ABSTRACT

In the canine isovolumetric left ventricle preparation, stimuli were applied to the efferent end of a transected cervical vagus nerve. The changes in left ventricular systolic pressure in response to vagal stimulation were compared under control conditions and when ventricular contractility was enhanced by various kinds of inotropic stimulation—left stellate ganglion stimulation, paired pacing, calcium chloride infusions, aminophylline infusions, and acetylstrophanthidin injections. Vagal stimulation under control conditions caused mean decreases in pressure in the various groups ranging from 13.2 to 16.2% of the control level. The depressant effect of vagal stimulation was potentiated during sympathetic stimulation, in confirmation of previous findings; the mean decrease in pressure produced by vagal stimulation during concurrent sympathetic stimulation varied from 20.1 to 20.8%. Calcium and aminophylline infusions had no significant effect on the ventricular response to vagal stimulation; the percent reductions in pressure caused by vagal stimulation were 18.1 and 15.5%, respectively. However, the response to vagal stimulation was markedly attenuated during paired pacing and after acetylstrophanthidin; the percent reductions in pressure caused by vagal stimulation were 5.0 and 9.4%, respectively. The potentiation of the response to vagal stimulation during increased cardiac sympathetic activity probably represents a specific adrenergic-cholinergic interaction.

ADDITIONAL KEY WORDS autonomic nervous system
inotropic stimuli sympathetic-parasympathetic interaction
cardiotonic agents adrenergic-cholinergic interaction paired pacing
aminophylline calcium acetylstrophanthidin cardiac glycosides

When ventricular myocardial contractility is enhanced by sympathetic neural activity, the negative inotropic effect of efferent vagal stimulation is potentiated (1). It has not yet been determined whether the greater efficacy of vagal stimulation is ascribable specifically to the increased sympathetic activity per se or whether it represents a general phenomenon that appears whenever myocardial contractility is enhanced.

In the experiments to be described, the ventricular myocardial responses to stimulation of a vagus nerve were compared under the following conditions: (1) control, (2) during concurrent cardiac sympathetic stimulation, and (3) while myocardial contractility was facilitated by paired pacing, calcium...
chloride infusions, aminophylline infusions, or acetylstrophanthidin injections.

**Methods**

Canine isovolumetric left ventricle preparations were made as described previously (1-3). Mongrel dogs were anesthetized with morphine sulfate (2 mg/kg, im), followed in 30 minutes by an intravenous infusion of urethane (400 to 800 mg/kg) and chloralose (40 to 60 mg/kg). A plastic cannula in the right cardiac chambers drained the venous return to a rotating-disc oxygenator. Blood from the oxygenator was pumped to an overflow reservoir at a hydrostatic level above the animal equivalent to about 100 mm Hg. To perfuse the coronary circulation, blood from this reservoir was conducted to the aortic arch through a cannula in the proximal segment of the ligated left subclavian artery. The aortic arch was ligated about 2 cm distal to the origin of the left subclavian artery.

Once total heart bypass was established, all possible reflex influences were precluded by sectioning both vagosympathetic trunks at the midcervical level, sectioning the communicating rami to both stellate ganglia, and rendering the central nervous system ischemic by ligating the right subclavian artery at its origin and both common carotid arteries at the midcervical level. Arterial occlusions at these levels destroy central nervous system function, but preserve peripheral vagal and sympathetic impulse conduction for considerably longer periods than if the brachiocephalic artery had been ligated at its origin from the aorta.

Left ventricular performance was assessed by measuring the pressure generated within a balloon inserted into the left ventricular cavity, as described previously (1-3). Pressure was registered on a Brush Mark 200 recorder by means of a Statham P23AA strain gauge. Atria and ventricles were paced synchronously by a Grass S-4 stimulator, with isolation unit. In 13 experiments, paired pacing was accomplished with the same stimulator and electrodes. The optimal delay between the paired stimuli was determined by trial and error in each experiment. Two other Grass S-4 stimulators with isolation units were used for efferent vagal and sympathetic stimulation.

