Diabetes-Induced Enhancement of Prostanoid-Stimulated Contraction in Mesenteric Veins of Mice
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Abstract—We investigated the influence of the diabetic state on the contractile response of longitudinal segments of isolated mesenteric vein to prostanoids and leukotriene (LT), and the contribution of the vascular endothelium to modulation of the contractile response was determined. The normal mesenteric vein and de-endothelialized veins of normal (ddY), diabetic KK-CAy and streptozotocin ddY mice (150 mg/kg, i.v., 6 weeks) were used. In the diabetic state, the contractions produced by noradrenaline (60 nM), high K+ solution (143.4 mM), and the thromboxane A2 analogue U-46619 (29 nM–29 mM) were not affected, and LTD4 (0.1 nM–1 μM)-induced contraction was suppressed. Contractions induced by prostaglandin (PG) E2 (0.2 μM–2 mM), PGF2α (0.3 μM–0.3 mM) and the prostacyclin derivatives PG12-Na (10–100 μM) and TRK-100 (0.2 μM–2 mM) were significantly enhanced in the presence of an intact vascular endothelium, but not in de-endothelialized segments. The increase in PGF2α (0.28 mM) contractions was dependent on age (correlation coefficient r=0.36, significant difference, P<0.05) and blood glucose (r=0.88, significant difference, P<0.01), but was independent of obesity. The contractile response to PGD2 (0.3–0.9 mM) was enhanced in both intact and de-endothelialized segments. These results indicate that the diabetic state affects prostanoid responses in an endothelium-dependent manner, except for the PGD2 response, which is independent of the endothelium.

Diabetes affects the properties of both the macro- and microvasculature, resulting in increased vascular permeability (1). As a consequence, vascular disease has been considered a complicating feature of the diabetic state (2). Recently, attempts to investigate the functions of vascular smooth muscle in diabetes mellitus were carried out using animal diabetes models, either genetically derived (3, 4) or chemically induced by alloxan (5–7) or streptozotocin (STZ, 8, 9) treatment. A significant decrease in prostacyclin production (10) and an increase in sensitivity to noradrenaline (NA, 11) have been reported in the aorta of diabetic mice and rats.

Evidence suggests that the endothelial cells modulate the responses of vascular smooth muscle to endogenous vasoconstrictor substances (12–14). The potentiation responses mentioned above were dependent on the endothelial cells. The present experiments were designed to investigate further the influence of diabetes on the responses of mesenteric vein preparations to prostanoids and leukotrienes in regards to the role of endothelial cells.

Materials and Methods
Normal (ddY, male, 7–8 week-old, 28–35 g), STZ (150 mg/kg, i.v.) treated (ddY, male, 4–12 weeks old, 24–40 g) and genetically KK-CAy (diabetic males: 24–35 weeks old, 26–40 g, female; 24–30 weeks old, 32–66 g, and prediabetic: 24–50 weeks old, 26–40 g) mice were used. The KK-CAy strain was bred in our laboratory by mating female KK mice with male KK-CAy mice (3). Animals with
blood glucose levels of less than 150 mg/dl (measured using a Beckman glucose analyzer) were considered to be normal (or pre-diabetic in the case of KK-CA\textsuperscript{+} mice), and those with glucose of more than 150 mg/dl considered to be diabetic.

