Role of β_2 Adrenergic Receptors in Human Atherosclerotic Coronary Arteries

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Background—Adrenergic regulation of coronary vasomotion is balanced between α_1-adrenergic–mediated (α_1-AR) constriction and β_2-adrenergic–mediated (β_2-AR) relaxation. This study aimed at assessing the role of β_2-ARs in normal, mildly atherosclerotic, and stenotic human coronary arteries.

Methods and Results—During intracoronary (IC) infusion of increasing doses of the β_2-AR agonist salbutamol (0.15, 0.3, and 0.6 μg/min) and the endothelial vasodilator acetylcholine (1, 3, and 10 μg/min), we measured (1) changes in lumen diameter (LD) by quantitative coronary angiography in 34 normal, 55 mildly atherosclerotic, and 42 stenotic coronary artery segments and (2) changes in average peak velocity (APV) and coronary blood flow (CBF) with the use of Doppler flow wire in 11 normal, 10 mildly atherosclerotic, and 11 stenotic coronary arteries. In 6 of 11 stenotic coronary arteries, the protocol was repeated after an IC bolus (12 μg/kg) of the α-adrenergic blocker phentolamine. In 6 of 11 normal coronary arteries, the protocol was repeated after an IC infusion (60 μmol/min) of N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide inhibitor. Neither salbutamol IC infusion nor acetylcholine significantly changed heart rate or blood pressure, whereas L-NMMA slightly increased blood pressure. In normal coronary arteries, salbutamol increased LD (LD max %: 11 ± 2, $P<0.05$), APV (APV max %: 53 ± 17, $P<0.05$), and CBF (CBF max %: 57 ± 17, $P<0.05$), whereas L-NMMA caused a blunted APV (APV max %: 27 ± 6, $P<0.05$) and CBF (CBF max %: 29 ± 6, $P<0.05$) response to salbutamol. In mildly atherosclerotic coronary arteries, the salbutamol increase in LD (LD max %: 10 ± 2, $P<0.05$), APV (APV max %: 33 ± 12, $P<0.05$), and CBF (CBF max %: 37 ± 12, $P<0.05$) was preserved. In stenotic coronary arteries, salbutamol induced a paradoxical reduction in LD (LD max %: -6 ± 2, $P<0.05$), APV (APV max %: -15 ± 9, $P<0.05$), and CBF (CBF max %: -15 ± 6, $P<0.05$), which was no longer observed after phentolamine. Acetylcholine increased LD (LD max %: 14 ± 3, $P<0.05$), APV (APV max %: 61 ± 20, $P<0.05$), and CBF (CBF max %: 67 ± 19, $P<0.05$) in normal coronary arteries. In mildly atherosclerotic coronary arteries, acetylcholine induced a significant reduction in LD (LD max %: -15 ± 2, $P<0.05$) and no changes in APV (APV max %: -6 ± 13, $P=$NS) and CBF (CBF max %: -10 ± 13, $P=$NS). In stenotic coronary arteries, acetylcholine significantly reduced LD (LD max %: -15 ± 3, $P<0.05$), APV (APV max %: -15 ± 9, $P<0.05$), and CBF (CBF max %: -15 ± 6, $P<0.05$).

Conclusions—In severely atherosclerotic coronary arteries, β_2-adrenergic vasodilatation is impaired, and this might contribute to alter the vasomotor balance, further precipitating myocardial ischemia during sympathetic activation. (***Circulation***. 2005;111:288-294.)

Key Words: receptors, adrenergic, alpha ■ receptors, adrenergic, beta ■ atherosclerosis ■ endothelium ■ acetylcholine

Adrenergic stimulation plays a key role in the regulation of coronary vasomotor tone, and it is modulated by atherosclerosis such that vasodilation is turned into vasoconstriction. Cold pressor test and mental stress, 2 common maneuvers causing adrenergic activation, induce vasodilation in normal coronary arteries and vasoconstriction in stenotic coronary arteries.[1,2]

