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Zatebradine, a Specific Bradycardic Agent, Enhances the Positive Inotropic Actions of Dobutamine in Ischemic Myocardium

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Objectives. This investigation determined whether attenuation of the tachycardia produced by dobutamine administration would improve perfusion and function distal to a severe coronary artery stenosis.

Background. Tachycardia adversely affects perfusion and function distal to a coronary artery stenosis. It is not known whether a specific bradycardic agent can improve blood flow and function in an ischemic zone during administration of dobutamine.

Methods. The effects of dobutamine (2, 5 and 10 μg/kg body weight per min) alone and in combination with zatebradine (0.5 mg/kg), a specific bradycardic agent, on hemodynamic status, segment shortening (ultrasound length transducers) and myocardial perfusion (microspheres) were studied in anesthetized dogs with severe left circumflex coronary artery stenosis.

Results. A 50% reduction in left circumflex coronary artery blood flow (58 ± 4 to 29 ± 2 ml/min [mean value ± SEM]) produced a decrease in systolic shortening in the ischemic zone. Only a dose of dobutamine that did not elevate heart rate (2 μg/kg per min) produced an increase in segment shortening in the ischemic zone. High doses of dobutamine (10 μg/kg per min) caused an increase in heart rate without improvement in function and a reduction in the subendocardial/subepicardial flow ratio (0.74 ± 0.06 to 0.48 ± 0.05). Zatebradine administered in the presence of dobutamine caused a decrease in heart rate, an increase in subendocardial/subepicardial blood flow ratio (0.48 ± 0.05 to 0.78 ± 0.09) and allowed an increase in ischemic zone segment shortening. When normalized for changes in heart rate, ischemic zone subendocardial flow increased by 123 ± 41% (0.39 ± 0.09 to 0.71 ± 0.12 ml/100 g per beat). Atrial pacing abolished the effects of zatebradine.

Conclusions. The present data suggest that the perfusion-contraction matching that accompanies a decrease in heart rate results in enhancement of inotropic stimulation of an ischemic zone. The actions of zatebradine are related to an increase in subendocardial blood flow per beat that allows improvement of regional contractile function.

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Dobutamine is a positive inotropic agent that acts to stimulate $beta_1$-, $beta_2$- and $alpha_1$-adrenoceptors (1,2) and is commonly used in patients with left ventricular systolic dysfunction and concomitant obstructive coronary artery disease. In the absence of myocardial ischemia, dobutamine augments cardiac output, myocardial inotropic state and coronary perfusion, with lesser effects on heart rate and blood pressure (3,4). The relative "inotropic selectivity" of dobutamine found at lower doses (1) is lost at higher doses as the positive chronotropic properties become increasingly manifest. In the presence of a flow-limiting coronary artery stenosis, increases in heart rate produced by dobutamine may serve to further aggravate an already compromised myocardial oxygen supply-demand balance and limit increases in regional contractile function. A decrease in diastolic perfusion time secondary to tachycardia may be especially deleterious to regional myocardial perfusion and function distal to a flow-limiting coronary stenosis (5,6). Previous studies (7,8) have documented that when inotropic stimulation with dobutamine is accompanied by an elevation of heart rate, regional maldistribution of coronary blood flow may occur. This is reflected in a redistribution of the normal transmural perfusion gradient, resulting ultimately in no change or even deterioration of contractile function in the ischemic zone.

Several compounds, termed specific bradycardic agents, have been developed that produce a reduction in heart rate. One such agent, zatebradine (UL-FS 49; 1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-[[2-(3,4-dimethoxyphenyl) ethyl]methylimino]-propyl]-2H-3-benzazepin-2-on-hydrochloride), has been shown to be one of the most efficacious and selective of these compounds (9). Zatebradine has markedly attenuated exercise-induced increases in heart rate in dogs (10,11), with resulting improvement in perfusion (11) and function (10,11) in an ischemic zone. Results of studies using zatebradine (12–14) and other specific bradycardic

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agents (13) in anesthetized dogs and pigs have also shown improvement in ischemic zone blood flow and function. Many (12–14), but not all (11), studies have suggested that the beneficial effects of zatebradine are solely due to a reduction in heart rate. The present investigation was designed to evaluate the effects of dobutamine on hemodynamic variables, regional myocardial blood flow and regional contractile function in anesthetized dogs with a severe coronary artery stenosis and to determine whether the actions of dobutamine could be favorably altered by selective attenuation of heart rate through concomitant administration of zatebradine.

Methods

Experimental preparation. All experimental procedures and protocols used in this investigation were reviewed by the Animal Care Committee of the Medical College of Wisconsin. All studies conformed to the Guiding Principles in the Care and Use of Animals of the American Physiological Society and the Guide for the Care and Use of Laboratory Animals (DHEW [DHHS] publication no. [NIH] 8523, revised 1985).

