Effects of Serotonin-Receptor Blockade on Angioplasty-Induced Vasospasm in an Atherosclerotic Rabbit Model

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Vasospasm occurs both in patients and animal models after angioplasty and may be associated with early closure of the dilated vessel. To investigate the mechanism of angioplasty-induced vasospasm, the effect of serotonin-receptor blockade with two serotonin (5-HT) antagonists, LY53857 and sergolexole, was examined in rabbits with focal femoral artery atherosclerosis. In preliminary studies, local infusion of 1-100 μg serotonin caused significant femoral artery vasoconstriction (p<0.05) in both normal and atherosclerotic rabbits. There was no significant difference in the degree of vasoconstriction induced by equal doses of serotonin in normal and atherosclerotic animals. Infusion of 10 μg serotonin produced a 23±5% decrease in luminal diameter in atherosclerotic femoral arteries. This was blocked by pretreatment with both 5-HT antagonists given separately in different animals before serotonin infusion (p<0.002). In contrast, LY53857 (sergolexole was not tested) had no significant effect on phenylephrine-induced vasoconstriction, confirming its specificity as an 5-HT-receptor antagonist. Balloon angioplasty of atherosclerotic vessels caused a significant increase in vessel diameter at the angioplasty site (45% increase from baseline diameter, p<0.05). This was associated with significant luminal narrowing both proximal (21% reduction from baseline, p<0.05) and distal (17% reduction from baseline, p<0.03) to the angioplasty site. These proximal and distal changes are most likely due to vasospasm, as there was no histological evidence of thrombus or dissection at these sites to explain the luminal narrowing. Pretreatment of animals with 10 mg LY53857 or 20 mg sergolexole blocked the proximal vasospasm (2.6±0.4 before versus 2.2±0.1 mm after angioplasty for LY53857, 2.1±0.4 before versus 2.1±0.4 mm after angioplasty for sergolexole; p=NS). Treatment with 20 mg LY53857 inhibited both proximal (2.3±0.1 before versus 2.2±0.2 mm after angioplasty, p=NS) and distal (1.7±0.1 before versus 1.6±0.2 mm after angioplasty, p=NS) vasospasm after angioplasty. Proximal (2.3±0.5 before versus 2.5±0.3 mm after) and distal (1.7±0.2 before versus 1.7±0.4 mm after) vasospasm was also prevented by pretreatment with 40 mg sergolexole. We conclude that 1) serotonin-induced vasoconstriction in normal and atherosclerotic rabbit femoral arteries is mediated in part by 5-HT receptors since it can be blocked by the specific antagonists LY53857 and sergolexole; 2) the specificity of LY53857 action is confirmed by its lack of inhibition of α-adrenergic-mediated vasoconstriction by phenylephrine infusion; and 3) 5-HT-receptor blockade inhibits angioplasty-induced vasospasm, suggesting that it may also be mediated by serotonin, perhaps released by platelets activated at the site of balloon angioplasty. Thus, this study suggests that 5-HT-receptor antagonists may be useful in preventing arterial spasm after angioplasty. (Arteriosclerosis and Thrombosis 1991;11:770-783)

The mechanism of acute closure after angioplasty is not definitely known. There is evidence that vasospasm occurs after balloon angioplasty in both humans and animals and it may be associated with acute closure. The mechanism by which angioplasty induces vasospasm is incompletely understood. Bates et al

From the Department of Medicine, Yale University School of Medicine/West Haven VA Medical Center, New Haven, Conn. Presented in part at the 51st Scientific Sessions of the American Heart Association, November 15, 1988, Washington, D.C., and at the 1990 National Meeting of the American Federation for Clinical Research, May 5, 1990, Washington, D.C. Supported by National Institutes of Health grant #RO1 HL-39467-01 (to M.D.E.) and the Veterans Administration Merit Review Grant (to M.D.E.). Address for reprints: Michael D. Ezekowitz, MD, PhD, Yale University School of Medicine, Internal Medicine/Cardiology 3FMP, 333 Cedar Street, New Haven, CT 06510. Received January 2, 1990; revision accepted January 17, 1991.
demonstrated that balloon dilatation of the left anterior descending coronary artery in dogs, at pressures sufficient to cause endothelial and medial damage, may induce vasospasm at distant sites, namely, in the left circumflex artery. Similar observations have been reported after human angioplasty. It has been shown that \( \alpha \)-adrenergic blockers, calcium channel blockers, and nitroglycerin do not completely prevent the induced vasospasm. Animal models have suggested that vasospasm is closely related to platelet deposition at the treated site, with release of vasoactive substances, including serotonin, from activated platelets.

