Increased Glucocorticoid Receptor and 11β-Hydroxysteroid Dehydrogenase Type 1 Expression in Hepatocytes May Contribute to the Phenotype of Type 2 Diabetes in *db/db* Mice

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Excess tissue glucocorticoid action may contribute to the hyperglycemia and insulin resistance associated with type 2 diabetes, but the associated mechanisms are poorly understood. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive 11-dehydrocorticosterone into active corticosterone, thus amplifying glucocorticoid receptor–mediated tissue glucocorticoid action, particularly in the liver. To examine the role of tissue glucocorticoid action in type 2 diabetes, we analyzed expression of glucocorticoid receptor and 11β-HSD1 and their regulation by endogenous hormones in vivo and in vitro in hepatocytes from *db/db* mice (a model of type 2 diabetes). We observed positive relations between expression of both glucocorticoid receptor and 11β-HSD1 in liver and insulin sensitivity and expression of PEPCK mRNA in *db/db* mice and *db/+* controls. Increased expression of glucocorticoid receptor and 11β-HSD1 in the liver of *db/db* mice was correlated with elevated circulating levels of corticosterone, insulin, and blood glu-cose. Treatment of *db/db* mice with glucocorticoid antagonist RU486 reversed the increases in the expression of glucocorticoid receptor and 11β-HSD1 within the liver and attenuated the phenotype of type 2 diabetes. Addition of corticosterone to *db/db* mouse primary hepatocytes activated expression of glucocorticoid receptor, 11β-HSD1, and PEPCK, and these effects were abolished by RU486. Incubation of primary hepatocytes with increasing concentrations of glucose caused dose-dependent increases in glucocorticoid receptor and 11β-HSD1 expression, whereas insulin did not affect the expression of 11β-HSD1 and glucocorticoid receptor in primary hepatocytes. These findings suggest that activation of glucocorticoid receptor and 11β-HSD1 expression within the liver may contribute to the development of type 2 diabetes in *db/db* mice. *Diabetes* 54:32–40, 2005

Insulin resistance is a significant risk factor for the development of type 2 diabetes and is frequently associated with obesity (1,2). Glucocorticoids are potent antagonists of insulin action and, when in excess, can induce insulin resistance and central obesity (Cushing's syndrome). Indeed, glucocorticoids stimulate hepatic gluconeogenesis and reduce the ability of insulin to inhibit glucose production, all of which are thought to be the major source of increased glucose production in type 2 diabetes (3,4). Glucocorticoids also promote the differentiation of preadipocytes into mature adipocytes and drive adipose tissue distribution and function, which is associated with the accumulation of body fat (5,6). Increased glucocorticoid production induces glucose intolerance and insulin resistance in genetically obese *db/db* and *ob/ob* mice and Zucker (*fa/fa*) rats (7–9). Pharmacological blockade of glucocorticoid receptor reduces glucocorticoid-related hyperglycemia and insulin resistance in these animal models and in patients with Cushing's syndrome (10–12), highlighting the importance of the interactions of glucocorticoids with glucocorticoid receptor in the development of type 2 diabetes and obesity (13,14). However, the actions of glucocorticoids on target tissues, such as liver and adipose tissue, are not necessarily dependent on circulating glucocorticoid levels but rather on their prereceptor metabolism, which is regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD1) (15,16).

11β-HSD1 is a low-affinity, NADP(H)-dependent dehydrogenase/oxoreductase that functions in most intact cells and tissues predominantly in oxoreduction of inactive cortisone to the active glucocorticoid receptor ligand cortisol and thus amplifies local glucocorticoid action (17,18). The importance of prereceptor metabolism of glucocorticoids is supported by the renal expression of 11β-HSD2, which inactivates glucocorticoids, thereby conferring aldosterone selectivity at the mineralocorticoid receptor (19). 11β-HSD2 activity is present in the kidney and colon (20,21), whereas the principle sites of 11β-HSD1 action are liver, adipose tissue, and muscle. In these tissues, 11β-HSD1 acts predominantly as a reductase to regenerate active glucocorticoids and thereby regulates

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DMEM, Dulbecco's modified Eagle's medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1.

access of glucocorticoids to the glucocorticoid receptor (22,23). Activation of 11β-HSD1 and glucocorticoid receptor could result in the production of excess tissue glucocorticoids and induction of glucocorticoid receptor–mediated local glucocorticoid action related to glucose homeostasis, insulin action, and adiposity, all of which are associated with development of type 2 diabetes and visceral obesity (24–26). In contrast, reduction of 11β-HSD1 expression, by either pharmacological inhibition or targeted gene disruption, prevents regeneration of active glucocorticoids from the inactive 11-keto forms, attenuates intrahepatic glucocorticoid action, and increases insulin sensitivity (27–29). Moreover, 11β-HSD1 knockout mice show reduced expression of mRNA for PEPCK, a crucial enzyme in hepatic gluconeogenesis, and are resistant to hyperglycemia when fed a high-fat diet (29). These studies point to the importance of hepatic 11β-HSD1–mediated synthesis of active glucocorticoids and glucocorticoid receptor occupancy in the pathogenesis of type 2 diabetes and obesity and suggest that tissue glucocorticoid actions may be effective targets for endogenous regulation and pharmacological inhibition in humans.

In the present study, we investigated the role of tissue glucocorticoids in glucocorticoid-related type 2 diabetes and obesity by examining the expression of glucocorticoid receptor and 11β-HSD1 in the liver of *db/db* mice. We also examined the effects of glucocorticoid receptor antagonist RU486 on prereceptor metabolism of corticosterone in the liver of *db/db* mice. Finally, we tested the direct effects of endogenous hormones and glucose on expression of glucocorticoid receptor and 11β-HSD1 in primary cultures of hepatocytes from *db/db* mice.

