Effects of Troglitazone on Hepatic and Peripheral Insulin Resistance Induced by Growth Hormone Excess in Rats

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It is well known that short-term growth hormone (GH) administration in humans and animals induces insulin resistance and glucose intolerance. The purpose of the present study was to clarify whether troglitazone, a new insulin-sensitizing drug of the thiazolidinedione class, counteracts the insulin antagonistic effects of recombinant human (rh) GH on glucose metabolism in rats. Male Wistar rats weighing 184 to 226 g were treated either with rhGH (n = 8) or rhGH plus troglitazone (n = 8). rhGH (20 IU/kg body weight/d) was given by subcutaneous injection twice daily for 2 days. Troglitazone was given at 100 mg/kg/d orally for 5 days before and 2 days during rhGH. Saline was injected to the control rats (n = 7). Euglycemic clamp studies with an insulin infusion rate of 8 mU/kg/min were performed in these rats after an overnight fast. Hepatic glucose output (HGO), glucose infusion rate (GIR), and glucose disappearance rate (GDR) were measured. Fasting levels of plasma glucose (6.6 ± 0.1, 6.1 ± 0.3, 6.5 ± 0.2 mmol/L), insulin (187.5 ± 24.1, 206.4 ± 24.1, 182.3 ± 31.0 pmol/L), and serum free fatty acid (FFA) (1.58 ± 0.18, 1.43 ± 0.16, 1.61 ± 0.25 mEq/L) were comparable among rats treated with rhGH, rhGH plus troglitazone, and controls, respectively. Basal HGO was also comparable among the three treatment groups. HGO was suppressed significantly during the hyperinsulinemic glucose clamp in control rats, but not in rhGH rats. When troglitazone was coadministered with rhGH, suppressibility of HGO during the glucose clamp was comparable to that of controls. GIR (13.5 ± 4.5 *v* 24.1 ± 4.1 mg/kg/min) and GDR (18.1 ± 5.8 *v* 30.3 ± 5.2 mg/kg/min) were decreased by rhGH treatment compared with control values. They returned to normal levels in rats treated with both rhGH and troglitazone (GIR, 22.4 ± 5.9; GDR, 24.7 ± 7.1). From these results, it is evident that rhGH treatment impaired insulin's ability to suppress HGO and stimulate peripheral glucose utilization. Troglitazone could block the insulin antagonistic effects of GH on hepatic glucose output and peripheral glucose utilization.

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RECOMBINANT GENE TECHNOLOGY has overcome the limitation of growth hormone (GH) supply and opened the door to new therapeutic uses for GH, including preservation of lean body mass during aging, modification of body composition, ovulation induction, treatment of osteoporosis, reversal of the catabolic effects of glucocorticoids, and acceleration of tissue repair after trauma or surgery.[1,2] In these settings, there is appropriate concern regarding adverse effects of GH on carbohydrate metabolism. Although physiological replacement of GH in GH-deficient children does not seem to carry any increased risk of diabetes arising during the treatment,[3] glucose intolerance is highly prevalent in patients with acromegaly, which is a chronic disease characterized by prolonged elevation of GH levels.[4] The effect of prolonged GH administration on carbohydrate metabolism in non–GH-deficient normal subjects is not known. Short-term administration of GH to normal subjects has been reported to impair insulin's ability to suppress hepatic glucose production and stimulate peripheral glucose utilization.[5,6] Thus, insulin resistance can be seen as a hallmark of the metabolic effects of GH.

