Effects of Troglitazone on Dexamethasone-Induced Insulin Resistance in Rats

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Troglitazone, a thiazolidinedione derivative, has been shown to counteract insulin resistance in obesity and non–insulin-dependent diabetes mellitus (NIDDM). To test its effects on dexamethasone-induced insulin resistance, we measured hepatic glucose production (HGP) and the insulin-stimulated glucose disposal rate (Rd) by a euglycemic-hyperinsulinemic glucose clamp technique coupled with 3-^{3}H-glucose infusion in male Wistar rats treated with low-dose dexamethasone ([LoDex] 0.05 mg/kg/d, n = 7), high-dose dexamethasone ([HiDex] 0.1 mg/kg/d, n = 7), or dexamethasone plus troglitazone (LoDex + T, n = 8; HiDex + T, n = 6). Dexamethasone was injected subcutaneously for 4 days. Troglitazone was administered orally at 20 mg/d for 3 days before and for 4 days along with the dexamethasone treatment. The glucose clamp study was performed after an overnight fast in chronically catheterized conscious rats with a continuous insulin infusion of 57.4 pmol/kg/min. Basal HGP was comparable among the control (45.8 ± 2.1 μmol/kg/min, n = 7), LoDex (47.9 ± 4.7 μmol/kg/min), LoDex+T (46.0 ± 2.6 μmol/kg/min), and HiDex+T (54.7 ± 3.4 μmol/kg/min) groups. It increased about twofold in the HiDex group (80.1 ± 5.2 μmol/kg/min, $P < .05$ *v* control). Under hyperinsulinemia, HGP was suppressed to a similar level in the control (11.3 ± 8.8 μmol/kg/min), LoDex (10.2 ± 8.4 μmol/kg/min), and LoDex+T (7.8 ± 7.9 μmol/kg/min) groups. The suppressive effect of insulin on steady-state HGP during the clamp was impaired in HiDex (63.7 ± 9.7 μmol/kg/min, $P < .05$) and HiDex+T (64.0 ± 6.5 μmol/kg/min, $P < .05$). Rd decreased 27% in LoDex (81.5 ± 5.8 μmol/kg/min, $P < .05$) and 36% in HiDex (71.3 ± 9.4 μmol/kg/min, $P < .05$) compared with the controls (111.4 ± 7.4 μmol/kg/min). Troglitazone prevented the decrease in Rd in LoDex+T (102.6 ± 5.7 μmol/kg/min), but not in HiDex+T (67.0 ± 6.4 μmol/kg/min). These results indicate that the development of peripheral insulin resistance was prevented by troglitazone in LoDex rats. Troglitazone may be a useful drug to treat steroid-induced diabetes.

TROGLITAZONE, a thiazolidinedione derivative, has been shown to reduce hyperglycemia and hyperinsulinemia effectively in animals with obesity and non–insulin-dependent diabetes mellitus (NIDDM)[1,2]. Several clinical trials have demonstrated its efficacy in humans with NIDDM[3-5] or impaired glucose tolerance.[6] The antidiabetic effects of troglitazone and other thiazolidinedione are clearly different from those of sulfonylureas in that they appear to work by either mimicking or enhancing insulin action without stimulating insulin secretion. Although the efficacy of troglitazone was documented initially in animal models with insulin resistance of genetic origin,[1] it may also be effective in ameliorating postnatally acquired insulin resistance such as that induced by diet[7] and hyperglycemia.[8,9] Thus, troglitazone may play a key role in the treatment of insulin-resistant diabetes of various etiologies, although not all insulin-resistant states respond to it.[10] Recently, in vitro studies demonstrated that englitazone[11,12] and pioglitazone,[12] the other members of the thiazolidinedione family, partially ameliorated the impairment of cellular glucose transport induced by dexamethasone. Therefore, the present study was designed to quantitatively measure the in vivo effects of troglitazone on dexamethasone-induced hepatic and peripheral (skeletal muscle and adipocyte) insulin resistance in rats.