RESEARCH DESIGN AND METHODS

Male C57BL/KsJ-obese (*db/db*) mice and their lean littermates (*db/+*) were purchased at 10 weeks of age from The Jackson Laboratory (Bar Harbor, ME) and allowed free access to tap water and standard laboratory diet. All animal experiments were approved by the Charles R. Drew University Humane Care and Use of Laboratory Animal Committee. *Db/db* mice and lean *db/+* controls were divided into four groups: *db/+* mice treated with vehicle, *db/+* mice treated with RU486, *db/db* mice treated with RU486, and *db/db* mice treated with vehicle. RU486 (also known as mifepristone; Sigma, St. Louis, MO) was first dissolved in 100% ethanol to a concentration of 50 μg/μl and then mixed with an equal volume of 0.9% NaCl. RU486 (25 mg/kg) or vehicle was given by intraperitoneal injection twice each day (at 0700 and 1900) for 2 or 3 weeks (12). Body weight and food intake were recorded daily. At the end of the second or third week after vehicle or RU486 administration, an insulin tolerance test was performed by intraperitoneal administration of 0.1 units of insulin/kg.

The liver was removed surgically under ether anesthesia and frozen immediately in liquid nitrogen. Blood samples were collected from each mouse, kept in an ice bath during processing, and then stored at −80°C until measurement of insulin, corticosterone, and glucose levels (30).

Primary cultures of hepatocytes. Hepatocytes were isolated from 12-week-old male *db/db* mice by a two-step collagenase perfusion procedure as described previously (30). Hepatocytes were then suspended in Dulbecco's modified Eagle's medium (DMEM)/F-12 with 10% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 0.125 μg/ml amphotericin B, 10^{-9} mol/l dexamethasone, and 10^{-9} mol/l insulin. Hepatocytes (1×10^6 cells) were plated in six-well dishes (coated with 0.05% collagen; Sigma) and incubated at 37°C for 4–6 h. Cells were then washed with PBS, and the medium was changed to DMEM/F-12 without fetal bovine serum. After 12 h, hepatocytes were treated with corticosterone (10^{-8} to 10^{-5} mol/l), insulin (10^{-5} mol/l), leptin (100–1,000 ng/ml), and progesterone (10^{-7} mol/l) in the presence or absence of RU486 (10^{-6} mol/l) for 24–72 h. In some experiments, medium was changed to glucose-free DMEM (Invitrogen, Carlsbad, CA), and cells were treated with varying concentrations of glucose (5–30 mmol/l) for 72 h.

Measurement of enzyme activity in vivo animals. The enzyme activity of 11β-HSD1 was determined by measuring conversion of corticosterone (B) to 11-dehydrocorticosterone (A), which reflects 11β-HSD1 protein levels and stable dehydrogenase activity, as reported previously (31). Briefly, the liver was homogenized in Krebs-Ringer buffer solution, and the protein concentration of each supernatant was measured by Bradford assay (Bio-Rad Protein Assay Kit; Biocal, Hercules, CA). Supernatants with constant protein concentrations were incubated with 2 μmol/l [^{3}H]B (New England Nuclear, Boston, MA), 4 mmol/l/l $NADP^+$, and Krebs-Ringer buffer solution (containing 0.2% glucose [pH 7.4]) at 37°C for 12 min in a shaking bath. On the basis of primary studies, the protein concentration was adjusted to ensure the linearity of product formation over a 15-min period. Steroids were extracted with ethyl acetate and separated by thin-layer chromatography in a chloroform-ethanol (9:1) system. The conversion of [^{3}H]B to [^{3}H]A was calculated from the radioactivity in each fraction.

Measurement of enzyme activity in vitro hepatocytes. 11β-HSD1 activity was examined by incubating intact isolated hepatocytes with 18 nmol/l unlabeled 11-dehydrocorticosterone (A) with 2 nmol/l [^{3}H]A as tracer or 25 nmol/l corticosterone with appropriate tritiated tracer as reported previously (30). Aliquots of culture medium from each well were removed at intervals between 1 and 24 h before and after addition of substrates. Steroids were extracted from 1 ml of culture medium and separated by thin-layer chromatography. Enzyme activity levels were estimated by counting of radioactivity. Protein concentrations were measured with Bio-Rad Protein Assay Kit. Enzyme activity was calculated as the percentage conversion rate of [^{3}H]A to [^{3}H]B. All experiments were performed on at least three separate animals.

Immunoblotting (Western blot). Liver tissues were homogenized on ice for 1 min in RIPA buffer (50 nmol/l Tris-HCl, 150 mmol/l NaCl, 1% sodium deoxycholate, 0.1% SDS, 100 mmol/l sodium orthovanadate, 1% Triton X-100, and EDTA-free protease inhibitors) to obtain total proteins from various treatment groups. Homogenates were centrifuged at 4°C at 12,000*g* for 10 min, and supernatants were collected. Protein concentrations were measured by Bradford assay. In primary culture of hepatocytes, cells were disrupted with lysis buffer that contained 1% SDS, 10 mmol/l Tris-HCl (pH 7.5), and protease inhibitors to obtain total cellular proteins. Fifty micrograms of total protein was electrophoretically resolved on 8% SDS-polyacrylamide gels and then transferred to nitrocellulose membrane. Blots were incubated overnight with anti-mouse glucocorticoid receptor α (1:400; Affinity BioReagents, Golden, CO). Blots were then washed five times for 5 min each with PBS-T and incubated with secondary antibody (anti-rabbit conjugated with horseradish peroxidase; 1:2,000) for 2 h. Quantitative image analysis was performed to determine the intensity of the protein signal and was expressed relative to the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