It has been advocated that a combination of endothelial dysfunction and increased α-adrenergic receptor responsiveness accounts for this phenomenon.[3,4] Consistently, pretreatment with α-adrenergic blockers is able to reverse this trend and prevent myocardial ischemia.[5,6] We have recently demonstrated in humans that intravenous dobutamine, an α_1-, β_1-, and β_2-adrenergic receptor agonist, vasodilates angiographically normal coronary arteries, whereas a lack of vasomotion to dobutamine was observed in stenotic coronary arteries. In these vessels, pretreatment with phentolamine restored dobutamine-induced vasomotion.[7]
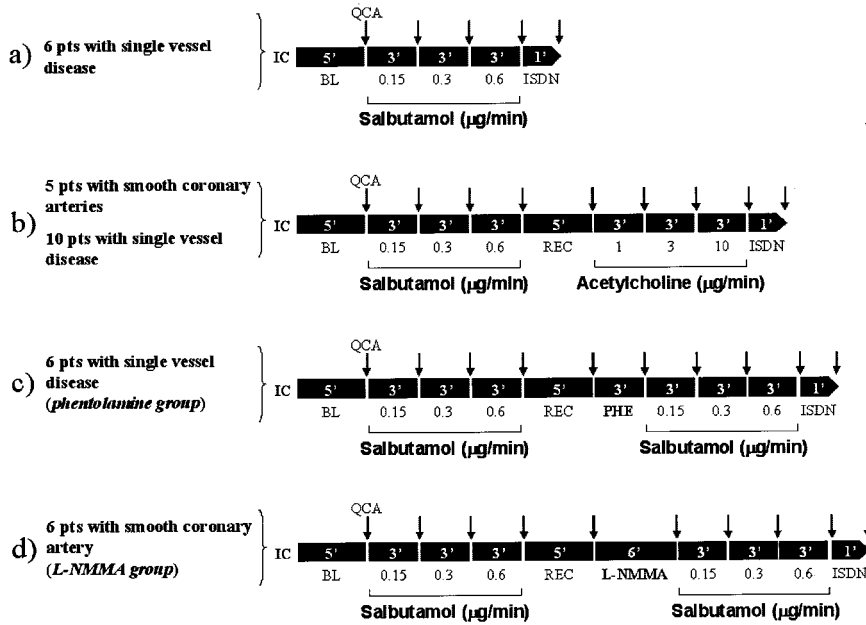
Figure 1. Diagrams of the study protocols. BL indicates baseline; REC, recovery; PHE, phentolamine; ISDN, nitrates. The arrows indicate the time points for acquisition of angiograms for quantitative coronary angiography measurements.

β_2-Adrenergic receptors (β_2-ARs) are expressed into coronary vascular wall and are important mediators of the adrenergic tone.[8–12] The present study aimed at assessing the role of β_2-ARs in normal coronary arteries and testing the hypothesis that the shift of the vasomotor balance toward constriction in atherosclerotic coronary arteries is a result of not only an enhanced α-adrenergic tone but also an impairment of the β_2-AR responsiveness.

Methods

Patients and Catheterization Protocol

The study population consisted of 33 patients with stable angina (Canadian Cardiovascular Society class II or III) and normal left ventricular ejection fraction referred for coronary angiography because of a positive or nonconclusive stress test; among these, 11 patients had normal coronary arteries and 22 had single-vessel disease. Cardiac medications, except for aspirin and statins, were withheld in all patients for at least 36 hours before the protocol.

A 6F sheath was introduced into the femoral artery, and a 6F guiding catheter was engaged into the coronary ostium. Five thousand units of heparin was given to all patients. On average, the duration of the protocol was 30 minutes, and no untoward effects or complications occurred. Informed consent was obtained from all patients before the diagnostic catheterization, in accordance with the protocol approved by the local ethics committees.

Experimental Protocols

Figure 1 summarizes the study protocols. All patients underwent the following protocol: after a baseline period of 5 minutes, an intracoronary (IC) administration of the selective β_2-adrenergic receptor agonist salbutamol was performed at 0.15, 0.3, and 0.6 μg/min at a constant infusion rate of 1.25 mL/min for 3 minutes for each dose. These doses of salbutamol were shown not to significantly affect cardiac inotropism and chronotropism.[13]

Five minutes after salbutamol infusion, 5 patients with angiographically normal coronary arteries and 10 patients with single-vessel disease received an IC administration of the endothelial vasodilator acetylcholine at 1, 3, and 10 μg/min at a constant infusion rate of 1.25 mL/min for 3 minutes for each dose.[14]

In another group of 6 patients with single-vessel disease, an IC bolus of the α-adrenergic blocker phentolamine (12 μg/kg) was given 5 minutes after salbutamol infusion, and 3 minutes later, the salbutamol protocol was repeated.[15]

In another group of 6 patients with angiographically normal coronary arteries, the salbutamol protocol was repeated after an IC infusion of the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA, 60 μmol/min, for 6 minutes).[16] All patients received an IC bolus of nitrates (300 μg) at the end of the protocols.

Data Acquisition

Data were recorded at every step of the protocols: at baseline, at the end of each dose of salbutamol, at the end of each dose of acetylcholine, at the end of L-NMMA infusion, and 3 minutes after phentolamine and nitrates (Figure 1).

