Response of Human Coronary Arteries to Serotonin After Injury by Coronary Angioplasty

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**Background.** Atherosclerotic stenoses that have exaggerated vasomotor responses are especially prone to restenosis after coronary angioplasty. Experimental studies show that vasomotor responses in normal vessels are altered by acute mechanical injury, an alteration that in part reflects changes in the functional characteristics of endothelium that has regenerated after injury.

**Methods and Results.** We examined, by quantitative coronary arteriography, the response of dilated and control coronary segments to intracoronary infusions of graded doses of serotonin, an endothelium-dependent vasoactive agent, and to intracoronary injection of isosorbide dinitrate, an endothelium-independent smooth muscle dilator in 15 patients who had undergone a single percutaneous transluminal coronary angioplasty procedure and who had no clinical features of variant angina. Dose-dependent constriction to serotonin occurred at all measured sites. The mean ± SEM diameter reductions, expressed as percent reduction in baseline diameter that was observed at proximal (18.1 ± 2.9, 18.8 ± 2.2) and distal (30.9 ± 4.4, 35.4 ± 5.3) control sites in the dilated and nondilated vessels, respectively, at the highest dose, were similar. The degree of constriction in distal segments was significantly (P < .01) greater than that in proximal segments. Total or subtotal occlusion occurred at the angioplasty site in 4 patients at the highest infused dose (10^−4 mol/L). The mean percent reduction in baseline diameter at previously dilated sites (53.8 ± 5.9) at this dose was significantly (P < .05) greater than that observed at the adjacent proximal control sites and similar to that observed at distal control sites. All segments dilated significantly after intracoronary injection of isosorbide dinitrate.

**Conclusions.** In dilated and nondilated vessels, serotonin caused significantly more marked constriction in distal than in proximal vessel segments. In dilated vessels, the vessel segments that had been subjected to angioplasty had a constrictor response to serotonin that was more marked than at adjacent proximal control sites and equivalent to that in the distal vessel segments. This enhanced constrictor response could be related to changes in endothelial cell function after regeneration or to hyperreactivity of smooth muscle cells at the angioplasty site. (Circulation. 1993;88[part 1]:2076-2085.)

**Key Words** • 5-hydroxytryptamine • circulation • stenosis • endothelium • vasospasm

High rates of restenosis have been reported when coronary angioplasty is performed on coronary segments that have exaggerated vasomotor responses. This observation was first reported in patients with variant angina. We and others demonstrated that when angioplasty was performed at stenotic sites that developed subtotal or total occlusion in response to the intravenous injection of methylergonovine before the procedure, the rate of restenosis was markedly greater than that observed at control dilated sites that had not demonstrated such an abnormal response. This observation has been extended to another group of patients that has an excess risk of restenosis after angioplasty. A recent prospective study in patients who had unstable angina and single-vessel disease demonstrated that a positive hyperventilation test before angioplasty, indicative of an abnormal vasomotor response at the stenotic site, was a powerful predictor of restenosis. Two these clinical observations suggest that the vasomotor properties of a stenosis may be of relevance to the subsequent occurrence of restenosis.

Experimental studies suggest that the performance of angioplasty may in itself be associated with alterations in vasomotor responses. In the pig model, coronary arterial injury is associated with persisting alterations in vasomotor responses with, in particular, an enhanced constrictor response to serotonin and to aggregating platelets that has been attributed to an acquired defect of endothelium-mediated vascular relaxation. This study was designed to compare the vasomotor response to serotonin, an endogenous platelet-derived vasoconstrictor and proaggregatory agent whose effect on smooth muscle is modulated by the endothelium, at arterial segments that had been subjected to coronary angioplasty with the response at control nondilated segments. We have previously shown that the constrictor response to serotonin in humans is more marked in distal than in proximal epicardial segments. We further showed that the degree of constriction induced by serotonin at stenotic sites in patients with stable angina

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was similar to that at adjacent proximal control sites. We therefore compared, by quantitative angiography, the responses of dilated vessel segments to intracoronary infusions of graded doses of serotonin with those of both proximal and distal control segments in the dilated vessel and in a nondilated vessel. We further compared these responses with those evoked by the intracoronary injection of isosorbide dinitrate, an endothelium-independent smooth muscle dilator.

