

Diabetes Mellitus-Induced Enhancement of Prostaglandin $F_{2\alpha}$ -Responses Is Inhibited by Lipoxygenase- but Not Cyclooxygenase-Inhibitors in Mesenteric Veins and Arteries of Mouse and Rat

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Received June 10, 1993 Accepted October 29, 1993

ABSTRACT—The mechanisms responsible for diabetes mellitus-induced enhancement of prostaglandin (PG) $F_{2\alpha}$ response were investigated in vascular smooth muscles isolated from diabetic mice and rats. Streptozocin (150 mg/kg, i.v. bolus, 6 week-elapsed)-ddY mice and (60 mg/kg, i.v. bolus)-Wistar rats and genetically diabetic GK-rats were used. The responses to $PGF_{2\alpha}$ were enhanced in small blood vessels such as mesenteric arteries (diabetic rats) and veins (diabetic mice) and they were reduced in large blood vessels such as the aorta and vena cava (diabetic rats). The enhanced response to $PGF_{2\alpha}$ in diabetic blood vessels was significantly inhibited by nordihydroguaiaretic acid (NDGA) (0.03 mM) and phenidone (0.05 mM), lipoxygenase inhibitors, cycloheximide (1 mg/kg, i.v.), a protein synthesis inhibitor and actinomycin D (2.8 mg/kg, i.v.), a RNA polymerase inhibitor, but neither inhibited by cyclooxygenase inhibitors, a thromboxane antagonist, nor Ca^{2+} antagonists. The $PGF_{2\alpha}$ response was also enhanced with aging alone, whereas the extent of enhancement was less than that with diabetes mellitus, and not significantly blocked by NDGA. These results demonstrate that diabetes mellitus-induced imbalance in the regulation of the eicosanoid metabolic pathways (suppressed cyclooxygenase and accelerated lipoxygenase) may cause the enhancement of $PGF_{2\alpha}$ -induced responses in small blood vessels.

Keywords: Prostaglandin $F_{2\alpha}$ response, Diabetic mesenteric vein and artery, Cyclooxygenase, Lipoxygenase, Lipoxygenase inhibitor

Diabetes mellitus produces vascular complications especially in the small blood vessels, causing microangiopathy, and produces alterations in vascular function, which are usually associated with aging (1). The enhanced prostaglandin (PG) $F_{2\alpha}$ responses are complicated by the positive correlation between the diabetic state and aging (2). In the previous paper, we reported that diabetes mellitus enhances $PGF_{2\alpha}$ -induced responses in an endothelium-dependent manner in the mesenteric veins of mice (2). Recently a decreased production of PGI_2 (3, 4) and increased productions of platelet thromboxane (TX) A_2 (5, 6) and leukotrienes C_4 and D_4 (7) have been reported. The present study was performed to investigate the mechanism of changes in prostanoids responses of diabetic and aged blood vessels. The alteration of $PGF_{2\alpha}$ responses by the diabetic state was also compared in small and large blood vessels.

MATERIALS AND METHODS

Animals and preparation

Streptozocin (STZ)-diabetic-ddY male mice (10-week-old, 31–37 g) and -Wistar male rats (6- to 57-week-old, 140–650 g), and GK male rats (8) (4- to 100-week-old, 101–470 g) were used. After injection of single doses (150 mg/kg and 60 mg/kg, i.v.) of STZ into ddY male mice (4-week-old) and Wistar male rats (4- or 6-week-old), 6 weeks and more than 1 week were allowed to elapse, respectively, before the animals were used for the experiments. The blood glucose levels of STZ-diabetic Wistar and diabetic GK rats were compared with age-matched control levels of nondiabetic Wistar rats (Fig. 2B). STZ-diabetic mice that showed blood glucose levels higher than 150 mg/dl in the fed state were used.

The mice were killed by decapitation. The rats were killed under anesthesia with sodium pentobarbital (50 mg/kg, i.p.). The vascular smooth muscles were rapidly

excised and cleaned of adhering fat and connective tissues. The longitudinal segments of mesenteric veins and arteries (2–3 mm below the portal vein, 10 mm long, 0.4–0.8 mm width, 4–8 mm² sectional area), vena cava (left superior portion, 5 mm long, 2 mm width, 10 mm² sectional area) and coronary artery (right proximal portion) were prepared. Longitudinal (10 mm long, 2 mm width) and circular (5 mm long, 2 mm width) segments of the aortic strip (5–10 mm below the aortic arch) were used.