Thiazolidinedione derivatives are a novel class of antidiabetic drugs known to act either by mimicking or enhancing insulin action in target tissues. These drugs were initially shown to improve insulin resistance in genetically obese animals, such as yellow KK and ob/ob mice, and Zucker fatty rats.[7,8] However, it was soon proved that they were also effective for insulin resistance induced by food[9,10] or secondary to hyperglycemia.[11] Recently, Towns et al[12] reported that pioglitazone, one of the thiazolidinedione drugs, decreased elevated plasma glucose and insulin levels in ob/ob mice treated with recombinant human (rh)GH without affecting the growth-promoting action of the hormone. The present study was designed to clarify whether troglitazone, which is the first thiazolidinedione derivative applied to clinical use, can improve insulin resistance induced by rhGH in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 184 to 226 g were housed individually in a cage with constant temperature (22 ± 2°C) and controlled lighting (light on from 5 AM to 5 PM). They had free access to food and water. They were allocated to one of the following three treatment groups: (1) GH treatment (n = 8), (2) GH and troglitazone treatment (n = 7), and (3) controls (n = 8). rhGH (Norditropin; a generous gift from Novo Nordisk, Bagsværd, Denmark) at a dose of 1 IU/100 g was administered subcutaneously in the morning and evening for 2 days. Saline instead of rhGH was injected to control rats. Saline or troglitazone (kindly provided by Sankyo, Tokyo, Japan) suspended in 0.5% carboxymethyl cellulose saline was given to the rats by gastric gavage twice daily for 5 days before and 2 days during rhGH treatment. The daily dose of troglitazone was two doses of 50 mg/kg each per day for a total of 100 mg/kg/d. After completion of studies with these three treatment groups of rats, we performed an additional experiment with normal rats (n = 5) treated with oral troglitazone and subcutaneous saline injection to examine whether troglitazone potentiates insulin action in intact animals.

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Submitted June 23, 1997; accepted January 15, 1998.

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0026-0495/98/4707-0003$03.00/0

Surgery

Four days before the glucose clamp study, indwelling catheters were placed in the rats. Under anesthesia with 50 mg/kg of pentobarbital sodium (Dinabot, Osaka, Japan) intraperitoneally, polyethylene catheters (Intramedic PE-10 and PE-50; Becton Dickinson, Sparks, MD) were inserted into the right carotid artery and jugular vein. The catheters were led around the neck and exteriorized through the skin at the vertex of the head. The catheters were filled with polyvinylpyrrolidone (PVP) solution containing 500 mg of PVP (K-90; Nakalai Tesque, Kyoto, Japan), 10 mg cefmetazone sodium (kindly provided by Sankyo), 1,000 U heparin sodium (Shimizu, Shizuoka, Japan), and 0.7 mL distilled water. The solution was aspirated before the clamp study. The carotid artery catheter was used for sampling blood, while the jugular catheter was used to infuse substrates into the animals.

Glucose clamp studies were performed in the morning following completion of GH treatment with overnight fasted conscious rats. Following basal blood sampling, a primed (6 μCi) and continuous (0.15 μCi/min) infusion of D-[3-^{3}H]-glucose (New England Nuclear, Boston, MA) was given for 60 minutes before and throughout the 120 minutes of the clamp study. Human regular insulin (Eli Lilly, Indianapolis, IN) infusion in a primed (287 pmol/kg/min for 30 seconds) and continuous (57.4 pmol/kg/min) fashion was given during the clamp. Plasma glucose was measured every 5 minutes, so as to adjust the unlabeled glucose infusion rate (GIR) to clamp plasma glucose at 6.1 mmol/L according to a reported algorithm.[13] Plasma samples were obtained at 50, 55, and 60 minutes of basal D-[3-^{3}H]-glucose infusion and at 90, 105, and 120 minutes during the clamp to measure plasma glucose, insulin, and the specific activity of D-[3-^{3}H]-glucose. Heparinized fresh whole blood, obtained by cardiac puncture from donor rats, was administered every 10 minutes to prevent intravascular volume depletion. The volume of blood transfusion was designed to replace quantitatively the total blood loss during the study.

Analytical Methods

Plasma glucose concentrations were determined with the glucose oxidase method using a Glucose Analyzer 2 (Beckman, Fullerton, CA). Plasma insulin level was measured by a double-antibody radioimmunoassay kit (Insulin Eiken, Tokyo, Japan) using rat insulin (a kind gift from Eli Lilly) as standard. The specific activity of plasma glucose was measured after deproteinization with 5% $ZnSO_4$ and 0.3N $Ba(OH)_2$ according to Somogyi's procedure. The plasma free fatty acid (FFA) value was measured according to standardized procedures (enzymatic method, NEFA-SS; Eiken).

Calculations

Hepatic glucose output (HGO) and glucose disposal rate (GDR) were calculated according to Steel's equations. All data are presented as the mean ± SE and statistical analyses were performed using one-way factorial ANOVA; pairwise comparisons were evaluated by means of Fisher's least significant difference (StatView; Abacus Concepts, Berkeley, CA) run on a Macintosh (Apple, Cupertino, CA) computer. Statistical significance was assumed at $P < .05$.