**Experimental Protocol**

In the experiments involving any of the nonadrenergic positive inotropic stimuli except acetylstrophanthidin, the effects of 30 seconds of unilateral efferent vagal stimulation (10 v, 2 msec, 15 pulses/sec) on left ventricular systolic pressure were usually determined eight times in the absence of sympathetic stimulation, four times before and four times after the positive inotropic stimulus. The percent reduction in pressure after each period of vagal stimulation was determined and the average was calculated. We previously found (1) that the percent reduction in pressure caused by a stimulus to a vagus nerve was constant for a given inotropic state of the ventricular myocardium. When the absolute magnitude of the pressure was varied over a wide range by changing the initial length of the myocardial fibers, the percent reduction induced by vagal stimulation remained constant (1).

Once before and once after the nonadrenergic positive inotropic stimulus in each experiment, the left stellate ganglion was stimulated at a frequency which would increase left ventricular systolic pressure to approximately twice the control value. The usual stimulation factors were 7 v, 2 msec, 2 to 3 pulses/sec. Once the steady-state response to cardiac sympathetic stimulation was attained, the changes in pressure in response to two successive 30-second periods of concurrent vagal stimulation were determined. The average of the four responses was calculated for each experiment, and represents the ventricular response to vagal stimulation during concurrent sympathetic excitation.

Additionally, the average response to two successive vagal stimulations was determined during the steady state of the nonadrenergic positive inotropic stimulus. Aminophylline (Searle), 10 mg/ml, or calcium chloride, 40 mg/ml, was administered at a constant rate by a Harvard infusion pump into the perfusion tubing proximal to the cannula in the left subclavian artery. The rate of infusion was adjusted to produce a steady-state left ventricular systolic pressure approximately twice the control level. The steady-state level was usually attained in 3 to 5 minutes. After the response to vagal stimulation was ascertained, the infusion was discontinued. The pressure then fell to or toward the control level, and the steady-state recovery level was usually reached in 3 to 5 minutes.

With paired pacing and with calcium chloride and aminophylline infusions, the effects were rapidly reversible, so that the influence of vagal stimulation before and after the positive inotropic stimulus could be determined. However, the effects of acetylstrophanthidin (San doz) were much more protracted. Therefore, the responses to vagal stimulation after acetyl strophanthidin (150 μg in 0.15 ml 47.5% ethanol) were compared with the response to vagal stimulation after injecting an equal volume of

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Effects of vagal stimulation on left ventricular systolic pressure under control conditions (B and F), during left stellate ganglion stimulation (C and G), and during paired pacing (E). Stimulation of the cardiac end of the cut right vagus nerve at 10 v, 2 msec, 15 pulses/sec, for 30 sec, is denoted by the downward deflection of the event marker in B, C, E, F, and G. Stimulation of the left stellate ganglion at 7 v, 2 msec, 2 pulses/sec, is denoted by the upward deflection of the event marker in C and G. A and D were recorded at a paper speed of 50 mm/sec to display the left ventricular pressure tracings under control conditions (A) and during paired pacing (D). All other panels were recorded at a paper speed of 0.5 mm/sec.

Results

PAIRED PACING

Figure 1 shows portions of the left ventricular pressure tracings from a representative experiment in which paired pacing was applied. Under control conditions (B), the stimulus to the right vagus nerve produced a 14% reduction in left ventricular systolic pressure. When the left stellate ganglion was stimulated, this pressure rose from 83...
mm Hg to a maximum of 154 mm Hg (C). Under these conditions, concurrent vagal stimulation caused a 17% decrease in left ventricular systolic pressure.

