Blood Pressure Lowering by Pioglitazone

Evidence for a Direct Vascular Effect

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Abstract

To examine potential mechanisms for the blood pressure–lowering action of the thiazolidinedione compound, pioglitazone (PIO), we studied the effects of the drug on blood pressure and insulin action in vivo and on vascular tissue in vitro. In vivo, PIO lowered blood pressure in fructose-fed and chow-fed rats to an extent that could not be explained by alterations in fasting plasma insulin or free magnesium concentrations or by alterations in whole-body insulin sensi-i9tivity. In vitro, PIO caused significant blunting of the contractile responses of aortic rings to NE, arginine vasopressin (AVP), and potassium chloride; the blunting of responses to NE was maintained after removal of the endothelium. To assess the potential importance of extracellular calcium to the vasodepressor effect of PIO, we measured contractile responses to NE in the absence of calcium, and then after acute restoration of calcium in the presence of NE. PIO had no effect on the contractile response in the absence of calcium. By contrast, PIO blunted by 42% the contractile response that occurred when the extracellular calcium supply was acutely restored in the presence of NE, suggesting that the blunting was mediated by blockade of calcium uptake by vascular smooth muscle. Such an effect was confirmed in cultured a7r5 vascular smooth muscle cells, which exhibited a brisk increase in intracellular calcium in response to AVP that was blocked by PIO in a dose-dependent fashion.

Our data indicate that PIO has a direct vascular effect that appears to be mediated at least in part by inhibition of agonist-mediated calcium uptake by vascular smooth muscle. The direct vascular effect may contribute to the blood pressure–lowering actions of PIO in vivo, because that effect could not be explained by alterations in whole-body insulin sensitivity. (*J. Clin. Invest.* 1995. 96:354–360.) Key words: hypertension • insulin sensitivity • vascular reactivity • calcium • norepinephrine

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1. *Abbreviations used in this paper:* NIDDM, non–insulin-dependent diabetes mellitus; PIO, pioglitazone.

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Introduction

Insulin resistance is a feature of several chronic disorders, including non–insulin-dependent diabetes mellitus (NIDDM),[1] obesity, atherosclerosis, and hypertension (1–5). The extent to which insulin resistance contributes to the pathogenesis of those disorders is controversial, in part because of a paucity of interventions that can specifically reverse insulin resistance. A new class of pharmacologic agents, the thiazolidinediones, has been reported to enhance insulin-mediated glucose disposal in animal models of diabetes and insulin resistance (6–14) and in humans with impaired glucose tolerance or NIDDM (15–17). These agents also have been reported to lower blood pressure and circulating triglyceride concentrations in insulin-resistant humans, monkeys, and rats (13, 14, 16, 17). Because the mechanism of action of thiazolidinediones is not fully understood, it is unclear whether the hemodynamic effects of thiazolidinediones are mediated indirectly, through changes in insulin action, or through other effects of these agents. The present study was carried out to determine whether the hemodynamic effects of one thiazolidinedione compound, pioglitazone (PIO), could be explained by the effect of the drug on insulin sensitivity in vivo and whether the drug has any direct actions on vascular tissue in vitro.

Methods

In vivo studies

Animals. Adult male Sprague-Dawley rats (Simonsen, CA) weighing 150–175 grams were housed singly with free access to food and water in an environmentally controlled vivarium (23±1°C, 30–40% humidity) with a 12-h light-dark photocycle. All procedures were approved by the Animal Care and Use Committee of the University of Southern California.

Experimental design. Animals were maintained on normal chow for 2 wk after arrival. During this time, baseline blood pressure measurements were made. Animals were then assigned randomly to one of four powdered experimental diets: (1) normal chow ("chow only"; $n = 17$), (2) a diet high in fructose ("fructose only"; $n = 17$), (3) the high-fructose diet plus PIO ("fructose + PIO"; $n = 17$), or (4) normal chow plus PIO ("chow + PIO"; $n = 11$). The caloric content of the normal chow (Teriad Rodent Diet 8604, Hardan Teklad, Madison, WI) was distributed as 58% carbohydrate (no fructose), 12% fat, and 30% protein. The high-fructose diet (Teklad 89054) contained 67% carbohydrate (98% of which was fructose), 13% fat, and 20% protein by calorie. PIO (kindly provided by the Upjohn Co., Kalamazoo, MI) was administered in the chow (400 mg PIO/kg chow resulting in a daily PIO intake of ~ 22 mg/kg body weight). Once animals were placed on their study diets, body weights and food intake were measured daily and tail cuff blood pressures were measured weekly. At the beginning of week 7, food was removed at 0800 h, and blood was taken by tail bleeding between 1500 and 1600 h for measurements of plasma glucose, insulin, and triglycerides. Blood was taken under similar conditions of fasting at the beginning of week 8 for plasma magnesium determinations. Hy-

perinsulinemic euglycemic clamps (described below) were performed after animals had completed 8 wk on the study diets.