The mice were killed by decapitation, and the mesenteric vein was immediately isolated and cleaned of adhering fat and connective tissues. The vein (10 mm long, 1 mm wide) was tied at both ends with silk threads and suspended in an organ bath (37°C) filled with normal Krebs solution (5 ml) of the following composition: 122 mM NaCl, 5.9 mM KCl, 15.5 mM NaHCO\textsubscript{3}, 1.2 mM MgCl\textsubscript{2}, 2.5 mM CaCl\textsubscript{2} and 11.5 mM glucose. The solution was continuously gassed with a mixture of 95% O\textsubscript{2} and 5% CO\textsubscript{2}. High K\textsuperscript{+} solution, 143.4 mM, was prepared by replacing NaCl with KCl and NaHCO\textsubscript{3} with KHC\textsubscript{2}O\textsubscript{3}. 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). A resting tension of 95 mg was applied in the control and diabetic veins. Changes in the isometric contractions were recorded in a linearorder (type WR 3701, Graphtec) equipped with a biophysiograph 180 system (San-ei) and an amplifier (6L5, San-ei). In some experiments, the endothelial cells were removed by opening the blood vessels and rubbing the inner surface with a cotton swab under a binocular microscope (15, 16). Removal of the vascular endothelium was confirmed by demonstration that the de-endothelialized segments pre-contracted by NA were not inhibited by acetylcholine (15, 16). The control blood vessels were de-endothelialized as well. The following compounds were used: PGD\textsubscript{2} (Funakoshi), PGE\textsubscript{2} (Fuji), TRK-100, PGF\textsubscript{2\alpha} (Kaken Seiyaku), PG\textsubscript{I\textsubscript{2}}-Na (Ono), U-46619 (Upjohn), LTD\textsubscript{4}-monomethylester (Paesel), NA (Sankyo), acetylcholine (Dai-ichi) and STZ (Sigma). PGE\textsubscript{2} and PGF\textsubscript{2\alpha} were dissolved in distilled water by brief sonication (UR-20P, Tomy Seiko) before use. TRK-100 and acetylcholine were also dissolved in distilled water, but were not sonicated. PGD\textsubscript{2} and PG\textsubscript{I\textsubscript{2}}-Na were dissolved in 0.1% ethanol. U-46619 was supplied in methylacetate, which was first removed by vacuum before it was dissolved in 0.1% ethanol at the required concentration. LTD\textsubscript{4} monomethylester was first hydrolyzed to LTD\textsubscript{4} using K\textsubscript{2}CO\textsubscript{3} (10 mg/0.2 ml distilled water) overnight at room temperature before use. STZ was dissolved in normal saline (0.9% NaCl) solution.

The contractile responses were expressed in terms of absolute tension (mg) or converted to percent of the maximal response. Statistical analyses were performed using the unpaired Student's t-test. Analysis of variance (Studentized range test) was used when comparing the different groups.

Results

Comparison of contractile response to K\textsuperscript{+}, noradrenaline and PGE\textsubscript{2\alpha} in KK-CA\textsuperscript{+} diabetes:
The maximum absolute contractions (mg tensions) to high K\textsuperscript{+} solution (143.4 mM), PGE\textsubscript{2\alpha} (0.28 mM) and NA (0.06 mM) were compared in mesenteric veins of normal (ddY), pre-diabetic and diabetic KK-CA\textsuperscript{+} mice (Fig. 1). These concentrations induced the maximal responses in the concentration-response curves (data not shown except that for PGE\textsubscript{2\alpha}). The tensions generated by high K\textsuperscript{+} solution and NA were not significantly different in any of the three preparations. However, the PGE\textsubscript{2\alpha}-induced contraction was significantly enhanced 1.5-fold in the diabetic state (Fig. 1).

In KK-CA\textsuperscript{+} male mice, the blood glucose
level increased progressively with increasing body weight and advancing age (17). Data regarding the influence of age were obtained for the PGF2α-induced contractions, and simultaneously, the blood glucose levels were measured. These three parameters were plotted in three dimensions, and the correlation coefficient was generated. The contractile responses to PGF2α were correlated with age and blood glucose levels in KK-CAY mice (Fig. 2). A blood glucose-dependent increase in PGF2α contraction was observed with a correlation coefficient (r)=0.88 (P<0.01). Similarly, the responses to PGF2α progressively increased with advancing age (r=0.36, P<0.05).

The contractile responses to the cumulatively added PGF2α (0.3 μM–0.3 mM) were compared in pre-diabetic male (blood glucose (BG) level: 134±5 mg/dl, body weight: 31.6±1.9 g) and non-diabetic obese female (BG level: 121±5 mg/dl, body weight: 44.3±3.9 g) KK-CAY mice. Both groups of mice were in the same age range (24–35 weeks) and did not show any significant differences in PGF2α response, although diabetic obese male mice (BG level: 314±24 mg/dl, 37.9±0.7 g) showed a significant increase in PGF2α response (Fig. 3).

