Excitation of Afferent Cardiac Sympathetic Nerve Fibers Induced by Vagal Stimulation

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SUMMARY
The effect of vagal stimulation on activity of afferent sympathetic nerve fibers from the dog's left ventricle has been examined. During partial constriction of the coronary artery, a brief electrical stimulation of the cervical vagus nerves resulted in a decrease in blood flow of the constricted artery, systolic bulge of the left ventricle, elevation of the ST segment of electrocardiogram and excitation of the afferent nerve fibers, which continued for up to 15 min. These changes were not produced without coronary artery constriction. Intravenous injection of phentolamine eliminated the decrease in blood flow, and suppressed systolic bulge, elevation of the ST segment and excitation of afferent fibers. Propranolol could not eliminate the decrease in blood flow while suppressed the other changes. Atropine eliminated all of these changes. The results indicate participation of adrenergic α-receptors in sustained decrease of coronary blood flow and excitation of afferent cardiac sympathetic nerve fibers which can be produced by a brief vagal excitation.

Additional Indexing Words:
Coronary constriction  Coronary blood flow  Systolic bulge  Phentolamine  Propranolol  Atropine

It is clinical evidence that anginal attack in a certain group of patients with coronary insufficiency occurs spontaneously.1)-7) In these patients, the attack is frequently associated with bradycardia and a fall in systemic blood pressure.2),3),6),7) In addition, the attack can be evoked by administration of cholinergic agents and can be prevented with atropine.2),7) The fact suggests participation of vagal augmentation in initiation of anginal attack.

Evidences indicate participation of afferent cardiac sympathetic nerve fibers in anginal pain in man8) and the pseudoadverse response in animals.9)-11) Although process of excitation of the afferent fibers during myocardial ischemia has been clarified considerably,11)-19) it is unclear whether and through what mechanisms vagal augmentation results in excitation of the afferent fibers which participate in nociception of the heart. This study was carried out...
to examine the effect of electrical stimulation of the cervical vagus nerves on activity of afferent sympathetic nerve fibers from the left ventricle.

**METHODS**

1. **Surgical Preparations**

   Experiments were performed on adult mongrel dogs under intravenous pentobarbital sodium anesthesia (35–40 mg/Kg). The trachea was intubated for artificial positive pressure respiration with air. The upper 8 ribs on the left side were removed. The anterior aspect of the heart was exposed by pericardiotomy. The proximal segment of the anterior descending branch of the left coronary artery was dissected free of surrounding tissues and a screw clamp was placed on it for constriction or occlusion.

   A catheter 1 mm in external diameter was introduced in retrograde fashion into a peripheral branch of the anterior descending branch to monitor the peripheral blood pressure. Another catheter was inserted into the right femoral artery to monitor the systemic blood pressure. In several occasions, a catheter was introduced into a small branch of the circumflex branch to monitor the pressure of the non-constricted coronary artery. A strain gauge arch was sewn to the anterior wall of the left ventricle distal to the screw clamp to measure the active force achieved by the region of the myocardium. The force was expressed as left ventricular tension.

2. **Recording Afferent Sympathetic Nerve Discharges**

   The left thoracic sympathetic trunk below the 4th communicating ramus, the ipsilateral upper 4 rami, the rostral limb of the left ansa subclavia and the left cervical vago-sympathetic trunk were transected. The 2nd or 3rd ramus was dissected into filaments using a dissecting microscope at 10–20 × magnification. One of the nerve filaments was placed on bipolar platinum-iridium electrodes connected to an a.c. coupled preamplifier. Another pair of stimulating electrodes were placed on ventral limb of the left ansa subclavia. The electrodes and nerve filament were covered with liquid paraffin warmed to 36–37°C.