Blood pressure measurements. Blood pressure was measured in two ways. Weekly measurements were made in all four study groups by an indirect tail cuff method (IITC Instruments Inc., Woodland Hills, CA) after 20 min of whole-body prewarming at 30°C. Animals were adapted to the blood pressure procedure three times on separate days before baseline blood pressures were obtained. Measurements were made by a technician who was blinded to the study diets. Ten measurements per animal were averaged for each weekly blood pressure determination.

To confirm the blood pressure lowering effect of PIO detected by tail cuff measurements (see below), rats fed normal chow with (n = 6) or without (n = 6) PIO for 3 wk had direct arterial pressure measurements. During the 3rd wk of the treatment period, animals were anesthetized with ketamine and xylazine, an incision was made in the neck over the left carotid artery, and a polyethylene catheter was inserted into the artery in the caudad direction. The catheter was secured in the artery with a suture and the proximal end of the catheter was tunneled subcutaneously and exteriorized over the occiput. On the 3rd d after catheter placement, the catheter was connected to a pressure transducer and digital pressure analyzer (BPA-100 Blood Pressure Analyzer, Micro-Med, Inc., Louisville, KY). After a 30-min period for habituation, mean arterial pressure was recorded every minute for 10 min while animals remained unrestrained in their cages. The average of the 10 readings was used as an individual measure of mean arterial pressure.

Glucose clamps. At least 3 d before glucose clamps, the distal third of each animal's tail was drawn through a hole in the cage bottom and secured there with a rubber stopper as described previously (18). On the day of glucose clamps, food was removed at ~ 0700 h and polyethylene catheters were placed percutaneously into the ventral tail artery and each lateral tail vein while animals were briefly restrained in a towel. After catheter placement (by 0800 h), animals were returned to their cages with tails secured as described above; they were free to move about and drink water during glucose clamps.

Starting at ~ 1200 h, animals received two sequential infusions of insulin (Humulin R, Eli Lilly Co., Indianapolis, IN) into one tail vein. The first infusion was given from 0 to 120 min at a rate of 1 mU · min^{-1} · kg body $weight^{-1}$. The second infusion was given from 121 to 280 min at 4 mU · min^{-1} · kg^{-1}. Plasma glucose concentrations were measured at 10-min intervals starting 30 min before the insulin infusion (40-μl blood samples). Plasma glucose was clamped at basal during insulin infusions using an infusion of dextrose (10% wt/vol in water). Blood samples (400 μl each) were drawn during the final 30 min of the low-dose insulin period (+100, 110, 120 min) and the high-dose insulin period (+260, 270, 280 min), and plasma from each period was pooled and stored at −20°C for insulin assay. Erythrocytes from the 400-μl blood samples were suspended in saline and reinfused at the end of each sampling period.

Because plasma insulin concentrations differed among study groups during the glucose clamps (see Results), whole-body sensitivity to infused insulin was calculated as the increase in the glucose infusion requirement between the final 30 min of the low-dose and high-dose insulin infusion periods, divided by the increase in plasma insulin between the two periods (dGINF/dINS).

Analytical techniques. Glucose was measured by glucose oxidase (Glucose Analyzer II, Beckman Instruments, Fullerton, CA). Insulin was measured by a charcoal precipitation RIA (19). The insulin concentrations of samples obtained during human insulin infusions were calculated using a human insulin standard; all other concentrations were calculated against a rat insulin standard (both standards from Novo Nordisk, Wilton, CT). Triglycerides were analyzed using enzymatic methods (Abbott VP Super System Blood Chemistry Analyzer, Chicago, IL) standardized against materials provided by the Centers for Disease Control (20). Total serum magnesium was measured by atomic absorption spectrophotometry (21), and serum ionized Mg^{2+} was measured by a Mg^{2+} selective ion electrode (Nova 8; Nova Biomedical, Waltham, MA) (22).

In vitro studies

Aortic rings. Abdominal aortae were removed from anesthetized male Sprague-Dawley rats weighing 250–300 grams as described previously (23). Residual blood was washed from the lumen of each vessel with cold Krebs solution. Vessels were placed in petri dishes filled with oxygenated Krebs-Henseleit buffer, and adherent fat and connective tissue were removed from vessels with the aid of a dissecting microscope. Aortae were cut into 3-mm-wide transverse rings and mounted under 0.5 grams resting tension on stainless steel prongs in a jacketed glass organ chamber containing Krebs-Henseleit bicarbonate buffer at pH 7.4, maintained at 37°C, with continuous oxygenation. Vessel tension was monitored isometrically using a strain guage transducer and pressure recorder equipped with a transducer amplifier. Rings were equilibrated for 60 min before exposure to pharmacologic agents.