Prostaglandin F2α response in the streptozotocin-diabetic state: Figure 4 shows the concentration-response relationship to PGF2α at various times after the injection of STZ.
third (126±3 mg/dl) day after STZ treatment, 
PGEF2α-induced contractions noticeably in-
creased, whereas blood glucose levels still 
remained in the normal range. At the start of 
the second week (399±11 mg/dl) and fourth 
week (382±16 mg/dl), marked hyper-
glycemia was observed in all mice, but there 
was no significant difference in PGF2α-
induced contractions compared to day 3 after 
injection. At 6 (456±24 mg/dl) and 8 (481 ± 
15 mg/dl) weeks after injection, the absolute 
maximal tensions progressively increased in 
parallel with blood glucose levels and with 
the duration of diabetes. The responses at a 
higher range of PGF2α concentrations in 
mice 6–8 weeks after STZ administration 
were significantly different from those in mice 
3 days after STZ administered. The curves at 
8 weeks were shifted to the left as compared 
with those at 6 weeks.

Comparison of various prostanoids, leuko-
triene and thromboxane responses in the 
streptozotocin-diabetic state: The intact mes-
enteric veins of normal (Fig. 5a) and STZ-
diabetic (Fig. 5b) ddY mice contracted when 
exposed to increasing concentrations of PG 
(D2, 0.3–0.9 mM; E2, 0.2 μM–2 mM; F2α, 0.3 
μM–0.3 mM), prostacyclins (PGI2-Na, 10–
Table 1. Diabetic state-induced alteration of contracting tension (mg) to eicosanoids in mesenteric veins of mice

| Compounds (mM) | Normal | Diabetic | Ratioa |
|----------------|--------|----------|--------|
| PGE2 (2.8)     | 4.0±1.2| 5.7±0.8* | 1.4    |
| PGF2α (0.28)   | 6.0±0.4| 8.7±0.9* | 1.5    |
| PGI2-Na (0.27) | 4.9±0.7| 9.0±1.4* | 1.8    |
| TRK-100 (0.24) | 2.4±0.1| 3.8±0.6* | 1.6    |
| PGD2 (0.28)    | 0.5±0.1| 1.7±0.4* | 3.4    |
| U-46619 (0.029)| 15.3±1.5| 14.7±1.5|        |
| LTD4 (0.001)   | 18.7±0.8| 8.6±1.3**| 0.5    |

The values are the means±S.E.M. (n=3-10). Significant differences from the normal values at *P<0.05 and **P<0.01 in diabetic mice (streptozotocin, 6-week elapsed) by the unpaired t-test. aRatio of the response of the diabetic vein to that of the normal vein, for each compound.

Table 2. Effects of de-endothelialization of mouse mesenteric veins on prostaglandin D2−, F2α−, U-46619- and leucotriene D4−induced contracting tension (mg)

| Compounds (mM) | unrubbed | Normal rubbed | Ratioa | unrubbed | Diabetic rubbed | Ratioa |
|----------------|----------|---------------|--------|----------|-----------------|--------|
| PGF2α (0.28)   | 6.9±0.8  | 7.1±1.4       | 1.2    | 8.7±0.9  | 5.9±0.4*        | 0.7    |
| PGD2 (0.28)    | 0.5±0.1  | 0.8±0.2       | 1.7    | 1.7±0.4  | 1.5±0.1         | 0.1    |
| U-46619 (0.029)| 14.7±1.2| 9.4±1.5*      | 0.6    | 14.7±1.5| 9.1±0.8*        | 0.6    |
| LTD4 (0.001)   | 18.7±0.8| 16.1±1.3      |        | 8.6±1.3  | 10.1±0.8        |        |

The values are the means±S.E.M. (n=3-10). Significance differences from the values of the un rubbed veins at *P<0.05 by the unpaired t-test. aRatio of the response of the de-endothelialized vein to that of the vein with intact endothelium, for each compound. bFrom streptozotocin treated mice (6-week elapsed).

