Impaired Arachidonic Acid–Mediated Activation of Large-Conductance Ca^{2+}-Activated K^+ Channels in Coronary Arterial Smooth Muscle Cells in Zucker Diabetic Fatty Rats

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We studied the arachidonic acid (AA)-mediated modulation of large-conductance Ca^{2+}-activated K^+ (BK) channels in coronary arterial smooth myocytes from lean control and Zucker Diabetic Fatty (ZDF) rats. A total of 1 μmol/l AA enhanced BK current by 274% in lean and by 98% in ZDF rats. After incubation with 10 μmol/l indomethacin, 1 μmol/l AA increased BK currents by 80% in lean and by 70% in ZDF rats. Vasoreactivity studies showed that the dilation of small coronary arteries produced by 1 μmol/l AA was reduced by 44% in ZDF rats. [^{3}H]6-keto-prostagladin $F_{1\alpha}$ ([^{3}H]6-keto-$PGF_{1\alpha}$,), the stable metabolite of prostacyclin (PGI_2), was the major [^{3}H]AA metabolite produced by coronary arteries of lean vessels, but ZDF vessels produced only 15% as much [^{3}H]6-keto-$PGF_{1\alpha}$. BK channel activation and vasorelaxation by iloprost were similar in lean and ZDF rats. Immunoblots showed a 73% reduction in PGI_2 synthase (PGIS) expression in ZDF vessels compared with lean vessels, and there was no change in cyclooxygenase (COX) and BK channel expressions. Real-time PCR studies showed that mRNA levels of PGIS, COX-1, and COX-2 were similar between lean and ZDF vessels. We conclude that PGI_2 is the major AA metabolite in lean coronaries, and AA-mediated BK channel activation is impaired in ZDF coronaries due to reduced PGIS activity. *Diabetes* 54:2155–2163, 2005

Cardiovascular diseases are the most important causes of morbidity and mortality in type 2 diabetic patients, who have a two- to fourfold increase in the risk of coronary artery disease (1). Metabolic, humoral, and hemodynamic factors all contribute to the development of vascular dysfunction (2). Endothelial dysfunction with altered activities of various endothelium-derived factors plays an integral role in diabetic vasculopathy (3). Arachidonic acid (AA) and its metabolites are important vascular tone modulators; however, their role in diabetes is not clear.

AA metabolites activate the vascular large-conductance Ca^{2+}-activated K^+ (BK) channels via direct and second-messenger mechanisms to produce vasorelaxation (4–6), and the generation of these vasoactive mediators involves three major enzymatic pathways. AA is converted by lipoxygenase (LOX) into leukotrienes and 12-hydroxyeicosatetraenoic acid (12-HETE), by cytochrome P450 (CYP) epoxygenase into epoxyeicosatrienoic acids and chain-terminal 20-HETE, and by cyclooxygenases (COX) into prostaglandins and thromboxanes (7). In the COX pathway, AA is converted to prostaglandin H_2 by COX-1, which is constitutively expressed in the most tissues, and by COX-2, which is an inducible isoform. Prostaglandin H_2 is further converted to prostacyclin (PGI_2) by PGI_2 synthase (PGIS), the main prostanoid produced in the vascular endothelium of many species, including humans (8). In addition, COX-1, COX-2, and PGIS are also present in vascular smooth muscle cells, producing PGI_2 in many vascular beds, including human coronary arteries (9,10). PGI_2 performs autocrine and paracrine functions by stimulating the PGI_2 receptor (IP) on the cell surface, leading to enhanced intracellular cAMP concentration. PGI_2 regulation appears to be altered in diabetes. Taylor (11) reported impaired PGI_2-induced vasorelaxation in the mesentery artery of type 1 diabetes. In type 2 diabetic patients with angiopathy, serum PGI_2 levels are decreased (12). The impaired endothelial dysfunction in diabetic rats can be restored by oral administration of a PGI_2 analog (13). Whether the reduced PGI_2 levels in diabetes would affect vascular ion channel regulation is unknown.

Because of its large conductance and high density in smooth muscle cells, the BK channel is an important determinant in the control of resting potential and vascular tone (14,15). In arterial smooth muscle, including those in human coronary arteries, BK channels are thought to be activated by Ca^{2+} "sparks" (15), giving rise to spontaneous transient outward currents and membrane hyperpolarization, and is thought to be a major mechanism in the regulation of arterial tone. BK channels are known to be

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6-keto-$PGF_{1\alpha}$, 6-keto-prostagladin $F_{1\alpha}$; 12-HETE, 12-hydroxyeicosatetraenoic acid; AA, arachidonic acid; BK, large-conductance Ca^{2+}-activated K^+; COX, cyclooxygenase; CYP, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; IBTX, iberiotoxin; IP, PGI_2 receptor; LOX, lipoxygenase; PGI_2, prostacyclin; PGIS, PGI_2 synthase.

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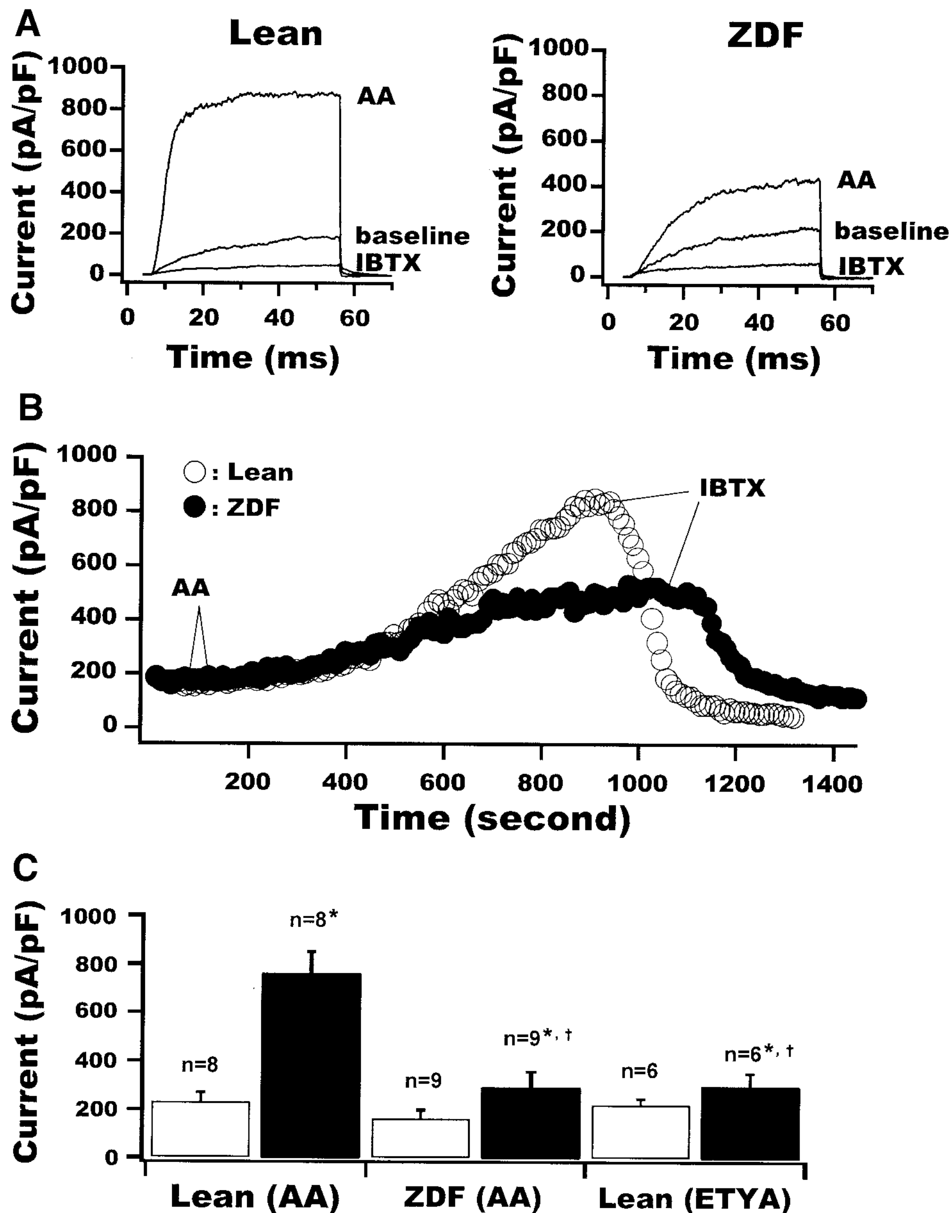

