Reduction of hepatic glucocorticoid receptor and hexose-6-phosphate dehydrogenase expression ameliorates diet-induced obesity and insulin resistance in mice

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Abstract

Intracellular glucocorticoid (GC) receptor (GR) function determines tissue sensitivity to GCs and strongly affects the development of type 2 diabetes and obesity. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) mediates intracellular steroid exposure to mouse liver GR by prereceptor reactivation of GCs and is crucially dependent on hexose-6-phosphate dehydrogenase (H6PDH)-generating NADPH system. Pharmacological inhibition of 11β-HSD1 improves insulin intolerance and obesity. Here, we evaluated the potential beneficial effects of 11β-HSD1 inhibitor carbenoxolone (CBX) in diet-induced obese (DIO) and insulin-resistant mice by examining the possible influence of CBX on the expression of GR, 11β-HSD1, and H6PDH in vivo and in vitro in hepatocytes. Treatment of DIO mice with CBX markedly reduced hepatic GR mRNA levels and reduced weight gain, hyperglycemia, and insulin resistance. The reduction of hepatic GR gene expression was accompanied by CBX-induced inhibition of both 11β-HSD1 and H6PDH activity and mRNA in the liver. Moreover, CBX treatment also suppressed the expression of both phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase enzyme (G6Pase) mRNA and improved hepatic [1, 2-3H] deoxy-D-glucose uptake in DIO mice. In addition, the treatment of primary cultures of hepatocytes with increasing concentrations of CBX led to a dose-dependent downregulation of GR mRNA levels, which correlated with the suppression of both 11β-HSD1 and H6PDH activity and their gene expression. Addition of CBX to primary hepatocytes also resulted in suppression of both PEPCK and G6Pase mRNA levels. These findings suggest that CBX exerts some of its beneficial effects, at least in part, by inhibiting hepatic GR and H6PDH expression.

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Introduction

Excess glucocorticoid (GC) production (Cushing’s syndrome) induces obesity and insulin resistance via activation of intracellular GC receptor (GR; Rizza et al. 1982, Friedman et al. 1993). The GR belongs to a cytoplasmic nuclear hormone receptor that is a steroid ligand-activated transcription factor (Carson-Jurica et al. 1990, Rosen & Miner 2005). In rodents, increased hepatic GR mRNA induces activation of the key hepatic gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) leading to hyperglycemia and insulin resistance in diabetic db/db mice and obese Zucker rats (Jenson et al. 1996, Stafford et al. 2001, Liu et al. 2005). Pharmacological evidence further validates that selective inactivation of GR improves insulin resistance and obesity in these diabetic animals and patients with Cushing’s syndrome (Okada et al. 1992, Sartor & Cutler 1996, Watts et al. 2005). Moreover, specific inactivation of hepatic GR reduces elevated glucose output and reduces hyperglycemia and insulin resistance in diabetic models (Opherk et al. 2004). These findings substantiate the importance of GR in the pathogenesis of insulin resistance and obesity, thereby implicating GR as a potential target for the treatment of type 2 diabetes and obesity.

Tissue GC availability to GR can be regulated at the prereceptor level by the 11β-hydroxysteroid dehydrogenases (11β-HSDs). 11β-HSD2 is largely expressed in the kidney and placenta, and is responsible for inactivating cortisol into cortisone and thus protecting the mineralocorticoid receptor (MR) occupation from cortisol (Liu...
et al. 1998, Seckl et al. 2000). By contrast, 11β-HSD1 is a bidirectional, NADP(H)-dependent microsomal enzyme that acts predominantly as a NADPH-dependent reductase to regenerate the active GR ligand corticosterone (cortisol in human) from inactive 11-dehydrocorticosterone (cortisone), and thereby amplifying local GC availability to GR in key target tissues, such as liver and adipose tissue (Jamieson et al. 1995, Bujalska et al. 1997, Kotelevtsev et al. 1997, Chapman & Seckl 2007). Enhanced 11β-HSD1 activity results in production of excess tissue GCs and induction of local GR activation leads to hepatic gluconeogenesis and adipocyte differentiation (Aoki et al. 2001, Masuzaki et al. 2001, Whorwood et al. 2002, Liu et al. 2005), all of which are associated with the development of metabolic syndrome and visceral obesity. Moreover, selective 11β-HSD1 inhibitors improve glucose intolerance and reduce food intake and weight gain in hyperglycemic mouse models, although these inhibitors are not yet available for clinical trials (Alberts et al. 2003, Hermanowski-Vosatka et al. 2005, Wang et al. 2006). These studies implicate the importance of 11β-HSD1 in the pathogenesis of metabolic syndrome and obesity. Crucially, the effects of 11β-HSD1 in the development of obesity and type 2 diabetes are dependent on its reductase activity, which requires NADPH as the cofactor to maintain its reductase activity. NADPH is regenerated by hexose-6-phosphate dehydrogenase (H6PDH) (Atanasov et al. 2004, Banhegyi et al. 2004), a microsomal enzyme located in the lumen of the endoplasmic reticulum (ER) and principally expressed in hepatocytes and adipocytes, sites of 11β-HSD1 and GR (Hewitt et al. 2005, McCormick et al. 2006). In these target tissues, H6PDH utilizes glucose-6-phosphate (G6P) and NADP to produce NADPH (Kimura et al. 1979, Stegeman et al. 1979), thus H6PDH is likely to be the crucial enzyme supplying NADPH for 11β-HSD1-induced amplification of tissue GR ligand cortisol/corticosterone production linked to the development of type 2 diabetes and obesity.