MATERIALS AND METHODS

Male Wistar rats weighing 180 to 250 g were housed individually in cages in a temperature-controlled room with a 12-hour light/dark cycle and free access to rodent food and water.

Drug Administration.

The rats were allocated to one of the following five treatment groups: (1) low-dose dexamethasone ([LoDex] 0.05 mg/kg/d, n = 7), (2) low-dose dexamethasone plus troglitazone ([LoDex+T] n = 8), (3) high-dose dexamethasone ([HiDex] 0.1 mg/kg/d, n = 7), (4) high-dose dexamethasone plus troglitazone ([HiDex+T] n = 6), and (5) control (n = 7). Dexamethasone sodium phosphate (Sankyo, Tokyo, Japan) was injected subcutaneously in the evening once daily for 4 days. Saline or 10 mg troglitazone (Sankyo) suspended in 0.5% carboxymethyl cellulose (Nacalai Tesque, Kyoto, Japan) saline was administered to each group of animals by gastric gavage twice daily for 3 days before and for 4 days along with the dexamethasone treatment. Control rats received a saline subcutaneous injection daily for 4 days and a saline gastric gavage twice daily for 7 days. Dexamethasone treatment resulted in decreased weight gain or even weight reduction in rats. Therefore, to match the body weight at the time of the glucose clamp study, rats that were 20 to 30 g heavier than the control rats were entered into the dexamethasone treatment group.

Glucose Clamp Study

A euglycemic-hyperinsulinemic glucose clamp study was performed with chronically catheterized conscious rats on the day after completing dexamethasone treatment. Surgery to place indwelling catheters in the rats was performed 3 days before the glucose clamp study. Polyethylene tubing (Intramedic PE-10 and PE-50; Becton Dickinson, Sparks, MD) filled with polyvinylpyrrolidone (PVP) solution consisting of 500 mg polyvinylpyrrolidone (K-90; Nacalai Tesque), 10 mg cefmetazole sodium (Sankyo), 1,000 U heparin sodium (Shimizu, Shizuoka, Japan), and 0.7 mL distilled water was inserted into the right jugular vein and right carotid artery under intraperitoneal pentobarbital sodium (Dynabot, Osaka, Japan) anesthesia. After the surgery, the rats received twice-daily subcutaneous injections of 10 mg cefmetazole until 24 hours before the experiment. Two hours before the clamp study, the overnight-fasted rats were placed in a plastic box at constant tempera-

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ture (20° to 24°C), where they were allowed to move freely. The arterial and venous catheters were aspirated to remove PVP solution. After obtaining a baseline blood sample from the arterial line, a primed (6 μCi) and constant infusion (0.15 μCi/min) of D-[3-^{3}H]-glucose (New England Nuclear, Boston, MA) was initiated through the venous line and continued for 180 minutes until the end of the clamp study. Blood was obtained at 50, 55, and 60 minutes for determination of basal plasma glucose and insulin and specific activity of labeled glucose. At 60 minutes, an infusion of human regular insulin (Eli Lilly, Indianapolis, IN) was started at a priming rate (287 pmol/kg/min) for 30 seconds and continued at a constant rate of 57.4 pmol/kg/min for 120 minutes. During the clamp, the plasma glucose level was measured every 5 minutes. A 30% unlabeled glucose infusion was started 5 minutes after commencement of the insulin infusion, and the infusion rate was adjusted according to the algorithm previously reported[13] to clamp plasma glucose at 6.1 mmol/L. When basal plasma glucose exceeded 6.1 mmol/L, plasma glucose was allowed to decrease to the clamp level over 10 to 20 minutes following initiation of the insulin infusion. Blood samples were drawn at 90, 105, and 120 minutes from the start of insulin infusion for measurement of steady-state plasma glucose and insulin and specific activity of labeled glucose. To prevent intravascular volume depletion, heparinized fresh whole blood obtained by cardiac puncture from the donor rats was administered every 10 minutes. The volume of blood transfusion was designed to quantitatively replace the total blood loss during the study.

