Accumulation of 5-Hydroxytryptamine Leads to Dysfunction of Adrenergic Nerves in Canine Coronary Artery Following Intimal Damage In Vivo

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Previous in vitro studies have demonstrated that coronary artery adrenergic nerves are a principal site of accumulation of 5-hydroxytryptamine released from aggregating platelets. The purpose of this study was to determine whether 5-hydroxytryptamine is accumulated by adrenergic nerves at sites of endothelial damage and platelet aggregation in vivo. Coronary artery 5-hydroxytryptamine content and response to in vitro adrenergic nerve stimulation were studied in dogs 24 hours following balloon catheter-induced intimal injury. 5-Hydroxytryptamine content was significantly increased in the catheter-damaged arteries, and there was a coincident decrease in the content of norepinephrine. The relaxation caused by acetylcholine was abolished in the catheter-injured arteries, indicating loss of this endothelial cell-mediated function. The normal β-adrenergic relaxation caused by nerve stimulation was inhibited, and in some cases, contractions resulted; these effects were prevented by serotonergic receptor antagonists. The sensitivity to exogenously added norepinephrine was unchanged, indicating that the changes in the response to nerve stimulation were not due to an altered smooth muscle response to the native neurotransmitter. These observations indicate that following intimal damage, which produces platelet aggregation on the luminal surface of the blood vessel, 5-hydroxytryptamine can assume a transmitter role in coronary artery adrenergic nerves and thereby cause their dysfunction. (Circulation Research 1987;61:829–833)

Release of vasoactive substances from aggregating platelets may contribute to the pathophysiology of unstable angina pectoris and coronary artery spasm. Studies in vivo have demonstrated that canine coronary arteries accumulate 5-hydroxytryptamine released from platelets aggregating at sites of endothelial damage. Our previous in vitro studies have suggested that platelet-released 5-hydroxytryptamine is accumulated principally in the adrenergic nerve endings via the amine uptake mechanism. Following in vitro loading of coronary artery adrenergic nerves with 5-hydroxytryptamine, the indoleamine is neurogenically released, and it counteracts the normal β-adrenergic action of the native transmitter, norepinephrine. The present studies were designed to determine whether accumulation of 5-hydroxytryptamine in adrenergic nerve endings occurs after endothelium damage in vivo and whether the amine causes dysfunction of the nerves.

Materials and Methods

Intimal Damage

The intimal surface of the left circumflex artery was damaged with a No. 2 French balloon embolectomy catheter (Edwards Laboratories, Inc., San Juan, Puerto Rico) in 10 mongrel dogs. A thoracotomy was performed under pentobarbital (30 mg/kg i.v.) and halothane (1%) anesthesia. Lidocaine (50-mg bolus followed by 0.1 mg/min i.v.) was given to prevent ventricular fibrillation. The balloon catheter was inserted via an arteriotomy in a ventricular coronary artery branch. Under visual guidance, the catheter was advanced retrograde into either the proximal or distal left circumflex coronary artery. The balloon was inflated (0.04 ml) and withdrawn along a length of artery a total of 3 times. The arteriotomy in the branch artery was ligated, the chest was closed, and the animal was allowed to recover from anesthesia. In 3 dogs, the distal and in 5 dogs, the proximal segment was injured with the catheter. In 2 dogs, the entire length of left circumflex coronary artery was injured, and control rings were obtained from the left anterior descending artery. Immediately prior to killing 24 hours later, Evans blue dye (400 mg) was injected intravenously so that during dissection of the artery from the surface of the heart, the location of catheter-induced damage was clearly visible from the cut ends of the vessel. In no case did occlusion of the left circumflex artery occur as a result of thrombosis. A small myocardial infarction was evident grossly in the distribution of the ligated branch artery. Light microscopy of catheter-injured arterial segments showed denudation of endothelial cells; no
mast cells were seen. Scanning electron micrographs demonstrated adhesion and spreading of platelets on the intimal surface.