Contractile responses were evaluated in two sets of experiments. In the first set, the incubation medium used for preincubation and during exposure to pharmacologic agents was standard Krebs-Henseleit bicarbonate buffer. Rings were preincubated for 60 min in the buffer and then exposed to NE (25–150 nmol/liter), KCl (28 mmol/liter), or arginine vasopressin (AVP; 0.66 nmol/liter) for sufficient time to achieve a stable contraction. Addition of each agonist was repeated twice to ensure a reproducible response, and then PIO dissolved in 0.1% DMSO was added to the incubation medium to achieve a final PIO concentration of 20 μg/ml and exposure to agonists was repeated in duplicate; rings were washed twice with fresh Krebs-Henseleit bicarbonate buffer between all exposures. For some of the NE experiments, endothelium was removed from aortic rings by gentle rubbing before the 60-min baseline equilibrium period.

In the second set of in vitro experiments, rings were preincubated in Krebs-Henseleit bicarbonate buffer without calcium for 60 min. Next, NE was added to the incubation medium to achieve a final concentration of 100 nmol/liter in the presence or absence of PIO, 20 μg/ml. After the contractile response attained a plateau, calcium was added to the medium to achieve a final concentration of 100 μmol/liter and tension was recorded after attainment of a new plateau. Rings were studied in duplicate (with and without PIO in alternating order), and contractile responses were expressed relative to the maximal contractile response observed in the presence of calcium and the absence of PIO.

Cultured smooth muscle cells. A7r5 cells derived from embryonic DB1X rat thoracic aortae were obtained from American Type Culture Collection (Rockville, MD). Cells were maintained in growth medium (DME with 10% FBS, 100 U/ml penicillin G, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin) and were incubated at 37°C in 100% humidity and 5% CO_2. Medium was replaced every 2 d. Confluent cells were detached by treating cells with 0.25% trypsin and 1 mM EGTA · 4Na in calcium- and magnesium-free balanced salt solution (GIBCO BRL, Gaithersburg, MD) for 4 min. Detached cells were centrifuged for 5 min at 1,000 rpm and suspended in Krebs solution containing 122 mM NaCl, 20 mM $NaHCO_3$, 5 mM KCl, 1.25 mM $MgCl_2$ · 1.6 mM $CaCl_2$, 5.5 mM glucose, 1% BSA, 0.03 mM EDTA · Na_2, and 0.1 mM L-ascorbic acid (pH 7.4) to achieve a final cell concentration of 10^6/ml. 1-ml aliquots of the cell suspensions were incubated with 4.5 mM Fluo-3 (Molecular Probes, Inc., Eugene, OR) for 30 min at 37°C. Cells were then washed twice with 4 ml of Krebs solution without BSA and resuspended in 1 ml of the same Krebs solution for fluorescence measurements. Intracellular fluorescence was recorded continuously (model F-2000 Fluorescence Spectrophotometer, Hitachi Instruments, San Jose, CA) for 2 min to assure a stable baseline reading, and then 0.5% DMSO was added to the incubation medium in the absence or presence of PIO at a final concentrations of 20, 50, or 100 μg/ml. 3 min later, AVP (20 nmol/l) (24) was added and continuous recording of fluorescence was continued for an additional 5 min. Intracellular calcium concentrations ($[Ca^{2+}]_i$) were calculated from the formula: $[Ca^{2+}]_i = K_d\,(F - F_{min})/(F_{max} - F)$, where K_d is 400 nm at vertebrate ionic strength, F is the fluorescence of the cell preparation under test conditions, F_{max} is the fluorescence after addition of 4 μM of the ionophore A23187 (Calbiochem, La Jolla, CA), and F_{min} in the fluorescence after addition of 2.5 mM $MnCl_2$ to quench both intracellular and extracellular dye fluorescence.

Statistical analysis

For in vivo studies, intergroup comparisons of body weights; food intake; fasting insulin, glucose, triglyceride and total and free magnesium concentrations; insulin sensitivity; and direct blood pressure measure-

Table I. Body Weights, Food Consumption, and Fasting Plasma Glucose, Insulin, and Triglyceride Concentrations of Four Groups Studied In Vivo

	Chow only (n = 17)	Fructose only (n = 17)	Chow + PIO (n = 11)	Fructose + PIO (n = 17)
Entire study				
Initial weight (grams)	296±5	296±4	310±5	297±6
Final weight (grams)	391±5	376±4*	404±4	396±7
Food consumption (kcal)	3,720±56	3,665±48	3,951±63†	3,947±74†
Week 7 on diets				
Fasting glucose (mmol/liter)	4.3±0.1	4.6±0.1	4.4±0.1	4.5±0.1
Fasting insulin (pmol/liter)	2.5±0.7	5.2±0.7*	2.5±0.8	2.2±0.6
Fasting triglycerides (mg/dl)	115±18	192±15**	114±26	92±19