Hemodynamic Data

Heart rate and blood pressure were digitally recorded (Marquette 1000 Ex, Mac-Laboratory System) during the entire study protocols.

Angiographic Data

Vessel dimensions and vessel dimension changes along the protocols were measured on angiograms, acquired according to the above steps, by quantitative coronary angiography, as previously described.[7] In each patient, an average of 3.8 segments were selected in one projection on the baseline angiogram. As shown in Table 1, coronary artery segments were pooled and analyzed separately as follows: (1) normal segments (n=34) from patients with angiographically smooth coronary arteries; (2) mildly atherosclerotic segments (n=55) from the adjacent coronary artery in patients with

TABLE 1.   Angiographic and Flow Data at Baseline

	Normal Vessels	Mildly Atherosclerotic Vessels	Stenotic Vessels
ALD, mm	2.7±0.3	2.4±0.2	2.5±0.3
MLD, mm	NA	NA	0.7±0.2
DS, %	NA	NA	72±4
APV, cm/s	19±1	17±2	17±2
CBF, mL/min	66±5	59±6	59±7

ALD indicates mean coronary artery diameter as measured at the reference segments; MLD, minimal lumen diameter; and % DS, percent diameter stenosis.

TABLE 2.   Clinical Characteristics of the Study Patients

	Control Patients (n=11)	CAD Patients (n=22)
Male/female	7/4	17/5
Age, y	58±4	62±3
Smoking	4 (36)	11 (50)
Diabetes	0 (0)	6 (27)
Dyslipidemia	3 (27)	10 (45)
Hypertension	3 (27)	13 (59)

Control patients are patients with normal coronary arteries; CAD patients are patients with single-vessel disease. Data are in absolute values; values in parentheses are percentages.

single-vessel disease; and (3) stenotic segments (n=42) from the stenotic coronary artery in patients with single-vessel disease.

The contrast medium used was the non-ionic, hypo-osmolar, monomer ioversol (Optiray 350, Guerbet).

Coronary Blood Flow

A 0.014-in flexible Doppler angioplasty guidewire (FloWire, Volcano Therapeutics Inc) was advanced in 11 normal coronary arteries of 11 patients with angiographically normal vessels. In 10 patients with single-vessel disease, the Doppler wire was positioned in the adjacent mildly atherosclerotic coronary artery (ie, nonstenotic), whereas in another 11 patients with single-vessel disease, the wire was positioned in the stenotic coronary artery. In one case, coronary blood flow (CBF) measurements could not be performed because of poor quality of the Doppler signal. The Doppler guidewire was connected to the 15-MHz pulsed Doppler velocimeter (ComboMap, Volcano Therapeutics Inc). Doppler velocity spectra were analyzed to measure average peak velocity (APV). CBF (mL/min) was calculated according to the following formula: $(\pi \times MLD^2)/(APV/8)$.

Statistical Analysis

Data are expressed as mean±SEM. Statistical comparison was made by ANOVA for repeated measurements to compare, within the patients, the effects of different doses of the drugs being tested. Post hoc analysis was performed with the Newman-Keuls test. Reproducibility among the measurements, calculated as previously described,[17] is as follows: (1) lumen diameter (LD), 5±3%; (2) APV at baseline, 5±5%; (3) APV at hyperemia, 4±4%; CBF, 7±6%. Statistical analysis was performed with Graph Pad Prism version 2.0. Probability values of $P>0.05$ were considered statistically not significant.

Results

Clinical and Hemodynamic Data

Clinical characteristics of the patients are shown in Table 2. Salbutamol IC infusion did not induce any significant change in heart rate (baseline heart rate: 73±3 bpm, to peak salbutamol heart rate: 76±3 bpm, P=NS) or in mean blood pressure (baseline mean blood pressure: 105±3 mm Hg, to peak salbutamol mean blood pressure: 105±3 mm Hg, P=NS). Salbutamol induced typical angina pectoris without significant ECG changes in 2 patients.