100 μM; TRK-100, 0.2 μM−2 mM), the thromboxane (TX) A2 stable analogue U-46619 (29 nM−29 μM) and LT (D4, 0.1 nM−1 μM). The mice had been rendered diabetic by injection of STZ to 4 weeks-old mice. The maximum absolute tension elicited by each eicosanoid, except for PGD2 and TRK-100 (Fig. 5b, Table 1), was compared in normal and diabetic mesenteric veins. In the diabetic state, the absolute tensions in response to prostanoids were all enhanced, whereas those produced by LTD4 were reduced. No significant change was observed in contractions induced by U-46619.

The responses to eicosanoid were compared in normal and diabetic mesenteric veins; data were obtained for both veins with intact vascular endothelium and de-endothelialized ones (Table 2). The mesenteric veins from either normal or diabetic mice pre-contracted with NA (0.6 μM) were similarly inhibited with acetylcholine (60 μM), provided the endothelium was intact. Loss of inhibition was observed after rubbing the inner surface of the vein to remove the endothelial cells (data not shown).

In mesenteric veins of normal mice, removal of endothelial cells did not affect the contractions induced by both PGF2α (0.28 mM), PGD2 (0.28 mM) and LTD4 (1 μM), and it significantly reduced the responses to U-46619 (29 μM) (Table 2). In diabetic mesenteric veins, removal of endothelial cells reduced the responses to PGF2α (0.28 mM) and U-46619 (29 μM), and it did not affect those to PGD2 (0.28 mM) and LTD4 (1 μM). The responses to PGE2 (2.8 mM), PGI2-Na (0.27 mM) and TRK-100 (0.24 mM) were also significantly reduced by de-endothelialization, where the response ratios of rubbed muscles to those of un rubbed muscles were 0.6, 0.4 and 0.4, respectively.

**Discussion**

We chose the mesenteric veins of diabetic male KK-CAT mice and STZ-mice as a diabetic disease model in the present study because the vein is an important blood vessel
that controls the blood flow in the liver together with the portal vein and because we have investigated a number of fundamental properties in the vascular smooth muscles of the above mice (17–20). The reactivity of vascular smooth muscles in diabetes is influenced by the duration of the diabetic state, the length of time that these animals were diabetic before sacrifice, body weight, and the cross-sectional areas of the control and diabetic veins. Severe hyperglycemia was observed in male KK-CA° mice, but not in females (3). The positive correlation between blood glucose levels and PGF$_{2\alpha}$-induced contraction is complicated by the positive correlation between age and the enhancement of PGF$_{2\alpha}$ contraction. However, the same phenomena were also observed in STZ-diabetic mice (6- and 8-week elapsed after STZ injection) as compared with age-matched mice. Obesity was noted in both males and females of the KK-CA° strain. The enhancement of PGF$_{2\alpha}$ response was independent of obesity because it was not observed in KK-CA° female mice without diabetes.

The injection of STZ significantly increased the contractile response to PGF$_{2\alpha}$ 3 days after injection, despite unaltered levels of blood glucose. At the start of the second week, the absolute tension in response to PGF$_{2\alpha}$ decreased, but this was not significant as compared to that obtained on day 3 after injection. After 6 and 8 weeks of STZ administration, PGF$_{2\alpha}$-induced contractions increased significantly, and this was dependent on blood glucose levels and the duration of diabetes. These results indicate that STZ not only induced the later development of diabetes but also exerted a direct initial effect on the mesenteric veins of mice. STZ at doses inducing no hyperglycemia has been reported to increase blood pressure in rats (21). STZ has been reported to be a powerful activator of guanylate cyclase in many rat tissues (22) and to elicit directly the vasodilation in rat aortic rings (23). No simple relationship between blood glucose levels and PGF$_{2\alpha}$ responses was found in STZ-administered mice. The initial effects of STZ may be caused by its direct action on blood vessels, and the later effects may be caused by the diabetic state.

Aside from PGF$_{2\alpha}$, other prostanoids and leukotrienes also contracted the intact mesenteric veins of mice in both the normal and diabetic states. This demonstrates the presence of receptors for each eicosanoid in the venous smooth muscle (24). The influence of diabetes on the synthesis of prostanoids has been reported, suggesting decreased prostacyclin production and increased TX synthesis (10, 25). In the present study, a significant increase in contractions by the diabetic state was observed only in the case of PGs (except for PGD$_2$) because the responses to high K$^+$ solution, the maximal concentration of NA, and TXA$_2$ were not significantly different in the veins of normal and diabetic mice. The response to LT was rather decreased by diabetes. Therefore, the above enhancement by diabetes was restricted to PGs related to the cyclooxygenase system.

