Biphasic Vasodilator Action of Troglitazone on the Renal Microcirculation

SHUJI ARIMA, KENTARO KOHAGURA, KAZUHISA TAKEUCHI, YOSHIHIRO TANIYAMA, AKIRA SUGAWARA, YUKIO IKEDA, MICHIAKI ABE, KEN OMATA, and SADAYOSHI ITO

Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University School of Medicine, Sendai, Japan.

Abstract. Recent studies have demonstrated that thiazolidinediones, novel antidiabetic compounds that improve the insulin sensitivity, lower BP and decrease urinary protein excretion. However, neither the target vasculature nor the underlying mechanism for their actions is well understood. In this study, the action of troglitazone (Tro), a thiazolidinedione compound, on the glomerular afferent (Af-Arts) and efferent (Ef-Arts) arterioles, crucial vascular segments to the control of glomerular hemodynamics, were directly examined. Rabbit Af-Arts and Ef-Arts were microdissected from the superficial cortex and perfused at constant pressure. Increasing doses of Tro (10^-8 to 10^-5 M) were added to both the bath and lumen of preconstricted arterioles. In Af-Arts, Tro caused dose-dependent and biphasic dilation. Tro at 10^-5 M increased the diameter by 28 ± 6% (n = 8, P < 0.01) until 20 min, with the diameter remaining at this level for 60 min, and then Tro began to dilate Af-Arts again. At 120 min, Tro at 10^-5 M further increased the diameter by 23 ± 4% (n = 6). Disrupting the endothelium had no effect on either dilation (n = 7 or n = 5). Pretreatment with SKF 96365 (50 μM), which inhibits both voltage- and receptor-operated calcium channels, abolished the early-phase dilation without affecting the late-phase dilation; 20 or 120 min after adding Tro at 10^-5 M, the diameter increased by 4 ± 2% (n = 7) or 28 ± 3% (n = 6), respectively. In contrast to Af-Arts, Tro caused monophasic dilation in Ef-Arts; Tro at 10^-5 M did not cause significant dilation until 80 min, and at 120 min the diameter increased by 37 ± 4% (n = 5). These results suggest that in the Af-Art Tro has biphasic endothelium-independent vasodilator action, which is partly mediated by an inhibition of calcium influx. This vasodilator action may play a role in the BP-lowering effect of Tro. In addition, by dilating the postglomerular Ef-Art, Tro may decrease the glomerular capillary pressure and hence the excretion of urinary protein.

There is increasing evidence that insulin resistance is a common feature of several frequent disorders such as non–insulin-dependent diabetes mellitus, obesity, atherosclerosis, and essential hypertension (1–3). Thus, many patients who have these diseases would be candidates for treatment with the thiazolidinediones (TZD), novel antidiabetic compounds that improve the insulin sensitivity (4,5). Indeed, besides their antidiabetic effects, TZD have been shown to lower BP in diabetic patients with hypertension (6–8) and to decrease albuminuria in diabetic rats (9,10) and patients with diabetic nephropathy (11). Although improvement of insulin resistance is proposed to be most responsible for these actions of TZD, direct vascular effect may also contribute to them, because several studies have demonstrated their vasodilator action in both experimental animals (12–14) and humans (15,16). In addition, Isshiki et al. (17) recently demonstrated that TZD ameliorate glomerular hyperfiltration in streptozotocin-induced insulin-deficient diabetic rats. Taken together, these studies suggest the possibility that TZD may exert their hypotensive or renoprotective action partly through decreasing the peripheral vascular resistance or the glomerular capillary pressures (Pgc), respectively. However, neither the target vasculature nor the underlying mechanism for these actions is well understood.

In this study, we directly examined the action of troglitazone (Tro), a TZD compound, on the renal arterioles. Thus, we isolated and microperfused rabbit afferent (Af-Arts) and efferent (Ef-Arts) arterioles, crucial vascular segments to the control of glomerular hemodynamics. We examined whether Tro causes vasodilation in these arterioles, and if so, the mechanism involved in the vasodilation.