**Methods**

**Patients**

We prospectively enrolled 17 consecutive patients scheduled for routine cardiac catheterization after percutaneous transluminal coronary angioplasty. In our institution, follow-up angiography is recommended to all patients after successful angioplasty and is currently performed in more than 85% of such patients. Patients who had undergone a first angioplasty procedure at a single site and who did not have a history of variant angina were considered eligible for the study. Two eligible patients with stable angina in whom diagnostic angiography demonstrated complex restenotic lesions were scheduled for immediate repeat angioplasty and were not studied.

The mean ± SEM age of the 15 patients we studied, 12 of whom were men, was 51.1 ± 2.8 years (range, 33 to 71 years). The angioplasty procedure reduced the mean severity of the dilated lesions from 77.0 ± 3.5% to 35.7 ± 2.2%. The clinical and angiographic characteristics of the patients at the time of angioplasty and at follow-up catheterization performed at a mean ± SEM of 6.4 ± 1.6 months after angioplasty are detailed in Table 1.

Informed consent for the discontinuation of therapy and for the intracoronary administration of serotonin was obtained from all the patients. The study protocol was approved by the Institutional Review Board of the University of Lille (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Lille). Regular antianginal medication was discontinued 48 hours before catheterization. All the patients were taking aspirin (100 to 300 mg daily), which was continued. Patients were allowed to use sublingual nitroglycerin as needed, but no study was performed within 3 hours of its administration.

**Drugs**

Serotonin creatinine sulfate was prepared by the Hammersmith Hospital Pharmacy (London, UK) and stored at −20°C in ampoules containing 5 mL of 10⁻² molar solution until just before use. The stock solution was diluted with normal (0.9%) saline to achieve final infused concentrations of 10⁻⁴ through 10⁻⁵ mol/L.

**Study Protocol**

Femoral arterial pressure, heart rate, and two ECG leads (V₁ and V₂) were recorded continuously throughout the study. After diagnostic arteriography, an optimal view was chosen to visualize the artery under study. A previous study has shown that random fluctuations or systematic changes caused by repeated infusions of saline have no significant effect on coronary diameter. We have shown that repeated injections, performed at 2-minute intervals, of the contrast medium used in this study have no significant effect on coronary diameter. The patients therefore received an infusion of vehicle solution (0.9% saline) followed by 2-minute infusions of serotonin creatinine sulfate (10⁻⁵ through 10⁻⁴ mol/L).

All infusions were administered through 8F Judkins catheters at room temperature at a rate of 1 mL/min with a syringe pump (Perfusor, Braun-Melsungen). An intracoronary bolus dose (2 mg) of isosorbide dinitrate diluted in normal (0.9%) saline was injected into the
study artery at the end of the protocol. Angiography was performed at baseline and after each infusion, after the injection of 6 to 8 mL of contrast medium (Radiopaque, Schering SA). Before each angiogram, the catheter was emptied to avoid the effects of bolus administration of serotonin.

**Quantitative Coronary Angiography**

The coronary arteriograms were analyzed with the CAESAR (Computer Assisted Evaluation of Stenosis and Restenosis) system, a computerized automatic analysis system. The 35-mm cine film was projected with a 35AX projector (Tagarno, Denmark), and the cine frame selected for analysis was scanned with a high-resolution video camera. The signal produced by the video camera was digitized and displayed on a video monitor. Regions of interest were chosen in the vessel, and a centerline was manually traced with a light pencil. The contours of the vessel were then automatically detected on the basis of the weighted sum of first and second derivative functions applied to the digitized brightness information. The diameter of the coronary catheter was used to convert the imaging data from pixels to millimeters. The mean diameters of proximal and distal reference segments and the minimum diameter of the stenotic segment were measured. We had previously determined the accuracy (defined as the signed difference between the measured and the true value) and the precision (defined as the standard deviation of these differences) of the CAESAR system in a study analyzing cine films of Plexiglas blocks containing precision-drilled models of coronary arteries filled with contrast medium. The accuracy was 0.07 mm, and the precision was 0.14 mm. In a separate study, we analyzed the intraobserver and interobserver variability of the CAESAR system in our institution. Ninety arterial segments from patients undergoing coronary angioplasty were analyzed by two independent observers and reanalyzed at a remote time. The mean intraobserver variation, expressed as the standard error of the estimate (SEE), was 0.10 mm. The interobserver variation (SEE) was 0.11 mm.

