Pertussis Toxin–Sensitive G Protein Mediates Coronary Microvascular Control During Autoregulation and Ischemia in Canine Heart

Tatsuya Komaru, Yan Wang, Kenjiro Akai, Kouichi Sato, Nobuyo Sekiguchi, Akihiko Sugimura, Toshinobu Kumagai, Hiroshi Kanatsuka, Kunio Shirato

Abstract GTP-binding regulatory proteins (G proteins) regulate various biological functions, but their participation in controlling coronary microvascular tone has not been established yet. The goal of the present study was to elucidate the role of pertussis toxin (PTX)-sensitive G protein in regulating coronary microvascular tone during autoregulation and ischemia. In 42 open-chest dogs, coronary arterial microvessels on the surface of the left ventricle were directly observed by epi-illuminated fluorescence microangiography using a floating objective system. PTX (300 ng/mL) was superfused onto the surface of the left ventricle for 2 hours to block Gα and Gβ protein in epi-myocardial coronary microvessels in vivo. PTX superfusion caused no change in the resting diameters of microvessels and significantly blocked the vasconstriction induced by BHT 920 (a selective α2-agonist). After pretreatment with PTX or its vehicle, the left anterior descending coronary artery (LAD) was occluded by a hydraulic occluder to reduce coronary perfusion pressure (CPP) in a stepwise fashion. A mild stenosis (CPP, 60 mm Hg), a severe stenosis (CPP, 40 mm Hg), and complete occlusion were sequentially produced. Coronary flow velocity in the LAD distal to the stenotic site was continuously monitored. In both PTX and vehicle groups, flow velocity did not significantly decrease during mild stenosis, proving that transmural coronary autoregulatory function was well preserved in the preparation. During severe stenosis and complete occlusion, the coronary flow velocity significantly decreased. In the vehicle group, microvessels <100 μm in diameter significantly dilated in response to the reduction in perfusion pressure (mild stenosis, 6.2±1.9%; severe stenosis, 21.1±4.4%; and complete occlusion, 16.8±5.9%; P<.05 versus baseline diameters). In the PTX group, microvessels did not dilate during each occlusion level (mild stenosis, −2.0±0.9%; severe stenosis, −3.9±1.9%; and complete occlusion, −13.4±2.9%; P<.05 versus vehicle group). PTX did not affect the microvascular dilation caused by nitroprusside. The present data indicate that PTX-sensitive G protein is crucially involved in microvascular control during autoregulation and ischemia. (Circ Res. 1994;75:556–566.)

Key Words • coronary circulation • guanine nucleotide regulatory protein • arterioles • microcirculation • vasodilation

The GTP-binding regulatory protein (G protein), which is a heterotrimer consisting of α, β, and γ subunits, ubiquitously exists in all eukaryocytes and has been well conserved in the process of biological evolution.1,2 The protein plays a pivotal role in the transduction of various biological signals from outside to inside the cell. Although G proteins were originally considered to consist of stimulatory (Gα) and inhibitory (Gβ) proteins that regulate adenyl cyclase activity, recent studies have shown that there are more kinds of G proteins and that they also regulate phospholipase C, phospholipase A2, several ion channels, and retinal cGMP phosphodiesterase as well as adenyl cyclase.1–6 Pertussis toxin (PTX) ADP-ribosylates α subunits of Gα and Gβ proteins and inhibits their functions.7 In the field of vascular physiology, several in vitro studies have implicated the role of PTX-sensitive G proteins in endothelium-dependent vasodilation by some agonists in conduit vessels.8–10 However, the physiological role of G protein in the control of coronary microvascular tone has not been established yet. In the present study, we developed an experimental model to inhibit PTX-sensitive G protein in coronary microcirculation in vivo, and we evaluated the involvement of PTX-sensitive G proteins in determining coronary microvascular tone during autoregulation and ischemia.