RESULTS

As shown in Table 1, fasting plasma glucose and insulin levels in the rats that received rhGH for 2 days and the rats treated with rhGH and troglitazone were not different from those in the control rats. Although the rats treated with rhGH and troglitazone showed slightly lower FFA levels than the other two groups of rats, the difference did not reach statistical significance.

In the fasting state, rats treated with rhGH alone or rhGH plus troglitazone showed comparable HGO to that of control rats. While the infusion rate of unlabeled glucose was adjusted to clamp plasma glucose levels at 6.1 mmol/L throughout the glucose clamp study, plasma glucose levels attained during the last 30 minutes of the clamp calculated as a mean of the values for 90, 105, and 120 minutes were comparable among rats treated with rhGH (6.3 ± 0.2 mmol/L), rats treated with rhGH plus troglitazone (5.7 ± 0.2 mmol/L), and control rats (6.1 ± 0.1 mmol/L). Steady-state plasma insulin levels during this period were also similar among the three groups of rats (rhGH, 1,260.8 ± 74.0 pmol/L; rhGH + troglitazone, 1,228.1 ± 87.7 pmol/L; controls, 1,339.9 ± 74.0 pmol/L). At this level of plasma insulin, HGO was suppressed significantly to 60.3% and 39.6% of the basal value in control rats and in rats treated with rhGH and troglitazone, respectively (Fig 1). Although a reduction to 75.1% of the basal level was noted in rhGH-treated rats, this was not statistically significant. Thus, rhGH treatment caused hepatic insulin resistance, and troglitazone seemed to prevent it.

Unlabeled GIRs during the clamp are shown in Fig 2, and the mean value for the last 30 minutes (90 to 120 minutes) was lower in rhGH-treated rats than in the other two groups of rats. The GDR calculated for the same period during the clamp was lower in rats that received rhGH than in controls. The GDR in rats treated with rhGH and troglitazone returned to the levels of control rats (Fig 3). Although these results suggest troglitazone prevented emergence of hepatic and peripheral insulin resistance following rhGH treatment, an additional study was

Table 1. Metabolic Characteristics of the Rats

	Body Weight (g)	FPG (mmol/L)	FPI (pmol/L)	FFA (mEq/L)
Control (n = 8)	203.0 ± 2.31	6.5 ± 0.2	182.3 ± 31.0	1.61 ± 0.25
rhGH (n = 8)	208.5 ± 3.98	6.6 ± 0.1	187.5 ± 24.1	1.58 ± 0.18
Troglitazone + rhGH (n = 7)	205.5 ± 4.69	6.1 ± 0.3	206.4 ± 24.1	1.43 ± 0.16

NOTE. Data are means ± SE.

Abbreviations: FPG, fasting plasma glucose; FPI, fasting plasma insulin.

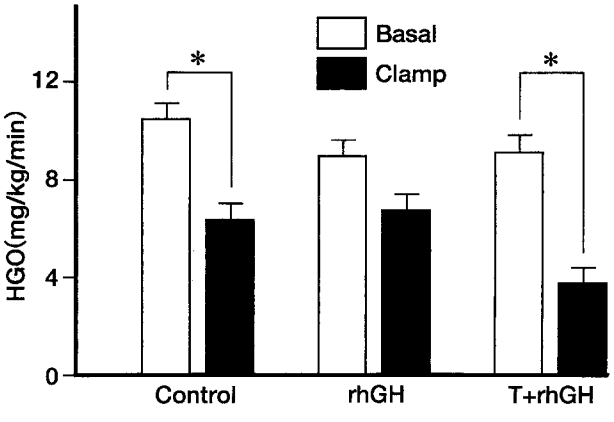

Fig 1. HGO during basal and glucose clamp conditions. *Significant difference from basal value by paired *t* test. Basal HGO was comparable between control rats and rats treated with either rhGH or troglitazone (T) + rhGH. During the clamp, HGO was suppressed in control and rhGH + T rats, but not in rhGH rats.