Similarly, acetylcholine did not change heart rate (peak acetylcholine heart rate: 74±4 bpm, P=NS) or mean blood pressure (peak acetylcholine mean blood pressure: 105±5 mm Hg, P=NS), and the intracoronary infusion could be completed in 5 patients with normal coronary arteries and in 7 patients with single-vessel disease. Because of coronary spasm and angina with ST-segment depression, acetylcholine
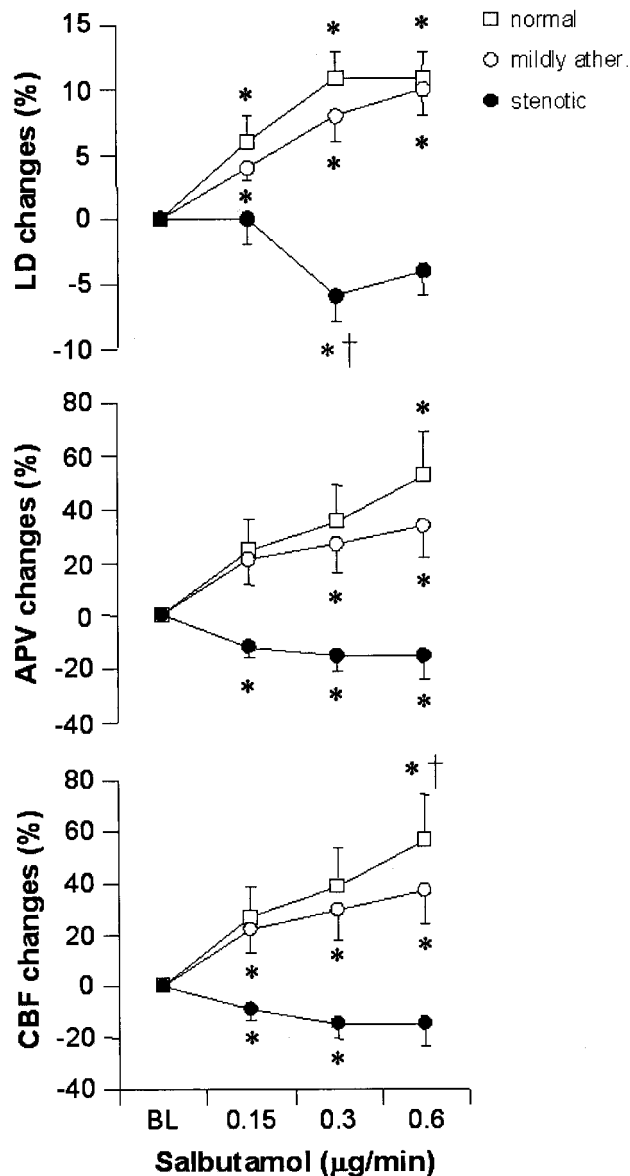

Figure 2. Top shows LD changes to salbutamol. Center shows APV changes to salbutamol. Bottom shows CBF changes to salbutamol. White boxes indicate normal segments or coronary arteries. White circles indicate mildly atherosclerotic segments or coronary arteries. Black circles indicate stenotic segments or coronary arteries. BL indicates baseline. *$P<0.05$ vs baseline. †$P<0.05$ vs preceding dose.

was stopped in 3 patients with single-vessel disease (at the first dose in 1 patient, at the second dose in 2 patients). Response at the maximal acetylcholine dose was considered for statistical comparison.

L-NMMA slightly increased mean blood pressure (peak mean blood pressure: 109±4 mm Hg, P=0.07 versus baseline), whereas no significant changes were observed in heart rate (peak heart rate: 78±4 bpm, P=NS).