Rubanyi et al demonstrated that coronary sinus blood from patients with coronary artery disease contained a soluble mediator that caused vasoconstriction of isolated canine coronary artery rings and that this effect could be blocked by methiothepin, a nonselective serotonin-receptor antagonist. More recently, Van den Berg et al demonstrated that patients with coronary artery disease had a significantly higher serotonin concentration difference between coronary sinus and aortic blood samples than did patients with minimal coronary artery disease. Similarly, Ashton et al showed that ketanserin, a relatively selective serotonin-receptor antagonist, could be used to block the cyclic flow variations seen in canine coronary arteries with concentric stenoses. However, serotonin has complex effects on arterial tone, with the overall effect influenced by resting tone and by the combined, sometimes contradictory, actions of serotonin at several different sites. The recent development of LY53857 and sergolexole, specific antagonists of the serotonin (S\(_2\)) receptor on the vascular smooth muscle cell, allows more direct evaluation of the role of serotonin as a mediator of angioplasty-induced vasospasm.

We have used rabbits with focal femoral artery atherosclerosis to evaluate the role of serotonin in angioplasty-induced vasospasm and to investigate the usefulness of LY53857 and sergolexole in blocking this process.

**Methods**

**Overview of the Experimental Design**

A series of studies was performed to investigate the role of serotonin in angioplasty-induced vasoconstriction. All studies conformed to the “Position of the American Heart Association on Research Animal Use” and were approved by the Yale University Animal Use and Care Committee. Initially, serotonin was infused into normal and atherosclerotic rabbits to test its ability to cause vasoconstriction and to establish a range of effective doses. Next, LY53857 and sergolexole, both specific S\(_2\)-receptor antagonists, were tested for their ability to block vasoconstriction caused by serotonin in atherosclerotic rabbits. Then the specificity of LY53857 as an S\(_2\)-receptor antagonist in this model was evaluated by assessing its inhibitory effect on phenylephrine (\( \alpha \)-adrenergic)-induced vasoconstriction. Finally, the ability of LY53857 and sergolexole to prevent angioplasty-induced vasoconstriction was tested in atherosclerotic rabbits.

**Induction of Focal Femoral Artery Atherosclerosis**

The method described by LeVeen et al was used to produce focal femoral atherosclerotic lesions in 8-10-lb New Zealand White rabbits. After induction of anesthesia with ketamine, 35 mg/kg (Ketaset, ketamine hydrochloride veterinary injection; Veterinary Products, Bristol Lab, Fort Dodge, Iowa) and xylazine 5 mg/kg (Rompun; Bayvet Division, Miles Laboratories, Inc., West Haven, Conn.) administered intramuscularly, a 1-2-cm segment of femoral artery 1 cm distal to the inguinal ligament was isolated. The isolated segments were ligated proximally and distally with silk ligatures and cannulated with a 27-gauge butterfly needle. A posterior vent was created by needle puncture. After the arterial segment was filled with 1 ml sterile saline, \( N_2 \) gas was infused via the butterfly needle through the segment at a flow rate of 80 ml/min for 8 minutes. This maneuver produced endothelial damage. The ligatures and needle were then removed, and hemostasis was achieved by direct compression. The treated segments were marked with metal surgical clips (Hemoclip, E. Weck & Co., Research Triangle Park, N.C.) and placed in the muscle lateral to the site, and the skin was approximated with a running subcuticular 4-0 Vicryl suture. Penicillin G 600,000 units was administered intramuscularly for prophylaxis of infection. The rabbits were then placed on a 2% cholesterol and 6% peanut oil diet (Dyets, Inc., Bethlehem, Pa.) for 28 days.

**Serotonin-Induced Vasoconstriction**

To evaluate the vasoconstrictive effects of serotonin on femoral arteries in vivo, six normal and seven atherosclerotic rabbits were anesthetized with ketamine and xylazine as described above. Anesthesia was maintained with an 8:1 solution of ketamine and xylazine administered intravenously. Each animal was placed in a perspex brace to standardize the position of the animals for subsequent angiographic studies. Rabbits were placed in a supine position with the hindlegs externally rotated and abducted and the knees fully extended. A midline neck incision was made, and the right carotid artery was isolated by blunt dissection. The vessel was then ligated cranially, and a 4F introducer was placed through an arteriotomy and advanced to the aortic arch under fluoroscopic guidance. After administration of 250 units heparin, a 0.014 USCI Veriflex guidewire (C.R. Bard, Inc., Billerica, Mass.) was advanced through the introducer to the distal abdominal aorta. A Medi-Tech 3.0-mm angioplasty catheter (Mansfield, Mass.) was then advanced over the guide wire to the distal abdominal aorta at the level of the L4–5 vertebral interspace. The wire was removed, and 1 ml 2% lidocaine was administered into the aorta. A baseline angiogram was obtained by manual injection of 8–9 ml 50% diatrizoate meglumine and diatrizoate...
sodium USP (Renographin 76, E.R. Squibb & Sons, Inc., Princeton, N.J.). Serotonin (5-hydroxytryptamine; Sigma Chemical Co., St. Louis, Mo.) was infused via the aortic catheter as a bolus at a dose of 1, 5, 10, 25, 50, or 100 μg, and a repeat angiogram was performed 5 minutes after infusion. Forty-five minutes after dosing, angiography was performed again to confirm return to baseline luminal diameter. A different dose of serotonin was then infused, with angiography repeated at 5 and 45 minutes as described above. Each animal received two to five doses of serotonin in this manner during an experiment.