Early studies have shown that coronary arterioles <100 μm in diameter dilate in response to the reduction in perfusion pressure.11–13 This response is considered to be an important defense mechanism that preserves coronary flow in the presence of coronary stenosis.14 Recent studies15,16 have reported that this microvascular response is mediated by the ATP-sensitive potassium channel. The mechanisms that modulate the activity of the channels during autoregulation and ischemia, however, remain to be determined. Although the ATP-sensitive potassium channel is regulated by the intracellular ATP level,17 many other factors have been known to affect the activity of the channel.18–24 G protein is also one of these possible modulators. Kirsch et al23 and Ito et al,25 in their patch-clamp studies, have provided evidence that the α subunit of Gβ protein opens the ATP-sensitive potassium channels in the cardiac myocyte, indicating that Gβ protein affects the open probability of the channel without the involvement of second
messengers. Accordingly, we tested the hypothesis that PTX-sensitive G protein mediates the microvascular dilution during autoregulation and ischemia.

Materials and Methods

General Preparation

Mongrel dogs of either sex (n=42; body weight, 4.4 to 10.5 kg; mean, 6.9±0.2 kg) were premedicated with ketamine (50 mg IM) and anesthetized with an intravenous injection of α-chloralose (60 mg/kg, Wako Chemicals). Additional doses of the anesthetic were given as necessary. The animals were intubated with a cuffed endotracheal tube and mechanically ventilated with a respirator (model Nsh-34RH, Harvard Apparatus). A positive end-expiratory pressure of 3 to 5 cm H2O was applied to prevent lung atelectasis. Arterial blood gases were kept within physiological ranges by adjusting the volume or frequency of ventilation or by application of supplemental oxygen. Intravenous infusion of sodium bicarbonate (50 mL; mean, 3000; pH 7.40) at a rate of 60 mL/h was maintained at 37°C and aortic pressure was served with a Statham strain-gauge transducer (model P23, Gould). A heat blanket. The right jugular vein was cannulated for administration of the drug and for the measurement of aortic pressure in the ascending aorta for the measurement of aortic pressure and PTX-sensitive G protein in vivo.

Apparatus). A positive end-expiratory pressure of 3 to 5 cm H2O was applied to prevent lung atelectasis. Arterial blood gases were kept within physiological ranges by adjusting the volume or frequency of ventilation or by application of supplemental oxygen. Intravenous infusion of sodium bicarbonate (50 mL; mean, 3000; pH 7.40) at a rate of 60 mL/h except during the superfusion of the heart. The inner diameter of the microvessels was measured by fluorescent microscopy, which was used as a contrast medium for the present study. The microvessel objective was used for the present study was a Leitz model PL-fl (×10; numerical aperture, 0.30).

Epi-illuminated fluorescence coronary microangiography. Heart rate was kept constant at 140 beats per minute by left atrial pacing after suppression of the sinus node with a local injection of 10% formaldehyde (0.3 to 0.5 mL) into the sinoatrial nodal area. The heart surface was kept moist throughout the experiment, thereby maintaining the area of interest in the microscopic field of view.

For the manipulation of the coronary perfusion pressure, the main trunk of the left anterior descending coronary artery (LAD) was dissected, and a hydraulic occluder was placed around the vessel. A small branch of LAD, which was distal to the occlusion point, was cannulated with a small catheter (outer diameter, 0.67 mm), and the distal coronary pressure was measured. The pressure was transmitted through a 20-MHz small suction-type Doppler probe20 placed on the point distal to the occluder, and the coronary flow velocity was measured by a pulsed Doppler flow velocimeter (model 100, Triton Technology Inc).

Image Acquisition System

For direct visualization of coronary microvessels in beating left ventricles, an intravital microscope system equipped with a floating objective, which was developed in our laboratory, was used. Details of this system have been previously reported.27,28 Briefly, a floating objective consists of a pair of convex lenses that transmit the real image to a standard microscope without any change in magnification. The lower part of the objective facing the heart surface can move perpendicularly in unison with the cardiac motion. The movable portion is lifted by an objective lifter that prevents its direct contact with the heart surface. The upper part of the floating objective was fixed to the stage of a standard microscope. The transmitted real images of the coronary microvessels were observed with a standard microscope. The microscope objective used for the present study was a Leitz model PL-fl (×10; numerical aperture, 0.30).