Materials and Methods
Isolation and Microperfusion of the Rabbit Af-Arts and Ef-Art

This study was performed in accordance with the Guide for Animal Experimentation, Tohoku University School of Medicine. We used methods similar to those described elsewhere (18–20) to isolate and microperfuse Af-Arts and Ef-Arts. Briefly, young male New Zealand white rabbits (1.5 to 2.0 kg body wt), fed standard rabbit chow and tap water ad libitum, were anesthetized with intravenous sodium pentobarbital (40 mg/kg) and given an intravenous injection of heparin (500
U). The kidneys were removed and sliced along the corticomedullary axis. Slices were placed in ice-cold minimum essential medium (Life Technologies BRL, Gaithersburg, MD) that contained 5% bovine serum albumin (Sigma Chemical, St. Louis, MO) and were microdissected under a stereomicroscope (SZH-10; Olympus, Tokyo, Japan), as described elsewhere. From each rabbit, a single superficial AF-Art and/or EF-Art with its glomerulus intact was microdissected. By use of a micropipette, the arteriole was transferred to a temperature-regulated chamber mounted on an inverted microscope (IMT-2; Olympus) with Hoffman modulation. The arteriole was cannulated with an array of glass pipettes as described elsewhere (18–20) and perfused with oxygenated medium 199 (Life Technologies BRL) that contained 5% bovine serum albumin. Intraluminal pressure was measured by Landis’ technique. A fine pipette was introduced into the arteriole through the perfusion pipette and was maintained at 60 mmHg in the case of AF-Arts. For EF-Art perfusion, an AF-Art was microdissected together with the glomerulus and attached EF-Art (250 to 300 μm in length). The AF-Art was cut short (~50 μm) and cannulated as described above, except that the perfusion pipette was advanced to the end of the AF-Art. The tip of the pressure pipette was placed just beyond the distal end of the AF-Art, and intraluminal pressure at this point was maintained at 50 mmHg throughout the experiment so as to eliminate the hemodynamic influences of the AF-Art (19,20). In a study elsewhere (19), we found that pressure in the EF-Art at a point 50 μm distal to the glomerulus was ~35 mmHg, the physiologic level, under these experimental conditions.

The bath was identical to the arteriolar perfusate, except that it contained 0.1% bovine serum albumin and was exchanged continuously. Microdissection and cannulation of the arteriole were completed within 90 min at 8°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. When the temperature was stable, a 30-min equilibration period was allowed before any measurements were taken. Images of the arteriole were displayed at magnifications up to ×1980 and recorded with a video system that consisted of a camera (CS520MD; Olympus), monitor (PVM1445MD; Sony, Tokyo, Japan), and video recorder (HR-S101; Victor, Tokyo, Japan). Using Hoffman modulation, we achieved a resolution of 0.6 μm with the ×40 objective lens. The diameter at the most responsive point was measured with an image-analysis system (VM-30; Olympus).

Experimental Protocols

Dose-Dependent Vasodilator Action of Tro on Preconstricted AF-Arts or EF-Arts. On the day of the experiment, a fresh solution that contained 10⁻² M Tro ([±]-5-14-(6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy)benzyl)-2,4-thiazolidinedione; Sankyo Co., Tokyo, Japan) or its vehicle was prepared in saline with 0.01% DMSO (Sigma), respectively. We first examined whether Tro causes dose-dependent vasodilation in renal arterioles. For this, AF-Arts or EF-Arts were preconstricted with norepinephrine (NE), because isolated arterioles have little intrinsic tone, making it difficult to observe their possible dilator responses. After the equilibration period, AF-Arts or EF-Arts were preconstricted by ~40% with NE (0.5 to 1.0 μM, Sigma), and then increasing doses of Tro (10⁻⁸ to 10⁻⁵ M) or its vehicle were added to both the bath and arteriolar perfusate. Luminal diameter was measured immediately before the addition of Tro (or its vehicle) and observed for 20 min at each dose. In a study elsewhere (21), we have demonstrated that NE at this concentration causes stable and sustained constriction of rabbit AF-Arts for >3 h (which is much longer than is needed to complete any experimental protocol performed in this study) in our experimental conditions.

Time-Dependent Vasodilator Action of Tro on Preconstricted AF-Arts or EF-Arts. We next examined the longer effect of 10⁻⁵ M Tro, because the plasma concentration of Tro in patients who receive Tro (although it is not available for clinical use now) maintains the μM level (at ~2 to 3 μM) for a long time (22). After the equilibration period, AF-Arts or EF-Arts were preconstricted by ~40% with NE, and then effects of 10⁻⁵ M Tro (or its vehicle) on the luminal diameter were observed for 120 min. Luminal diameter was measured every 20 min.

