Differential Vasoactive Effects of the Insulin Sensitizers Rosiglitazone (BRL 49653) and Troglitazone on Human Small Arteries In Vitro

Adrian B. Walker, Ebrahim K. Naderali, Paula D. Chattington, Robin E. Buckingham, and Gareth Williams

BRL 49653 (rosiglitazone) and troglitazone are thiazolidinedione insulin-sensitizing agents, which are undergoing clinical evaluation as treatments for NIDDM. Potential side effects of thiazolidinediones include edema and hemodilution. Although the underlying mechanisms are presently unclear, animal and human studies have demonstrated a vasodilator action of troglitazone, which could in theory cause fluid retention. This in vitro study compared the direct vasodilator effects of troglitazone and BRL 49653 in small arteries (n = 44) from human subcutaneous fat. In arterial rings with a functioning endothelium and preconstricted with norepinephrine (NE; 6 µmol/l), troglitazone (n = 22 vessels), but not BRL 49653 (1–100 µmol/l), caused a concentration-related relaxation (69.4 ± 5.2% at 100 µmol/l; P < 0.01). In the presence of indomethacin (IM; 10 µmol/l; n = 12), this vasorelaxant effect of troglitazone was abolished (P < 0.01 vs. troglitazone alone) and replaced by enhanced vasoconstriction (58.5 ± 39.5% over the NE baseline) similar in magnitude to that produced by troglitazone vehicle (ethanol) alone (n = 16; NS vs. ethanol vehicle). By contrast, BRL 49653 (100 µmol/l; n = 22) and an equivalent volume of ethanol alone (n = 12) caused similar degrees of vasoconstriction (18.7 ± 14.6 and 22.5 ± 8.0%, respectively; NS). In the presence of IM (10 µmol/l; n = 10), the vasoconstrictor effect of BRL 49653 was enhanced (41.5 ± 14.4%), although not significantly (NS vs. BRL 49653 alone or ethanol alone). Additional studies in Wistar rat arteries showed a similar vasodilator effect of troglitazone that was not inhibited by L-NAME (100 µmol/l). The α-tocopherol moiety alone had no vasorelaxant effect at concentrations up to 300 µmol/l. Thus, in human arterial resistance vessels in vitro, BRL 49653 does not possess the direct, IM-sensitive vasorelaxant action of troglitazone. This vasodilation could, in theory, permit transmission of systemic pressure to the capillary bed.
***Diabetes* 47:810–814, 1998**

From the Diabetes and Endocrinology Research Group (A.B.W., E.K.N., G.W.), University of Liverpool; the Royal Liverpool University Hospital (P.D.C.), Liverpool; and SmithKline Beecham Pharmaceuticals (R.E.B.), Welwyn, Wales, U.K.

Address correspondence and reprint requests to Dr. Adrian B. Walker, Diabetes and Endocrinology Research Group, University Clinical Departments at Aintree, Fazakerley Hospital, Longmoor Lane, Liverpool L9 7AL U.K.

Received for publication 16 June 1997 and accepted in revised form 26 January 1998.

ACh, acetylcholine; IM, indomethacin; NE, norepinephrine; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptor; PSS, physiological salt solution.

There is growing interest in the potential use of the thiazolidinedione insulin-sensitizing agents, e.g., troglitazone, BRL 49653, and pioglitazone, as therapy for patients with NIDDM (1,2). In addition, the potential of troglitazone to prevent progression from impaired glucose tolerance to NIDDM is being studied in the Non-Insulin-Dependent Diabetes Primary Prevention Trial (3).

Thiazolidinediones enhance insulin sensitivity in animal models of insulin resistance (3–6), and reduction in the compensatory hyperinsulinemia is thought to be a consequence of this. More recently, troglitazone has been shown to have similar insulin-sensitizing effects in human NIDDM (7) and in obese, insulin-resistant, but nondiabetic subjects (2).