Carbenoxolone (CBX) is a derivative of the licorice ingredient glycyrrhetic acid and has been shown to be a potent inhibitor of both 11β-HSD1 and 11β-HSD2. The beneficial effect of 11β-HSD1 inhibition on insulin tolerance has been reported in both healthy men and type 2 diabetic patients using CBX despite impaired cortisol inactivation within MR target tissues via inhibition of 11β-HSD2 (Monder et al. 1989, Walker et al. 1995, Andrews et al. 2003). Similarly, CBX attenuated hepatic insulin tolerance and improved lipid metabolism in rodents (Saegusa et al. 1999, Nuotio-Antar et al. 2007), although it did not reduce insulin resistance in obese Zucker rats (Livingstone & Walker 2003). Moreover, CBX also diminished the metabolism of 7-ketocholesterol related to inhibition of 11β-HSD1 in hepatocytes (Schweizer et al. 2004). These findings further demonstrate the efficacy of CBX in the control of type 2 diabetes. However, the CBX effects on the expression of GR and H6PDH and its contribution to improvement of obesity and insulin resistance have not yet been reported. Here, we evaluated the effects of CBX on the expression of GR and H6PDH in diet-induced obese (DIO) mice that are insulin resistant. We also assessed whether CBX would limit the corticosterone availability to mouse hepatic GR and the subsequent effects on glucose homeostasis and insulin tolerance in these mice. Finally, we explored the influence of CBX on 11β-HSD1, GR and H6PDH expression, and activity in primary cultures of hepatocytes.

Materials and methods

Animal experiments

Male C57BL/6 mice were obtained at 6 weeks of age from Charles River Laboratories (Bar Harbor, ME, USA) and housed in a room illuminated daily from 0700 to 1900 h (12 h light:12 h darkness cycle) with free access to water and standard laboratory chow. All mice were maintained in accordance with the Institutional Animal Care and Use Committee at The Charles Drew University (Los Angeles, California, USA). Mice developed DIO with insulin resistance following a high-fat diet consisting of 58% of calories from fat (D12331; Research Diets Inc., New Brunswick, NJ, USA) for 30 weeks before the initiation of treatment. Control mice were fed a standard diet consisting of 11% fat (D12328; Research Diets). Mice in each diet group were treated with CBX (25 mg/kg) dissolved in water or a matched volume of water via oral gavage for 2 weeks. This pharmacological dose of CBX is similar to previous doses used in rats and mice (Saegusa et al. 1999, Livingstone & Walker 2003). Food intake and body weight were measured before the initiation of the treatment and on the last day of the treatment. At the end of the second week after vehicle or CBX administration, non-fasting blood samples were collected into heparin tubes between 0900 and 1000 h and then stored at –80 °C until measurement of blood glucose, insulin, and corticosterone concentrations.