   Stimuli were delivered by an isolation unit connected to a square-wave stimulator. Pulses were monophasic and 1.0 msec in duration. The evoked action potentials and the time base were displayed on the screen of a cathode ray oscilloscope from which photographic records were made. The distance between the stimulating and recording electrodes was measured by a caliper. Classification of the fibers was made by conduction velocity.\(^5\)\(^{14,20,21,22}\) The fibers which had conduction velocities between 4.5 and 26 M/sec were classified as myelinated Aδ fibers and the fibers which had conduction velocities less than 2 M/sec were classified as unmyelinated C fibers.\(^5\)\(^{13,15}\)

   After recording each action potential, the anterior wall of the left ventricle was tapped with the blunt tip of a glass rod 1 mm in diameter in order to examine whether the fibers in the filament respond to tapping. When no fiber responded to tapping, the anterior descending branch was occluded for a few minutes. When a fiber responded to tapping or occlusion, the fiber was called afferent left ventricular fiber.
The right cervical vago-sympathetic trunk was transected and the vagus trunk was dissected free of the sympathetic trunk. The cut peripheral end of the vagus trunk was placed on bipolar electrodes and a train of square-wave electrical pulses (1 msec, 20 HZ, 20 v, and for 10 sec) was applied on it before and during coronary constriction or occlusion in 23 fiber preparations in 23 dogs. The action potentials evoked by vagal stimulation were recorded with peripheral blood pressure of the coronary artery and left ventricular tension on continuous film strips. At the same time, the action potentials were triggered to a square-wave generator and the output was integrated by a pulse-integrator. The height of the integrated record indicated the number of the action potential per second and was expressed as impulse/sec. Classification of the fibers that responded to vagal stimulation was made by comparing the spike height, spike duration, and configuration of vagally evoked potentials with those evoked by stimulation of the ansa subclavia.14)

3. Recording Efferent Sympathetic Nerve Discharges
The left inferior cardiac nerve which run the junction of the left atrium and the pulmonary veins was transected and the cut central end was placed on bipolar electrodes to measure the action potentials of the efferent sympathetic nerves to the heart in 5 dogs. The right cervical vagus nerve was stimulated with the same train of electrical pulses to observe its effect on efferent sympathetic nerves to the heart.

4. Recording Coronary Blood Flow
A segment of the anterior descending branch proximal to the screw clamp was dissected free of surrounding tissues and a magnetic flowmeter was placed on it in 37 dogs. In 16 of these dogs, vagal stimulation was performed before and during partial constriction of the anterior descending branch. The remaining dogs were not used for vagal stimulation, but were used for examination of the relationship between the peripheral blood pressure and blood flow of the constricted branch. In this series of experiments, a monopolar electrode was fixed to the anterior wall of the left ventricle to measure surface ventricular electrocardiogram. The femoral arterial pressure pulses were used to trigger a square-wave generator and the output was integrated by a pulse-integrator to measure heart rate.

5. Administrations of Chemicals
Vagal stimulation was performed before and after the injections of 0.5 mg/Kg phentolamine into the left jugular vein in 4 dogs which were used for recording afferent discharges. In our preliminary study, the injections of the agent during coronary constriction frequently resulted in ventricular fibrillation. Therefore, the peripheral blood pressure of the constricted coronary artery which was reduced by the agent was elevated to the level of before the injection by constriction of the descending thoracic aorta. This procedure prevented occurrence of ventricular fibrillation.

Vagal stimulation was performed before and 7–10 min after the injections of 0.3 mg/Kg propranolol in 4 dogs which were used for recording the afferent discharges. Vagal stimulation was also performed before and after the injections of 0.5 mg/Kg atropine in 3 dogs which were used for recording the afferent discharge.

