Type 1 11β-Hydroxysteroid Dehydrogenase Mediates Glucocorticoid Activation and Insulin Release in Pancreatic Islets*

Received for publication, August 31, 2000
Published, JBC Papers in Press, September 5, 2000,
DOI 10.1074/jbc.C000600200

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Metabolic transformation of glucocorticoid hormones constitutes a determinant of their cell-specific effects. The most important reaction for this class of steroids is the reversible C11 keto/β-hydroxyl conversion between receptor-binding 11β-OH steroids and the nonbinding 11-oxo compounds, carried out by 11β-hydroxysteroid dehydrogenases (11β-HSDs). In this study, we determined the role of glucocorticoid conversion by 11β-HSD in pancreatic islets and its function in the regulation of insulin release. Pancreatic islets isolated from ob/ob mice display type 1 11β-hydroxysteroid dehydrogenase activity, i.e. in intact cells the reductive reaction prevails, leading from dehydrocorticosterone to corticosterone. Expression of type 1 11β-HSD mRNA was detected by reverse transcriptase-polymerase chain reaction in islets isolated from ob/ob mice and also from human tissue. Incubation of β-cells in the presence of 11-dehydrocorticosterone leads to a dose-dependent inhibition of insulin release, indicating cellular activation of 11-dehydrocorticosterone to the receptor ligand, further confirmed by reporter gene assays. Inhibition of 11β-HSD activity by carbenoxolone reverses inhibition of insulin release. The presence of 11β-HSD in islets supports the concept that reactivation of inert circulating hormone precursors in a cell-specific manner plays a major role in glucocorticoid physiology in rodents and man.

Glucocorticoid (GC) hormones play a critical role in the regulation of carbohydrate metabolism. They increase hepatic glucose production through gluconeogenesis by induction of key enzymes like phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (1–4). Furthermore, they decrease insulin sensitivity in skeletal muscle and adipose tissue. Both GC-induced effects, increased hepatic glucose production and insulin resistance, result in increased plasma insulin levels. However, this is attenuated by a direct inhibitory effect of glucocorticoids on insulin release from pancreatic β-cells as suggested by in vitro and in vivo studies (5–13).

GCs mediate their effects through specific intracellular receptors present in almost all cell types including β-cells. The glucocorticoid receptor belongs to the superfamily of nuclear hormone receptors, which function as ligand-activated transcription factors (14–16). Several studies point out that circulating levels of steroid, cellular expression of receptors, and intracellular metabolic GC conversion determine whether GC gain access as active ligands to their receptors (17). In this respect, it has been established that the metabolic biotransformation at the 11β-hydroxy/11-oxo function of glucocorticoid hormones by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD, EC 1.1.1.146) is important in GC physiology and determines cell-specific modulation of glucocorticoid and mineralocorticoid effects (17–19). Thus far, two 11β-HSD isozymes (11β-HSD-1 and 11β-HSD-2) have been characterized in detail (17–19). 11β-HSD-1 acts in vivo mainly as a NADPH-dependent reductase, thereby activating GCs from circulating 11-oxo precursors (cortisone in humans, and 11-dehydrocorticosterone in rodents) to the respective 11β-OH receptor ligands (cortisol, corticosterone) (20, 21). The type 2 isozyme (11β-HSD-2) functions in vivo and in vitro exclusively as a high affinity (Kₐ, 10 nm), NAD⁺-dependent dehydrogenase of adrenal glucocorticoids. It inactivates “active” cortisol to cortisone, thereby “protecting” the mineralocorticoid receptor from occupancy by cortisol (22–24). The importance of the type 1 enzyme for hepatic regulation of glucose metabolism by “reactivating” cortisone to cortisol has been demonstrated by a mouse “knockout” model (25) and other in vivo and in vitro studies (26, 27).

The intention of the present investigation was to evaluate the occurrence of 11β-HSD in Langerhans’ islets of the pancreas and, if present, to assess the role of this enzyme in β-cell glucocorticoid physiology and insulin release.

EXPERIMENTAL PROCEDURES

Animals, Human Pancreatic Specimen, Isolation of Islets, and Cell Culture—ob/ob mice, 10–12 months old and weighing 50–60 g, were kept as described (12). Pancreatic tissue was removed and digested with collagenase under continuous shaking in Hanks’ balanced salt solution at 37 °C. Islets were collected under stereomicroscopy and incubated in RPMI medium supplemented with glucose. Human pancreatic tissue was obtained through routine surgery. Immediately after resection, islets were prepared by collagenase treatment, and islets were collected under stereomicroscopy.

