Increased Cyclooxygenase-2 Expression and Prostaglandin-Mediated Dilation in Coronary Arterioles of Patients With Diabetes Mellitus

Tamás Szerafin,* Nóra Erdei,* Tibor Fülop, Eniko T. Pasztor, István Édes, Akos Koller, Zsolt Bagi

Abstract—Based on findings of experimental models of diabetes mellitus (DM) showing increased expression of vascular cyclooxygenase-2 (COX-2), we hypothesized that in patients with DM changes in COX-2–dependent prostaglandin synthesis affect vasomotor responses of coronary arterioles. Arterioles were dissected from the right atrial appendages obtained at the time of cardiac surgery of patient with DM(+) or without documented diabetes DM(−). Isolated arterioles (89±15 μm in diameter) were cannulated and pressurized (at 80 mm Hg), and changes in diameter were measured with video microscopy. After spontaneous tone developed [DM(−): 32±7%; DM(+) : 37±5%; P=NS], arteriolar responses to bradykinin were investigated. Dilations to bradykinin (0.1 nmol/L to 1 μmol/L) were significantly (P<0.05) greater in DM(+) than DM(−) patients (10 nmol/L: 77±10% versus 38±14%). In both groups, dilations were similar to the NO-donor, sodium nitroprusside. In arterioles of DM(+), but not those of DM(−), patients’ bradykinin-induced dilations were reduced by the nonselective COX inhibitor indomethacin or by the selective COX-2 inhibitor NS-398 (DM(+) at 10 nmol/L: to 20±4% and 29±7%, respectively). Correspondingly, a marked COX-2 immunostaining was detected in coronary arterioles of DM(+), but not in those of DM(−) patients. We conclude that in coronary arterioles of diabetic patients bradykinin induces enhanced COX-2–derived prostaglandin-mediated dilation. These findings are the first to show that in humans diabetes mellitus increases COX-2 expression and dilator prostaglandin synthesis in coronary arterioles, which may serve to increase dilator capacity and maintain adequate perfusion of cardiac tissues. (Circ Res. 2006;99:e12-e17.)

Key Words: diabetes mellitus ■ human coronary arteriole ■ endothelium ■ bradykinin ■ cyclooxygenase 2 ■ prostacyclin

Development of macrovascular diseases, such as atherosclerotic plaque formation and atherothrombosis, are common among patients with diabetes mellitus contributing to the increased incidence of acute myocardial infarction.1 However, much less is known regarding the specific alterations in vasomotor responses of coronary microvessels of patients with diabetes,2 which could lead to disturbed regulation of myocardial blood flow. Changes in the vasomotor function of coronary resistance arterioles have particular importance, because reduced coronary flow reserve has been previously reported in diabetic patients, despite intact vascular morphology.3,4 Development of endothelial dysfunction likely represents an early manifestation of diabetes-related macro- and microvascular complications.5 Correspondingly, experimental studies on animals have revealed that dilator mechanisms intrinsic to coronary vascular wall are impaired in diabetes mellitus.6–9 Recent studies on animal models of diabetes mellitus have suggested a pivotal role for alterations in cyclooxygenase-2 (COX-2)–dependent synthesis of prostaglandins affecting vasodilator mechanisms.10,11 In the canine coronary circulation, COX-2–derived prostacyclin contributed to the agonist-induced dilator responses.12

Less, if any, is known regarding the role of prostaglandins in human heart, although COX-2 seems to play an important role in prostacyclin biosynthesis in several organs and tissues in healthy humans.13 Interestingly, it was shown that in patients with atherosclerosis, COX-2 is widely expressed in the atherosclerotic plaques and in the arterial wall.14 However, it is not known if COX-2 expression is altered in the human coronary arterioles and whether it has an impact on prostaglandin-mediated coronary vasomotor responses in patients with diabetes mellitus. Based on the above we hypothesized that COX-2 expression is increased in coronary arterioles of patients with diabetes mellitus, and it modifies agonist-induced prostaglandin-mediated vasomotor responses.
Materials and Methods

Patient Characteristics

All protocols were approved by the Ethical Committee of the University of Debrecen, Medical and Health Science Center. All patients were given written information about the experimental use of human specimen. Patients who underwent coronary bypass or valve replacement surgery were chosen. Patients were divided into two groups, with or without documented diabetes mellitus.

