Effects of Ciglitazone on Blood Pressure and Intracellular Calcium Metabolism

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Ciglitazone is the prototype of the thiazolidinedione class of compounds currently being developed for the treatment of insulin resistance and non-insulin-dependent diabetes. The effects of thiazolidinediones on blood pressure and cell calcium metabolism are not well defined. In the obese Zucker rat, a widely studied model of insulin resistance associated with mild hypertension, we investigated the effects of ciglitazone on plasma insulin levels and mean arterial pressure. We also evaluated the effects of ciglitazone on the changes in cytosolic calcium induced by platelet-derived growth factor in A172 human glioblastoma cells and rat A10 vascular smooth muscle cells. Oral administration of ciglitazone, approximately 45 mg/kg per day for 4 weeks, induced significant reductions in plasma insulin levels ($p<0.001$) and blood pressure ($p<0.05$). Ciglitazone was also found to significantly attenuate the capacity of platelet-derived growth factor BB homodimer to induce sustained increases in intracellular free calcium. These findings suggest that thiazolidinediones may offer a novel pharmacological approach to the treatment of hypertension, and raise the possibility that these compounds may affect blood pressure not only by affecting insulin metabolism but also by modifying the cell calcium response to pressor agents, growth factors, or both. (Hypertension 1993;21:1020-1023)

KEY WORDS • insulin • insulin resistance • platelet-derived growth factor • atherosclerosis • hypertension, essential

Numerous epidemiological and clinical studies have shown that hypertension is often associated with obesity, glucose intolerance, and non-insulin-dependent (type II) diabetes mellitus. Blood pressure tends to correlate positively with hyperinsulinemia, and lean, nondiabetic hypertensive individuals appear to be characterized by hyperinsulinemia and insulin resistance. These observations suggest that hypertension is an insulin-resistant state, and it has been proposed that insulin resistance, per se, is a pathogenetic determinant of increased blood pressure. Furthermore, hyperinsulinemia stimulates proliferation of vascular smooth muscle, modifies ion transport in a variety of tissues, enhances sympathetic nervous activity, and promotes renal retention of sodium. These are all potential mechanisms whereby disordered insulin metabolism might increase blood pressure.

The thiazolidinediones are a class of compounds being developed for the treatment of non-insulin-dependent diabetes mellitus. These drugs increase insulin sensitivity in insulin-resistant states and reverse the associated hyperinsulinemia, hyperlipidemia, hypercholesterolemia, and hyperglycemia. Given the possibility that insulin resistance and hyperinsulinemia contribute to hypertension, we hypothesized that administration of a thiazolidinedione might decrease blood pressure. To test this hypothesis, we determined whether the prototype thiazolidinedione, ciglitazone, could decrease blood pressure in the fatty Zucker rat, a rodent model of insulin resistance and mild hypertension. Because agonist-induced changes in cell calcium metabolism have been proposed to play important roles in the pathogenesis of hypertension and atherosclerosis, we also determined the effects of ciglitazone on increases in intracellular free calcium induced by platelet-derived growth factor (PDGF).

Methods

Whole Animal Studies

Two groups of 6-week-old female obese Zucker rats were purchased from Charles River Laboratories, Inc., Wilmington, Mass. Previous studies have shown that these rats are characterized by mild hypertension, insulin resistance, and hyperinsulinemia. The experimental group was fed a standard rat diet containing 0.05% (wt/wt) ciglitazone; the control group was given the same diet without ciglitazone. The rats were individually housed in metabolic cages, and daily food intake was measured for each rat. Twenty-four-hour urine collections were obtained for measurements of urine output and urine sodium concentration (flame photometry). The amount of ciglitazone ingested by the experimental group was 1.1 to 1.9 μmol/kg body wt per day over the duration of the study. After 4 weeks, mean arterial pressure was measured with rats in the unanesthetized,
unrestrained state through indwelling femoral arterial catheters as previously described.8 Blood pressure measurements were obtained 4 hours after rats had recovered from methoxyflurane anesthesia and were fully awake and resting comfortably in their cages. After blood pressure measurements were obtained, blood was collected for determination of plasma insulin concentration by standard radioimmunoassay techniques. Blood samples were obtained 8 hours after cessation of food intake. Ciglitazone was a gift from The Upjohn Co., Kalamazoo, Mich., where the insulin assays were also performed.

Cellular Studies

Effects of ciglitazone on platelet-derived growth factor–induced changes in intracellular calcium. The methods used for growth and maintenance of A172 human glioblastoma cells and rat A10 vascular smooth muscle cells and for measurement of intracellular free calcium concentration ([Ca$^{2+}$]) have been described in detail elsewhere.9 This cell line was chosen because it lacks voltage-sensitive calcium channels, and we had previous experience with the effects of PDGF on [Ca$^{2+}$] metabolism in these cells.9 PDGF was chosen because of its well-known effects on vascular smooth muscle contraction, cell proliferation, and chemotaxis.10 The PDGF used in these studies was the purified BB homodimer (G. Pierce, Amgen Biologicals, Thousand Oaks, Calif.).

Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and seeded at low density in microscope slide chambers and grown to confluence. The cells were washed, starved by incubation in serum-free DMEM for 3 to 4 hours at 37°C, and loaded with the fluorescent intracellular calcium indicator indo 111 by adding 3 μmol/L indo 1 acetoxymethyl ester (indo 1-AM) for 30 minutes. The effects of PDGF (0.67 nmol/L) on the intracellular calcium signal were studied in control cells and in cells pretreated with ciglitazone. This concentration of PDGF was chosen because it lies within the physiological range and is approximately twice the half-maximal concentration on the PDGF dose–response curve.9 Ciglitazone treatment consisted of its addition from a stock solution in dimethyl sulfoxide simultaneously with indo 1-AM. Final concentrations of the drug and dimethyl sulfoxide were 6.3 μmol/L and 10 mmol/L, respectively. After incubation for 30 minutes at 37°C, the medium containing indo 1-AM (with or without ciglitazone) was removed, and the cells were washed three times and incubated in fresh serum- and phenol red–free DMEM for an additional 45 minutes to ensure complete hydrolysis of indo 1-AM intracellularly. To confirm that ciglitazone did not cause a nonspecific increase in membrane calcium leak, we performed separate experiments in which the intracellular calcium signal was monitored in cells incubated in 2 mmol/L manganese chloride. The fluorescence intensity of intracellular indo 1 was subjected to image analysis on an ACAS 470 laser microspectrofluorometric imaging cytometer (Meridian Instruments Inc., Okemos, Mich.).9 Relative changes in [Ca$^{2+}$], are expressed as changes in the ratio of the fluorescence intensities at 405 and 485 nm, the emission maxima for the indo 1–Ca$^{2+}$ complex and the free indo 1, respectively.

Results

Effects of Ciglitazone on Plasma Insulin and Blood Pressure in Fatty Zucker Rats

Oral administration of ciglitazone to obese Zucker rats caused a 55% reduction in the mean plasma insulin concentration (Figure 1A). The reduction in plasma insulin was highly significant (p<0.001 by t test) and of a magnitude similar to that previously reported in ciglitazone-treated obese rats by Fujita et al.7 Although we did not measure plasma concentrations of glucose, previous investigators have found that in fatty Zucker rats, the reductions in plasma insulin induced by ciglitazone are not accompanied by increases in circulating concentrations of glucose.7

Oral administration of ciglitazone to obese Zucker rats caused a modest but statistically significant (p<0.05 by t test) reduction in blood pressure (Figure 1B). The blood pressures of the ciglitazone-treated fatty Zucker rats were similar to those of normotensive Lewis rats and lean Zucker rats.8 There was no significant difference between mean body weight and food intake (measured weekly) of control versus ciglitazone-treated rats (data not shown).
The urine output of rats treated with ciglitazone (97±8 mL/wk) was greater than that of controls (80±5 mL/wk, p<0.05 by t test). However, the urine concentration of sodium was lower in the ciglitazone-treated animals, so urinary excretion of sodium was not different between control rats and those treated with ciglitazone. Whether the difference in urine output was related to an effect of ciglitazone on fluid intake or on renal function is unknown.

**Effects of Ciglitazone on the Platelet-Derived Growth Factor-Induced [Ca²⁺]i Signal in A172 Cells**

The effect of PDGF on [Ca²⁺]i in A172 cells is shown in Figure 2A and represents the composite signal obtained by scanning eight adjacent cells. The vertical bar indicates the time at which PDGF (0.67 nmol/L) was added to the well containing the cells. There was an initial delay followed by a sharp rise in [Ca²⁺], to a peak extending to a plateau phase lasting approximately 3 minutes, followed by a gradual decay in the calcium signal. In our previous studies of the effects of PDGF on [Ca²⁺], in A172 cells, the presence of EGTA in the bathing medium eliminated the secondary sustained [Ca²⁺] phase without affecting the initial [Ca²⁺] peak, suggesting that this initial peak is caused by the release of calcium from intracellular stores. Restoration of the secondary phase by adding excess Ca²⁺ extracellularly led to the conclusion that this phase of the PDGF Ca²⁺ signal was the result of Ca²⁺ entry across the plasma membrane. Our previous work showed that PDGF does not depolarize A172 cells but rather imposes a slight hyperpolarized state.⁹

The effect of ciglitazone (6.3 µmol/L) on the [Ca²⁺]i transient signal evoked by PDGF is shown in Figure 2B. Treatment with ciglitazone had no significant effect on the resting or peak value of [Ca²⁺], attained after addition of PDGF (0.67 nmol/L). However, the secondary phase of the calcium signal, the portion mediated by Ca²⁺ influx across the plasma membrane (via the opening of PDGF-operated Ca²⁺ channels), was abolished by this concentration of ciglitazone. The result shown in Figure 2B represents the composite signal obtained by scanning 10 adjacent cells.

