Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm

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ABSTRACT We injected acetylcholine (ACh), the neurotransmitter of the parasympathetic nervous system, into the coronary arteries of 28 patients with variant angina. Injection of 10 to 80 μg ACh into the coronary artery responsible for the attack induced spasm together with chest pain and ST segment elevation or depression on the electrocardiogram in 30 of the 32 arteries of 25 of the 27 patients. The injection of 20 to 100 μg ACh into the coronary artery not responsible for the attack in 18 patients resulted in various degrees of constriction in most of them, but no spasm in any of them. After intravenous injection of 1.0 to 1.5 mg atropine sulfate, the injection of ACh into the coronary artery responsible for the attack did not induce spasm or attack in any of the nine coronary arteries injected in eight patients. We conclude that the intracoronary injection of ACh induces coronary spasm and attack in patients with variant angina and that the activity of the parasympathetic nervous system may play a role in the pathogenesis of coronary spasm. We also conclude that the intracoronary injection of ACh is a useful test for provocation of coronary spasm.

Circulation 74, No. 5, 955-963, 1986.

It is now widely accepted that spasm of an epicardial coronary artery (coronary spasm) plays an important role in the pathogenesis, not only of variant angina, but also of other forms of resting angina, some types of exertional angina, and types of acute myocardial infarction. However, the precise mechanism(s) by which coronary spasm occurs remains to be elucidated. Coronary spasm occurs most often when patients are at rest, and is usually not provoked by exercise in the daytime. We have shown that an attack of variant angina or coronary spasm can be induced by subcutaneous injection of methacholine, an analogue of acetylcholine (ACh), which is the neurotransmitter of the parasympathetic nervous system, and that the attack can be suppressed by atropine, a parasympatholytic agent, in some patients with variant angina. Subsequently Endo et al. confirmed our results. On the basis of this fact and the fact that the activity of the parasympathetic nervous system is enhanced at rest and is suppressed by physical activity, we postulated that the activity of parasympathetic nervous system might be related to the pathogenesis of variant angina or coronary spasm.

It has been shown that stimulation of the parasympathetic (vagus) nerve or intracoronary injection of ACh causes coronary vasodilatation, as demonstrated by an increase in coronary blood flow in dogs, and that ACh dilates isolated epicardial coronary arteries in dogs and monkeys. In humans subcutaneous injection of methacholine causes profound dilatation of systemic vasculature, resulting in a transient fall in blood pressure and compensatory tachycardia due to reflex sympathetic discharge. We therefore speculated that coronary spasm might be induced by sympathetic discharge by way of α-adrenergic stimulation. Recently, however, reports have appeared that show that ACh contracts strips of human coronary arteries obtained from hearts of transplant recipients or cadav-
ers.\textsuperscript{17, 19, 20} Moreover, we observed that the intracoronary injection of ACh caused constriction of the coronary artery injected in most adult subjects with angiographically normal coronary arteries and no ischemic heart disease.\textsuperscript{21}

The present study was designed to determine whether ACh, the neurotransmitter of the parasympathetic nervous system, causes coronary spasm when injected directly into the coronary arteries of patients with variant angina.

**Methods**

**Patients.** Twenty-eight patients with variant angina admitted to the Kumamoto University Hospital were studied. Their age, sex, electrocardiographic changes during attack, and results of coronary arteriography are listed in table 1. All patients had attacks of chest pain associated with ST segment elevation on the electrocardiogram occurring at rest, usually from midnight to early morning. In five patients (Nos. 1, 3, 12, 21, and 23) attacks were sometimes associated with ST segment elevation in the precordial leads and at other times they were associated with ST elevation in inferior leads. No patient had a history of myocardial infarction, asthma, or active peptic ulcer. All except two patients (Nos. 1 and 7) had spontaneous attacks within 1 month of the study; patients 1 and 7 had been on dilatiazem and were free from attacks for 5 and 11 months, respectively. All drugs except nitroglycerin were stopped for at least 3 days before the study; nitroglycerin was also stopped at least 2 hr before the study. Written informed consent was obtained from each patient.

