In this case, local hyperkalemia and acidosis would appear to be the cause of the VF, in a manner similar to the induction of VF after myocardial infarction [45–47]. The ischemic zone in studies of coronary occlusion in dogs has been shown to be the first part of the heart to fibrillate [48]. The increased incidence of ST segment elevation after high-dose isoproterenol implies myocardial ischemia may be a contributor to the VF observed in these experiments. Sympathetic over-activity that can have additional local cardiac effects was therefore necessary to induce VF.

One of the physiologic features of the autonomic/hypoxia-driven VF was sudden and marked dilation of the LV wall. This finding is consistent with a prior porcine study that showed an increase in end-diastolic length of the ischemic cardiac region following coronary occlusion was associated with a higher likelihood of developing VF. This held true for animals given nitroglycerin or no drug (i.e., it was independent of whether the animal was experiencing stretching of the myocardium; [49]). Dilation and hypoperfusion of a given area of myocardium was important for induction of VF in our experiments. In our study, an EDD increased beyond its baseline in rats experiencing VF was observed. However, an EDD increase also occurred for bradyarrhythmias. Taken together with the sudden systolic dysfunction in rats experiencing VF (Fig. 3, d1–d2), it appears that sudden mechanical dysfunction may be a contributing cause to ventricular arrhythmias such as VF.

Animals experiencing VF could maintain a higher BP and oxygen saturation than those with non-sinus bradycardia. Oxygen saturation may be an important factor in VF induction and evidence of synchrony. Indeed, the VF amplitude in cardiac over-paced rats (which were mechanically ventilated) was more than twice that of rats which experienced autonomic/hypoxia-driven VF [50], and oxygen availability may be related to the VF amplitude. These observations point to a specific set of conditions necessary for VF: the heart must both be maintaining its function and suffer from a sudden (perhaps local) mechanical dysfunction for VF to occur, and the level of tissue oxygenation needs to be compromised, but not too much. Outside of these conditions, bradyarrhythmias ensue.

Autonomic derangements may contribute to SUDEP [51], and they are certainly common in epilepsy [33, 52]. A combination of these autonomic derangements with global cardiac hypoxia can produce VF. The conditions are very specific, however, so the types of autonomic derangements occurring during seizures, which include activation of both parasympathetic and sympathetic divisions, even with hypoxemia, are unlikely to precipitate VF [31, 33]. Indeed, vagal activity, even a little, is cardioprotective against VF, and seizures cause increases in activity in both branches of the ANS [33].

Defibrillatory mechanism of VNS

NO has been implicated to have anti-fibrillatory effects independent of muscarinic action [25–27]. The data presented from our studies were that the combination of L-NAME and atropine blocked the defibrillatory effect of the vagus whereas L-NAME alone had no effect on defibrillation efficacy. Our data support acetylcholine rather than NO as mediating the protective effects of VNS. Disparate study findings between this and prior studies may be related to the choice of animal model (rabbit vs. rat).

A number of possibilities have been suggested for chronic benefits of ACh exposure, including (1) ACh decreases inflammation and thus decrease arrhythmias by association [53], (2) VNS prevents loss of connexin-43 during myocardial infarction [54], or (3) ACh induces decreased TIMP and MMP-9 expression in cardiac myocytes [53]. The mechanism by which VNS defibrillates is its ability to bring about cardiac standstill via cholinergic efferents of the vagus nerve. Essentially, the heart is forced to "reset" to a proper rhythm, as in electric cardioversion.

Ventricular fibrillation can occur via a variety of mechanisms. Surawicz offered the following catalogue for its causes: (1) extrinsic stimuli (e.g., electrical stimuli), (2) modify entire myocardium (e.g., KCl infusion), and (3) modify part of the myocardium (topical application of a drug like aconitine on the myocardium, reperfusion of ischemic myocardium) [29]. The forms of VF induced in this study can be classified as either extrinsic (electrical stimulation) or intrinsic (autonomic activation/hypoxia, KCl, aconitine). This study has shown that VNS can be used to defibrillate a variety of induced VF in rats across both categories. Right and left VNS appear to have similar VNS efficacy rates. VNS was ineffective at converting rhythm for animals that underwent KCl infusions, since the high concentration of potassium likely rendered VNS ineffective. VNS also failed in the presence of aconitine, a drug that blocks nerve function by poisoning voltage-sensitive sodium channels [55].