Body weights and food were measured between 0800 and 1000 h. Initial and final weights were measured before and after 8 wk on the study diets. Food consumption was measured daily and is expressed as total kcal consumed during 8-wk study period. Plasma for glucose, insulin, and triglycerides was obtained from cut tail tips between 1500 and 1600 h after removal of food at 0800 h at the beginning of the 7th wk on the diets. * $P < 0.03$, ** $P < 0.001$ vs. other three groups; † $P < 0.05$ vs. same diet without PIO.

ments were compared by ANOVA with Fisher's LSD for the post hoc comparisons. Weekly tail cuff blood pressure measurements were compared by repeated measures ANOVA and Fisher's LSD.

For in vitro studies, contractile responses of aortic rings to single concentrations of AVP and KCl in the presence and absence of PIO were compared by unpaired two-tailed Student's t tests. ANOVA was used to compare contractile responses of aortic rings to multiple concentrations of NE, responses in the presence and absence of calcium, and peak intracellular calcium concentrations of a7r5 cells in response to AVP. All data are presented as means±SEM.

Results

In vivo studies

The fructose-only group gained less weight than the animals in the other three groups ($P < 0.03$; Table I), which gained similar amounts of weight during the 3-wk study period. The reduced weight in the fructose-only group occurred despite a total energy intake that was similar to the chow-only group (Table I). The two groups that received PIO consumed more calories than the other two groups ($P < 0.005$; Table I).

Fasting plasma glucose concentrations did not differ significantly among the four experimental groups at week 7 (Table I). Fasting insulin and triglyceride concentrations were increased approximately twofold in the fructose-only animals compared with the other three groups (Table I).

Plasma glucose concentrations measured during the low-dose and high-dose insulin infusion periods of the glucose clamps did not differ significantly among the four study groups (Table II). Likewise, there were no significant intragroup differences in glucose levels between the two clamp periods. Plasma insulin concentrations during the low-dose insulin period were highest in the fructose-only group and lowest in the chow + PIO group; insulin concentrations differed significantly only between those two groups. During the high-dose insulin infusions, plasma insulin concentrations were higher in the fructose-only and fructose + PIO groups than in the two groups that had not received a high-fructose diet ($P < 0.01$; Table II), suggesting reduced whole-body insulin clearance in the two fructose-fed groups. Insulin sensitivity, measured as the slope of the line relating plasma insulin concentrations to glucose infusion rates during the human insulin infusions, was lower in the fructose-only group than in the other three groups ($P < 0.03$; Fig. 1), which demonstrated similar insulin-mediated changes in glucose requirements during the clamps.

Systolic blood pressures measured by tail cuff did not differ significantly among the four study groups before randomization to their experimental diets (Fig. 2). In animals randomized to chow only, systolic pressure increased significantly during the first 4 wk after randomization but did not increase further during weeks 5–8 (Fig. 2). Animals randomized to the fructose-only diet exhibited a similar increase in systolic pressure during weeks 1–4 and a further increase during weeks 5–8, so that the pressures were significantly higher in the fructose-only than in the chow-only group during weeks 5–8 (137±2 vs. 130±2 mmHg, $P < 0.001$; Fig. 2). PIO prevented the rise in systolic blood pressure that occurred over 8 wk in both the chow-only and the fructose-only groups (Fig. 2). As a result, by weeks 5–8 the two PIO-treated groups had systolic blood pressures that were similar (119±3 for chow + PIO and 121±4 mmHg for fructose + PIO; P = NS) and that were significantly lower than pressures in their respective dietary control groups (fructose only and chow only; Fig. 2).

The effect of PIO to prevent the age-related rise in blood

Table II. Plasma Glucose and Insulin Concentrations during Glucose Clamps in the Four Groups Studied In Vivo

Infusion period	Diet	Plasma glucose	Plasma insulin
		mmol/liter	*pmol/liter*
Low-dose insulin	Chow only	7.8±0.1	7.8±0.3
	Fructose only	7.8±0.1	8.7±0.6
	Chow + PIO	7.7±0.1	6.8±0.3*
	Fructose + PIO	7.7±0.1	7.5±0.4
High-dose insulin	Chow only	7.8±0.1	17.7±0.5
	Fructose only	8.0±0.1	21.3±0.7**
	Chow+PIO	7.9±0.1	18.7±0.9
	Fructose+PIO	7.9±0.2	20.7±0.8**

Data are averages obtained during the final 30 min of the low-dose and high-dose insulin infusion periods of euglycemic clamps performed after 8 wk on the experimental diets. ** $P < 0.01$ vs. respective chow-fed group; * $P < 0.02$ vs. fructose only.