Angiographic Data

As shown in Figure 2 (top), salbutamol IC infusion induced a significant dose-response increase in LD in normal segments (LD max %: 11±2, $P<0.05$ versus baseline). In mildly
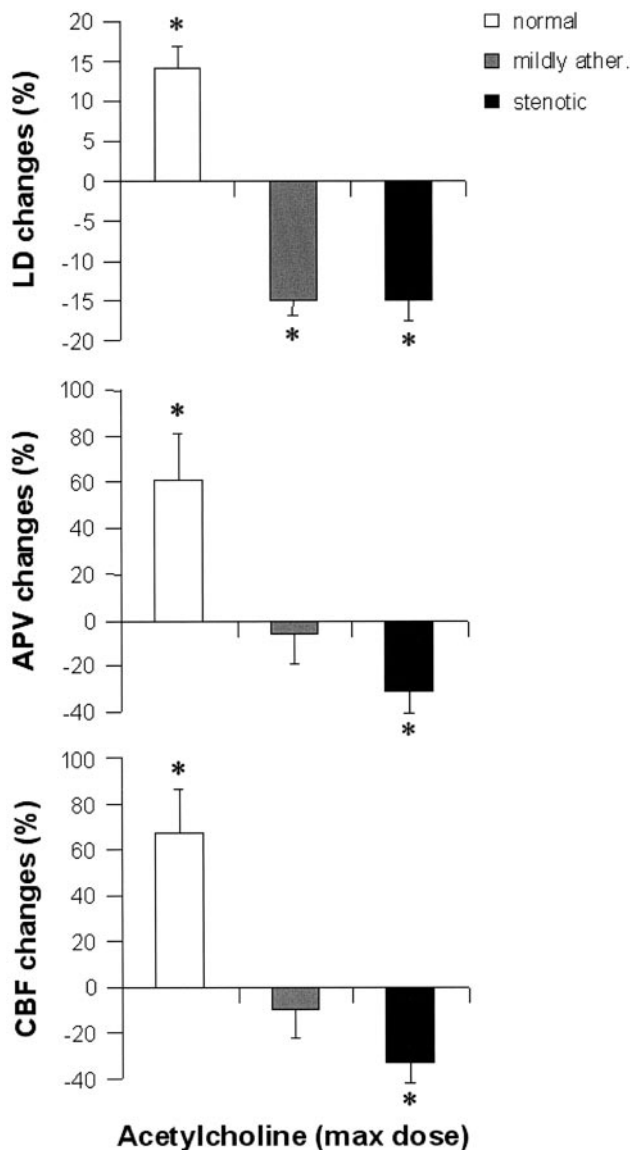
Figure 3. Changes in LD (top), APV (center), and CBF (bottom) at maximal achieved dose of acetylcholine are shown in normal segments or coronary arteries (white bars), in mildly atherosclerotic segments or coronary arteries (gray bars), in stenotic segments or coronary arteries (black bars). *$P<0.05$ vs baseline.
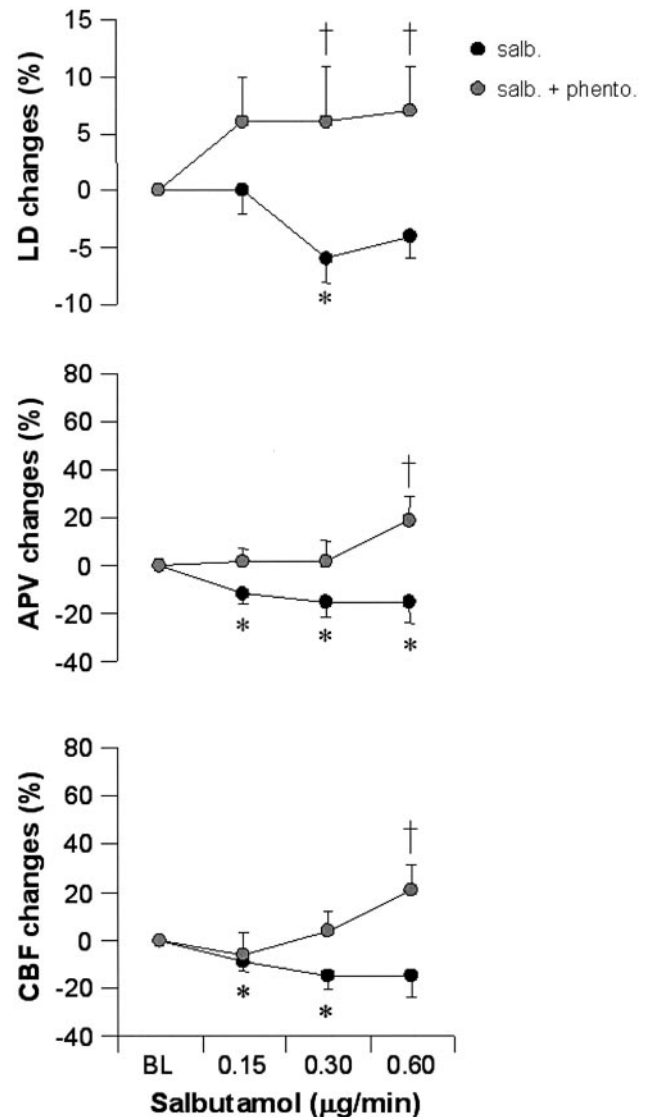

Figure 4. Changes in LD (top), APV (center), and CBF (bottom) to salbutamol (salb.) before (black circles) and after an IC bolus of phentolamine (phento.) (gray circles), in stenotic segments or coronary arteries. *$P<0.05$ vs baseline. †$P<0.05$ vs respective dose of salbutamol before phentolamine.

atherosclerotic segments, the increase in LD to salbutamol was comparable (LD max %: 10 ± 2, $P<0.05$ versus baseline) to normal segments. In stenotic segments, a small constriction was observed only at 0.3 μg/min of salbutamol (LD max %: -6 ± 2, $P<0.05$ versus baseline).

