Muscarinic Effects of Vagosympathetic Trunk Stimulation on the Repetitive Extrasystole (RE) Threshold

STEPHEN H. RABINOWITZ, M.D., RICHARD L. VERRIER, PH.D., AND BERNARD LOWN, M.D.

SUMMARY The precise mechanism of the vagus in opposing adrenergic influences on cardiac vulnerability has not been defined. In the present investigation, cholinergic stimulation was produced by administering the selective muscarinic agent methacholine (MCh) and ventricular vulnerability was assessed by measuring the repetitive extrasystole (RE) threshold. MCh produced a sustained 98 ± 12% (P < 0.0001) increase in the RE threshold; the increase was abolished by concurrent infusion of atropine (0.2 mg/kg). When MCh was administered during beta-adrenergic blockade with propranolol, however, no further increase in RE threshold occurred beyond that resulting from propranolol alone. Infusion of norepinephrine (NE), in a dose insufficient to provoke systemic hypertension, significantly lowered the vulnerable period threshold. This decrease was completely abolished by concurrent vagosympathetic stimulation (VS). It is concluded that VS affects ventricular vulnerability through its muscarinic action and that the protection against VF is in part due to modulation of neural and humoral beta-adrenergic inputs.

THE INFLUENCE OF THE VAGOSYMPATHETIC TRUNK on the occurrence of ventricular arrhythmias has been studied for over 100 years. In 1859, Einbrodt1 employed an inductorium to provoke ventricular fibrillation (VF) in dogs. He measured VF threshold by changing the distance between inductorium coils. The delivered current was increased by approximating these coils. In the control state, VF was provoked at a distance between the coils of 90 cm. During vagal stimulation, the spacing could be narrowed to 30 cm before VF occurred. Einbrodt concluded that the vagus was protective against VF. Later investigators extended these observations to other ventricular arrhythmias. It has been demonstrated that vagosympathetic trunk stimulation,2-6 as well as vagomimetic drugs,7-11 protect against, while vagotomy2, 3, 14-16 and vagolytic drugs8, 15-19 predispose to ventricular arrhythmias. Contrary observations, in which vagal stimulation provoked ventricular arrhythmias, have also been recorded.20-22 Recently, attention has been refocused on the action of the vagus on ventricular vulnerability to VF. Vagal stimulation has been shown to increase the vulnerable period threshold, as well as protect the acutely ischemic canine heart against arrhythmias.23-26 Kent et al.27 have demonstrated anatomic cholinergic pathways in the ventricular conduction system by which this presumed effect is mediated. However, Kolman et al.28 failed to observe a rise in vulnerable period threshold for VF in the absence of enhanced sympathetic activity. These workers ascribed vagal protection in the acutely ischemic heart to antagonism of the well known enhancement of both sympathetic neural as well as humoral activity which follows coronary artery occlusion.29 Thus, two diametrically opposed views are currently being entertained; namely, that the vagus exerts a direct effect on ventricular vulnerability, and conversely, that this action is indirect and only operates with increasing levels of sympathetic tone. These disparate interpretations require clarification. Resolution of this problem is complicated by the fact that testing for cardiac vulnerability by repeatedly provoking VF and defibrillating is associated with enhanced sympathetic neural and humoral activity. This may thereby predetermine a response to the vagus on susceptibility to VF. A solution to this problem is suggested by the observation of a number of investigators30-36 who have found that repetitive extrasystoles precede the occurrence of VF. Recently, Matta et al.36 have systematically investigated the relationship between the repetitive extrasystole (RE) and the VF threshold under diverse experimental conditions. Single electrical pulses, 2 msec in duration, were delivered at 1-2 msec intervals during the ventricular vulnerable period. Repetitive extrasystoles consistently occurred when 66% of the fibrillatory current was delivered. The timing in the cardiac cycle for eliciting repetitive extrasystoles was coincident with that for inducing VF. The relationship between repetitive extrasystole and VF thresholds was maintained under a variety of autonomic conditions including stellate ganglion stimulation, vagal excitation, and beta-adrenergic blockade. In the present study, the RE threshold technique was employed for defining the mechanism of vagal action on ventricular vulnerability.