Experimental protocol

The smooth muscle segment was tied at both ends with silk threads and suspended in an organ bath (5 ml) filled with Krebs solution (37°C): 122 mM NaCl, 5.9 mM KCl, 15.5 mM NaHCO₃, 1.2 mM CaCl₂ and 11.5 mM glucose. The solution was continuously gassed with 95% O₂ and 5% CO₂. One end of the preparation was tied to the base of the tissue bath, while the other end was connected to a transducer (U-gauge, type UL-2-240; Minebea, Nagano). The tissue was allowed to equilibrate for 30–60 min at 95–100 mg of a predetermined passive tension and to develop an optimal active tension.

Changes in the isometric contractions were recorded in

a linearecorder (Recti-Horiz-8K; NEC-San-ei, Tokyo) equipped with a biophysigraph 180 system (NEC-San-ei) and an amplifier (6L5, NEC-San-ei). The cumulative concentration-response curves for PGF_{2α} (0.28 μM–0.28 mM) were obtained by adding the drugs directly to the tissue bath. Each concentration of PGF_{2α} was allowed to react with the muscle for 1–2 min.

Drugs

The following compounds were used: PGF_{2α} (Kaken Seiyaku, Kyoto), verapamil (Knoll, Ludwigshafen, FRG), nitrendipine (Yoshitomi Seiyaku, Osaka), ryanodine and aspirin (Wako, Osaka), ONO-3708 (Ono, Osaka) (9), cycloheximide, actinomycin D, indomethacin, nordihydroguaiaretic acid (NDGA), 1-phenyl-3-pyrazolidone (phenidone) and streptozocin (STZ) (Sigma, St. Louis, MO, USA). PGF_{2α} was dissolved in distilled water by brief sonication (UR-20P; Tomy Seikou, Tokyo) before use. Nitrendipine was dissolved in 20% *N,N*-dimethylformamide. Indomethacin and NDGA were dissolved in 0.15% ethanol by brief sonication. Aspirin and phenidone were dissolved in distilled water. Cycloheximide, actinomycin D and STZ were dissolved in normal saline (0.9% NaCl).