Determination of Insulin Release—Insulin release from islets was determined by incubating batches of three islets for 60 min at 37 °C in 300 μl of Krebs-bicarbonate buffer (pH 7.4, supplemented with 2 mM bovine serum albumin) under basal (3.3 nm), and stimulatory (8.3 and 16.7 nm) glucose concentrations. Prior to the study of insulin release, islets were cultured in RPMI 1640 with 11 mM glucose in the presence or absence of the 11β-hydroxysteroid dehydrogenase inhibitor carbenoxolone; TLC, thin layer chromatography; LSC, liquid scintillation counting; bp, base pair(s).

* This study was supported by grants from the European Community (BIO4CT97–2123), Swedish Medical Research Council (13X-3532, 4X-3766, 72X-00034, and 13X-2819), Novo Nordisk Fonden, Denmark, the Nordins Insulin Foundation committee, Karolinska Institutet, and Pharmacia Corporation (to U. C. T. O.), Sweden. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: GC, glucocorticoid; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 11-DHC, 11-dehydrocorticosterone; CBX, carbenoxolone; CBG, corticosterone-binding globulin; PCR, polymerase chain reaction; RIA, radioimmunoassay; RT-PCR, reverse transcriptase-PCR; TLC, thin layer chromatography; LSC, liquid scintillation counting; bp, base pair(s).
inhibition of DHC reduction by CBX in intact islet preparations. 25 m
Km
3

hydrogenase in intact pancreatic islets isolated from ob/ob mice.

11-dehydrocorticosterone plus 100,000 cpm tracer (reduction). Medium

11-hydroxysteroid dehydrogenase as a function of substrate concen-
tration. Analysis of kinetic parameters by regression analysis reveals a

range from 8.5 to 43 pmol/25 islets

11-dehydrocorticosterone as substrates, low levels of
derived from 10 to 500 nM and further supplemented with

tracer steroid. After incubation reaction mixtures were extracted with a

5-fold volume of ethyl acetate after the addition of excess unlabeled
corticosterone and 11-DHC (5 μl of 10 mg/ml solution). The organic

phase was dried under nitrogen, redissolved in methanol, transferred to

silica TLC plates, and steroids were separated using a mobile phase of
dichlormethane/acetone (4:1, v/v). Substrates and products were de-
dected by UV illumination (254 nm). Spots detected were cut out and
eluted into scintillation fluid, and the fractional conversion into product
was determined by LSC.

RT-PCR—Total RNA from liver and isolated islets was prepared
using the RNAzol RNA preparation kit. First-strand cDNA synthesis
was performed with random hexanucleotide primer mixtures, total
RNA (1.0 μg/10 μl reaction), and 200 units/10 μl Moloney murine
leukemia virus reverse transcriptase (MMLV-RT, Life Technologies,
Inc.) for 45 min at 40 °C followed by a 5-min denaturation at 95 °C. PCR
with thermostable polymerase (Taq polymerase, Stratagene) was carried out
using mouse-specific 11β-HSD-1 (29) (sense, 5′-TTA TGA AAA AAT
ACC TCC TCC C; antisense, 5′-CTT GTA TCT CCA GGG CGC ATT C),
human-specific 11β-HSD-1 (sense, 5′-ATG CTC CAA GGA AAG AAA
GTG ATT GTA ACA GGG GCC; antisense, 5′-CTA CTT TGA TCT CCA GGG CGC
ATT C), human-specific 11β-HSD-1 (sense, 5′-TGA CGT TCG GTC AAC G; antisense, 5′-CAT
GTA GGC CAT GAG GTC) primer sets. Denaturation was at 95 °C (1
min), annealing at 52 °C (1 min), with extension at 72 °C (1 min) for a
total of 40 cycles. Resulting products (human 11β-HSD-1, 5,000 bp;
mouse 11β-HSD-1, 729 bp; GAPDH, 965 bp) were analyzed on 1%
TAE agarose gel electrophoresis (ethidium bromide staining) of ob/ob mouse and human tissues.