Seven adult male or female mongrel dogs weighing 18 to 28 kg were fasted overnight, anesthetized with sodium pentobarbital (25 mg/kg body weight) and sodium barbital (200 mg/kg) and ventilated by a respirator (Ohio Medical Products, Airco Inc.) with room air enriched with oxygen. Respiratory rate and tidal volume were adjusted to maintain arterial blood gases within normal physiologic limits (pH 7.35 to 7.45; partial pressure of carbon dioxide, 30 to 35 mm Hg; partial pressure of oxygen, 85 to 100 mm Hg). Body temperature was monitored and maintained at 37.5 ± 1°C by a heating pad and servomechanical controller.

Arterial and left ventricular systolic and diastolic pressures were recorded using a 7F dual-pressure transducer-tipped catheter (SPR-277, Millar) passed through the left carotid artery into the ascending aorta and left ventricle, respectively. The peak positive first derivative of left ventricular pressure (dP/dt), an index of global contractility, was obtained by electronic differentiation of the left ventricular pressure pulse. A triangular wave of known slope was used to calibrate the differentiator. A 7F thermodilution pulmonary artery catheter (American Edwards Laboratories) was advanced into the main pulmonary artery from the jugular vein. Cardiac output was determined by the thermodilution technique with the use of a cardiac output computer (series 7000, Marquette Electronics Inc.) and recorded as the average of three determinations.

The left femoral vein and artery were catheterized for drug administration and for withdrawal of reference arterial blood samples used in determination of myocardial tissue blood flow, respectively. A thoracotomy was performed in the left fifth intercostal space. The lung was retracted and the heart suspended in a pericardial cradle. A 1.0- to 1.5-cm segment of the proximal left circumflex coronary artery was carefully dissected from the surrounding tissue, and an electromagnetic flow probe (Statham SP7515, Gould Instruments), was placed around the vessel for measurement of coronary blood flow. A micrometer-driven mechanical occluder was positioned distal to the flow probe so that no branches were present between the probe and the occluder. The occluder was used to determine zero blood flow and to produce a noncircumferential stenosis of a constant 3-mm length. A catheter was placed in the left atrium for the injection of radioactive microspheres.

Myocardial segment function (percent systolic shortening) was measured in the regions perfused by the left anterior descending and left circumflex coronary arteries by pairs of piezoelectric crystals. The crystals were inserted (10 to 15 mm apart and 7 to 9 mm deep) into the subendocardium of the center of the normal (left anterior descending artery) and ischemic (left circumflex artery) perfusion territories parallel to fiber orientation. The leads were connected to an ultrasound amplifier that transformed the sound pulse into an electrical signal proportional to the distance between the crystals. The crystals were precalibrated, and the tracings were monitored with an oscilloscope (model 2215A, Tektronix Inc.). Using left ventricular dP/dt, end-systolic length was determined at maximal negative dP/dt, and end-diastolic length was determined just before the onset of systole. The lengths were normalized according to the method described by Theroux et al. (15). Percent segment shortening was calculated by the use of the following equation: Percent segment shortening = [(End-diastolic length − End-systolic length)/End diastolic length] × 100. The depth of each crystal was verified at the completion of each experiment.

The regional distribution of myocardial blood flow was determined by using the radioactive microsphere technique. Carbonized plastic microspheres (15-μm diameter) (Dupont) labeled with cerium-141, chromium-51, niobium-95 or ruthenium-103 were obtained as 1 mCi of nuclide in 5 ml of isotonic saline solution to which 1 drop of Tween 80 was added to minimize aggregation. The mixture was agitated before injection in a vortex mixer (Cole-Palmer, model 4722) for 15 min. Approximately 2 to 4 × 10^6 microspheres were injected into the left atrium as a bolus followed by a saline wash. A few seconds before each microsphere injection, a timed collection of reference flow from the femoral artery was started (precalibrated Harvard infusion/withdrawal pump, model 1941, Harvard Apparatus) and maintained at a constant rate (7 ml/min) for 3 min.

The electrocardiogram, aortic and left ventricular systolic and diastolic pressures, left ventricular dP/dt, phasic and mean coronary blood flows and regional segment function in normal and ischemic zones were continuously recorded on a polygraph (model 7, Grass Instrument Co.). Diastolic and mean coronary vascular resistances were calculated by dividing diastolic and mean aortic blood pressures by diastolic and mean coronary blood flows, respectively.

The heart was electrically fibrillated and immediately removed at the conclusion of each experiment. The left

Table 1. Effects of Dobutamine and Zatebradine Administration on Systemic Hemodynamic Variables