**TABLE 1**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr/sex)</th>
<th>ECG changes during attack</th>
<th>Coronary arteriogram after NTG (%) stenosis</th>
<th>Coronary arteriogram after ACh (%) stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} ) or II, III, a( V_{1-4} )</td>
<td>100% S 11</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>50/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{1-4} )</td>
<td>100% S 3</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>52/F</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} ) or II, III, a( V_{1-4} )</td>
<td>100% S 7</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>59/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>99% S 3</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>41/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>99% S 2</td>
<td>90% S 6</td>
</tr>
<tr>
<td>6</td>
<td>40/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>90% S 1\textsuperscript{a}</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>54/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>100% S 6</td>
<td>25% S 6</td>
</tr>
<tr>
<td>8</td>
<td>61/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>100% S 13 \textsuperscript{a}</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>58/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>25% S 13\textsuperscript{a}</td>
<td>Normal</td>
</tr>
<tr>
<td>10</td>
<td>54/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>99% S 2</td>
<td>25% S 2</td>
</tr>
<tr>
<td>11</td>
<td>66/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>100% S 2</td>
<td>25% S 2</td>
</tr>
<tr>
<td>12</td>
<td>50/M</td>
<td>ST ( \uparrow ) in II, III, a( V_{5,6} )</td>
<td>100% S 2</td>
<td>25% S 2</td>
</tr>
</tbody>
</table>

**Coronary arteriography.** Coronary arteriographic examinations (Sones technique\textsuperscript{22}) were done in the morning while patients were in the fasting state. After control coronary arteriograms of the left coronary artery in the right anterior oblique projection and of the right coronary artery in the left anterior oblique projection were obtained by injection of 8 ml of Urografin 76 (Schering AG), a temporary pacemaker was inserted into the right ventricle of each patient and the pacing rate was set at 40 beats/min. Relations between focus spot, the patient, and height of image tube were kept constant.

Acetylcholine chloride (Daichi Seiyaku) dissolved in 5 ml of warmed 0.9% saline in incremental doses of 10, 20, 30, 50, 80, and 100 \( \mu \)g was then injected into the coronary artery presumed to be responsible for the attack, as judged by the leads in which ST segment elevation on the electrocardiogram occurred. A coronary arteriogram was obtained when ST segment changes and/or chest pain appeared or 3 min after each injection. When
Results

The intracoronary injection of ACh caused a transient decrease in heart rate that was dose dependent and tended to be more marked when the injection was into the right than when it was into the left coronary artery. The temporary pacemaker set at 40 beats/min was activated in all patients after the injection of 50 μg ACh into the right coronary artery and in 14 of the 24 patients after the injection of 100 μg ACh into the left coronary artery. The transient reduction in arterial blood pressure was associated with severe bradycardia.

Injection of ACh into the coronary artery responsible for the attack. Injection of ACh into the coronary artery presumed to be responsible for ST segment elevation induced spasm, chest pain, and ST segment changes on the electrocardiogram in 30 of the 32 arteries in 25 of the 27 patients (table 1). Coronary spasm was associated with ST segment elevation in 23 patients and with ST segment depression in five patients. Coronary spasm associated with ST segment changes appeared from 30 sec to 3 min after injection of ACh and disappeared spontaneously or after injection of nitroglycerin into the artery involved in spasm. In five patients (Nos. 1, 3, 12, 21, and 23) whose attacks were sometimes associated with ST segment elevation in the precordial leads and at others with ST elevation in the inferior leads, spasm appeared in the right coronary artery when that artery was injected and in the left coronary artery when it was injected.

The dose of ACh required for provocation of spasm was 10 to 50 μg in the case of the right coronary artery and 10 to 80 μg in the case of the left coronary artery. The segments of coronary artery in which spasm appeared are shown in table 1 according to the classification of American Heart Association Committee Report.23

In two patients (Nos. 22 and 25) whose attacks were associated with ST segment elevation in the inferior leads, injection of 50 μg ACh into the right coronary artery caused 50% and 75% reduction of the diameter of the artery, respectively, but no chest pain or ST segment changes appeared.