FIG. 1. ***A*: K^+ currents at baseline, with 1 μmol/l AA, and with 100 nmol/l IBTX in coronary myocytes from lean and ZDF rats. Holding potential = −60 mV, testing potential = 100 mV. *B*: Time course of the effects of 1 μmol/l AA on K^+ current in lean and ZDF cells. Lines indicate the time of application of AA and IBTX. *C*: Densities of IBTX-sensitive K^+ currents before (□) and after (■) exposure to 1 μmol/l AA in lean and ZDF cells and to 1 μmol/l 5,8,11,14-eicosatetraynic acid in lean cells. *P < 0.05 vs. baseline; †P < 0.05 vs. lean (AA).**

modulated by various endogenous vasoactive agents (16, 17) and are targets of modulation by endothelium-derived hyperpolarizing factors. β-Adrenergic stimulation activates BK channels through both cAMP-dependent protein kinase and direct G protein mechanisms (18). Similarly, nitric oxide (NO) can activate BK channels through cGMP-dependent protein kinase and through direct effects (19,20). In contrast, stimulation of protein kinase C inhibits the BK channels (21,22). However, the effect of type 2 diabetes on BK channel regulation has not been examined in detail.

In this study, we examined AA-mediated small coronary vasorelaxation and BK channel activation in coronary arterial smooth muscle cells in ZDF rats, which exhibit many features of human type 2 diabetes, including obesity, glucose intolerance, insulin resistance, hyperinsulinemia, hyperglycemia, hypercholesterolemia, hypertriglyceridemia, and moderate hypertension (23). We found that coronary vasorelaxation and BK channel activation by AA were significantly reduced in ZDF rats. COX is the major pathway for AA metabolism in both lean and ZDF coronary vessels, with PGI_2 being the major product. However, PGI_2 production is decreased substantially in ZDF coronary arteries due to reduced PGIS protein, even though PGIS mRNA expression is normal. We believe that this study is the first report demonstrating activation of BK channels by metabolites derived from conversion of AA in native vascular smooth muscle cells and an impairment of this process in diabetic rats. These results provide new insight into the mechanisms of vascular dysfunction in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Male Zucker Diabetic Fatty (ZDF) rats (Gmi-*fa/fa*) and their control lean rats (Gmi-*fa*/+ or +/+) were obtained from Charles River Laboratories (Wilmington, MA) at 6–8 weeks old. All the rats were housed in the animal care facility at the Mayo Clinic. They received a Purina 3008 modified mouse/rat diet, according to a protocol approved by the Animal Use Committee, Mayo Foundation. At the time of experiment (~4 weeks of hyperglycemia >300 mg/dl), the average weight and blood glucose levels were 346.4 ± 13.3 g and 155.3 ± 10.5 mg/dl in lean rats and 404.9 ± 4.4 g and 447.4 ± 11 mg/dl (n = 34, P < 0.05 vs. lean) in ZDF rats, respectively.

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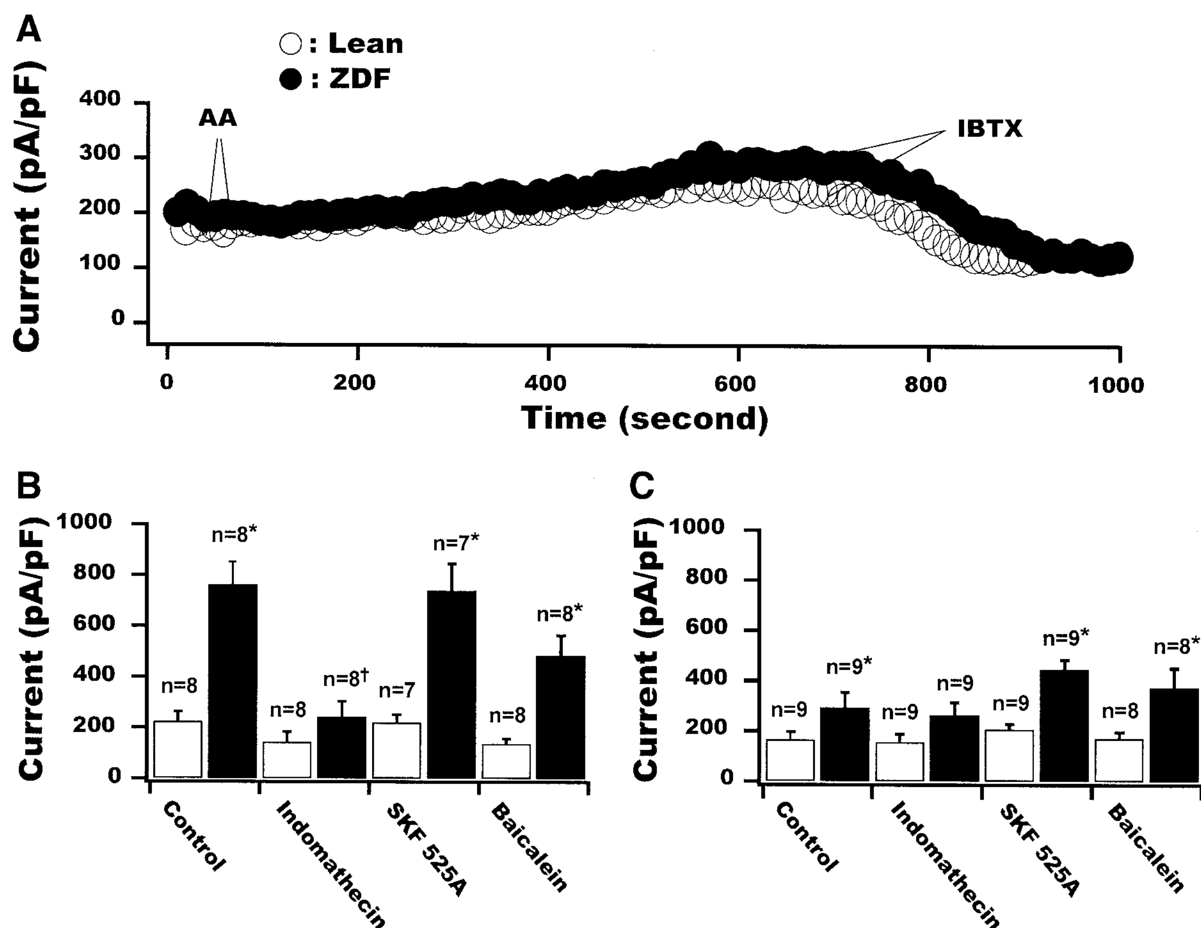