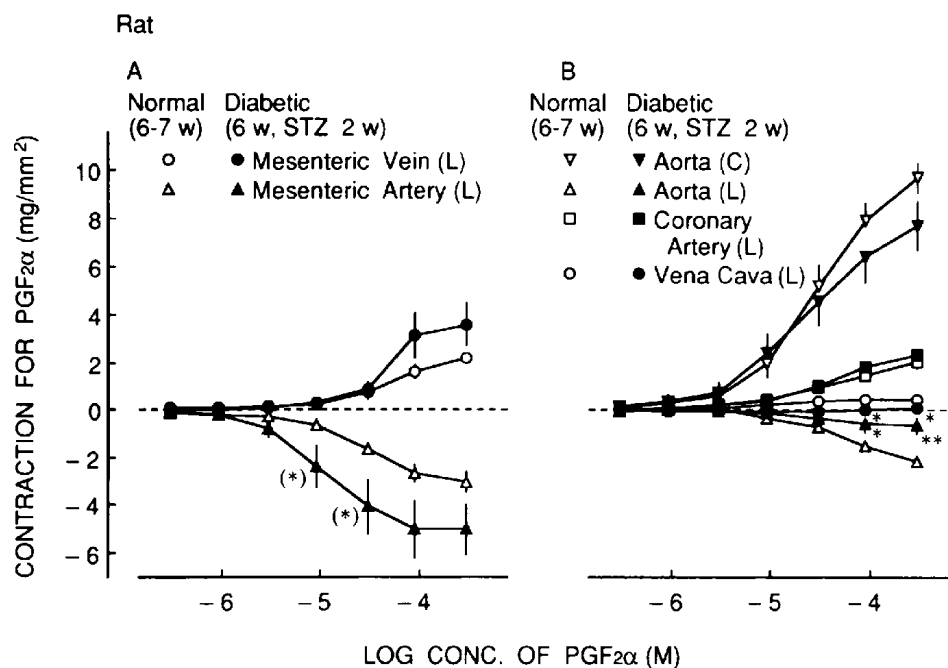


Fig. 1. Diabetic state-induced PGF_{2α} responses in isolated longitudinal mesenteric veins (circles) and arteries (triangles) (A), circular aorta (reversed triangles), longitudinal aorta (triangles), coronary artery (squares) and vena cava (circles) (B) of Wistar male rats, in the normal state (open symbols) (6- to 7-week-old) and 2-week-elapsd diabetic state (closed symbols) after injection of streptozocin (STZ, 60 mg/kg, i.v.) (6-week-old). Log cumulative concentration (PGF_{2α})-response (mg tension per mm² sectional area of segments) curves are plotted. L: longitudinal segments, C: circular segments. The values are means ± S.E.M. (n=3–6). **P<0.01 and *P<0.05: compared with normal rats (unpaired Student's *t*-test, two-tailed or one-tailed (statistics indicated in parentheses)).

Data analysis

The contractile responses were expressed in terms of tension (mg) or tension per sectional area of tissue (mg/mm²). Statistical analyses were performed using the unpaired Student's *t*-test, range test after ANOVA when comparing the different groups of data, and in part paired Student's *t*-test.

RESULTS

PGF_{2α} responses of small blood vessels are enhanced, but those of large blood vessels are reduced, by diabetes mellitus in rats

The responses of rat small and large blood vessels to PGF_{2α} were changed in the opposite direction by the diabetic state. Figure 1 shows a comparison of the concentration-response curves to PGF_{2α} in small and large blood vessels. The STZ-diabetic state tended to enhance the PGF_{2α}-contraction in the mesenteric veins (longitudinal segments) and enhanced the PGF_{2α}-induced apparent relaxation of the mesenteric artery (longitudinal segments) of rats (Fig. 1A). On the other hand, the diabetic state reduced the PGF_{2α}-induced responses in the longitudinal muscles of the vena cava and aorta, but not the coronary artery (Fig. 1B). The responses of aortic circular muscles tended to be reduced by the diabetic state (Fig. 1B).

PGF_{2α}-responses are enhanced to a greater extent by diabetes mellitus than by aging alone in longitudinal muscles of mesenteric arteries in rats

The maximal contraction of mesenteric veins (longitudinal) and the maximal decrease in tension of mesenteric arteries (longitudinal) were changed progressively with aging (Fig. 2A). The PGF_{2α} responses were reduced in mesenteric veins but enhanced in mesenteric arteries with aging. However, the apparent relaxation by PGF_{2α} in mesenteric arteries were enhanced more rapidly and greatly in diabetic rats than in normal age-matched Wistar rats. The maximal enhancement was obtained at the age of 33 weeks in both rats. The levels were reduced thereafter in GK rats but sustained still at 57 weeks in age-matched Wistar rats.

The relationships between aging and blood glucose levels in the fed state were compared among the normal and STZ-Wistar male rats and GK male rats (Fig. 2B). A severe hyperglycemia (more than 300 mg/dl) was observed in STZ-mice from 1 to 4 weeks after application of STZ. GK rats (8-week-old) showed mild (150–300 mg/dl) or severe hyperglycemia (more than 300 mg/dl). The alteration of PGF_{2α}-induced responses occurred with some time lag after the rise in blood glucose levels.