RNA extraction and semiquantitative RT-PCR. Total RNA was extracted from hepatocytes with a single-step extraction method (RNAzol B; Invitrogen). Equal amounts of RNA from each sample were reverse transcribed and then amplified by PCR, with primers specific for mouse 11β-HSD1 (forward 5′-TTGATGGCAGTTATGAAAAAT-3′, reverse 5′-TACAGAAGTATCAGGCAGGAC-3′), glucocorticoid receptorα (forward 5′-TGCTATGCTTTGCTCCTGATCTG-3′, reverse 5′-TGTCAGTTGATAAAACCGCTGC-3′), PEPCK (forward 5′-AGCCTCGACAGCCTGCCCCAGG-3′, reverse 5′-CCAGTTGTTGACCAAAGGCTTTT-3′), and 18S RNA (forward 5′-GTAACCCGTTGAACCCCATT-3′, reverse CCATCCAATCGGTAGTAGCG-3′). 18S RNA was included as an internal control. PCR products were visualized on 2% agarose gels by ethidium bromide staining, and gels were photographed under ultraviolet light. Relative gene expression was quantified by densitometry with the Eagle Eye II Quan titation System (Stratagene, La Jolla, CA) and was normalized to the signals of 18S cDNA. The amplification conditions were 30 cycles (for 11β-HSD1), 32 c ycles (for glucocorticoid receptorα), 25 cycles (for PEPCK), and 24 cycles (18S RNA) of denaturation for 1 min at 94°C; annealing for 1 min at 55°C; and extension for 1 min at 72°C. These conditions were shown in preliminary studies to result in amplification within the linear range.

Statistical analysis. All values are expressed as the mean ± SE. Correlations were tested by linear regression or multiple linear regression analysis. Comparisons between the different experimental groups were performed by using either the Student's *t* test or ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Characterization of glucocorticoid receptor and 11β-HSD1 expression in db/db mice. As shown in Table 1, *db/db* mice had very high body weights and serum levels of corticosterone and insulin. The mean blood glucose level of *db/db* mice was significantly higher than that of *db/+* controls ($P < 0.001$). 11β-HSD1 activity in the liver of

TABLE 1
Body weight, blood glucose, serum insulin, and plasma corticosterone

	db/+ RU486	*db/+* vehicle	*db/db* vehicle	*db/db* RU486
Body weight (g)	27 ± 1.8	30 ± 2.5	52 ± 4.5*	46 ± 3.0
Blood glucose (mg/dl)	147 ± 8.0	154 ± 7.0	520 ± 37*	180 ± 11†
Insulin (ng/ml)	14 ± 2.2	16.3 ± 2.8	137 ± 9*	130 ± 12
Corticosterone (ng/ml)	232 ± 24‖	37 ± 4.4	256 ± 40§	332 ± 43‡

Data are mean ± SE; n = 6/group. *$P < 0.001$ vs. *db/+* vehicle; †$P < 0.01$ vs. *db/db* vehicle; ‡$P < 0.05$ vs. *db/db* vehicle; §$P < 0.005$ vs. *db/+* vehicle; ‖$P < 0.01$ vs. *db/+* vehicle.

db/db mice was increased significantly to 48% over that of *db/+* mice ($P < 0.001$; Fig. 1*A*). Quantitative RT-PCR analysis revealed that the increase in enzyme activity occurred in parallel with 11β-HSD1 mRNA expression, which increased to twofold over that of *db/+* animals (Fig. 1*B*). Similarly, glucocorticoid receptor mRNA expression in the liver of *db/db* mice was markedly higher than that of *db/+* mice ($P < 0.005$; Fig. 2*A*). This was confirmed by Western blot analysis, which revealed higher levels of glucocorticoid receptor protein expression in the liver of *db/db* mice in comparison with that of *db/+* controls ($P < 0.001$; Fig. 2*B*). When both *db/+* controls and *db/db* mice were examined together, there was a positive correlation between 11β-HSD1 activity and glucocorticoid receptor mRNA expression within the liver ($P < 0.001$). The level of PEPCK mRNA in the liver of *db/db* mice was 1.7-fold higher than that of *db/+* mice (Fig. 3*B*). Moreover, the changes in hepatic 11β-HSD1 activity were positively correlated with all blood glucose levels, hepatic PEPCK mRNA expression, and insulin sensitivity in *db/+* controls and *db/db* mice ($P < 0.001$).

Effects of RU486 treatment on the phenotype of type 2 diabetes in *db/db mice*. RU486 treatment for 3 weeks reduced blood glucose levels ($P < 0.01$) with no significant changes in insulin levels and body weight in comparison with values in vehicle-treated *db/db* mice. In contrast, RU486 treatment significantly increased plasma corticosterone levels in *db/db* and *db/+* mice in comparison with levels in their vehicle-treated controls ($P < 0.01$; Table 1). However, RU486 treatment had no significant effects on plasma glucose and insulin levels with no significant reduction of body weight in *db/+* mice (Table 1). Treatment of *db/db* mice with RU486 for 3 weeks significantly reduced hepatic 11β-HSD1 activity to 60% less than that of vehicle-treated *db/db* mice ($P < 0.005$); this decrease in enzyme activity occurred in parallel with decreased hepatic 11β-HSD1 mRNA expression in RU486-treated *db/db* mice (Fig. 1). Treatment of *db/db* mice with RU486 for 3 weeks also reduced both hepatic glucocorticoid receptor mRNA and protein expression to levels similar to those in the vehicle-treated *db/+* mice (Fig. 2). Similarly, PEPCK mRNA expression in the liver of RU486-treated *db/db* mice was 53% less than that of vehicle-treated *db/db* controls ($P < 0.005$; Fig. 3*B*). Moreover, treatment of *db/db* mice with RU486 for 3 weeks increased the glucose-lowering effect of insulin in comparison with that observed in *db/db* mice that were treated with vehicle (Fig. 3*A*). In addition, a shorter treatment of *db/db* mice with RU486 for 2 weeks also reversed the phenotype of type 2 diabetes in *db/db* mice with simultaneous reduction in 11β-HSD1 and glucocorticoid receptor expression within the liver (data not shown). However, treatment of *db/+* mice with RU486 did not exert significant effects on the hepatic 11β-HSD1 and glucocorticoid receptor expression with no change in PEPCK mRNA levels.