			Dobutamine				
	Prestenosis	Post-stenosis	2 μg/kg per min	5 μg/kg per min	10 μg/kg per min	Zatebradine (0.5 mg/kg body wt) + Dobutamine (10 μg/kg per min)	Zatebradine (0.5 mg/kg body wt) + Pacing + Dobutamine (10 μg/kg per min)
Heart rate (beats/min)	125 ± 2	125 ± 2	130 ± 2	152 ± 4*	166 ± 5*	97 ± 7*†	166 ± 5*
MAP (mm Hg)	100 ± 5	106 ± 5	111 ± 6	119 ± 7*	120 ± 6*	105 ± 5†	119 ± 5*
LVSP (mm Hg)	115 ± 5	120 ± 5	129 ± 6	140 ± 7*	140 ± 7*	136 ± 8*	138 ± 5*
LVEDP (mm Hg)	6 ± 1	7 ± 1	6 ± 1	6 ± 1	6 ± 1	7 ± 1	6 ± 1
Rate-pressure product (mm Hg·beat/min·10^3)	14.4 ± 0.8	15.0 ± 0.9	16.9 ± 0.9	21.2 ± 1.1*	23.1 ± 1.4*	12.8 ± 0.9†	22.9 ± 1.2*
+dP/dt (mm Hg/s)	1,729 ± 107	1,768 ± 111	2,118 ± 46*	2,261 ± 24*	2,275 ± 37*	2,220 ± 23*	2,271 ± 28*
Cardiac output (liters/min)	3.0 ± 0.2	3.0 ± 0.2	3.8 ± 0.4	4.4 ± 0.5*	4.9 ± 0.6*	3.8 ± 0.4†	4.9 ± 0.6*
Stroke volume (ml/beat)	24 ± 1	24 ± 1	29 ± 3	29 ± 3	30 ± 3	40 ± 5*†	29 ± 3
SVR (dynes·s·cm^{-5})	2,715 ± 163	2,910 ± 177	2,485 ± 251*	2,280 ± 236*	2,080 ± 1,220*	2,290 ± 240*	2,190 ± 230*

*$p < 0.05$ versus post-stenosis values. †$p < 0.05$ versus dobutamine (10 μg/kg per min). Values presented are mean value ± SEM. LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; +dP/dt = peak positive first derivative of left ventricular pressure; rate-pressure product = product of heart rate × left ventricular systolic pressure; SVR = systemic vascular resistance; wt = weight.

circumflex coronary artery was then cannulated at the site of the stenosis, and india ink (5 ml) was injected into the artery to darken the area of myocardium distal to the stenosis. The heart was washed with saline solution and fixed in formalin for 24 h. The left ventricle was separated from the remainder of the heart and divided into stained and unstained regions. Multiple tissue samples were obtained from the central zone of each region. The tissue samples were subdivided into subepicardial, midmyocardial and subendocardial layers of approximately equal weight (0.3 to 0.6 g). The samples were weighed and placed in scintillation vials. The activity of each isotope was determined in duplicate at four energy windows in a gamma counter (Auto-Gamma 5000 series, Packard Instrument Co.). Similarly, the activity of each isotope in the reference blood samples was also determined. The true activity of each isotope in the tissue sample was measured by correcting for energy overlap. Myocardial blood flow (Q_m [ml/min per g]) was calculated from the equation $Q_m = Q_r \cdot C_m / C_r$, where Q_r = rate of withdrawal (ml/min) of the reference blood sample; C_r = true activity (counts/min) of the reference blood sample; and C_m = true activity (counts/min per g) of the myocardial tissue sample. Myocardial blood flow values of tissue samples from ischemic and normal areas were pooled for calculation of flow in the subepicardium, midmyocardium and subendocardium of each region.

Experimental protocol. After surgery, the preparation was allowed to stabilize for 30 min. A stenosis of the left circumflex coronary artery sufficient to reduce mean coronary blood flow by 50% was produced by the mechanical occluder. In all experiments, hemodynamic data were measured before and 15 min after application of the coronary artery stenosis. Radioactive microspheres were injected after a stable level of stenosis had been achieved. Hemodynamic changes were measured during three doses of dobutamine (2, 5 and 10 μg/kg per min). Each infusion rate was allowed to reach steady state conditions for a period of 15 min. Microspheres were injected during the high dose for measurement of myocardial blood flow. Intravenous zatebradine (0.5 mg/kg, intravenously) was administered during the high dose of dobutamine, and hemodynamic data, segment function and myocardial perfusion were again measured. Heart rate was then increased to the level present before zatebradine administration by means of atrial pacing, and the final measurement of tissue flow was obtained. In all experiments, at least 15 min was allowed after drug administration or atrial pacing before data collection to ensure a stable hemodynamic state.

Drugs. Dobutamine and zatebradine were dissolved in 0.9% saline solution and freshly prepared on the day of each experiment.

Statistical analysis. The transmural distribution of coronary blood flow (subendocardial/subepicardial blood flow ratio) is expressed as the ratio of flow per gram of subendocardium to flow per gram of subepicardium. Unless otherwise specified, statistical analysis of results was carried out by analysis of variance with repeated measures, and when a significant overall effect was detected, the Bonferroni modification of the *t* test was applied to compare single mean values. The Student paired *t* test was utilized to compare post-stenosis control flows in the normal and ischemic zones. Differences were considered significant at $p < 0.05$. All results are presented as mean value ± SEM.