Microvascular Diameter Measurement

The microvascular images including the plasma layer were enhanced by fluorescein isothiocyanate dextran (30 mg/mL in saline, 0.2 mL), which was injected via the catheter placed in the left atrium each time fluorescent images were obtained. The inner diameter of arterial microvessels was measured on a high-resolution monitor screen (model C1846-01, Hamamatsu Photonics), which was used as a contrast medium for the present study. The microvessel objective was used for the present study was a Leitz model PL-fl (×10; numerical aperture, 0.30).

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superfused at doses of 1, 10, and 100 μmol/L (for 5 minutes at each dose), and hemodynamic variables and the microvascular images were obtained at the end of each application of BHT 920. In dogs pretreated with PTX, endothelin 1 (1 nmol/L) was topically applied after the application of BHT 920 to exclude the possibility that PTX impairs the microvascular contractile apparatus. Blood gases and pH were measured during superfusion of PTX or vehicle and at the end of the experiments.

In another 13 dogs, the effect of PTX on microvascular responses to endothelin 1 was investigated (protocol 1b). After control measurement of monitored variables, PTX (7 dogs) or vehicle (6 dogs) was superfused for 2 hours in the same manner as described above. Thereafter, endothelin 1 (10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, and 10^{-8} mol/L) was sequentially superfused (5 minutes for each concentration). Hemodynamic variables and microvascular diameters were measured every 30 minutes during PTX or vehicle superfusion and at the end of each endothelin 1 superfusion.

In protocol 2 (14 dogs), the involvement of PTX-sensitive G protein in the regulation of coronary microvascular tone during the reduction in perfusion pressure was investigated. After control measurement of monitored variables, PTX (7 dogs) or vehicle (7 dogs) was superfused in the same manner as in protocol 1 for 2 hours. Thereafter, coronary perfusion pressure was reduced in a stepwise fashion to 60 mm Hg (mild stenosis) and to 40 mm Hg (severe stenosis), followed by complete occlusion. Hemodynamic variables, microvascular diameters, and coronary flow velocity data were collected after 5 minutes at each occlusion level. Blood gases and pH were measured every 30 minutes during superfusion of PTX or vehicle and at the end of the experiments. In this protocol, only vessels <100 μm were investigated, because early studies have shown that only small arterioles dilate in response to a reduction in perfusion pressure.

In protocol 3 (3 dogs), the effect of PTX on microvascular responses to sodium nitroprusside was investigated to test whether PTX has a nonspecific effect on microvascular dilator responses. Nitroprusside was sequentially superfused at doses of 1, 10, and 100 μmol/L (5 minutes for each concentration). Thereafter, PTX was superfused in the same fashion as in protocol 1. After pretreatment with PTX, each dose of nitroprusside was again applied.

In all of these protocols, additional doses of α-chloralose (=10 mg/kg) were usually injected every half hour to maintain enough anesthetic level during the 2-hour superfusion of PTX or vehicle. Microvascular diameters, hemodynamic variables, or blood gas data were measured more than 20 minutes after administration of the anesthetic.

**Table 1. Hemodynamic Variables, Blood pH, and Blood Gases During Protocol 1a (12 Dogs)**

| Vehicle/Perfusate | min | BHT 920, μmol/L | 1 nmol/L
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<td>115±4</td>
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<tr>
<td>Diastolic aortic pressure, mm Hg</td>
<td>94±5</td>
<td>94±5</td>
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<tr>
<td>Mean aortic pressure, mm Hg</td>
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<td>106±4</td>
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<td>102±6</td>
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<td>106±3</td>
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<td>PO₂, mm Hg</td>
<td>97±3</td>
<td>99±4</td>
<td>100±4</td>
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BHT 920 is a selective α-agonist. Values are mean±SEM.
for paired samples modified by the Bonferroni multicomparison method was applied to detect the significant changes.\textsuperscript{29} To compare the percent changes in microvascular diameters between PTX and vehicle groups, Student's t-test for unpaired samples was applied. At $P < .05$, the differences were accepted as significant.

