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.

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 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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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 during its passage through the coronary vascular bed. We believe this is a reasonable assumption. However, the results presented below do not depend appreciably on the validity of this assumption. In the first group of animals the NE concentrations in the arterial blood were only 18 ± 3% (SE) and 15 ± 3% of the levels in the coronary sinus blood during sympathetic stimulation alone at 2 and 4 Hz, respectively. In the second group of animals the NE concentrations in the arterial blood were 13 ± 3% and 17 ± 3% of the concentrations in the coronary sinus blood during sympathetic stimulation alone before and after atropine, respectively. Under each of these conditions, moreover, superimposition of vagal stimulation had no detectable effect on the NE concentration in the arterial blood. Hence, computing NE overflow either on the basis of the coronary sinus NE concentration or on the basis of the atrioventricular concentration difference would not substantially influence the overall results.

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%.

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.
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).

Coronary sinus blood flow increased from a mean control

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

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

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

![Figure 3](http://circres.ahajournals.org/)

**Figure 3** 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 rise 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. 10-16 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, 23 and (2) it averted the changes in ventricular preload which are associated with the marked changes in atrial contractility produced by vagal stimulation. 15, 24 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.

The change in CF did not reflect an alteration in cardiac pacing. The atria and ventricles were paced synchronously 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).

The data obtained in our study support the contention of Löffelholz and Muscholl 24 that the vagally mediated antagonism of the cardiotoxic effects of sympathetic stimulation is accomplished, at least partially, by a reduction in the rate of release of NE. The overflow of NE into the coronary sinus reflects the balance between NE release and subsequent reuptake by the sympathetic postganglionic nerve terminals. In the present study, no effort was made to determine whether the vagally mediated reduction in NE overflow (Figs. 2 and 3) represents diminished liberation or enhanced reuptake of this neurotransmitter. However, it has been shown for the perfused rabbit heart that acetylcholine does not block the uptake of NE. 26 It is highly probable, therefore, that the reduced overflow of NE during vagal stimulation in our experiments reflects diminished release rather than increased reuptake.

The experiments of Löffelholz and Muscholl which employed vagal stimulation were restricted to atrial tissue. 2-4 we have extended their findings to the cardiac ventricles. In our experiments, only a small fraction of the NE appearing in the coronary sinus blood during sympathetic stimulation originated in the left atrium; the vast majority was derived from ventricular tissue. The atria do receive an abundant sympathetic innervation; per unit of weight, they are probably more richly innervated than the ventricles. The myocardial catecholamine concentration is an index of the density of postganglionic sympathetic terminals, 30-34 and it has been amply demonstrated that the catecholamine concentration in the left atrium is 2 to 3 times as great as that in the left ventricle. 32-33 However, the drainage of venous blood from the left atrium into the coronary sinus is only a small fraction of that derived from the left ventricle. Furthermore, the coronary blood flow to the left atrium is only about 3% or 4% of the total coronary blood flow. 35 A significant fraction of this small flow does not drain into the coronary sinus, but rather enters the left atrial cavity through Thebesian channels. 44 Hence, most of the norepinephrine that overflows into the coronary sinus must be derived from nerve terminals in the ventricular walls, and only a small fraction is derived from atrial nerve endings. Therefore, in the present study the changes in NE overflow caused by vagal stimulation must reflect changes taking place predominantly in the ventricular walls. Undoubtedly, similar alterations also are occurring in the atrial walls, but they can account for only a small fraction of the NE that overflows into the coronary sinus.

Therefore the reduction in ventricular CF evoked by vagal stimulation which is shown in this study (Figs. 2 and 3) must have been caused, at least in part, by a reduction in the rate of release of NE. The blockade of this effect by atropine (Fig. 3) supports the contention of Muscholl and his collaborators 26-28 that there are muscarinic inhibitory receptors on the terminal sympathetic nerve fibers. The data we have presented in this paper show that such muscarinic receptors must be located on the sympathetic terminals in the walls of the ventricles as well as in other regions of the heart. Also, the vagal and sympathetic nerve endings must lie in close enough proximity to each other so that the acetylcholine released from the vagal endings can combine with the muscarinic receptors on the sympathetic terminals.

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Triggered Activity in Cardiac Muscle Fibers of the Simian Mitral Valve

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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 cause the types of cardiac arrhythmias that usually are attributed to reentry.

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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