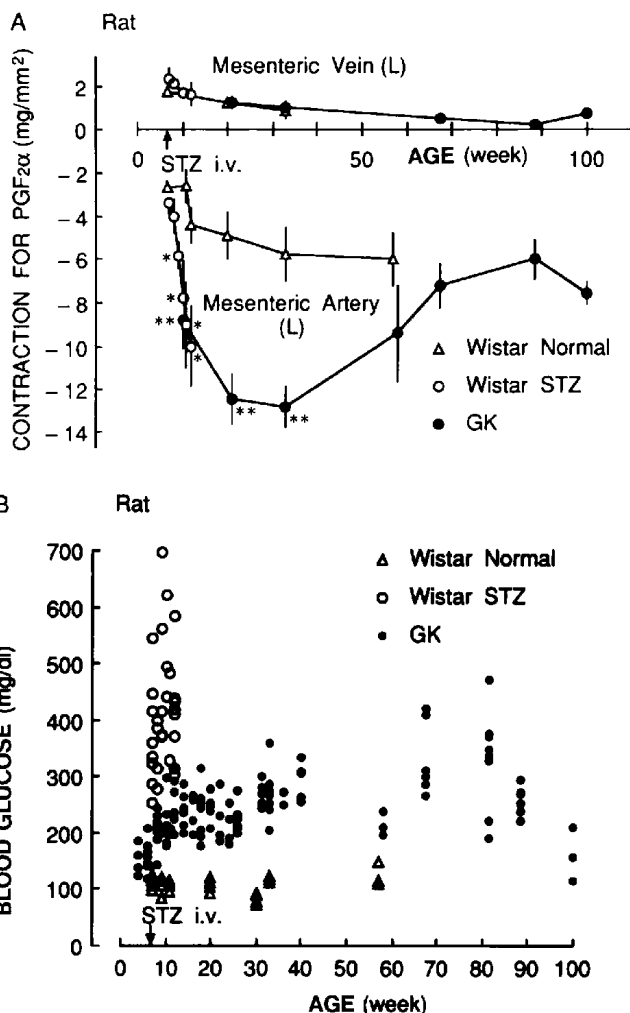


Fig. 2. The alteration of diabetic state-induced PGF_{2α} (0.28 mM)-responses compared with aging alone in isolated longitudinal mesenteric veins (upper three lines) and arteries (lower three lines) (A) and blood glucose levels with age (B) of Wistar-streptozocin (STZ) rats (open circles) and GK rats (closed circles) compared with Wistar-normal rats (triangles). The tensions are expressed as in Fig. 1. The values are means \pm S.E.M. ($n=3-6$). The STZ-Wistar rats were obtained after injection of streptozocin (60 mg/kg, i.v. bolus, at the 7-week-age depicted with an arrow). ** $P < 0.01$ and * $P < 0.05$: compared with Wistar normal rats (unpaired Student's *t*-test).

Nordihydroguaiaretic acid and phenidone, lipoxygenase inhibitors, inhibit PGF_{2α}-induced contraction enhanced by diabetes mellitus in mesenteric veins of mice and rats

To elucidate the mechanisms of the enhanced PGF_{2α} response in isolated diabetic mesenteric veins (longitudinal segments), the effects of nordihydroguaiaretic acid (NDGA) and phenidone, lipoxygenase inhibitors were investigated. NDGA (0.03 mM) significantly suppressed the diabetic state-induced enhancement of apparent relaxation by PGF_{2α} in the longitudinal mesenteric arteries of GK-rats (10-week-old) but not in those of aged (58-week-

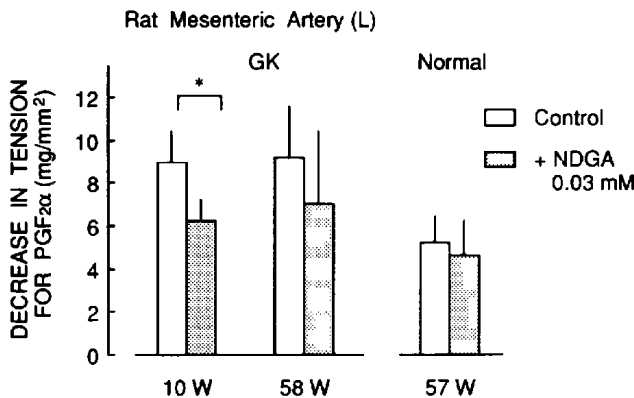


Fig. 3. Inhibition of a lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA), on the enhancement of apparent relaxation (decrease in tension) by $\text{PGF}_{2\alpha}$ (0.28 mM) in isolated longitudinal mesenteric artery of diabetic GK rats at 10 weeks of age, which was found at 58 weeks of age; Wistar normal rats at 57 weeks of age also showed no such inhibition. The tensions (mg/mm^2) were measured before (open column) or after 1-hr exposure (shaded column) to NDGA (0.03 mM). The values are means \pm S.E.M. ($n=3$). * $P<0.05$: compared with the values without NDGA (unpaired Student's t -test).

old) GK rats (Fig. 3). The $\text{PGF}_{2\alpha}$ -induced decrease in tension was not enhanced by age (57-week-old) and not affected by NDGA in normal Wistar rat (Fig. 3). Figure 4 shows the concentration-response curves for $\text{PGF}_{2\alpha}$ (0.28 μM –0.28 mM) with or without NDGA in normal and STZ-diabetic muscles of ddY mice. The diabetic state developed greater contractile forces in response to $\text{PGF}_{2\alpha}$ as previously reported (2). NDGA (0.03 mM) and phenidone (0.05 mM) significantly suppressed the diabetic state-induced enhancement of $\text{PGF}_{2\alpha}$ responses (Fig. 4 and Table 1). However, NDGA (0.03 mM) only tended to suppress the $\text{PGF}_{2\alpha}$ response in mouse normal muscles as shown in Fig. 4. Phenidone (0.05 mM) also more markedly suppressed it in the diabetic state (the decrease in tension: 5.87 ± 0.71 mg) than in the normal state (1.46 ± 0.25 mg) ($P<0.01$, $n=5$).

