Effect of Vagal Stimulation on the Overflow of Norepinephrine into the Coronary Sinus during Cardiac Sympathetic Nerve Stimulation in the Dog

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SUMMARY In anesthetized dogs with the chest open, supramaximal stimulation of the left cardiac sympathetic nerves at 2 and 4 Hz produced an increase of 40-50% in ventricular contractile force (CF) and of 40-65% in coronary sinus blood flow. At these frequencies of stimulation, norepinephrine (NE) overflow into the coronary sinus was 29.8 ± 5.1 (SE) and 54.9 ± 13.2 ng/min, respectively. Concurrent, supramaximal vagal stimulation, at a frequency of 15 Hz, had no significant effect on coronary sinus blood flow, but caused a 25% reduction in CF and a 30% decrease in NE overflow. The changes in CF and NE overflow evoked by vagal stimulation were prevented by atropine. These results are consistent with the hypothesis that there are muscarinic receptors on the postganglionic sympathetic terminals in the walls of the ventricles. Acetylcholine released during vagal stimulation combines with these receptors, causes a reduction in the liberation of NE, and thereby attenuates the positive inotropic response.

COMPLEX interactions between the sympathetic and parasympathetic innervations of the heart are known to occur. One type of cardiac autonomic interaction has been termed "accentuated antagonism"; it is characterized by a progressive attenuation of the cardiac effects of activity in one division of the autonomic nervous system as the level of antagonistic activity in the other division is increased. Two types of mechanisms have been proposed to account for accentuated antagonism: (1) a cholinergically mediated reduction in the quantity of norepinephrine (NE) released in response to a given level of cardiac sympathetic activity, and (2) a cholinergically mediated reduction in the cardiac response to a given quantity of released NE.

Our present series of experiments was designed to test the first hypothesis. Previous experiments by Löffelholz and Muscholl on isolated, perfused rabbit hearts demonstrated that an infusion of acetylcholine caused a reduction in the quantity of NE released in response to a given stimulus to the cardiac sympathetic nerves. In a subsequent study on isolated rabbit atria, these investigators showed that the quantities of acetylcholine liberated during vagal stimulation were adequate to curtail the rate of release of NE during sympathetic activity. In that study, the ventricles were removed because it was thought that "their vagal innervation is poor." However, numerous studies have shown that vagal stimulation does have a significant negative inotropic effect on ventricular contractility. Furthermore, it has been demonstrated that this effect is more pronounced the greater the background level of cardiac sympathetic activity. In the present study, we have conducted tests to determine whether this sympathetic-vagal interaction, as it involves ventricular contractility, is at least partially dependent on a vagally mediated reduction in NE release at sympathetic postganglionic terminals.

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Methods

All experiments were conducted on mongrel dogs anesthetized with sodium pentobarbital, 30 mg/kg, iv. To minimize the effects of withdrawal of blood for chemical determinations, dextran was infused continuously.

The chest was opened through the 4th intercostal space. Heparin, 500 units/kg, was injected intravenously to prevent blood coagulation, and a modified Morawitz cannula was introduced into the coronary sinus through the azygos vein. The tip of the cannula was fixed in position by a suture placed around the coronary sinus, within 1 cm of its ostium. Consequently, most of the coronary sinus blood was drained by the cannula. The coronary venous blood was conducted from this cannula through the extracorporeal probe of an electromagnetic flowmeter (Biotronix) and was returned via the right external jugular vein. Right ventricular force was recorded by a Walton-Brodie strain gauge arch sutured about halfway between the apex and base of the heart and about 1 or 2 cm lateral to the anterior descending coronary artery. The two feet of the arch were aligned in parallel to the anterior descending coronary artery. Contractile force, femoral arterial blood pressure, and coronary sinus flow were recorded on a Brush Mark 260 oscillograph.