Measurements of Biogenic Amines

5-Hydroxytryptamine and norepinephrine were assayed by high performance liquid chromatography with electrochemical detection in acid extracts of arterial segments (10–20 mm length) adjacent to those in which physiologic responses were measured. Segments were washed (3 × 5 minutes) in physiological saline at 4°C and extracted for 1 hour at 4°C in 0.5 ml, 0.1 N acetic acid containing 1 g/l sodium metabisulfite, 50 mg/l EDTA, 1 μl 1% dithiothreitol, 5 μl 1% cysteine, 100 μl 10⁻⁶ M n-methyl 5-hydroxytryptamine, and 100 μl 10⁻⁹ M 3,4-dihydroxybenzylamine (as internal standards). The extracts were filtered (0.45 μm) and injected into chromatographs consisting of a 7.5-cm Altex Ultrasphere 3-μm ODS reverse phase column (LC-4B electrochemical detector, Bioanalytical Systems, Lafayette, Ind.) and a glassy carbon electrode (+0.6 V versus Ag/AgCl). Solvent delivery was by way of a Beckman 110B or Eldex AA pump. The solvent for 5-hydroxytryptamine consisted of 6 ml/1 concentrated acetic acid, 40 ml/l acetone, 13.6 g/l sodium acetate, 50 mg/l EDTA, and 30 mg/l sodium octyl sulfate; that for norepinephrine consisted of 30 ml/l methanol, 13.6 g/l sodium acetate, 3.84 g/l citric acid, 50 mg/l EDTA, and 250 mg/l sodium octyl sulfate. Identification was by injected standards, and quantitation was by measured peak height; results are reported as picogram per milligram of tissue blotted wet weight. Recoveries of 5-hydroxytryptamine and norepinephrine averaged 88 and 98%, respectively.

Organ Chamber Experiments

Rings of coronary artery (6 mm length) were suspended in organ chambers filled with physiological saline, and isometric tension was recorded following equilibration of the rings at the optimal resting tension for contraction. To observe relaxations, the rings were contracted with prostaglandin F₂α (2 × 10⁻⁶ M) prior to obtaining responses to nerve stimulation, acetylcholine, or norepinephrine. Functional integrity of the endothelium in each ring segment was determined by the concentration-dependent relaxation caused by acetylcholine. Relaxations caused by exogenous norepinephrine or acetylcholine were obtained by half-log increases in organ chamber concentration (10⁻⁸–10⁻⁶ M). Adrenergic nerves were activated by transmural electric field stimulation (10 V, 0.2-msec duration, 0.5–16 Hz). All responses were obtained in the presence of indomethacin (3 × 10⁻³ M) because this agent is required for in vitro measurements of the response of canine coronary arteries to adrenergic nerve stimulation. To compare the effects of in vivo and in vitro exposure to 5-hydroxytryptamine, control segments of coronary artery obtained from dogs not receiving catheter-injury were incubated with the amine. Rings were mounted in organ chambers and incubated in 5-hydroxytryptamine (10⁻⁶ M) for 2 hours. They were then rinsed repeatedly for 1 hour before being contracted with prostaglandin F₂α and stimulated electrically. Some rings were treated identically, but methiothepin (10⁻⁷ M) was added 1 hour prior to electrical stimulation. Additional segments of coronary artery were similarly incubated in the presence and absence of 5-hydroxytryptamine (10⁻⁴ M) for 1 hour, and extracted for analysis of biogenic amines. All responses to electrical stimulation of noninjured and catheter-injured rings and of rings incubated with 5-hydroxytryptamine were prevented by tetrodotoxin (10⁻⁷ M).

Data Analysis

Responses to acetylcholine and norepinephrine are expressed as a percent of the maximal relaxation caused by sodium nitroprusside (10⁻⁶ M) added immediately after the highest concentration of agonist. IC₅₀s are determined by least-squares estimation of the concentration causing 50% relaxation and are reported as the logarithm of the concentration. Relaxations caused by electrical stimulation are expressed as a percent of the contractions produced by prostaglandin F₂α (2 × 10⁻⁶ M); these contractions were not significantly different in noninjured or catheter-injured segments or in segments incubated in 5-hydroxytryptamine (6.8 ± 0.9, 7.5 ± 2.2, and 7.2 ± 1.3 g). Statistical comparisons of mean ± SEM of single data points or IC₅₀s were made using the Student’s t test. Significance was accepted for p < 0.05.