Results

Aortic pressure and blood gas data in protocol 1a are shown in Table 1. Aortic pressure did not change significantly, and the blood gases and pH were kept within physiological values throughout the experiment.

Microvascular diameters during superfusion with PTX or vehicle in protocol 1a are shown in Fig 1. During vehicle superfusion, diameters of either large vessels ($n=11$; range, 104 to 247 $\mu$m) or small vessels ($n=11$; range, 43 to 98 $\mu$m) did not change, indicating the stability of the microvascular preparation. PTX did not cause any change in the microvascular diameters of large vessels ($n=9$; range, 105 to 241 $\mu$m) or small vessels ($n=10$; range, 45 to 98 $\mu$m).

In the absence of PTX, BHT 920 constricted large vessels in a dose-dependent fashion, whereas it did not produce any constriction in small vessels (Figs 2 and 3). According to the regression analysis, the size dependence of the microvascular responses to BHT 920 was significant at doses of 10 and 100 $\mu$mol/L (Fig 2). PTX abolished the BHT 920–induced vascular constriction (Fig 3). Endothelin 1 constricted the microvessels even with PTX pretreatment, indicating that the contractile apparatus of microvascular smooth muscle had been preserved.

During protocol 1b, hemodynamic variables and blood gases were stable (Table 2). Microvascular diameters did not change during superfusion of vehicle (large vessels [$n=8$]: range, 105 to 275 $\mu$m; control, 169±20 $\mu$m; 30 minutes, 168±19 $\mu$m; 60 minutes, 169±19 $\mu$m; 90 minutes, 168±19 $\mu$m; and 120 minutes, 166±19 $\mu$m; and small vessels [$n=9$]: range, 45 to 91 $\mu$m; control, 67±4 $\mu$m; 30 minutes, 69±5 $\mu$m; 60 minutes, 67±4 $\mu$m; 90 minutes, 68±5 $\mu$m; and 120 minutes, 67±4 $\mu$m) or PTX (large vessels [$n=8$]: range, 130 to 250 $\mu$m; control, 169±14 $\mu$m; 30 minutes, 169±13 $\mu$m; 60 minutes, 166±12 $\mu$m; 90 minutes, 163±13 $\mu$m; and 120 minutes, 167±11 $\mu$m; and small vessels [$n=8$]: range, 55 to 97 $\mu$m; control, 75±5 $\mu$m; 30 minutes, 74±6 $\mu$m; 60 minutes, 73±5 $\mu$m; 90 minutes, 74±6 $\mu$m; and 120 minutes, 75±6 $\mu$m) as was the case in protocol 1a. Endothelin 1 produced concentration-dependent vasoconstriction in both large and small vessels, and PTX superfusion did not affect vasoconstriction (Fig 4), indicating that PTX superfusion does not affect microvascular constriction in a nonspecific manner.

Table 3 shows the aortic pressure, blood gases, and distal coronary pressure in protocol 2. Aortic pressure
did not significantly change, and the blood gases and pH were kept within physiological values throughout the experiment. No dogs died during the reduction in perfusion pressure. Microvascular diameters did not change during the superfusion of vehicle (11 vessels: range, 44 to 96 μm; control, 71±5 μm; 30 minutes, 72±5 μm; 60 minutes, 73±5 μm; 90 minutes, 71±5 μm; and 120 minutes, 71±5 μm) or PTX (12 vessels: range, 44 to 99 μm; control, 66±5 μm; 30 minutes, 67±6 μm; 60 minutes, 66±5 μm; 90 minutes, 66±5 μm; and 120 minutes, 66±5 μm) as was the case in protocol 1. The coronary perfusion pressure—coronary flow velocity relation during the reduction in perfusion pressure in protocol 2 is shown in Fig 5. In both vehicle and PTX groups, mild stenosis (perfusion pressure, 60 mm Hg) did not cause any significant changes in coronary flow velocity, indicating well-preserved transmural autoregulatory control of the coronary vascular beds. Severe stenosis (perfusion pressure, 40 mm Hg) and complete occlusion caused a significant decrease in coronary flow velocity, indicating that these occlusion levels represented the myocardial ischemic condition.