Injection of ACh into the coronary artery responsible for the attack after atropine. Atropine sulfate, 1.0 mg, was given intravenously to seven patients (Nos. 2, 8, 18, 21, 23, 26, and 28) after ACh-induced attacks and spasm had been demonstrated and disappeared without nitroglycerin. In one patient (No. 6) 1.5 mg atropine sulfate was given intravenously because marked bradycardia occurred during control coronary arteriography. After administration of atropine, ACh was injected into the nine coronary arteries responsible for attacks in doses of up to 100 μg in the case of the left coronary artery and up to 50 μg in the case of the right coronary artery. After atropine, coronary spasm, ST segment changes, and chest pain were not induced by ACh.

Injection of ACh after phentolamine. Phentolamine did not suppress ACh-induced coronary spasm or attack in any of the five patients who received this drug.

Injection of saline into the coronary artery responsible
for the attack. Injection of warmed 0.9% saline into the coronary artery responsible for the attack did not change the diameter of the artery injected in any of the four patients and the possibility that coronary spasm might have been induced by the intracoronary injection of inert solutions was thus excluded.

Injection of ACh into the coronary artery not responsible for the attack. Injection of ACh in doses of 20, 30, 50, 80, and 100 μg into the left coronary artery and in doses of 20, 30, and 50 μg into the right coronary artery not responsible for the attack in 18 patients caused various degrees of constriction. There was 25% to 75% reduction of diameter in 10 of the 20 left coronary arteries after intracoronary injection of 100 μg of ACh and in two of the four right coronary arteries after intracoronary injection of 50 μg of ACh. Thus, half of the coronary arteries not responsible for attacks constricted significantly in response to ACh. However, there were some arteries that did not narrow and in fact dilated. The response of the arteries to ACh was not uniform along the course of the same artery and also differed among the arteries. Neither chest discomfort nor ST segment changes appeared after this procedure in any of these patients.

Response of noninjected coronary artery to the intracoronary injection of ACh. In 10 patients in whom arteriographic examination of the noninjected and injected coronary arteries was done after the intracoronary injection of ACh, no change in diameter was observed in the noninjected coronary artery.

There was a highly significant difference (p < .001) in the incidence of coronary spasm induced by ACh in patients given atropine and those not given atropine. However, there was no difference in the incidence of ACh-induced coronary spasm in patients given phentolamine and those not given phentolamine. There was a highly significant difference (p < .001) in the incidence of coronary spasm after ACh and after saline were injected into the coronary artery responsible for the attack. There was also a highly significant difference (p < .001) in results of injection of ACh into the coronary artery responsible for the attack and results of injection into the coronary artery not responsible for the attack.

Results in representative patients are shown in figures 1 to 5. In patient 1, who had attacks associated with ST segment elevation in lateral chest and inferior leads, 20 μg ACh in the left coronary artery induced chest pain associated with ST segment elevation in leads I and V4-6, 1 min after its injection. Coronary arteriographic examination at this time showed that spasm appeared and completely occluded the left circumflex artery at the proximal site (segment I1), as shown by the arrow in figure 1, A, but did not appear at the right coronary artery (figure 1, B). Figure 2 shows electrocardiographic results and arterial blood pres-

**FIGURE 1.** Coronary arteriograms from patient 1. Injection of 20 μg ACh into the left coronary artery induced spasm at the proximal site of the left circumflex artery as shown by the arrow in A, but no spasm was demonstrated in the right coronary artery (B). Injection of 20 μg ACh into the right coronary artery, on the other hand, induced diffuse spasm of the right coronary artery, as shown by the small arrows in D, but no spasm was demonstrated in the left coronary artery, including the left circumflex artery (C).
Control

During

Attack

After

Attack

ECG aVF
V_{46}

BP

mmHg

L-ACh 20 μg

1.0 sec

YN51M

FIGURE 2. Electrocardiogram (ECG) and arterial blood pressure before and after injection of 20 μg ACh into the left coronary artery in patient 1. Heart rate and arterial blood pressure did not change significantly after the injection but ST segment elevation in leads I and V_{46} (not shown) associated with chest discomfort appeared 1 min after the injection. ST segment elevation and chest discomfort disappeared spontaneously in 30 sec. Arterial blood pressure was monitored through a Sones catheter in the aorta and could not be monitored during injection of ACh into the coronary artery because the same catheter was used for the injection. BP = arterial blood pressure; L-ACh = acetylcholine injected into the left coronary artery.