Acetylcholine IC infusion significantly increased LD in normal segments (LD max %: 14 ± 3, $P<0.05$ versus baseline), whereas a significant reduction of LD was observed in mildly atherosclerotic (LD max %: -15 ± 2, $P<0.05$ versus baseline) and stenotic segments (LD max %: -15 ± 3, $P<0.05$ versus baseline) (Figure 3, top).

Phentolamine IC bolus did not change LD (LD %: 2 ± 3, $P=$NS versus baseline). After phentolamine, salbutamol did not significantly change LD in mildly atherosclerotic segments (LD max %: 9 ± 4, $P<0.05$ versus baseline; $P=$NS

versus before phentolamine) or in stenotic segments (LD max %: 6 ± 3, $P=$NS versus baseline) (Figure 4, top).

IC infusion of L-NMMA was associated with a small reduction in LD of normal segments (LD %: -5 ± 3, $P<0.05$ versus baseline). After L-NMMA, the dose-dependent increase in LD induced by salbutamol was unchanged (LD max %: 9 ± 2, $P=$NS versus baseline) (Figure 5, top).

IC nitrates significantly increased LD in normal segments (LD %: 39 ± 6, $P<0.05$ versus baseline) and to a minor extent in mildly atherosclerotic (LD %: 26 ± 3, $P<0.05$ versus baseline) and in stenotic segments (LD %: 13 ± 2, $P<0.05$ versus baseline).

CBF Data

In normal coronary arteries, salbutamol significantly increased APV (APV max %: 53 ± 17, $P<0.05$ versus baseline)
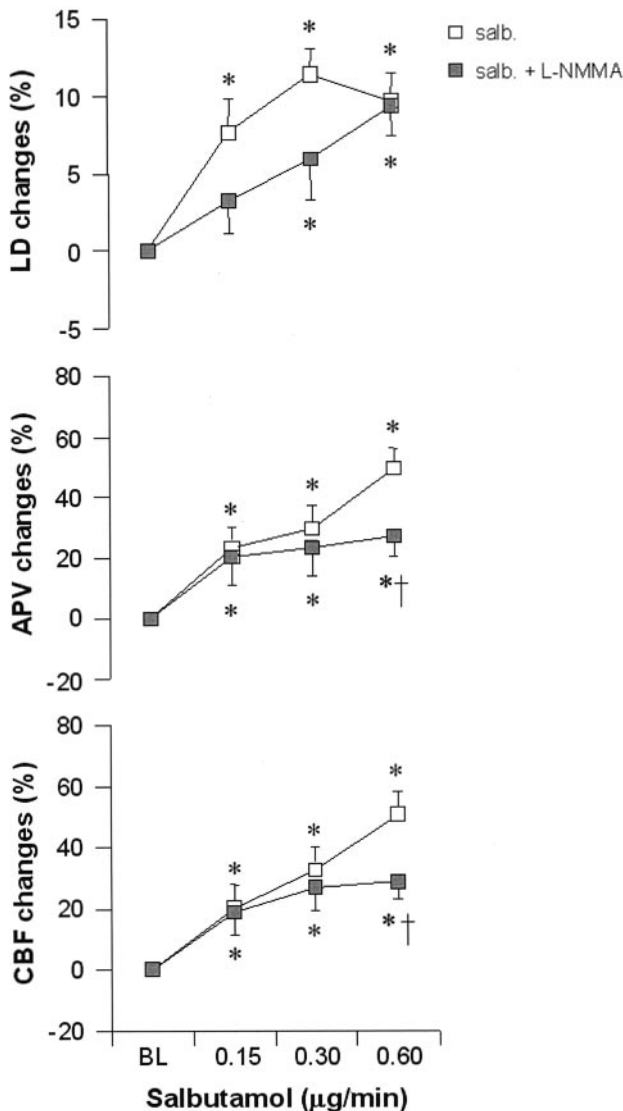
Figure 5. Changes in LD (top), APV (center), and CBF (bottom) to salbutamol before (white boxes) and after an IC infusion with L-NMMA (gray boxes), in normal segments or coronary arteries. *$P<0.05$ vs baseline. †$P<0.05$ vs respective dose of salbutamol before L-NMMA.

and CBF (CBF max %: 57 ± 17, $P<0.05$ versus baseline). In mildly atherosclerotic coronary arteries, the salbutamol-induced increases in APV (APV max %: 33 ± 12, $P<0.05$ versus baseline) and CBF (CBF max %: 37 ± 12, $P<0.05$ versus baseline), even though less pronounced, were not significantly different compared with normal coronary arteries. In stenotic arteries, salbutamol infusion was associated with a significant reduction in APV (APV max %: -15 ± 9, $P<0.05$ versus baseline) and CBF (CBF max %: -15 ± 6, $P<0.05$ versus baseline) (Figure 2, center and bottom).