The cervical vagosympathetic trunks were transected and shielded bipolar palladium electrodes were applied to the cardiac ends of both nerves. Tight ligatures were applied about the upper poles of both stellate ganglia; previous experiments have shown that, in the dog, this procedure virtually abolishes the tonic sympathetic discharge to the heart. A shielded bipolar electrode was placed about the left ansa subclavia. The left side was selected for stimulation because it has been demonstrated that the positive chronotropic effects of stimulating the left cardiac sympathetic fibers are small compared to those caused by right-sided stimulation. The ansa subclavia was stimulated by means of a Grass S4 stimulator with supramaximal (usually 8 V) rectangular pulses, 1 msec in duration, for each of two periods of 15 minutes. There was a rest period of 10 minutes between the two periods of sympathetic stimulation.
Two sets of experiments were conducted. For the first we used a group of seven dogs which weighed, on the average, 20.3 ± 0.6 (SEM) kg; their hearts weighed 155 ± 9 g. Frequencies of 2 and 4 Hz were used during the 15-minute periods of sympathetic stimulation; the order of application of these two frequencies was selected by chance in each experiment. For the second group of experiments we used six dogs which weighed, on the average, 21.8 ± 0.8 kg; their hearts weighed 153 ± 8 g. During both 15-minute periods of sympathetic stimulation we used a frequency of 2 Hz. However, during the 10-minute rest period between the two stimulation periods, atropine sulfate (1 mg/kg, iv) was injected. Just before and just after the second period of stimulation we verified that this dose of atropine was adequate to abolish completely the bradycardia or asystole produced by strong vagal stimulation.

Throughout each period of sympathetic stimulation (except in the experiments conducted after the injection of atropine), the atria and ventricles were paced synchronously at a constant rate that just exceeded the rate produced by sympathetic stimulation. Each of the 15-minute periods of sympathetic stimulation was subdivided into five consecutive 3-minute periods. The first, third, and fifth such periods consisted of the sympathetic stimulation alone, at either 2 or 4 Hz. During the second and fourth periods, the vagi also were stimulated. The two vagal electrodes were connected in parallel to the stimulus isolation unit of a Grass S4 stimulator. The stimuli were supramaximal (usually 10 V), 1 msec in duration, and applied at a frequency of 15 Hz.

NE was assayed in arterial and coronary sinus blood by the fluorometric methods of Anton and Sayre and Laverty and Taylor. Blood samples were collected at the end of each 3-minute interval during the two 15-minute periods of cardiac neural stimulation. The rate of endogenous NE overflow during neural stimulation was computed as the product of the coronary sinus blood flow and the NE concentration in the coronary sinus blood. In computing the rate of NE overflow, it was assumed that the small quantity of NE in the coronary arterial blood was completely cleared in 3 minutes.

The composite data for the seven animals in the first series of experiments are presented in Figure 2. The heights of the open bars represent the mean values of the various responses during the three alternate 3-minute periods of sympathetic stimulation alone. For each animal the values during these three periods were averaged. The heights of the diagonally hatched bars represent the mean values of the responses during combined vagal and sympathetic stimulation. In each experiment, the values of the two periods of combined stimulation were averaged. The paired t-test was used to determine the significance of the various differences; hence, the standard errors of the various mean values are not incorporated in the figure.

During sympathetic stimulation alone at a frequency of 2 Hz, CF increased by 47.3 ± 6.2% (SE). When the vagi were stimulated also, there was a consistent reduction in CF from the peak attained with sympathetic stimulation alone (Fig. 2). When the differences in CF during sympathetic stimulation alone and during combined vagal and sympathetic stimulation were measured for each animal, the mean difference for the entire group was found to be 10.4 ± 1.2% (SE) of the mean control CF ($P < 0.001$).

During sympathetic stimulation alone at 4 Hz, CF increased by 38.9 ± 9.3% above the control value. This value was somewhat less than that obtained with stimulation at 2 Hz. This result is unusual, in that during preliminary observations an immediate increase in stimulation frequency from 2 to 4 Hz always was followed by an increase in CF, and a reduction in stimulation frequency from 4 to 2 Hz was always followed by a decrease in CF. The smaller response at 4 Hz than at 2 Hz observed in Figure 2 was mainly attributable to the tendency for the response to become

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

**Figure 1** The changes in right ventricular contractile force during cardiac neural stimulation in a representative experiment. Beginning at arrow 1, the left ansa subclavia was stimulated at 8 V, 1 msec, 2 Hz, for 15 minutes; 3 minutes later (arrow 2) both vagosympathetic trunks were also stimulated at 10 V, 1 msec, 15 Hz for a train duration of 3 minutes; arrow 3 marks the end of that period of vagal stimulation. Atria and ventricles were paced synchronously at a frequency of 160 beats/min.