FIGURE 3. Electrocardiogram (ECG) and arterial blood pressure before and after injection of 20 μg ACh into the right coronary artery in patient 1. After the injection transient bradycardia associated with a fall in arterial blood pressure appeared and the temporary pacemaker set at 40 beats/min was activated. Seventy seconds later marked ST segment depression in the inferior leads appeared, which disappeared after sublingual administration of 0.3 mg nitroglycerin. Arterial blood pressure was measured as in figure 2. BP = arterial blood pressure; R-ACh = acetylcholine injected into the right coronary artery.
segment depression in the inferior leads, without ST segment changes in the lateral chest leads, and associated with chest pain appeared (figure 3). The coronary arteriogram obtained at this time demonstrated diffuse spasm of the right coronary artery (figure 1, D), but no spasm of the left coronary artery (figure 1, C). Thus, spasm appeared in the artery into which ACh was injected and not in the contralateral artery, irrespective of hemodynamic changes induced by ACh injection. Spasm and ST segment depression disappeared promptly after sublingual administration of 0.3 mg nitroglycerin. In patient 2, whose attack was associated with ST segment elevation in inferior leads, injection of 100 μg of ACh into the left coronary artery caused transient bradycardia associated with a reduction in arterial blood pressure, as shown in the upper panel of figure 4, but neither spasm of the left or right coronary artery nor ischemic ST segment changes appeared (figure 4, top, and figure 5, A). However, when 20 μg of ACh was injected into the right coronary artery, spasm associated with ST segment elevation in the inferior leads appeared after 90 sec, although heart rate and arterial blood pressure remained almost unchanged (figure 4, middle, and figure 5, B). Thus, hemodynamic changes were not necessarily associated with ACh-induced coronary spasm. The spasm and ST segment elevation disappeared spontaneously within 30 sec (figure 5, C), and 1.0 mg atropine sulfate was given intravenously. Five minutes after the administration of atropine, 50 μg ACh was injected into the right coronary artery. Neither changes in heart rate or arterial blood pressure nor spasm or ischemic ST segment changes appeared after the injection, as shown in figure 4, bottom, and figure 5, D. Thus, atropine blocked the action of ACh.

**Discussion**

The present study shows that the injection of ACh, the neurotransmitter of the parasympathetic nervous system, into the coronary artery responsible for an
attack of variant angina induces spasm of the artery that is associated with chest pain and ST segment changes. Moreover, the injection of ACh into the coronary artery not responsible for the attack also resulted in significant constriction of the artery in half of the arteries in this study.

The intracoronary injection of ACh in a high dose caused transient, severe bradycardia accompanied by a fall in blood pressure, particularly when administered into the right coronary artery, an effect to be expected in view of the fact that the sinus and atroventricular nodes are perfused by coronary arteries, particularly by the right coronary artery. This may have triggered a reflex sympathetic discharge, which might have been responsible for coronary spasm and vasoconstriction by way of α-adrenergic receptors. However, the fact that spasm and vasoconstriction occurred in the artery into which ACH was injected even in the absence of noticeable hemodynamic changes and did not occur in the contralateral artery even when hemodynamic changes were severe strongly suggests that spasm and vasoconstriction were caused by a direct cholinergic muscarinic effect of ACh and not by a reflex sympathetic discharge. The fact that the administration of phentolamine, an α-adrenergic-blocking agent, did not suppress ACh-induced coronary spasm and vasoconstriction in any of the patients and the fact that the intracoronary injection of ACh did not induce coronary spasm in any of the patients who had been given atropine, an antimuscarinic drug, also support this contention.

However, the possibility that prejunctional effects of ACh on adrenergic nerve endings inhibited release of norepinephrine and thus limited the sympathetic β-adrenergic relaxation of the coronary artery (as shown in isolated canine epicardial coronary arteries) cannot be excluded by our results.