Methods

Healthy mongrel dogs of either sex, weighing 10 to 28 kg, were anesthetized intravenously with 8 mg/kg of sodium methohexitol (Lilly) and 100 mg/kg of alpha-chloralose (Sigma Chemical) 10% w/v in polyethylene glycol 400. Additional alpha-chloralose 50 mg/kg was given when required to maintain the level of anesthesia during the experiment. Electrical testing of the heart was conducted no less than 30 min after anesthetic administration. The animals were ventilated with 100% oxygen via a cuffed endotracheal tube by means of a Harvard respiratory pump. The pump was adjusted to maintain the arterial blood pH at 7.35-7.45. A
polyethylene catheter was inserted into the right femoral artery for sampling arterial blood and for monitoring blood pressure. Both femoral veins were catheterized for administering anesthetic and other drugs specified below. A Statham P23dB transducer, Hellige preamplifier, and American Optical oscilloscope were used to record systemic arterial pressure. Mean pressures were obtained by electrically integrating the pulsatile output of the transducer. The system was calibrated using a mercury manometer standard.

Cardiac Testing

Electrical testing of the heart was accomplished using an intracavitary lead system, as previously reported.28, 30, 31 The unit consisted of two catheters which were bound together side by side with silk suture. One catheter was used for ECG recording (Elecatht, 5 mm × 1 mm electrodes, 8 mm apart) and the other (Medtronic, 5 mm × 3 mm electrodes, 16 mm apart) to deliver both pacing and testing impulses to the heart. The recording catheter terminated 30 mm proximally to the tip of the pacing catheter. The catheter unit was positioned under fluoroscopic control at the right ventricular apex via a jugular vein. The distal electrode of the recording catheter and a left femoral needle electrode were used to provide a continuous oscilloscope display (American Optical) of the intracavitary electrogram. A Medtronic pacemaker was employed to deliver rectangular pulses of 2 msec duration at 280 msec intervals. This rate (214 beats/min) was required to override spontaneous heart rates following vagotomy. The pacemaker current was adjusted to twice the mid-diastolic threshold. The distal pole of the pacemaker catheter was made cathodal. Testing impulses were generated by a specially designed device consisting of a pacemaker-triggered time delay circuit and a constant current pulse generator with an electrically isolated output.

Determination of the RE threshold was accomplished in the following manner: With heart rate maintained constant, a testing cycle lasting 4 sec was established. This was comprised of pacing for 3 sec, followed by delivery of a test impulse, and a 1 sec pause, after which pacing was resumed. In successive testing cycles, beginning with the midportion of the T wave, test impulses were delivered progressively earlier by 1 msec intervals. A 2 mA rectangular stimulus of 2 msec duration was employed for this exploration. If no RE resulted, the interval was rescaned in 1 ma current increments. Once a stimulus current of 20 ma was reached, 2 ma increments were employed. The RE threshold was taken to be the minimum current intensity at which RE responses occurred in two out of three trials.

The occurrence of VF was uncommon using this method; however, in the rare instance when it developed, the heart was defibrillated within 15 seconds by a D.C. pulse (200 Wsec capacitor discharge from a Lown cardioverter) delivered through a pair of aluminum plates (100 cm²) which were previously fastened on either side of the thorax.

Vagosympathetic Trunk Stimulation (VS)

The cervical vagosympathetic trunks were sectioned bilaterally 2 cm below the level of the carotid bifurcation in 19 dogs. The distal cut ends of the vagi were mounted on insulated bipolar electrodes. A rest period of 10 min was allowed for complete recovery of the vulnerable period threshold following vagal manipulations.16 Nerve stimulation was accomplished using rectangular pulses of 5 msec duration, 40 Hz and 3–15 V. Stimulation voltages were independently adjusted so that asystole was produced with either right- or left-sided vagal excitation. Repetitive extrastyle threshold was determined before, during, and after bilateral VS. Heart rate was maintained constant at 214 beats/min during threshold testing.