Drugs

Final molar concentrations in the organ chamber are reported. Acetylcholine chloride, cyproheptadine, Evans blue dye, 5-hydroxytryptamine creatinine sulfate, indomethacin, norepinephrine hydrochloride, and prostaglandin F₂α (Tris salt) were obtained from Sigma Chemical Co., St. Louis, Mo. Methiothepin maleate was generously provided by Hoffmann-LaRoche, Nutley, N.J.

Results

Arterial Content of 5-Hydroxytryptamine and Norepinephrine

The content of 5-hydroxytryptamine was 10 ± 6.0 pg/mg in noninjured coronary artery segments and was increased significantly to 171 ± 56 pg/mg in catheter-injured segments (p < 0.025, n = 6). There was a coincident decrease from 600 ± 46 to 355 ± 31 pg/mg (p < 0.01) in the arterial content of norepinephrine in catheter-injured segments compared with noninjured segments from the same hearts.

After incubating control coronary arteries in vitro for 2 hours with 5-hydroxytryptamine (10⁻⁶ M), the arterial content of the indoleamine was significantly increased to 354 ± 72 pg/mg compared with 8.5 ± 5.7 pg/mg in segments incubated similarly but in the absence of 5-hydroxytryptamine (n = 5). The content of norepinephrine of coronary arteries from these control dogs (1,210 ± 200 pg/mg) was greater than that...
in noninjured arteries from dogs that received catheter-injury, but the difference was not statistically significant (p<0.1). In vitro exposure of coronary arteries to, and accumulation of, 5-hydroxytryptamine did not significantly affect the arterial content of norepinephrine (1,140±190 pg/mg).

**Physiologic Responses**

All of the catheter-injured rings failed to respond to acetylcholine (10⁻³–10⁻⁶ M). All of the noninjured control rings relaxed in response to acetylcholine (log IC₅₀, -7.1 ± 0.2).

When catheter-injured coronary rings were contracted by prostaglandin F₂α in 2 of 10 dogs, electric stimulation at 0.5–2 Hz caused further contractions, and attenuated relaxations occurred at higher frequencies (Figure 1). The contractions were prevented and the relaxations were increased when the responses were repeated in the presence of the serotonergic receptor antagonists, cyproheptadine (10⁻⁶ M, Figure 1) or methiothepin (10⁻⁶ M). In contrast, all noninjured coronary segments relaxed in response to electric stimulation.

In coronary arteries from the remaining 8 dogs, electric stimulation caused relaxations that were significantly inhibited in catheter-injured rings compared with those in the noninjured arteries (Figure 2). The maximal relaxation caused by 16-Hz stimulation in injured rings was 71 ± 11% of the contraction caused by prostaglandin F₂α, which was significantly less than that in noninjured rings (105 ± 8.6%, p<0.05). Following the addition of methiothepin (10⁻⁶ M), the relaxations caused by electric stimulation were not significantly different in balloon-injured and noninjured rings (Figure 2). The response to 16-Hz stimulation increased significantly to 91 ± 11% (p<0.05) in the presence of methiothepin in injured rings, while no significant effect of the antagonist on the response to 16 Hz was observed in the noninjured controls (104 ± 79%). At 2-Hz stimulation, the relaxation of injured and noninjured coronary artery rings in control solution was not significantly different. However, the relaxation increased by 37 ± 12% following methiothepin in noninjured rings, whereas in injured rings, the response increased significantly more (by 134 ± 48%, p<0.05) following the antagonist. All relaxations in injured and noninjured rings were blocked by propranolol (10⁻⁶ M).

Catheter-induced injury did not affect the sensitivity to relaxations caused by exogenously added norepinephrine. The log IC₅₀ in noninjured rings was -7.5 ± 0.2, and that in injured segments was -7.6 ± 0.2.