Troglitazone and pioglitazone have been shown to lower elevated arterial pressure in rat models of hypertension (8–10), and a blood pressure–lowering effect has been observed with troglitazone in humans (2).

Pioglitazone and troglitazone have been reported to cause dilation of peripheral blood vessels in animal studies (4,10). Skin blood flow was increased by subcutaneous injection of troglitazone in dexamethasone-induced diabetic obese Zucker rats and control Wistar rats; however, no such effect was seen with pioglitazone or BRL 49653, and the troglitazone-induced increase in skin blood flow was inhibited by indomethacin (IM) (4). Troglitazone increased prostaglandin (PG)I_2 production from isolated aortic rings and increased PGI_2 and PGE_2 production by 3T6 fibroblasts (4), further suggesting that the increased skin blood flow was mediated by locally generated vasodilator PGs. Pioglitazone has been shown to lower blood pressure when given to Sprague-Dawley rats, and it has a direct effect to blunt the contractile response of aortic rings to norepinephrine (NE), arginine vasopressin, and KCl in vitro; this effect of pioglitazone was absent when Ca^{2+} was removed from the medium (10). Further work by Zhang et al. (11) suggested that the vascular effects of pioglitazone are due to inhibition of the depolarization-induced inward flux of Ca^{2+} into vascular smooth muscle cells. These data suggest that both troglitazone and pioglitazone possess properties other than binding to and activating the peroxisome proliferator-activated receptor (PPAR)-γ, the molecular target now widely believed to be central to the insulin-sensitizing action of the thiazolidinediones (12,13).

The possibility that side effects of thiazolidinediones, unrelated to their primary action as insulin-sensitizing agents, will also be present in humans is a subject of considerable scientific and therapeutic interest. In the case of troglitazone, the

only thiazolidinedione for which substantial clinical data are published, there are suggestions that mild fluid retention, manifested as edema, is found in a variable proportion of patients (14–18). It is possible that these effects have a hemodynamic origin, as some patients exhibit a fall in mean arterial pressure and calculated peripheral resistance, as well as an increase (presumably baroreceptor-reflex mediated) in cardiac index and stroke volume index (19).

In the present study, we have compared troglitazone and BRL 49653 for their ability to relax small arteries from human subcutaneous adipose tissue, as such an action may expose the capillary bed to the systemic blood pressure.

RESEARCH DESIGN AND METHODS

Small resistance arteries (n = 44; diameter ~250 µm) were dissected from subcutaneous fat removed from patients (n = 7) undergoing laparotomy for cholecystectomy under general anesthetic. All patients were otherwise healthy and receiving no medication preoperatively, received the same anesthetic agents (thiopentone sodium induction and isoflurane inhalation maintenance), and gave written informed consent. The study had approval by the Royal Liverpool University Hospital Trust Ethics Committee.

Acetylcholine (ACh), NE, and IM (Sigma, Poole, U.K.) were made up as fresh base solutions in physiological salt solution (PSS; composition [in mmol/l]: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026, glucose 5.5) for each experiment. Troglitazone and BRL 49653 (SmithKline Beecham) were dissolved in 96% ethanol.

The subcutaneous fat was immediately placed in cold PSS (4°C) that had been gassed with 5% CO_2 in O_2. Arteries were dissected free of fat and connective tissue and mounted on two 40-µm diameter stainless-steel wires in an automated myograph (Cambustion, Cambridge, U.K.), based on the principle of the Mulvany myograph (20), and gassed continuously with 5% CO_2 in O_2.

After 1-h equilibration, the length-tension characteristics for each vessel were determined using the law of Laplace ($P = T/r$; where P is the transmural pressure, T is the tension, and r is the vessel radius). Each vessel was then adjusted to the normalized diameter, i.e., that which it would achieve at rest in vivo under a transmural pressure of 100 mmHg. This has previously been shown to be the diameter at which the greatest force is generated (21). The myograph computer calculated the target tension each vessel should develop in response to a maximal stimulus.

