Acute Effects of Troglitazone on In Vivo Insulin Action in Normal Rats

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Troglitazone is a newly developed antidiabetic agent that shows hypoglycemic effects in insulin-resistant animal models and non–insulin-dependent diabetic humans. To determine whether this drug could affect in vivo insulin action acutely, insulin-stimulated glucose utilization was measured with the euglycemic glucose clamp technique before, during, and after troglitazone infusion (20 μg/min) in normal rats. Hepatic glucose production (HGP) was measured with a tracer-dilution technique (D-[3-^{3}H]-glucose). At 18-pmol/kg/min insulin infusion rate, steady-state glucose disposal rate (GDR) was significantly increased during troglitazone infusion versus control vehicle infusion (162 ± 6.1 *v* 142.3 ± 4.4 μmol/kg/min, $P < .02$). The glucose infusion rate (GIR) required to maintain euglycemia increased shortly (10 to 20 minutes) after initiation of troglitazone infusion and was significantly greater until 30 minutes after cessation of the drug versus the vehicle infusion. At 9-pmol/kg/min insulin infusion rate, HGP was significantly decreased during troglitazone infusion as compared with control vehicle infusion (21.7 ± 3.5 *v* 39.5 ± 3.7 μmol/kg/min, $P < .02$). These results indicate that troglitazone can acutely increase in vivo insulin action in normal rats, and some possible mechanisms are discussed.

THIAZOLIDINEDIONES represent a new class of oral hypoglycemic agents that exert effects by enhancing insulin action in vitro[1] and by improving insulin resistance in vivo in insulin-resistant animal models[2] and in man.[3,4] Troglitazone is a member of this new class of drugs and exerts potent glucose-lowering effects in insulin-resistant diabetic animals[5] and in non–insulin-dependent (type II) diabetic human subjects.[3,4] Thus, when insulin-resistant diabetic animals or patients are treated with the drug, a number of beneficial effects have been reported, including reduction in glucose, insulin, and triglyceride levels, improvement in insulin resistance, and a decrease of elevated rates of hepatic glucose production (HGP).[3,5] However, in long-term treatment studies, one cannot deduce which effects are primary and which are secondary, nor do these studies provide major insights into the mechanisms of action. Consequently, in the present study we examined the acute effects of intravenous infusion of troglitazone, and determined whether its ability to enhance insulin action was direct and whether skeletal muscle and/or liver were involved.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (200 to 225 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and housed individually in separate cages in an environmentally controlled room with a 12-hour light/dark cycle (light 6 AM to 6 PM). They were fed normal pelleted laboratory chow (Harlan Teklad, Madison, WI) and water ad libitum. After 1 week of acclimation, they were anesthetized with ketamine 50 mg/kg, xylazine 4.8 mg/kg, and acepromazine 1 mg/kg intramuscularly. A midline ventral incision was made in the anterior neck, and the right jugular vein and left carotid artery were catheterized with small Tygon tubing (ID 0.015 and OD 0.030 in; Norton, Akron, OH). The catheters were tunneled subcutaneously around the side of the neck to the back of the head, exteriorized through the skin, and filled with heparin solution. Catheters were flushed every other day until the glucose clamp study. The average body weight at the time of glucose clamp studies was 245 ± 4 g (N = 24).

Euglycemic Glucose Clamp Studies

After allowing at least a 1-week postoperative recovery period, euglycemic-hyperinsulinemic clamp studies were performed. All studies were conducted in the morning after a 12-hour overnight fast. Animals were placed in a restraining cage (the animals had been accustomed to the cage previously), and extension tubings were attached to the jugular vein by an adapter so that glucose, insulin, D-[3-^{3}H]-glucose, and troglitazone could be infused simultaneously into the jugular vein. The carotid catheter was used for blood sampling. A primed-continuous infusion of D-[3-^{3}H]-glucose 0.07 μCi/min (17.70 Ci/mmol; New England Nuclear, Boston, MA) in saline was administered throughout the study to determine overall rates of glucose metabolism,[7] and HGP was calculated according to Steele's equation.[8] After a 60-minute basal period, a primed-continuous infusion of human regular insulin (Eli Lilly & Co, Indianapolis, IN) at the rate of either 18 or 9 pmol/kg/min was administered throughout the study, and plasma glucose concentration was kept constant at 5.6 mmol/L by a variable infusion of 20% dextrose solution. Troglitazone was dissolved in dimethylsulfoxide/phosphate-buffered saline at a final concentration of 0.77 mg/mL and infused at a rate of 20 μg/min. The infusion protocol consisted of three consecutive phases: phase I was a 90-minute infusion of insulin at either 18 or 9 pmol/kg/min, by which time steady-state insulin levels and glucose disposal rates (GDRs) are reached; phase II was a 60-minute simultaneous infusion of insulin 18 or 9 pmol/kg/min plus troglitazone 20 μg/min or vehicle dimethylsulfoxide/phosphate-buffered saline; and phase III was another 60-minute period of insulin infusion alone. Animals were killed on completion of the experiments, and this study was approved by the Animal Subjects Committee of the University of California, San Diego.