The response of the dilated segment was quantified by measuring the minimum diameter of the segment at baseline and after each intervention. The mean diameters of proximal and distal control segments in the dilated vessel, identified by their distance from side branches or from the origin of the vessel, were also determined. For angioplasty procedures on the left coronary artery, the mean diameters of control proximal and distal segments in the nondilated vessel after each intervention were also determined. In the patient who developed complete occlusion and in the three patients who developed subtotal occlusion at the site of the previous angioplasty after infusion of the highest dose of serotonin, the diameter of the control segment distal to the site of angioplasty could not be measured.

**Statistical Analysis**

All data are expressed as mean±SEM unless otherwise indicated. A one-way ANOVA with a design for repeated measures followed by two-tailed paired Student's t tests with the Bonferroni correction was used for comparison of sequential changes in the absolute values of coronary artery diameters and for comparison of hemodynamic parameters after infusion of graded doses of serotonin. Two-tailed Student's t tests for paired observations were used to test differences in percent reduction in coronary diameter between segments at the maximum infused dose of serotonin. Linear regression analysis was used to determine whether a relation existed between changes in lumen diameter from angioplasty to follow-up and the constrictor response to serotonin at the dilated segments.

**Results**

**Hemodynamic Parameters**

There were no significant changes in heart rate or in systolic arterial blood pressure associated with intracoronary infusion of saline or of incremental concentrations of serotonin.

**Responses of Coronary Vessel Segments**

The changes observed in the vessel segments we studied are detailed in Table 2 and Figs 1 through 3. The intracoronary infusion of 0.9% saline was not associated with significant changes in epicardial luminal diameter.

The repeated-measures ANOVA to evaluate segment and dose demonstrated significant differences for both
Responses of Proximal and Distal Control Segments

The mean percentage reduction in lumen diameter compared with baseline at the highest infused dose (10^{-4} mol/L) that was observed at control sites proximal to the dilated stenosis did not differ significantly from that observed at control sites of similar diameter in nondilated arteries. The mean maximal percentage reduction in lumen diameter compared with baseline was significantly (P<.01) greater at distal (30.9±4.4% in the dilated vessel, 35.4±5.3% in the nondilated vessel) control sites than at proximal (18.1±2.9% in the dilated vessel, 18.8±2.2% in the nondilated vessel) control sites. The degree of constriction was similar at corresponding sites in the dilated and nondilated vessels (Figs 1 and 3).

Response at the Dilated Site

Total (in 1 patient) or subtotal (in 3 patients) occlusion occurred at the site of the previous angioplasty in 4 of the 15 patients studied after infusion of the highest dose of serotonin (10^{-4} mol/L). The mean percentage constriction at the dilated site was significantly (P<.05) greater than that observed at proximal control sites and slightly but not significantly greater than that observed at distal control sites. The angiographic findings in 2 of the patients we studied are illustrated in Figs 4 and 5.

The constrictor responses that were observed in individual patients at the prestenotic and stenotic sites after infusion of the highest dose of serotonin are illustrated in Fig 2. The degree of constriction at the dilated site was similar whether or not restenosis (defined as the presence of >50% luminal narrowing at the dilated site at follow-up assessed by quantitative coronary angiography) had occurred.

To further investigate whether there was a relation between the degree of constriction to serotonin and the tendency to restenosis in the segments, we determined whether there was a relation between the absolute change in minimal lumen diameter at the dilated site after infusion of the highest dose of serotonin and the absolute change in lumen diameter between angioplasty and follow-up angiography. There was no correlation (r=.23, P=NS) between the two variables (Fig 6).