Representative microvascular images of protocol 2 are shown in Fig 6. In the vehicle group, arterioles dilated in response to complete coronary occlusion. In the PTX group, this dilation did not occur. Individual microvascular responses and group data in this protocol are shown in Figs 7 and 8, respectively. Mild stenosis, severe stenosis, and complete occlusion caused dilation of the arterioles in the vehicle group (mild stenosis: 6.2±1.9%, P<.05 versus baseline; severe stenosis: 21.1±4.4%, P<.05 versus baseline; and complete occlusion: 16.8±5.9%, P<.05 versus baseline). In the PTX group, the microvascular dilation induced by reductions in perfusion pressure was abolished. In particular, complete occlusion produced a decrease in the diameter of arterioles, which may have undergone passive change because of the reduction in the distending pressure.

Table 2. Hemodynamic Variables, Blood pH, and Blood Gases During Protocol 1b (13 Dogs)

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<th>90</th>
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Values are mean±SEM.
Aortic pressure did not significantly change, and the blood gases and pH were kept within physiological values during protocol 3 (Table 4). Nitroprusside produced microvascular dilation in both large vessels (n=6; range, 111 to 182 μm) and small vessels (n=5; range, 42 to 96 μm) before the PTX pretreatment (Fig 9). By the end of the superfusion with PTX, the microvascular diameters returned to control levels both in large vessels (control, 133±10 μm; end of superfusion, 130±9 μm) and small vessels (control, 64±9 μm; end of superfusion, 65±11 μm). The application of PTX did not affect the nitroprusside-induced vasodilation (Fig 9). These results indicate that PTX superfusion does not inhibit microvascular dilator responses in a nonspecific manner.

Discussion
The most important findings provided by the present study are as follows: (1) Superfusion of PTX effectively and selectively blocks coronary microvascular constriction by an α2-agonist, indicating in vivo blockade of the PTX-sensitive G protein. (2) The blockade of PTX-sensitive G protein abolished microvascular dilation in response to a reduction in perfusion pressure, indicating that vasodilation during autoregulation and ischemia is critically mediated by the PTX-sensitive G protein.

The present study is the first report to show the involvement of the PTX-sensitive G protein in the microvascular defense mechanism in physiological and pathological conditions.

Critique of Methodology
The general preparation of the present study was quite stable in terms of hemodynamics, blood gases, and blood pH, as shown in Tables 1 through 4. Coronary circulation also remained in physiological condition in terms of a well-preserved autoregulatory defense mechanism, as shown in Fig 5.

To maintain the physiological condition of epimyocardial coronary microvessels, the cardiac surface was always kept wet with Krebs’ solution warmed to 37°C. Although the two needles were inserted into the mid-