11β-HSD1 and glucocorticoid receptor expression in primary cultures of hepatocytes. Primary hepatocytes from *db/db* mice showed <4% conversion of [^{3}H]B to [^{3}H]A n the dehydrogenase direction with 95% conversion of [^{3}H]A to [^{3}H]B in the reductase direction within 24 h, indicating that 11β-HSD1 functions predominantly as a reductase in intact *db/db* mouse hepatocytes. As shown in Fig. 4*A*, treatment of primary cultures of hepatocytes with a corticosterone concentration (10^{-6} mol/l) similar to what occurs in *db/db* mice in vivo significantly increased 11β-HSD1 activity ($P < 0.01$). The increase in enzyme activity was paralleled by semiquantitative RT-PCR showing that corticosterone resulted in a 1.5-fold increase over control levels in 11β-HSD1 mRNA levels in primary cultures of hepatocytes (Fig. 4*B*). Moreover, treatment of primary cultures of hepatocytes with increasing doses of corticosterone (5×10^{-7}-10^{-5} mol/l) led to dose-dependent in-

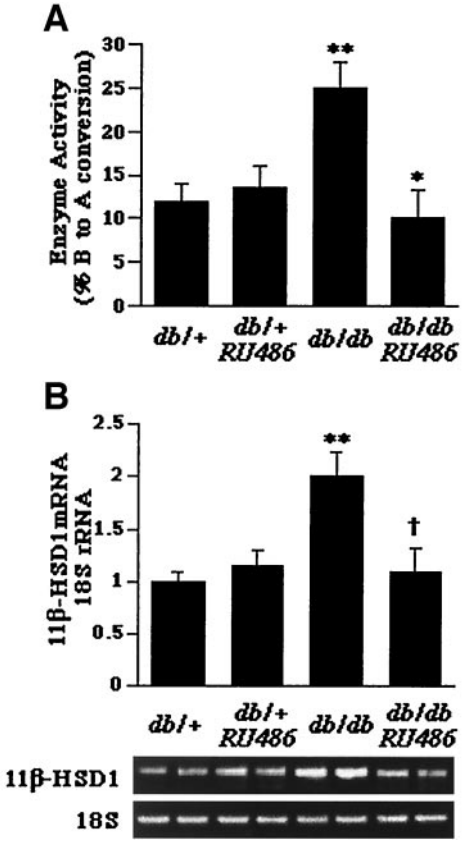

FIG. 1. Comparison of 11β-HSD1 activity (*A*) and mRNA expression (*B*) in the livers of *db/+* mice that were treated with vehicle (*db/+*), *db/+* mice that were treated with RU486 (*db/+* RU486), *db/db* mice that were treated with vehicle (*db/db*), and *db/db* mice that were treated with RU486 (*db/db* + RU486) for 3 weeks. *A*: Enzyme activity expressed as percentage conversion of [^{3}H]B to [^{3}H]A. *B*: Expression and relative quantification of enzyme mRNA levels expressed relative to the amount of 18S rRNA. Values are means ± SE; n = 6 mice/group. **$P < 0.001$ vs. *db/+* controls; *$P < 0.001$ vs. *db/db* mice; †$P < 0.01$ vs. *db/db* mice.

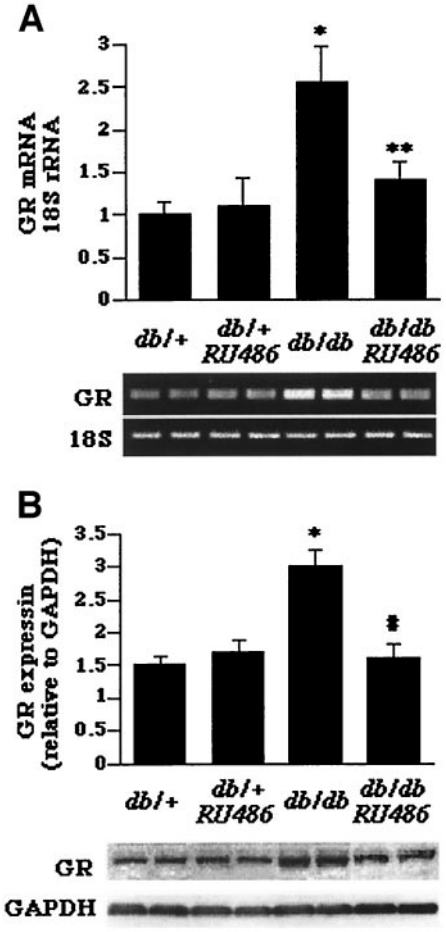

FIG. 2. The expression of glucocorticoid receptor mRNA (*A*) and protein (*B*) in the livers of *db/+* mice that were treated with vehicle (*db/+*), *db/+* mice that were treated with RU486 (*db/+* RU486), *db/db* mice that were treated with vehicle (*db/db*), and *db/db* mice that were treated with RU486 (*db/db* + RU486) for 3 weeks. *A*: Expression and relative quantification of glucocorticoid receptor mRNA levels expressed relative to the amount of 18S rRNA. *B*: Expression and relative quantification of glucocorticoid receptor protein levels expressed relative to the amount of GAPDH. Values are means ± SE; n = 5–6 mice/group. *P < 0.001 vs. *db/+* controls; **P < 0.005 vs. *db/db* mice; #P < 0.01 vs. *db/db* mice.

creases in 11β-HSD1 mRNA expression (data not shown). Corticosterone treatment of primary hepatocytes also induced glucocorticoid receptor mRNA expression, which was increased 1.8-fold in comparison with that of controls (Fig. 5*A*). This induction in glucocorticoid receptor mRNA levels by corticosterone in primary hepatocytes was paralleled by changes in glucocorticoid receptor protein expression, as shown by Western blot analysis (Fig. 5*B*). Treatment of hepatocytes with both corticosterone and RU486 (10^{-6} mol/l) abolished the corticosterone-mediated increases in both 11β-HSD1 and glucocorticoid receptor expression in comparison with hepatocytes with corticosterone alone (Figs. 4 and 5). Moreover, corticosterone-induced expression of PEPCK mRNA in primary hepatocytes was reduced significantly by RU486 (Fig. 4*C*). However, RU486 treatment for 72 h did not have a significant effect on either 11β-HSD1 or glucocorticoid receptor expression in primary hepatocytes (Figs. 4 and 5). Similarly, no change in 11β-HSD1 activity or mRNA expression was observed at 72 h in cells that were exposed to a higher concentration of insulin (10^{-5} mol/l; Fig. 4). Moreover, leptin (100–1000 ng/ml) treatment did not significantly influence 11β-HSD1 and glucocorticoid receptor expression in *db/db* mouse hepatocytes (data not shown). In addition, a higher concentration of progesterone (10^{-7} mol/l) than what occurs naturally in *db/db* mice did not significantly influence 11β-HSD1 activity or mRNA levels in primary cultures of hepatocytes (Figs. 4 and 5).