Analytical Procedures

Plasma glucose level was measured by the glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA), and plasma insulin level was measured by a double-antibody

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radioimmunoassay.[9] Plasma glucose specific activity was measured in duplicate after deproteination with perchloric acid.[10]

All data are expressed as the mean ± SE, and statistical analyses were performed with Student's unpaired *t* test. All studies used the euglycemic clamp technique in chronically catheterized conscious rats.

RESULTS

The basic study design was to infuse insulin at either 9 or 18 pmol/kg/min for a 90-minute period, at which point steady-state stimulation of glucose disposal was achieved. At this point, a concomitant infusion of troglitazone (20 μg/min) or vehicle was begun and maintained for 60 minutes; after 60 minutes of combined insulin plus troglitazone or vehicle infusion, troglitazone or vehicle was discontinued and the insulin infusion was maintained constant for a further 60 minutes. During this time, glucose was maintained at euglycemic values and insulin concentrations remained constant. Glucose and insulin values during glucose clamp studies are listed in Table 1. Steady-state glucose and insulin levels were constant during all three phases of the infusion studies at both 9- and 18-pmol/kg/min infusion rates.

During the 18-pmol/kg/min insulin infusion, the glucose infusion rate (GIR) required to maintain euglycemia during the clamp study reached a steady state at 60 minutes, and when troglitazone was coinfused with insulin, GIR increased shortly (10 to 20 minutes) after initiation of the drug (Fig 1A). The difference in GIR between troglitazone and vehicle groups became statistically significant from 30 minutes after initiation of the drug onward until 30 minutes after cessation of the drug. GIR gradually declined after troglitazone infusion was stopped, but still did not return to predrug levels at 60 minutes after cessation. During the control vehicle infusion, no change in GIR was observed through the three phases of the study. Isotopically measured steady-state incremental GDR above the basal rate

Table 1. Glucose (mmol/L) and Insulin (pmol/L) Levels During Glucose Clamp Studies

Insulin Infusion Rate	Glucose Clamp Study			
	Basal	Phase I	Phase II	Phase III
18 pmol/kg/min				
Troglitazone (n = 11)				
Plasma glucose	6.3 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.7 ± 0.2
Plasma insulin	30 ± 6	456 ± 24	426 ± 30	468 ± 24
Vehicle (n = 4)				
Plasma glucose	7.5 ± 0.2	5.7 ± 0.2	5.6 ± 0.1	5.7 ± 0.2
Plasma insulin	26 ± 8	448 ± 18	432 ± 24	452 ± 30
9 pmol/kg/min				
Troglitazone (n = 5)				
Plasma glucose	7.2 ± 0.3	5.8 ± 0.2	5.7 ± 0.2	5.7 ± 0.2
Plasma insulin	22 ± 6	118 ± 24	120 ± 18	126 ± 18
Vehicle (n = 4)				
Plasma glucose	6.8 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	5.8 ± 0.3
Plasma insulin	24 ± 6	122 ± 18	116 ± 18	124 ± 24

NOTE. Data are the mean ± SE. During clamp studies, insulin was infused throughout the three phases of the study. In phases I and III, only insulin was infused, and in phase II, insulin plus troglitazone or vehicle was infused. Values were not different between troglitazone and vehicle groups at both 18- and 9-pmol/kg/min insulin infusion.