Inhibition of Serotonin-Induced Vasoconstriction
To determine whether the vasoconstrictive effects of serotonin were mediated by the \( S_2 \) receptors LY53857 and sergolexole, specific \( S_2 \)-receptor antagonists (provided by Eli Lilly and Co., Indianapolis, Ind.) were tested in atherosclerotic rabbits. The rabbits were anesthetized and instrumented as described above. After a baseline angiogram, 1 mg LY53857 (group A, \( n=5 \)) or 2 mg sergolexole (group B, \( n=6 \)) and 10 μg serotonin, a dose previously shown to consistently produce significant vasoconstriction, were infused sequentially through the aortic catheter. Five minutes later, angiography was repeated to assess the effect of the infusion on angiographic luminal diameter. Forty-five minutes after infusion, a repeat angiogram was obtained to confirm return to baseline dimensions. To confirm that the tested vessels reacted normally to the serotonin infusion, 10 μg serotonin was then infused alone via the aortic catheter, and angiograms were repeated at 5 and 45 minutes. Finally, to determine whether the inhibitors had an independent effect on vessels, 1 mg LY53857 (group A) or 2 mg sergolexole (group B) alone was infused via the catheter. Angiograms were obtained at 5 and 45 minutes after infusion.

Phenylephrine-Induced Vasoconstriction
To confirm that LY53857 was a specific \( S_2 \)-receptor antagonist in this model and did not prevent \( \alpha \)-adrenergic-mediated vasoconstriction, we tested the response of atherosclerotic rabbits to phenylephrine with or without pretreatment with LY53857. The rabbits were anesthetized and instrumented as described previously. After a baseline angiogram was obtained, the animals were infused either with 10 mg LY53857 immediately before infusion of 0.06 mg phenylephrine (five rabbits) or with 0.06 mg phenylephrine alone (six rabbits). Angiography was repeated 5 minutes after infusion.

Angioplasty-Induced Vasospasm
To assess the effects of LY53857 and sergolexole on angioplasty-induced vasospasm, 35 atherosclerotic rabbits were anesthetized and instrumented as described. After a baseline angiogram was obtained, 5, 10, or 20 mg LY53857 or 20 or 40 mg sergolexole (\( n=5 \) in each treatment group) was infused as a bolus via the angioplasty catheter. In six rabbits, a second angiogram was obtained 10 minutes after infusion of LY53857 but before proceeding with angioplasty to confirm the lack of any significant effect of this agent on baseline luminal diameter. Four of these rabbits received 20 mg LY53857; the remaining two were treated with 10 mg LY53857.

A 0.014-in. guide wire was then placed across one of the femoral artery lesions. The angioplasty catheter was advanced over the wire until the balloon was centered across the area of angiographic stenosis. The 2.5-mm balloon was then inflated to 10 atm pressure by use of a hand indeflator (Advanced Cardiovascular Systems, Inc., Mountain View, Calif.) for three 1-minute inflation periods, with 1-minute intervals between each inflation. Position and diameter of the inflated balloon were confirmed radiographically. At the completion of the dilatation protocol the catheter tip was withdrawn into the iliac artery, and 1 ml 2% lidocaine was administered through the catheter into the angioplastied vessel according to our standard protocol. The catheter was then withdrawn to the distal abdominal aorta at the level of the L4–5 vertebral interspace, and a repeat angiogram was obtained 10 minutes after the final balloon dilatation.

The LY53857 control group (control group A) consisted of five atherosclerotic rabbits that were subjected to an identical angioplasty protocol as the experimental group but that were not pretreated with LY53857. A second group of five atherosclerotic rabbits (control group B) was randomized to treatment with a placebo injection of sterile water in place of pretreatment with sergolexole.