FIG. 2. ***A*: Time course of the effect of 1 μmol/l AA on K^+ current in lean and ZDF cells preincubated with 10 μmol/l indomethacin for 1 h. BK current densities in lean (*B*) and ZDF (*C*) cells before (□) and after (■) exposure to 1 μmol/l AA, with control, preincubated with 10 μmol/l indomethacin, 5 μmol/l SKF525A, or 10 μmol/l baicalein. *P < 0.05 vs. baseline; †P < 0.05 vs. control.**

Electrophysiology studies. Single smooth muscle cells were enzymatically isolated from small coronary arteries as described previously (5). Whole-cell K^+ currents were elicited continuously with a holding potential of −60 mV and a testing potential of −100 mV at 30-s intervals, filtered at 2 kHz, and sampled at 20 kHz with an AxoClamp 200B amplifier and pCLAMP 8 software. The pipette solution contained 140 mmol/l KCl, 10 mmol/l HEPES, 1 mmol/l $MgCl_2$, 5 mmol/l Na_2ATP, 0.5 mmol/l Na_2GTP, 1 mmol/l EGTA, and 0.018 mmol/l $CaCl_2$ (200 nmol/l free Ca^{2+}), pH 7.35. The bath solution contained 145 mmol/l NaCl, 4 mmol/l KCl, 1 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$, 10 mmol/l HEPES, and 10 mmol/l glucose, pH 7.4. The iberiotoxin (IBTX)-sensitive (100 nmol/l) current was determined and referred to as the BK current. All experiments were conducted at room temperature (22°C).

Videomicroscopy. Small coronary arteries (~100–200 μm in diameter) were isolated, and vasoreactivity was measured as described previously (24). Vessels were mounted in an organ chamber with Krebs solution that contained 118.3 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 25 mmol/l $NaHCO_3$, and 11.1 mmol/l glucose, pH 7.4. Only the vessels that showed >85% relaxation with 10^{-4} mol/l nitroprusside and 35% constriction with 60 mmol/l KCl were used.

Western blot analysis. Western blotting was performed as previously described (25). Isolated coronary arteries from ~3–6 pairs of lean and ZDF rats were homogenized, electrophoresed, transferred to nitrocellulose membranes, then immunoblotted with goat anti-mouse COX-1 and COX-2 antibodies (1:1,000 dilution, Santa Cruz Biotechnology, CA), rabbit anti-bovine PGIS antibodies (1:50, Cayman Chemical, Ann Arbor, MI), and rabbit anti-human BK channel antibodies (custom made, 1:500). Blots were also probed with anti-actin antibodies (1:500, Santa Cruz) as loading controls. The blots were developed with enhanced chemiluminescence solution and exposed to Biomax MR films. Optical density of the bands was analyzed by Scion Image software (Scion, Frederick, MD). Protein expression was expressed as relative abundance normalized to actin.

RNA isolation and cDNA synthesis. Total RNA from small coronary arteries was isolated using RNeasy Mini Kit (QIAGEN, Valencia, CA) and treated with deoxyribonuclease. RNAs were reverse transcribed using the ThermoScript RT-PCR System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Quantitative real-time PCR. Real-time semiquantitative PCR analyses were performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Primers for the COX-1, COX-2, PGIS, and for the reference gene (ribosomal protein L32) were selected from published sequences. For rat COX-1: forward, 5′-CCTCACCAGTCAATCCCTGT-3′ and reverse, 5′-AGGTGGCATTCACAAACTCC-3′; for COX-2: forward, 5′-GATTGACAGCCCACCAACTT-3′ and reverse, 5′-CGGGATGAACTCTCTCCTCA-3′; and for PGIS: forward, 5′-TGTCCATGCAGAGCTGAAAC-3′, reverse, 5′-TTCCCTCCTGTCTGCCATAG-3′. Using these primers, PCR products of the expected size were obtained, and no other products were amplified (data not shown). Duplicate samples were analyzed and quantitative results were obtained by normalizing the target signal to the L32 signal (forward primer: 5′-GAAACTGGCGGAAACCCA-3′ and a reverse primer: 5′-GGATCTGGCCCTTGAATCTTC-3′) (20,26).

Eicosanoid production in coronary arteries of lean and ZDF rats. Coronary arteries from two lean and two ZDF rats were dissected and collected in 1 ml modified Dulbecco's modified Eagle's medium (DMEM) containing 0.1 μmol/l BSA (DMEM/BSA) on ice. The vessels were incubated for 1 h at 37°C with 1 ml of DMEM/BSA containing 5 μmol/l [5,6,8,9,11,12,14,15-^{3}H]AA (1 μCi/nmol). Incubations were terminated by removing the media and extracting the lipids. Radioactive metabolites of AA were separated by reverse-phase high-performance liquid chromatography (6). The elution profile consisted of water/formic acid at pH 4.0 and an acetonitrile gradient that increased from 30 to 57% over 30 min, increased from 57 to 65% over 20 min, maintained at 65% for 5 min, and then increased to 100% acetonitrile for 6 min. Radioactivity of the column effluent was measured by an in-line flow scintillation detector. Retention times of the radiolabeled components were compared with those of standards.