Each vessel was constricted twice using KPSS (in which the NaCl of PSS was replaced by 125 mmol/l KCl) for 2 min, followed by a 10-min rest period. Any vessel failing to reach its predetermined target tension was discarded.

Endothelial viability was assessed by preconstricting vessels with 6 µmol/l NE; when a plateau tension was achieved (~2 min), the vessels were relaxed by exposure to a stepped ACh concentration in the range of 10 nmol/l to 10 µmol/l. Vessels failing to achieve at least 65% relaxation with ACh were assumed to be damaged and were discarded.

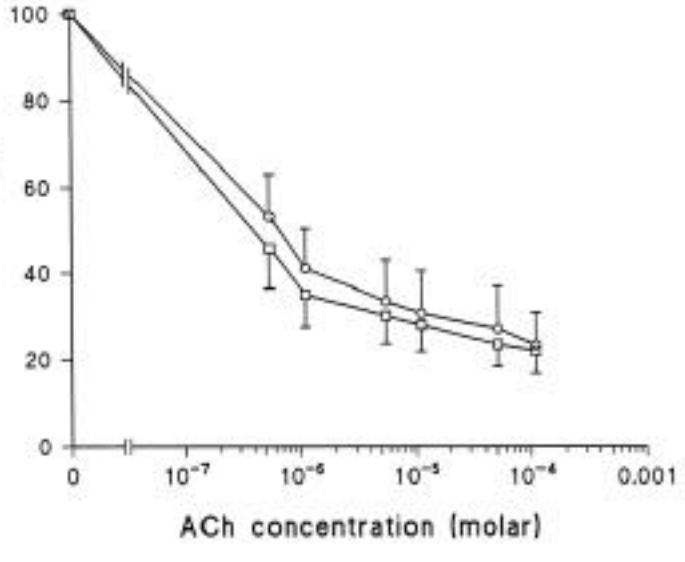

FIG. 1. ACh dose-relaxation curves in NE-preconstricted arteries before exposure to BRL 49653 or troglitazone. ○, BRL 49653 (n = 22); □, troglitazone (n = 22). NS between curves.

After a 30-min rest period, the vessels were again preconstricted with 6 µmol/l NE and then exposed either to troglitazone (n = 22) or to BRL 49653 (n = 22; 1–100 µmol/l), added to the bath in half-log-molar increments. Subsequently, to further study the possible mechanism of troglitazone-induced vasorelaxation, on completion of the relaxation-response curve to these agents, a number of the vessels were washed three times and incubated for 15 min in drug-free PSS. IM (10 µmol/l) was subsequently added; the vessels were incubated for a further 15 min; and then the cycle of preconstriction with NE, stepped addition of thiazolidinedione and washing to remove the drugs, was repeated. Finally, after 60 min of incubation in drug-free PSS, the vessels were preconstricted with NE and exposed to ethanol vehicle alone, added in volumes equivalent to those used during earlier stepped addition of thiazolidinedione, to determine the vasoactive effects attributable to ethanol vehicle alone.

Wistar rat arteries. In addition, we studied the effects of BRL 49653 and troglitazone in mesenteric arteries from 10-week-old, out-bred male Wistar rats (n = 6) in the same system. After being primed twice with KPSS, vessels were contracted first with KPSS and then exposed to BRL 49653 or troglitazone (1–100 µmol/l). After washing out the bath, vessels were then contracted with 6 µmol/l NE and again exposed to BRL 49653 or troglitazone first in the absence and then in the presence of N^G-nitro-L-arginine methyl ester (L-NAME) (100 µmol/l). In calcium-free PSS (i.e., PSS without the addition of $CaCl_2$), the arteries were contracted with 6 µmol/l NE and then exposed to BRL 49653, troglitazone, or ethanol vehicle. After contraction with 6 µmol/l NE, arteries were exposed to α-tocopherol (Sigma), freshly dissolved in distilled water, 100–300 µmol/l.