Glucose and insulin tolerance test (ITT)

Fourteen days following CBX or vehicle treatment, mice were fasted overnight, and a basal blood sample was taken from a tail vein and then injected with 2 g/kg D-glucose (25% stock solution in saline) intraperitoneally. Blood samples were drawn at 15-, 30-, 60-, and 120-min intervals after the glucose injection. For the ITT, animals were fasted for 8 h, and blood samples were drawn at different times following insulin injection (0-75 U/kg, i.p.; Novolin R; Eli Lilly).
Hormonal assays

Blood glucose levels were determined by the glucose oxidase method. Serum corticosterone was determined by RIA using mouse corticosterone as a standard (ICN Biomedicals, Costa Mesa, CA, USA). Serum insulin levels were measured by RIA using rat insulin as a standard (Crystal Chemicals, Chicago, IL, USA).

2-Deoxy-glucose uptake in liver

Fourteen days following CBX or vehicle treatment, mice were fasted for 8 h, then anesthetized with sodium nembutal (100 mg/kg i.p.; Witczak et al. 2007), and [1, 2-3H]deoxy-D-glucose (2-[-3H]DG; NEN Life Science Products, Boston, MA, USA; 1.0 Ci/kg body weight) was administered via the jugular vein (Ho et al. 2004). After 1 h, animals were killed, and their livers were removed, cleaned, weighed, and solubilized with tissue Solubilizer (Amersham). Radioactivity was measured in liver samples using a liquid scintillation counter.

Primary cultures of hepatocytes and drug treatment

Primary hepatocytes were isolated from DIO mice by a two-step collagenase perfusion procedure as previously described (Liu et al. 2003, 2006) and seeded onto collagen-coated dishes in Dulbecco’s Modified Eagle’s Medium (DMEM)/F12 medium (Invitrogen Life Technologies) with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin supplemented with dexamethasone (10^-9 M) and insulin (10^-9 M). After 4 h incubation, the cells were then washed with PBS and treated with CBX (10^-7–10^-4 M) for 12–72 h.

Immunoblotting analysis (western blot)

Protein was extracted from liver tissues and primary hepatocytes by homogenization with ice-cold radio immunoprecipitation assay (RIPA) buffer as previously described (Schweizer et al. 2004, Liu et al. 2005). Protein concentrations were measured with the Bradford assay (Bio-Rad protein assay kit). For western blot detection of GR and H6PDH protein expression, proteins were boiled with sample buffer containing 1% SDS, Tris–HCl (10 mmol/l (pH 7.5)), loaded and separated on 8 or 10% SDS polyacrylamide gels and then electrophoretically transferred to Hybond-electrochemiluminescence (ECL) nitrocellulose membranes. Membranes were blocked in 5% skim milk (Bio-Rad) for 30 min and then incubated with a polyclonal anti-GR antibody (1:1000, Affinity BioReagents, Golden, CO, USA) and mouse antihuman H6PDH antibody (1:500, Novus Biological, Littleton, CO, USA) overnight at 4°C. The bound primary antibody was visualized using ECL system (Amersham Biosciences). Membranes were then exposed to film or scanned, and densitometry was performed to quantify the intensity of the GR and H6PDH protein signal using Eagle Eye II Quantitation System.

Analysis of metabolic gene expression

Total RNA was prepared from hepatocytes with a single-step extraction method (RNAzol B; Invitrogen), RNA samples were quantified by spectrophotometry, and integrity was assessed by 1-5% agarose gel electrophoresis and ethidium bromide staining. RNA from each sample was reverse transcribed and then amplified by PCR with primers specific for mouse GR (forward: 5′-TGCTATGCTTTGCTCTGACT-3′, reverse: 5′-TGTGCAAGTTGATAAAAAGCCTG-3′), PECK (forward: 5′-AGCTGACAGCCTGCCCCAGG-3′, reverse: 5′-CCAGTTGGTACAAAAAGGCTTT-3′), G6P enzyme (G6Pase) (forward: 5′-AAGACTCCTCCAGGACTCGTT-3′, reverse: 5′-TACGAGGTAGAATCCAAAGCGCGG-3′), H6PDH (forward: 5′-AGCCACTCTCTCATCCAGG-3′, reverse: 5′-ATGTGAGCGGTGGAGATCA-3′), 11β-HSD1 (forward: 5′-TTCGATGCAATTTATGAAGGAATT-3′, reverse: 5′-TACAGAAGTATACGGCAGG-3′), and 18S RNA (Liu et al. 2003, 2006). The amplification conditions of GR, 11β-HSD1, H6PDH, PECK, and G6Pase were optimized in preliminary studies to result in amplification within the linear range. PCR products were visualized on agarose gels by ethidium bromide staining and gels were photographed under u.v. light. Relative gene expression was quantified by densitometry with the Eagle Eye II Quantitation System (Stratagene, La Jolla, CA, USA) and was normalized to the signal of 18S rRNA.