Symptoms and ECG Changes

Eight patients developed chest pain during infusion of the highest concentration of serotonin, with associated ECG changes in seven (ST segment elevation in two, depression in five). In four of these patients, the symptoms were associated with total (in one) or subtotal
sclerotic vessels. Our results significantly that atherosclerotic coronary vessels to the intracoronary infusion of serotonin and showed that such infusion caused dose-dependent epicardial vasoconstriction and that the constrictor effect, assessed by quantitative coronary angiography, was much more marked in distal than in proximal epicardial vessels. Indeed, the most striking angiographic feature after infusion of serotonin was the intense constriction of the tertiary branches of the major vessels and of collateral vessels that were too small to evaluate by quantitative angiographic techniques. These findings suggested that there were regional differences in the response to serotonin in the human coronary circulation. Golino et al also demonstrated that serotonin infusion caused dose-dependent epicardial constriction. In addition, they showed that infusion of serotonin at doses that caused only a modest reduction in epicardial cross-sectional area was associated with significant reductions in coronary blood flow, suggesting that serotonin has a particularly potent constrictor effect on vessels too small to be seen angiographically.

In a subsequent study, we showed that these regional differences in the response to serotonin may be related to differences in the distribution of serotonin receptor subtypes in proximal compared with distal epicardial vessels. Ketanserin, a selective antagonist at the S2 receptor subtype, inhibited the constrictor response to serotonin in proximal but not in distal epicardial vessels in patients with atherosclerosis who had chronic stable angina.

We also reported the response of discrete primary coronary stenoses to intracoronary infusion of serotonin. In patients with stable effort angina, a similar degree of constriction occurred at stenotic and at prestenotic segments at the maximal infused dose (10⁻⁴ mol/L) of serotonin. By contrast, we found that in patients with variant angina who had vasospasm superimposed on a single discrete epicardial stenosis, there was a marked hyperreactivity to the constrictor effects of serotonin at the site of the lesion. Total or subtotal epicardial occlusion at the stenotic site occurred at infused doses of serotonin that caused only moderate constriction at the adjacent prestenotic site.

The results of the present study once again demonstrate that serotonin is a potent vasoconstrictor of atherosclerotic coronary vessels and that its effects are most marked in the distal epicardial vasculature. They also demonstrate, however, that the intracoronary infusion of serotonin is consistently associated with a greater degree of constriction at the site of a previous angioplasty than at the adjacent proximal nondilated site; indeed, at 4 of the 15 lesions studied, total or subtotal epicardial occlusion occurred at the dilated site.

Potential Mechanisms of Serotonin-Induced Hyperconstriction and Spasm

The mechanisms of this enhanced constrictor response may be multifactorial and cannot be completely
Fig 4. Angiographic findings in a patient who presented with stable angina pectoris and who had undergone angioplasty on a proximal right coronary artery lesion. The angiogram immediately before angioplasty (A) shows a discrete proximal right coronary artery stenosis. At follow-up angiography, mild restenosis had developed (B). After infusion of serotonin (10^-4 mol/L for 2 minutes), there was subtotal occlusion at and just distal to the site of the previous angioplasty, with diffuse constriction of the smallest visible distal branches (C). The patient developed typical angina, and there was ST segment elevation on the ECG.
Fig 5. Angiographic findings in a patient who presented with unstable angina pectoris and who had undergone angioplasty on an isolated proximal left anterior descending lesion. The angiogram immediately before angioplasty (A) shows a discrete proximal left anterior descending stenosis. At follow-up angiography, restenosis had not developed (B). After infusion of serotonin (10^{-4} mol/L for 2 minutes), moderate focal constriction developed at the site of the previous angioplasty, with mild diffuse constriction of the distal branches of the left anterior descending coronary artery (C). There was no constriction of the left circumflex artery or of its branches. The patient did not develop chest pain, and there was no change in the ECG.
The reduced relaxations evoked by serotonin and bradykinin are caused by a receptor-mediated endothelium-dependent release of nitric oxide, which relaxes the vessel. It is therefore possible that the hyperreactive response to serotonin that we have documented at the site of previous angioplasty compared with the response at the adjacent nondilated proximal control site reflects a further functional impairment of the endothelium at such sites related to the previous injury.

It is also possible that the hyperconstriction at the site of previous angioplasty reflects an enhanced reactivity of smooth muscle cells. Histological studies after experimental angioplasty demonstrate migration of smooth muscle cells into the intima and subsequent proliferation. A recent experimental study showed that endothelial injury is associated with a change in smooth muscle cell phenotype that may lead to an increased sensitivity to serotonin and to thromboxane.