The effects of PDGF and ciglitazone on various parameters relating to [Ca²⁺]i are summarized in Table 1. The data were obtained by independently evaluating each of the 8 and 10 cells used to generate Figures 2A and 2B, respectively. The data in Table 1 confirm the lack of a significant effect of ciglitazone on the prepeak delay or the initial calcium peak response. On the other hand, the prolonged postpeak elevation in [Ca²⁺]i was virtually eliminated at this concentration of ciglitazone (Table 1 and Figure 2B). The addition of excess Ca²⁺ to the bathing medium failed to restore the secondary phase of the PDGF-induced Ca²⁺ response blocked by ciglitazone.

In studies in cells incubated with manganese, we found no evidence suggesting an effect of ciglitazone on membrane leakiness to calcium. The addition of manganese chloride to cells treated with ciglitazone was associated with a small, continuous decrease in fluorescence similar to that observed in cells not treated with ciglitazone (data not shown). Experiments carried out in cultured A10 rat vascular smooth muscle cells yielded results similar to those obtained with the A172 cells, namely, that ciglitazone eliminated the secondary sustained phase of the calcium transient induced by PDGF without affecting the initial peak response (data not shown). The effect of ciglitazone appeared to be maximal at the concentration used (6.3 µmol/L).

**Discussion**

In the insulin-resistant obese Zucker rat, we have found that oral administration of ciglitazone reduces...
plasma concentrations of insulin and reduces blood pressure. In a preliminary study, Fujiwara et al. reported that administration of the thiazolidinedione CS-045 to obese Zucker rats also decreases blood pressure. Although these findings are consistent with the hypothesis that hyperinsulinemia, insulin resistance, or both may contribute to increased blood pressure, they do not prove a causal relation between disordered insulin metabolism and hypertension. It is possible that the effects of thiazolidinediones on blood pressure are independent of those on insulin metabolism. In fact, Kotchen and colleagues have recently reported that oral administration of pioglitazone can decrease blood pressure in the one-kidney, one clip renal hypertensive rat, a model of high blood pressure that is not thought to be dependent on disordered insulin metabolism. It is also possible that some of the effects of thiazolidinediones on insulin metabolism are secondary to thiazolidinedione-induced metabolic changes leading to increased tissue perfusion and increased glucose uptake.

In the present study, we found that pretreatment of human A172 glioblastoma cells and rat aortic smooth muscle cells with ciglitazone can block the secondary phase calcium signal evoked by PDGF. Agonist-mediated calcium entry into mammalian cells occurs via multiple mechanisms, most notably increased conductance of Ca^{2+} ions through voltage-operated and receptor-operated calcium channels. In a previous study in A172 cells, we found that the initial peak in cytosolic calcium induced by PDGF is the result of release of Ca^{2+} from intracellular stores and that the second phase of the calcium signal evoked by PDGF is due to influx of calcium from the extracellular environment. Because the calcium signal generated by PDGF in A172 cells is unaffected by concentrations of verapamil, diltiazem, or nifedipine, known to inhibit voltage-operated calcium channels, it appears that the PDGF-induced influxes of calcium may involve a receptor-operated calcium channel.

The current findings suggest that ciglitazone blocks the portion of the PDGF-stimulated calcium transient that is mediated by receptor-operated calcium channels in the plasma membrane of A172 human glioblastoma cells. The fact that the other parameters of the calcium signal remained unaffected also suggests that the effect of ciglitazone is relatively selective for the ionic mechanisms that govern the secondary phase of the calcium signal. Based on the experiments with manganese, the changes in intracellular calcium induced by ciglitazone do not appear to be secondary to a nonspecific effect on membrane leakiness to calcium. It remains to be determined whether the effect of ciglitazone on the second phase calcium response is due to direct blockade of a plasma membrane calcium channel or blockade of some distal step in the transduction process that initiates the second phase. The present observations raise the possibility that thiazolidinediones may lower blood pressure not just by affecting insulin metabolism but also by attenuating calcium-dependent contractile and proliferative responses to vasoactive agonists such as PDGF.

PDGF may play important roles in thrombus formation, vascular smooth muscle cell proliferation and migration, and myointimal hyperplasia, processes critical to the pathogenesis of coronary heart disease. Given that ciglitazone can attenuate the PDGF-induced increases in intracellular calcium and given its capacity to ameliorate other major risk factors for cardiovascular disease (i.e., hypertension, insulin resistance, and hyperlipidemia), the thiazolidinediones may represent a particularly potent tool for the prevention of coronary artery disease. If ciglitazone and related compounds do prove capable of blocking PDGF receptor-operated calcium channels, the thiazolidinediones may represent candidates for a novel class of calcium antagonists. At the moment, the precise mechanisms whereby thiazolidinediones affect cell calcium metabolism are unknown and require more detailed investigation.

References
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