Vagal stimulation was performed before and after the injections of 0.5 mg/Kg phentolamine, 0.5 mg/Kg propranolol, and 0.5 mg/Kg atropine in 5, 5 and 2 dogs which were used for recording coronary blood flow, respectively.
RESULTS

1. The Effect of Vagal Stimulation on Afferent Fiber Discharges

Application of square-wave electrical pulses (1 msec, 20 Hz, 20 v, and for 10 sec) to the cut peripheral end of the right cervical vagus trunk was performed in 23 fiber preparations. Twenty fibers were classified as myelinated Aδ fibers and the other 3 as unmyelinated C fibers. All of the fibers were excited by occlusion of the anterior descending branch of the left coronary artery. The mean peripheral blood pressure of the occluded branch was 8.7 ± 2.5 (mean ± SD) mmHg.

Vagal stimulation before coronary constriction caused a fall of as much as 70% of the control value in mean coronary blood pressure and a decrease in heart rate. Immediately after cessation of stimulation, both coronary and systemic blood pressures returned to the control level and overshot. Heart rate increased and left ventricular tension was augmented (Fig. 1). However, no significant increase in impulse frequency of the afferent fibers was ob-

![Fig. 1. The effect of electrical stimulation of the right cervical vagus trunk (ST) on activity of an Aδ fiber from the left ventricle before (A) and during constriction of the anterior descending branch of the left coronary artery (B). From the top channel: Integrated action potentials, peripheral blood pressure of the anterior descending branch which was expressed as coronary blood pressure, heart rate calculated from the systemic blood pressure pulses, systemic blood pressure and left ventricular tension measured from a strain gauge arch.](image-url)
Fig. 2. The effect of vagal stimulation on activity of an A\(\delta\) fiber during coronary artery constriction. The mean C.B.P. before constriction was 103 mmHg. From the top photograph: Before stimulation; immediately after cessation of stimulation; 19 and 53.5 sec after cessation of stimulation. The downward arrows indicate QRS of electrocardiogram. Downward motion of L.V.T. during the phase of systole (3rd and 4th photographs) indicates systolic bulge.

served after cessation of stimulation (Figs. 1 and 3). Quantitative examination of impulse frequency during stimulation could not be made due to stimulation artifact.

After control stimulation experiments, the anterior descending branch was constricted so as to reduce its peripheral blood pressure below the control level but over the level of during occlusion. Constriction of the branch caused an abrupt fall in its peripheral blood pressure. The pressure, however, rose up gradually and attained a stable level in a few minutes. This stable level was called the level caused by constriction. The magnitude of fall in pressure was expressed as percent fall. Left ventricular tension was also reduced by constriction, however, it was gradually restored. Vagal stimulation was performed after the coronary blood pressure became stable. Percent fall in mean coronary blood pressure during constriction was 60–85 in 10 fiber preparations. In these preparations, the magnitude of fall in systemic blood pressure and decrease in heart rate during vagal stimulation were not different from those of control stimulation experiments. Although systemic blood pressure returned to the control level immediately after cessation of stimulation, coronary blood pressure did not return to the level of before stimulation and maintained a lower level for up to 7 min. Left ventricular tension was
reduced throughout the phase of systole, indicating development of systolic bulge. The bulge developed 0–24 sec after cessation of stimulation and continued for up to 6 min. All of the fibers tested began to excite with develop-

Fig. 3. The relationship between percent fall in mean peripheral blood pressure of the constricted coronary artery and the effect of vagal stimulation on activity of afferent nerve fibers. ST = vagal stimulation. c = afferent fiber discharges before stimulation. Latency for development of systolic bulge and its duration are shown in the figure.
ment of systolic bulge and continued to excite even after systolic bulge was replaced by normal modality of myocardial contraction (Figs. 1, 2, and 3). Excitation of the A\(\delta\) fibers was synchronous with each cardiac beat as shown in Fig. 2. On the contrary, excitation of the C fibers was irregular and independent. Details on modality of excitation have been reported elsewhere.\(^{13-15}\)

When percent fall in coronary blood pressure caused by constriction was smaller, development of systolic bulge was less frequent and excitation of the afferent fibers was less obvious (Fig. 3).