The responsiveness of the coronary artery to ACh may depend on preexisting coronary tone and it is therefore important to control systemic hemodynamics and determinants of coronary tone in studies with this drug. In a previous report we showed that the intracoronary injection of ACh in the same doses as those used in the present study resulted in constriction of the coronary artery in adult subjects in whom hemodynamics were stable and the heart rate and arterial blood pressure were kept constant by right ventricular pacing. The present study was not performed under conditions of constant heart rate and arterial blood pressure because right ventricular pacing would have made it
difficult to interpret ischemic electrocardiographic changes in patients with variant angina. Also, right atrial pacing was impossible due to the atrioventricular block caused by ACh. Nevertheless, injection of ACh into the coronary artery responsible for the attack induced spasm in 30 of the 32 arteries in 25 of the 27 patients with variant angina and induced coronary vasoconstriction in the two arteries in the remaining two patients. Thus, the coronary artery responsible for attacks in patients with variant angina is very sensitive to ACh and constricts abnormally in response to this agent.

We now routinely use intracoronary injections of ACh to provoke spasm in patients suspected of having coronary spasm. The attack induced by ACh is of short duration, probably because ACh is rapidly destroyed by acetylcholinesterase in vivo. Also, intracoronary injection allows provocation of spasm in the right and left coronary arteries separately in patients who have spasm in both coronary arteries. No adverse effects of the intracoronary injection of ACh were observed, with the exception of transient bradycardia that were easily converted by a pacemaker that had been inserted in the right ventricle.

Coronary spasm occurs most often in patients at rest, particularly from midnight to early morning, and is usually not provoked by exercise in the daytime. It is also well known that the activity of the parasympathetic nervous system is enhanced at rest and is suppressed by physical activity. Our results show that the intracoronary injection of ACh, the neurotransmitter of the parasympathetic nervous system, induces coronary spasm and attack in patients with variant angina. Although the intracoronary injection of ACh is a laboratory artifact that may not mimic the highly selective release of ACh at synaptic membrane sites containing specialized synaptic channels, there is a possibility that the activity of parasympathetic nervous system can play a role in the pathogenesis of an attack of variant angina or coronary spasm. The fact that atropine, a parasympathetic blocking agent, suppressed the ACh-induced attack in the present study and that it prevented attacks in some patients with variant angina, as reported in our previous studies, also supports this concept. However, it must be admitted that cholinergic blockade with atropine does not necessarily prove that there is a neuronal effect of the parasympathetic nervous system, since atropine will act nonselectively on most muscarinic receptors, including those of endothelial cells and muscle cells.

Recently, Furchgott and Zawadzki reported that ACh can reproducibly relax arterial strips in vitro if care is taken to preserve the endothelium during the preparation of the strips. Contraction was observed only when the endothelium was inadvertently removed, suggesting that the direct response of the smooth muscle in the absence of endothelium is contraction. They and others have postulated that a factor that initiates the relaxation of vascular smooth muscle is released by the endothelial cells when they are exposed to ACh.

Most patients with variant angina are over the age of 40, as shown in this series and others. This may indicate that coronary spasm does not usually occur in the young and normal coronary artery, and is somehow related to coronary atherosclerosis. It is known that endothelial injury and proliferation of smooth muscle cells are essential in the pathogenesis of atherosclerosis. It is thus possible that the coronary arteries that are involved in spasm in response to ACh may be in various stages of atherosclerosis with absent or dysfunctional endothelium, although some may appear normal angiographically. There are recent reports that show that atherosclerotic rabbit or canine coronary arteries have increased sensitivity to the vasoconstrictor effect of ergonovine or serotonin.

Alternatively, ACh may cause constriction of epicardial coronary arteries in humans. It has been shown that there is a marked variation in the response to several agonists among animal species. Ginsburg et al. showed that ACh and its analogue, carbachol, contract strips of human coronary arteries obtained from the hearts of transplant recipients. Subsequently, other investigators reported the same results in human coronary arteries from cadavers. However, it is not known whether the endothelium of coronary arteries in these studies was completely normal or not. Apparently, further studies are needed to determine whether ACh or the activity of the parasympathetic nervous system constricts the normal epicardial coronary artery in vivo in humans.

References
PATHOPHYSIOLOGY AND NATURAL HISTORY—VARIANT ANGINA


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_Circulation_. 1986;74:955-963
doi: 10.1161/01.CIR.74.5.955

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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