Analytical Procedures

Plasma glucose levels were measured by the glucose oxidase method (Glucose Analyzer 2; Beckman, Fullerton, CA), and plasma insulin levels were measured by a double-antibody radioimmunoassay (Eiken, Tokyo, Japan) using rat insulin (Eli Lilly) as a standard. Plasma glucose specific activity was measured after deproteinization with 5% $ZnSO_4$ and 0.3N $Ba(OH)_2$ according to Somogyi's procedure.[14]

Calculations

Hepatic glucose production (HGP) and the glucose disposal rate (Rd) were calculated using Steele's equation.[15] All data are shown as the mean ± SE, and statistical analyses were performed using one-way factorial ANOVA and multiple-comparison tests (StatView II; Abacus) on a Macintosh (Apple, Cupertino, CA) computer. Statistical significance was assumed at $P < .05$.

RESULTS

There were no significant differences among the treatment groups in terms of body weight (Table 1).

Fasting plasma glucose levels were comparable among the control, LoDex, and LoDex+T groups. HiDex caused fasting hyperglycemia ($P < .05$). During the clamp, steady-state plasma glucose values were almost equal among the groups except for HiDex, in which it was slightly higher than in the other groups ($P < .05$). This is due to the failure of plasma glucose to decrease to the clamp level in some of the HiDex rats even in the absence of exogenous glucose infusion. Fasting plasma insulin levels were comparable statistically among the control, LoDex, and LoDex+T groups. But troglitazone tended to decrease fasting plasma insulin in LoDex rats, although not significantly. Fasting plasma insulin values were significantly higher in HiDex rats with or without troglitazone treatment. Steady-state plasma insulin levels reached approximately 1,700 pmol/L, with no significant difference among the treatment groups.

Basal HGP was comparable between the control rats (45.8 ± 2.1 μmol/kg/min) and LoDex rats (47.9 ± 4.7 μmol/kg/min) or LoDex+T rats (46.0 ± 2.6 μmol/kg/min; Fig 1). However, HiDex resulted in significantly increased basal HGP (80.1 ± 5.2 μmol/kg/min). When troglitazone was added to HiDex, basal HGP (54.7 ± 3.4 μmol/kg/min) was not significantly different from the control value. Hyperinsulinemia during the clamp suppressed HGP in LoDex rats (10.2 ± 8.4 μmol/kg/min) or LoDex+T rats (7.8 ± 7.9 μmol/kg/min) to a degree similar to that in control rats (11.3 ± 8.8 μmol/kg/min). In HiDex rats, HGP was resistant to the suppressive effects of hyperinsulinemia during the clamp (HiDex, 63.7 ± 9.7 μmol/kg/min; HiDex+T, 64.0 ± 6.5 μmol/kg/min; $P < .05$ *v* control group). Addition of troglitazone to the dexamethasone treatment did not enhance the effect of insulin to suppress HGP during the clamp.

The mean glucose infusion rate (GIR) during the last 30 minutes of the clamp, which was used to calculate the glucose Rd, was lower in the LoDex group (71.3 ± 7.3 μmol/kg/min, $P < .05$) than in the control group (100.6 ± 11.0 μmol/kg/min; Fig 2). The GIR (94.8 ± 33.6 μmol/kg/min) in LoDex+T rats was comparable to the control value. In the HiDex group, the GIR (6.5 ± 4.7 μmol/kg/min, $P < .05$ *v* control group) was markedly decreased, and it was unaltered by administration of troglitazone (3.0 ± 1.1 μmol/kg/min, $P < .05$ *v* control group).