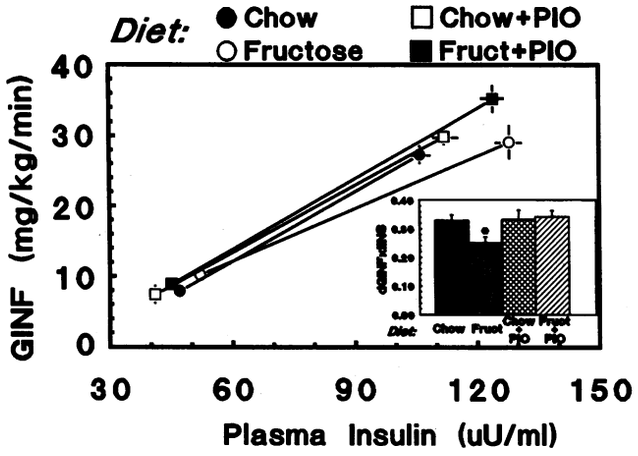

Figure 1. Relationships between exogenous glucose requirements (*GINF*) and plasma insulin concentrations (*INS*) during euglycemic clamps in four groups studied in vivo. Data are from the low-dose insulin infusion period (left set of four symbols) and the high-dose insulin period (right set of four symbols). Slopes of the lines connecting the symbols represent whole-body insulin sensitivity (dGINF:dINS; mg/kg/min per μU/ml), which is compared among the four study groups in the inset. * $P < 0.03$ vs. other three groups.

pressure in chow-fed rats was confirmed by direct carotid arterial measurements in a separate group of animals studied for 3 wk. Weekly tail cuff measures of systolic pressure in those rats were consistent with the pattern shown in Fig. 2; pressures were lower in the PIO-treated group within 2 wk. Direct measurements of mean arterial pressure at the end of the 3rd wk of treatment revealed a significantly lower value in PIO-treated animals than in animals that received chow alone (100±5 vs. 111±2 mmHg; $P < 0.02$).

Plasma total magnesium concentrations were lower in the fructose-only than in the chow-only group (Table III). PIO treatment lowered plasma total magnesium concentrations in both groups, so that the fructose + PIO group had the lowest plasma total magnesium concentrations overall (Table III). As was true for total magnesium, ionized magnesium concentrations were lower in the fructose-only than in the chow-only group (Table III). PIO tended to increase plasma ionized magnesium, in contrast to the effects of the drug on total magnesium concentrations. As a result, the fructose + PIO and chow + PIO groups had plasma ionized magnesium concentrations that were similar to the chow-only group (Table III).

In vitro studies

Aortic rings. PIO did not alter the resting tension of intact or denuded aortic rings (Fig. 3). Sequential exposure of intact rings to NE at concentrations of 25–150 nmol/liter produced dose-dependent vasoconstriction that was blunted by 29–41% during exposure to PIO, 20 μg/ml (Fig. 3, *top*). Blunting of similar magnitude (32–56%) was observed when endothelium-denuded rings were exposed to the same range of NE concentrations in the presence of PIO (Fig. 3, *bottom*). The full NE response was restored to intact and denuded rings after removal of PIO from the incubation medium (not shown). Contractile responses to 28 *m*mol/liter KCl (242±19 vs. 542±20 grams increase in tension; $P < 0.02$) and 0.66 *n*mol/liter AVP (264±19 vs. 345±5 grams increase in tension; $P < 0.02$) were also blunted by PIO, 20 μg/ml. Thus, PIO attenuated the effect of three different agonists in rat aortic rings through a mechanism that, at least in the case of NE, did not depend on the presence of an intact endothelium.

After a 1-h preincubation in calcium-free medium, the contractile response of intact rings to NE (100 nmol/liter) was only 17% of the response observed after acute addition of calcium to the same preparation in the presence of NE (Fig. 4). PIO did not alter the contractile response to NE in the absence of calcium but reduced by 42% the response that occurred after acute addition of calcium (Fig. 4).