**Table 3. Hemodynamic Variables, Blood pH, and Blood Gases During Protocol 2 (14 Dogs)**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle/Pertussis Toxin, min</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Vehicle group (7 dogs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic aortic pressure, mm Hg</td>
<td>116±4</td>
<td>116±4</td>
<td>115±4</td>
<td>116±3</td>
</tr>
<tr>
<td>Diastolic aortic pressure, mm Hg</td>
<td>94±5</td>
<td>94±5</td>
<td>92±5</td>
<td>91±5</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>105±4</td>
<td>106±5</td>
<td>103±4</td>
<td>102±4</td>
</tr>
<tr>
<td>Coronary perfusion pressure distal to stenosis, mm Hg</td>
<td>104±5</td>
<td>105±5</td>
<td>102±4</td>
<td>101±4</td>
</tr>
<tr>
<td>pH</td>
<td>7.40±0.01</td>
<td>7.40±0.01</td>
<td>7.38±0.01</td>
<td>7.38±0.01</td>
</tr>
<tr>
<td>Pco2, mm Hg</td>
<td>32±1</td>
<td>33±1</td>
<td>33±1</td>
<td>33±1</td>
</tr>
<tr>
<td>Po2, mm Hg</td>
<td>95±3</td>
<td>95±3</td>
<td>92±4</td>
<td>91±2</td>
</tr>
<tr>
<td>Pertussis toxin group (7 dogs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic aortic pressure, mm Hg</td>
<td>115±6</td>
<td>116±6</td>
<td>117±5</td>
<td>116±4</td>
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<tr>
<td>Diastolic aortic pressure, mm Hg</td>
<td>90±5</td>
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<tr>
<td>Mean aortic pressure, mm Hg</td>
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<tr>
<td>Coronary perfusion pressure distal to stenosis, mm Hg</td>
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<td>99±5</td>
<td>99±4</td>
<td>99±3</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.01</td>
<td>7.42±0.01</td>
<td>7.39±0.01</td>
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</tr>
<tr>
<td>Pco2, mm Hg</td>
<td>34±0</td>
<td>34±1</td>
<td>34±1</td>
<td>35±1</td>
</tr>
<tr>
<td>Po2, mm Hg</td>
<td>103±6</td>
<td>97±5</td>
<td>102±5</td>
<td>104±4</td>
</tr>
</tbody>
</table>

LAD indicates left anterior descending coronary artery. Values are mean±SEM.
Fig 5. Graph showing coronary perfusion pressure–coronary flow velocity relation during reduction in perfusion pressure. Coronary flow velocity was normalized by the values at the end of superfusion with vehicle or pertussis toxin. In both the pertussis toxin group (●, seven dogs) and the vehicle group (○, seven dogs), autoragulation of coronary flow was preserved, because coronary flow velocity at the perfusion pressure of mild stenosis (60 mm Hg) was not significantly different from baseline values. Severe stenosis (40 mm Hg) and complete occlusion significantly decreased the flow velocity. There was no difference between the two groups. Values are mean±SEM. *P<.05 vs baseline.

myocardium to continuously observe the coronary microvessels in vivo, previous studies have shown that their effect on coronary microvasculature and coronary flow is minimal.30,31 Previous studies from our laboratory have shown that the microvascular preparation possesses physiological and pharmacological reactivity.26,32-34 The environment of the observed area in the present study can be considered as stable and nearly physiological during experiments for the following reasons: (1) The microvascular diameters were stable during the superfusion with the vehicle of PTX. (2) The microvascular dilation in small arterioles in response to a reduction in perfusion pressure was well preserved. (3) The pharmacological vasomotion of the microvessels in response to several agents such as BHT 920, endothelin 1, and nitroprusside was preserved.

In the present study, fluorescence-labeled dextran was injected, and ultraviolet light was applied each time fluorescent vascular images were obtained. The ultraviolet light might have caused some microvascular damage. The exposure period, however, was <10 seconds for each image, and the exposure was intermittent. Reed and Miller35 have shown that intermittent exposure to ultraviolet light for short periods does not lead to microvascular injury, whereas continuous exposure for a long period evokes microvascular injury such as macromolecular leakage and stasis of capillary blood flow. No thrombus formation or stasis of blood flow was observed in the present study. Furthermore, Lamping et al36 have shown that microvascular diameters measured in fluorescent images by use of fluorescein isothiocyanate dextran and ultraviolet light are almost identical to the ones measured in polarized images. Their method to enhance coronary microvessels was almost identical to our method, indicating that our methodology for fluorescence coronary microangiography is appropriate.