Measurement of hepatic 11β-HSD1 and H6PDH activity

Hepatic 11β-HSD1 activity was measured after homogenization as previously described (Liu et al. 2005). The protein concentration was measured by Bradford assay (Bio-Rad Protein Assay Kit Biocal). After extraction, supernatants were incubated with 2 μmol/l [3H]corticosterone (B) in the presence of 200 μM NADP+ (for 11β-dehydrogenase activity) using 0.25 mg protein/ml for 10 min at 37°C. Steroids were extracted with ethyl acetate, separated by thin layer chromatography (TLC) and quantified by scintillation counting. The percentage of interconversion of [3H]B and [3H]11-DHC was calculated from the radioactivity in each fraction. H6PDH enzyme activity was carried out by spectrophotometric measurement of NADPH production in the presence of 1–2 mM glucosamine-6-phosphate and NADP using absorbance at 340 nm using a

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spectrophotometer (Ultrospec 2100; Amersham Biosciences). The microsomal pellet was obtained by centrifugation of the 10 000 g supernatant for 1 h at 100 000 g. Homogenized tissue protein (200 μg) or protein from microsomes (20 μg) was incubated in 100 mM glycine buffer solution (containing 1% BSA (pH 100) and 0.5–5 mM glucosamine-6-phosphate (Ropson & Powers 1998, Nammi et al. 2007), 1–5 mM NADP) at room temperature for 0–5 min as described (Welberg et al. 2000). Specific activities were calculated and expressed as micromoles of NADPH production per minute per milligram of protein.

Analysis of 11β-HSD1 and H6PDH activity in primary hepatocytes

11β-HSD1 has been shown to be exclusively a 11β-reductase in primary cultures of mouse and rat hepatocytes (Jamieson et al. 1995, Liu et al. 2005), thus 11β-HSD1 reductase activity was measured in primary cultures of DIO mouse hepatocytes by measuring the rate of interconversion of [3H]11-DHC and [3H]B. Briefly, the cells were incubated with 2 nmol/l [3H]11-DHC with 18 nmol/l unlabeled 11-DHC at 10–30 min as previously reported (Liu et al. 2003, 2005). Steroids were extracted from 1·0 ml culture medium with ethyl acetate and separated by TLC. Protein concentrations were measured with Bio-Rad Protein Assay Kit. Enzyme activity levels were determined by counting of radioactivity. For the H6PDH activity in vitro, 20 μg protein extracts from primary hepatocytes were incubated with 2 mM G6P as substrates in 100 μl total volume of glycine buffer (pH 10·0) with 0·5 mM NADP as a cofactor (Stegeman & Klotz 1979, Nammi et al. 2007). The changes in absorbance at 340 nm were measured over 25 min at 5-min intervals, and the relative H6PDH activity was expressed as above described.

Statistical analyses

All data are expressed as the mean ± s.e.m. The normality of the distribution of data was established using Wilks–Shapiro test, and outcome measures between groups were compared by Student’s t-test. To compare multiple groups, one-way ANOVA is used. If ANOVA reveals significant differences, then individual group comparisons were performed by using the Newman–Keul’s post hoc test. The differences among groups were considered significant at P<0·05.

Results

The effects of 11β-HSD1 inhibitor CBX on body weight, blood glucose, serum insulin, and corticosterone

As shown in Table 1, DIO mice had increased body weight and elevated serum levels of insulin and corticosterone compared with chow-fed lean controls. The mean blood glucose levels of DIO mice were markedly higher than those of lean controls (P<0·001). Treatment of DIO mice with CBX for 2 weeks significantly reduced the blood glucose levels, the body weight, and the average food intake (P<0·01 versus respective vehicle-treated groups; Table 1). There was a trend toward a decrease in serum corticosterone levels with no significant effect on insulin levels in DIO mice after CBX treatment. CBX treatment had no significant effects on body weight, food intake, blood glucose, insulin, and corticosterone levels in lean mice as compared with vehicle-treated controls (Table 1).