Methacholine (MCh) Infusion

Twenty-three dogs with intact vagus nerves were employed. The muscarinic agent, acetyl-(D,L)-beta-methylcholine chloride (J.T. Baker Co.) in normal saline solution was infused intravenously using a Harvard infusion pump. The infusion was started at 1 µg/kg/min and was increased in 1 µg/kg/min steps at 30 min intervals up to a maximum rate of 5 µg/kg/min. This infusion schedule was found to produce maximal changes in RE threshold. Determinations of heart rate, cardiac rhythm, arterial blood pressure and RE threshold were made at each infusion level (i.e., after 30 min at a given infusion rate). The highest RE threshold attained was noted in each animal.

The effect of an intravenous injection of the selectively muscarinolytic drug, atropine sulfate (0.2 mg/kg, Lilly), on the MCh-induced changes in RE threshold was studied in four dogs. Atropine was administered during the peak effect of MCh on the RE threshold. MCh infusion was maintained constant throughout. Threshold determinations were made immediately after injection of atropine and at 2 min intervals until the RE threshold recovered to the pre-injection level.

In 11 dogs, beta-adrenergic blockade with intravenous propranolol (0.25 mg/kg, Inderal, Ayerst) was induced prior to MCh infusion. This dose of propranolol is sufficient to block humoral and most neural adrenergic influences without altering myocardial membrane stability.30 MCh infusion was instituted 10 min after propranolol administration. The RE threshold response to MCh in these beta-blockaded animals was studied as outlined above. When the experiment required more than 2 hours, an additional 0.15 mg/kg of propranolol was given.

Norepinephrine (NE) Infusion and VS

The effect of NE infusion on the RE threshold was studied in 16 vagotomized dogs. Norepinephrine bitartrate (Levophed, Winthrop) in normal saline was infused intravenously at rates of 0.25, 0.5, and 1.0 µg (base)/kg/min. An infusion period of 10 min was allowed prior to testing the effect of NE on RE threshold. In eight animals, the blood pressure response to NE was not controlled, whereas in the remaining eight dogs, the blood pressure rise was prevented by controlled exsanguination according to the method of Mundschau et al.49 An expandable polyethylene bladder containing 100 ml of heparinized saline was placed in a pressurized reservoir of 50 liters capacity. The bladder was connected to cannulas placed in the femoral arteries. Air pressure around the bladder was maintained at the animal's control arterial pressure by a regulated compressor (Cordis).
The objective of controlling blood pressure in these latter experiments was to examine the effect of NE on the RE threshold in the absence of catecholamine-induced hypertension. These diverse studies were accomplished with and without bilateral VS.

The results were analyzed using Students' t-test for paired data. The criterion of significance was \( P < 0.05 \).

**Results**

**Vagosympathetic Trunk Stimulation (VS) and the RE Threshold**

The effect of VS on the RE threshold was studied in 19 dogs. The increase in RE threshold during vagal stimulation was 24 ± 6\% (mean ± SEM; range from 19 ± 2 ma to 24 ± 2 ma) \( (P < 0.001) \).

**Methacholine (MCh) and the RE Threshold**

The RE threshold response to muscarinic stimulation produced by intravenous MCh was investigated in 23 dogs. MCh infusion was associated with a nearly immediate fall in arterial blood pressure (mean pressure decreased 20 to 50 mm Hg) which was followed within 10 sec by a sinus tachycardia, presumably of reflex origin. The heart rate and pressure responses to MCh showed considerable individual variation. Cardiac arrhythmias, including atrial fibrillation, various degrees of heart block, and asystole (rate maintained by pacing) were also noted within the first few minutes following MCh infusion. These arrhythmias, as well as the alterations in heart rate and arterial blood pressure, generally stabilized within 15 to 20 min after the onset of MCh infusion. The heart rate stabilized at 141 ± 11 beats/min (control 138 ± 8, NS) and the mean arterial blood pressure stabilized at 86 ± 9 mm Hg (control 130 ± 6, \( P < 0.0001 \)).

The effect of MCh on the RE threshold was examined during this period of relative hemodynamic stability. MCh resulted in a maximal increase of 98 ± 12\% \( (P < 0.0001) \) in the vulnerable period threshold (fig. 1). This usually occurred at an infusion rate of 4 \( \mu \)g/kg/min. The threshold remained elevated as long as MCh infusion was continued, which was five hours in the longest case. In no case was tachyphylaxis noted. Upon cessation of infusion, the threshold returned to the control value within 20 min.