As in some balloon-injured rings, coronary rings exposed to 5-hydroxytryptamine (10⁻⁶ M) for 2 hours in vitro contracted when stimulated from 0.5–2 Hz and demonstrated decreased relaxations at higher frequencies (Figure 3). Relaxation to 16-Hz stimulation of these coronary artery rings (10±3.7%, n=6) was
5-hydroxytryptamine (10⁻⁶ M) and 1 hour rinsing; and stimulation of coronary adrenergic nerves following exposure to 5-hydroxytryptamine in vitro. Responses are shown of three rings of coronary artery from the same heart, a, Control coronary artery ring; b, ring following 2 hours incubation with 5-hydroxytryptamine (10⁻⁶ M) and 1 hour rinsing; and c, ring similarly incubated with 5-hydroxytryptamine but treated with methiothepin (10⁻⁶ M) prior to electric stimulation. In each case, the smooth muscle is contracted with prostaglandin F₂α (2 × 10⁻⁴ M); after the preparation stabilized, electric stimulation from 0.5 to 16 Hz was performed. Contractions were observed in response to 0.5—4 Hz of the ring incubated with 5-hydroxytryptamine alone, while similar relaxations occurred in the control ring and in that treated with methiothepin.

Contractions were most evident at low frequencies of electric stimulation and were prevented by cyproheptadine⁶ or methiothepin. The similarity between the contractions of coronary rings exposed to 5-hydroxytryptamine in vivo and in vitro and their prevention by the serotonergic antagonists indicates that the mediator of the altered nerve function observed in the catheter-injured rings is 5-hydroxytryptamine. That the contractions caused by electric stimulation were prevented by tetrodotoxin further indicates that 5-hydroxytryptamine is present in adrenergic nerves 24 hours following in vivo intimal damage and that neuronal release of the newly acquired transmitter mediates the contractions as it does in rings incubated with the indoleamine.⁶ Contractions were not observed in all coronary arteries after in vivo intimal damage. This may be due to the barrier between the lumen and the adrenergic nerves provided by the thickness of the arterial wall as well as to monoamine oxidase present in coronary arterial smooth muscle.⁷ This barrier was partially circumvented in the in vitro studies in which the coronary arteries were immersed in platelets⁶ or in 5-hydroxytryptamine and in which a greater amount of the indoleamine was accumulated.

Discussion

As observed in this and previous studies, damage to the endothelium induced by a balloon catheter in vivo leads to platelet aggregation on the intimal surface of the coronary artery⁶⁻¹² and to accumulation by the artery of 5-hydroxytryptamine.⁴⁻⁵ It is likely that the accumulated 5-hydroxytryptamine originates from the platelets, because platelets are numerous at the site of injury and no mast cells were observed histologically. Furthermore, platelet-released 5-hydroxytryptamine is accumulated by coronary arteries in vitro with similar functional consequences.⁶

The contractions of catheter-injured coronary arteries during electric stimulation are very similar to those observed following exposure of dog coronary arteries to the concentration of platelets present in plasma,⁶ or to the micromolar concentration of 5-hydroxytryptamine released in the former in vitro experiments. The

FIGURE 3. Example of contractions caused by in vitro electric stimulation of coronary adrenergic nerves following exposure to 5-hydroxytryptamine in vitro. Responses are shown of three rings of coronary artery from the same heart. a, Control coronary artery ring; b, ring following 2 hours incubation with 5-hydroxytryptamine (10⁻⁶ M) and 1 hour rinsing; and c, ring similarly incubated with 5-hydroxytryptamine but treated with methiothepin (10⁻⁶ M) prior to electric stimulation. In each case, the smooth muscle is contracted with prostaglandin F₂α (2 × 10⁻⁴ M); after the preparation stabilized, electric stimulation from 0.5 to 16 Hz was performed. Contractions were observed in response to 0.5—4 Hz of the ring incubated with 5-hydroxytryptamine alone, while similar relaxations occurred in the control ring and in that treated with methiothepin.

significantly more inhibited than in catheter-injured coronary segments. The relaxation to 16 Hz of rings incubated with 5-hydroxytryptamine and treated with methiothepin (87 ± 5.2%, n = 6) was not significantly different from balloon-injured rings treated with the antagonist.