Chemicals. Unless otherwise mentioned, all chemicals used were obtained from Sigma-Aldrich (St. Louis, MO). β-Diethyl-aminoethyldiphenylpropylac-

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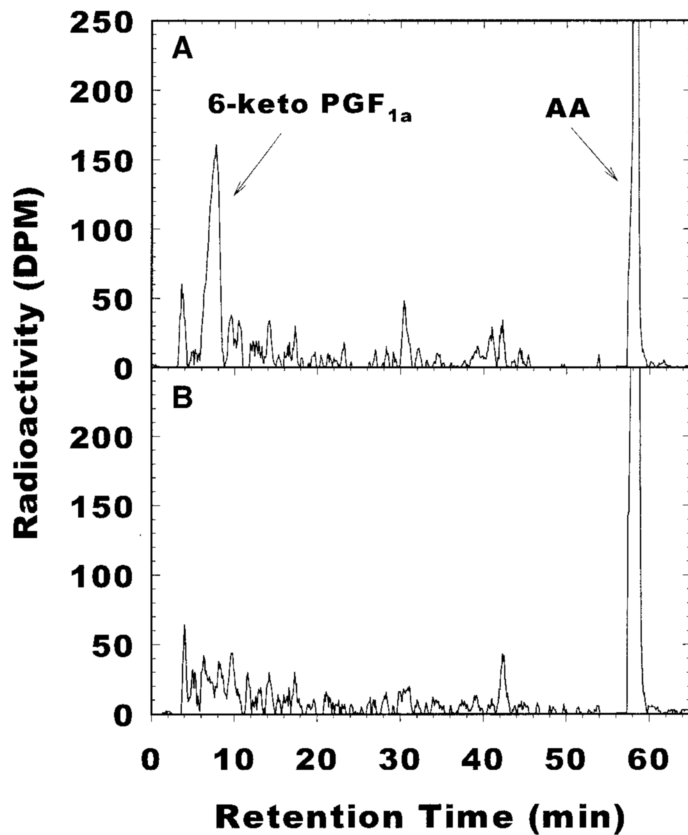

FIG. 3. AA metabolites produced by coronary arteries from lean (*A*) and ZDF (*B*) rats. Isolated vessels were incubated with 5 × 10^{-6} mol/l [^{3}H]AA (specific activity 1 μCi/nmol) for 1 h at 37°C. Lipids were extracted and analyzed by reverse-phase high-performance liquid chromotography. The major peak at 7.5 min had the same retention time as a 6-keto-$PGF_{1\alpha}$ standard.

etate (SKF525A) was purchased from BIOMOL (Plymouth Meeting, PA). Iloprost was obtained from Cayman.

Statistical analysis. Data are presented as means ± SE. Student's *t* test was used to compare data between two groups. Paired *t* test was used to compare data before and after treatment. Data from multiple groups were compared using one-way ANOVA followed by Tukey post hoc test with SigmaStat software (Systat Software, Point Richmond, CA). Statistical significance was defined as $P < 0.05$.

RESULTS

Altered AA regulation of coronary arterial BK channels in type 2 diabetes. At baseline, isolated coronary arterial smooth muscle cells from lean and ZDF rats have similar BK current densities (179.8 ± 9.5 in lean vs. 178.0 ± 6.9 pA/pF in ZDF rats, holding potential = −60 mV, testing potential = 100 mV, $n = 80$). The averaged cell capacitance was 6.92 ± 0.14 pF ($n = 108$) in lean and 6.90 ± 0.13 pF in ZDF ($n = 108$, P = NS) rats, respectively, and was within the range reported in similar cells (27). Figure 1*A* shows representative whole-cell K^+ channel currents in coronary arterial smooth muscle cells from lean and ZDF rats. The effect of 1 μmol/l AA on K^+ currents peaked after ~13–18 min and was inhibited by 100 nmol/l IBTX (Fig. 1*B*). AA (1 μmol/l) increased the IBTX-sensitive BK channel currents by 274.4 ± 40.5%, from 226.5 ± 38.7 to 761.6 ± 90.1 pA/pF ($n = 8$, $P < 0.05$ vs. baseline), in lean rats and by 97.6 ± 37.4%, from 166.1 ± 32.1 to 294.5 ± 61.0 pA/pF ($n = 9$, $P < 0.05$ vs. baseline and vs. lean), in ZDF rats, suggesting that the AA-mediated BK channel regulation was impaired in ZDF rats. In comparison, direct superfusion of 1 μmol/l 5,8,11,14-eicosatetraynic acid, a nonmetabolized cogener of AA, only increased the BK currents by 33.1 ± 12.7%, from 224.5 ± 25.1 to 298.1 ± 40.1 pA/pF in lean cells ($n = 6$, $P < 0.05$ vs. AA) (Fig. 1*C*). These findings suggest that enzymatic metabolism is required for AA-mediated BK channel activation, and the direct fatty acid effect is minimal.

In coronary smooth muscle cells from lean rats, incubation with 10 μmol/l baicalein (a LOX inhibitor) or with 5 μmol/l SKF525A (a CYP epoxygenase inhibitor) had no significant effect on BK channel activation by AA. In contrast, after incubation with 10 μmol/l indomethacin (a COX inhibitor), 1 μmol/l AA increased BK currents by only 79.9 ± 14.3%, from 144.5 ± 40.7 to 243.8 ± 61.5 pA/pF ($n = 8$, $P < 0.05$ vs. control) (Fig. 2), suggesting that AA activates BK channels mainly through metabolites of the COX pathway in lean coronary arterial myocytes. Incubation with pathway inhibitors in ZDF cells did not alter AA-mediated BK channel activation (Fig. 2), suggesting that the production of AA metabolites by the COX pathway might be impaired.

Reduced PGI_2 production in coronary arteries of ZDF rats. The major radiolabeled metabolite produced by the lean coronary arteries was 6-keto-prostagladin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a stable product of PGI_2. This is represented by the 7.5-min peak in the high-performance liquid chromotography chromatogram (Fig. 3). In 1 h, the lean coronary arteries converted 2.7% of the available [^{3}H] AA to PGI_2, whereas ZDF vessels produced only 14.7% as much [^{3}H]6-keto-$PGF_{1\alpha}$ (1,401 pmol/g wet weight in lean vs. 206 pmol/g wet weight in ZDF rats; the total wet weight of coronary arteries from two lean rats was 65.9 mg and that from two ZDF rats was 48.3 mg). These results demonstrate that the conversion to PGI_2 through COX is the major metabolic pathway for AA in the small coronary arteries in lean rats and that this important process is impaired in ZDF rats, a finding consistent with our electrophysiological results.