A7r5 cells. Acute exposure of a7r5 cells to AVP at a final concentration of 20 nmol/liter resulted in an 11-fold increase in intracellular free calcium, from 171±9 to 1,873±279 nmol/

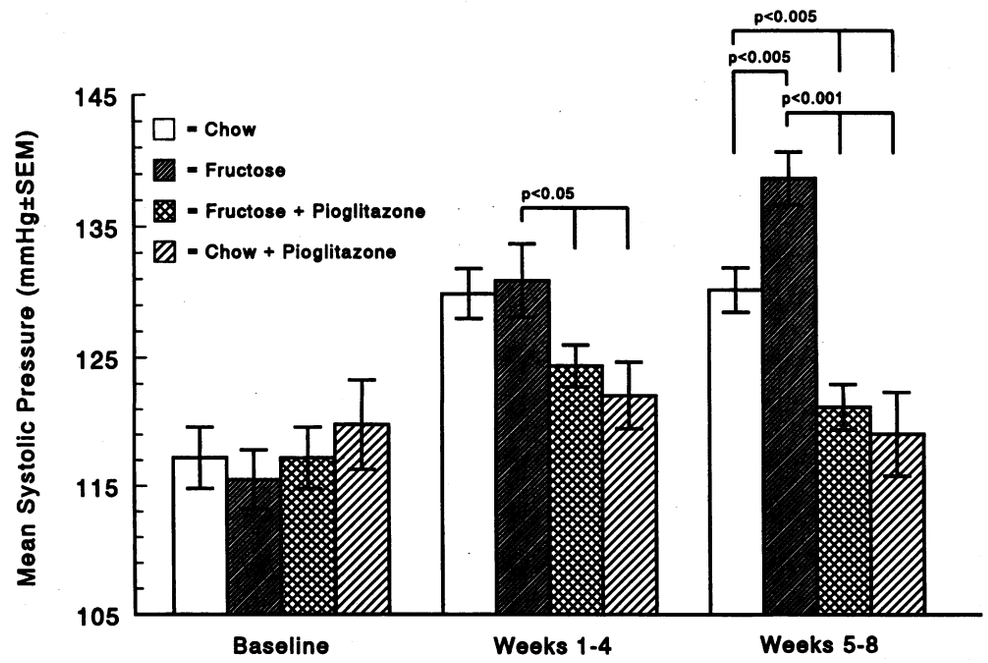

Figure 2. Systolic blood pressure measured weekly by tail cuff in the four groups studied in vivo. The left set of bars depicts baseline blood pressures measured on three separate days before assignment to the experimental diets. The middle and right sets of bars depict blood pressures during weeks 1–4 and 5–8 on the diets, respectively.

Table III. Total and Ionized Plasma Magnesium Concentrations in Four Groups Studied In Vivo

	Chow only	Fructose only	Chow + PIO	Fructose + PIO
Total Mg (mmol/liter)	0.629±0.004	0.579±0.013*	0.600±0.013*	0.525±0.017*†
Ionized Mg^{2+} (mmol/liter)	0.267±0.030	0.183±0.016*	0.279±0.023†	0.244±0.023†

Plasma was obtained from cut tail tips between 1500 and 1600 h after removal of food at 0800 h at the beginning of the 8th wk on the experimental diets. Total magnesium was measured by atomic absorption spectrophotometry and ionized magnesium by a Mg^{2+} sensitive electrode. * $P < 0.05$ vs. chow only; † $P < 0.05$ vs. fructose only.

liter. PIO in concentrations of 20, 50, and 100 μg/ml caused a dose-dependent reduction in peak calcium to responses that were 67%, 53%, and 19%, respectively, of the peak response observed in the absence of PIO (Fig. 5).

Discussion

The results of the present investigation indicate that PIO has a direct vasodepressor effect on vascular smooth muscle. The effect was suggested by our in vivo studies, which revealed that chow-fed and fructose-fed animals given PIO exhibited reductions in arterial blood pressure that could not be explained by alterations in whole-body insulin sensitivity or fasting insulin and glucose concentrations. The vasodepressor effect was confirmed by our in vitro studies of aortic rings, which revealed blunted contractile responses to three different vasoconstrictors. The blunting could not be explained by alterations in nitric oxide synthesis or action, because PIO blunted contractile responses to NE in the absence of the endothelium. That observation distinguishes PIO from both insulin and IGF-1, which blunt agonist-mediated contraction of aortic rings through an endothelium-dependent mechanism (23). The fact that PIO blunted contractile responses to NE in the presence but not in the absence of calcium, combined with previous observations (25, 26) that PIO and related compounds block agonist-mediated calcium uptake by vascular smooth muscle cells, suggests that the vasodepressor action was mediated by a blockade of agonist-mediated calcium uptake. Indeed, our studies of intracellular free calcium concentrations in a vascular smooth muscle cell line revealed a dose-dependent inhibition of AVP-mediated calcium uptake or release in those cells. Taken together, our data from whole animals, aortic rings, and vascular smooth muscle cells indicate that PIO lowered blood pressure through a mechanism that was not dependent on enhanced insulin sensitivity and that likely involved a direct action on vascular smooth muscle to reduce contractility by inhibiting agonist-mediated calcium uptake and/or intracellular release.