Effect of Tro on Angiotensin II (AngII) Action in AF-Arts. We found that Tro at the μM level causes significant vasodilation in NE-preconstricted renal arterioles (see the Results section). To exclude the possibility that Tro specifically attenuates NE-induced vasoconstriction rather than causing vasodilation in renal arterioles, we next examined whether Tro attenuates AngII-induced vasoconstriction in AF-Arts. Because AngII (at least at high concentrations) causes a transient vasoconstriction in AF-Arts under our experimental conditions (18), preconstriction induced by AngII may not persist long enough for the experiment. Thus, we examined the effect of Tro pretreatment on AngII-induced vasoconstriction rather than examining the action of Tro in AngII-preconstricted AF-Arts. After the equilibration period, 10⁻³ M Tro or its vehicle was added to both the bath and arteriolar perfusate. Twenty or 120 min later, increasing doses of AngII (10⁻¹¹ to 10⁻⁸ M, Sigma) were added to both the bath and arteriolar perfusate. The luminal diameter was measured immediately before the addition of AngII and observed for at least 10 min at each dose.

Effect of the Endothelium Disruption on Tro-Induced Dilation in AF-Arts. We next examined the possible contribution of the endothelium to Tro-induced dilation in AF-Arts. After the equilibration period, AF-Arts were perfused for 10 min with perfusate that contained both 2% guinea pig complements (Sigma) and antibodies against human factor VIII–related antigen (14.29 mg/ml, Atlantic Antibodies, Stillwater, MN). This was followed by a 20-min washout period during which AF-Arts were perfused with perfusate that contained neither antibodies nor complements. We have demonstrated elsewhere that this treatment selectively disrupts endothelial cells without altering the function of vascular smooth-muscle cells (VSMC) (23). After disrupting the endothelium, AF-Arts were preconstricted by ~40% with NE, and the dose-dependent or time-dependent vasodilator action of Tro were examined as in protocol 1 or 2, respectively. At the end of each experiment, we confirmed that AF-Arts did not dilate in response to acetylcholine (10 μM, Sigma), an endothelium-dependent vasodilator.

Effect of Calcium-Channel Blockade on Tro-Induced Dilation in AF-Arts. Kawasaki et al. (14) recently reported that Tro dilates coronary artery by inhibiting the calcium (Ca²⁺) influx to VSMC. Thus, we next examined the possible contribution of Ca²⁺ influx through the receptor- or voltage-operated Ca²⁺ channels to the Tro-induced vasodilation in AF-Arts. After the equilibration period, AF-Arts were treated with SKF 96365 (50 μM, BIOMOL, Plymouth Meeting, PA), an inhibitor of receptor- and voltage-operated Ca²⁺ channels (24). Thirty minutes later, AF-Arts were preconstricted by ~40% with NE, and the dose-dependent or time-dependent vasodilator action of Tro were examined as in protocol 1 or 2, respectively.

Statistical Analyses

Values were expressed as mean ± SEM, and all statistical analyses were carried out with absolute values. Paired t test was used to examine whether the diameter at a given concentration differed from the control or preconstricted value within each group. ANCOVA was
used to examine whether dose-response curves differed between groups, and a two-sample t test was used to examine whether the change in diameter at a given concentration differed between groups. \( P < 0.0125 \) (0.05/4) was considered significant, with the use of Bonferroni’s adjustment for multiple comparisons.