Some limitations exist in drawing inferences from our VNS defibrillation results in rats. VF can spontaneously revert to normal sinus rhythm, so one might question whether cardioversion was driven by VNS. By allowing runs of VF that were several seconds or more, we believe we have tested VNS on relatively stable runs of VF. Moreover, the inability to defibrillate when atropine was added provides evidence that VNS was indeed defibrillating VF. Another limitation is our use of rats for this study. It remains to be seen if VNS defibrillation is effective in larger animals, since the anatomical details of autonomic innervation differ and larger hearts defibrillate with less ease than smaller hearts [56]. Notwithstanding the properties of different heart sizes, we have demonstrated the principle that VNS defibrillation can occur, and as such it may have use in other animals, including humans.

Defibrillation in humans

Defibrillation by existing devices (both internal and external) functions by completely depolarizing membranes of the cardiac myocytes, specifically during the relative refractory period [57]. Large electric shocks are most effective at defibrillating, though pacing (70/min) can also be used to entrain the area of the heart responsible for fibrillation [57, 58]. We suggest that VNS defibrillation has a number of advantages over existing implantable technologies. First, while it requires surgery for placement of the electrodes on vagus nerve, no electrodes enter the heart. Implantable devices that are placed via catheter to reach the ventricular lining in one or more locations have elements that cross one or more valves of the heart. Irritation of the lining and the valve edges can lead to complications that will not occur from an entirely extra-cardiac device. Second, the stimulus parameters can be configured and tested much more simply and safely. Vagus nerve stimulation parameters can be set to produce a short period of cardiac standstill (the equivalent of one or two missed beats), and the train length extended with the safe understanding that the longer trains will be effective. This is compared with the current techniques of inducing a period of fibrillation by electrically stimulating the heart [59], and then configuring the parameters of the implantable defibrillation to convert the arrhythmia. In fact, the prescribing physician may elect to deliver a low frequency continuous train of vagus nerve stimuli to provide protection of the heart from arrhythmias without significant impact of afferent nerve fiber activation [53].

Current implantable cardioverter-defibrillators are reported to have perfect or nearly perfect efficacy given sufficiently high current [59, 60]. The defibrillation method described here was perfectly effective when VNS was configured to produce a period of cardiac standstill. Defibrillation attempts were termed failures if VF ended spontaneously at some time after VNS ended or if fibrillation never ended. Of the rats studied, one rat died from VF, and this rat had been administered L-NAME and atropine, which rendered VNS ineffective. Thus, VNS is highly effective as a defibrillator provided it is configured to produce cardiac standstill, and this configuration can be safely established at the time of implant and checked routinely for consistency. VNS has been used for a variety of conditions, including epilepsy, depression, and heart failure [61]. VNS has even been suggested as of use as a preventative measure for patients with coronary disease [53, 62]. Right VNS is even currently being evaluated for patients with advanced heart failure since it has been shown to reverse ventricular remodeling [61]. Because there is risk in delivering external electrical shocks to such patients, VNS can be used as a defibrillator in those with existing implanted vagus nerve stimulators.

Tachyarrhythmias during or after VNS

While a brief period of tachycardia was commonly noted after the cessation of a vagal train, brief ventricular tachycardia (VT) was observed in two rats during the period of cardiac standstill (an example shown in Fig. 7),