Results

Hemodynamic actions of dobutamine and zatebradine. Systemic hemodynamic data are summarized in Table 1. Stenosis of the left circumflex coronary artery produced no changes in systemic hemodynamic status. Administration of

Table 2. Effects of Dobutamine and Zatebradine on Coronary Hemodynamic Variables

	Prestenosis	Post-stenosis	Dobutamine 2 μg/kg per min	Dobutamine 5 μg/kg per min	Dobutamine 10 μg/kg per min	Zatebradine (0.5 mg/kg body wt) + Dobutamine (10 μg/kg per min)	Zatebradine (0.5 mg/kg body wt) + Pacing + Dobutamine (10 μg/kg per min)
Diastolic coronary blood flow (ml/min)	84 ± 4*	49 ± 4	42 ± 4	44 ± 5	40 ± 5	40 ± 5	39 ± 4
Mean coronary blood flow (ml/min)	58 ± 4*	29 ± 2	30 ± 2	31 ± 3	29 ± 3	29 ± 3	29 ± 3
Diastolic coronary vascular resistance (ru)	1.1 ± 0.1*	2.4 ± 0.3	2.6 ± 0.3	2.7 ± 0.4	2.9 ± 0.3	2.4 ± 0.2	3.0 ± 0.2
Mean coronary vascular resistance (ru)	1.8 ± 0.2*	3.8 ± 0.9	3.9 ± 0.4	4.0 ± 0.5	4.2 ± 0.4	3.8 ± 0.4	4.3 ± 0.4

*$p < 0.05$ versus post-stenosis value. Values presented are mean value ± SEM. ru = resistance units; wt = weight.

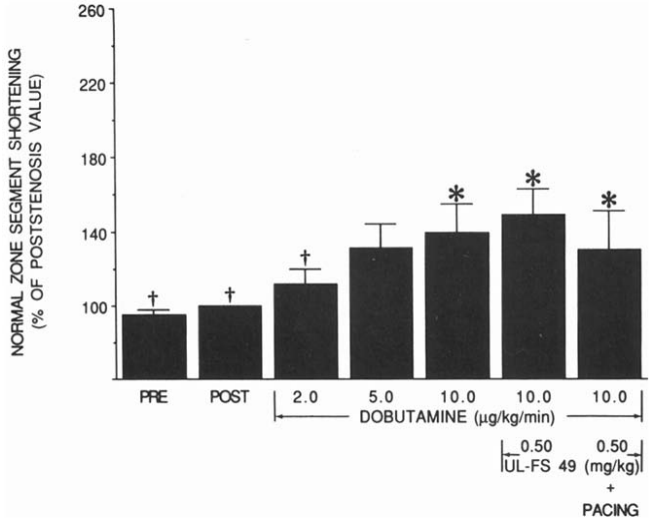
dobutamine caused dose-related increases in heart rate, left ventricular systolic pressure, rate-pressure product, left ventricular peak positive dP/dt and cardiac output. Administration of zatebradine during the high dose of dobutamine produced a significant reduction in heart rate. The decrease in heart rate was accompanied by a reduction in mean arterial pressure. Left ventricular systolic pressure and peak positive dP/dt were unchanged by zatebradine. Cardiac output was reduced by zatebradine compared with the high dose of dobutamine, but stroke volume was significantly increased. Atrial pacing to the heart rate present before zatebradine administration abolished these hemodynamic changes. The effects of dobutamine and zatebradine on coronary hemodynamic variables are presented in Table 2. Application of a severe stenosis to the left circumflex coronary artery produced a reduction in blood flow of approximately 50%. Administration of dobutamine or zatebradine, or both, caused no change in diastolic or mean coronary blood flow or coronary vascular resistance.

Effects of dobutamine and zatebradine on regional myocardial function. Changes in systolic shortening in the normal and ischemic zones are depicted in Figures 1 and 2, respectively. Hemodynamic variables and segment length recorded in a typical dog during dobutamine administration in the absence or presence of zatebradine are shown in Figure 3. Contractile function of the normal zone was increased by dobutamine in a dose-related fashion. Administration of zatebradine or atrial pacing had no effect on segment shortening of the normal zone. A significant reduction in function of the ischemic zone occurred coincident with application of the stenosis. Administration of low doses of dobutamine (2 μg/kg per min) produced significant improvement in ischemic zone function. Higher doses of dobutamine, however, produced no change in ischemic zone function. Administration of zatebradine was associated with an increase in segment shortening in the ischemic zone. The improvement in function was abolished by atrial pacing to rates present before zatebradine administration.

Effects of dobutamine and zatebradine on myocardial blood flow. Alterations in regional myocardial blood flow are summarized in Table 3. Blood flow to the normal zone was increased by dobutamine. Zatebradine decreased blood flow slightly, and atrial pacing restored blood flow to levels comparable to those present during the high dose of dobutamine. No change in the subendocardial/subepicardial blood flow ratio was produced by dobutamine, zatebradine or atrial pacing in the normal zone (Fig. 4).

Blood flow to all layers of the ischemic zone (subepicardial, midmyocardial and subendocardial), including subendocardial flow per beat, was significantly ($p < 0.05$) reduced by the stenosis compared with flow in the normal zone. Subepicardial blood flow in the ischemic zone was signifi-

Figure 1. Segment shortening data in the normal (left anterior descending coronary artery) zone before (PRE) left circumflex coronary artery stenosis; after (POST) stenosis; and after administration of dobutamine 1) alone, 2) simultaneously with zatebradine (UL-FS 49), and 3) simultaneously with zatebradine and atrial pacing. *$p < 0.05$ versus post-stenosis values. †$p < 0.05$ versus dobutamine alone (10 μg/kg body weight per min).



