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Initial Predominance of the Oxidative Activity of Type I 11β-Hydroxysteroid Dehydrogenase in Primary Rat Leydig Cells and Transfected Cell Lines

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ABSTRACT: Glucocorticoids suppress testosterone production in Leydig cells. The level of glucocorticoid action is set within the Leydig cell by the number of glucocorticoid receptors and by the activity of 11β-hydroxysteroid dehydrogenase (11βHSD). This enzyme acts either as an oxidase inactivating glucocorticoid or as a reductase amplifying its action. It is currently unknown whether extracellular conditions might cause 11βHSD oxidative and reductive activities in Leydig cells to change inversely or independently. The aim of the present study was to determine whether extracellular conditions set in vitro by various culture time and media components, such as glucose and pyruvate, affect the relative rates of 11βHSD oxidation and reduction. Primary rat Leydig cells and cell lines (COS1 and CHOP cells) transfected with 11βHSD-I complementary DNA (cDNA), were incubated with 25 nmol/L (physiologic range) or 500 nmol/L (stress range) concentrations of radiolabeled substrates, corticosterone or 11-hydrocorticosterone, for 0 to 24 hours. Oxidative activity predominated over reductive activity under initial conditions when product formation increased linearly with time. For example, in Dulbecco's modified Eagle medium/F12 medium (containing 5.5 mmol/L glucose), the peak ratio of oxidation to reduction (with 1 denoting equivalence of oxidative and reductive activities) was 5.5 in rat Leydig cells, 19.7 in COS1 cells, and 20.8 in CHOP cells. Glucose stimulated reductive activity but did not change the predominant direction of 11βHSD catalysis at earlier times. In COS1 cells transfected with 11βHSD-I cDNA, oxidative activity rapidly increased during the first 2 hours of the incubation, then gradually decreased while reductive activity increased steadily. The relative ratio of oxidation to reduction rapidly declined to less than 0.5 at 6 hours, and thus the favored direction of catalysis changed from oxidation to reduction. However, in transfected CHOP cells, 11βHSD oxidative activity rapidly increased during the first 2 hours and continued to increase for 24 hours. The ratio of oxidative to reductive activity rapidly declined but kept above 1 in CHOP cells for 24 hours, and the favored direction of catalysis remained predominantly oxidative. These results revealed that 11βHSD-I is a predominant oxidase initially in Leydig cells and 2 cell lines, and that the oxidative activity is gradually lost over time. The data suggest that type I 11βHSD is a predominant oxidase in Leydig cells in vivo.

Key words: Transfection, CHOP cells, COS1 cells.

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Glucocorticoids acting through the glucocorticoid receptor induce a variety of responses in cells, including proliferation, differentiation, and apoptosis (Chrousos et al, 1992). Leydig cells contain glucocorticoid receptors and are the primary target of glucocorticoid action in the testis (Ortlip et al, 1981; Stalker et al, 1989; Schultz et al, 1993). Because glucocorticoid-induced suppression of testosterone production is mediated by glucocorticoid receptors (Hales and Payne, 1989; Monder et al, 1994; Gao et al, 1996), the intracellular concentration of glucocorticoid within the Leydig cell should set the magnitude of its effect. In this regard, 11β-hydroxysteroid dehydrogenase (11βHSD) is an enzymatic determinant of glucocorticoid action in Leydig cells because this enzyme can control the intracellular concentration of active glucocorticoid. 11βHSD acts as an oxidase to protect Leydig cells from glucocorticoid-induced suppression of testosterone production by inactivating corticosterone (Monder et al, 1994). On the other hand, type I 11βHSD, the only isoform known to be present in rat Leydig cells, has been shown to act primarily as a reductase when expressed in tissues such as the liver or under pathologic conditions such as chronic stress (Jamieson et al, 1995; Nyska et al, 1998). In Leydig cells, however, a predominantly reductive 11βHSD would, if present, serve to amplify the action of glucocorticoid, resulting in a lower rate of testosterone production. Two isoforms of 11βHSD have been identified. Type I 11βHSD (11βHSD-I) is an NADPH-dependent oxidoreductase (Lakshmi and Monder, 1988; Agarwal et al, 1989; Rajan et al, 1995; Zhou et al, 1995) and is reported to be exclusively reductive in liver and transfected cell lines (Duperrex et al, 1993; Low et al, 1994; Jamieson et al, 1995). Type II (11βHSD-II) is NAD-dependent and exclusively oxidative (Rusvai and Naray-Fejes-Toth, 1993; Albiston et al, 1994; Agarwal et al, 1995). Previous studies have shown that rat Leydig cells contain only 11βHSD-I and do not express 11βHSD-II (Phillips et al, 1989; Li et al, 1996; Roland

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and Funder, 1996; Ge et al, 1997a). However, intact rat Leydig cells and Leydig cell microsomes express 11βHSD activity that is predominantly oxidative when measured with linear rates of product formation under physiologic conditions (Gao et al, 1997; Ge et al, 1997b). This observation runs contrary to results obtained with liver cells, COS7 cell lines, and toad bladder cell lines transfected with 11βHSD-I complementary DNA (cDNA; Duperrex et al, 1993; Low et al, 1994; Jamieson et al, 1995; Leckie et al, 1998).