BK channel activation by iloprost is not altered in ZDF. We examined the direct effect of iloprost, a stable analog of PGI_2, on BK currents in lean and ZDF coronary arterial smooth myocytes (Fig. 4*A*). In lean cells, 1 μmol/l iloprost increased the BK currents by 145.4 ± 18.6%, from a baseline of 121.7 ± 27.2 to 307.3 ± 74.5 pA/pF ($n = 6$, $P < 0.05$ vs. baseline). Iloprost had a similar effect in ZDF cells, increasing the BK currents by 146.1 ± 30.5%, from a baseline of 116.8 ± 36.5 to 283.0 ± 101.5 pA/pF ($n = 5$, $P < 0.05$ vs. baseline, P = NS vs. lean). These results indicate that the PGI_2 signaling mechanism is intact and BK channel response to PGI_2 is preserved in ZDF rats (Figs. 4*B* and *C*). Compared with the AA effects on BK channel activation, the onset of the iloprost effects occurred promptly and reached peak effects within 5 min. In addition, we tested the effects of the cAMP inhibitor Rp-cAMP to further investigate the effects of the PGI_2 signaling pathway on BK channel activation. After preincubation with 100 μmol/l Rp-cAMP, a membrane-permeable cAMP inhibitor, 1 μmol/l AA produced only a 67.1 ± 7.0% increase in BK currents in lean cells ($n = 6$, $P < 0.05$ vs. lean without Rp-cAMP pretreatment), from a baseline of 199.1 ± 18.2 to 336.6 ± 42.2 pA/pF, and only 22.9 ± 8.4% BK channel

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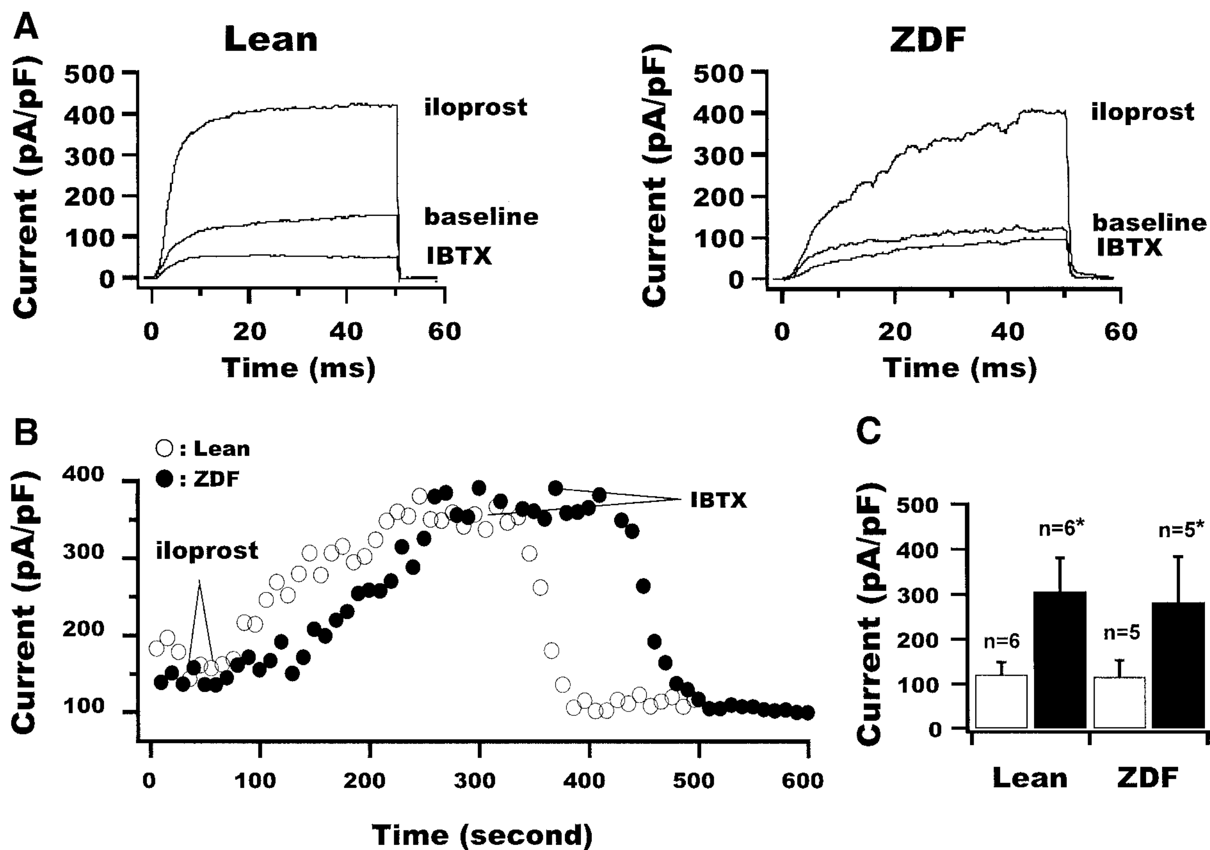

FIG. 4. *A*: K^+ currents from lean and ZDF cells at baseline, after application of 1 μmol/l iloprost and 1 μmol/l iloprost plus 100 nmol/l IBTX. *B*: Time course of the effects of iloprost on K^+ currents in lean and ZDF cells. *C*: BK current densities before (□) and after (■) exposure to 1 μmol/l iloprost in lean and ZDF cells. $^*P < 0.05$ vs. baseline.

enhancement in ZDF cells ($n = 4$, P = NS vs. ZDF without Rp-cAMP pretreatment), from 175.9 ± 11.4 of baseline to 233.3 ± 19.2 pA/pF (Fig. 5). The percentage increases of BK currents in response to 1 μmol/l AA were significantly blocked by Rp-cAMP in lean cells (Fig. 5*D*), but Rp-cAMP had no effects in ZDF cells ($n = 4$, P = NS vs. ZDF control). The level of BK channel activation attained after Rp-cAMP incubation was similar to that observed in lean cells preincubated with 10 μmol/l indomethacin and in ZDF cells with or without Rp-cAMP (Fig. 2). These results indicated that the AA-mediated activation of BK channel activities in lean cells is PGI_2 and cAMP dependent, and this important channel modulation mechanism is impaired in ZDF rats.

AA-mediated dilation of small coronary arteries is impaired in ZDF rats. AA dose-dependently dilated small coronary arteries of lean rats, but this was impaired in ZDF vessels at all doses tested (Fig. 6*A*). In ZDF vessels, 1 μmol/l AA produced 44% less relaxation than in lean vessels (26.7 ± 1.8% relaxation in ZDF vessels, $n = 6$, vs. 47.5 ± 4.1% in lean, $n = 5$, $P < 0.05$). After a 30-min incubation with 10 μmol/l indomethacin, 1 μmol/l AA produced only 21.0 ± 3.1% relaxation in lean vessels ($n = 5$, $P < 0.05$ vs. lean baseline) and 25.3 ± 2.0% relaxation in ZDF vessels ($n = 6$, P = NS vs. ZDF baseline), suggesting AA-mediated vasorelaxation in lean is dependent on COX activity, but such regulation is impaired in ZDF vessels. However, 1 μmol/l iloprost produced a similar amount of relaxation in the lean and ZDF vessels (34.7 ± 3.6% in lean, $n = 7$ and 32.2 ± 1.1% in ZDF, $n = 5$, P = NS) (Fig. 6*B*), suggesting the impairment of the AA effects in ZDF was due to the reduced production of PGI_2.