Pancreatic islets were isolated

by collagenase treatment and dispersed as described above. The cells
were resuspended in RPMI 1640 culture medium (Life Technologies,
Inc.) containing 11 mM glucose supplemented with 10% fetal bovine
serum, 100 international units/ml penicillin and 100 μg/ml streptomycin.
Cells were grown in 6-well plates, incubated at 37 °C in 5% CO2,
and transfected by LipofectAMINE Plus (Life Technologies, Inc.) with
400 ng of a (GRE)-tk-Luc reporter plasmid (30). The following day, fresh
medium was added containing either 100 nM 11-DHC or 100 nM corti-

costerone with or without 5 μM CBX at final concentration. After 16 h,
cells were lysed and analyzed for luciferase activity. Each experimental
condition was analyzed in triplicate; values represent the mean ± S.D.
of 2 experiments.

RESULTS

11β-Dehydrogenase Activity in Pancreatic Islets—Islets of
Langershans prepared from ob/ob mice display 11β-dehydro-
steroid dehydrogenase activity (Fig. 1, A and B). In intact cells,
using corticosterone and 11-DHC as substrates, low levels of
dehydrogenase but high levels of 11-keto reductase activity
could be detected (Fig. 1A), thus presenting features of the type
1 (i.e. liver-type) 11β-hydroxysteroid dehydrogenase isozyme.
Enzymatic constants for 11-oxo reduction were determined in

oxidized (CBX; 0, 0.5, 1.0, and 5.0 μM), followed by a 30-min incubation
in Krebs-bicarbonate buffer with 3.3 mM glucose. Insulin release was
determined by use of a radioimmunoassay (RIA) (12). Samples were run
in triplicate, and the number of experiments was n = 4–6.

11β-Hydroxysteroid Dehydrogenase and 11-Oxo-reductase Assays—
Oxidative and reductive conversions of corticosterone and 11-dehydro-
corticosterone (11-DHC), respectively, were analyzed by incubating
intact islet preparations in the presence of 100,000 cpm [3H]corticosterone
(specific activity 2.2 terabecquerel/mmol) or 100,000 cpm [3H]de-

11β-HSD Regulates Insulin Release

FIG.1. Glucocorticoid metabolism by 11β-hydroxysteroid dehydro-
genase in intact pancreatic islets isolated from ob/ob mice. A, islets were incubated for 20 h in RPMI medium supplemented
with 50 nM corticosterone plus 100,000 cpm tracer (oxidation) or 50 nM
11-dehydrocorticosterone plus 100,000 cpm tracer (reduction). Medium
and cells were extracted, and relative product formation was deter-
mined by TLC followed by LSC. Blanks indicate experiments performed
in the absence of islets. B, reduction of 11-dehydrocorticosterone by islet
11β-hydroxysteroid dehydrogenase as a function of substrate concen-
tration. Analysis of kinetic parameters by regression analysis reveals a

Km of 92 nM and a Vmax of 8.5 (mean Kmax, 97.5 ± 24.3; mean Vmax, 24.0;
range from 8.5 to 43 pmol/25 islets × 12 h). n = 5 experiments. C, inhibition of DHC reduction by CBX in intact islet preparations. 25 islets
were incubated at 100 nM DHC concentration in the absence (first column, DHC) or presence of 5 μM CBX (DHC + 5.0 CBX) or 20 μM CBX
(DHC + 20.0 CBX).

FIG.2. RT-PCR analysis by 1% TAE-agarose electrophoresis
(ethidium bromide staining) of ob/ob mouse and human tissues.
A, mouse tissues (liver, pancreatic islets); B, human tissue (pancrea-
ritic islets). Total RNA from liver and pancreatic islets was subjected to
first-strand cDNA synthesis followed by PCR with 11β-HSD-1-specific
(A, lanes 4 and 5; B, lane 3) or GAPDH-specific (A, lanes 1 and 2; B, lane
1) primer sets, resulting in DNA products of 729 (mouse 11β-HSD-1),
819 (human 11β-HSD-1), and 965 bp (GAPDH) in size. A, lanes 1 and 4;
liver; lanes 2 and 5, islets. A, lane 3 and B, lane 2, negative control
(cDNA omitted). M, 1-kb DNA ladder standard (Life Technologies, Inc.).