In the experiment, additional stimuli were applied to the vagus in the presence and absence of stellate stimulation, but the records are not included in Figure 1. Then paired pacing was instituted (D and E). Initially, the pressure increased to over 200 mm Hg, but it gradually decreased. Two successive stimuli to the vagus (E) decreased pressure by only 5.1 and 5.2%, respectively. When the level of pressure after vagal stimulation was different from that before, as in this example because of its gradual decline during paired pacing, the percent reduction caused by vagal stimulation was calculated on the basis of the average of values just before and just after each vagal stimulation.

When paired pacing was discontinued (arrow, E), left ventricular systolic pressure decreased to about 50 mm Hg, which was considerably below the control level prior to paired pacing. Thereafter, the pressure gradually improved over the next several minutes. Similar instances of depressed contractility following paired pacing have been reported previously (5, 6). As shown in F, which was recorded 6 minutes after cessation of paired pacing, vagal stimulation diminished pressure by 14%, which is virtually identical to the response displayed in B, prior to paired pacing. During subsequent left stellate ganglion stimulation (G), vagal stimulation reduced systolic pressure by 23%.

The composite data from the 13 experiments in which paired pacing was employed are summarized at the left in Figure 2. Vagal stimulation, in the absence of sympathetic stimulation or paired pacing, resulted in a mean reduction of 13.2% ± 1.8 (SE) in left ventricular systolic pressure. During stellate ganglion stimulation, vagal stimulation reduced the pressure by a mean value of 20.1 ± 2.2%, a significantly greater effect (P < 0.005). Conversely, during paired pacing, the effect of vagal stimulation was significantly attenuated (P < 0.005), reducing pressure by a mean of only 5.0 ± 1.5%. Stellate stimulation increased it by 82.9 ± 8.9 mm Hg, and paired pacing produced a mean rise of 91.2 ± 10.6 mm Hg in this series of 13 experiments.

CALCIUM

The composite data for the 12 experiments in which CaCl₂ was infused are displayed in the middle section of Figure 2. In this series, the average reduction of left ventricular sys-

![Graph](https://example.com/graph.png)
Systolic pressure by vagal stimulation under control conditions was 16.2 ± 2.3%. Stellate ganglion stimulation resulted in a mean increase of 82.3 ± 7.8 mm Hg. During this sympathetic stimulation, vagal stimulation caused a 20.3 ± 2.2% reduction of pressure, which was significantly greater than that produced under control conditions (P < 0.005). Infusion of CaCl₂ raised pressure 90.3 ± 10.0 mm Hg. The average reduction in pressure produced by vagal stimulation during the infusion of CaCl₂ was 18.1 ± 2.5%. This was somewhat greater than the value obtained under control conditions, but the difference was of questionable significance statistically (0.1 > P > 0.05). The percent reduction in pressure by vagal stimulation during stellate ganglion stimulation was significantly greater than that elicited during the CaCl₂ infusion (P = 0.05).

**FIGURE 3**

Effects of vagal stimulation on left ventricular systolic pressure under control conditions (B and E), during left stellate ganglion stimulation (C and F), and after acetylstrophanthidin (G). Left vagal stimulation (10 v, 2 msec, 15 pulses/sec for 30 sec) is denoted by the downward deflection of the event marker in panels B, C, E, F, and G. Left stellate ganglion stimulation (7 v, 2 msec, 4 pulses/sec) is denoted by the upward deflection of the event marker in panels C and F. At the arrow in D, 0.15 ml of 47.5% ethanol was injected into the coronary arterial perfusion line. At the arrow in G, acetylstrophanthidin, 150 µg in 0.15 ml of 47.5% ethanol, was injected into the coronary arterial perfusion line. A and the right-hand segment of G were recorded at a paper speed of 50 mm/sec to display the left ventricular pressure curves under control conditions (A) and after acetylstrophanthidin (G). Paper speed in all other records was 0.5 mm/sec.
AMINOPHYLLINE

The composite data from the 11 animals in which aminophylline was infused are shown in Figure 2. Stimulation of the vagus under control conditions resulted in a 16.1 ± 2.1% mean decrease in pressure. Infusion of aminophylline caused a mean increase of 75.6 ± 6.4 mm Hg. During the infusion of aminophylline, vagal stimulation reduced pressure by 15.5 ± 2.1%, which was not significantly different from the control effect (P > 0.1). Stellate ganglion stimulation raised pressure by 79.8 ± 9.0 mm Hg. During this stimulation, vagal stimulation diminished pressure by 20.4 ± 2.4%, which was significantly greater than the effect of vagal stimulation during control conditions (P < 0.005).