Because the favored direction of 11βHSD is a major determinant for glucocorticoid action in Leydig cells, we examined whether extracellular conditions can modulate the favored direction of 11βHSD catalysis in primary rat Leydig cells and in cell lines transfected with 11βHSD-I cDNA. Specifically, we sought to analyze the influence of in vitro conditions, including culture time and media components, on relative rates of 11βHSD oxidation and reduction. Intracellular oxidation-reduction regulation, which has general importance for the control of cell growth and enzymes of intermediary metabolism (Powis et al, 1995; Sundaresan et al, 1995), should also be a determinant of 11βHSD activities. Because 11βHSD-I oxidation and reduction require the pyridine cofactors, $NADP^+$ and NADPH, respectively, the oxidation-reduction balance established by $NADP^+$/NADPH levels is a set point for the enzyme's favored direction of catalysis. Metabolism of glucose via glucose-6-phosphate dehydrogenase is the rate-limiting step for the production of NADPH (Yoshimoto et al, 1973; Kletzien et al, 1994; Pandolfi et al, 1995). In turn, pyruvate metabolism via lactate dehydrogenase establishes the level of NAD^+ (Park et al, 1998), which activates 11βHSD-II. Because the NAD^+-dependent 11βHSD-II is not expressed in Leydig cells, modulation of pyruvate concentrations is not expected to affect 11βHSD activity in this cell type. Therefore, the relative importance of the glucose-6-phosphate dehydrogenase and lactate dehydrogenase pathways for Leydig cell 11βHSD activities was assessed by depleting glucose and pyruvate from the culture medium.

Materials and Methods

Chemicals

[1,2,6,7-*N*-^{3}H]Corticosterone (specific activity 88 Ci/mmol) was purchased from Dupont-New England Nuclear (Boston, Mass). 11-Dehydro[1,2,6,7-*N*-^{3}H]corticosterone was prepared from [^{3}H]corticosterone, as described earlier (Lakshmi and Monder, 1985). Corticosterone and 11-dehydrocorticosterone were purchased from Steraloids (Wilton, NH). $NADP^+$ and NADPH, D-glucose, and sodium pyruvate were purchased from Sigma Chemical Co (St Louis, Mo). High-glucose Dulbecco's modified Eagle medium (DMEM), low-glucose DMEM, DMEM/F12 (1:1), and minimum essential media α (MEM) were purchased from GIBCO (GIBCO BRL Products, Life Technologies, Gaithersburg, Md). Thin-layer chromatography plates (Baker-flex) were obtained from J.T. Baker Inc (Phillipsburg, NJ). The 1.2-kb insert of 11βHSD-I (p11DH-I) was generously provided by Dr. P. White (University of Texas Southwestern Medical Center, Dallas, Tex). An expression vector, pcDNA I, was purchased from Invitrogen (San Diego, Calif).

Leydig Cell Isolation

Leydig cells were isolated from the testes of adult Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) by centrifugal elutriation and Percoll density gradient centrifugation, according to the method of Klinefelter et al (1993). The purity of cells isolated by this method was evaluated by histochemical staining for 3β-HSD activity, with 0.4 mmol/L etiocholanolone as the steroid substrate (Payne et al, 1980). Enrichment of Leydig cells was typically to 92% to 96% purity.

Culture of Leydig Cells and Cell Lines

In order to analyze the effects of constituents in culture media on 11βHSD in rat Leydig cells and COS1 and CHOP cells transfected with 11βHSD-I cDNA, the optimized media previously published for each of the 3 cell types were first examined: DMEM/F12 (1:1) supplemented with 1% bovine serum antigen (BSA) for rat Leydig cells, DMEM supplemented with 10% fetal calf serum (FCS) for COS1 cells, and MEM supplemented with 10% FCS for CHOP cells (Heffernan and Dennis, 1990). Leydig cells were cultured at 34°C with 5% O_2 and 5% CO_2, and COS1 and CHOP cells were cultured at 37°C with 5% CO_2. Glucose- and pyruvate-free DMEM also was used to determine the possible effects of glucose and pyruvate on 11βHSD activities in all 3 cell types. The cells were preincubated for 1 hour, with the media depending on experimental requirements, and they were subsequently incubated for assay of 11βHSD activities.