Type 1 11β-HSD Regulates Insulin Release
human islets (Fig. 2)

same set of experiments was performed with RNA isolated from corticosterone by 11

number. The enzymatic activation of dehydrocorticosterone to 3

product formation/25 islets

insulin release by 11-DHC in the absence (control) or presence of 0.5, 1.0, and 5.0 m M carbenoxolone. Insulin release was determined by analyzing the incubation medium for insulin by RIA. n = 5 experiments.

intact islet preparations. The apparent K_m determined was 97.5 ± 24.3 ns, with a V_m ranging from 8.5 to 45 pmol of product formation/25 islets × 12 h, (mean, 24.0) (Fig. 1B), with the variation due to inevitable deviations in islet size and cell number. The enzymatic activation of dehydrocorticosterone to corticosterone by 11β-HSD could be inhibited by the synthetic compound carbenoxolone (Fig. 1C). Moreover, homogenates from islets, prepared by detergent extraction in buffered 1% Triton X-100, displayed characteristics of type 1 11β-HSD in disrupted tissues; this was detected as elevated NADP+-dependent 11β-OH dehydrogenase activity compared with NADPH-dependent 11-oxo reduction of glucocorticoids (data not shown).

Type 1 11β-Hydroxysteroid Dehydrogenase Isozyme in β-Cells—In agreement with functional characteristics obtained, 11β-HSD-1 expression in ob/ob pancreatic islets was detected by RT-PCR (Fig. 2A). An RT-PCR product of a size identical to that from liver was obtained using specific mouse primer sets (29), indicating expression of type 1 11β-HSD in pancreatic islets. The same set of experiments was performed with RNA isolated from human islets (Fig. 2B). Similarly, a specific product was amplified by PCR using human-specific primer sets, pointing to expression of 11β-HSD-1 in human islet tissue.

Pancreatic Type 1 11β-HSD and Insulin Release—To assess its physiological function in pancreatic tissue, the role of 11β-HSD in intracellular glucocorticoid activation and the effect on insulin release was evaluated. In a first series of experiments, the role of 11-DHC in insulin release was determined (Fig. 3A). Glucose-dependent insulin release was demonstrated at several glucose concentrations (3.3, 8.3, and 16.7 m M) showing the intact response to glucose stimulation of the islet preparations. The presence of 11-DHC at 50 and 500 m M in the medium inhibited insulin release in a dose-dependent fashion in all experimental settings (e.g. at 6.7 m M glucose from 302 milli-units of insulin/3 islets/h to 60 milliunits insulin/3 islets/h at 500 m M 11-DHC; Fig. 3A), indicating intracellular conversion of the nonbinding receptor ligand 11-DHC to the active compound corticosterone by 11β-HSD. The inhibitory effect of 11-DHC was similar but lower (about 12%) compared with corticosterone, in agreement with the kinetic data obtained. In a second set of experiments, using the 11β-HSD inhibitor compound carbenoxolone, almost complete and dose-dependent reversal of inhibition of insulin release could be achieved (Fig. 3B) at 5 m M inhibitor concentration (94.4 versus 39.4% without CBX). These data indicate that 11-oxo reduction of 11-keto glucocorticoids in islets is necessary for the inhibition of insulin release. The 11-DHC-mediated pathway of intracellular signaling indeed proceeds through glucocorticoid receptor activation (Fig. 4). Islets were transfected with a GC-responsive luciferase reporter gene construct and incubated in the presence of 100 n M 11-DHC in the presence or absence of 5 m M CBX. 11-DHC-treated cells showed an increase in the activity of the GC response element reporter gene, whereas coincubation with CBX substantially reduced transcriptional activity. This result suggests that 11-DHC-mediated cellular effects are achieved through metabolic activation to the receptor ligand corticosterone by the enzyme 11β-HSD-1.

FIG. 3. Inhibition of insulin release by 11-dehydrocorticosterone in islet preparations from ob/ob mouse. A, islets incubated in the presence of low (3.3 m M), medium (8.3 m M), or high (16.7 m M) glucose. Insulin release was measured by RIA in the absence (control) or presence (sample) of 50 or 500 m M 11-DHC. B, relative inhibition of insulin release by 11-DHC in the absence (control) or presence of 100 m M 11-DHC and in the presence of 0.5, 1.0, and 5.0 m M carbenoxolone. Insulin release was determined by analyzing the incubation medium for insulin by RIA. n = 5 experiments.

DISCUSSION

In this study, we aimed at defining the possible role of 11β-HSD-mediated GC metabolism in pancreatic islets in relation to insulin release. The novel finding was that 11β-HSD-1 is present in pancreatic islets of humans and ob/ob mice as assessed by RT-PCR and plays a key role in GC-mediated inhibition of insulin release.