The results of semiquantitative RT-PCR analysis for 11β-HSD1 and glucocorticoid receptor mRNA expression in primary hepatocytes that were treated with glucose are shown in Figs. 6*B* and 7*A*. Treatment of primary cultures of hepatocytes with increasing doses of glucose (5–30 mmol/l) led to dose-dependent increases in 1β-HSD1 mRNA expression ($P < 0.01$; Fig. 6*B*). The increases in mRNA levels were consistent with results of analyses of enzyme activity that showed 1.25- to 2-fold increases in 11β-HSD1 activity in *db/db* mouse hepatocytes in comparison with control levels (Fig. 6*A*). Similarly, in comparison with control levels, levels of glucocorticoid receptor mRNA expression in primary cultures of hepatocytes after treatment with glucose at increasing concentrations were increased by 30–41% ($P < 0.01$; Fig. 7*A*). In addition, treatment of primary hepatocytes with glucose resulted in marked dose-dependent increases in glucocorticoid receptor protein levels (Fig. 7*B*).

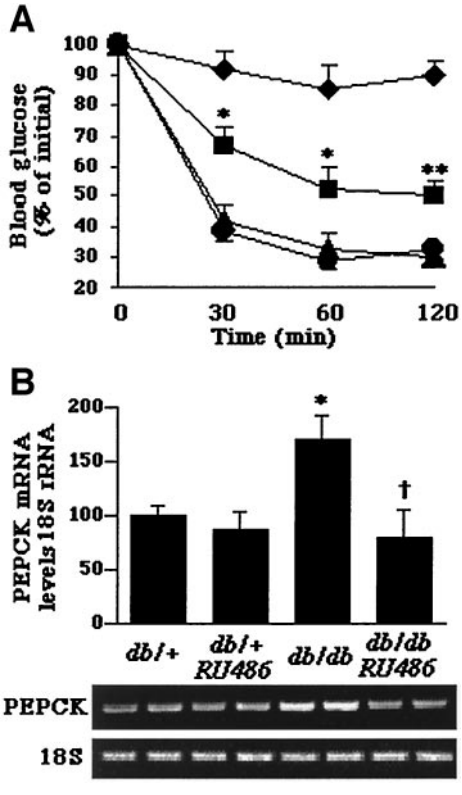

FIG. 3. *A*: Insulin tolerance test in *db/+* mice that were treated with vehicle (●; *db/+*), *db/+* mice that were treated with RU486 (▲; *db/db*), *db/db* mice that were treated with vehicle (◆; *db/db*), and *db/db* mice that were treated with RU486 (■; *db/db* + RU486) for 3 weeks. Blood samples were collected from the tail at the indicated time, and glucose levels were measured. *B*: PEPCK mRNA expression and relative quantification of PEPCK mRNA levels in livers of *db/+* mice that were treated with vehicle (*db/+*), *db/+* mice that were treated with RU486 (*db/+* RU486), *db/db* mice that were treated with vehicle (*db/db*), and *db/db* mice that were treated with RU486 (*db/db* + RU486). Values are means ± SE; n = 4–5 mice/group. *P < 0.01 vs. *db+* controls; **P < 0.001 vs. *db/db* mice; †P < 0.005 vs. *db/db* controls.