**Results**

**Dose-Dependent Vasodilator Action of Tro on Preconstricted Af-Arts or Ef-Arts**

NE decreased luminal diameter of Af-Arts or Ef-Arts from 17.3 ± 0.5 or 15.6 ± 0.6 μm to 9.9 ± 0.4 (n = 8) or 9.8 ± 0.7 (n = 5) μm, respectively. As shown in Figure 1, Tro caused dose-dependent dilation in Af-Arts; significant dilation was observed from 10\(^{-6}\) M, which began to cause dilation from 10 min, and the diameter increased by 2.0 ± 0.5 μm (or 19 ± 5%, \( P < 0.01 \)) at 20 min. Tro at 10\(^{-5}\) M also began to cause dilation from 10 min, and the diameter increased by 2.7 ± 0.6 μm (or 28 ± 6%) at 20 min. In contrast, when observed for 20 min at each dose, Tro did not cause any dilation in Ef-Arts; the dilation from 10 min, and the diameter increased by 2.0 ± 0.5 μm, respectively. At 10 min, the diameter increased by 2.7 ± 0.6 μm (or 28 ± 6%) at 20 min. In contrast, when observed for 20 min at each dose, Tro did not cause any dilation in Ef-Arts; the change in luminal diameter induced by Tro at 10\(^{-5}\) M was 0.7 ± 0.3 μm (at 20 min). We confirmed that Tro vehicle had no effect on the luminal diameter of preconstricted Af-Arts (n = 5) or Ef-Arts (n = 3), either.

**Time-Dependent Vasodilator Action of Tro on Preconstricted Af-Arts or Ef-Arts**

NE decreased luminal diameter of Af-Arts or Ef-Arts from 18.2 ± 0.8 or 15.4 ± 0.5 μm to 9.9 ± 0.3 (n = 6) or 9.9 ± 0.5 (n = 5) μm, respectively. As in the case observed in protocol 1, 10\(^{-5}\) M Tro increased the diameter of Af-Arts by 2.1 ± 0.5 μm (or 21 ± 4%, \( P < 0.01 \)) at 20 min, and the diameter remained at this level until 80 min (Figure 2). Thereafter, Tro began to cause dilation again, and at 100 or 120 min (compared with that observed at 20 min), the diameter increased further by 2.3 ± 0.7 (or 21 ± 7%) or 2.8 ± 0.4 μm (or 23 ± 4%), respectively. At 120 min, the luminal diameter of Af-Arts reached 14.8 ± 0.6 μm (4.8 ± 0.5 μm or 48 ± 4% increase, compared with the preconstricted level). When we gave Tro >120 min, no more dilation was observed (n = 4). In Ef-Arts, 10\(^{-5}\) M Tro began to cause significant (\( P < 0.01 \)) dilation from 80 min, and the diameter increased by −3.6 ± 0.3 μm (or 37 ± 4%) at 120 min. Thus, it became clear that therapeutic concentrations of Tro have biphasic or monophasic vasodilator action on the Af-Arts or Ef-Arts, respectively. When observed for 120 min, Tro vehicle had no effect on the luminal diameter of preconstricted Af-Arts (n = 3) or Ef-Arts (n = 3), either.

**Effect of Tro on AngII Action in Af-Arts**

In vehicle-treated Af-Arts (basal diameter 17.1 ± 0.7 μm; n = 6), AngII caused dose-dependent constriction; significant constriction was observed from 10\(^{-11}\) M, and the diameter decreased by 12.2 ± 0.8 μm (71 ± 6%) at 10\(^{-8}\) M. Pretreatment with 10\(^{-5}\) M Tro for 20 or 120 min did not affect basal luminal diameter; the diameter before and after the treatment was 17.2 ± 0.4 and 17.3 ± 0.4 μm (n = 8) or 18.1 ± 0.5 and 18.3 ± 0.4 μm (n = 5), respectively. However, as shown in Figure 3, pretreatment with Tro significantly (\( P < 0.01 \)) attenuated vasoconstrictor action of AngII on Af-Arts; AngII at 10\(^{-8}\) M decreased the diameter only by 8.6 ± 1.4 (50 ± 8%) or 6.2 ± 1.1 μm (34 ± 6%) in Af-Arts treated with Tro for 20 or 120 min, respectively. We also confirmed that pretreatment with 10\(^{-5}\) M Tro for 120 min significantly (\( P < 0.01 \)) attenuates vasoconstrictor action of 10\(^{-8}\) M AngII on Ef-Arts at (n = 4).