Construction of Expression Plasmid and Transfection

An expression plasmid was constructed to express rat 11βHSD-I cDNA by the 1265–base pair *Eco*RI fragment of p11DH-I (Agarwal et al, 1989), which included the entire coding region. This fragment was ligated into a pcDNA I expression vector via an *Eco*RI site. The *Escherichia coli* transformants carrying an insert were selected by colony hybridization, and a clone with the insert in the correct orientation relative to the vector's T7 promoter was identified by restriction mapping.

All transfections were carried out on 80% confluent cultures in 35-mm, 6-well plates. Aliquots of 1 μg 11βHSD-I pcDNA I were transfected into mammalian COS1 and CHOP cells with the FuGENE Transfection Reagent (Boehringer Mannheim Corp, Indianapolis, Ind) according to manufacturer's protocol. Cells were allowed to grow for 24 hours in media containing 10% FCS. Then media were removed and replaced with medium-containing radiolabeled substrates added for 11βHSD activity assays.

Assay of Relative 11βHSD Enzymatic Activity

Relative 11βHSD oxidative activity was determined by measuring the rate of conversion of corticosterone to 11-dehydrocorticosterone. Triplicate samples were incubated with the reaction

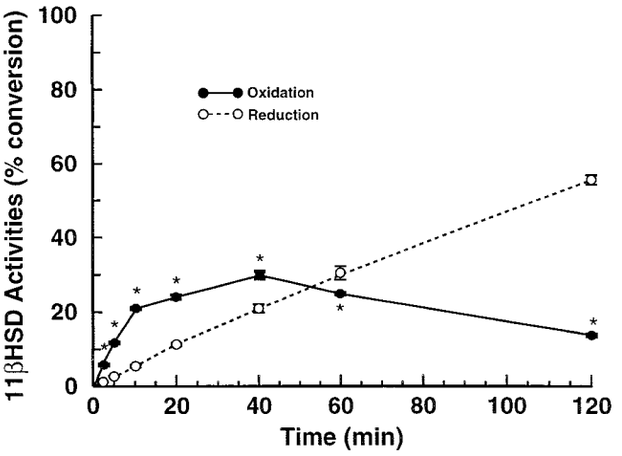

Figure 1. 11β-hydroxysteroid dehydrogenase (11βHSD) oxidative and reductive activities in intact rat Leydig cells. Aliquots of 0.1×10^6 intact rat Leydig cells were incubated with 25 nmol/L [^{3}H]corticosterone (oxidation) or [^{3}H]11-dehydrocorticosterone (reduction) in Dulbecco's modified Eagle medium (DMEM)/F12 medium are analyzed for different periods. Values are stated as the mean ± SEM (n = 3). Asterisks indicate significant differences between 11βHSD oxidative and reductive activities at each time point at $P < .05$.

mixture prepared in media that contained 0.2 μCi [1,2,6,7-*N*-^{3}H]corticosterone and unlabeled corticosterone, at a final concentration of 25 nmol/L or 500 nmol/L. The relative 11βHSD reductive activity was determined similarly, except that [1,2,6,7-*N*-^{3}H]11-dehydrocorticosterone and unlabeled 11-dehydrocorticosterone were used as the substrate. When relative 11βHSD oxidative and reductive activities were measured in cultured intact cells over time, 0.5-mL aliquots of culture medium were removed without replacement for assay at timed intervals. The steroids were then extracted from the samples with ethyl acetate, and the organic layer was dried under nitrogen. The steroid residues were chromatographed on thin-layer chromatography plates in chloroform:methanol (9:1), and the radioactivity was measured with a radiometric scanner (System 200/AC3000, Bioscan Inc, Washington, DC). The percentage of conversion of corticosterone to 11-dehydrocorticosterone and 11-dehydrocorticosterone to corticosterone was calculated by dividing the radioactive counts identified as 11-dehydrocorticosterone (or corticosterone, respectively) by the total counts.

Data Analysis

In each experiment, data were obtained from triplicate assays, and the results were expressed as the mean ± SEM. Statistical analysis of the changes in 11βHSD oxidative and reductive activities was performed by Kruskal-Wallis analysis of variance (ANOVA) followed by multiple comparisons to identify significant differences between groups (Sokal and Rohlf, 1995).