PGIS protein expression is reduced in coronary arteries of ZDF rats. To determine the cause of reduced PGI_2 production in ZDF coronary arterial smooth muscle cells, we assessed the expression of COX pathway enzymes that regulate PGI_2 synthesis. Immunoblot analysis showed that the amounts of COX-1, COX-2, and BK channel proteins present in coronary arteries were similar between lean and ZDF rats (Fig. 7). In contrast, there was 73% less PGIS protein in the coronary vessels of ZDF rats (0.78 ± 0.05 in lean vs. 0.21 ± 0.07 in ZDF, a 3.7-fold difference, $n = 3$, $P < 0.05$ vs. lean) (Fig. 7*B*). These results indicate that the reduced AA conversion to PGI_2 in ZDF coronary arteries was due to reduced PGIS enzyme, and these findings are consistent with the high-performance liquid chromotography analysis of [^{3}H]AA metabolites (Fig. 3).

To determine whether the reduced PGIS protein in ZDF coronary arteries is due to reduced transcriptional expression, we measured the mRNA levels of the enzymes in the COX pathway that regulate PGI_2 production. Real-time PCR analysis showed that the relative abundance of mRNA of COX-1 (0.96 ± 0.17, $n = 6$), COX-2 (0.75 ± 0.22, $n = 6$), and PGIS (0.95 ± 0.18, $n = 6$, P = NS vs. lean for all) in ZDF coronary arteries were comparable with those in lean vessels. These results suggest that the reduced PGIS enzyme in ZDF rats is not the result of reduced mRNA expression but may be due to impaired translation or increase in enzyme turnover.

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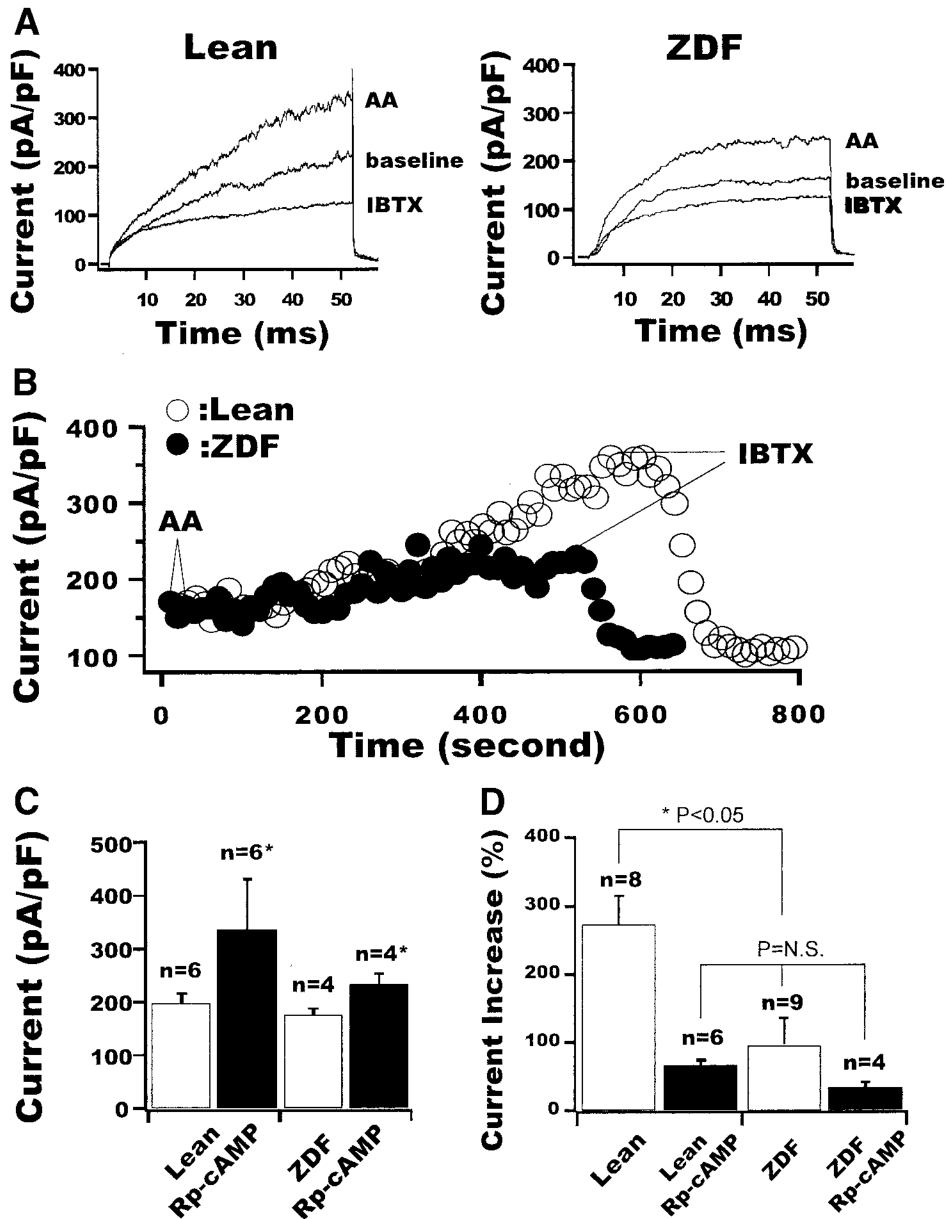

FIG. 5. ***A*****: K^+ currents at baseline, with 1 μmol/l AA and with 1 μmol/l AA plus 100 nmol/l IBTX from lean and ZDF cells after incubation with 100 μmol/l Rp-cAMP for 1 h. *B*: Time course of typical experiments in lean and ZDF cells preincubated with 100 μmol/l Rp-cAMP for 1 h. *C*: Effects of AA on BK current densities in lean (□) and ZDF (■) cells preincubated with 100 μmol/l Rp-cAMP. **P* < 0.05 vs. baseline; †*P* < 0.05 vs. ZDF. *D*: The percentage effects of AA on BK channel activations from lean (□) and ZDF (■) cells with and without incubation with Rp-cAMP. **P* < 0.05.**

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DISCUSSION

The present study demonstrates that the vascular smooth muscle BK channels can be directly activated by AA metabolites generated by the smooth muscle cells. The following major findings are observed in the lean and ZDF rats. *1*) AA can be rapidly converted into active metabolites that activate BK channels in coronary arterial smooth muscle cells of lean control rats. *2*) AA-induced BK channel activation is mainly mediated by PGI_2. *3*) Coronary BK channel activation and coronary relaxation are impaired in ZDF rats due to reduced PGI_2 production. *4*) PGIS protein expression is significantly reduced in ZDF coronary arteries even though the PGIS mRNA expression is normal. These findings suggest that impaired AA-mediated BK channel activation might contribute to the vascular dysfunction observed in type 2 diabetes.