Occlusion of the anterior descending branch caused systolic bulge in all preparations. Excitation of the afferent fibers was also produced by occlusion. Vagal stimulation during occlusion caused a further augmentation of systolic bulge and excitation of the afferent fibers (Fig. 3).

2. The Effect of Vagal Stimulation on Coronary Blood Flow

In order to clarify the mechanisms for the long-lasting systolic bulge and excitation of the afferent sympathetic nerve fibers induced by a brief vagal stimulation during coronary artery constriction, the effect of vagal stimulation on blood flow through the constricted coronary artery was examined.

Vagal stimulation before coronary constriction caused an abrupt decrease

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**Fig. 4.** The effect of electrical stimulation of the right cervical vagus trunk (1 msec, 20 Hz, 20 \(v\), and for 10 sec) on peripheral blood pressure of anterior descending branch (ADB) and the circumflex branch (CB), left ventricular tension and blood flow of the anterior descending branch. Upward arrows indicate stepwise constriction of the anterior descending branch. The downward arrows indicate vagal stimulation.
Fig. 5. Upper half: The relationship between percent fall in mean coronary blood pressure (MCBP) and percent decrease in coronary blood flow (CBF) induced by constriction. Open circles = left ventricular tension not affected by constriction. Crosses = reduced systolic development of left ventricular tension. Solid circles = systolic bulge produced. Control MSBP = control mean systemic blood pressure. Control MCBP = control mean coronary blood pressure.

Lower half: The relationship between percent fall in mean coronary blood pressure and percent decrease in coronary blood flow induced by constriction in the preparations which were used for vagal stimulation. Open circles = systolic bulge not produced by vagal stimulation. Solid circles = systolic bulge produced by vagal stimulation.
in coronary blood flow. The flow, however, returned to the control level and overshot immediately after cessation of stimulation. When the coronary blood pressure was lowered by constriction, on the contrary, the blood flow did not return to its level of pre-stimulation and continued to decrease for several minutes (Fig. 4). Responses of systemic blood pressure and the pressure of the non-constricted coronary artery were not different from those of the control stimulation experiments. The decrease in coronary blood flow was accompanied by systolic bulge and a fall in coronary blood pressure. Surface electrocardiogram was recorded in several preparations. Elevation of ST segment and an increase in amplitude of T wave were observed after

Fig. 6. Upper half: The relationship between percent fall in mean coronary blood pressure induced by constriction and the duration of decrease in blood flow of the constricted coronary artery induced by vagal stimulation.

Lower half: The relationship between percent fall in mean coronary blood pressure induced by constriction and the duration of systolic bulge induced by vagal stimulation. In this half are included the results in the preparations which were used for recording afferent fiber discharges.
cessation of control stimulation. Their duration was 8-42 sec. During constriction, on the contrary, the duration of elevation of ST segment was up to 12 min and the amplitude of T wave was reduced after cessation of stimulation (Fig. 7).

The relationship between percent fall in mean coronary blood pressure and percent decrease in coronary blood flow induced by constriction was examined. The relationship was expressed as $Y \text{(percent fall in mean coronary blood pressure)} = 16.6 X^{0.3528} \text{(percent decrease in coronary blood flow)}$. Systolic bulge was produced when percent fall in mean coronary blood pressure became over 75. On the other hand, vagal stimulation produced systolic bulge when percent fall in mean peripheral coronary blood pressure became over 25 (Fig. 5). The duration of decrease in coronary blood flow induced by vagal stimulation became longer when percent fall in mean coronary blood pressure induced by constriction became larger. Also, the duration of systolic bulge induced by vagal stimulation became longer when percent fall in mean coronary blood pressure induced by constriction became larger (Fig. 6). The relationship between the duration of decrease in coronary blood flow and the duration of systolic bulge induced by vagal stimulation was expressed as $Y \text{(duration of systolic bulge)} = 1.213 X \text{(duration of decrease in coronary blood flow)} - 0.1819$, $r = 0.9407$.