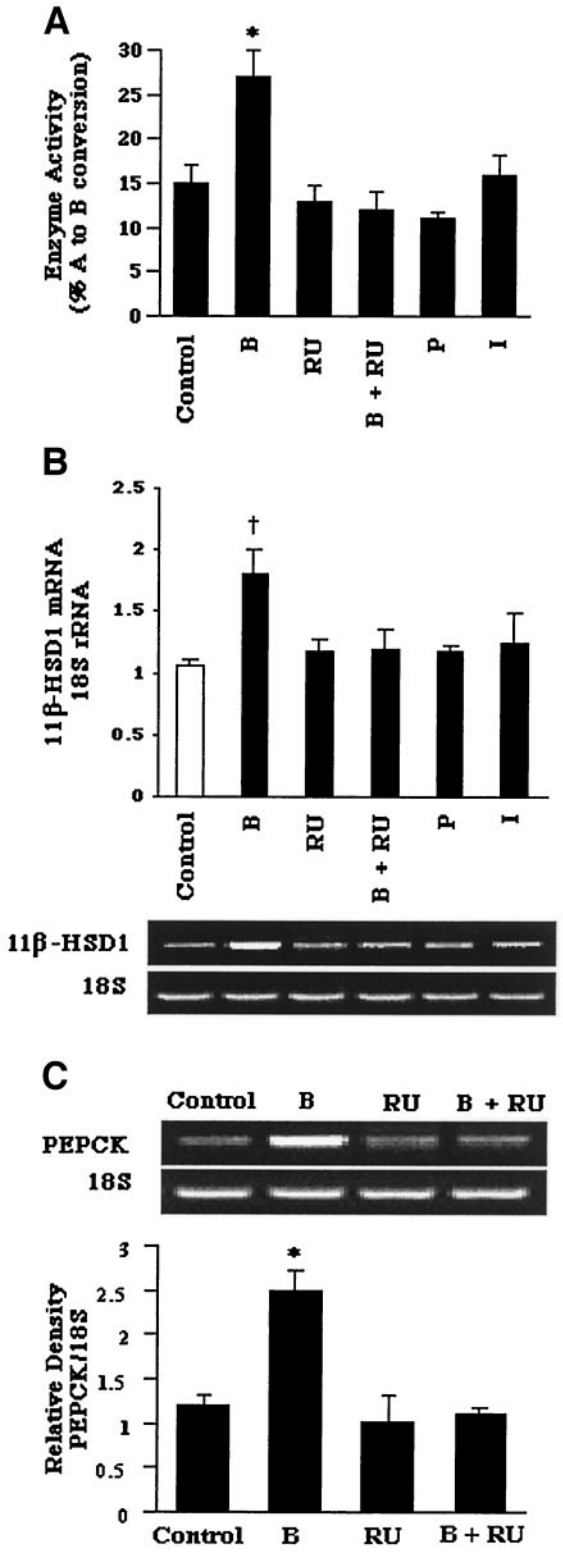

FIG. 4. Effects of corticosterone on the expression of 11β-HSD1 activities (*A*) and mRNA levels (*B*) and PEPCK mRNA expression (*C*) in primary cultures of hepatocytes from *db/db* mice. *A*: Enzyme activity expressed as percentage conversion of [^{3}H]A to [^{3}H]B. *B* and *C*: Expression and relative quantifications of 11β-HSD1 and PEPCK mRNA levels were determined by semiquantitative RT-PCR and are expressed relative to the amount of 18S rRNA. Hepatocytes were incubated in media with indicated concentrations of corticosterone (10^{-6} mol/l), RU486 (10^{-6} mol/l), progesterone (10^{-7} mol/l), and insulin (10^{-5} mol/l) for 72 h. Values are the means ± SE from three to four separate culture preparations. *P < 0.01 vs. controls; †P < 0.001 vs. controls.

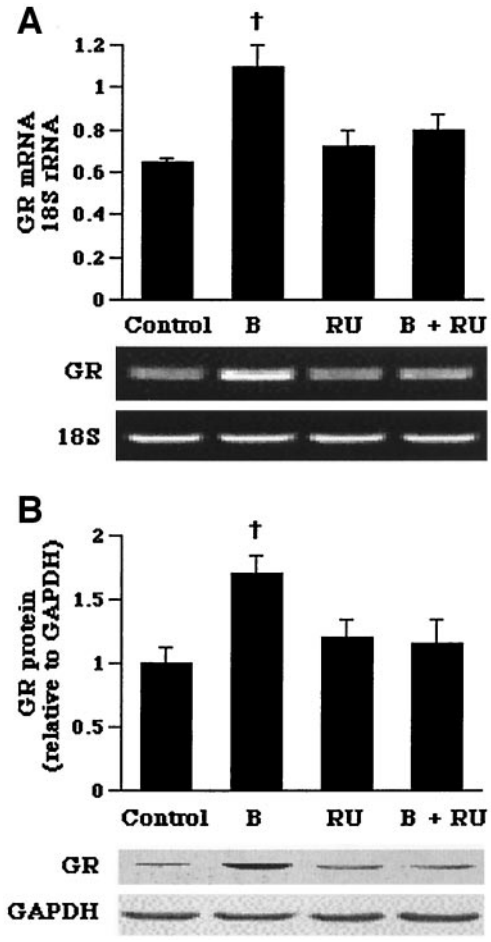

FIG. 5. Effects of corticosterone on the expression of glucocorticoid receptor mRNA (*A*) and protein levels (*B*) in primary cultures of hepatocytes from *db/db* mice. *A*: Expression and relative quantification of glucocorticoid receptor mRNA levels expressed relative to the amount of 18S rRNA. *B*: Expression and relative quantification of glucocorticoid receptor protein levels expressed relative to the amount of GAPDH. Hepatocytes were incubated in media with indicated concentrations of corticosterone (10^{-6} mol/l) and RU486 (10^{-6} mol/l) for 72 h. Values are the means ± SE from three separate culture preparations. †P < 0.01 vs. controls; *P < 0.001 vs. controls.

DISCUSSION

Accumulating evidence suggests that glucocorticoids play fundamental roles in the development of type 2 diabetes and obesity, acting mainly through glucocorticoid receptor, which confers tissue-specific responsiveness to circulating corticosteroids and thus mediates glucocorticoid-related obesity and insulin resistance. Patients with glucocorticoid excess (Cushing's syndrome) have impaired glucose tolerance and develop type 2 diabetes and visceral obesity (32,33). In *ob/ob* and *db/db* mice, removal of adrenal steroids or treatment with the glucocorticoid antagonist RU486 reduces hepatic gluconeogenesis and attenuates or even reverses the glucose tolerance and insulin resistance (34–36). Importantly, tissue glucocorticoids can be activated at the prereceptor level by 11β-HSD1, which regulates the availability of corticosterone for binding to intracellular glucocorticoid receptor and thus determines the tissue-specific action of glucocorticoids. In fact, altered tissue glucocorticoid action has been implicated as a pathophysiological mediator of type 2 diabetes and obesity through tissue-specific dysregulation of 11β-HSD1 and

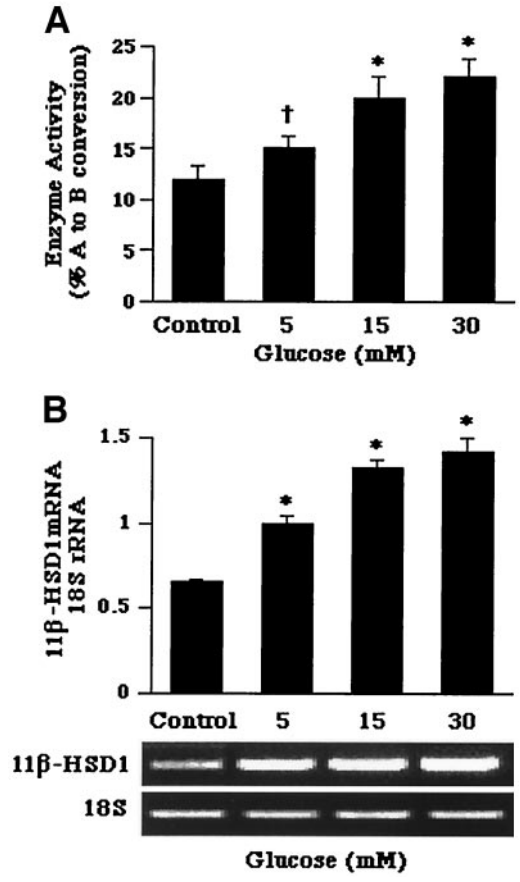

FIG. 6. Effects of glucose on 11β-HSD1 activity (*A*) and mRNA expression (*B*) in primary cultures of hepatocytes from *db/db* mice. *A*: Enzyme activity expressed as percentage conversion of [^{3}H]A to [^{3}H]B. *B*: 11β-HSD1 mRNA levels were determined by semiquantitative RT-PCR and expressed relative to the amount of mRNA found in controls. Hepatocytes were incubated in glucose-free media with indicated concentrations of glucose (5–30 mmol/l) for 72 h. Values are the means ± SE from three separate culture preparations. †$P < 0.05$ vs. controls; *$P < 0.01$ vs. controls.