Isolation of Coronary Arterioles

With the use of microsurgical instruments and an operating microscope, the coronary arteriole (∼1 mm in length) from the right atrial appendage was isolated and cannulated, as described previously.2 The cannulated arteriole was connected with silicone tubing to a pressure servo control system (Living Systems Instrumentation) to set the intraluminal pressure to 80 mm Hg. Changes in arteriolar diameter were continuously recorded with a digital camera (CFW1310; Scion Corp), connected to a microscope (Nikon, Eclipse 80i).

Experimental Protocols

During an incubation period of 1 hour, a spontaneous myogenic tone developed in the isolated coronary arterioles in response to the intraluminal pressure of 80 mm Hg. In the first series of experiments, cumulative concentrations of the endothelium-dependent dilator bradykinin (0.1 nmol/L to 1 μmol/L) and the endothelium-independent dilator sodium nitroprusside (SNP; 1 nmol/L to 10 μmol/L) were administered to the arterioles and changes in diameter were measured. The same protocols were repeated in the presence of indomethacin (10 μmol/L, for 30 minutes), a nonspecific inhibitor of the COX. Arterioles were also incubated in the presence of the selective COX-2 inhibitor, NS-39810 (10 μmol/L, for 30 minutes) and agonist-induced arteriolar responses were obtained again.

Immunohistochemistry

Atrial appendages from DM(−) (n=8) and DM(+) patients (n=8) were embedded and frozen in OCT compound (Tissue Tek, Electron Microscopy Sciences). Acetone-fixed consecutive sections (10 μm thick) were immunolabeled with a polyclonal anti–COX-2 primary antibody (dilution 1:100; Cayman Chemicals). Immunostainings were visualized using the avidin-biotin horseradish peroxidase visualization systems (Vectastain kit; Vector Laboratories), stained with DAB. Immunofluorescent labeling was performed with primary antibody was omitted. Images of the sections were collected with a digital camera (CFW 1310C; Scion Corp) connected to a Nikon Eclipse 80i microscope.

Data Analysis

Data are expressed as means±SEM. Agonist-induced arteriolar responses and myogenic tone were expressed as changes in arteriolar diameter as a percentage of the maximal dilation defined as the passive diameter of the vessel at 80 mm Hg intraluminal pressure in a Ca2+-free medium, as described previously.10 Statistical analyses were performed using GraphPad Prism Software by two-way repeated-measures ANOVA followed by Tukey post hoc test or Student t test as appropriate. Data were fitted assuming the sigmoid dose–response curves and the negative logarithm to base 10 of the ED50 was calculated. Multivariate analysis (logistic regression or MANOVA, as appropriate) were performed to examine the influence of age, sex, and underlying diseases on bradykinin-induced vasodilation and ED50 using NCSS Software. P<0.05 was considered statistically significant.