**Effect of Endothelium Disruption on Tro-Induced Dose-Dependent Dilation in Af-Arts**

Treatment with complements and antibodies against factor VIII–related antigen did not alter luminal diameter of Af-Arts,
versus vehicle-treated arterioles. In vehicle-treated afferent arterioles, angiotensin II caused dose-dependent constriction. Pretreatment with 10^{-5} M Tro for 20 or 120 min significantly attenuated vasoconstrictor action of angiotensin II. ○, vehicle-treated arterioles; ●, arterioles treated with 10^{-5} M Tro for 20 min; and ▲, arterioles treated with 10^{-5} M Tro for 120 min. P < 0.01 versus vehicle-treated arterioles.

possible mechanisms for which have been discussed elsewhere (23); the diameter before and after the treatment was 17.0 ± 0.4 and 16.9 ± 0.6 μm, respectively (n = 7). As reported elsewhere (23), disruption of the endothelium with this procedure did not affect vasoconstrictor action of NE added to the bath; NE decreased the diameter to 10.3 ± 0.3 μm, a level similar to that observed in protocol 1. As shown in Figure 4, disruption of the endothelium had no effect on Tro-induced dose-dependent dilation in Af-Arts. In these arterioles, Tro (added for 20 min at each dose) began to cause significant dilation from 10^{-6} M (2.0 ± 0.4 μm or 20 ± 4%), and the diameter increased by 2.7 ± 0.6 μm (27 ± 6%) at 10^{-5} M.

Figure 3. Effect of Tro pretreatment on the vasoconstrictor action of angiotensin II in afferent arterioles. In vehicle-treated afferent arterioles, angiotensin II caused dose-dependent constriction. Pretreatment with 10^{-5} M Tro for 20 or 120 min significantly attenuated vasoconstrictor action of angiotensin II. ○, vehicle-treated arterioles; ●, arterioles treated with 10^{-5} M Tro for 20 min; and ▲, arterioles treated with 10^{-5} M Tro for 120 min. # P < 0.01 versus luminal diameter before the addition of angiotensin II. * P < 0.05, ** P < 0.01 versus vehicle-treated arterioles.

Effect of Endothelium Disruption on Tro-Induced Time-Dependent Dilation in Af-Arts

The diameter before and after the treatment with complements and antibodies against factor VIII–related antigen was 18.1 ± 0.7 and 17.1 ± 0.5 μm, respectively (n = 5). NE decreased the diameter to 10.3 ± 0.6 μm, a level similar to that observed in protocol 2. As shown in Figure 5, disruption of the endothelium had no effect on Tro-induced time-dependent dilation in Af-Arts, either. In these arterioles, 10^{-5} M Tro increased the diameter of Af-Arts by 1.9 ± 0.4 μm (or 20 ± 4%, P < 0.01) at 20 min, and the diameter remained at this level until 80 min. Thereafter, Tro began to cause dilation again, and at 120 min the diameter increased further, by 2.8 ± 0.7 μm (or 23 ± 6%), compared with that observed at 20 min.

Figure 4. Effect of endothelial disruption (De-Endo) on Tro-induced dose-dependent vasodilation in preconstricted afferent arterioles. Endothelium disruption with antibodies against factor VIII–related antigen and complements had no effect on Tro-induced dose-dependent dilation. ○, nontreated arterioles; ●, De-Endo arterioles. * P < 0.01 versus luminal diameter before the addition of Tro.

Effect of SKF 96365 on Tro-Induced Dose-Dependent Dilation in Af-Arts

Pretreatment with 50 μM SKF 96365 did not affect basal luminal diameter; the diameter before and after the treatment was 16.5 ± 0.5 and 16.7 ± 0.7 μm (n = 7). NE (0.5 to 1.0 μM) decreased the diameter to 10.0 ± 0.2 μm. As shown in Figure 6, Tro did not cause any dilation in such arterioles (observed for 20 min at each dose); the change in luminal diameter induced by 10^{-5} M Tro was 0.5 ± 0.3 μm (at 20 min). Thus, pretreatment with SKF 96365 abolished the dose-dependent early-phase vasodilator action of Tro in Af-Arts.

Figure 5. Effect of De-Endo on 10^{-5} M Tro-induced time-dependent vasodilation in preconstricted afferent arterioles. Endothelium disruption with antibodies against factor VIII–related antigen and complements had no effect on Tro-induced time-dependent dilation. ○, nontreated arterioles; ●, De-Endo arterioles. * P < 0.01 versus luminal diameter before the addition of Tro.
μM) decreased the diameter to 10.0 ± 0.2 μm. As shown in Figure 7, 10−5 M Tro began to cause significant dilation from 100 min. At 100 or 120 min, the diameter increased by 3.0 ± 0.4 (or 30 ± 4%) or 3.4 ± 0.2 μm (or 34 ± 3%), respectively. Compared with that observed at 20 min (10.5 ± 0.3 μm), the diameter increased by 2.5 ± 0.4 (or 24 ± 4%) or 2.9 ± 0.2 μm (or 28 ± 3%), respectively. Thus, pretreatment with SKF 96365 did not affect the late-phase vasodilator action of Tro on Af-Arts.