Results

Direction of 11βHSD Catalysis in Intact Rat Leydig Cells

The effects of the culture conditions on 11βHSD oxidative and reductive activities were evaluated through analysis of intact rat Leydig cells cultured in an optimized medium, DMEM/F12 (1:1) supplemented with 1% BSA (Klinefelter et al, 1993). When enzyme activities were measured in this medium after a short incubation with substrates (Ge et al, 1997b), 11βHSD was predominantly oxidative. However, when enzyme activities were measured after a long incubation with substrates (over 3 hours), the predominant activity was reductive (Leckie et al, 1998). This discrepancy led us to assess the effects of culture time on 11βHSD oxidative and reductive activities. 11βHSD oxidative and reductive activities were measured with 25 nmol/L (physiologic range) and 500 nmol/L (stress range) substrate concentrations. As shown in Figure 1, at 25 nmol/L substrate concentration, 11βHSD oxidative activity in intact Leydig cells had 2 phases: an initial rapid increase followed by a gradual decline. Reductive activity, however, increased gradually but continuously over time. In the presence of 500 nmol/L substrate, 11βHSD oxidative activity initially increased, reaching a plateau, whereas reductive activity increased continuously over time (Table 1). These trends resulted in 11βHSD activity shifting from predominant oxidation (with a oxidation to reduction ratio > 1) under initial incubation conditions, to predominant reduction (oxidation to reduction ratio < 1) after periods lasting longer than 40 minutes in the presence of either 25 or 500 nmol/L substrates. These results indicate that the direction of 11βHSD oxidation and reduction changes over time during incubations in vitro.

Table 1. *Time course of 11β − hydroxysteroid dehydrogenase (11βHSD) oxidative and reductive activities in intact rat Leydig cells measured in Dulbecco's Modified Eagle Medium (DMEM)/F12 and DMEM depleted with glucose and pyruvate (DMEM-Glu-Pyr)*

	11βHSD (% Conversion)*	
Time (min)	Oxidation	Reduction
DMEM/F12		
5	5.16 ± 0.22	0.88 ± 0.81
10	7.34 ± 0.35	4.99 ± 0.61
20	7.67 ± 1.15	6.88 ± 1.37
40	8.49 ± 0.23	13.26 ± 0.18
60	10.67 ± 0.23	15.15 ± 0.75
120	11.64 ± 0.79	21.19 ± 1.58
DMEM-Glu-Pyr		
5	12.24 ± 0.27	0.09 ± 0.87
10	14.90 ± 0.21	1.37 ± 0.05
20	25.02 ± 1.91	0.00 ± 0.28
40	40.75 ± 1.94	1.10 ± 0.30
60	39.04 ± 1.46	0.60 ± 0.44

* Data are expressed as means ± SE (n = 3).

Effects of Medium Components on 11βHSD Activities in Rat Leydig Cells

The energy substrates, glucose and pyruvate, contribute to the regulation of intracellular oxidation-reduction sta-

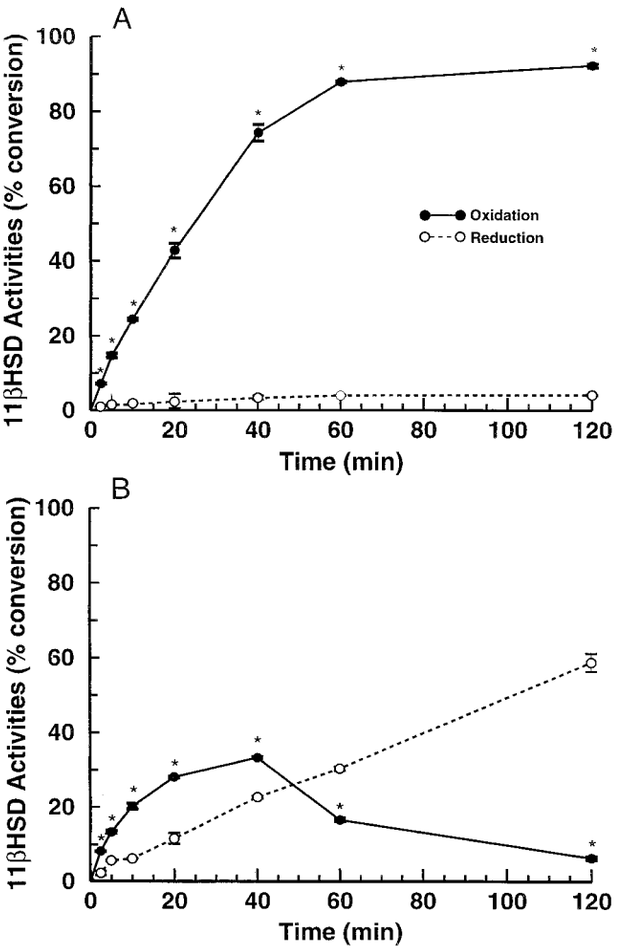

Figure 2. Influence of in vitro conditions on 11βHSD oxidative and reductive activities in intact rat Leydig cells. Aliquots of 0.1×10^6 intact rat Leydig cells were incubated with 25 nmol/L [^{3}H]corticosterone (oxidation) or [^{3}H]11-dehydrocorticosterone (reduction) in glucose-, pyruvate-depleted DMEM media **(A)** or DMEM medium supplemented with 1000 mg/L glucose and 110 mg/L sodium pyruvate **(B)**. Values are stated as mean ± SEM (n = 3). Asterisks indicate significant differences between 11βHSD oxidative and reductive activities at each time point at $P < .05$.