In addition to the direct vascular actions of PIO in vitro, the drug changed circulating magnesium concentrations in a direction that may have contributed to the lower blood pressure observed in PIO-treated animals, particularly during fructose-feeding. The high-fructose diet that we used is known to be relatively low in magnesium compared with standard rat chow, and the high-fructose diet lowers circulating magnesium con-

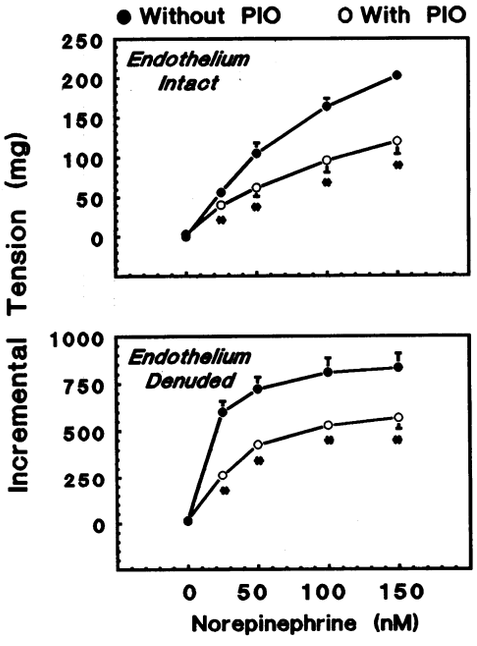

Figure 3. Effect of PIO on contractile responses of rat aortic rings to NE. Intact (*top*) and endothelium-denuded (*bottom*) rings from normal rats were preincubated for 60 min and then exposed to NE (25–150 nmol/liter) in the absence or presence of PIO, 20 μg/ml. Responses are expressed relative to a resting tension of 0.5g, which was unchanged after the addition of PIO alone (NE = 0 nmol/liter). * $P < 0.02$ vs. "without PIO" at same NE concentration. Note scale differences in *y*-axis between top and bottom panels.

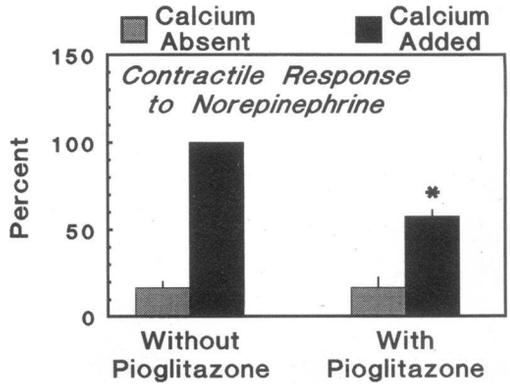

Figure 4. Effect of PIO on contractile responses of intact aortic rings to NE in the absence or presence of calcium. (*Left*) Bars depict plateau tension above basal observed when intact aortic rings were exposed continuously to NE (100 nmol/liter) in calcium-free medium, followed by acute addition of calcium to the medium to achieve a final concentration of 100 μmol/liter. (*Right*) Bars depict analogous experiments carried out in the presence of PIO, 20 μg/ml. A total of eight rings were studied with and without PIO in alternating order. Contractile responses are expressed relative to the full response observed for each ring in the presence of calcium and the absence of PIO. * $P < 0.002$ vs. "without PIO."

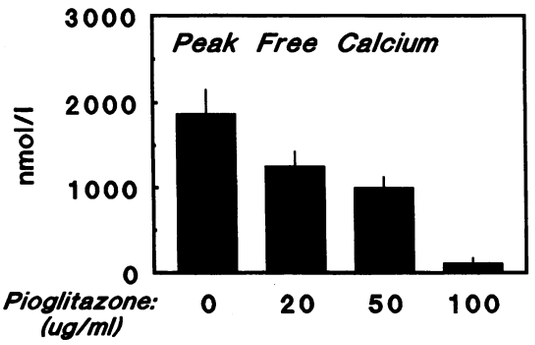

Figure 5. Effect of PIO on peak intracellular free calcium responses of a7r5 vascular smooth muscle cells to AVP, 20 nmol/liter. Free calcium was measured by Flou-3 during constant exposure to PIO at the doses indicated. PIO caused a dose-dependent reduction in peak calcium responses ($P < 0.04$ by analysis of linear tends).

centrations (21). Moreover, dietary magnesium supplementation ameliorates insulin resistance and elevated blood pressure in this fructose-fed model (21). Low magnesium concentrations have been associated with insulin resistance, elevated blood pressure, and enhanced vascular reactivity in other rat strains (27) and in humans (28–31), whereas magnesium administration has been shown to reduce vascular reactivity to pressors such as angiotensin II in normal men (31). Thus, restoration of serum ionized magnesium concentrations by PIO may have contributed to a reduction of vascular reactivity and a lowering of blood pressure during fructose feeding in the present study. However, two observations suggest that restoration of circulating ionized magnesium concentrations did not fully explain the hemodynamic actions of PIO in vivo. First, blood pressure was lower in the fructose-fed group that received PIO than in the chow-only group, despite the fact that plasma ionized magnesium concentrations were similar in those two groups. Second, PIO prevented the normal rise in blood pressure in chow-fed rats, but the drug did not increase plasma ionized magnesium concentrations in that group. Thus, alterations in circulating magnesium concentrations cannot fully explain the blood pressure patterns that we observed during PIO administration in vivo. Whether PIO had a direct effect to increase intracellular magnesium concentrations in vascular smooth muscle, as has been demonstrated recently for adipocytes studied in vitro (32), remains to be determined.