The sensitivity of the mesenteric vein preparations to PGD$_2$ was increased in diabetic mice, whereas that of LTD$_4$ was decreased, independent of an intact vascular endothelium. The change in the responses of above the two eicosanoids may be due to an alteration of their receptors.

The contractions in response to PGF$_{2\alpha}$, PGE$_2$, PGD$_2$, PGI$_2$-Na, TRK-100 in both normal and diabetic vascular muscle were dependent on the presence of an intact vascular endothelium. The response to LTD$_4$ was independent, and the response to U- 46619 (a TXA$_2$ stable analogue) was dependent on endothelial cells in both normal and diabetic muscles. In the diabetic state, increases in absolute tension in response to PGs (except for PGD$_2$) were dependent on an intact vascular endothelium. However, diabetes had no influence on TXA$_2$-induced contractions. It has been shown that in isolated coronary arteries, hypoxia and anoxia cause the endothelial cells to release vasoconstrictor substances that augment the contractile response to PGF$_{2\alpha}$ (14, 26). The present study suggests that diabetes may accelerate the release of a constrictor substance from the endothelial cells of venous smooth muscles, which may then diffuse to adjacent vascular smooth muscle and enhance
the contractile response to PGs. The effect of diabetes on the release of EDRF from the arteries has been investigated (27). Venous smooth muscles in general do not release or do not respond to EDRF. The present study, however, suggests that the endothelium of diabetic mouse mesenteric vein apparently releases some vasoactive substances, although we have not yet identified the vasoconstrictor substance released from the endothelial cells in the diabetic state.

Recently, the attenuation of endothelium-dependent relaxation has been reported in the aorta from diabetic rats (28). The reasons for the different influences of the diabetic state on the types of vessels from which the responses to eicosanoids were obtained remain to be elucidated.

We concluded that the enhancement of prostanoid (except for PGD2)-induced contraction in mesenteric veins is related to the severity of the diabetic state and is endothelial cell-dependent, although diabetes apparently has no influence on the response to high K+, NA and TXA2. The contractile responses to PGD2 were enhanced, whereas those produced by LTD4 were reduced, independent of the presence of an intact vascular endothelium.