In 2 preparations, vagus trunk was stimulated with electrical pulses (1 msec, 5 Hz, 10 v. and for 10 sec). The fall in systemic blood pressure during stimulation was very slight and the decrease in heart rate was up to 25% of the control value. In these experiments, the coronary blood flow increased during stimulation. After cessation of stimulation, the blood flow decreased slightly and systolic bulge was of short duration.

3. The Effect of Phentolamine, Propranolol, and Atropine on Decrease in Coronary Blood Flow Induced by Vagal Stimulation

Vagal stimulation was performed during constriction of the coronary artery before and after the intravenous injection of 0.5 mg/Kg phentolamine in 5 dogs. The peripheral blood pressure of the partially constricted coronary artery which was reduced by phentolamine, was elevated to the level of before the injection by constriction of the descending thoracic aorta. The mean coronary pressure before the injection was 31.2 ± 4.0 mmHg. The pressure was 29.6 ± 2.8 mmHg after aortic constriction. The decrease in coronary blood flow during vagal stimulation was not affected, but the decrease after cessation of stimulation was eliminated by pretreatment with phentolamine (Fig. 7). The fall in coronary blood pressure, development of systolic bulge and elevation of ST segment of electrocardiogram were also suppressed by pretreat-
Fig. 7. A: Before coronary artery constriction. B: Vagal stimulation during coronary artery constriction. C: Vagal stimulation after administration of 0.5 mg/Kg phenolamine. The descending thoracic aorta was occluded to elevate the coronary blood pressure to the level of before the administration of the agent. Systemic blood pressure fell due to aortic occlusion. Heart rate was not recorded. The downward arrows indicate vagal stimulation.
Pretreatment with 0.5 mg/Kg propranolol could not eliminate the decrease in coronary blood flow induced by vagal stimulation, however, it suppressed development of systolic bulge and elevation of ST segment of electrocardiogram. On the other hand, pretreatment with 0.5 mg/Kg atropine eliminated all of the changes induced by vagal stimulation.

4. The Effect of Phentolamine, Propranolol, and Atropine on Excitation of Afferent Nerve Fibers Induced by Vagal Stimulation

The effect of vagal stimulation during coronary artery constriction was examined before and after administration of 0.5 mg/Kg phentolamine. Excitation of the afferent fibers was suppressed by the agent in all preparations. Pretreatment with propranolol also suppressed excitation of the fibers induced by vagal stimulation (Fig. 8). On the other hand, pretreatment with 0.5 mg/Kg atropine eliminated excitation of the fibers induced by vagal stimulation.

5. The Effect of Vagal Stimulation on Activity of the Efferent Sympathetic Nerves to the Heart

![Fig. 8. The effect of vagal stimulation on activity of an Aδ fiber during coronary artery constriction. A: Before the administration of propranolol. B: After the administration of the agent.](image-url)
After transection of the left cervical vago-sympathetic trunk, the action potentials of the efferent fibers in the left inferior cardiac nerves were recorded. The anterior descending branch was not constricted. Excitation of the efferent fibers occurred during vagal stimulation. Excitation began with a fall in systemic blood pressure.

**DISCUSSION**

The results in this study indicate that a brief vagal stimulation can result in a sustained decrease in blood flow of the coronary artery and sustained excitation of the afferent cardiac sympathetic nerve fibers provided when the coronary artery is partially constricted.

The decrease in blood flow of the partially constricted coronary artery occurred during vagal stimulation and again after cessation of vagal stimulation. Since the decrease in both phases was eliminated by pretreatment with atropine, it is likely that cholinergic fibers in the vagus nerves played a major role in initiation of the decrease.

The decrease in coronary blood flow that occurred during vagal stimulation was eliminated by atropine, but was not affected by phentolamine nor by propranolol. In addition, an increase in flow was produced by weak stimulation during which the decrease in heart rate was very small and the fall in systemic blood pressure was slight. Therefore, the decrease in coronary blood flow during vagal stimulation with strong electrical pulses may have been due to extreme bradycardia which was associated with severe hypotension.