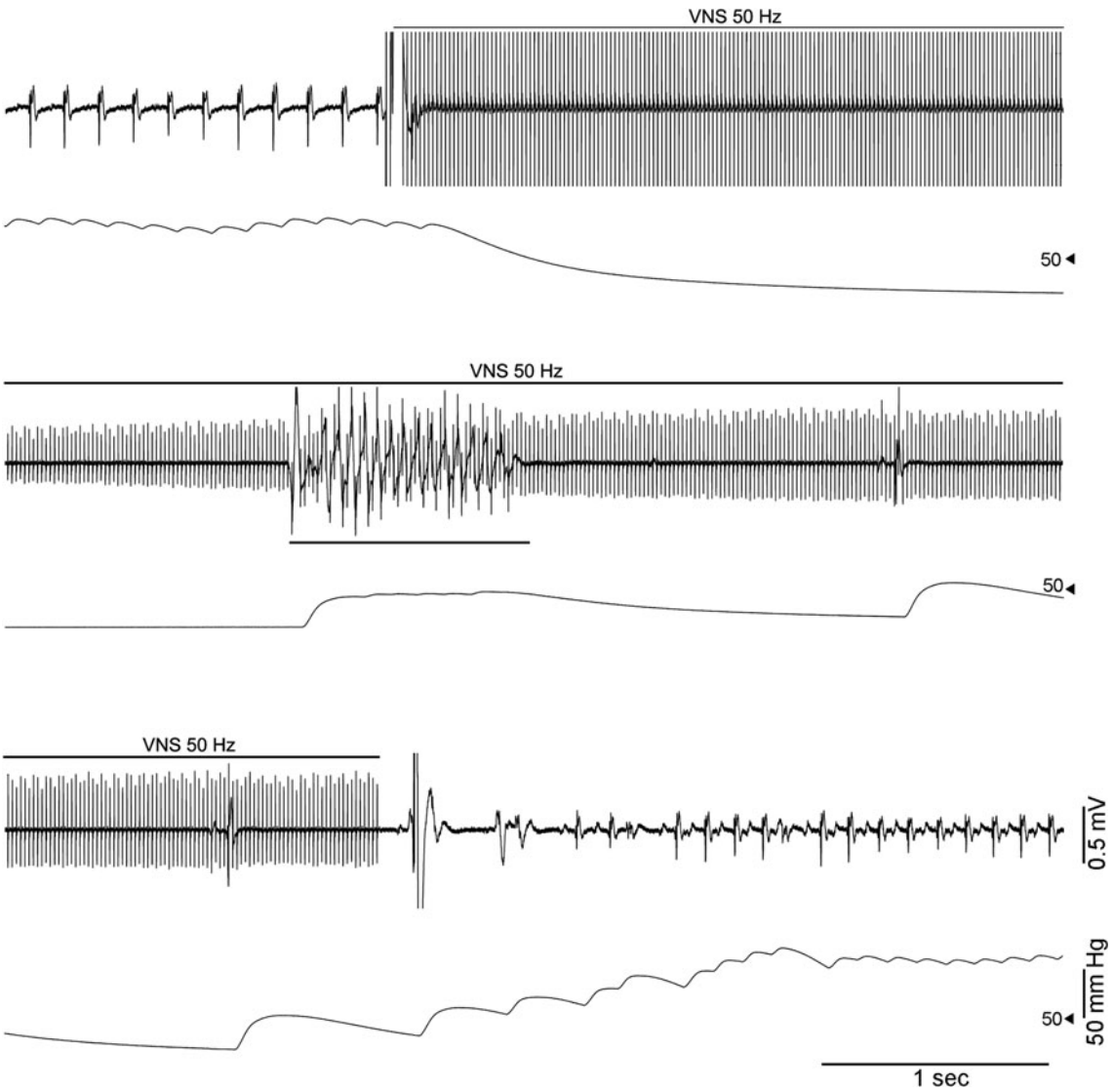

Fig. 7 Ventricular tachyarrhythmia during VNS. EKG and BP tracings for a rat that experienced ventricular tachycardia during a 50-Hz vagal train. The rat had been administered isoproterenol 29 mg/kg and underwent vagotomy while its airway was clear. The period of VT is *underlined*. Note the change in BP during the arrhythmia

and in one rat immediately after the vagal train. The rats had each undergone vagotomy and had been infused with high-dose isoproterenol. One of the two rats with VT during cardiac standstill underwent severe hypoxemia during this period. In this last case, it is possible that the rat underwent a ventricular tachyarrhythmia by the above method of induction, yet this was stopped by the vagal train. In fact, it may be that both cases of VT during cardiac standstill may be the result of the mechanism discussed above and that the vagal train acted as the dead space by stopping respiration. It may, however, also be possible to explain these cases of VT as an inability of the vagal train to stop a specific local population of myocytes from firing action potentials. This would allow for the driving of ventricular rhythm by this population, yielding VT and not VF. The rat which experienced VT subsequent to a vagal train may be an extreme case of the post-stimulus tachycardia that we have observed.

Others have shown that vagal input to the heart is actually pro-arrhythmic in diseases like Brugada syndrome, where increased vagal activity can cause VF [63–65], and long QT3 syndrome, where increased vagal activity leads to irregular bradyarrhythmias and eventually death [63]. However, our findings show that vagal input to the heart is not necessarily entirely protective even in normal hearts. Thus, a "vagal storm" that does not affect the entire myocardium may result in VT, and after-effects of extreme vagal action may be severe ventricular arrhythmias. This paradoxical effect of vagal activity may be significant in SUDEP, in contrast to autonomic/hypoxemia-induced VF.

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Conflict of interest None.

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