**Results**

Figure 1 is a record of the effects of sympathetic and vagal stimulation on myocardial contractile force (CF) in a representative experiment. Sympathetic stimulation (beginning at arrow 1), increased CF by 48% above the control value. With concomitant vagal stimulation (arrow 2), CF fell to 30% above control (panel C). On the cessation of vagal stimulation (arrow 3), CF increased again to 42% above control (panel D). Thus, the increment in CF above control during combined sympathetic and vagal stimulation was only about 65% of the average increment during the preceding and following periods of sympathetic stimulation alone. In other words, vagal stimulation appeared to reduce the positive inotropic effect of a given level of sympathetic stimulation by about 35%.
stimulation at 2 and 4 Hz, and during supramaximal vagal sinus blood flow, and norepinephrine overflow in seven dogs during periods 2 and 4.

The responses to sympathetic stimulation (unshaded bars) in each experiment are the averages of the responses during periods 1, 3, and 5. The vagi were stimulated concomitantly with the sympathetic nerves during periods 2 and 4.

The responses to combined neural stimulation (diagonally hatched bars) in each experiment are the averages of the responses during periods 2 and 4. The responses to combined neural stimulation (diagonally hatched bars) in each experiment are the averages of the responses during periods 2 and 4.

The coronary sinus blood flow increased from a mean control value of about 29% of the control level in this second group of animals during sympathetic stimulation alone. With the addition of vagal stimulation, the mean reduction in CF was 10.8 ± 2.3% of the mean control CF (P < 0.001, by the paired t-test).

Coronary sinus blood flow increased as described in the legend for Figure 2. The mean right ventricular contractile force, coronary sinus blood flow, and norepinephrine overflow in seven dogs during a prestimulation control period, during supramaximal sympathetic stimulation at 2 and 4 Hz, and during supramaximal vagal stimulation at 15 Hz combined with sympathetic stimulation. In each animal sympathetic stimulation at each frequency was continuous for 15 minutes but was subdivided into five consecutive 3-minute observation periods. The responses to sympathetic stimulation (unshaded bars) in each animal are the averages of the responses during periods 1, 3, and 5. The vagi were stimulated concomitantly with the sympathetic nerves during periods 2 and 4.

Throughout the 15-minute period of continuous sympathetic stimulation alone at 2 Hz, the increment in CF was well maintained. At 4 Hz, however, there was usually a progressive decline in CF. Such progressive reductions in response to sympathetic stimulation at the middle to upper levels of the physiological frequency range have been observed previously.

Of much greater relevance to the present study is the response to superimposed vagal stimulation. During continuous sympathetic stimulation at 4 Hz, just as at 2 Hz, the addition of vagal stimulation produced a consistent reduction in CF. For the entire group of animals the mean difference in CF during sympathetic stimulation alone and during combined vagal and sympathetic stimulation was 10.2 ± 4.2% of the control CF (P = 0.025). Thus, the mean difference in CF produced by vagal stimulation during sympathetic stimulation at 4 Hz was virtually the same as the difference found for sympathetic stimulation at 2 Hz (10.4%).

The coronary sinus blood flow increased substantially during sympathetic stimulation; the increment was greater at 4 Hz than at 2 Hz (P = 0.05). However, superimposing vagal stimulation did not significantly alter the coronary sinus blood flow at either stimulation frequency.

The overflow of NE into the coronary sinus was 29.8 ± 5.1 ng/min during sympathetic stimulation alone at 2 Hz. The mean difference in NE overflow during sympathetic stimulation alone and during combined vagal and sympathetic stimulation was 9.9 ± 2.8 ng/min (P = 0.001, by the paired t-test). Thus the superimposition of vagal stimulation decreased the NE overflow by about one-third of the rate found during sympathetic stimulation alone. The rate of NE overflow was 54.9 ± 13.2 ng/min during sympathetic stimulation alone at 4 Hz. This rate was about twice that which was found at a frequency of 2 Hz. The mean difference in NE overflow during sympathetic stimulation alone at 4 Hz and during combined sympathetic and vagal stimulation was 16.1 ± 2.6 ng/min (P < 0.001), a reduction of about 29% of the value during sympathetic stimulation alone.

In the second series of experiments there were two 15-minute periods of sympathetic stimulation at 2 Hz, separated by a 10-minute rest period. Atropine sulfate, 1 mg/kg, was given near the beginning of the rest period, and cardiac pacing was discontinued. The responses to neural stimulation are displayed in Figure 3. Before atropine, the responses resembled those during sympathetic stimulation at 2 Hz in the first series (Fig. 2). CF increased by 24.1 ± 5.9% (SE) above the control level in this second group of animals during sympathetic stimulation alone. With the addition of vagal stimulation, the mean reduction in CF was 10.8 ± 2.3% of the mean control CF (P < 0.001, by the paired t-test).