Differing from the decrease in coronary blood flow during stimulation, the decrease that occurred after cessation of stimulation was eliminated by phentolamine, but was not by propranolol. Several factors can be pointed out for this decrease: excitation of the adrenergic β-receptors in the coronary artery due to reflex excitation of cardiac sympathetic nerves,23) excitation of the α-receptors due to released epinephrine-like substance caused by released acetylcholine from the cholinergic nerve terminals in the heart,24)-29) direct action of acetylcholine on coronary smooth muscles, and decrease in collateral blood flow supply due to hypotension. In this study, efferent cardiac sympathetic nerves were excited by vagal stimulation probably due to hypotension. In addition, the decrease in blood flow was eliminated by pretreatment with an α-receptor blocking agent with phentolamine. The fact indicates that excitation of the adrenergic α-receptors in the coronary arterial wall due to reflex excitation of the efferent cardiac sympathetic nerves, has played a role in the decrease of coronary blood flow. It is unlikely that muscarinic action
of acetylcholine contributed to the decrease since phentolamine eliminated the decrease. It is also unlikely that decrease in blood flow was due to decreased collateral blood supply since the flowmeter was placed on the coronary artery close to the region of constriction and since the decrease was eliminated by phentolamine. However, it remains to be elucidated whether acetylcholine-induced release of epinephrine-like substance$^{28,29}$ and/or adrenergic fibers in the vagus trunk$^{24}$ have also participated in the decrease in blood flow of the constricted coronary artery.

Coronary artery constriction was necessary for production of sustained decrease in coronary blood flow that occurred after cessation of vagal stimulation. It is likely that the $\beta$-receptors in the coronary arterial wall could not produce effective vasodilator effect because of coronary dilatation due to autoregulation.$^{30,31}$ Consequently, the vasoconstrictor effect of the $\alpha$-receptors was unmasked, leading to contraction of the coronary artery, decreased coronary blood flow and more severe ischemia of the constricted area of the left ventricular wall.

Pretreatment with a $\beta$-adrenergic receptor blocking agent with propranolol could not eliminate the decrease in coronary blood flow that was produced by vagal stimulation while it suppressed systolic bulge, elevation of the ST segment of electrocardiogram and excitation of the afferent nerve fibers. The result suggests that the increased cardiac work load and energy requirements due to excitation of the $\beta$-receptors in the myocardium$^{32}$ have also participated in production of more severe ischemia and excitation of the afferent fibers.

Several seconds of latency was required for development of systolic bulge, ischemic electrocardiographic changes, and excitation of the afferent nerve fibers. This latency may have been due to the time required to consume the stored energy in the myocardium since the latency was not different from that induced by complete coronary constriction.$^{11,15}$

The afferent cardiac sympathetic nerve fibers cause reflex excitation of the efferent sympathetic nerves to the heart and the kidney and accordingly cause a rise in systemic blood pressure.$^{11,33-35}$ In addition, they have an action as nociceptor of the heart.$^{10,11}$ They are composed of myelinated $A\delta$ and unmyelinated $C$ fibers.$^{14}$ The fibers can participate in production of anginal pain in man$^{81}$ as well as the pseudoadflective response in animals.$^{91-111}$ Their response to mechanical and chemical stimuli and modalities of excitation during myocardial ischemia have been reported elsewhere.$^{111-121,15,17,18}$ In this study, it was revealed that modality of excitation of the afferent nerve fibers was the same as that of during complete coronary artery constriction.$^{15}$

Clinical studies suggest contribution of the vagus nerves to initiation of
the attack in a certain group of patients with variant form of angina pectoris.\textsuperscript{2,7} In these patients, reversible coronary artery constriction has been demonstrated by angiography.\textsuperscript{1} However, it remains to be elucidated whether an identical mechanism participates in the clinical and experimental events.

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