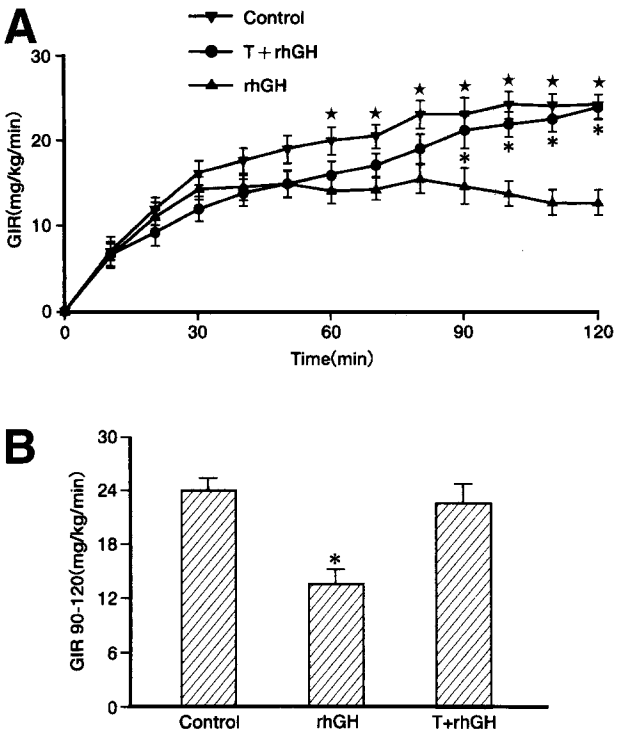

Fig 2. (A) Unlabeled GIRs during the clamp. GIR was decreased in rhGH-treated rats and there were significant differences between rhGH-treated rats and rats treated with T + rhGH. Significant difference between rhGH and control (*) or T + rhGH (*) by Fisher's test. (B) Mean values of GIR from 90 to 120 minutes (GIR 90-120). GIR 90-120 was decreased in rhGH-treated rats. Rats treated with T + rhGH showed normal values. *Significant difference from controls by ANOVA.

performed to determine whether troglitazone specifically counteracts the diabetogenetic effects of rhGH or whether potentiation of insulin action by troglitazone is nonspecific and can be seen in intact animals. Glucose clamp studies were performed in five normal rats (body weight, 204.4 ± 5.9 g) after they received troglitazone 20 mg/d for 7 days. HGO values of these

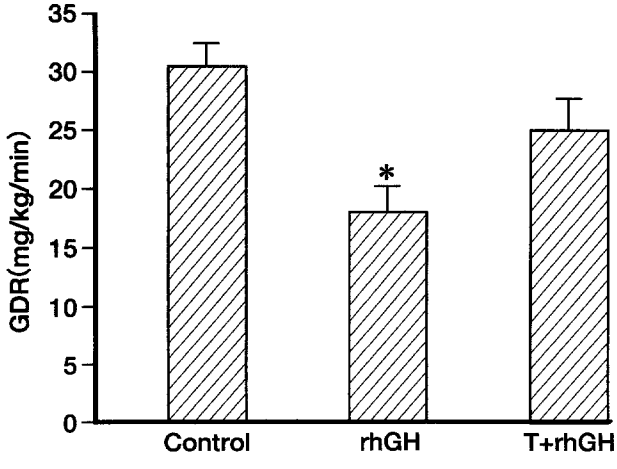

Fig 3. GDR decreased in rhGH-treated rats, but there was no difference between rats treated with T + rhGH and controls. *Significant difference from controls by ANOVA.

rats in the basal state (11.3 ± 1.8 *v* 10.4 ± 0.6 mg/kg/min, not significant [NS]) and during the hyperinsulinemic clamp (8.24 ± 0.7 *v* 6.28 ± 0.8 mg/kg/min, NS) were similar to the values of control rats. Neither infusion rate of unlabeled glucose (25.4 ± 2.2 *v* 24.1 ± 1.4 mg/kg/min, NS) nor GDR (35.5 ± 2.3 *v* 30.3 ± 1.8 mg/kg/min, NS) was significantly different from the respective values of control rats.