Statistical analysis. Results were analyzed by Scheffé's multiple analysis, two-way analysis of variance using Arcus Pro-II computer software (Medical Computing, Aughton, U.K.). Differences were considered statistically significant when $P < 0.05$. Data are presented throughout as means ± SE.

RESULTS

Human arteries. There was no significant difference in diameters between vessels assigned to exposure to troglitazone (278.5 ± 14.6 µm) or to BRL 49653 (263.6 ± 18.7 µm; P = 0.4). Moreover, vessels in the two groups displayed comparable endothelium-dependent relaxation when exposed to ascending concentrations of ACh. The maximum relaxation response was 77.7 ± 7.6% for the vessels assigned to BRL 49653 and 78.1 ± 5.2% for those subsequently exposed to troglitazone (NS between groups; Fig. 1).

Addition of troglitazone (22 vessels) induced a rapid-onset (20–30 s latency), concentration-related relaxation response over the range 10–100 µmol/l. For each addition of troglitazone to the bath, responses plateaued after approximately 2 min. At the highest concentration, the relaxation was 69.4 ± 5.2% (Fig. 2). In the presence of IM (n = 12), the vasorelaxant action of troglitazone was abolished, and instead there was further constriction of the arteries (58.5 ± 39.5% at 100 µmol/l; $P < 0.01$ vs. troglitazone alone). This increment in vascular tone was not significantly different from that seen with ethanol vehicle alone (n = 16; Fig. 2).

In contrast to troglitazone, increasing concentrations of BRL 49653 did not cause vasorelaxation (n = 22; Fig. 3), only further vasoconstriction (18.7 ± 14.6% above baseline) similar to that caused by ethanol alone (n = 12; 22.5 ± 8.0%; NS). In the presence of IM, the vasoconstrictor response to BRL 49653 was slightly but not significantly augmented (n = 10; 41.5 ± 14.4% above the NE baseline; NS vs. BRL 49653 alone or ethanol alone).

Wistar rat arteries. BRL 49653, as in the human vessels, caused no relaxation of either KPSS or NE-preconstricted arteries in either PSS or calcium-free PSS (data not shown).

Troglitazone caused relaxation of both KPSS and NE-preconstricted arteries between 1 and 100 µmol/l, with a maximum of 96.3 ± 1.0% relaxation (Fig. 4). The presence of L-NAME (100 µmol/l) had no effect on the relaxation, with a

maximum 94.5 ± 0.9% relaxation with 100 µmol/l troglitazone (Fig. 4). In calcium-free PSS, troglitazone caused a similar degree of relaxation: maximal 94.8 ± 1.5% with 100 µmol/l troglitazone (Fig. 4).

There was no relaxation observed with α-tocopherol at concentrations up to 300 µmol/l; higher concentrations resulted in the compound coming out of solution in the organ bath.

DISCUSSION

In the present study, troglitazone caused concentration-related relaxation of NE-preconstricted human subcutaneous resistance arteries in vitro, and the response was inhibited by the cyclooxygenase inhibitor IM. These data suggest that in human small arteries, troglitazone-induced vasodilation is PG mediated. The observations in this study are consistent with the earlier report that troglitazone increases skin blood flow in both normal rats and dexamethasone-induced diabetic obese Zucker rats and that this effect is also prevented by IM (4). In rat aortic rings, troglitazone has been shown to augment production of PGI_2 (4), although no attempt was made in the present study to identify the vasodilator prostanoid. Song et al. (22) have recently demonstrated that in the tail artery from Wistar rats, troglitazone decreased NE-induced contractile responses by a mechanism not dependent on nitric oxide production but associated with inhibition of Ca^{2+} currents in cultured vascular smooth muscle cells. These results do suggest some similarity between the effects of troglitazone on blood vessels from the rodent and humans in vitro. We also showed no effect of L-NAME on troglitazone-induced relaxation in rat vessels; however, our vessels also relaxed after NE-preconstriction in calcium-free PSS, suggesting that a mechanism other than that causing closure of smooth muscle calcium channels is in operation. It may be that more than one mechanism explains the vasodilation seen with troglitazone and thus the apparent difference between our observation and those of Song et al. (22); however, like them we propose that the mechanism does not involve nitric oxide release. There is no evidence from our study to suggest that the α-tocopherol moiety of troglitazone is vasoactive, even at concentrations threefold higher than the maximum concentration of troglitazone studied.