glucocorticoid receptor. Transgenic mice overexpressing adipose 11β-HSD1 have increased intra-adipose corticosterone levels and glucocorticoid receptor expression and develop visceral obesity, hypertension, and insulin resistance when fed a high-fat diet (24). This increased expression seems also to be of pathogenic importance as transgenic mice overexpressing 11β-HSD1 selectively in the liver exhibit insulin resistance, although without obesity (37). In contrast, 11β-HSD1 knockout mice are resistant to diet- or stress-induced hyperglycemia and have reduced activation of the key hepatic gluconeogenic enzymes (29). In humans, increased hepatic 11β-HSD1 is thought to be an important factor in the development of insulin resistance syndrome through induction of local cortisol production and glucocorticoid receptor occupancy (26). Similarly, in patients with hypothalamic obesity, the conversion of cortisone to cortisol is enhanced through induction of 11β-HSD1 activity (38). In contrast, inhibition of hepatic 11β-HSD1 increased insulin sensitivity and reduced expression of PEPCK, a key enzyme in gluconeogenesis in humans as well as rats (27,28). These findings suggest that 11β-HSD1–enhanced intrahepatic glucocorticoid action may play an important role in the pathogenesis of type 2 diabetes. Consistent with these findings, we observed that the phenotype of type 2 diabetes in *db/db* mice was associated with the activation of expression of glucocorticoid receptor and 11β-HSD1 in the liver. This elevated expression of hepatic glucocorticoid receptor and 11β-HSD1 corresponded to increased hepatic PEPCK mRNA expression, high blood glucose, insulin resistance, and obesity, suggesting that increased local glucocorticoid action in the liver may contribute to the development of type 2 diabetes in *db/db* mice.

Leptin-resistant *db/db* mice are a widely used animal model characterized by genetic leptin receptor deficiency, which results in hypercortisolism-related insulin resistance, obesity, and many other biochemical and pathological features shared by human type 2 diabetes (12,36). The elevated circulating levels of corticosterone and insulin in *db/db* mice were confirmed here and are thought to be key factors in the control of their type 2 diabetes and obesity. In the present study, we observed that the induction of glucocorticoid receptor and 11β-HSD1 expression in the liver of *db/db* mice was associated with the elevated circulating levels of corticosterone. This was confirmed by

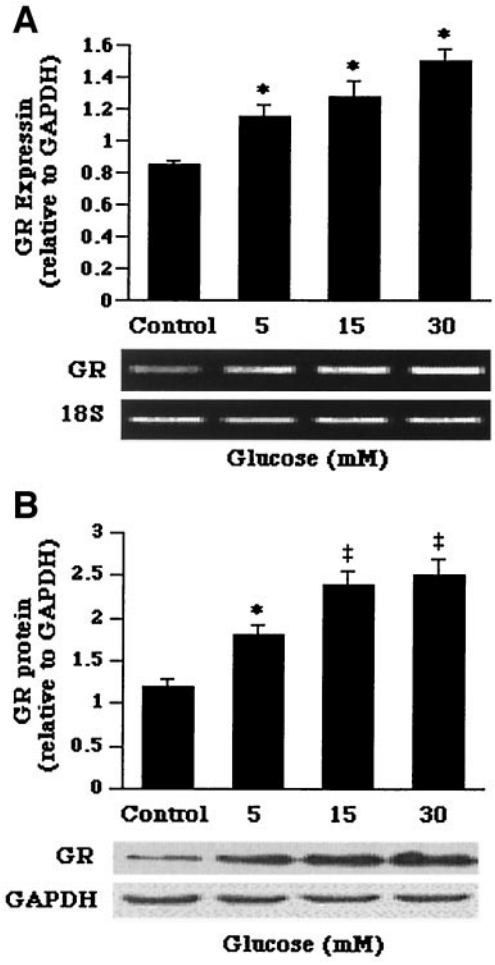

FIG. 7. Effects of glucose on the expression of glucocorticoid receptor mRNA (*A*) and protein levels (*B*) in primary cultures of hepatocytes from *db/db* mice. *A*: Expression and relative quantitation of glucocorticoid receptor mRNA levels expressed relative to the amount of 18S rRNA. *B*: glucocorticoid receptor protein levels were determined by Western blot analysis and expressed relative to the amount of GAPDH found in controls. Hepatocytes were incubated in glucose-free media with indicated concentrations of glucose (5–30 mmol/l) for 72 h. Values are the means ± SE from three separate culture preparations. *$P < 0.01$ vs. controls; ‡$P < 0.001$ vs. controls.