**Atropine and Effects of MCh**

The effect of administering the selectively muscarinicolytic agent, atropine, on the MCh-induced increase in RE threshold was examined in four of the 23 dogs. In all four animals, atropine returned the threshold to, or below, the value observed prior to MCh infusion (fig. 1). This occurred immediately after atropine administration. The RE threshold usually recovered to the MCh-induced level within 6 to 10 min after the atropine injection. MCh infusion was maintained constant throughout.

**Propranolol Administration**

Beta-adrenergic blockade was induced with propranolol prior to MCh infusion in 11 dogs. Propranolol resulted in a 76 ± 25\% \( (P < 0.01) \) increase in RE threshold. In these animals, MCh infusion produced no further increase in the RE threshold (fig. 2).

**Effect of VS on RE Threshold during Norepinephrine (NE) Infusion**

To explore vagal and humoral adrenergic interactions on ventricular vulnerability, the effect of VS on the RE threshold during NE infusion was studied in 16 dogs. In the eight dogs in which the blood pressure response to NE was not controlled, NE at infusion rates of 0.25 and 0.5 \( \mu \)g/kg/min decreased the threshold by 18 ± 5\% \( (P < 0.025) \) and 35 ± 13\% \( (P < 0.025) \) respectively. Norepinephrine at 1.0 \( \mu \)g/kg/min did not significantly alter the threshold (fig. 3, upper panel). This was the only rate which produced a significant increase in mean systemic arterial blood pressure. In the eight dogs in which the pressor response was abolished by controlled exsanguination, NE produced a significant decrease in threshold at all infusion rates tested. Vagal stimulation applied during NE infusion restored the RE threshold to the control level. This occurred irrespective of the NE infusion rate (fig. 3, lower panel).

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Effect of the muscarinic agent methacholine (MCh) on the repetitive extrasystole (RE) threshold in 23 dogs. Infusion of MCh produced a 98 ± 12\% increase in the RE threshold. This increase in threshold was abolished by the concurrent administration of the selectively muscarinicolytic agent atropine in four dogs. \( (1 ± \text{the standard error of the mean}).\)

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Repetitive extrasystole (RE) threshold response to MCh during beta-adrenergic blockade with propranolol in 11 dogs. Propranolol \( (P) \) infusion (0.25 mg/kg) was associated with a 76 ± 25\% increase in threshold as compared with control. Subsequent administration of MCh produced no further change in ventricular vulnerability.
Discussion

Stimulation of the vagosympathetic trunk has been shown by Kent et al. to increase substantially the fibrillation threshold of the normal, as well as of the acutely ischemic heart. The antifibrillatory effect was ascribed to a direct action of the cholinergic neurotransmitter on the specialized conducting system. However, the vagus is a mixed nerve. Stimulation of the cervical vagus may elicit nicotinic and adrenergic as well as muscarinic effects. Moreover, Kolman et al. in our laboratory, recently demonstrated that the effect of vagal stimulation on ventricular vulnerability is significantly related to the prevailing level of adrenergic tone. It therefore becomes relevant to determine which component of vagal action affects changes in cardiac vulnerability. The present study thus had two objectives: to assess muscarinic vagal action on the vulnerable period threshold, and to define muscarinic-adrenergic interactions on this myocardial property.

Vagal stimulation in the absence of adrenergic activation produced only a moderate increase (24 ± 6%) in the vulnerable period threshold. The vagus-induced changes in vulnerability are comparable to those obtained by Kolman et al. in open-chested dogs. It is unclear whether the increase in threshold observed during vagal stimulation in the absence of stellate ganglion stimulation or norepinephrine infusion reflects a direct effect of the vagus on ventricular vulnerability or is due to antagonism of tonic adrenergic activity. The fact that beta-adrenergic blockade with propranolol increased the vulnerable period threshold in the resting condition suggests that tonic adrenergic activity was indeed operative in the present study.