### Table 1. Patient Demographics, Diseases, and Medications

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DM(−)</th>
<th>DM(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male, n (%)</td>
<td>7 (53.85)</td>
<td>5 (41.67)</td>
</tr>
<tr>
<td>Age</td>
<td>60±14</td>
<td>59±8</td>
</tr>
<tr>
<td>Underlying disease, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>0 (0)</td>
<td>2 (16.67)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0 (0)</td>
<td>10 (83.33)</td>
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<tr>
<td>Hypertension</td>
<td>9 (69.23)</td>
<td>11 (91.67)</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>6 (46.15)</td>
<td>5 (41.67)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>3 (23.08)</td>
<td>8 (66.67)</td>
</tr>
<tr>
<td>Angina</td>
<td>3 (23.08)</td>
<td>7 (58.33)</td>
</tr>
<tr>
<td>Premyocardial infarction</td>
<td>2 (15.38)</td>
<td>3 (25.00)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>0 (0)</td>
<td>3 (25.00)</td>
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<tr>
<td>Heart failure</td>
<td>0 (0)</td>
<td>1 (8.33)</td>
</tr>
<tr>
<td>Valve disease</td>
<td>11 (84.62)</td>
<td>5 (41.67)</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>Aspirin</td>
<td>3 (23.08)</td>
<td>5 (41.67)</td>
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<tr>
<td>Lipid lowering</td>
<td>6 (46.15)</td>
<td>5 (41.67)</td>
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<td>Insulin</td>
<td>0 (0)</td>
<td>3 (25.00)</td>
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<td>Oral antidiabetics</td>
<td>0 (0)</td>
<td>5 (41.67)</td>
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<tr>
<td>β-blockers</td>
<td>9 (69.23)</td>
<td>8 (66.67)</td>
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<td>ACE inhibitor</td>
<td>7 (53.85)</td>
<td>5 (41.67)</td>
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<td>Diuretics</td>
<td>8 (61.54)</td>
<td>7 (58.33)</td>
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<tr>
<td>Anticoagulants</td>
<td>9 (69.23)</td>
<td>5 (41.67)</td>
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<tr>
<td>Calcium-channel blockers</td>
<td>4 (30.77)</td>
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<td>Surgical procedures, n (%)</td>
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<tr>
<td>Coronary artery bypass graft</td>
<td>2 (15.38)</td>
<td>8 (66.67)</td>
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<tr>
<td>Valve replacement</td>
<td>11 (84.62)</td>
<td>5 (41.67)</td>
</tr>
<tr>
<td>Other causes</td>
<td>1 (7.69)</td>
<td>1 (8.33)</td>
</tr>
</tbody>
</table>

Data are means±SD. n indicates the No. of patients studied.

Results

Vasomotor Responses of Coronary Arterioles

Patient demographics are summarized in Table 1. In the isolated coronary arterioles a spontaneous tone developed in response to 80 mm Hg intraluminal pressure; there were no significant differences between the active (DM(−): 87±17 and DM(+) 77±8 μm) and passive (in Ca2+ free solution, DM(−): 129±15 and DM(+) 144±7 μm) diameters and the calculated myogenic tone (DM(−): 32±7 and DM(+) 37±5%). During the protocols vasoactive agents were applied consecutively after a 20-minute incubation period, and the basal tone was the same at the time of applications of various drugs. Bradykinin (0.1 nmol/L to 1 μmol/L) elicited substantial dilation in coronary arterioles, which was significantly greater in DM(−) patients (Figure 1a), with −log[ED50] values of 8.46±0.18 and 7.77±0.22 (P<0.05), respectively. In both groups, dilations were similar in response to the NO-donor sodium nitroprusside (SNP; 1 nmol/L to 10 μmol/L; Figure 1b), with −log[ED50] values of 7.3±0.28 and 7.23±0.34 (P=NS), respectively. In arterioles of DM(+) patients bradykinin-induced dilations were reduced to the control level by the nonselective COX inhibitor.
indomethacin (Figure 2b). Also, the selective COX-2 inhibitor NS-398 significantly reduced bradykinin-induced dilations in DM(−) arterioles, and in the presence of COX-2 inhibitor there was no significant difference between the responses of two groups (at bradykinin concentrations of 10 nmol/L, 0.1 μmol/L, 1 μmol/L, DM(−) versus DM(+) 33±9 versus 29±7%, 63±10 versus 64±12%, 83±7 versus 70±15%, respectively; Figure 2c and 2d). Neither indomethacin nor NS-398 significantly affected bradykinin-induced responses in arterioles of DM(+) patients (Figure 2a and 2c).