ACETYLSYROPHANTHIDIN

Because the effects of acetylstrophanthidin were not reversible within the time limits of the experimental preparation, the experimental design was somewhat different from that used for the other positive inotropic agents. The effect of vagal stimulation after acetylstrophanthidin was compared with that before, either in the same animal or in a different animal.

The tracings in Figure 3 illustrate the influence of acetylstrophanthidin on the efficacy of vagal stimulation in a representative experiment. Initially, vagal stimulation decreased pressure by 20% (B); During stellate ganglion stimulation, the decrease was 26% (C). Administration of 0.15 ml of the solvent (47.5% ethanol) caused a transient reduction in pressure (arrow, D). Five minutes later, vagal stimulation under control conditions (E) decreased pressure by 18%, but during concurrent sympathetic stimulation (F), the decrease was 21%.

In six animals, vagal stimulation 5 minutes after 0.15 ml of 47.5% ethanol decreased pressure by an average of 12.9%; the decrease before ethanol was 13.9% (Fig. 4). This difference was not statistically significant. These observations provide control data for the effects not only of the small volume of ethanol but also for the passage of a specific time period (5 minutes) in this preparation. Vagal stimulation during sympathetic excitation in these same experiments reduced pressure 18.8%, which was significantly greater (P < 0.005) than that during control conditions (13.9%).

In a separate series of six animals, 150 μg acetylstrophanthidin, dissolved in 0.15 ml of 47.5% ethanol, was injected instead of the ethanol alone (Fig. 4). Before acetylstrophanthidin was given, vagal stimulation alone decreased pressure by an average of 14.8%; during concurrent sympathetic excitation, the decrease was 20.8% (P < 0.005). Five minutes after acetylstrophanthidin was
given, vagal stimulation decreased pressure by only 9.8%, significantly less than before acetylstrophanthidin \( P < 0.005 \).

In five of the six animals given the control injection of 47.5% ethanol, 150 \( \mu \)g of acetylstrophanthidin was subsequently administered. An example is shown in G of Figure 3; acetylstrophanthidin was injected at the arrow. From a preinjection level of 81 mm Hg, pressure rose to 111 mm Hg within 3 minutes after the injection; at 5 minutes, vagal stimulation reduced pressure by only 13%; reductions under control conditions were 20% \( (B) \) and 18% \( (E) \).

At the bottom left of Figure 4 are the composite data from the five experiments in which acetylstrophanthidin was administered after the control observations of the results of ethanol alone. Prior to acetylstrophanthidin, vagal stimulation alone decreased pressure by an average of 12.8%; during concurrent sympathetic excitation, the decrease was 20.8% \( (P < 0.005) \). Five minutes after acetylstrophanthidin administration, vagal stimulation reduced pressure 9.0%, less than the effect before acetylstrophanthidin, a difference of only borderline significance \( (P = 0.07) \), probably because of the small sample size.

The data from all experiments in which acetylstrophanthidin was given were combined, and the composite results are also shown in Figure 4 \( \text{(bottom right)} \). The average reduction in pressure \( (20.8 \pm 2.5\%) \) produced by vagal stimulation during sympathetic excitation was significantly greater \( (P < 0.005) \) than that produced by vagal stimulation alone \( (13.9 \pm 1.9\%) \), whereas 5 minutes after acetylstrophanthidin the average reduction \( (9.4 \pm 1.7\%) \) was significantly less \( (P < 0.005) \). The mean increase in pressure produced by acetylstrophanthidin was 62.0 \( \pm 7.1 \) mm Hg, whereas that produced by stellate stimulation was 90.0 \( \pm 8.3 \) mm Hg.