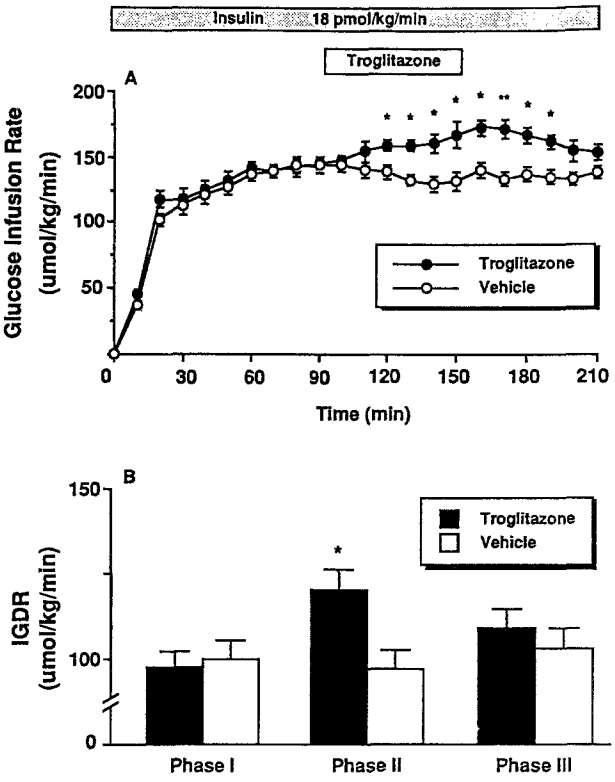

Fig 1. Effect of troglitazone on insulin action in vivo. (A) GIR required to maintain euglycemia during glucose clamp study at insulin infusion rate of 18 pmol/kg/min. GIR rapidly increased upon initiation of troglitazone infusion and gradually decreased after cessation of the drug. *$P < .05$ *v* vehicle, **$P < .01$ *v* vehicle. (B) IGDR above the basal rate during glucose clamp study. IGDR was significantly greater during troglitazone infusion versus vehicle infusion. *$P < .02$ *v* vehicle.

(IGDR) was significantly greater during troglitazone infusion as compared with vehicle infusion (120.5 ± 5.7 *v* 97.4 ± 5.5 μmol/kg/min, $P < .02$; Fig 1B). At these insulin levels, HGP was completely suppressed throughout the study. Thus, short-term intravenous administration of troglitazone leads to a prompt increase in insulin-stimulated GDR.

At this relatively high level of insulin infusion, HGP was completely suppressed, and we were thus unable to assess the effects of troglitazone on insulin's ability to inhibit glucose production. Consequently, additional studies were performed at a lower insulin infusion rate (9 pmol/kg/min), which should lead to only partial inhibition of hepatic glucose metabolism. During this lower-dose insulin infusion, HGP was only minimally inhibited during the first insulin period, but was significantly suppressed ($P < .02$) by 45% when troglitazone was added to the infusate as compared with the vehicle infusion period (Fig 2). Once the drug was stopped and insulin continued, HGP increased again to predrug levels. At this lower insulin infusion rate, there was no significant change in GDR during the study.

DISCUSSION

Troglitazone is a member of the thiazolidinedione class of hypoglycemic compounds, which exert biologic effects by

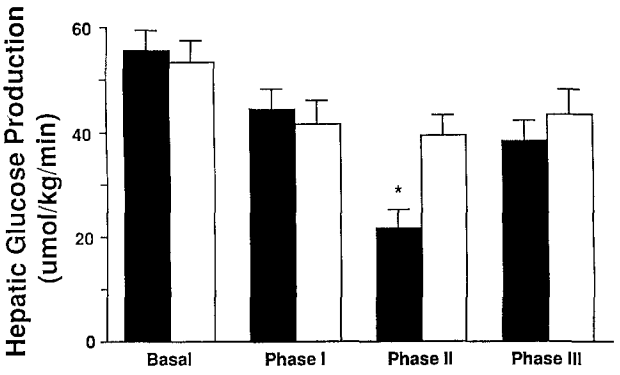

Fig 2. Steady-state HGP during glucose clamp study at the lower insulin infusion rate (9 pmol/kg/min). HGP was significantly decreased during (■) troglitazone infusion *v* (□) vehicle infusion. *$P < .02$ *v* vehicle.