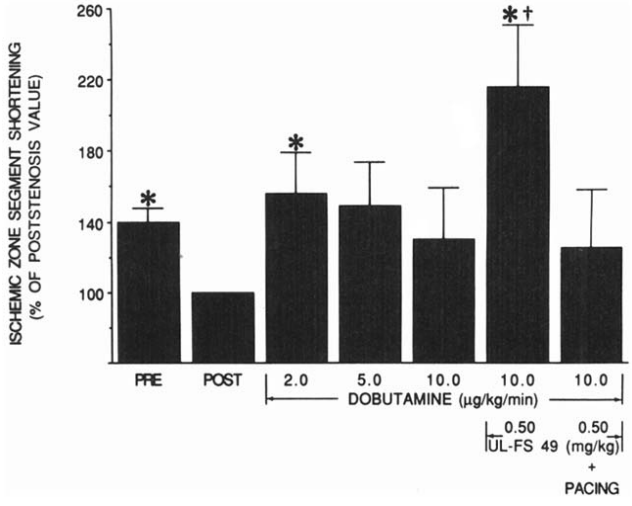


Figure 2. Segment shortening data in the ischemic zone before left circumflex coronary artery stenosis; after stenosis, and after administration of dobutamine 1) alone, 2) simultaneously with zatebradine, and 3) simultaneously with zatebradine and atrial pacing. Abbreviations and symbols as in Figure 1.

cantly increased by dobutamine without alteration of midmyocardial or subendocardial blood flow. Thus, the subendocardial/subepicardial flow ratio was significantly reduced by dobutamine (Fig. 5). Zatebradine returned the ischemic zone transmural blood flow ratio to values present before dobutamine infusion. The effect of zatebradine on the transmural distribution of coronary flow was abolished by atrial pacing (Fig. 5).

Discussion

The findings from this investigation demonstrate that the positive inotropic actions of dobutamine in ischemic myocardium can be enhanced by the concomitant administration of zatebradine, a specific bradycardic agent. The results also show the adverse effects of dobutamine-induced increases in heart rate on regional myocardial perfusion and segment function and the improvement in perfusion and function attainable when the positive chronotropic properties of this agent are eliminated. The improvement in ischemic zone perfusion and function produced by zatebradine were eliminated by atrial pacing, suggesting that the beneficial effects of zatebradine were due solely to a reduction in heart rate.

A new class of anti-ischemic drugs termed specific bradycardic agents have been developed that produce dose-related reductions in the rate of sinus node discharge (9,16). One such agent is a substituted benzothiophene, structurally related to verapamil, known as zatebradine. This compound reduces heart rate without direct alterations in inotropic or lusitropic state or vascular tone (9,12,13,16,17). Previous investigations in conscious dogs have demonstrated that zatebradine improves perfusion and function in a collateral-dependent zone during exercise (11). Similar improvements in ischemic zone perfusion and function produced by zatebradine have also been found in experimental models using anesthetized dogs (12) and pigs (14).

Inotropic stimulation of ischemic myocardium. In the present investigation, dobutamine administration produced dose-related increases in heart rate, mean arterial pressure, rate-pressure product, left ventricular peak positive dP/dt and cardiac output and a decrease in systemic vascular resistance. Similar hemodynamic changes caused by dobutamine have been found in previous studies using anesthetized dogs (18,19). In the present and previous (8,20,21) investigations, dobutamine produced progressive increases in percent segment shortening in nonischemic regions, accompanied by uniform increases in blood flow to the sub-