Acetylcholine significantly increased APV (APV max %: 61 ± 20, $P<0.05$ versus baseline) and CBF (CBF max %: 67 ± 19, $P<0.05$ versus baseline) in normal coronary arteries. In mildly atherosclerotic coronary arteries, no significant changes in APV (APV max %: -6 ± 13, $P=$NS) or in CBF (CBF max %: -10 ± 13, $P=$NS) were observed after acetylcholine. In stenotic coronary arteries, acetylcholine reduced

both APV (APV max %: -31 ± 9, $P<0.05$ versus baseline) and CBF (CBF max %: -33 ± 9, $P<0.05$ versus baseline) (Figure 3, center and bottom).

In stenotic coronary arteries, phentolamine IC did not significantly change APV (APV max %: -14 ± 8, $P=$NS) or CBF (CBF max %: -16 ± 9, $P=$NS). After phentolamine, salbutamol did not significantly change APV (APV max %: 19 ± 10, $P=$NS versus baseline) or CBF (CBF max %: 21 ± 11, $P=$NS versus baseline) (Figure 4, center and bottom).

In normal coronary arteries, IC infusion of L-NMMA reduced the APV (APV %: -15 ± 4, $P<0.05$ versus baseline) and CBF (CBF %: -22 ± 8, $P<0.05$ versus baseline). After L-NMMA, salbutamol-induced increases in APV (APV max %: 27 ± 6, $P<0.05$ versus baseline) and CBF (CBF max %: 29 ± 6, $P<0.05$ versus baseline) were blunted but remained significant (Figure 5, center and bottom).

IC nitrates significantly increased APV and CBF in normal (APV %: $26\pm5\%$ and CBF %: $34\pm6\%$, $P<0.05$ versus baseline) and mildly atherosclerotic coronary arteries (APV %: 23 ± 6 and CBF %: 29 ± 6, $P<0.05$ versus baseline). In stenotic coronary arteries, nitrates did not change APV (APV %: 2 ± 8, $P=$NS) or CBF (CBF %: 5 ± 8, $P=$NS).

Discussion

The present study evaluated β_2-adrenergic receptor response at the macrocirculatory (lumen dimension changes) and at the microcirculatory level (APV and calculated CBF changes). In normal coronary arteries, a significant vasodilation to β_2-adrenergic receptor stimulation was observed at both levels that appears to be partially endothelium-mediated. In mildly atherosclerotic coronary arteries, this vasodilation is reduced but preserved. In stenotic coronary arteries, salbutamol IC infusion induced a paradoxical vasoconstriction that is no longer observed after phentolamine.

β-Adrenergic Receptors in Normal Coronary Arteries

The coronary vasomotor balance is such that β_1- and β_2-adrenergic receptors contribute to the vasodilatory drive, opposing the vasoconstrictive forces represented primarily by α_1-adrenergic receptor hyperresponsiveness and endothelial dysfunction. Both β_1- and β_2-adrenergic receptors are expressed on coronary endothelial and vascular smooth muscle cells,[8–10] even though β-adrenergic receptors expressed on these latter cells have been reported to play a prevalent role.[12,18] Furthermore, the distribution of β-adrenergic receptors in the coronary circulation is heterogeneous, β_2-adrenergic receptors being more important for the regulation of the coronary resistance vessels.[11,12,18,19] Consistently, our data in normal coronary arteries showed a significant increase in LD of the epicardial vessel and even more in APV, with a net increase in CBF of $>50\%$. In these coronary arteries, acetylcholine induced a significant vasodilation, suggesting a preserved endothelial function. Inhibition of nitric oxide synthase with L-NMMA did not affect salbutamol vasodilation at the epicardial level, but the microcirculatory response was attenuated, suggesting that there is an endothelium-

dependent component to β_2-adrenergic–mediated vasodilation in this compartment.

β-Adrenergic Receptors in Atherosclerotic Coronary Arteries

In atherosclerotic coronary arteries, adrenergic stimulation by cold pressor test and mental stress is known to induce paradoxical vasoconstriction.[1,2] In addition, we have recently demonstrated that intravenous dobutamine, an α_1-, β_1-, and β_2-adrenergic receptor agonist, is associated with blunted vasodilation in mildly atherosclerotic coronary arteries and lack of vasomotion in stenotic coronary arteries.[7] The present data have been obtained with local (IC) infusion of doses of salbutamol that did not change heart rate or blood pressure.