AA modulates vascular tone via its vasoactive metabolites, and products from all three metabolic pathways are known to modulate the vascular K^+ channels. The BK channels in coronary arterial smooth muscle cells are potently activated by 12-HETE, epoxyeicosatrienoic acids, dihydroxyeicosatrienoic acid (the metabolite of epoxyeicosatrienoic acids), and prostaglandin E_2 (4–6,28), whereas those in cerebral arterial smooth muscle cells are inhibited by 20-HETE (29). BK channel activation is implicated in IP receptor–mediated vasorelaxation (30), but PGI_2 activation of vascular BK channels was not directly demonstrated. AA-mediated vasorelaxation also shows diverse species- and vascular bed–dependent schemes. In human coronary arterioles, the AA effects are mediated via the CYP epoxygenase pathway (31), while AA-induced vasorelaxation in porcine coronary vessels is COX dependent (32). In rat small mesenteric arteries, the AA effects are mainly contributed by 12-HETE, a product of LOX (33), and this pathway is also impaired in ZDF rats (24). Our results indicate that the BK channel in coronary arterial smooth myocytes from lean rats is mainly activated by PGI_2. These findings illustrate that the main metabolite

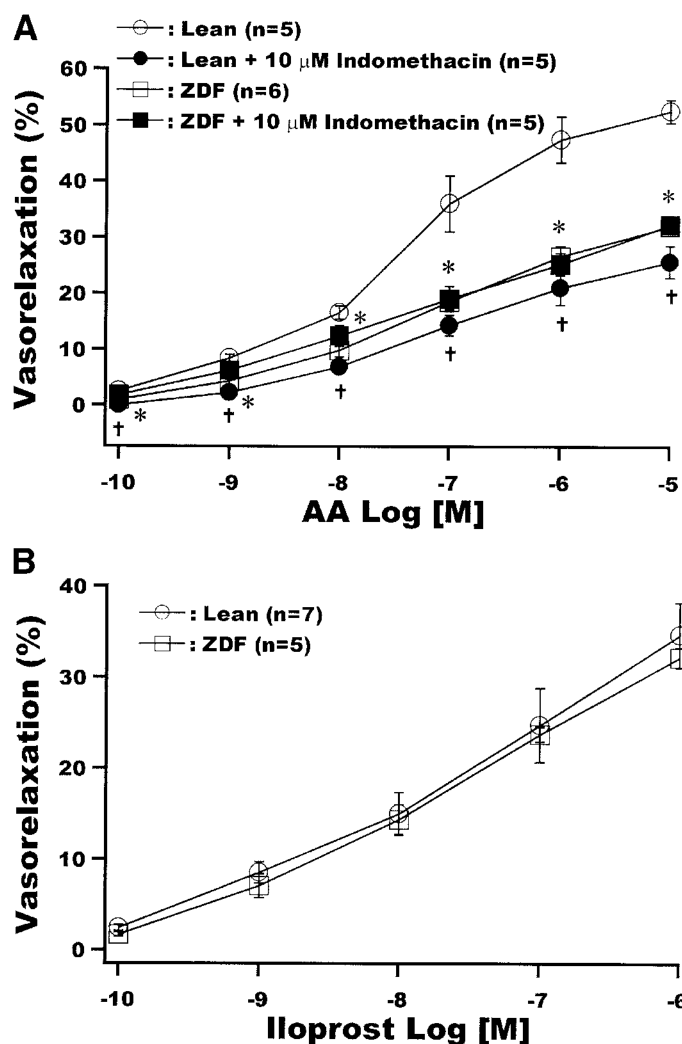

FIG. 6. ***A*****: Effect of AA on relaxation of coronary arteries from lean and ZDF rats with and without a 30-min incubation with 10 μmol/l indomethacin. $*P < 0.05$ vs. lean for ZDF; $†P < 0.05$ vs. lean for lean pretreated with indomethacin.** ***B*****: Effect of iloprost on relaxation of small coronary arteries from lean and ZDF rats.**

produced from AA varies depending on species and vascular bed.

PGI_2 is the major product of COX-catalyzed metabolism of AA in vascular endothelium (34). PGI_2 is also synthesized by vascular smooth muscle cells, including those from human coronaries (9,10). Lipopolysaccharides (*E. coli* O26:B26) evoked a 300-fold increase in PGI_2 release from bovine aortic smooth muscle cells, suggesting that vascular smooth muscle is capable of generating a large amount of PGI_2 (9). In our [^{3}H]AA metabolism study, we did not determine the tissue of origin of PGI_2 synthesis, but it is likely that both endothelial and smooth muscle cells contributed. The impaired [^{3}H]AA conversion to PGI_2 in ZDF coronary arteries is consistent with the impaired COX-dependent AA-induced BK channel activation, suggesting that PGIS activity in smooth muscle cells is diminished in type 2 diabetes.

In inside-out patches from rabbit pulmonary arterial smooth myocytes, 50 μmol/l AA increased the BK channel opening probability by 9.7-fold (35). This suggests that AA can directly activate BK channels. However, our whole-cell current recordings obtained with 1 μmol/l AA, a more physiologically relevant concentration, indicate that the effects on BK channel activation are mainly mediated by AA metabolites. First, the AA effects had a slow onset and reached maximal activation only after 13–18 min, suggesting metabolic conversion of AA might be involved. Second, the AA effects were reduced substantially by indomethacin and by the cAMP inhibitor Rp-cAMP, suggesting the IP receptor–mediated cAMP-dependent signaling pathway is involved. Third, the nonmetabolized AA cogener, 5,8,11,14-eicosatetraynic acid, only produced 33% of the AA effects, suggesting that conversion to active metabolites accounts for most of the BK activation effects. Therefore, our results indicate that the AA-induced BK channel activation in coronary arterial smooth muscle cells requires the integrity of the COX pathway and is dependent on PGI_2 signaling. In ZDF rats, this important regulatory mechanism is profoundly impaired, and this may contribute importantly to the abnormal regulation of vascular tone observed in diabetes.

Immunoblot analysis corroborated the biochemical findings that PGI_2 production is significantly reduced in type 2 diabetes, showing that ZDF coronary arteries contain 75% less PGIS protein compared with lean vessels. Hence, the reduced COX pathway activity is caused by the decrease in PGIS protein in the ZDF coronary vessels. The noted absence of a corresponding increase in the formation of LOX or CYP products when the ZDF coronary arteries were incubated with [^{3}H]AA indicates that the reduced COX pathway activity was not associated with a compensatory increase of activity in the other two pathways.