Also, dilations to SNP were not significantly different after incubation with indomethacin or NS-398 in both groups of vessels (at 10⁻⁵ mol/L, DM(−) after indomethacin: 88±6%, after NS-398: 86±9%, after NS-398: 82±10%, P=NS from the responses of DM(−)). Incubation of vessels with COX inhibitors did not significantly affect the basal coronary arteriolar diameters during the course of experiments (arteriolar diameters before and after indomethacin in DM(−): 89±9 and 94±8 μm, and in DM(+) 73±6 and 81±12 μm; before and after NS-398, in DM(−): 87±17 and 88±6 μm, and in DM(+) 82±8 and 80±5 μm).

By multivariate analysis, only the presence of diabetes, independent of other risk factors and comorbidities, predicted the enhanced arteriolar dilation to bradykinin (10 nmol/L) and alteration in ED₅₀ value (Table 2). In contrast to bradykinin-induced responses, no underlying diseases were associated with dilation to SNP and its ED₅₀ value (not shown).

**Immunohistochemistry**

No apparent immunostaining for COX-2 was detected in arterioles of DM(−) patients, but COX-2 staining was markedly enhanced in the coronary arterioles from DM(+) patients. COX-2 immunostaining was localized throughout the arteriolar wall, both in endothelial and smooth muscle layers, as detected by costaining of COX-2 and smooth muscle α-actin (Figure 3).

**Table 2. Multivariate Analysis of the Influence of Underlying Diseases, Sex, and Age on Bradykinin-Induced Dilation and Calculated ED₅₀ Values**

<table>
<thead>
<tr>
<th></th>
<th>% Dilation to Bradykinin (10⁻⁸ mol/L)</th>
<th>ED₅₀</th>
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<tbody>
<tr>
<td></td>
<td>n=25</td>
<td>n=25</td>
</tr>
<tr>
<td>Coefficient</td>
<td>SE</td>
<td>P</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6.51 ± 3.19</td>
<td>0.04*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.2 ± 2.37</td>
<td>0.93</td>
</tr>
<tr>
<td>Coronary disease</td>
<td>0.76 ± 1.84</td>
<td>0.68</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>-1.01 ± 1.85</td>
<td>0.56</td>
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<tr>
<td>MIcardial infarction</td>
<td>2.14 ± 2.35</td>
<td>0.36</td>
</tr>
<tr>
<td>Valve diseases</td>
<td>-2.72 ± 2.11</td>
<td>0.2</td>
</tr>
<tr>
<td>Male</td>
<td>0.85 ± 1.84</td>
<td>0.64</td>
</tr>
<tr>
<td>Age</td>
<td>-0.54 ± 0.1</td>
<td>0.81</td>
</tr>
</tbody>
</table>

* indicates the No. of patients studied. *P<0.05

**Figure 1.** Changes in diameter of coronary arterioles isolated from nondiabetic (DM(−), n=13) and diabetic patients (DM(+), n=12) in response to cumulative doses of bradykinin (a) and sodium nitroprusside (SNP; b). Data are means±SEM. Asterisks indicate significant differences (P<0.05).

**Figure 2.** Changes in diameter of coronary arterioles isolated from nondiabetic (DM(−), n=5 to 6) and diabetic patients (DM(+), n=5 to 6) in response to cumulative doses of bradykinin, before and after incubation with indomethacin (a and b) or NS-398 (c and d). Data are means±SEM. Asterisks indicate significant differences (P<0.05).

**Table 2. Multivariate Analysis of the Influence of Underlying Diseases, Sex, and Age on Bradykinin-Induced Dilation and Calculated ED₅₀ Values**
be also well established.5 Much less is known regarding the dependent dilation of the large conductance arteries seems to depend on the EDHF-mediated dilations were preserved in the diabetic group. Because the reduction in bradykinin-induced dilations in the diabetic group, but it significantly reduced bradykinin-induced responses to the control level in coronary arterioles of diabetic patients. Furthermore, selective inhibition of COX-2 also reduced bradykinin-induced coronary dilations in the diabetic group, but had no effect on responses of vessels of nondiabetic group. Because the reduction in bradykinin-induced dilations by the selective COX-2 inhibitor was the same as indomethacin-induced inhibition, we suggest that in coronary arterioles of diabetic patients bradykinin elicits dilator prostaglandin.