Microvascular Constriction by α2-Agonist

The validity of our method of applying PTX as a blockade of G, and G, proteins in coronary microvessels was tested by observing the effect of PTX on α2-agonist–induced vasoconstriction. BHT 920 is one of the most specific α2-agonists.37 The present data indicate that coronary microvessels >100 μm constrict in response to BHT 920 in the resting condition. However, a recent study by Chilian38 has shown that the α2-agonist BHT 933 did not constrict coronary microvessels in the resting condition and that it constricted the vessels only under the low perfusion pressure. The discrepancy can be explained by the difference in the route of drug administration. Chilian administered the α2-agonist by intracoronary infusion, whereas BHT 920 was applied

Fig 6. Representative microvascular images in protocol 2. In the vehicle group (A and B), complete occlusion of the left anterior descending coronary artery (B) produced marked arteriolar dilatation compared with baseline (A). In contrast, in the pertussis toxin group (C and D), complete occlusion (D) did not produce any dilatation compared with baseline (C). Bars indicate 100 μm.
application of α-agonists better mimics the effect of sympathetic nervous stimulation than intraluminal application, because the nerve endings of vessels are located in the abluminal side. In our study, small arterioles <100 μm did not constrict in response to BHT 920. This may have resulted from the compensation by autoregulatory dilation produced by upstream constriction.

**Treatment With PTX In Vivo**

PTX has been widely used to block Gα and Gβ proteins.1–7 PTX consists of A-protomer and B-oligomer. A-protomer is a catalytic subunit that is responsible for the ADP-ribosylation of G proteins; B-oligomer plays a key role in binding of the toxin with cell plasma membrane and in transferring A-protomer into the cell.8 The effects of α2-agonists on vascular smooth muscle, including both vasodilatation via endothelium-derived relaxing factor and direct vasoconstriction, have been known to be mediated by Gα protein.8,9 In the present study, 2-hour superfusion with PTX effectively blocked the α2-adrenergic constriction in coronary microvessels, whereas the contractile responses to endothelin were preserved (Fig 3). Accordingly, it is likely that the superfusion of PTX effectively blocked the microvascular responses mediated by Gβ and Gα proteins. Fleming et al42 have reported that intravenous injection of PTX 48 hours before experiments effectively catalyzed the endogenous ADP-ribosylation of Gα and Gβ proteins in the sarcolemma of excised hearts. Lipton et al43 have recently reported that intravenously infused PTX inhibited the changes in vascular resistance caused by α2-agonist in pulmonary vascular beds. But neither of these studies evaluated the hemodynamic effect of the treatment. Our preliminary experiments with dogs showed that the intravenous treatment with PTX caused severe hemodynamic impairment, such as severe diastolic hypotension (unpublished observation). Therefore, in the present study, we topically applied the PTX to block the PTX-sensitive mechanisms in coronary microvasculature to avoid the involvement of the other various biological effects of PTX on the systemic circulation and other organs.

PTX did not change the coronary microvascular resting diameters. This phenomenon indicates that the PTX-sensitive mechanism is not significantly involved in

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**Fig 7.** Scatterplots showing individual arteriolar responses during mild stenosis (A: perfusion pressure, 60 mm Hg), severe stenosis (B: perfusion pressure, 40 mm Hg), and complete occlusion (C). • indicates pertussis toxin (300 ng/mL) group (12 vessels); ○, vehicle group (11 vessels).

**Fig 8.** Bar graphs showing the effect of pertussis toxin on the dilation of coronary arterioles <100 μm in diameter produced by the reduction in perfusion pressure. In the vehicle group (open bars, 11 vessels), mild stenosis, severe stenosis, and complete occlusion dilated the arterioles. On the other hand, in the pertussis toxin group (solid bars, 12 vessels), arteriolar dilation was abolished. Values are mean±SEM. *P<.05 vs vehicle group.
determining the resting coronary microvascular tone or that the involvement of the PTX-sensitive mechanism is compensated by other backup mechanisms.

PTX ADP-ribosylates the α subunit of Gi protein, leading to the uncoupling of the Gi protein with its receptors.7 PTX also has been reported to block Gi protein and transducin.1–6 Among these G proteins, Gi and Gs proteins have been known to exist in the heart.64–65 We cannot draw a conclusion about which G protein is responsible for the microvascular control observed in the present study.