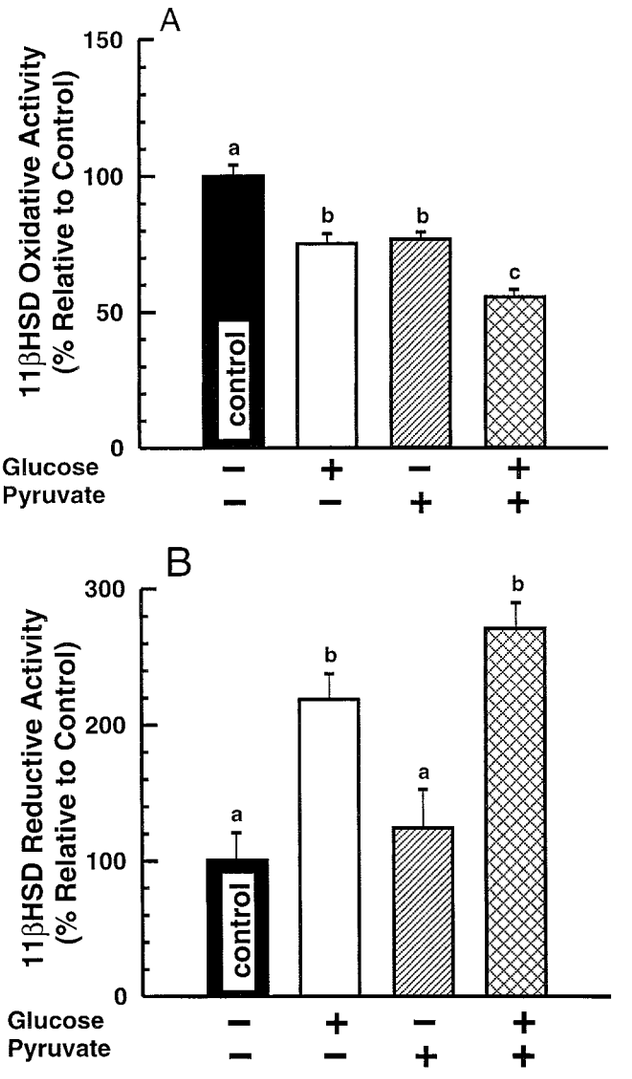

Figure 3. Effects of glucose and pyruvate on 11βHSD oxidative and reductive activities in intact rat Leydig cells. Aliquots of 0.1×10^6 intact rat Leydig cells were incubated with **(A)** 25 nmol/L [^{3}H]corticosterone (oxidation) or **(B)**[^{3}H]11-dehydrocorticosterone (reduction) in glucose- and pyruvate-depleted DMEM (control) or DMEM media supplemented with glucose (1000 mg/L) or sodium pyruvate (110 mg/L), or both, for 10 minutes. Values are stated as mean ± SEM (n = 3). Shared letters indicate significant differences between groups at $P < .05$.

tus. Because DMEM/F12 (1:1) medium contains 5.5 mmol/L D-glucose and 1 mmol/L sodium pyruvate, we evaluated whether the biphasic oxidation of 11βHSD reported above could be eliminated by culture of Leydig cells in the absence of glucose and pyruvate. Leydig cells were incubated in DMEM free of glucose and sodium pyruvate during measurement of 11βHSD oxidative and reductive activities, and low (25 nmol/L) and high (500 nmol/L) substrate concentrations were investigated. Depletion of these components caused 11βHSD to become exclusively oxidative, and reductive activity was lowered to near undetectable levels (Figure 2a and Table 1). Inclusion of 5.5 mmol/L D-glucose and 1 mmol/L sodium pyruvate in DMEM restored 11βHSD oxidative and reductive activities to levels that were similar to unaltered DMEM/F12 (Figure 2b). This suggested that 1 or both of these components is needed for maintenance of 11βHSD reductive activity.

In order to evaluate the relative importance of glucose versus pyruvate for 11βHSD function, 11βHSD oxidative and reductive activities were analyzed in DMEM free of glucose and pyruvate, with medium supplemented with either glucose (5.5 mmol/L) or sodium pyruvate (1 mmol/L) or with medium with glucose plus sodium pyruvate. As shown in Figure 3, the addition of either glucose or sodium pyruvate reduced 11βHSD oxidative activity, and

Table 2. *Effects of different media on 11β-hydroxysteroid dehydrogenase (11βHSD) oxidative and reductive activities in COS1 cells transfected with type I 11βHSD cDNA**