Altered vascular ionic channel activities have been reported in both type 1 and type 2 diabetes, but the two types of diabetes are associated with different changes. In type 1 diabetic rats, the L-type Ca^{2+} channels in vascular smooth muscle cells showed increased sensitivity to cAMP and hyperglycemia (36), and BK channel sensitivity to carbon monoxide is reduced (37). In addition, after 24 h of exposure to high glucose (23 mmol/l), rat coronary smooth muscle cells showed impaired activity in the voltage-gated K^+ channels (4-aminopyridine sensitive) (38). Impaired ATP-sensitive K^+ channel activity has also been implicated in the diabetic human coronary artery, but direct characterization of the channel was not performed (39). In rats that developed insulin resistance on high-fructose diet, there was a reduction in BK currents in mesenteric arteries, but the channel sensitivity to voltage or Ca^{2+} activation was unaltered (40). In the present study, the response of the BK channel to iloprost was comparable between lean and ZDF coronary smooth muscle cells, suggesting that the defect in ZDF rats is upstream of the IP receptor signaling cascade, and this defect is not associated with altered channel properties or channel expression. These results indicate that PGIS is primarily responsible for the impaired AA-induced BK channel activation in ZDF rats.

PGI_2 is now known to also perform intracrine functions by interacting with the cytoplasmic and perinuclear peroxisome proliferator–activated receptors, promoting their translocation to the nucleus and thereby modulating gene expression and apoptosis by binding to the peroxisome proliferator response element (41). We do not know whether this genomic action of PGI_2 contributes to the

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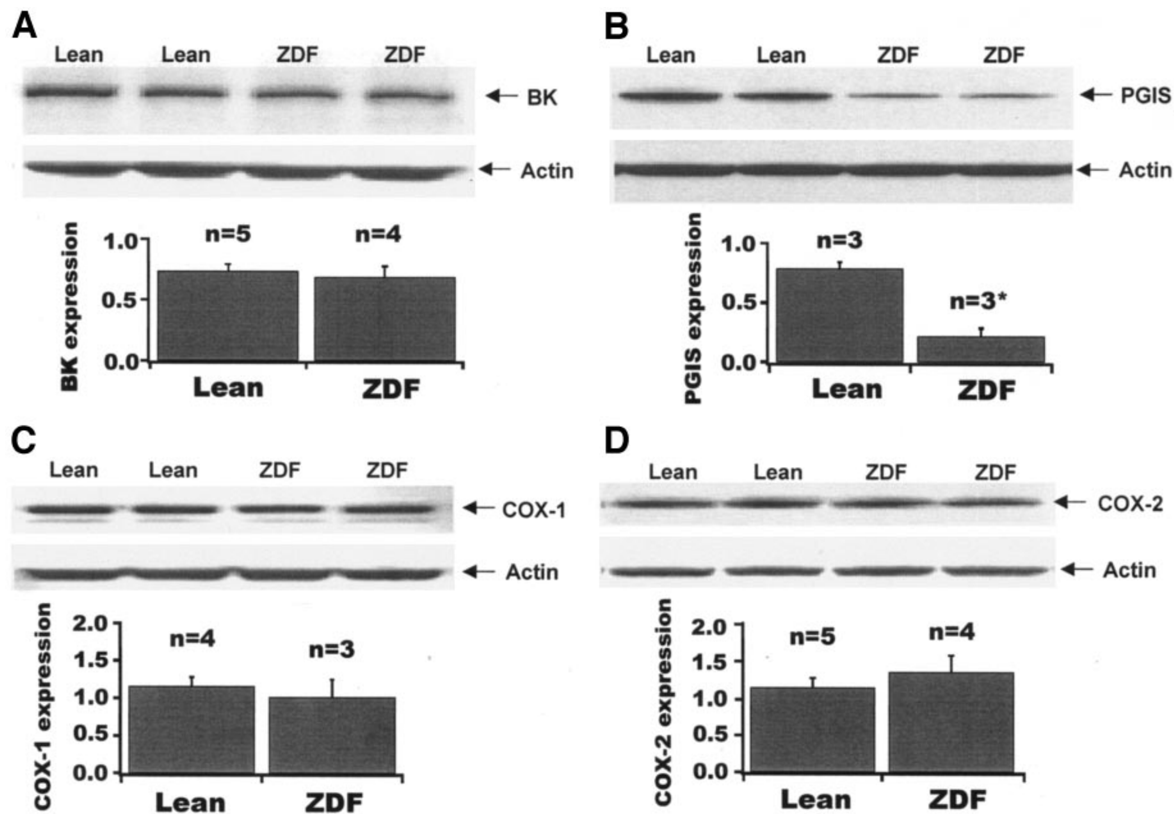

FIG. 7. Immunoblot analysis of BK channels (*A*), PGIS (*B*), COX-1 (*C*), and COX-2 (*D*) in coronary arteries from lean and ZDF rats. Actin served as the loading control. Results are expressed as relative abundance normalized to actin. **P* < 0.05 vs. lean.

impaired BK channel regulation in ZDF rats; however, BK channel expression and PGIS mRNA levels are unaltered. Moreover, in the time frame of the experiments in this study, we believe the results pertain mainly to the IP receptor–mediated effects. Indeed, incubation with Rp-cAMP resulted in significant inhibition of the AA-mediated BK channel activation in coronary smooth muscle cells of lean rats but not in ZDF cells. Furthermore, iloprost produced the same amount of BK current activation in lean and ZDF cells, consistent with a defect in PGI_2 production and not in cAMP signaling. However, 1 μmol/l iloprost produced 38% less BK current activation than 1 μmol/l AA. This may reflect that the extracellular applied synthetic PGI_2 analog is not as potent or efficient as endogenously produced PGI_2. Yet, we cannot rule out the possibility that the AA effect on BK channel activation is more complicated than is currently understood and other regulatory mechanisms may be involved. However, our ion channel results are in agreement with those from the coronary vasoreactivity studies, suggesting the defects noted in single cells also occur in diabetic vessels.

The mechanism of reduced PGIS in ZDF rats is unclear. When human aortic endothelial cells were cultured in high glucose, an increase in tyrosine nitration of PGIS with a decrease in its activity was noted (42). These effects were prevented by the protein kinase C inhibitor calphostin C, which prevented reactive oxygen species formation, restored NO release, and reduced PGIS tyrosine nitration (43). This is a plausible mechanism for the reduced PGIS activity in ZDF coronary arterial smooth muscle cells, since tyrosine nitration is known to enhance proteolytic degradation of proteins (44).

In summary, our results show that in addition to altered endothelial function, impaired smooth muscle function also occurs in type 2 diabetes. Abnormalities in these non-endothelial-dependent mechanisms of vasorelaxation may contribute importantly to the vascular dysfunction in type 2 diabetes.

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