**Discussion**

The fractional reduction in left ventricular systolic pressure caused by efferent vagal stimulation was consistently greater with than without an appreciable background of sympathetic neural activity, in confirmation of previous findings \( (1) \). However, when ventricular myocardial contractility was enhanced by paired pacing, by calcium chloride or aminophylline infusions, or by acetylstrophanthidin injections, the depressant effect of vagal stimulation was not potentiated. On the contrary, with paired pacing and acetylstrophanthidin, the influence of vagal stimulation was actually attenuated. It is probable, therefore, that the greater influence of vagal activity on ventricular performance during concurrent sympathetic activity represents a specific adrenergic-cholinergic interaction, rather than a nonspecific response that develops whenever ventricular contractility is enhanced.

The observed adrenergic-cholinergic interaction may be mediated in two ways. First, the norepinephrine released at the sympathetic terminals might in some manner potentiate the negative inotropic effects of the parasympathetic activity. Second, the released acetylcholine might partially block the positive inotropic effects of the concurrently liberated norepinephrine. Experiments have been described which show that both mechanisms are possible, but the relative importance of each remains to be established.

Sympathetic potentiation of parasympathetic influences on the heart may be produced by changes in potassium levels; cardiac sympathetic stimulation produces a sudden, brief uptake of potassium by the heart \( (7) \), and the responsiveness of the cardiac pacemaker to vagal stimulation is quite sensitive to the level of extracellular potassium \( (8) \). An alternative explanation of this sympathetic potentiation is that catecholamines enhance the excitability of parasympathetic ganglia \( (9) \). Studies of end-plate potentials in skeletal muscle strongly suggest that epinephrine increases the quantity of acetylcholine released per nerve impulse in motor nerve terminals \( (10) \). If this is true of cardiac parasympathetic ganglia or at the postganglionic vagal terminals, an
augmented cardiac response to parasympathetic activity would result, even if the level of activity in preganglionic parasympathetic pathways remained constant.

The other major possibility, a partial adrenergic blockade by acetylcholine, has been advanced previously (11, 12) and has been correlated with certain metabolic changes, notably in relation to glycogen metabolism and cyclic AMP. Acetylcholine is a potent inhibitor of the glycogenolytic action of epinephrine in guinea pig hearts (13). Sympathetic stimulation increases phosphorylase a activity in rat heart, and this effect is antagonized by acetylcholine (14). Catecholamines accelerate the formation of cyclic AMP in certain preparations of heart muscle, and this is counteracted by acetylcholine (15). The correlation between myocardial cyclic AMP and the contractile state of the heart strongly suggests that cyclic AMP may play a direct or indirect role in the development of the inotropic response (16, 17), and in the adrenergic-cholinergic interaction as well.

The mechanisms responsible for the attenuation of the response to vagal stimulation during paired pacing and after acetylstrophanthidin administration have not been established. In addition to diminishing the efficacy of vagal stimulation, paired pacing results in a decreased responsiveness to norepinephrine or calcium gluconate infusions (18). However, the diminished responsiveness to these positive inotropic agents is probably related to the fact that paired pacing itself induces a nearly maximal increase in contractility (18).

The digitalis glycosides interact with many chemical agents, notably the cations, potassium and calcium (19-21), and certain sympathomimetic and sympatholytic agents (22-24). The attenuated response of the ventricular myocardium to vagal stimulation after acetylstrophanthidin in the present study is the reverse of the phenomenon observed at the cardiac pacemaker, where the response to vagal stimulation is potentiated by the cardiac glycosides (25-28). Evidently, different mechanisms are involved in the cholinergic interactions with acetylstrophanthidin at the pacemaker and myocardial cells.

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