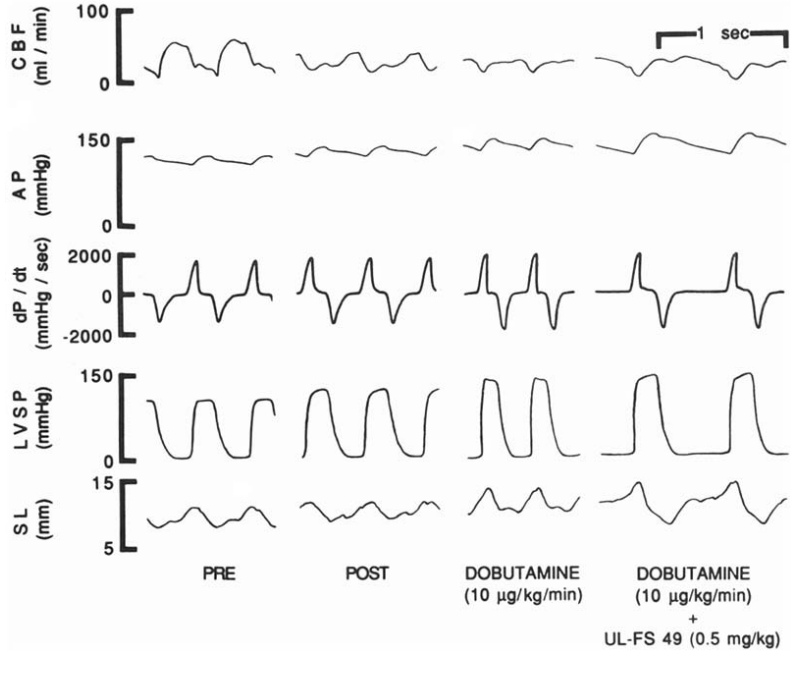


Figure 3. Typical recording of coronary blood flow (CBF), aortic pressure (AP), first derivative of left ventricular pressure (dP/dt), left ventricular systolic pressure (LVSP) and segment length (SL) before and after coronary artery stenosis and after administration of dobutamine alone and simultaneously with zatebradine (UL-FS 49). All traces were recorded at the same paper speed (50 mm/s). Other abbreviations as in Figure 1.

Table 3. Effects of Dobutamine and Zatebradine Administration on Regional Myocardial Perfusion

	Post-stenosis	Dobutamine (10 μg/kg body wt per min)	Dobutamine (10 μg/kg body wt per min) + Zatebradine (0.5 mg/kg body wt)	Dobutamine (10 μg/kg body wt per min) + Zatebradine (0.5 mg/kg body wt) + Pacing
Normal zone (ml/min per g)				
Subepicardium	1.26 ± 0.22	2.07 ± 0.25*	1.79 ± 0.25*	1.95 ± 0.20*
Midmyocardium	1.07 ± 0.19	1.69 ± 0.29*	1.55 ± 0.20*	1.61 ± 0.26*
Subendocardium	1.02 ± 0.12	1.55 ± 0.14*	1.62 ± 0.17*	1.57 ± 0.20*
Transmural	1.11 ± 0.17	1.77 ± 0.21*	1.65 ± 0.19*	1.71 ± 0.21*
Subendocardial flow per beat (ml/100 g per beat)	0.82 ± 0.10	0.95 ± 0.09	1.69 ± 0.22*†	0.95 ± 0.11
Ischemic zone (ml/min per g)				
Subepicardium	0.81 ± 0.09	1.27 ± 0.17*	0.87 ± 0.11†	1.08 ± 0.19
Midmyocardium	0.68 ± 0.09	0.75 ± 0.14	0.72 ± 0.12	0.60 ± 0.09
Subendocardium	0.60 ± 0.07	0.69 ± 0.14	0.68 ± 0.12	0.52 ± 0.08
Transmural	0.70 ± 0.08	0.89 ± 0.14	0.76 ± 0.11	0.73 ± 0.11
Subendocardial flow per beat (ml/100 g per beat)	0.48 ± 0.06	0.39 ± 0.09	0.71 ± 0.12*†	0.32 ± 0.05

*$p < 0.05$ versus post-stenosis values. †$p < 0.05$ versus dobutamine (10 μg/kg per min). Values presented are mean value ± SEM. wt = weight.

endocardium and subepicardium. Regional function and perfusion in the normal region were unaffected by zatebradine.

A stenosis sufficient to reduce rest coronary blood flow by 50% (as assessed by the electromagnetic flow probe) was used in our study. This degree of stenosis resulted in a decrease in subendocardial blood flow compared with that in the normal region, with a concomitant depression of contractile function. Only doses (2 μg/kg per min) of dobutamine that produced no change in heart rate increased shortening in the ischemic zone in the absence of zatebradine. Higher doses of dobutamine were accompanied by increases in heart rate but no change in ischemic zone segment shortening. Comparison with previous experimental studies investigating the effect of dobutamine on ischemic zone function is difficult because of differences in the severity of coronary stenoses utilized and the different doses of dobutamine applied.

In anesthetized dogs with total coronary artery occlusion, dobutamine in doses larger (20 μg/kg per min) than those used in the present study increased ischemic zone blood flow (21). However, this same dose produced an increase in ST segment elevation and may have increased the extent of ischemic myocardial injury (21). Vatner and Baig (20) demonstrated in anesthetized dogs with complete coronary oc-

Figure 4. Normal zone (left anterior descending coronary artery) subendocardial/subepicardial blood flow ratio (ENDO/EPI) after left circumflex coronary artery stenosis application and after administration of dobutamine 1) alone, 2) simultaneously with zatebradine (UL-FS 49), and 3) simultaneously with zatebradine and atrial pacing.