The enhancement of $\text{PGF}_{2\alpha}$ -induced responses in diabetic muscles was suppressed by neither aspirin (0.2 mM) and indomethacin (0.2 mM), cyclooxygenase inhibitors (Table 1), nor ONO-3708 (0.003 μM), a TXA_2 antagonist (data not shown). The concentrations used were effective enough to cause inhibitions of cyclooxygenase and an antagonistic action against TXA_2 , respectively. Verapamil (2 μM), nitrendipine (6 μM) and ryanodine (1 μM), Ca^{2+} antagonists, did not affect the enhanced $\text{PGF}_{2\alpha}$ responses in diabetic muscles (data not shown).

Protein synthesis inhibitors suppress $\text{PGF}_{2\alpha}$ responses enhanced by diabetes mellitus in mesenteric veins of mice

We investigated whether the STZ-diabetic state-induced

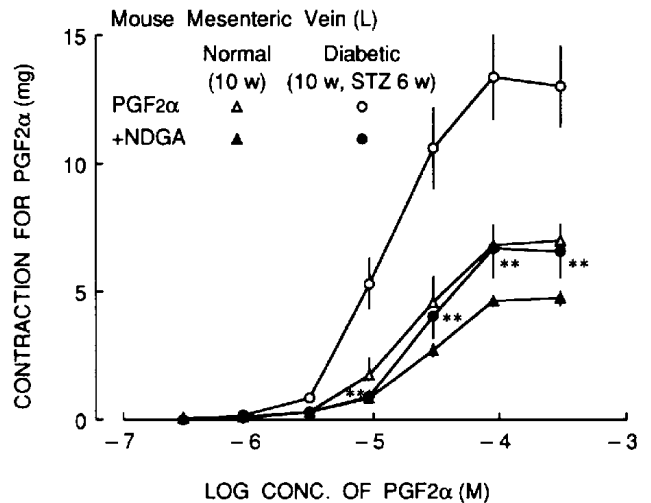


Fig. 4. Inhibition of enhanced $\text{PGF}_{2\alpha}$ responses by nordihydroguaiaretic acid (NDGA, 0.03 mM) in longitudinal mesenteric veins of streptozocin (STZ)-diabetic ddY mice (circles) compared with normal mice (10-week-old) (triangles). STZ-diabetic mice (10-week-old; blood glucose level, 330–700 mg/dl) were used at 6 weeks after the injection of STZ (150 mg/kg, i.v. bolus). Log cumulative concentration ($\text{PGF}_{2\alpha}$)-tension (mg) curves are plotted in the absence (open symbols) and presence (closed symbols) of NDGA (1-hr pretreatment). The values are means \pm S.E.M. ($n=3-5$). ** $P<0.01$: significant difference from the values without NDGA by Student's range test after ANOVA.

Table 1. Effects of cyclooxygenase inhibitors and lipoxygenase inhibitors on $\text{PGF}_{2\alpha}$ -induced contractions in mesenteric veins of diabetic mice

Compounds	Concentration (mM)	Tension (mg)	
		untreated	treated
Aspirin	0.2	15.3 ± 1.9	14.9 ± 1.7
Indomethacin	0.2	11.8 ± 2.8	10.9 ± 2.2
NDGA	0.03	13.0 ± 1.6	$6.6 \pm 1.0^{**}$
Phenidone	0.05	13.5 ± 2.2	$7.6 \pm 1.9^{**}$

The changes in the isometric contractions to $\text{PGF}_{2\alpha}$ (0.28 mM) were measured before (untreated) and after (treated) 1-hr exposure of the mesenteric veins of diabetic mice (streptozocin, 6 week-elapsd) to the enzyme inhibitors. The values are the means \pm S.E.M. ($n=3-5$). Significant difference from the untreated value at ** $P<0.01$ by the Studentized range test or the paired t -test. NDGA: nordihydroguaiaretic acid.

enhancement of $\text{PGF}_{2\alpha}$ responses was due to the increase in protein synthesis. Cycloheximide (1 mg/kg, i.v.), an inhibitor of protein synthesis, and actinomycin D (2.8 mg/kg, i.v.), a RNA polymerase inhibitor, were injected into mice 8 hr before the isolation of mesenteric veins. Cycloheximide suppressed the $\text{PGF}_{2\alpha}$ responses in either diabetic or normal muscles, whereas actinomycin D suppressed only the responses in diabetic muscles (Table 2).