Interestingly, there is also evidence of a vasorelaxant action of troglitazone in humans (19). In that study, designed to detect cardiac hypertrophy by echocardiography in NIDDM patients during prolonged treatment with troglitazone (800 mg per day), there was evidence of a fall in calculated peripheral resistance at 12, 24, 36, and 48 weeks. Our results demonstrating a vasodilator effect of troglitazone on human resistance arteries in vitro provide a possible explanation for the results observed in vivo during long-term therapy. It is unknown at present whether the vasodilator action of troglitazone in humans can be antagonized by cyclooxygenase inhibition in vivo.

Blood flow in the microvascular circulation is normally controlled by a variety of neural, humoral, and paracrine mechanisms and has been extensively studied in IDDM. Hemodynamic abnormalities observed in the microcirculation of patients with IDDM include an increase in blood flow in the retina, kidney, and skin; this increase may predate the development of established microvascular complications.

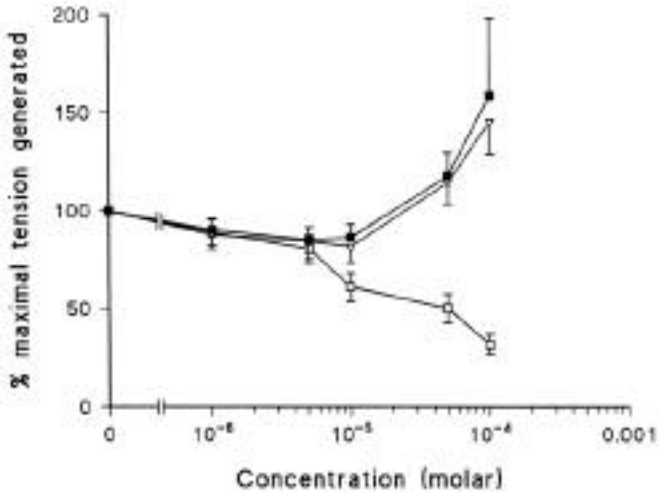

FIG. 2. Troglitazone dose-response curve, with and without IM (10^{-5} mol/l). □, troglitazone; ■, troglitazone and IM ($P < 0.01$ vs. troglitazone alone); ▽, ethanol vehicle (NS vs. troglitazone and IM).

Microcirculatory function is less easily studied early in NIDDM, as there is often a long preclinical phase before the diagnosis of diabetes is made. Basal capillary pressure is not raised, and it has been proposed that the microvasculature is relatively protected from the effects of transmitted systemic blood pressure by changes in the precapillary resistance arteries, possibly due to the insulin-resistant state that precedes overt NIDDM (23–25). Although a vasodilator and thus blood pressure–lowering effect of the thiazolidinediones may be beneficial, it could theoretically be disadvantageous to dilate the precapillary resistance arteries and allow transmission of arterial blood pressure to the capillary circulation, with possible acceleration of microangiopathy.

Insulin has been shown to act as a vasodilator in a number of vascular beds (26,27), and this appears to be due to an effect of insulin to stimulate nitric oxide release (28–30).