11β-HSD1 inhibitor CBX improved the glucose homeostasis and insulin tolerance in DIO mice

Glucose tolerance test revealed that treatment of DIO mice with CBX decreased the glucose intolerance observed in vehicle-treated DIO mice (Fig. 1A). This was confirmed by the ITT, which showed that the glucose-lowering effects of insulin were significantly improved in CBX-treated DIO mice in comparison with that observed in vehicle-treated DIO mice after insulin challenge (Fig. 1B). Consistent with these observations, CBX when compared with vehicle increased 2-[3H]DG uptake by 1·8-fold in DIO mice (P<0·001). Similarly, the hepatic 2-[3H]DG uptake was increased by 1·5-fold after treatment with CBX in lean compared with

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All values are mean ± s.e.m. *P<0·001 versus lean vehicle. †P<0·01 versus DIO vehicle. ‡P<0·005 versus lean vehicle.

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vehicle-treated controls (Fig. 2A). Quantitative RT-PCR analysis showed that CBX treatment resulted in a 12.5% \((P<0.05)\) decrease in hepatic PEPCK mRNA expression levels in lean mice and a 27% \((P<0.01)\) decrease in hepatic PEPCK mRNA expression levels in DIO mice versus their respective vehicle-treated mice (Fig. 2B).

Hepatic G6Pase mRNA expression decreased by 1.2- \((P<0.05)\) and 1.25-fold \((P<0.01)\) respectively in lean and DIO mice after CBX treatment (Fig. 2C).

The effects of CBX treatment on 11\(\beta\)-HSD1 and GR gene expression in DIO mice

CBX as compared with vehicle treatment decreased hepatic 11\(\beta\)-HSD1 activity by 27% \((P<0.01)\) in lean mice and by 63% \((P<0.001)\) in DIO mice (Fig. 3A).

This was confirmed by quantitative RT-PCR analysis, which showed that CBX treatment decreased hepatic 11\(\beta\)-HSD1 mRNA by 1.3-fold \((P<0.05)\) in lean mice and by 1.6-fold \((P<0.01)\) in DIO mice (Fig. 3B). Similarly,
the GR mRNA expression of liver tissue was reduced by 1.32- and 1.4-fold after treatment with CBX in both lean and DIO mice (P<0.001 versus respective vehicle-treated mice; Fig. 3C). Western blot analysis revealed that the hepatic GR nuclear protein levels were reduced by 1.2- and 1.6-fold in both lean and DIO mice after CBX treatment (P<0.01 and P<0.005 versus respective vehicle-treated mice; Fig. 3D).

**CBX-induced inhibition of hepatic 11β-HSD1 and GR expression is associated with suppression of H6PDH activity and gene expression**

Parallel to the decrease in 11β-HSD1 activity and GR expression, hepatic H6PDH activity was reduced by 35% (P<0.01) in DIO mice after CBX treatment compared with vehicle-treated controls (Fig. 4A). Quantitative RT-PCR analysis revealed that CBX decreased hepatic H6PDH mRNA levels by 2.4-fold (P<0.001) in DIO mice (Fig. 4B). Moreover, CBX treatment also reduced the levels of both H6PDH activity (P<0.05) and its mRNA expression (P<0.05) in lean mice compared with those of vehicle-treated controls.

**The effects of CBX in target gene expression in primary cultures of hepatocytes**

To confirm our observations *in vivo*, we treated primary hepatocytes from DIO mice with CBX and measured GR mRNA and 11β-HSD1. As shown in Fig. 5A, the treatment of primary cultures of hepatocytes with increasing doses of CBX led to a 1.1- to 1.5-fold decrease in GR mRNA levels compared with those of controls. Similarly, the level of the 11β-HSD1 reductase activity in primary cultures of hepatocytes following treatment with CBX at increasing concentrations was decreased by 50–76% in comparison with control levels.
Moreover, this decrease in 11β-HSD1 reductase activity by CBX in primary hepatocytes was paralleled by changes in 11β-HSD1 mRNA expression, as shown by quantitative RT-PCR analysis (Fig. 5C). In addition to these suppressive effects, quantitative RT-PCR analysis also revealed that CBX treatment of hepatocytes resulted in a dose-dependent decrease in the PEPCK mRNA expression in comparison with that of controls (Fig. 6A). Moreover, a dose-dependent decrease in G6Pase mRNA expression was also observed in primary hepatocytes after CBX treatment (Fig. 6B).