DISCUSSION

We demonstrated that administration of rhGH at a dose of 20 IU/kg/d for 2 days in male Wistar rats produced a significant decrease in steady-state GIR and the GDR during a hyperinsulinemic glucose clamp. Insulin-mediated suppression of HGO during the clamp was also impaired. These results clearly indicate the emergence of both hepatic and peripheral insulin resistance following rhGH administration in rats. Fasting plasma glucose and insulin levels did not differ between rats treated with rhGH and those not treated. Fasting plasma insulin levels have been elevated in most,[14-17] but not all[18,19] studies that have demonstrated insulin resistance after GH treatment in humans and animals. The study reported by Ørskov et al[18] is fully consistent with our results. They demonstrated, using a glucose clamp technique, an impaired ability of insulin to suppress HGO and to stimulate peripheral glucose uptake, as well as decreased glucose-induced glucose uptake in the face of unaltered fasting plasma glucose and insulin levels in human subjects who received a continuous infusion of rhGH (40 ng/kg/min) for the preceding 9 hours. The plasma insulin response to GH administration is probably dependent on variables such as dose of GH, length of treatment, and mode of administration. Unaltered fasting plasma glucose and insulin levels may not guarantee unaltered insulin action in humans and animals receiving short-term GH administration.

GH-induced hepatic and peripheral insulin resistance was no longer seen when rats were pretreated with troglitazone. As mentioned earlier, the potency of thiazolidinediones to counteract the diabetogenic actions of GH has been suggested by the findings that pioglitazone reverted elevated plasma glucose and insulin levels to pretreatment values in GH-treated ob/ob mice.[12] The present study confirmed the drug action to counteract GH-induced insulin resistance. We started troglitazone administration in advance of GH treatment, because a lapse of several days to 2 to 3 weeks had been reported to be necessary for the full effects of the drug emerge.[8,20] It is highly probable that the drug is not only effective for the prevention, but also for the correction, of established insulin resistance, since in the aforementioned study, whether the initiation of pioglitazone administration preceded or followed GH treatment did not produce different results.[12] Interestingly, in the same study, the growth-promoting action of GH was not affected by pioglitazone. The combination of GH and thiazolidinedione may have a therapeutic advantage of maximally promoting growth while minimizing the diabetogenic potency of GH.

It has been suggested that some of the insulin-antagonistic effects of GH may be induced by increased lipolysis and a subsequent elevation in plasma FFA levels, which ultimately inhibits glucose uptake and oxidation.[21] It has also been argued that many of the effects of thiazolidinedione drugs on glucose

metabolism are secondary to reduced lipid availability.[10] However, these explanations seem unlikely to be the case. In the present study, the fasting serum FFA level was not elevated after 2 days of treatment with rhGH, nor was it affected by troglitazone.

GH is well known to possess insulin-like, as well as insulin-antagonistic action.[22] Consistent with its insulin-like effect, GH has recently been reported to share some of the early steps of intracellular signaling pathways with insulin, that is, following interaction with its receptor, GH stimulates tyrosyl phosphorylation of insulin receptor substrate-1 and -2 and association of these phosphorylated proteins with phosphatidylinositol 3-kinase, which is believed to play a pivotal role in the mechanism of insulin action.[23] On the other hand, the molecular mechanisms of the insulin-antagonistic effect remain unclear. Insulin receptor kinase activity was unaltered in adipose tissues taken from pigs treated with porcine GH for 7 days[24] and increased in the liver of rats that harbored GH-secreting tumors.[25] The cellular content of GLUT-1, but not GLUT-4, was reduced in cultured 3T3-F442A adipose cells incubated with GH.[26] In contrast, neither GLUT-1 nor GLUT-4 gene expression was altered in skeletal muscles from rats with a GH excess.[16,27] The mechanism by which thiazolidinediones exert their insulin-sensitizing effects has been addressed by a number of investigators in various conditions with insulin resistance. In these studies, almost every step in the insulin-signaling pathway, including insulin receptor kinase,[28] insulin receptor substrate-1,[29] phosphatidylinositol 3-kinase,[30,31] and glucose transporters,[32-34] has been proposed as a possible target of the drug action. Nevertheless, it is unknown whether these are the primary events or secondary phenomena reflecting the efficiency of the whole pathway of insulin action. In conclusion, troglitazone offsets the hepatic and peripheral insulin resistance induced by GH excess. Coadministration of troglitazone may be a useful strategy in the new use of GH in subjects who are not GH-deficient.

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