References

1 Vandana, H. and Brecher, P.: Glucose and fatty acid metabolism in normal and diabetic rabbit cerebral microvessels. Am. J. Physiol. 252, E648–E653 (1987)
2 Longhurst, P.A. and Head, R.J.: Responses of the isolated perfused mesenteric vasculature from diabetic rats: The significance of appropriate control tissues. J. Pharmacol. Exp. Ther. 235, 45–49 (1985)
3 Kimura, M., Suzuki, J. and Amemiya, K.: A genetically diabetic model "KK-CAY" for a pharmacological assay. Endocrinol. Japon. 26, 185–196 (1979)
4 Rosenblum, W.I., El-Sabban, F. and Loria, R.M.: Platelet aggregation in the cerebral and mesenteric microcirculation of mice with genetically determined diabetes. Diabetes 30, 89–92 (1981)
5 Fortes, Z.B., Garcia Leme, J. and Scivoletto, R.: Influence of diabetes on the reactivity of mesenteric microvessels to histamine, bradykinin and acetylcholine. Br. J. Pharmacol. 78, 39–48 (1983)
6 Fortes, Z.B., Garcia Leme, J. and Scivoletto, R.: Vascular reactivity in diabetes mellitus: Role of the endothelial cell. Br. J. Pharmacol. 79, 771–781 (1983)
7 Illorach, M.A.S., Böhm, G.M. and Garcia Leme, J.: Decreased vascular reactions to permeability factors in experimental diabetes. Br. J. Exp. Pathol. 57, 747–754 (1976)
8 Harrison, H.E., Reece, A.H. and Johnson, M.: Decreased vascular prostacyclin in experimental diabetes. Life Sci. 23, 351–356 (1978)
9 Ramanadham, S., Lyness, W.H. and Tenner, T.E., Jr.: Alterations in aortic and tail artery reactivity to agonists after streptozotocin treatment. Can. J. Physiol. Pharmacol. 62, 418–423 (1984)
10 Rosenblum, W.I. and Hirsh, P.D.: Some interrelationships between glucose levels, thromboxane production and prostacyclin production in normal and diabetic mice. Prostaglandins 27, 111–118 (1984)
11 MacLeod, K.M. and McNeill, J.H.: The influence of chronic experimental diabetes on contractile responses of rat isolated blood vessels. Can. J. Physiol. Pharmacol. 63, 52–57 (1985)
12 Hickey, K.A., Rubanyi, G., Paul, R.J. and Highsmith, R.F.: Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. Am. J. Physiol. 248, C550–C566 (1985)
13 Miller, V.M. and Vanhoutte, P.M.: Endothelium-dependent contractions to arachidonic acid are mediated by products of cyclooxygenase. Am. J. Physiol. 248, H432–H437 (1985)
14 Vanhoutte, P.M., Rubanyi, G.M., Miller, V.M. and Houston, D.S.: Modulation of vascular smooth muscle contraction by the endothelium. Annu. Rev. Physiol. 48, 307–320 (1986)
15 De Mey, J.G. and Vanhoutte, P.M.: Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. J. Physiol. (Lond.) 316, 347–355 (1981)
16 Furchgott, R.F. and Zawadzki, J.V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288, 373–376 (1980)
17 Amemiya, K., Suzuki, J. and Kimura, M.: Mechanism of action of pronase on chronic proliferative inflammation including granuloma angiogenesis in mice. Folia Pharmacol. Japon. 88, 279–288 (1985) (Abs. in English)
18 Kimura, M., Suzuki, J., Amemiya, K. and Yamada, T.: Involvement of glucocorticoid in insulin-induced angiogenesis of adjuvant pouch granuloma in diabetic mice. J. Pharmacobiodyn. 10, 266–271 (1987)
19 Kimura, M., Amemiya, K. and Suzuki, J.: Insulin-induced granuloma tissue formation and angiogenesis in alloxan-treated diabetic mice. Endocrinol. Japon. 34, 55–63 (1987)  
20 Kimura, I., Matsui, T. and Kimura, M.: Increase in basal pulse rate and blood pressure by the diabetic state in KK-CAY mice, alloxan-mice and streptozotocin-mice. Japan. J. Pharmacol. 46, 93–96 (1988)  
21 Kawashima, H., Igarashi, T., Nakajima, Y., Akiyama, Y., Usuki, K. and Ohtake, S.: Chronic hypertension induced by streptozotocin in rats. Naunyn Schmiedebergs Arch. Pharmacol. 305, 123–126 (1978)  
22 Vesely, D.L., Graves, W.R. and Lo, T.M.: Isolation of a guanylate cyclase inhibitor from the Balsam pear (Momordica charantia abreviata). Biochem. Biophys. Res. Commun. 77, 1294–1299 (1977)  
23 Thomas, G. and Ramwell, P.W.: Streptozotocin: A nitric oxide carrying molecule and its effect on vasodilation. Eur. J. Pharmacol. 161, 279–280 (1989)  
24 Kimura, I., Kimura, M. and Pancho, L.R.: Modulation of eicosanoid-induced contraction of mouse and rat blood vessels by gingerols. Japan. J. Pharmacol. 50, 253–261 (1989)  
25 Jeremey, J.Y., Mikhailidis, D.P. and Dandona, P.: Simulating the diabetic environment modifies in vitro prostacycin synthesis. Diabetes 32, 217–221 (1983)  
26 Rubanyi, G.M. and Vanhoutte, P.M.: Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. J. Physiol. (Lond.) 364, 45–56 (1985)  
27 Harris, K.H. and MacLeod, K.M.: Influence of the endothelium on contractile responses of arteries from diabetic rats. Eur. J. Pharmacol. 153, 55–64 (1988)  
28 Oyama, Y., Kawasaki, H., Hattori, Y. and Kanno, M.: Attenuation of endothelium dependent relaxation in aorta from diabetic rats. Eur. J. Pharmacol. 132, 75–78 (1986)