**PTX-Sensitive Mechanism and Microvascular Response to Reduction in Perfusion Pressure**

Previous studies have shown that a reduction in coronary perfusion pressure causes dilation in arterioles <100 μm in diameter.11–13 The present data are consistent with these reports. Komaru et al15 and a previous report from our laboratory38 have shown that glibenclamide, which is a potent and selective blocker of ATP-sensitive potassium channels, abolished the microvascular dilation in response to a reduction in perfusion pressure, indicating the critical participation of the channel in microvascular control in autoregulation and ischemia.

However, the mechanism that modulates the open probability of the channels in microvessels has not yet been determined. The depletion of ATP was originally considered to be the mediator of the opening,46 but it is unlikely that the ATP level in tissue is depleted during autoregulation. Even in the ischemic condition, the tissue ATP level is not rapidly depleted.47 Therefore, it is unlikely that intracellular ATP mediates the open probability in coronary microvessels.

Recent evidence21,25 has shown that Gi protein can open the ATP-sensitive potassium channel. Ito et al25 have stated the possibility that Gi protein may also open the channel in some cases. Since both of these studies are patch-clamp studies, the opening of the ATP-sensitive potassium channel by Gi or Gs is unlikely to be mediated by second messengers. It is probable that the reduction in perfusion pressure activates Gi or Gs protein, which is coupled with ATP-sensitive potassium channels, leading to arteriolar dilation for the maintenance of coronary blood flow.

Another possible role of PTX-sensitive Gi protein in the regulation of coronary microvessels is that Gi or Gs protein in the microvascular endothelium, not in the smooth muscle cell, is activated by a reduction in perfusion pressure, leading to a release of endothelium-derived relaxing factor and to an increase in arteriolar conductance. Smith and Canty48 have recently raised the possibility that nitric oxide plays a role in autoregulatory mechanisms in conscious dogs. Furthermore, Kubo et al49 have demonstrated that intracellular cGMP, which is increased by nitric oxide, activates the ATP-sensitive potassium channels in the vascular smooth muscle.

The agonist that mediates the microvascular responses via PTX-sensitive Gi protein during autoregulation and ischemia has not been determined. Adenosine might be a possible mediator. In the cardiomyocyte, the A1 receptor has been shown to couple to Gi protein.51 In vascular tissue, however, vasodilation is mainly mediated by the A2 receptor, and Furukawa et al52 have recently demonstrated that adenosine-mediated vasorelaxation is not affected by PTX. Dole et al51 and Hanley et al52 have independently demonstrated that adenosine deaminase...
does not alter autoregulatory gain. Furthermore, Komaru et al. have recently shown that neither adenosine receptor blocker nor adenosine deaminase inhibits arteriolar dilation during low perfusion pressure.

In the present study, the activation of PTX-sensitive G protein by BHT 920 produced microvascular constriction. On the other hand, the present study has demonstrated that the PTX-sensitive G protein mediates autoregulatory microvascular dilation. This means that PTX-sensitive G protein can mediate both vasoconstriction and vasodilation. The functional specificity of the G protein is determined by the receptors and effectors to which particular G proteins link. G protein is known to couple with various effectors, including adenyl cyclase, potassium channels, phospholipase C, and phospholipase A2. It has been suggested that one G protein can couple with more than one effector at the same time, resulting in the regulation of more than one effector function. Accordingly, it is very likely that PTX-sensitive G protein activation leads to diverse biological effects and that the G protein function differs from receptor to receptor and from tissue to tissue. Clinical Implications

G proteins are located in the cell membrane and are susceptible to insults such as hyperlipidemia and diabetes mellitus. Shimokawa et al. have reported the dysfunction of PTX-sensitive G protein in coronary vascular endothelium in epicardial conduit coronary arteries with atherosclerosis. Furthermore, Gawler et al. have shown that the expression of G protein is abolished in hepatocytes of animal models with diabetes mellitus induced by streptozotocin or alloxan. If it occurs at the coronary microvascular level, the dysfunction of PTX-sensitive G protein may be responsible for an impaired defense mechanism during ischemia. Previous works have demonstrated that autoregulatory flow control is impaired in several organs in diabetes mellitus. The present data may provide new insights in organ flow control in various diseased conditions.

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