Table 2. Effects of protein synthesis inhibitors on PGF_{2α}-induced contraction in mesenteric veins of normal and diabetic mice

	Tension (mg)		
	untreated	cycloheximide	actinomycin D
Normal	5.5 ± 0.3	3.5 ± 0.2**	6.1 ± 1.0
Diabetic	11.8 ± 0.7**	7.5 ± 1.5 [†]	6.3 ± 0.6 ^{††}

The mesenteric veins of diabetic mice (streptozocin, 6 week-elapased) were isolated 8 hr after the intravenous injection of cycloheximide (1 mg/kg) or actinomycin D (2.8 mg/kg). The values are the means ± S.E.M. (N=4–6). Significant differences from the data of untreated normal muscles at **P<0.01 and from the data of untreated diabetic muscles at [†]P<0.01 and ^{††}P<0.05 by the unpaired *t*-test.

DISCUSSION

Individuals with diabetes mellitus develop vascular complications with aging, and this disorder produces microangiopathy and alters vascular functions (1). The reactivity of vascular smooth muscles in diabetes mellitus is influenced by the duration of the diabetic state. In the previous study, we have reported that prostanoid responses are enhanced in an endothelium-dependent manner in the mesenteric veins of diabetic mice (2). The present results demonstrated that diabetes mellitus affected PGF_{2α} responses increasingly in small blood vessels (mesenteric veins and arteries), but decreasingly in large blood vessels (smooth muscles of aorta and vena cava), and did not have any effect in the coronary artery. The microangiopathy in diabetes mellitus could be observed in the PGF_{2α} responses of small blood vessels such as the mesenteric vein and artery.

Even aging itself when not complicated by diabetes mellitus causes alterations in vascular function. In the mesenteric arteries of normal rats, the PGF_{2α}-induced responses were enhanced with aging. However, the extent of enhancement with aging alone was far less than that with diabetes mellitus.

To investigate the involvement of eicosanoid metabolic pathways in the diabetes mellitus-induced enhancement of PGF_{2α} responses, cyclooxygenase- (10) and lipoxygenase- (11, 12) inhibitors were used. Aspirin or indomethacin (cyclooxygenase inhibitors) did not affect the enhanced PGF_{2α} responses, but they were markedly suppressed by the lipoxygenase inhibitor NDGA and the mixed type-inhibitor phenidone, suggesting the involvement of lipoxygenase. A TXA₂ antagonist did not influence the enhanced PGF_{2α} responses. The diabetic blood vessels generate less prostacyclin via cyclooxygenase than the control (3, 4). In diabetes mellitus, the vasoconstriction via lipoxygenase-catalyzed reactions induced the change of response by arachidonic acid (13). Phospholipase A₂

which elicits arachidonic acid from the plasma membrane is activated by Ca²⁺ (14). The enhanced PGF_{2α} responses by diabetes mellitus was not related to phospholipase A₂ because several Ca²⁺ antagonists did not change the PGF_{2α} responses. The above results suggest that the diabetic state in mesenteric blood vessels 1) may suppress cyclooxygenase, which is linked to the up-regulation of prostaglandin receptors, causing the enhancement of PGF_{2α} responses, and 2) may accelerate lipoxygenase, which is linked to the down-regulation of leukotriene receptors, reducing leukotriene responses. We previously have reported that leukotriene D₄-induced responses are rather reduced by diabetes mellitus in mouse mesenteric veins (2). The present results that cycloheximide and actinomycin D suppressed the enhanced PGF_{2α} responses support the above mechanism.

In conclusion, the diabetic mellitus-induced imbalance in the regulation of the eicosanoid metabolic pathways (suppressed cyclooxygenase and accelerated lipoxygenase) may cause the enhancement of PGF_{2α}-induced responses in small blood vessels.

Acknowledgments

We are grateful to Ms. Leonora Rivera Pancho and Ms. Yumiko Matsuura for their skillful technical assistance and thank Kaken Seiyaku for their kind gift of PGF_{2α}.

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