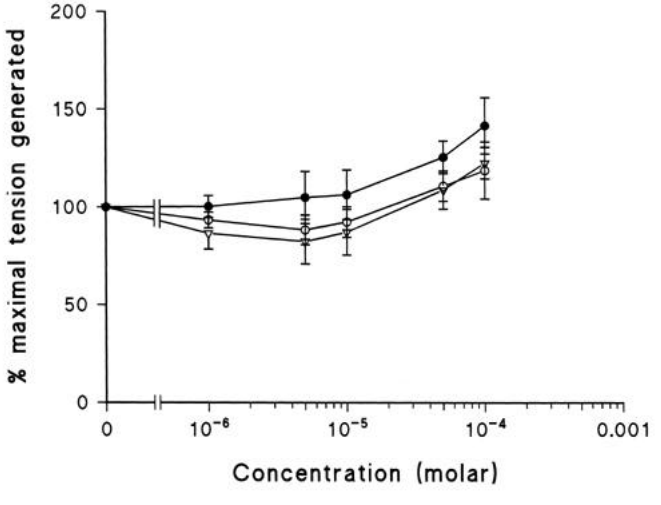

FIG. 3. BRL 49653 dose-response curves, with and without IM, compared with vehicle alone. ○, BRL 49653; ●, BRL 49653 and IM (10^{-5} mol/l); ▽, ethanol vehicle alone.

Insulin-induced vasodilation is impaired in subjects with insulin resistance due to obesity (31), NIDDM (32), and essential hypertension (33). It is possible that thiazolidinediones may lower blood pressure by enhancing any tonic vasodilator response to insulin, thereby reducing peripheral arterial resistance independent of, and possibly in addition to, any direct vasodilator action. In addition, by reducing plasma insulin levels, these drugs may reduce the potential blood pressure–raising actions of insulin, such as renal sodium reabsorption (8) and increased sympathetic activity (34,35).

It has been suggested that peroxisome proliferators could bind to fatty acid binding protein and displace fatty acids, which are then able to activate PPAR (36). If this were the case, then troglitazone may particularly stimulate arachidonic acid release, which would act as a substrate for cyclooxygenase, with enhanced PG production and the vasodilation observed in this study. However, it is not clear why this is not also seen with BRL 49653.

From this study, BRL 49653 does not exhibit vasorelaxant properties in human resistance arteries in vitro. Because no hemodynamic data have yet been published on BRL 49653, it is not possible to say whether the results observed in vitro translate to an absence of vasodilation in vivo. For the present, our findings constitute an interesting distinction between the drugs; however, it is premature to say whether these differences will also form a basis for distinguishing between the agents in terms of adverse events or theoretical benefits or disadvantages in the treatment of patients with NIDDM.

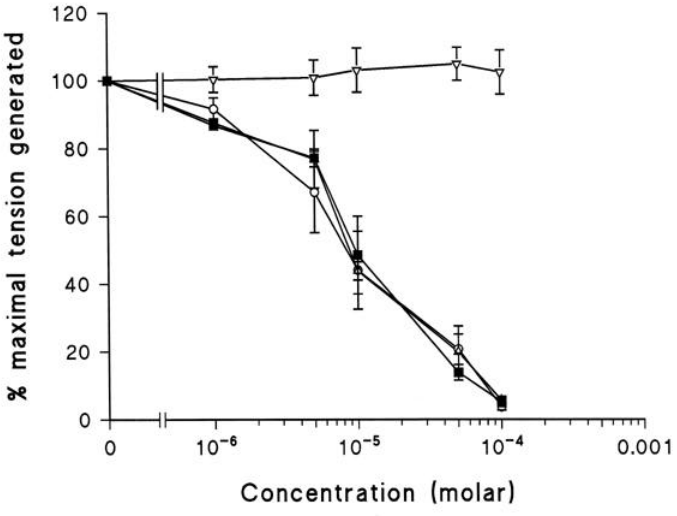

FIG. 4. Wistar rat mesenteric arteries. ■, troglitazone dose-response curve in PSS; △, troglitazone dose-response curve in calcium-free PSS; ○, troglitazone dose-response curve with 100 µmol/l L-NAME in PSS; ▽, ethanol vehicle in calcium-free PSS.