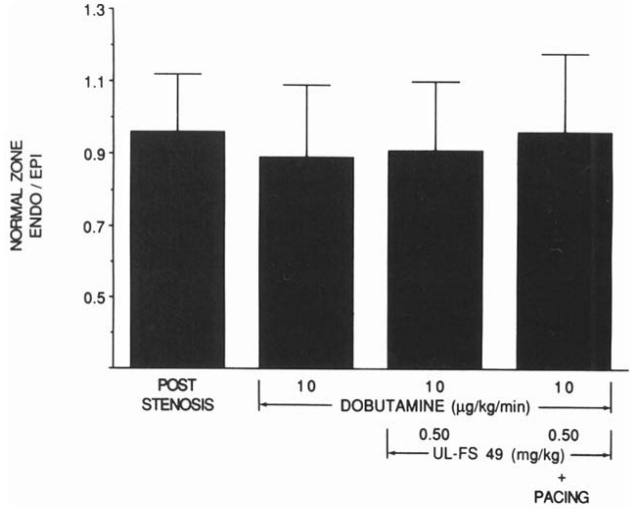
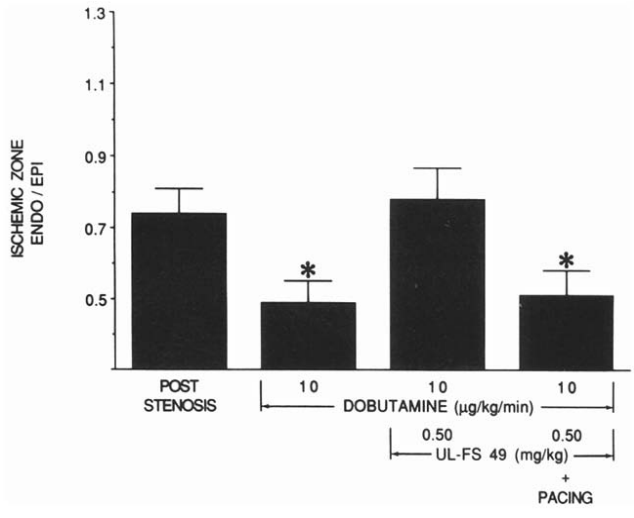


Figure 5. Ischemic zone subendocardial/subepicardial blood flow ratio after left circumflex coronary artery stenosis application and after administration of dobutamine 1) alone, 2) simultaneously with zatebradine (UL-FS 49), and 3) simultaneously with zatebradine and atrial pacing. Abbreviations and symbols as in Figures 1 and 4.



clusion that myocardial perfusion in the ischemic zone was unchanged by dobutamine, provided that heart rate was unchanged. Tissue flow, subendocardial/subepicardial flow ratio and regional myocardial function were depressed if tachycardia was produced. In the present study, utilizing a severe coronary stenosis but with persistent anterograde flow, dobutamine (10 μg/kg per min) increased only subepicardial flow, whereas midmyocardial and subendocardial perfusion remained unchanged in the ischemic zone. Regional function was not improved at doses that produced increases in heart rate. Similar results were found by McGillem et al. (19). Dobutamine (10 μg/kg per min) failed to improve ischemic zone segment shortening in anesthetized dogs with a coronary stenosis sufficient to produce a >80% reduction of reactive hyperemia.

A relative or absolute decrease in subendocardial blood flow with an associated reduction in the subendocardial/subepicardial blood flow ratio has been demonstrated not only with dobutamine (8,18), but also with dopamine, isoproterenol and norepinephrine (8,22) after exercise (11) and during atrial pacing (6). Autoregulatory mechanisms preserve approximate equality of flows per gram across the ventricular wall as coronary perfusion pressure changes. However, these autoregulatory mechanisms may fail, and subendocardial ischemia may ensue when pressure distal to a coronary stenosis decreases below ~70 mm Hg (23). Dobutamine (10 μg/kg per min) has previously been shown to reduce perfusion pressure distal to a coronary stenosis and to decrease the subendocardial/subepicardial blood flow ratio (8). Tachycardia (24) and exercise (25) may produce an adverse shift in the lower limit of subendocardial autoregulation, as manifested by the onset of subendocardial ischemia at a higher distal coronary artery pressure. In the present study, although higher doses of dobutamine did not cause regional function to deteriorate below control (poststenosis) levels, the failure of regional wall function to improve was secondary to persistence of a subendocardial perfusion deficit.

Control of heart rate during inotropic stimulation. After zatebradine administration, contractile function of the ischemic segment was improved dramatically by dobutamine. Zatebradine produced a decrease in heart rate without change in left ventricular peak positive dP/dt or function of the control region. The decrease in heart rate produced by zatebradine during the infusion of dobutamine was associated with a marked increase in function of the ischemic zone. Potential reasons for the improvement in regional contractile function relate to a favorable alteration in myocardial oxygen supply-demand balance. Previous investigations have shown that beta-adrenergic blocking agents, the non–beta-blocker *N*-dimethyl propranolol, as well as the specific bradycardic agents alinidine, AQ-AH 208 and zatebradine, favorably redistribute ischemic zone flow to the subendocardium (12,13,26,27). Mechanisms by which this redistribution of flow could occur include an increase in diastolic perfusion time; restoration of autoregulation, particularly in the subepicardium of the ischemic zone; and direct effects on collateral blood flow.

Regional myocardial blood flow. The majority of myocardial blood flow occurs during diastole, and subendocardial blood flow and perfusion distal to a coronary obstruction are almost exclusively diastolic (28). As a consequence, should diastolic perfusion time be reduced, the subendocardium will receive the least flow (28). Interventions that prolong diastole will proportionately increase perfusion to the subendocardium (5). It is well documented that regional myocardial function is closely related to the level of subendocardial blood flow. A linear relation exists between the level of inner wall perfusion and regional wall function (29). This relation has been termed perfusion-contraction matching, whereby regional function is dependent on and matched to subendocardial blood flow under steady state conditions (14,30). Indolfi et al. (14) normalized subendocardial blood flow for heart rate (subendocardial blood flow per beat) and described a good correlation between perfusion and regional contractile function.