To gain further insight into the effects of CBX on 11β-HSD1 reductase activity and GR mRNA expression, the levels of both H6PDH protein and mRNA expression were determined in primary hepatocytes treated with different concentrations of CBX for 48 h. Western blot analysis showed that H6PDH protein expression in primary hepatocytes was decreased in a dose-dependent manner by CBX treatment (Fig. 7A). The decrease in protein levels was consistent with the results of quantitative RT-PCR analysis that demonstrated that CBX decreased H6PDH mRNA levels by 1.2- to 1.6-fold in primary hepatocytes (Fig. 7B). Similar to our

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findings on 11β-HSD1 reductase activity, CBX (10 μM) treatment also decreased NADPH production in the presence of glucosamine-6-phosphate as a substrate, demonstrating that H6PDH activity was decreased in these intact cells after CBX treatment as compared with that of vehicle-treated controls (Fig. 7C).

**Discussion**

In this study, we found that oral administration of the 11β-HSD1 inhibitor CBX suppressed hepatic GR gene expression and reduced the diet-induced obesity and insulin resistance in mice. Moreover, CBX-induced reduction in GR expression was accompanied by the suppression of both PEPCK and G6Pase mRNA expression.
expression and induction of hepatic \(2^{[3]}\text{H}\text{DG} \) uptake, indicating that reduction of hepatic GR expression may be involved in the antidiabetic actions of CBX in DIO mice. Consistent with these findings, our works with hepatocytes showed that CBX reduced the expression of both GR and PEPCK mRNA in primary hepatocytes from DIO mice. This is supported by previous reports demonstrating that CBX inhibited GR mRNA levels in hypothalamic paraventricular nucleus in rats (Welberg et al. 2000), although no direct evidence exists about the CBX-induced alteration of GR mRNA in diabetic rodents. Our findings indicate that CBX could exert its beneficial effects in DIO mice, at least in part, through inhibition of hepatic GR mRNA expression. Inhibition of hepatic GR expression by CBX could reduce the ability of the tissues to respond to excess circulating GCs and attenuate GR-induced activation of both PEPCK and GCs-antagonizing insulin action. This could reduce hepatic gluconeogenesis and improve hypercortisolemia-related glucose intolerance, insulin resistance, as well as food intake in DIO mice.

The observed inhibitory effects of CBX on GR mRNA paralleled the suppression of hepatic 11β-HSD1 expression and improved food intake, weight gain, and insulin resistance in DIO mice. Our cell work further demonstrated that CBX directly suppressed 11β-HSD1 reductase activity and its gene expression in primary hepatocytes and correlated with the suppression of GR and PEPCK mRNA expression. These results show that CBX inhibited mouse hepatic 11β-HSD1 reductase activity at the transcriptional level, thereby reducing the intracellular active steroids exposure to mouse liver GR by lowering corticosterone regeneration. Our results are in agreement with previous studies reporting that CBX is a potent 11β-HSD1 inhibitor through the reduction of 11β-HSD1 mRNA (Saegusa et al. 1999) and exerts some beneficial effects in patients with type 2 diabetes as well as in rodents (Walker et al. 1995, Andrews et al. 2003, Nuotio-Antar et al. 2007). This is supported by a recent study demonstrating that inhibition of recombinant human 11β-HSD1 gene in intact transfected cells by selective inhibitors was able to prevent the subsequent cortisol-induced activation of GR (Schuster et al. 2006). Similarly, we recently reported that RU486 blocks GR activation and liver GC regeneration, thereby attenuating the phenotype of type 2 diabetes in db/db mice (Liu et al. 2005). These data support our suggestion that CBX-induced inhibition of 11β-HSD1 in hepatocytes may be an important metabolic signaling that interferes with ligands supply to the GR and thus GR-mediated local GC action. In addition, epididymal adipose 11β-HSD1 activity was decreased by 27% \((P<0.01)\) in DIO mice after CBX (Liu et al. unpublished observations), consistent with a recent clinical study demonstrating that CBX inhibits adipocyte 11β-HSD1 activity and limits GCs exposure of the human adipose tissue (Tomlinson et al. 2007). Thus, the possibility that lessening of adipose GC reproduction and GR activation must also be considered as a potential mechanism in the beneficial effects of CBX in DIO mice.