Administering the muscarinic agent methacholine (MCh) resulted in a 98% increase in repetitive extrasystole (RE) threshold. Enhancement in RE threshold reflects a similar degree of alteration in cardiac vulnerability to VF. As early as 1935, Hoff and Nahum reported that the susceptibility to VF resulting from alternating current shock in the cat was substantially reduced by MCh. Our studies have demonstrated that the MCh protection against VF is completely abolished by atropine, thus providing further support for the interpretation that vagal action on cardiac vulnerability is mediated through muscarinic action.

Are the muscarinic effects of MCh on ventricular vulnerability likewise influenced by the level of adrenergic activity? In propranolol-treated animals, MCh did not exert any additional enhancing action on the RE threshold beyond that achieved by the propranolol alone. The increase in threshold by MCh thus appears to result from a significant extent from antagonism of adrenergic action. This view is consistent with the studies of Hoff and Nahum who showed that, in cats presensitized with either benzol or chloroform inhalation, MCh afforded protection against catecholamine-induced ventricular arrhythmias including VF. An antagonistic effect of MCh on arrhythmias provoked by catecholamines has also been demonstrated in man.

The present findings do not explain the quantitative difference in the vagal and MCh effects on ventricular vulnerability. The following possibilities deserve further consideration: 1) vagal effects may be blunted by nicotinic and adrenergic effects which do not attend cholinergic stimulation with MCh; 2) there may be muscarinic cholinergic receptors which are not innervated by the vagus nerve; 3) the quantities of acetylcholine released by electrical stimulation of the vago sympathetic trunk may be different from those released during physiological activation of cholinergic pathways to the heart.

Cellular and biochemical studies indicate that muscarinic inhibition of sympathetic effects on the heart may be accounted for by inhibition of release of neurotransmitter from sympathetic nerve endings or by attenuation of response to norepinephrine (NE) action at the receptor level. We have shown that vagal stimulation can completely prevent the effect of infused NE on ventricular vulnerability. Thus, a muscarinic effect at a receptor site may be the basis for this interaction on cardiac vulnerability. This helps explain the findings of Kent et al. which appear to contradict our observations. In dogs treated with the catecholamine depleting drug 6-hydroxydopamine, these workers noted that vagal stimulation continued to augment the VF threshold. Although 6-hydroxydopamine significantly reduces myocardial NE content, it also supersensitizes the heart to NE and
increases adrenomedullary catecholamine turnover. It is conceivable, therefore, that the basis for the vagal effect on ventricular vulnerability in these “catecholamine depleted” animals was due to muscarinic antagonism of NE released from the adrenal gland.

The effect of vagal stimulation on ventricular vulnerability appears to differ from the action on cardiac chronotropy and inotropy. Whereas a vagally-induced change in vulnerable period threshold did not occur during beta-adrenergic blockade, others have found that pretreatment with propranolol did not completely preclude the effects of vagal stimulation on myocardial contractility and chronotropic properties. It is unclear whether this represents a fundamental difference in mechanism or merely a quantitative difference in the degree of beta-adrenergic blockade required to preclude a given cardiovascular effect of vagal antagonism to the residual adrenergic tone.

The present study provides additional insights related to the influence of pharmacologic doses of NE on ventricular vulnerability. The prevailing view is that promulgated by Hoffman, Han and their respective coworkers, and holds that exogenous NE induces only transient decreases in the vulnerable period threshold. Han et al. administered 2–3 μg/kg/min of NE and ascribed the brief reduction of threshold to the inhomogeneous myocardial distribution of the neurotransmitter at the very inception of the infusion. Once myocardial levels became uniform, NE was devoid of effect. Our findings permit a differing interpretation. We noted that when the pressor response to NE was prevented by controlled exsanguination, a significant and sustained reduction in the vulnerable period threshold occurred. Moreover, when suppressor doses of NE (0.25 to 0.5 μg/kg/min) were employed, a consistent effect on cardiac vulnerability was still present. This lower infusion level coincides with the maximal secretory rate of the adrenal medulla during acute experimental coronary artery occlusion in the dog. The most likely explanation for the failure of previous investigators to demonstrate a sustained reduction of threshold by NE relates to the employment of excessive and unphysiologic doses of this humoral agent. The ensuing pressor effect resulted in reflex withdrawal of sympathetic neural tone which cancelled the direct action of NE on the myocardium. In this regard, Verrier et al. have shown that acute arterial hypertension produced by phenylephrine or aortic obstruction raised the fibrillation threshold, an effect which can be abolished by baroreceptor denervation or beta-adrenergic blockade.