In the present study, zatebradine caused no change in subendocardial flow in the ischemic region, but percent segment shortening dramatically improved. When normalized for heart rate, however, subendocardial blood flow per beat was significantly increased by zatebradine. Furthermore, the failure of segment shortening to be increased by high doses of dobutamine before zatebradine administration corresponded with an unchanged normalized subendocardial blood flow. Expressing myocardial perfusion in this manner may account for changes in both myocardial oxygen supply and demand. This emphasizes that the functional response to bradycardia in an ischemic zone may result from modification of both oxygen demand (fewer contractions per minute) and a substantial increase in subendocardial blood flow per beat (increased oxygen availability), thus allowing augmented regional contraction. In the present investigation, regional myocardial oxygen consumption was not directly measured. It is generally held that bradycardia will decrease myocardial oxygen consumption, but this may not apply equally to the subepicardium and subendocardium. Reduced contractions per minute would decrease myocardial oxygen consumption, but improvement in contractile function during bradycardia would be expected to increase oxygen consumption per beat. Prediction of the effects of bradycardia on ischemic myocardial oxygen consumption is complex, and until practical methods for measuring regional oxygen consumption become available, the relative contributions of bradycardia-induced changes in myocardial oxygen demand and supply toward improved regional contractile performance remain speculative.

As diastolic perfusion time decreases, a selective decrease in subendocardial vascular resistance occurs to maintain uniform net transmural perfusion through autoregulation (31). This compensatory mechanism may fail in the presence of a coronary artery obstruction. A decrease in diastolic perfusion time or distal coronary perfusion pressure, or

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both, may exhaust vasodilator reserve in the subendocardium, with a resultant "vertical steal" (an increase in subepicardial perfusion at the expense of the subendocardium). Several beta-blocking agents have been shown to increase distal perfusion pressure and to reduce the calculated resistance of a coronary stenosis (26,27,32). A reduction in myocardial oxygen demand by beta-blockers may restore the ability of the subepicardial vasculature to autoregulate, thus diverting flow to the more intensely ischemic subendocardium. In the present investigation, zatebradine caused a decrease in subepicardial flow of the ischemic zone and an increase in the subendocardial/subepicardial blood flow ratio. Distal coronary artery perfusion pressure was unmeasured. However, the dominant factor causing improvement in regional function in the present and previous (14) studies was the prolonged diastolic perfusion time, which increased subendocardial flow per beat. Left ventricular end-diastolic pressure remained unchanged throughout the course of these experiments. Previous studies in anesthetized dogs with a significant coronary artery stenosis have also demonstrated that left ventricular end-diastolic pressure remains unaltered after dobutamine infusion (8,19). Although increased diastolic filling as a consequence of diastolic prolongation would be expected, an increase in left ventricular end-diastolic pressure was not observed after zatebradine.

The present investigation was performed in barbiturate-anesthetized dogs, resulting in an elevated heart rate (125 beats/min) in the control state. In the absence of a subsequent positive chronotropic stimulus (e.g., dobutamine), baseline tachycardia itself would be expected to enhance the beneficial actions of zatebradine compared with drug administration during lower heart rates. Had the control heart rate been lower, it is possible that the peak heart rates achieved with dobutamine would have been less, thereby reducing the subsequent beneficial effect of zatebradine. Additionally, with slower control heart rates, post-stenosis ischemic zone segment shortening might be a greater percent of prestenosis control values. This would tend to reduce the percent improvement that could be produced by zatebradine.

Conclusions. The results of this investigation demonstrate that the perfusion-contraction matching that accompanies the decrease in heart rate produced by zatebradine administration allows improved post-stenotic myocardial function during inotropic stimulation despite a persistent reduction in subendocardial blood flow. The improvement in regional function is entirely abolished by atrial pacing. These findings underscore the detrimental effect of tachycardia on post-stenotic myocardial function. The actions of zatebradine are likely to be due solely to a reduction in heart rate, leading to fewer active contractions per unit time (decreased oxygen consumption) and a substantial increase in subendocardial blood flow per beat (increased oxygen availability), thus allowing augmented regional contraction.

Clinical implications. Specific bradycardic agents such as zatebradine represent a unique therapeutic modality for the treatment of cardiovascular disease. Zatebradine markedly reduces heart rate without depressing myocardial contractility, unmasking alpha-adrenergic vasoconstrictor mechanisms in the coronary vasculature or directly affecting vasomotor tone in the periphery. Thus, in select clinical circumstances, zatebradine may have important advantages over agents such as beta-adrenergic receptor antagonists or slow calcium channel blocking agents. The ability to augment subendocardial blood flow per beat and regional function in an ischemic zone during administration of dobutamine is of distinct clinical interest. When inotropic support is required for patients with coronary artery disease, the addition of a specific bradycardic agent may reduce the extent of ischemia caused by positive chronotropic stimulation or allow higher doses of these agents to be used, or both.

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