ameliorating insulin resistance and thereby improving insulin action.[11] The drug exerts potent glucose- and insulin-lowering effects in several obese, hyperinsulinemic, diabetic animal models such as KK mice, C57BL6J-ob/ob mice, and Zucker fatty rats, and a relatively rapid (within 2.5 hours) glucose-lowering effect of troglitazone in diabetic KK mice has been reported.[2] In addition, clinical studies have shown that troglitazone improves insulin resistance and decreases plasma glucose and insulin levels in non–insulin-dependent diabetic subjects.[3]

Although this drug demonstrates clinical efficacy in man and is undergoing extensive evaluation in clinical trials, relatively little is known about its mechanisms of action. For example, in long-term treatment studies of man or animals, it is not possible to ascertain which of the effects of the drug are primary and which are secondary. Since increased hepatic glucose output, due at least partly to hepatic insulin resistance, and decreased insulin-mediated skeletal muscle glucose uptake, also due to insulin resistance, are two characteristic features of non–insulin-dependent diabetes mellitus,[12] we sought to determine whether intravenous infusions of troglitazone could exert short-term effects on liver and skeletal muscle insulin action in vivo. Thus, insulin was infused at the rate of either 18 or 9 pmol/kg/min in a group of normal rats for 210 minutes; during the middle 60-minute period of this insulin infusion, a simultaneous infusion of troglitazone 20 μg/min or vehicle was also administered.

Our results show that troglitazone infusion increased the overall in vivo GDR at 18-pmol/kg/min insulin infusion. These effects were seen within 10 to 20 minutes after initiation of drug administration, demonstrating the short-term effect of troglitazone to potentiate insulin-stimulated glucose disposal. During the third phase of the infusion protocol, troglitazone infusion was discontinued but insulin administration was maintained. During this period, GDRs decreased slightly but did not return to predrug levels, indicating that the insulin-potentiating effect of troglitazone lasted at least 60 minutes after cessation of drug administration.

Since approximately 80% of overall in vivo insulin-stimulated glucose disposal involves uptake into skeletal muscle, one can infer from our glucose clamp studies that troglitazone augments insulin-stimulated glucose uptake into skeletal muscle. This finding would be completely consistent with recent reports from Horikoshi et al,[13] which demonstrate that troglitazone is able to potentiate insulin-stimulated glucose uptake into the perfused rat hindlimb.

During the higher-dose insulin infusion, HGP was completely suppressed, and we were therefore unable to assess the effects of troglitazone on hepatic glucose metabolism. However, at the lower rate of insulin infusion (9 pmol/kg/min) HGP was only partially suppressed, and during the period of simultaneous troglitazone plus insulin administration, suppression of HGP was 45% greater than for control vehicle infusion.

Although we cannot rule out the possibility that the short-term hepatic effect of troglitazone may be due to an effect on gluconeogenic substrate availability, it is more likely that troglitazone directly augments insulin's ability to suppress HGP under these circumstances.[14] There were no significant changes in GDR during the study at this lower rate of insulin infusion. This suggests that troglitazone needs an insulin concentration high enough to stimulate GDR before insulin-potentiating effects are manifested.

The cellular mechanisms underlying these drug-related effects are not elucidated by the present studies. However, since troglitazone rapidly improves insulin's biologic effects to stimulate glucose disposal and suppress HGP, one must postulate that the site of action of this drug is rather early in the insulin-action cascade and common to both of these effects of insulin in these two different tissues, or that the drug has multiple sites of action such that it could enhance these two different biologic effects.

In summary, we have demonstrated that troglitazone exerts rapid in vivo effects to potentiate insulin action in liver and skeletal muscle. Thus, the drug augmented insulin's effect to suppress hepatic glucose output at the same time it increased insulin's ability to stimulate glucose disposal. Given the rapidity of these actions, it is likely that both represent direct effects of the drug on these two different tissues. Since increased hepatic glucose output and decreased insulin-stimulated glucose disposal are characteristic features of non–insulin-dependent diabetes mellitus, these studies emphasize potential beneficial effects of this drug on the metabolic defects present in this disease.

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