Time and Media	11βHSD (% Conversion) Oxidation	Reduction
0.5 hours		
DMEM + FCS	11.38 ± 1.14 a†	1.99 ± 0.51 a
DMEM/F12 + BSA	8.97 ± 0.44 a	0.45 ± 0.01 b
DMEM-Glu-Pyr	10.97 ± 1.13 a	0.25 ± 0.01 b
1 hour		
MEM + FCS	10.93 ± 0.64 a	2.81 ± 0.39 a
DMEM/F12 + BSA	14.28 ± 0.24 a	3.04 ± 0.24 a
DMEM-Glu-Pyr	14.99 ± 2.15 a	2.04 ± 0.18 a
2 hours		
MEM + FCS	12.92 ± 0.52 a	14.51 ± 0.99 a
DMEM/F12 + BSA	32.45 ± 1.42 b	10.61 ± 2.10 ab
DMEM-Glu-Pyr	22.10 ± 0.06 c	8.51 ± 0.68 b
4 hours		
MEM + FCS	12.51 ± 0.52 a	47.02 ± 0.18 a
DMEM/F12 + BSA	25.85 ± 1.69 b	43.55 ± 1.25 a
DMEM-Glu-Pyr	26.79 ± 0.62 b	31.28 ± 1.51 b
6 hours		
MEM + FCS	4.08 ± 0.82 a	57.71 ± 5.02 a
DMEM/F12 + BSA	9.92 ± 0.56 a	52.96 ± 2.30 a
DMEM-Glu-Pyr	10.87 ± 4.34 a	38.96 ± 5.02 a

* 11βHSD oxidative and reductive activities were examined in COS1 cells transfected with 11βHSD-I cDNA in Dulbecco modified Eagle medium + 10% fetal calf serum (DMEM + FCS), DMEM/F12 + 1% bovine serum albumin (DMEM/F12 + BSA), and glucose- and pyruvate-free DMEM (DMEM-Glu-Pyr) from 0.5 hrs to 6 hrs.

† Values are means ± SEM (n = 3). The shared alphabet letters designate groups at each time points that were not different at $P < 0.05$.

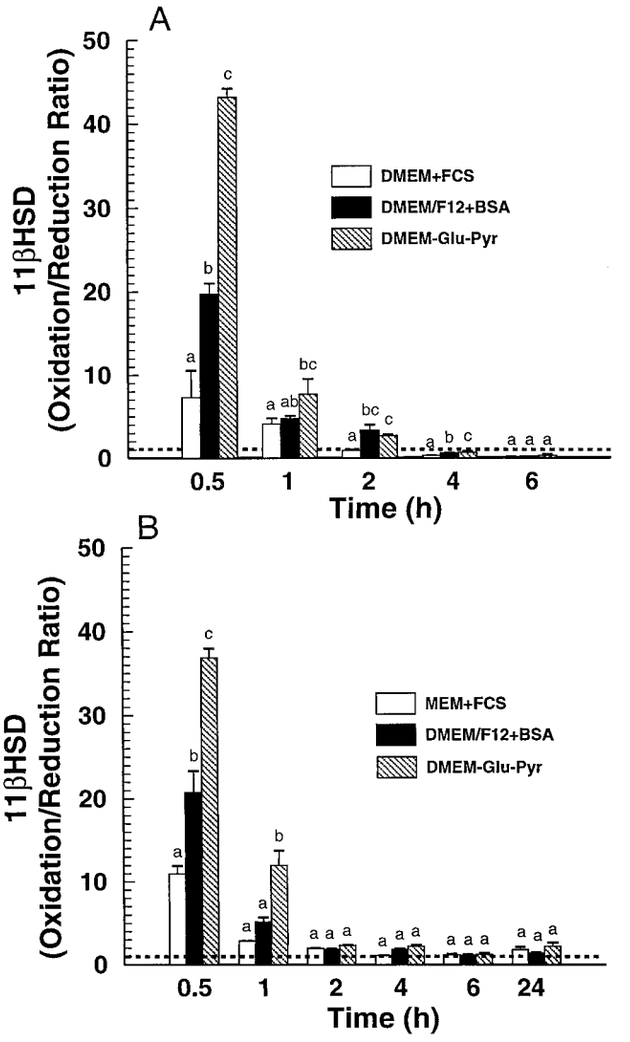

Figure 4. The ratio of 11βHSD oxidation to reduction in intact COS1 and CHOP cells transfected with 11βHSD-I complementary DNA (cDNA). When 80% confluent, COS1 **(A)** and CHOP **(B)** cells were transfected with 1 μg 11βHSD-I pcDNA. After 24 hours, the medium was removed, and both cell lines were incubated with 25 nmol/L [^{3}H]corticosterone (oxidation) or [^{3}H]11-dehydrocorticosterone (reduction) in DMEM/F12 + 1% bovine serum antigen (DMEM + BSA), and glucose- and pyruvate-free DMEM (DMEM-Glu-Pyr) except high glucose DMEM + 10% fetal calf serum (FCS) (DMEM + FCS) for COS1 cells and minimum essential media (MEM) + 10% FCS (MEM + FCS) for CHOP cells. The dashed line marks the 1:1 ratio of oxidation to reduction, indicating when the 2 activities were equivalent. Values are stated as mean ± SEM (n = 3). Shared letters designate groups that were not different at $P < .05$.

the effect was synergistic when both components were added together. Glucose induced 11βHSD reductive activity, and sodium pyruvate did not induce 11βHSD reductive activity. This indicated that 11βHSD reductive activity in Leydig cells is subject to regulation by glucose metabolism.