More than a decade ago, Nitenberg et al showed that despite the presence of angioarchitecturally normal coronary arteries and normal left ventricular systolic function, the coronary flow reserve is reduced in diabetic patients.3 Given that, it has been suggested that in diabetes mellitus epicardial atherosclerosis may not be the primary cause resulting in abnormalities in coronary flow reserve, but rather this is attributable to reduction in dilator capacity of coronary vessels.3,4 In this context, in animal models of diabetes mellitus, reduced agonist- and flow-induced7,15 endothelium-dependent dilations of coronary arterioles were shown. In humans with diabetes, presence of reduced endothelium-dependent dilation of the large conductance arteries seems to be also well established.5 Much less is known regarding the effect of diabetes on endothelial function of microvessels, especially in the human coronary circulation. In isolated coronary arterioles obtained from diabetic patients, Guterman and his colleagues have found a reduced dilation in response to hypoxia, and proposed that impairment of ATP sensitive K+ -channel activation may be the underlying mechanism for this alteration.9 Also, in the simultaneous presence of NO synthase and COX inhibitors they have found that bradykinin-induced endothelium-derived hyperpolarizing factor (EDHF)-mediated dilations were preserved in the diabetic coronary arterioles.9 It is known that, in addition to NO and EDHF,16 bradykinin-induced coronary arterial responses can be mediated by dilator prostaglandins, such as prostacyclin.17 Bradykinin-induced prostacyclin release in the coronary circulation seems to be especially important when other vasodilator pathways, such as NO-mediated mechanisms, are inhibited.17

Prostaglandins play key roles in the vascular homeostasis, including antithrombotic, antiproliferative, and vasodilator effects.18–20 Interestingly, it has been recently proposed that both in animals12 and in humans COX-2 is the predominant source of vascular prostacyclin synthesis.13 Given that, an enhanced COX-2–derived prostacyclin synthesis might have a particular importance under pathological conditions, when COX-2 expression is significantly increased.14,21 In a mouse model of diabetes mellitus, recent studies have found that vascular COX-2 expression is markedly increased, contributing to the altered prostaglandin-mediated responses of large arteries and skeletal muscle arteries.10,11 On the basis of the aforementioned it seems logical to hypothesize that vascular expression of COX-2 is enhanced in human coronary arterioles in diabetes, which may modulate prostaglandin-mediated responses.

Role of COX-2-Derived Prostaglandins in Enhanced Coronary Arteriolar Dilation in Diabetes

In the present study, pharmacological inhibitors of prostaglandin synthesis were used to reveal the role of prostaglandin in mediation of bradykinin-induced dilation, of isolated coronary arterioles. We found that nonselective inhibition of COXs by indomethacin did not significantly affect bradykinin-induced arteriolar dilation in the nondiabetic group, but it significantly reduced bradykinin-induced responses to the control level in coronary arterioles of diabetic patients. Furthermore, selective inhibition of COX-2 also reduced bradykinin-induced coronary dilations in the diabetic group, but had no effect on responses of vessels of nondiabetic group. Because the reduction in bradykinin-induced dilations by the selective COX-2 inhibitor was the same as indomethacin-induced inhibition, we suggest that in coronary arterioles of diabetic patients bradykinin elicits dilator prostaglandin release primarily via stimulating COX-2. In addition to the functional experiments, immunohistochemical studies were performed to detect alteration in the arteriolar expression of COX-2. Compared with nondiabetic patients we have found a marked COX-2 immunostaining in coronary arterioles of diabetic patients, which was localized both to the endothelial and smooth muscle layers of arteriolar wall. These findings are in accordance with our previous observations obtained in an animal model of diabetes mellitus10 and extend them to that of human diabetes. Collectively, our results suggest that in coronary arterioles of patients with diabetes mellitus increased COX-2 expression may contribute to an enhanced synthesis/release of dilator prostaglandin, most likely prostacyclin, on stimulation.