It is well known that 11β-HSD1 amplification of tissue cortisol/corticosterone regeneration is critically dependent on H6PDH activity. In the ER, H6PDH catalyzes G6P and NADP to regenerate NADPH, thereby playing an important role in determining 11β-HSD1 reductase activity in liver and adipose tissues (Kimura et al. 1979, Stegeman et al. 1979, Atanasov et al. 2004, Banhegyi et al. 2004, Hewitt et al. 2005, McCormick et al. 2006). In the present study, we found that treatment of DIO mice with CBX inhibited hepatic 11β-HSD1 activity and simultaneously reduced H6PDH activity and its gene expression. To our knowledge, this is the first report showing that CBX influenced hepatic H6PDH mRNA expression and activity. This was further confirmed by our results in mouse primary hepatocytes showing that CBX suppressed both H6PDH protein and its mRNA levels and reduced NADPH production accompanied by the reduction of 11β-HSD1 reductase activity. Our findings suggest that the ability of CBX to inhibit hepatic 11β-HSD1 activity may be associated with the suppression of H6PDH-mediated NADPH production. It further confirms recent findings that describe the importance of H6PDH in the control of 11β-HSD1 as a distinct oxoreductase (Banhegyi et al. 2004, Hewitt et al. 2005, McCormick et al. 2006). This is also indirectly supported by a recent study showing that many patients with clinically apparent 11β-HSD1 reductase deficiency actually have mutations in the gene coding for H6PDH and are unable to regenerate NADPH from NADP by catalyzing the conversion of G6P to 6-phosphogluconolactone (Draper et al. 2003). Similarly, hepatic 11β-HSD1 reductase activity was impaired in H6PDH knockout mice through inactivation of local NADPH regeneration and these mutant mice exhibited fasting hypoglycemia (Lavery et al. 2006).

Although the underlying mechanism of these observations is not entirely clear, the current study observed that CBX led to reduction of hepatic H6PDH activity that is correlated with hypoglycemia by the suppression of the key gluconeogenic enzyme PEPCK and G6Pase expression in DIO mice. Our cell work demonstrating that CBX treatment caused a dose-dependent suppression of both PEPCK and G6Pase mRNA levels and corresponded to the reduction of H6PDH activity and its gene expression in mouse primary hepatocytes. Suppression of the PEPCK and G6Pase expression by CBX could reduce hepatic gluconeogenesis sufficiently to decrease the accumulation of hepatic glycogen and circulating glucose levels, thereby preventing the primary synthesis...
pathway of G6P from glycogen and glucose, leading to the reduction of G6P generation in mouse hepatocytes. This is crucial to inhibition of the ability of H6PDH utilizing NADP to regenerate NADPH in ER (Draper et al. 2003). Our findings are consistent with recent studies reporting that lack of G6P availability to H6PDH is associated with impaired hepatic 11β-HSD1 reductase activity in hypoglycemic mice (Chou et al. 2002). Moreover, CBX inhibition of hepatic 11β-HSD1 reductase activity itself decreased NADP regeneration from NADPH in ER by preventing the conversion of 11-dehydrocorticosterone to corticosterone, thereby impairing an effective pathway for supplying NADP as cofactor to H6PDH linked to the reduction of H6PDH activity (Ferguson et al. 1999). These findings agree with the notion that H6PDH activity is dependent on luminal NADP availability, which can be regenerated by oxidizing luminal NADPH in a reaction catalyzed by 11β-HSD1 (Hino & Minakami 1982, Csala et al. 2006). Our data demonstrate that inhibition of 11β-HSD1 may play an important role in control of CBX-induced suppression of hepatic H6PDH in DIO mice, although further studies using specific 11β-HSD1 inhibitors would be expected to provide additional evidence. Therefore, CBX-mediated inhibition of H6PDH might result from an indirect action reducing the availability of both G6P and NADP to H6PDH in liver.

In summary, our study demonstrates that CBX effectively inhibited hepatic 11β-HSD1 activity and exerted beneficial effects including suppression of the diet-induced insulin resistance and obesity in DIO mice. These results also showed that the beneficial effects of CBX in DIO mice may be mediated, at least in part, through interference with the GR and H6PDH expression in the liver. These studies raise the possibility that functional cooperative manipulation of both H6PDH and 11β-HSD1 within target tissues may be a better approach to control insulin resistance and obesity. Thus, in addition to the novel roles of CBX in the suppression of both GR and H6PDH expression, 11β-HSD1 inhibitors could be considered as a potential pharmacotherapeutic agent for the treatment of type 2 diabetes and metabolic syndrome.

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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