Termination of Experiment and Acquisition of Histological Samples
After angioplasty, four animals in each control group, five treated with 5 mg LY53857, four treated with 20 mg LY53857, and 10 treated with sergolexole were pressure perfused and killed for histological analysis. A vertical midline abdominal incision was made, and the aorta was isolated by blunt dissection. The proximal portion was ligated, and a 4F cannula was inserted above the iliac bifurcation and held in place by a silk suture. The distal arterial system was then perfused at a pressure of 100 mm Hg with 50 ml saline, followed by 100 ml 3% glutaraldehyde at room temperature. At the time of perfusion, the animal was killed with an intravenous injection of 3 ml sodium pentobarbital, (65 mg/ml) (Nembutal, Abbott Labs, North Chicago, Ill.). A 4–8-cm section of the angioplasted artery was removed en bloc and preserved in 3% glutaraldehyde for light microscopy. Four-micron to 6-μm sections were cut from the proximal to the distal end and color coded to identify their location relative to one another. Each section was stained with hematoxylin and eosin and Richardson's combination of Verhoff's elastic and Gomori's trichrome stains.19

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Figure 1. Plot of response (percent decrease in diameter as \(1 - \frac{\text{diameter after infusion}}{\text{diameter before infusion}}\)) of nonangioplastied normal (---) and atherosclerotic (----) vessels to infusions of serotonin (mcg). Best-fit logarithmic curves are illustrated for normal (solid line; percent decrease = \(13.111 + 10.138 \times \log_{10}\text{serotonin}; r = 0.68\)) and atherosclerotic responses (dashed line; percent decrease = \(11.237 + 11.967 \times \log_{10}\text{serotonin}; r = 0.63\)). Serotonin doses above 10 \(\mu\)g consistently produced vasoconstriction.

**Data Analysis**

All angiograms were read by two observers blinded to treatment. Intraluminal diameters were measured with the aid of electronic calipers (Brown and Sharpe, Athol, Mass.). Measurements were made at the distal aorta (L5-6 intervertebral space), at the proximal femoral artery (femoral head), at the site of the atherosclerotic lesion or angioplasty or at a comparable level of the femoral artery in normal rabbits, and at the distal femoral artery (the midfemur). The diameter of the inflated angioplasty balloon during the final inflation was also measured to determine the balloon-to-vessel ratio. A 1-cm grid placed at the level of the femoral artery for all angiograms allowed correction for differences in magnification between angiograms. The measurements obtained from each observer were averaged for analysis. Measurements between readers correlated well \((r=0.917)\), with a mean difference of 0.13±0.40 mm (mean±SD) between values obtained from the two observers. Measured diameters for groups are presented as mean±SEM.

To allow comparison of the responses of vessels and vessel segments with different baseline diameters, change in baseline diameter, defined as \(1 - \frac{\text{diameter after treatment}}{\text{baseline diameter}}\), was calculated after each drug infusion or angioplasty. Responses of a group of rabbits to different treatments were compared by Student's \(t\) test for paired observations with a Bonferroni correction for multiple comparisons. Responses of rabbits in different treatment groups were compared by Tukey's honestly significant difference test for post hoc comparison of multiple means. \(p<0.05\) was considered statistically significant.

Histological sections from segments proximal and distal to as well as at the angioplasty sites were examined by two observers blinded to the rabbits' treatment. Particular attention was directed toward the presence of thrombus or dissection in the vessel wall.

**Results**

**Serotonin-Induced Vasoconstriction (Figure 1)**

Six normal and seven atherosclerotic rabbits were infused with doses of serotonin ranging from 1 to 100 \(\mu\)g. Significant vasoconstriction was seen with doses as low as 1 \(\mu\)g \((p<0.05)\), with higher doses inducing greater degrees of vasoconstriction as illustrated in Figure 1.

Figure 1 confirms that there was no significant difference in the response of normal and atherosclerotic rabbits to a given dose of serotonin, as measured by Student's \(t\) test for unpaired observations, although such a difference may be masked in part by the small number of observations and the high interanimal variability of responses.

**Inhibition of Serotonin-Induced Vasoconstriction (Figure 2)**

Infusion of 10 \(\mu\)g serotonin alone in five atherosclerotic rabbits resulted in a 23±5% decrease in
luminal diameter at the atherosclerotic site compared with baseline, as illustrated in Figure 2A. However, infusion of 1 mg LY53857 followed by 10 μg serotonin in the same rabbits produced no significant decrease from baseline diameter (1 ± 7%). This was significantly different from the vasoconstriction seen with serotonin alone (p < 0.002). Infusion of LY53857 (1 μg) alone likewise produced no significant change in luminal diameter from baseline (3 ± 7%). In a second group of five rabbits, infusion of a 10-μg dose of serotonin resulted in a decrease in luminal diameter to 1.2 ± 0.2 mm from a baseline of 1.4 ± 0.3 mm (p < 0.05), as illustrated in Figure 2B. When 10 μg serotonin was given after treatment with 2 mg sergolexole in the same rabbits, however, there was no significant decrease from baseline (1.4 ± 0.3 versus 1.4 ± 0.3 mm). This differed significantly from the response seen after infusion of serotonin alone (p < 0.01). Infusion of 2 mg sergolexole alone likewise produced no significant change in luminal diameter (1.3 ± 0.2 versus 1.4 ± 0.2 mm at baseline).