Acknowledgment

The authors acknowledge the design and construction of electronic equipment by Mr. Nicholas Jordan, and the technical assistance of Messrs. George LeBrun and Samuel Rivers.

References

2. Garrey WE: Some effects of cardiac nerves upon ventricular fibrillation. Am J Physiol 21: 283, 1908
3. Levy AG: The exciting causes of ventricular fibrillation in animals under chloroform anesthesia. Heart 4: 319, 1913
7. MacWilliam JA: Fibrillar contraction of the heart. J Physiol (Lond) 8: 296, 1887
36. Matta RJ, Verrier RL, Lown B: The repetitive extrasystole as an index of
Interobserver Variability in Coronary Angiography

Leonard M. Zir, M.D., Stephen W. Miller, M.D., Robert E. Dinsmore, M.D., John P. Gilbert, Ph.D., and J. Warren Harthorne, M.D.

SUMMARY Four experienced coronary angiographers (two radiologists and two cardiologists) independently assessed the location and degree of coronary artery stenosis, and the location and degree of left ventricular wall motion abnormalities in 20 coronary angiograms.

Marked interobserver variability was noted in quantifying percent coronary artery stenosis and degree of left ventricular wall motion abnormalities. For example, in only 13/20 (65%) of the coronary angiograms did all observers agree about the significance of a stenosis (defined as greater than 50% in diameter luminal narrowing) in the proximal or mid left anterior descending coronary artery.

DESPITE GENERAL ACCEPTANCE among physicians that coronary cineangiography provides an objective, accurate method of diagnosing the presence and severity of coronary artery disease, its precision has not been fully examined. The present communication addresses one potential limitation of coronary angiography, namely, interobserver variability.

Methods

Four experienced coronary angiographers from the Massachusetts General Hospital independently evaluated 20 coronary angiograms for the presence and location of coronary artery stenosis and location and degree of left ventricular wall motion abnormality. Although the background and experience of the four angiographers differed in some ways, each angiographer has performed or interpreted at least 1500 coronary angiograms. Two observers, JWH, a cardiologist, and RED, a radiologist, introduced coronary angiography to the Massachusetts General Hospital in 1967. The other two observers, LMZ, a cardiologist, and SWM, a radiologist, are senior members of the Catheterization Laboratory staff of the Massachusetts General Hospital. The 20 good quality coronary angiograms used in this study were selected by a senior X-ray technician from the 600 coronary angiograms performed at the Massachusetts General Hospital from January to June, 1974. Starting with the first coronary angiogram performed in January, 1974, approximately every twentieth coronary angiogram was selected for analysis. The senior X-ray technician, who is in a large part responsible for angiographic quality control in our laboratory, was instructed to select only angiograms of good quality. In this study good quality was based upon the visual recognition of an acceptable diagnostic coronary angiogram. The optimal resolution of our cineangiographic system was measured by placing a star phantom on the image intensifier screen and exposing the phantom image on 35 mm Dupont SF-2 film. The cine of the star phantom was projected on a Tajarno-35 projector and demonstrated a resolution of 2 line pairs per mm.

None of the observers participated in the selection of the angiograms and none of the angiograms were of patients followed clinically by the observers. As one or more of the observers may have interpreted the angiograms prior to the present study, the angiograms were blinded as to patient vulnerability to ventricular fibrillation. Am J Physiol: 1976 (in press)
44. Hoff HE, Nahum LH: The role of adrenaline in the production of ventricular rhythms and their suppression by acetyl-beta-methylcholine chloride. J Pharmacol Exp Ther 52: 235, 1934
Muscarinic effects of vagosympathetic trunk stimulation on the repetitive extrasystole (RE) threshold.
S H Rabinowitz, R L Verrier and B Lown

Circulation. 1976;53:622-627
doi: 10.1161/01.CIR.53.4.622

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1976 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on
the World Wide Web at:
http://circ.ahajournals.org/content/53/4/622