Dubey et al. (33) reported that PIO inhibited the effect of insulin, EGF, and FCS to induce both proliferation and ^{3}H-thymidine incorporation in a primary culture of arteriolar smooth muscle cells isolated from the preglomerular region of rat kidneys. Such an inhibitory effect, if operative in other vessels, could have contributed to the prevention of age-associated increases in blood pressure that we observed in our in vivo studies. However, we have not been able to demonstrate an inhibition by PIO of serum-mediated induction of protooncogenes or early growth response genes (c-*fos* and Erg-1) or serum-mediated stimulation of ^{3}H-thymidine incorporation in an established rat vascular smooth muscle cell line (A10 from American Type Culture Collection) or in an established mouse mesangial cell line (34) (Law, R., and Hsueh, W., unpublished observation). Thus, the effect of PIO to inhibit vascular proliferation will require further investigation before such an effect can be implicated in lowering of blood pressure that we observed in vivo.

Lee et al. (14) recently reported that troglitazone, a thiazolidinedione compound that is related chemically to PIO, maintained normal insulin action in rats fed a high-fructose diet for 3 wk. Those investigators also reported that tail cuff systolic blood pressures in fructose-fed rats remained similar to chow-fed rats, in contrast to the exaggerated lowering of systolic pressure that we observed when fructose-fed or chow-fed rats received PIO for 8 wk. The lowering of blood pressure during PIO administration was confirmed in chow-fed animals in our studies by direct arterial measurements after only 3 wk of drug administration. The reasons for the apparent interstudy differences in patterns of blood pressure are not clear, although differences in the ages of the rats that were studied and in the specific thiazolidinedione compounds that were administered may have contributed to the different blood pressure patterns.

Our findings and other reports of the effects of thiazolidinediones on blood pressure and insulin action may provide some important clues regarding the association between insulin resistance and hypertension. Blood pressure lowering has been reported during thiazolidinedione administration in a variety of settings characterized by normal as well as reduced insulin action on glucose metabolism. Specifically, thiazolidinediones have lowered blood pressure in several settings characterized by insulin resistance: obese Zucker rats (25); Dahl salt-sensitive rats (10, 33); fructose-fed rats (14, and present report); and humans with obesity, impaired glucose tolerance, or NIDDM (15–17). In each of those settings, the blood pressure–lowering effect was associated with an improvement in insulin action, suggesting that the two effects of thiazolidinediones might be linked mechanistically. By contrast, data from normal rats presented herein and data from Zhang et al. (10) and Dubey et al. (33) in rats with renovascular hypertension indicate that PIO can also lower blood pressure in rat models that are not characterized by whole-body insulin resistance. PIO did not alter insulin action in those rat models or in lean Wistar rats (11, 12). Thus, it appears that chronic administration of thiazolidinedione compounds frequently but not invariably (33) reduces blood pressure through a mechanism that may be related to inhibition of calcium entry into cells. The drugs also enhance insulin action in many animal models of insulin resistance and in insulin-resistant humans, but they have limited effects on insulin action in the absence of insulin resistance. Because increased cytosolic calcium has been associated with insulin resistance in vivo (35, 36) and in vitro (37), it is possible that inhibition of calcium entry into cells is involved not only in the hemodynamic actions of thiazolidinediones but also in the insulin-sensitizing effects of those drugs, particularly in states of insulin resistance. That possibility remains to be tested but, if proven, would provide one mechanistic link between insulin resistance and hypertension (38).

In summary, our findings indicate that PIO has direct vascular actions that may explain, at least in part, the blood pressure lowering that has been observed during chronic administration of the drug and related compounds in vivo. Whether the vascular effects of thiazolidinedione compounds contribute to their ability to improve insulin action in states of insulin resistance, perhaps by improving blood flow to insulin sensitive tissues (39, 40), and whether blockade of calcium entry is involved in the insulin-sensitizing effects of these drugs remains to be determined. Nevertheless, the dual impact of these agents on blood pressure and insulin action suggests they will have great utility in the treatment of conditions that are characterized by insulin resistance and hypertension.

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