using isolated hepatocytes from *db/db* mice in which corticosterone increased the expression of glucocorticoid receptor and 11β-HSD1. Our present results are consistent with those of earlier reports that dexamethasone and cortisol increase 11β-HSD1 activity in rat hepatocytes and human stromal adipocytes (39,40). Moreover, sequence analysis of the 11β-HSD1 gene revealed that there is a putative glucocorticoid response element present in the promoter region of 11β-HSD1 gene (41). However, it is unclear whether glucocorticoids directly or indirectly regulate 11β-HSD1 as glucocorticoids regulate C/EBP transcription, which has been showed to be a potent activator of 11β-HSD1 gene in hepatoma cells and mouse liver (42). These data support our suggestion that the activation of 11β-HSD1 expression within liver may be associated with the actions of circulating glucocorticoids in *db/db* mice. The close relationship between the levels of glucocorticoid receptor protein and 11β-HSD1 activity and their mRNA expression in response to exposure to the glucocorticoid receptor-ligand corticosterone in our study are consistent with possible regulation of 11β-HSD1 and glucocorticoid receptor in *db/db* mouse hepatocytes by corticosterone at the level of transcription. Our results indicate that glucocorticoids may positively modulate their own prereceptor metabolism and that hypercortisolism-related hyperglycemia and insulin resistance in *db/db* mice may be mediated, at least in part, through activation of 11β-HSD1 and glucocorticoid receptor expression in hepatocytes. Hepatic activation of 11β-HSD1 may elevate both intrahepatic corticosterone and circulating glucocorticoid levels, all of which lead to induction of hepatic gluconeogenesis and insulin resistance in *db/db* mice. This is supported by findings in animal models and in obese humans indicating that tissue-specific activation of 11β-HSD1 and glucocorticoid receptor may contribute to the development of type 2 diabetes and obesity (19,26,38).

It is widely known that the role of RU486 in type 2 diabetes is associated with its ability to block both glucocorticoid receptor and progesterone receptor. In humans, RU486 has been used to treat hypercortisolemia associated with Cushing's syndrome and other conditions in which the endogenous glucocorticoid receptor is hyperactivated through increased glucocorticoid levels (43). Pharmacological blockade of glucocorticoid receptor by RU486 antagonizes high-fat diet–induced type 2 diabetes and reduces hyperglycemia in *db/db* mice and obese Zucker rats (35,36,44). In the present study, we found that treatment of *db/db* mice with RU486 reduces the phenotype of type 2 diabetes and simultaneously reduces expression of 11β-HSD1 and glucocorticoid receptor in the liver. Moreover, we observed that RU486 treatment reverses corticosterone-induced expression of 11β-HSD1 and glucocorticoid receptor in primary hepatocytes from *db/db* mice, which is consistent with early reports that glucocorticoid-induced upregulation of 11β-HSD1 can be blocked by RU486 in human amniotic fibroblasts and muscle cells (45,46). These findings indicate that the beneficial effects of RU486 on the phenotype of type 2 diabetes in *db/db* mice are not necessarily limited to blocking of both glucocorticoid receptor and progesterone receptor but may also be mediated, at least in part, through endogenous inactivation of 11β-HSD1 and glucocorticoid receptor expression in the liver. Although blockade of progesterone activity by RU486 may be involved in the reduction of blood glucose levels in *db/db* mice (44), progesterone did not affect expression of 11β-HSD1 in primary hepatocytes from *db/db* mice, consistent with a previous study showing that progesterone has no effect on 11β-HSD1 activity in rat hepatocytes (45,47,48). This is also consistent with earlier reports that the liver is not a major target tissue for progesterone, and it supports our hypothesis that the blocking effect of RU486 on corticosterone's activation of 11β-HSD1 expression in hepatocytes from *db/db* mice is very likely through glucocorticoid receptor rather than progesterone receptor.

RU486 treatment also reversed the hyperglycemia and reduced hepatic 11β-HSD1 and glucocorticoid receptor expression observed in *db/db* mice. Indeed, earlier studies showed that hyperglycemia might contribute to elevated circulating corticosterone or cortisol levels (49,50). We recently reported that hyperglycemia altered local glucocorticoid metabolism via modulation of renal 11β-HSD1 activity in experimental diabetic animals (51). In the present study, we observed that hyperglycemia correlated with the activation of 11β-HSD1 and glucocorticoid receptor expression in the liver of *db/db* mice. Moreover, we found that physiological concentrations of glucose increased 11β-HSD1 activity at the transcriptional level in *db/db* mouse hepatocytes through induction of its gene expression. The direct effect of glucose on 11β-HSD1 indicates that glucose is an important metabolic signal that increases intrahepatic corticosterone production, thereby increasing circulating glucocorticoid levels, and that hyperglycemia in *db/db* mice may be involved in the activation of 11β-HSD1 and glucocorticoid receptor–mediated local glucocorticoid action in the liver. In contrast, reduced blood glucose levels after RU486 treatment may reduce the activation of 11β-HSD1 and glucocorticoid receptor expression in the liver of *db/db* mice. Although we observed an association between insulin resistance and expression of 11β-HSD1 and glucocorticoid receptor in the liver of *db/db* mice, we did not find that insulin directly altered 11β-HSD1 and therefore did not modulate active corticosterone exposure to glucocorticoid receptor in hepatocytes from *db/db* mice. This could be interpreted that insulin exerts species- and tissue-specific differential regulation of 11β-HSD1. Insulin increases 11β-HSD1 in human skeletal muscle cells (46), decreases 11β-HSD1 in rat hepatocytes (39), and has no effect on 11β-HSD1 in human adipose stromal cells (40). In addition, we did not observe the direct effects of leptin on 11β-HSD1 and glucocorticoid receptor in *db/db* mouse hepatocytes, all of which are consistent with our earlier report (30). We conclude that increased intrahepatic glucocorticoid action through activation of 11β-HSD1 and glucocorticoid receptor expression may be associated with both the actions of circulating glucocorticoids and glucose.

In summary, we showed that increased expression of glucocorticoid receptor and 11β-HSD1 in liver may be an important component in the development of type 2 diabetes in *db/db* mice. We also found that the activation of 11β-HSD1 and glucocorticoid receptor expression in the liver of *db/db* mice are mediated, at least in part, through the actions of elevated circulating levels of glucocorticoids and blood glucose. The benefits of RU486 in type 2

diabetes in *db/db* mice may be associated with the endogenous inactivation of local glucocorticoid action through reducing expression of 11β-HSD1 and glucocorticoid receptor in the liver. These findings suggest that tissue-specific modulation of intrahepatic glucocorticoid action may be an effective target for treatment of type 2 diabetes in humans.

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