Direction of 11βHSD Catalysis in Intact Cell Lines Transfected with 11βHSD-I cDNA

Given that 11βHSD-I is the only isoform of this enzyme known to be expressed by rat Leydig cells (Phillips et al, 1989; Li et al, 1996; Roland and Funder, 1996; Ge et al, 1997a), and in light of the present data showing that culture time and medium components affect 11βHSD activities, it was necessary to determine whether the cell type in which the enzyme is expressed affects 11βHSD-I oxidative and reductive activity levels. COS1 cells, which have the same origin as COS7 cells, were analyzed because 11βHSD-I was reported to be exclusively reductive after transfection into the COS7 cell line (Duperrex et al, 1993). COS1 cells were evaluated in their optimized cell culture medium, DMEM supplemented with 10% fetal calf serum, and with the 2 media that had been tested in the previous experiments, DMEM/F12 with 1% BSA and DMEM-glucose-pyruvate. In all 3 media, 11βHSD oxidative activity exhibited a biphasic trend in COS 1 cells, increasing rapidly at first and then decreasing (Table 2), with a shift from predominant oxidation to reduction (Figure 4).

The CHOP cell line, originally derived from CHO

Table 3. *Effects of different media on 11β hydroxysteroid dehydrogenase (11βHSD) oxidative and reductive activities in CHOP cells transfected with type I 11βHSD cDNA**

Time and Media	11βHSD-I (% Conversion) Oxidation	Reduction
0.5 hours		
MEM + FCS	3.47 ± 0.07 a†	0.32 ± 0.07 a
DMEM/F12 + BSA	6.20 ± 0.35 b	0.30 ± 0.03 a
DMEM-Glu-Pyr	9.04 ± 0.23 c	0.25 ± 0.01 a
1 hour		
MEM + FCS	9.87 ± 0.10 a	3.46 ± 0.06 a
DMEM/F12 + BSA	10.75 ± 0.35 a	2.13 ± 0.22 b
DMEM-Glu-Pyr	10.36 ± 0.46 a	0.91 ± 0.15 c
2 hours		
MEM + FCS	11.45 ± 0.31 a	10.62 ± 0.15 a
DMEM/F12 + BSA	15.49 ± 1.69 a	8.56 ± 1.29 ab
DMEM-Glu-Pyr	13.93 ± 1.15 a	6.07 ± 0.31 b
4 hours		
MEM + FCS	25.73 ± 0.67 a	23.85 ± 1.42 a
DMEM/F12 + BSA	31.82 ± 2.36 a	17.33 ± 0.47 b
DMEM-Glu-Pyr	33.65 ± 0.89 a	15.07 ± 0.26 b
6 hours		
MEM + FCS	33.13 ± 0.94 a	28.46 ± 2.44 a
DMEM/F12 + BSA	32.79 ± 1.19 a	28.87 ± 1.99 a
DMEM-Glu-Pyr	28.84 ± 4.18 a	23.08 ± 0.89 a
24 hours		
MEM + FCS	61.13 ± 6.15 a	35.13 ± 2.62 a
DMEM/F12 + BSA	54.14 ± 4.26 a	39.59 ± 1.66 a
DMEM-Glu-Pyr	56.99 ± 3.27 a	28.65 ± 5.83 a

* 11βHSD oxidative and reductive activities were examined in CHOP cells transfected with 11βHSD-I cDNA in modified Eagle medium + 10% fetal calf serum (MEM + FCS), Dulbecco modified Eagle medium/F12 + 1%BSA (DMEM/F12 + BSA) and glucose-and pyruvate-free DMEM (DMEM-Glu-Pyr) from 0.5 hrs to 6 hrs.

† Values are means ± SE (n = 3). The shared alphabet letters designate groups at each time point that were not different at $P < 0.05$.

cells, has oxidative and reductive activities after transfection with 11βHSD-I (Agarwal et al, 1989). In contrast to COS1 cells, however, CHOP cells did not have a biphasic trend for 11βHSD oxidative activity (Table 3), and oxidative activity was always predominant despite a decline in the relative ratio of oxidation to reduction (Figure 4). These results suggested that cellular context and incubation time modulate 11βHSD-I oxidative and reductive activities.