The ob/ob mouse, an animal model of non-insulin-dependent diabetes mellitus, exhibits hyperglycemia, hyperinsulinemia,
and obesity. The animals are obese already at 3–4 weeks of age, and a significant difference between lean and obese animals in blood glucose and plasma insulin concentration is observed at 4–5 weeks. Islets of ob/ob mice are often used to study β-cell functions because these islets contain about 90% β-cells. We previously demonstrated that treatment of ob/ob mice with the synthetic GC dexamethasone decreases insulin release (12). These observations were extended by studies using transgenic mice overexpressing the GC receptor in β-cells (12–13), highlighting the importance of GC signaling in insulin release.

In the present study, we investigated the role of 11β-HSD-1 in respect to insulin release from islets of ob/ob mice. For this purpose, we evaluated the inhibitory effect of 11-DHC on insulin release in the presence and absence of the 11β-HSD inhibitor CBX. In the absence of CBX, 11-DHC markedly inhibited insulin release, whereas a reversal of this effect was noted in the presence of CBX, indicating an important role of 11β-HSD-1 in the regulation of insulin release.

We extended these novel findings by characterizing 11β-HSD in ob/ob islets and identified 11β-HSD-1 as the islet isoform. As shown earlier in hepatic or neural tissues (21, 31), 11β-HSD-1 functions in close to in vivo models, i.e. in primary culture, continuous cell lines, or transfected intact cells, as a reductase, thereby activating 11-oxo glucocorticoids to the GC receptor binding 11β-hydroxy hormones. However, upon disruption of intact cells, oxidative activity appears, indicating a labile character of the enzyme (17–18, 32). Interestingly, the apparent $K_m$ of the reductive activity differs between in vitro (tissue homogenates, recombinant material) and close to in vivo (intact cells) situations by about an order of magnitude. As determined in vitro, the affinity constant is in the low μM range, but in intact cells it appears to be between 100 and 400 nM as noted in this and other studies (33, 34). These features, in vivo reductive activity and oxidative and reductive activities in disrupted cells, were also observed in our experiments and, along with the RT-PCR data, clearly demonstrate the presence of 11β-HSD-1 in islets from ob/ob mice. It is also apparent that 11β-HSD-1 expression is not restricted to islets from this animal model of obesity. Rather, expression is detected in human islets, as determined in this study, or in further mammalian species, as indicated by the presence of activity in homogenized rat pancreatic tissue (35) and intact rat islets,² pointing to a role similar to that in ob/ob mice. Furthermore, it is now possible on these grounds to explain earlier data obtained from pancreas perfusion studies, demonstrating that cortisone was able to mediate inhibition of insulin release (36).

The results of this and other studies have several important consequences. First, 11β-HSD-1 expression and a functional role in tissues critically involved in glucose and carbohydrate metabolism and homeostasis (i.e. liver, adipose tissue, pancreatic islets) are now established (25–27, 37). In all cases, the tissue-specific effects are related to intracellular reductive activation of 11-oxo glucocorticoids via 11β-HSD-1 to GC receptor ligands, followed by tissue-specific induction or repression of GC controlled genes. This concept implies that circulating cortisone or 11-dehydrocortisone, which can be activated in a tissue-specific manner via 11β-HSD-1 to its receptor ligands.

To investigate the importance of this isoform in vivo, mice were produced with targeted disruption of the 11β-HSD-1 gene (25). These animals were unable to convert inert 11-DHC to corticosterone in vivo. Despite compensatory adrenal hyperplasia and increased adrenal secretion of corticosterone, homozygous mutants had attenuated activation of the key hepatic gluconeogenic enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. These 11β-HSD-1 knockout mice were found to resist hyperglycemia provoked by obesity or stress, suggesting that this effect was due to attenuation of gluconeogenesis. This hypothesis is strongly supported by studies in man, demonstrating that carbamazepine enhances insulin sensitivity and decreases hepatic glucose production (26). The present study suggests that the resistance to hyperglycemia in 11β-HSD-1−/− mice was probably also partially mediated by improved insulin release. Accordingly, 11β-HSD-1 appears to be an interesting target for the development of novel approaches in the treatment of type 2 diabetes mellitus.

Acknowledgments—Critical discussions with L. Abramhsen are gratefully acknowledged.

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² A. Khan and U. Oppermann, unpublished data.