Phenylephrine-Induced Vasoconstriction (Figures 3 and 4)

Infusion of 0.06 mg phenylephrine reduced proximal femoral diameter from 1.9 ± 0.1 to 1.4 ± 0.1 mm (26 ± 3% reduction), distal femoral diameter from 1.5 ± 0.1 to 1.18 ± 0.08 mm (22 ± 2%), and lesion di-
FIGURE 3. Angiograms of vessels in two rabbits before and after infusion of phenylephrine, demonstrating lack of effect of LYS3857 on α-adrenergic-mediated vasoconstriction. Panel A: Baseline angiogram of vessels in an atherosclerotic rabbit. L, atherosclerotic lesion; P, segment of femoral artery proximal to lesion; D, femoral artery distal to atherosclerotic site. Panel B: Angiogram of same rabbit 5 minutes after infusion of 0.06 mg phenylephrine, with marked vasoconstriction throughout femoral arteries bilaterally. Proximal femoral artery diameter decreased from 2.0 to 1.6 mm on the right and from 2.2 to 1.7 mm on the left. Distal femoral artery diameter decreased from 1.8 to 1.3 mm on the right and from 1.8 to 1.4 mm on the left. Panel C: Baseline angiogram of vessels in a second atherosclerotic rabbit. Note that the right femoral artery is occluded. Panel D: Angiogram of the same rabbit 5 minutes after infusion of 10 mg LYS3857 followed by 0.06 mg phenylephrine. There is marked vasoconstriction of the aorta and left femoral artery, with near obliteration of the lumen at the atherosclerotic site. Left femoral artery diameter decreased proximally from 1.7 to 1.3 mm and distally from 1.5 to 0.9 mm. Early filling of left femoral vein (small arrows) and inferior vena cava (large arrow) with contrast is also shown.

Ameter from 1.13±0.08 to 1.05±0.08 mm (7±3%). Infusion of 10 mg LYS3857, a 10-fold higher dose of the agent than was used to block serotonin-induced vasoconstriction, immediately before phenylephrine administration had no significant effect on phenylephrine-induced vasoconstriction, as illustrated in the representative angiograms of Figure 3. The responses are summarized in Figure 4.

Angioplasty-Induced Vasospasm (Figures 5–8)

Thirty-five atherosclerotic rabbits were treated with three 1-minute balloon inflations at 10 atm 1 minute apart. This resulted in a significant increase in diameter at the site of atherosclerotic narrowing in each treatment group and in both control groups (Figure 5). In contrast, there were concomitant decreases in the measured luminal diameters both proximal (2.4±0.1 versus 1.9±0.1 mm, p<0.001) and distal (1.8±0.1 versus 1.5±0.1 mm, p<0.03) to the angioplasty site in the five rabbits not receiving LYS3857 (control group A, Figures 6A and 7A) and in the five rabbits receiving sterile water injections in place of treatment with sergolexole (control group B, Figures 6B and 7B). Pretreatment with 5 mg LYS3857 did not significantly affect proximal

FIGURE 4. Bar graph of response of atherosclerotic vessels to infusion of 0.06 mg phenylephrine alone (●, n=6) or immediately after infusion of 10 mg LYS3857 (■, n=5) at proximal, distal, and atherosclerotic (lesion) femoral artery sites. Values are expressed as mean±SEM percent luminal narrowing (1−[diameter after infusion/diameter before infusion]). There was no significant difference in the magnitude of vasoconstriction at any site with LYS3857 infusion compared with infusion of phenylephrine alone.
(2.4±0.1 versus 1.9±0.1 mm, \( p<0.05 \)) or distal (1.74±0.07 versus 1.26±0.07 mm, \( p<0.001 \)) spasm after angioplasty. This is illustrated in Figure 8 with angiograms from a representative experiment. With 10 mg LY53857, there was no significant proximal spasm (2.6±0.4 versus 2.2±0.1 mm). There was, however, a significant decrease in distal diameter (1.74±0.05 versus 1.3±0.1 mm, \( p<0.002 \)), indicating that distal spasm was not inhibited. When animals were pretreated with 20 mg LY53857, both proximal and distal spasms were inhibited (Figures 6A, 7A, and 8). Similarly, after pretreatment with 20 mg sergolexole, there was no significant proximal vasospasm after angioplasty (2.1±0.4 versus 2.1±0.4 mm) (Figure 6B). There was, however, a significant decrease in distal vessel diameter (1.6±0.3 versus 1.3±0.2 mm, \( p<0.05 \)), consistent with vasospasm (Figure 7B). Both proximal and distal vasospasms were prevented by pretreatment with 40 mg sergolexole. Thus, higher doses of LY53857 and sergolexole were needed to block distal as opposed to proximal spasm.
**Histology (Figure 9)**