Those coronary arteries that did not exhibit serotonergic contractions did manifest a significant attenuation of the relaxation caused by adrenergic nerve stimulation. Stimulation of canine coronary adrenergic nerves in vitro has previously been shown to release norepinephrine that acts primarily at β-adrenoceptors that mediate relaxation of coronary smooth muscle.³ The decreased relaxation of catheter-injured arteries was not due to changes in responsiveness of coronary smooth muscle adrenoceptors, because the response of the arteries to exogenously added norepinephrine was not affected. The role of 5-hydroxytryptamine in the attenuated relaxations caused by nerve stimulation is indicated by the action of methiothepin. Methiothepin has been shown to antagonize receptors that mediate smooth muscle contraction of the dog coronary artery caused by 5-hydroxytryptamine.³ The antagonist is specific in this action as other mediators of coronary artery contraction are unaffected.¹³ Methiothepin has no significant effect on relaxations caused by 16-Hz stimulation of control and noninjured coronary artery rings. Thus, the increase caused by the drug in the 16-Hz response of the catheter-injured arteries is consistent with an action of 5-hydroxytryptamine at smooth muscle serotonergic receptors that limits the β-adrenergic response to nerve stimulation. The similar effect of methiothepin on the response to 16 Hz of coronary rings exposed to 5-hydroxytryptamine in vitro further supports this action of the antagonist. Methiothepin blocks both α₁ and serotonergic presynaptic receptors that limit the release of norepinephrine at low but not at high frequencies.¹³ The α₁-receptor activity of the antagonist explains why the drug increases relaxations of control and noninjured coronary rings to 2-Hz stimulation. However, the greater effect of methiothepin on the response to 2 Hz in catheter-injured than in noninjured rings also suggests that
activation of serotonergic receptors limits the response of catheter-injured coronary rings at the lower frequency. Although a decrease in norepinephrine release at lower frequencies by an action of 5-hydroxytryptamine at prejunctional receptors cannot be excluded in catheter-injured coronary rings, this did not occur after exposure to the indoleamine in vitro. Thus, the contractions observed in catheter-injured coronary rings following methiothepin suggest that 5-hydroxytryptamine is the sole mediator of the alteration in nerve function observed under these experimental conditions.

The loss of arterial content of norepinephrine in catheter-injured arteries may have occurred by catheter-induced neural injury or by displacement from the nerve endings by 5-hydroxytryptamine. The latter mechanism may be less likely because it did not occur during in vitro exposure to 5-hydroxytryptamine during which the content of the indoleamine increased to a greater extent. The decrease in norepinephrine content could have contributed to lessening the β-adrenergic response to nerve stimulation of catheter-injured arteries but was evidently not of primary importance since the β-adrenergic response to electric stimulation of the injured arteries was not significantly different from that of noninjured arteries in the presence of methiothepin. There may have been an overall decrease in the content of norepinephrine in the coronary arteries from dogs receiving catheter-injury that may have been related to the surgery or anesthesia, although this did not affect the response of these arteries to nerve stimulation compared to those of control vessels. As judged by the similar contractions caused by prostaglandin F₂α, the catheter-injury, the surgery, or the exposure to 5-hydroxytryptamine did not significantly affect smooth muscle contractility.

Immediately following endothelial cell removal, coronary arteries are hypersensitive to the contractile effects of 5-hydroxytryptamine and platelets and platelet-released products can interfere with the function of adrenergic nerves. The present observations demonstrate that in the first 24 hours following endothelial cell denudation in vivo, 5-hydroxytryptamine can be accumulated by coronary arteries and can cause adrenergic nerve dysfunction, contributing to abnormal coronary artery reactivity.

Mural thrombi have been recognized to play an important role in clinical exacerbations of human atherosclerotic coronary artery disease manifested by angina pectoris, coronary artery spasm, and myocardial infarction. Indeed, coronary arteries from patients with ischemic heart disease contain high concentrations of 5-hydroxytryptamine. The present study, in conjunction with our previous work, suggests the platelet as the origin of and the adrenergic nerve as an important site of accumulation of 5-hydroxytryptamine. Pathologic alterations in neurotransmitter content could be important in causing neurogenically mediated coronary vasoconstriction.

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