Discussion

In this study, we examined the vascular action of Tro, a TZD compound, on the Af-Arts and Ef-Arts, crucial vascular segments to the control of glomerular hemodynamics. We found that Tro at therapeutic concentrations (although not available for clinical use now) causes biphasic or monophasic vasodilation in preglomerular Af-Arts or postglomerular Ef-Arts, respectively. In Af-Arts, Tro at μM concentrations increased the diameter by ~50%, which represents an ~80% decrease in vascular resistance (the vascular resistance is proportional to the reciprocal of fourth power of radius). Because Af-Arts account for most of the preglomerular vascular resistance, and an increase in their vascular resistance contributes to the pathogenesis of essential hypertension (25), this vasodilator action may play an important role in the BP-lowering effect of Tro (6,7). This notion is consistent with findings that have suggested the possibility that Tro lowers BP through its vasodilator action (8,16). In addition, through its vasodilator action on the Ef-Art, Tro would decrease the P_{GC}. Because glomerular hypertension is now believed to be responsible, at least in part, for the development of glomerular dysfunction (such as glomerular hyperfiltration and albuminuria) in diabetes (26,27), this vasodilator action may partly account for the renoprotective effect of Tro observed in patients with diabetic nephropathy (11).

In this study, vasodilation was observed in isolated arterioles perfused without insulin, which means that Tro has a vasodilatory effect on renal arterioles independent of its influence on the insulin sensitivity. To study Tro’s vasodilator mechanism(s), we first examined the possible role of the endothelium, because studies have shown that Tro improves endothelial function or endothelium-dependent vasodilation in humans (16,28). We found that disruption of the endothelium had no effect on either dilation in Af-Arts, which demonstrates that both vasodilator mechanisms are endothelium-independent. These results are consistent with in vitro findings of Song et al. (13) or Kawasaki et al. (14) that Tro dilates endothelium-removed rat tail artery or porcine coronary artery, respectively. Although the reason why Tro causes endothelium-dependent vasodilation only in humans (or in vivo) is unclear, there are several possibilities other than species or vascular difference. First, in vivo Tro may augment the vasodilator action of insulin, which causes vasodilation partly through stimulation of the endothelial NO release (29,30). This possibility is supported by the finding of Kotchen et al. (31) that pioglitazone, another TZD, attenuates the NE-induced contraction of rat aorta by unmasking a latent endothelium-dependent vasodilator action of insulin. Second, antioxidant properties of the α-tocopherol moiety, which is contained in Tro (32,33), may be involved in the difference. It has been demonstrated that α-tocopherol given to humans causes a reduction in the reactive oxygen species generation (34). Thus, it may be that by decreasing reactive oxygen species generation, especially O₂⁻, which reduces the bioavailability of NO, Tro may exert endothelium-dependent and NO-dependent vasodilator action under conditions with increased oxidative stress. We have demonstrated elsewhere that high glucose, which induces oxidative stress, constricts rabbit Af-Art by decreasing basal NO release (21). Ohishi and Carmines (35) also demonstrated that NO activity is decreased in rat Af-Arts during the hyperfiltration stage of diabetes. Taken together, it is possible that by decreasing...
reactive oxygen species generation, Tro may increase the NO level and induce endothelium-dependent vasodilation in Af-Arts exposed to high glucose (or in diabetic Af-Arts). However, because disruption of the endothelium had no effect, it is unlikely that antioxidant properties are involved in Tro’s vasodilator action observed in this study (under conditions without increased oxidative stress).