The atherosclerotic process produced in this model was focal, with normal vessels appearing adjacent to the atherosclerotic segments, with a separation of less than 10 μm. As illustrated in Figure 9, there was marked intimal hyperplasia, with 50–75% cross-sectional luminal narrowing in the most stenotic segments. The plaque consisted of a cap of myointimal cells and fibrous tissue. At the base of the intimal plaque, there was an accumulation of lipid-laden macrophages, or foam cells. These foam cells largely replaced the muscular elements of the normal media and, in many cases, they infiltrated the adventitia as well.

After angioplasty there was evidence of splitting of the intima (dissection) with rupture of the internal elastic lamina. The dissection extended into the media, but the external elastic lamina remained intact in all cases. There was no evidence of hemorrhage in the adventitia. In treated sites, there was evidence of thrombus formation in angioplastied animals in control group A (three of four), control group B (one of four), and animals pretreated with 5 mg (two of five) or 20 mg (two of four) LY53857 or 40 mg (one of five) sergolexole. No thrombus was seen in the five animals treated with 20 mg sergolexole. Dissection was noted in all groups as well (control group A, three of five; control group B,
three of four; 5 mg LY53857, three of five; 20 mg LY53857, four of four; 20 mg sergolexole, two of five; 40 mg sergolexole, five of five). There was no evidence of thrombus or dissection in sections proximal or distal to the site of angioplasty.

**Discussion**

The major findings of this study are that 1) local infusion of serotonin caused similar degrees of vasoconstriction in both normal and atherosclerotic rabbit femoral arteries in anesthetized animals; 2) serotonin-induced vasoconstriction was blocked by treatment with LY53857 or sergolexole; 3) LY53857 did not prevent phenylephrine-induced vasoconstriction, confirming its specificity as an $S_2$-receptor antagonist; 4) significant vasospasm occurred both proximal and distal to the dilated sites after balloon angioplasty; and 5) angioplasty-induced vasospasm was inhibited by pretreatment of animals with LY53857 or sergolexole.

The effects of serotonin on arterial tone are complex. Serotonin-induced vasoconstriction can be mediated by direct stimulation of the $S_2$ class of receptors on vascular smooth muscle, presynaptic facilitation of sympathetic neurotransmitter release, displacement of norepinephrine from adrenergic receptors, or direct stimulation of $\alpha$-adrenergic receptors. Opposing this action is the vasodilatory effect of serotonin mediated...
FIGURE 8. Angiograms of vessels in two rabbits before and after angioplasty, demonstrating effect of LY53857 on angioplasty-induced vasoconstriction. Top of figure is cephalad. Panel A: Preangioplasty angiogram of rabbit pretreated with 5 mg LY53857. L, atherosclerotic lesion; P, segment proximal to lesion; D, segment distal to lesion. Note that right femoral artery is totally occluded. Panel B: Angiogram after angioplasty, with luminal narrowing due to spasm at proximal and distal sites. Spasm is most marked at distal site. Thus, LY53857 at this low dose was not effective. Panel C: Preangioplasty angiogram of rabbit before treatment with 20 mg LY53857. Panel D: Illustrates that spasm was prevented after angioplasty at proximal and distal sites. Note that lesion site is dilated versus preangioplasty angiogram, indicating successful angioplasty. Also, right femoral artery is totally occluded.

by $S_2$ receptors on vascular endothelium. The net effect of serotonin on a vascular system depends on resting tone and the sum of the individual effects of serotonin at each of these sites.

We found that serotonin was a potent vasoconstrictor of both normal and atherosclerotic rabbit femoral arteries, with as much as a 40% reduction in luminal diameter from baseline with a dose of 100 $\mu$g serotonin. There was no significant difference between normal and atherosclerotic vessels in response to serotonin in vivo, although such a difference may, in part, have been masked by the wide variability of the response seen and the small number of animals studied. In contrast, others have shown an enhanced contractile response to serotonin in atherosclerotic hypercholesterolemic rabbits and monkeys. Both the reduced production of endothelium-derived relaxation factor and an increase in the number of serotonin receptors in the atherosclerotic vessel wall have been proposed as mechanisms of this effect in rabbits.