Comparison With Previous Studies

Several studies have looked at the vasomotor responses of epicardial vessels subjected to percutaneous transluminal coronary angioplasty. Quyyumi et al. studied the effects of methylergonovine in 14 consecu-
tive patients 6 weeks to 3 months after successful single-vessel angioplasty. Intravenous infusion of ergonovine provoked focal spasm at the previously dilated site in 5 patients: the mean reduction in lumen diameter at the dilated site was 51% in the patients who developed spasm; by contrast, the reduction in luminal diameter at the adjacent proximal control site was consistently <20%. By contrast, el-Tamimi et al19 recently demonstrated that the intracoronary injection of ergonovine 1 week after angioplasty in 12 consecutive patients who had undergone single-vessel coronary angioplasty caused a degree of constriction at the angioplasty site similar to that at control nondilated sites. The explanation for these apparently contradictory observations may be related to the time period that elapsed between the angioplasty procedure and the performance of the provocative tests. In the dog, it has been demonstrated that large coronary vasomotion in response to ergonovine does not change at all from 1 to 10 days after endothelial denudation,20 whereas at 1 month after denudation, there is a marked hyperreactivity to the constrictor effects of ergonovine at the dilated site.21

Another recent study demonstrated that the constrictor response to intracoronary infusion of acetylcholine was similar at dilated sites and at nondilated proximal control sites 1 week after coronary angioplasty in humans.22 However, the degree of constriction of the control segments distal to the angioplasty site was significantly greater than that observed in the distal segments of a control nondilated artery. In the present study, the constrictor response that was observed in some patients at control segments distal to the dilated site was greater than that observed at the corresponding control sites in the nondilated artery. Although the mean degree of constriction response of the control segments distal to the angioplasty site was not significantly different from that observed in the distal segments of a control nondilated artery, it was of course not possible to assess the behavior of the distal segment in the dilated vessel in the subgroup of patients who developed severe constriction at the angioplasty site. Further studies that use lower doses of serotonin in conjunction with Doppler measurements of coronary flow may help to clarify the effect of angioplasty on the response of small coronary vessels to serotonin.

Potential Limitations

There are methodological difficulties in comparing the responses of coronary segments that have differing absolute diameters. The use of percent change relative to baseline for comparisons between segments magnifies changes that occur in smaller coronary segments. Our previous work suggests that the constrictor response to serotonin is greater in distal than in proximal coronary segments, perhaps related to regional differences in the distribution of serotonin receptor subtypes. Thus, the demonstration that previously angioplastied sites constrict much more than adjacent proximal control segments and to the same extent as distal segments is consistent with a real difference in the behavior of such segments in response to serotonin. Furthermore, total or subtotal epicardial occlusion occurred after serotonin infusion at 4 of the 15 angioplasty sites. We have never observed such a response in proximal or distal sites, in stenotic or in nonstenotic vessels, in patients who had stable ischemic heart disease. Finally, the similar degree of constriction to serotonin at angioplasty sites that had <50% or >50% stenosis at control angiography and the lack of correlation between the constrictor response to serotonin and the loss in lumen diameter between angioplasty and follow-up suggest that the observed responses cannot be explained by geometric or lesion-related factors alone.

Conclusions

The results of this study demonstrate that after the acute mechanical injury associated with coronary angioplasty, there is a more marked constrictor response to serotonin at the site of such injury than at nondilated sites. This could be a result of an alteration in the functional properties of endothelium that has regenerated after injury, of a hyperreactivity of vascular smooth muscle cells at the site of the previous angioplasty, of a combination of these factors, or of other as yet unelucidated factors. The demonstration that an enhanced constrictor response to an endothelium-dependent vasoactive agent occurs after angioplasty raises the possibility that altered vasomotor reactivity related to the angioplasty procedure itself may be one of the factors that facilitates the process of restenosis. This possibility is consistent with the results of clinical studies that have demonstrated that the presence of coronary vasospasm induced by ergonovine 6 months after successful coronary angioplasty was frequently accompanied by restenosis,1 and that the presence of an abnormal vasomotor response before angioplasty is strongly predictive of subsequent restenosis.2

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Response of human coronary arteries to serotonin after injury by coronary angioplasty.
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