In this setting, we found that in (1) mildly atherosclerotic coronary arteries, β_2-adrenergic receptor vasodilation is reduced but preserved, in accordance with previous data supporting a prevalent role of β_2-adrenergic receptors of vascular smooth muscle cells.[12,20] In these coronary arteries, acetylcholine induced a paradoxical vasoconstriction, suggesting the presence of some degree of endothelial dysfunction already at this stage of atherosclerosis. Conversely, IC nitrates induced a significant vasodilation, corroborating the finding of a preserved function of the vascular tunica media. In (2) stenotic coronary arteries, salbutamol, unexpectedly, induced a reduction of both LD and APV, with a consequent decrease in CBF; yet during salbutamol IC infusion, 2 patients of 16 with stenotic coronary arteries had clinical symptoms of angina. This apparent paradox could be explained by a salbutamol α-adrenergic–mediated effect. In fact, salbutamol may have stimulated presynaptic β_2-adrenergic receptors, with subsequent norepinephrine release.[13,21] Consistently, after pretreatment with phentolamine, an α_1-/α_2-adrenergic receptor blocker, vasoconstriction to salbutamol is no longer observed. Not surprisingly, acetylcholine induces a paradoxical vasoconstriction in these coronary arteries, demonstrating endothelial dysfunction. The IC bolus of nitrates induced a significant vasodilation of the stenotic epicardial vessel, demonstrating that vasodilatory response to direct smooth muscle cell stimulation is preserved.

Clinical Implications

Previous data from Kern et al[22,23] have suggested that β-blockers (intravenous propranolol) could potentiate coronary vasoconstriction in patients with coronary artery disease undergoing a cold pressor test because of an unopposed α-adrenergic vasomotor tone. On the contrary, Gaglione et al[24] showed a significant vasodilation of stenotic coronary arteries after intracoronary propranolol in patients undergoing supine bicycle stress testing. Finally, Bortone et al[25] have shown that propranolol administered intravenously is able to significantly decrease coronary luminal area of both normal and stenotic coronary arteries at rest but conversely can induce coronary vasodilation during exercise. They concluded that the reduction in myocardial oxygen consumption and the prevention of exercise-induced stenosis vasoconstriction might explain the beneficial effect of β-blocker treatment in most patients with coronary artery disease. Our data further extend these observations by supporting an impairment of

coronary β_2-adrenergic receptors in stenotic coronary arteries. In this condition, a direct influence of β-blockers on the coronary vasomotor tone is less effective, and most of the beneficial effect might be consequent to the reduced myocardial oxygen consumption.

Some early reports showed that a therapy with nebulized salbutamol in asthmatic patients could induce myocardial ischemia and unmask coronary artery disease.[26,27] This finding was explained primarily by an increase in myocardial O_2 consumption secondary to an increase in heart rate induced by salbutamol. Our data propose a novel mechanism potentially explaining salbutamol-provoked ischemia. In fact, at doses not able to increase heart rate or blood pressure, salbutamol induces a paradoxical vasoconstriction in stenotic coronary arteries, which in 2 patients led to clinical symptoms of angina.

Limitations

The paradoxical constriction of the microcirculation to salbutamol could alternatively be explained by a fall of the CBF downstream from the stenosis, secondary to the vasoconstriction of the epicardial vessel. Despite this limitation, the lack of a significant vasomotion to salbutamol after α-blockade does still support the notion that β_2-adrenergic receptors are impaired at microcirculatory level.

The attenuation of salbutamol-induced vasodilation after L-NMMA could be a result of the inhibition of the endothelial β_2-adrenergic receptor component or, less specifically, of the inhibition of a flow-mediated component. Our data do not allow us to distinguish between these 2 components, even though Sun et al[18] have reported, in isolated human coronary arterioles, a preserved vasodilation to norepinephrine after nitric oxide synthase inhibition and mechanical removal of the endothelium.

Conclusions

The present study confirms that in normal coronary arteries, there is an important role for β_2-adrenergic receptor–mediated coronary vasomotion that is partially mediated by the endothelium, especially at the microcirculatory level. In the presence of mild atherosclerosis, β_2-adrenergic receptor vasodilation is preserved. In stenotic coronary arteries, an impairment of β_2-adrenergic receptor response is associated with a paradoxical vasoconstriction to salbutamol that is α-adrenergic–mediated. The present findings suggest that an impairment of coronary β_2-adrenergic receptor response also contributes to alter the vasomotor balance in the setting of coronary atherosclerosis, further precipitating myocardial ischemia during sympathetic activation.

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