The glucose Rd during the hyperinsulinemic clamp was decreased 27% and 36%, respectively, in LoDex (81.5 ± 5.8 μmol/kg/min, $P < .05$) and HiDex (71.3 ± 9.4 μmol/kg/min, $P < .005$), compared with the control (111.4 ± 7.4 μmol/kg/min; Fig 2). Therefore, dexamethasone caused insulin resistance in peripheral glucose utilization. In LoDex+T and HiDex+T rats, the glucose Rd in the former (102.6 ± 5.7 μmol/kg/min) was comparable to the value in control rats, and in the latter it was lower than the control value (67.0 ± 6.4 μmol/kg/min). These results indicate that troglitazone prevented the development of peripheral insulin resistance in LoDex, but was ineffective against HiDex.

Table 1. Metabolic Characteristics of the Rats

Characteristic	Control	LoDex	HiDex	LoDex+T	HiDex+T
No. of rats	7	7	7	8	6
Body weight (g)	209 ± 5.6	222 ± 7	202 ± 3.8	211 ± 6.7	214 ± 7.1
Plasma glucose (mmol/L)					
Fasting	7.0 ± 0.4	7.5 ± 0.3	9.4 ± 0.8*	7.7 ± 0.3	8.6 ± 0.3*
Steady-state	6.2 ± 0.1	5.8 ± 0.1	6.8 ± 0.3*	6.3 ± 0.1	6.5 ± 0.3
Plasma insulin (pmol/L)					
Fasting	85 ± 17	204 ± 34	391 ± 85*	119 ± 17	374 ± 85*
Steady-state	1,683 ± 204	1,717 ± 102	1,904 ± 204	1,513 ± 51	1,972 ± 119

*$P < .05$ τ control.

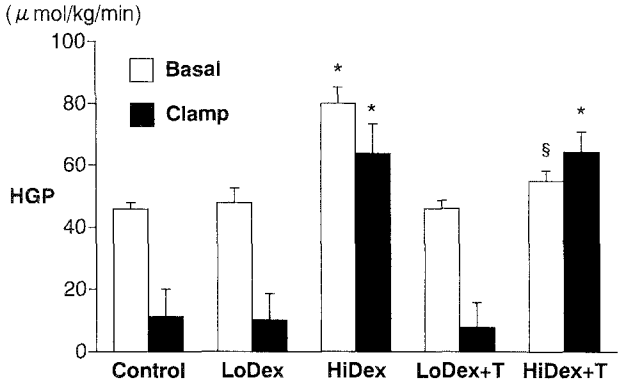

Fig 1. HGP in the basal state and during the clamp. *$P < .05$ *v* control, §$P < .05$ *v* HiDex.

DISCUSSION

Dexamethasone administration at a dose of 0.05 mg/kg/d (designated as LoDex) to rats for 4 days decreased the whole-body glucose Rd by 27% during the hyperinsulinemic-euglycemic clamp while HGP was suppressed to the same degree as in the control rats. In the rats in which troglitazone was coadministered with dexamethasone, the glucose Rd during the clamp was comparable to the value in control rats. Thus, troglitazone prevented the development of peripheral insulin resistance in rats treated with dexamethasone. As described in the Methods, to match the body weight at the time of the glucose clamp study, rats that were 20 to 30 g heavier than the controls before the treatment were entered into the dexamethasone treatment group. Even if they have comparable body weight at the time of study, decreased food intake and/or increased energy consumption that result in decreased weight gain may affect comparisons of dexamethasone-treated rats and controls. It has been reported that pioglitazone[12] and englitazone[11,12] improved the impairment of 2-deoxyglucose uptake in 3T3-L1 adipocytes[11] treated with dexamethasone and in soleus muscle[12] from dexamethasone-treated rats. The present study confirms that these effects of thiazolidinediones observed at the level of cellular glucose transport can be seen in vivo by measurement of whole-body glucose disposal, with troglitazone sharing the effects of pioglitazone and englitazone.