REFERENCES

1. Saltiel AR, Olefsky JM: Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 45:1661–1669, 1996
2. Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky JM: Improvement in glucose tolerance and with insulin resistance in obese subjects treated with troglitazone. *N Engl J Med* 331:1188–1193, 1994
3. National Institutes of Health: Non-Insulin-Dependent Diabetes Primary Prevention Trial. *NIH Guide Grants Contracts* 22:1–20, 1993
4. Fujiwara T, Oshawa T, Miyamoto M, Ushiyama S, Matsuda K, Horikoshi H: Troglitazone (CS-045) acutely increases skin blood flow in dexamethasone-induced diabetic obese Zucker rats and normal rats (Abstract). *Diabetes* 44 (Suppl. 1):72A, 1995
5. Stevenson RW, Hutson NJ, Krupp MN, Volkmann RA, Holland GF, Eggler JF, Clark DA, McPherson RK, Hall KL, Danbury BH, Gibbs EM, Kreutter DK: Actions of novel antidiabetic agent englitazone in hyperglycemic hyperinsulinemic ob/ob mice. *Diabetes* 39:1218–1227, 1990
6. Oakes ND, Kennedy CJ, Jenkins AB, Laybutt DR, Chisholm DJ, Kraegen EW: A new antidiabetic agent, BRL 49653, reduces lipid availability and improves insulin action and glucoregulation in the rat. *Diabetes* 43:1203–1210, 1994
7. Suter SL, Nolan JJ, Wallace P, Gumbiner B, Olefsky JM: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. *Diabetes Care* 15:193–203, 1991
8. Yoshioka S, Nishino H, Shiraki T, Ikeda K, Koike H, Okuno A, Wada M, Fujiwara T, Horikoshi H: Antihypertensive effects of CS-045 treatment in obese Zucker rats. *Metabolism* 43:75–80, 1993
9. Kaufman LN, Peterson MM, DeGrange LM: Pioglitazone attenuates diet-induced hypertension in rats. *Metabolism* 44:1105–1109, 1995
10. Buchanan TA, Meehan WP, Jeng YY, Yang D, Chan TM, Nadler JL, Scott S, Rude RK, Hseuh WA: Blood pressure lowering by pioglitazone: evidence for a direct vascular effect. *J Clin Invest* 96:354–360, 1995
11. Zhang F, Sowers JR, Ram JL, Standley PR, Peuler JD: Effects of pioglitazone on calcium channels in vascular smooth muscle. *Hypertension* 24:170–175, 1994
12. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkinson WO, Willson TM, Kliewer SA: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPAR-γ). *J Biol Chem* 270:12953–12956, 1995
13. Berger J, Bailey P, Biswas C, Cullinan CA, Doebber TW, Hayes NS, Saperstein R, Smith RG, Leibowitz MD: Thiazolidinediones produce a conformational change in peroxisome-proliferator-activated receptor-γ: binding and activation correlate with antidiabetic actions in *db/db* mice. *Endocrinol* 137:4189–4195, 1996
14. Kuzuya T, Iwamoto Y, Kosaka K, Takebe K, Yamanouhchi S, Kasuga M, Kajinuma H, Akanuma Y, Yoshida S, Shigeta Baba S: An early phase II clinical trial of a new oral hypoglycemic drug, CS-045, in patients with non-insulin-dependent diabetes mellitus. *J Clin Ther Med* 9 (Suppl. 3):3–19, 1993
15. Akanuma Y, Kosaka K, Kuzuya T, Shimizu N, Shigeta Y, Kaneko T: Clinical evaluation of a new oral hypoglycemic agent CS-045 in patients with non-insulin-dependent diabetes mellitus poorly controlled by diet alone: a dose-finding study by dose increasing method. *J Clin Ther Med* 9 (Suppl. 3):19–37, 1993
16. Akanuma Y, Kosaka K, Kuzuya T, Shimizu N, Shigeta Y, Kaneko T: Clinical evaluation of a new oral hypoglycemic agent CS-045 in patients with non-insulin-dependent diabetes mellitus poorly controlled by sulphonylureas: a dose-finding study by dose increasing method. *J Clin Ther Med* 9 (Suppl. 3):39–60, 1993
17. Kosaka K, Kuzuya T, Akanuma Y, Shigeta Y, Kaneko T: Clinical evaluation of a new oral hypoglycemic agent CS-045 in patients with non-insulin-dependent diabetes mellitus poorly controlled by diet alone: a double-blind, placebo-controlled study. *J Clin Ther Med* 9 (Suppl. 3):61–93, 1993
18. Berkowitz K, Peters R, Kjos SL, Goico J, Marroquin A, Dunn ME, Xiang Azen S, Buchanan TA: Effect of troglitazone on insulin sensitivity and pancreatic β-cell function in women at high risk for NIDDM. *Diabetes* 45:1572–1579, 1996
19. Ghazzi MN, Perez JE, Antonucci TK, Driscoll JH, Huang SM, Faja BW, the Troglitazone Study Group, Whitcomb RW: Cardiac and glycemic benefits of troglitazone treatment in NIDDM. *Diabetes* 46:433–439, 1997
20. Mulvany MJ, Halpern W: Mechanical properties of smooth muscle cells in situ. *Nature* 260:617–619, 1976
21. Mulvany MJ, Halpern W: Contractile properties of small arterial resistance vessels in spontaneously hypertensive rats. *Circ Res* 41:19–26, 1977
22. Song J, Walsh MF, Igwe R, Ram JL, Barazi M, Dominguez LJ, Sowers JR: Troglitazone reduces contraction by inhibition of vascular smooth muscle cell Ca^{2+} currents and not endothelial nitric oxide production. *Diabetes* 46:659–664, 1997
23. Jaap AJ, Donohoe M, Shore AC, Tooke JE: Limited microvascular vasodilation in patients with acromegaly (Abstract). *J Endocrinol* 37 (Suppl.):45, 1993
24. Jaap AJ, Tooke JE: The pathophysiology of microvascular disease in non-insulin-dependent diabetes (Editorial). *Clin Sci* 89:3–12, 1995
25. Sandeman DD, Pym C, Green E, Seamark C, Shore AC, Tooke JE: Microvascular vasodilation in feet of newly-diagnosed non-insulin-dependent diabetic patients. *BMJ* 302:1122–1123, 1991
26. Baron AD: Hemodynamic actions of insulin. *Am J Physiol* 267:E187–E202, 1994
27. McNally PG, Lawrence IG, Watt PAC, Hillier C, Burden AC, Thurston H: The effect of insulin on the vascular reactivity of isolated resistance arteries taken from healthy volunteers. *Diabetologia* 38:467–473, 1995

28. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511–2515, 1994
29. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. *J Clin Invest* 94:1172–1179, 1994
30. Walker AB, Savage MW, Dores J, Williams G: Insulin-induced attenuation of noradrenaline-mediated vasoconstriction in resistance arteries from Wistar rats is nitric oxide dependent. *Clin Sci* 92:147–152, 1997
31. Laakso M, Edelman SV, Brechtel G, Baron AD: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. *J Clin Invest* 85:1844–1852, 1990
32. Laakso M, Edelman SV, Brechtel G, Baron AD: Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–1083, 1992
33. Feldman RD, Briebier GS: Insulin-mediated vasodilatation: impairment with increased blood pressure and body mass. *Lancet* 342:707–709, 1993
34. Natali A, Quinones GA, Santoro D, Pecori N, Taddei S, Salvetti A, Ferrannini E: Relationship between insulin release, antinatriuresis and hypokalaemia after glucose ingestion in normal and hypertensive man. *Clin Sci* 85:327–335, 1993
35. Anderson EA, Balon TW, Hoffman RP, Sinkey CA, Mark AL: Insulin increases sympathetic activity but not blood pressure in borderline hypertensive humans. *Hypertension* 19:621–627, 1992
36. Canon JR, Eacho PI: Interaction of LY171883 and other peroxisome proliferators with fatty acid binding protein isolated from rat liver. *Biochem J* 280:387–391, 1991