There are several factors that may explain the apparent discordance between our findings and those of other investigators. The demonstration of endothelium-dependent relaxation frequently requires preconstriction of vessels with another agonist. In our anesthetized rabbits, it is likely that the studied vessels are maximally dilated at baseline, as demonstrated by their lack of further dilatation to infused nitroglycerin. Thus, any vasodilatory effect of low-dose serotonin on normal vessels may be masked. Similarly, others have reported that the $S_2$-mediated contractile effects of serotonin may predominate in vivo and serotonin-induced endothelium-dependent relaxation may not be apparent unless unmasked by an $S_2$-receptor antagonist. In addition, there is some evidence that dietary treatment of atherosclerotic monkeys can restore endothelium-dependent relaxation to normal. The atherosclerotic rabbits we used to study serotonin-induced vasoconstriction had been fed with standard rabbit chow for an average of 32 days before testing with serotonin. To the extent that this normalization of endothelium-dependent relaxation can occur in atherosclerotic rabbits, it is possible that the atherosclerotic vessels tested could react normally to serotonin infusion.

Serotonin, however, can cause vasoconstriction via either $\alpha$-adrenergic or $S_2$ receptors in vascular tissue. We therefore used LY53857 and sergolexole, two potent and highly selective competitive $S_2$-receptor antagonists with no significant agonist activity, to contract vascular smooth muscle to determine whether the vasoconstrictive effects of serotonin
were mediated by α-adrenergic or serotonin receptors. We found that pretreatment of animals with 1 mg LY53857 or 2 mg sergolexole completely inhibited the vasoconstrictor effect of 10 μg serotonin infused locally, thus demonstrating that the vasoconstriction was an S2-mediated effect. The lack of effect of LY53857 on phenylephrine-induced vasoconstriction confirmed the specificity of this agent's action on the S2 receptor in the atherosclerotic rabbit.

In our model, there was a 21% proximal and a 17% distal luminal narrowing compared with baseline immediately after angioplasty in control animals. Analysis of histological samples from the treated vessels confirmed that the observed narrowing was not due to the presence of thrombus or dissection, which were only seen at the site of dilatation. Despite this significant vasospasm proximal and distal to the site of angioplasty, there was no significant vasospasm seen at the site of balloon dilatation. This is consistent with the findings of others in porcine, rabbit, and human models and may be due to medial damage during stretch injury and resultant smooth muscle paralysis. We have shown previously in this model that dilatation to 10 atm with an appropriately sized balloon causes moderate degrees of medial necrosis and patchy areas of acellularity in the treated areas and that this may render the dilatation site unresponsive to ergonovine-induced vasoconstriction. Of note, treated sites can regain some responsiveness to vasoconstrictors with time, particularly to serotonin. Others have suggested that treated sites do retain the ability to vasoconstrict when treated with appropriately sized balloons at relatively low inflation pressures.

The mechanism by which vasospasm occurs in adjacent segments is unclear, but animal studies have suggested that it is related to platelet deposition and activation, with release of serotonin, thromboxane A2, and other potential vasoconstrictors at the site of angioplasty. Such studies have clearly demonstrated that platelet deposition occurs at the site of angioplasty, particularly within the first 2 hours after dilatation. Aspirin and heparin therapy are only partially effective in blocking this platelet deposition. Both serotonin and thromboxane A2 have been suggested as potential vasoconstrictor mediators released by activated platelets. Golino et al and Ashton et al have shown that either S2-receptor blockade with LY53857 or thromboxane A2 blockade with SQ29548 was effective in blocking the cyclic flow variations seen in extrinsically stenosed coronary arteries in anesthetized dogs. Similarly, Anderson et al demonstrated that cyclic flow variations occurring in canine coronary arteries after angioplasty could be blocked by either of these agents. These studies suggest that both serotonin and thromboxane A2 are important physiological mediators of vasospasm but that blockade of either agent's action may be sufficient to interrupt the process. However, while coronary sinus levels of the stable metabolite thromboxane B2 can be shown to increase in the setting of acute occlusion after coronary angioplasty in patients, vasospasm can be documented in the absence of such changes.

Pretreatment of animals with 20 mg LY53857 or 40 mg sergolexole immediately before angioplasty was effective in blocking distal as well as proximal vasospasm after angioplasty. In contrast, a dose of 10 mg LY53857 or 20 mg sergolexole prevented the proximal luminal narrowing but did not significantly inhibit the distal vasospasm seen after angioplasty. The higher dose requirement to block distal compared with proximal spasm may reflect higher local concentrations of serotonin distal to the angioplasty site as a result of serotonin released by platelets at the angioplasty site being carried distally by blood flow. The dose of inhibitor needed to block angioplasty-induced vasospasm
was 20-fold higher than that needed to block the vasoconstrictive effect of infused serotonin. This may likewise reflect sustained high local concentrations of serotonin after angioplasty, possibly due to ongoing release as platelets are activated at the site of injury.

In addition to blocking the vasoconstrictive effect of serotonin on vessels, serotonin-receptor antagonists have also been shown to inhibit platelet deposition in stenotic canine coronary arteries. This may result in improved distal patency of the treated vessel after angioplasty, both by preventing the physical occlusion due to platelet deposition and by inhibiting release of serotonin from platelets that might otherwise become activated at the angioplasty site. However, our histological study confirmed thrombus formation at the angioplasty site in animals pretreated with LY53857 or sergolexole as well as in control animals not treated with these agents.