The mean right ventricular contractile force, coronary sinus blood flow, and norepinephrine overflow in six dogs before and after the intravenous administration of atropine sulfate, 1 mg/kg. The left cardiac sympathetic nerves were stimulated supramaximally at 2 Hz for 15 minutes both before and after atropine. Each stimulation period was subdivided into five intervals, as described in the legend for Figure 2.
level of 30.0 ± 2.9 ml/min to a mean value of 44.4 ± 4.0 ml/min during sympathetic stimulation alone. With concurrent vagal stimulation the mean blood flow decreased slightly to 41.1 ± 3.5 ml/min. The NE overflow increased from close to zero to a mean value of 33.2 ± 3.8 ng/min during sympathetic stimulation alone. The mean reduction in the rate of NE overflow was 11.2 ± 2.1 ng/min ($P < 0.001$) during concurrent vagal stimulation.

As shown in the right half of Figure 3, the changes produced by vagal stimulation all were abolished by atropine. Sympathetic stimulation alone at 2 Hz resulted in a 69.2 ± 17.4% increase in CF. The much greater increase in CF after atropine probably can be attributed to the absence of cardiac pacing. The atria and ventricles were paced synchronously before atropine because strong vagal stimulation would ordinarily produce marked sinus bradycardia and third-degree atrioventricular block. After atropine, pacing was no longer necessary. Of greater relevance to the present study, the reduction in CF ordinarily evoked by concurrent vagal stimulation was abolished by atropine. Similarly, after atropine the increase in coronary sinus blood flow evoked by sympathetic stimulation was unaffected by vagal stimulation. The rate of NE overflow increased after atropine from near zero to a level of 25.8 ± 5.4 ng/min during sympathetic stimulation. This value was less than that observed before atropine, although the difference was not significant statistically ($P = 0.3$). Concurrent vagal stimulation produced no appreciable reduction in NE overflow (a change of only 0.2 ± 0.9 ng/min).

Discussion

The vagally induced reduction in ventricular CF observed in our present study confirms the results of numerous previous experiments. The changes in CF in our study probably reflect a true negative inotropic vagal effect on the ventricular myocardium, because most of the other principal experimental variables that could alter CF were either held constant or were observed not to change appreciably during vagal stimulation. Synchronous atrioventricular pacing prevented adventitious changes in ventricular CF in two ways: (1) it prevented the changes in contractility which are associated with the force-frequency relationship, and (2) it averted the changes in ventricular preload which are associated with the marked changes in atrial contractility produced by vagal stimulation. The coronary blood flow during combined vagal and sympathetic stimulation was not significantly different from that during sympathetic stimulation alone (Fig. 2). Therefore, the vagally induced change in CF could not be attributed to a concomitant change in coronary blood flow. Finally, there was no significant change in arterial blood pressure when vagal stimulation was superimposed on cardiac sympathetic stimulation.

As argued previously, the vagal stimulation which is shown in this study (Figs. 2 and 3) must be derived from nerve terminals in the ventricular walls, but they can account for only a small fraction of the NE that overflows into the coronary sinus blood.

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Triggered Mitral Valve Activity/Wit and Cranefield

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SUMMARY The action potential of cardiac fibers in the anterior mitral valve leaflet of the monkey heart is followed by an after-hyperpolarization. The addition of catecholamines causes a delayed after-depolarization to follow the after-hyperpolarization. The amplitude of the after-depolarization increases as the stimulus cycle length is decreased, or after premature stimulation, and as a result can reach threshold to yield nondriven, sustained rhythmic activity which we term triggered activity. This sustained rhythmic activity can be terminated by a single, appropriately timed, premature stimulus. The amplitude of the action potentials of mitral valve fibers is increased by catecholamines; the amplitude and rate of depolarization are depressed by verapamil. The amplitude of the action potentials is little affected by tetrodotoxin (TTX) but the maximum rate of depolarization is reduced by TTX. The delayed after-depolarization induced by catecholamines is abolished by verapamil, as is triggered activity. These observations suggest that mitral valve fibers generate slow response action potentials, that triggerable sustained rhythmic activity may be a property of the slow response and that such activity may cause the types of cardiac arrhythmias that usually are attributed to reentry.

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WE HAVE described, in Purkinje fibers exposed to sodium-free solutions, a form of sustained rhythmic activity that can be initiated and sometimes terminated by a single stimulus and that is probably not reentrant.1-4 The driven action potential that triggers such activity is followed by an early after-hyperpolarization and a delayed after-depolarization.

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