The pathological importance and the underlying mechanisms responsible for the enhanced COX-2 expression in coronary arterioles of diabetic patients, however, are not fully understood. Recently, investigators raise the hypothesis that during the development diabetes mellitus adaptive mechanisms may compensate for the impaired vascular function. For example, loss of NO mediation of dilation has been
shown to be associated with an enhanced EDHF mediation in several pathological conditions. In addition, at the site of atherosclerotic plaques, a specific involvement of the upregulated COX-2 enzyme and consequent increased prostacyclin production has been proposed, which may limit platelet aggregation and thrombus formation. Also, a specific role for COX-2-derived prostaglandins has been proposed in flow-induced adaptive vascular remodeling in an animal model of atherosclerosis.

In line with these ideas, our present data suggest that in human coronary arteries upregulation of COX-2 and bradykinin-induced release of dilator prostaglandins, despite the likely presence of disturbed microcirculation, may serve as an adaptive mechanism aiming to reduce the potentially detrimental effects of diabetes on coronary blood flow to cardiac tissue.

We speculate that the underlying mechanism(s) responsible for the upregulation of COX-2 in diabetes are oxidative stress and low grade inflammation, which both have been recently implicated in the pathogenesis of diabetes mellitus. In an animal model of diabetes mellitus superoxide production in coronary arterioles was significantly increased, whereas in diabetic patients, Guzik et al have demonstrated that vascular production of superoxide is enhanced. Interestingly, it has been found that the enhanced superoxide production was associated with increased COX-2 expression in high glucose–treated mesangial cells and endothelial cells in culture.

In the present study, presence of other risk factors and comorbidities should also be considered to contribute to the observed alterations in coronary arterioles. However, multivariate analysis revealed that only the presence of diabetes, independent of other risk factors and comorbidities, predicted the enhanced coronary dilation to bradykinin. It should be noted that in our study population there was a higher proportion of coronary artery disease in the diabetic group, therefore we cannot entirely exclude the possible impact of coronary artery disease on diabetic microvascular alterations. Also, at this age it is likely that both nondiabetic and diabetic patients had some degree of coronary heart disease without it being clinically apparent.

Clinical Relevance of Upregulated COX-2 in Coronary Arterioles

Our present findings may have particular clinical importance for several reasons. In humans, endogenous release of basal and flow-stimulated bradykinin contributes substantially to the dilator responses of coronary vessels. Particularly, the beneficial effect of angiotensin converting enzyme (ACE) inhibitors is, in part, ascribed to the enhanced levels of bradykinin in the vasculature. However, at this time it is uncertain whether ACE inhibitors have beneficial effects on the coronary micro vessels in humans with diabetes. On the other hand, recent studies on patients with cardiovascular risk factors reported controversial findings regarding the safety of use of nonsteroid antiinflammatory drugs, including selective COX-2 inhibitors. These findings have drawn a great attention to prostaglandins produced by the vascular endothelium. Our present findings raise the hypothesis that pharmacological inhibition of the prostaglandin synthesis in the arteriolar wall may adversely affect coronary vasodilator responses in patients with diabetes. However, to our best knowledge there are no previous clinical studies addressing the effect of COX inhibition with respect to myocardial events in diabetic patients. Further studies are needed to elucidate the specific effects of various pharmacological interventions on the regulation of coronary arteriolar diameter; hence tissue perfusion in diabetes mellitus.

Taken together, this study is the first to show that in humans with diabetes there is an increased COX-2 expression and augmented prostaglandin-mediated bradykinin-induced dilation in coronary arterioles, which may represent a compensatory mechanism aiming to maintain an appropriate blood supply of the myocardium.

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Disclosures

None.

References


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