While angiographic evidence of acute thrombosis occurs in less than 10% of failed angioplasties,22 there is often overlap between the incidence of thrombosis and vasospasm that makes it difficult to implicate one or the other as the primary cause of acute closure.1 Acute closure resulting from severe spasm, thrombosis, and/or dissection accounts for as much as 44% of primary angiographic failures in patients in some studies.42 Treatment with aspirin and calcium channel antagonists does not completely inhibit the occurrence of spasm acutely after angioplasty.3 This may be a reason for the lack of efficacy of these agents in altering the ultimate rate of restenosis seen both in animals43 and humans.44,45 Our rabbit model affords distinct advantages in studying this process, given the potential for direct correlation between angiographic and histological findings after angioplasty. Using this model, we have found that acute thrombotic occlusion by either angiographic or histological criteria is rare.32

While percutaneous transluminal coronary angioplasty is highly effective in achieving acute patency of stenosed coronary arteries,42 the relatively high rate of restenosis within the first 6 months26,47 is a major limitation of the procedure. The etiology of restenosis after angioplasty is likely to be multifactorial, but platelets have been implicated as mediators of this process on several levels. The potential roles for platelets in restenosis include 1) thrombus formation with subsequent reorganization; 2) release of vasoactive substances such as serotonin and thromboxane A2, causing vasoconstriction with a resultant decrease in blood flow; and 3) release of chemotactic and mitogenic substances inducing migration and proliferation of smooth muscle cells at the treated site, including platelet-derived growth factor.48-49 While thrombus formation was not prevented in this study, LY53857 and sergolexole did significantly block the vasospasm seen at proximal and distal sites after angioplasty. To the extent that either LY53857 or sergolexole does inhibit platelet deposition at the angioplasty site, there may be a reduction in platelet-derived growth factor and other chemotactic and mitogenic mediators that may be involved in the intimal and smooth muscle proliferation responsible for restenosis. The long-term effects of LY53857 or sergolexole on restenosis in this model have not been evaluated.

Limitations of the Rabbit Model of Angioplasty

Rabbits provide a useful model for the study of angioplasty of atherosclerotic vessels.32,50,51 Our technique for inducing focal atherosclerosis is modified from that described by LeVeen et al.18 It combines endothelial damage by air dessication locally with a high-lipid (2% cholesterol, 6% peanut oil) diet for 1 month. This consistently results in focal fibrocellular atherosclerotic plaques, with a 50–75% reduction in luminal cross-sectional area, which are suitable for angioplasty. The acute and chronic changes after angioplasty in this model45,52 are similar to those described in the limited available material from humans.52

As with all experimental models of human atherosclerosis, there are important differences between the induced atherosclerotic lesions in the rabbit and those seen in humans. The calcification, extensive fibrosis, and necrosis seen in advanced human atherosclerosis are not present in this model. Furthermore, these rabbits develop hypercholesterolemia to an extent rarely seen in humans (>1,000 mg/dl). In general, foam cells are more prominent in the induced lesions in rabbits compared with those in human atherosclerosis. Rabbits do have significantly higher intraplatelet levels of serotonin than do humans, but LY53857 and sergolexole were still effective blockers of induced vasospasm in this model. It is possible that because of the lower content of serotonin in human platelets, less serotonin is released in humans after angioplasty. Thus, the effect of a serotonin-receptor inhibitor may be less pronounced.

Implications

We have demonstrated in a rabbit model of focal femoral atherosclerosis that serotonin causes significant vasoconstriction. This action can be blocked by \( \text{S}_{2} \)-receptor blockade with LY53857 or sergolexole. Similarly, \( \text{S}_{2} \)-receptor blockade prevented the proximal and distal vasospasm seen after balloon angioplasty. This suggests that angioplasty-induced vasoconstriction may be mediated by serotonin released from activated platelets deposited at the dilatation site. We speculate that specific \( \text{S}_{2} \)-receptor antagonists may be useful as adjunctive therapy to prevent acute closure secondary to vasospasm after angioplasty in patients, and thus are worthy of further evaluation.

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References
28. Ashton JH, Golino P, McNatt JM, Buta LM, Willerson J: Serotonin S3 and thromboxane A{sub 2}-prostaglandin H2 receptor blockade provide protection against epinephrine-induced

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Effects of serotonin-receptor blockade on angioplasty-induced vasospasm in an atherosclerotic rabbit model.
S L Sigal, J Gellman, I J Sarembock, P J LaVeau, Q S Chen, H S Cabin and M D Ezekowitz

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