In the present study, when the dose of dexamethasone was doubled to 0.1 mg/kg/d, the beneficial effects of troglitazone were no longer seen. Although this may imply a weak potency of the drug in this model of insulin resistance, there remains the possibility that a higher dose and/or longer treatment of troglitazone than currently used may overcome the effects of HiDex. As to the effects on hepatic glucose metabolism, HiDex increased basal HGP and impaired the effect of insulin to suppress HGP during the glucose clamp. Increased basal HGP itself in the face of elevated plasma insulin is indicative of insulin resistance. Hepatic insulin resistance may be caused by the known effects of dexamethasone on the liver, including induction of several gluconeogenic enzymes or increased hepatic uptake of precursors for gluconeogenesis.[16] In addition, in view of the recent recognition that the effect of insulin on HGP is at least partly secondary to the effects of insulin on peripheral tissues,[17] hepatic insulin resistance in HiDex rats may be mediated to some extent by dexamethasone-induced peripheral insulin resistance. In this setting, troglitazone treatment prevented the increase in basal HGP in HiDex rats. On the other hand, the impaired suppression of HGP during the clamp was not ameliorated by troglitazone. The prevention of the increase in basal HGP may suggest that troglitazone was able to block the development of hepatic insulin resistance; however, the failure to enhance the effect of insulin to suppress HGP during the clamp indicates the opposite. These results do not allow a straightforward interpretation. It is possible that troglitazone enhances the effect of submaximal but not maximal or near-maximal concentrations of insulin. Due to the lack of a dose-response study, we cannot draw a firm conclusion about the effect of troglitazone on dexamethasone-induced hepatic insulin resistance.

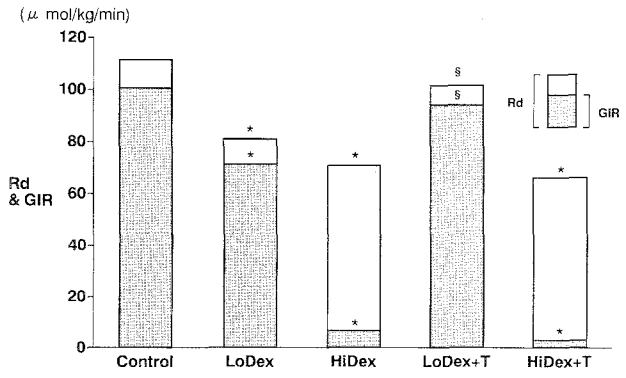

Fig 2. Mean GIR during steady state and glucose Rd during the clamp. *$P < .05$ *v* control, §$P < .05$ *v* LoDex.

The molecular mechanisms by which thiazolidinediones exert insulin-mimicking or sensitizing effects have been extensively studied. Possible sites of their action in insulin-signaling pathways proposed from a number of studies in various conditions with insulin resistance include an increased amount and/or function of insulin receptor tyrosine kinase,[18] insulin receptor substrate-1,[19] phosphatidylinositol 3-kinase,[20,21] and glucose transporters.[22-24] Investigators who focused on the effects of thiazolidinediones in glucocorticoid-induced insulin resistance found that they improve insulin-stimulated cellular glucose transport without altering the content of GLUT4 in soleus muscle.[12] The protection of insulin receptor substrate-1 from downregulation by dexamethasone may account at least partly for the amelioration of insulin resistance.[19] Whether these mechanisms are primarily responsible for the drug actions or are secondary to the improved efficiency of the whole pathway of insulin action remains unclear. Recently, it has been reported that thiazolidinediones bind to peroxisome proliferator–activated receptor-γ (PPAR-γ), which then modulates adipocyte differentiation.[25] Since there is a correlation between the binding affinity to PPAR-γ and the antidiabetic potency of the thiazolidinediones, it is speculated that PPAR-γ may mediate the drug actions.[26]

In conclusion, we have confirmed the efficacy of troglitazone in dexamethasone-induced insulin resistance in vivo. Troglitazone may hold promise in the treatment of glucocorticoid-induced diabetes.

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