We next examined whether Tro dilates Af-Arts by blocking the Ca^{2+} influx pathway (voltage-operated Ca^{2+} channel and/or receptor-operated Ca^{2+} channel) as it does in other vascular beds (13,14). We found that pretreatment with SKF 96365, which inhibits both voltage-operated and receptor-operated Ca^{2+} channels (24), abolished the early-phase dilation without affecting the late-phase dilation, which suggests that Tro induces rapid vasodilation of Af-Arts by decreasing intracellular calcium concentration ([Ca^{2+}]_{i}) of VSMC by blocking Ca^{2+} influx pathway. This notion may explain why Tro did not cause rapid vasodilation in Ef-Arts, because mechanisms of calcium mobilization are quite different between Af-Arts and Ef-Arts (for example, functional expression of voltage-operated Ca^{2+} channel is dense or sparse in Af-Arts or Ef-Arts, respectively) (36,37). Although the specific Ca^{2+} channel involved is unclear, our finding elsewhere (20) that complete blockade of the voltage-operated Ca^{2+} channel abolishes NE-induced preconstriction in Af-Arts suggests that this Ca^{2+} channel was not completely blocked by SKF 96365 in this study (because NE produced sustained preconstriction). Thus, blockade of the receptor-operated Ca^{2+} channel may play an important role in Tro-induced rapid vasodilation in Af-Arts.

The mechanism for the Tro-induced late-phase dilation is so far unclear; however, there are several possibilities. First, Tro may stimulate the production of some endothelium-independent vasodilator(s). Second, peroxisome proliferator-activated receptor γ, a nuclear receptor that is activated by Tro (38) and functionally expressed in diverse cell types, including mesangial cells (39) and VSMC (40,41), may be involved. However, there is no direct evidence that activation of proliferator-activated receptor γ causes vasodilation, although we have recently reported that 15-deoxy-Δ12,14-prostaglandin J_{2}, an endogenous ligand for proliferator-activated receptor γ (42), causes vasodilation in rabbit Af-Arts (43). Further studies are required to clarify the mechanisms by which Tro causes vasodilation in renal microcirculation.

As mentioned above, the early stages of diabetes mellitus are characterized by an increase in P_{GC}, which is now believed to contribute to the pathogenesis of diabetic glomerulopathy (26,27). Thus, agents that normalize P_{GC} are most likely to slow the progression of renal damage. In this respect, angiotensin-converting enzyme inhibitors or AngII receptor antagonists, which decrease P_{GC} by dilating Ef-Arts, are known to retard or prevent the progression of renal damage (44–46). In this study, we found that Tro also exerts vasodilator action on Ef-Arts, which suggests that Tro may decrease P_{GC} and afford renal protection when used in patients with diabetes (although it is not available for clinical use now) independent of its insulin-sensitizing (or plasma glucose reducing) action. Indeed, when compared with metformin, Tro profoundly suppressed albuminuria in patients with diabetes, even though it decreased plasma glucose less efficiently than metformin (11). Furthermore, it has been reported that Tro inhibits high glucose-induced proliferation of VSMC (47) or glomerular mesangial cells (17) by inhibiting the activation of protein kinase C. Thus, in addition to its hemodynamic actions, Tro may exert renoprotective effects by inhibiting the protein kinase C activity in diabetes.

In summary, we found that Tro at therapeutic concentrations dilates both Af-Arts and Ef-Arts. These vasodilator actions may contribute to the BP lowering or renoprotective effects of Tro, respectively. In addition to these vascular actions, Tro would exert beneficial cardiovascular effects in patients with diabetes by ameliorating the insulin resistance, a common cause of multiple risk-factor syndrome (1–3). Although Tro has been withdrawn from the market because of its hepatotoxicity, it seems likely that Tro is very useful in the prevention or treatment of diabetic complications (such as atherosclerosis or nephropathy) in patients with diabetes. Studies that examine whether other clinically available TZD may exert similar vascular actions on the glomerular microcirculation are required.

Acknowledgments

This work was supported in part by a research grant for cardiovascular research (11C-4) from the Ministry of Health and Welfare of Japan and Grants 12470208, 12877162, and 13770594 from the Ministry of Science, Education and Culture, Japan. The authors thank Miss Hiroko Kato for her experimental assistance.

References

41. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM: 15-deoxy-\(\alpha,\beta,\gamma,\delta\)-prostaglandin \(J_2\) is a ligand for the adipocyte determination factor PPAR \(\gamma\). *Cell* 83: 803–812, 1995
42. Arima S, Takeuchi K, Kohagura K, Taniyama Y, Sugawara A, Ikeda Y, Omata K, Ito S: Biphasic vasodilator action of trogl-