In both cell lines, during initial incubation conditions in which product formation is linear, 11βHSD activities were affected by the composition of the culture medium (Tables 2 and 3). Glucose- and pyruvate-depleted media yielded a higher ratio of 11βHSD oxidation to reduction in both COS1 and CHOP cells during the first hour of incubation (Figure 4). These data demonstrated that type I 11βHSD oxidative activity in both COS7 and CHOP cells initially predominated over reductive activity.

Discussion

The favored direction of 11βHSD controls the magnitude of glucocorticoid action in Leydig cells by acting as an oxidase to protect Leydig cells from the deleterious effects of excessive glucocorticoid concentrations. Alternatively, by favoring the reductase, for example prior to puberty (Ge et al, 1997b), 11βHSD could amplify glucocorticoid action. The results of the present study demonstrated that the favored direction of 11βHSD-I catalysis in intact cells is dependent on cell culture duration and cell type. Although 11βHSD activities were seen to change dramatically during culture in vitro, oxidation predominated over reductive activity initially at physiologic (25 nmol/L) and stress level (500 nmol/L) concentrations of substrate.

During in vitro culture of intact rat Leydig cells, with all media tested, 11βHSD behaved as a predominant oxidase when measured at initial time points. These results corroborated previous studies of 11βHSD with intact Leydig cells in short-term (5-minute) assays (Ge et al, 1997b). However, more recently, Leckie et al (1998) reported the predominance of 11βHSD reductase in rat Leydig cells. The discrepancy probably results from the longer duration of cell culture used by this group. As shown in the present study, the predominant direction of 11βHSD started shifting from oxidation to reduction after 40 minutes. Therefore, a transition in the favored direction of 11βHSD catalysis may not be observed with sampling at the end of a 3-hour incubation, leaving only the predominant reductase activity. Similar changes of 11βHSD direction occur in established cell lines transfected with 11βHSD-I. In earlier studies (Duperrex et al, 1993; Low et al, 1994), 11βHSD-I was shown to be an exclusive reductase in COS7 and toad bladder cells when substrates were incubated with cells for 24 hours or more. This agreed with our findings for COS1 cells during incubations lasting over 4 hours, in which the 11βHSD-I was predominantly reductive. Because COS7 cells are derived from the same parent cell line as COS1 cells, the similarity of the results is not surprising.

The present study advances the hypothesis that 11βHSD in intact primary rat Leydig cells and transfected cell lines is predominantly oxidative. The initially rapid catalysis of 11βHSD oxidation is possibly caused by the high-affinity, low-capacity component of 11βHSD oxidative activity in Leydig cells (Ge et al, 1997a). In contrast, the low-affinity, high-capacity 11βHSD reductive activity predominates after an extended duration of Leydig cell culture. In the in vivo situation, the catalytic product of 11βHSD does not accumulate but diffuses, or it is transported away. In contrast, catalytic product accumulates with increasing time in vitro. It is likely that the

initial 11βHSD-I reaction catalyzed in vitro reflects the in vivo situation, and that longer-term assays of the enzyme in vitro do not. Further support for the hypothesis that 11βHSD oxidative activity predominates in Leydig cells in vivo comes from our observations on the Leydig cell cytotoxin, ethane dimethanesulfonate (EDS). EDS-induced destruction of Leydig cells eliminates oxidative activity but does not affect 11βHSD reductive activity in the whole testis (Ge and Hardy, unpublished data). Because the reductive 11βHSD activity remained after EDS-induced Leydig cell destruction, we speculate that another enzyme capable of catalyzing this reaction is present in the seminiferous tubule.

Results from the present study demonstrated that glucose and pyruvate are regulatory factors for 11βHSD oxidative and reductive activity. Depletion of glucose and pyruvate from the culture medium suppressed 11βHSD reductive activity to nearly undetectable levels in Leydig cells. The effects of glucose on the favored direction of 11βHSD in Leydig cells were also demonstrated by Ferguson et al (1999). In the present study, depleting glucose and pyruvate from the medium increased 11βHSD oxidative activity and decreased reductive activity in COS1 and CHOP cells during the first 2 hours of culture, but it had no effect on enzyme activities at later times. However, glucose and pyruvate were not critical components in the medium for maintaining type I 11βHSD activities, because depletion of glucose and pyruvate did not change type I 11βHSD activities at later times. The explanation for glucose and pyruvate effects on 11βHSD oxidative and reductive activities probably lies in the regulation of intracellular levels of NADPH. 11βHSD-I requires $NADP^+$ and NADPH as cofactors, and the oxidation-reduction balance between $NADP^+$ and NADPH should modulate the 2 enzyme activities. Intracellular NADPH is derived from the metabolism of glucose by a rate-limiting step catalyzed by glucose-6-phosphate dehydrogenase (Yoshimoto et al, 1973; Kletzien et al, 1994; Pandolfi et al, 1995). Depletion of glucose will result in lower rates of glucose metabolism, thereby decreasing intracellular NADPH levels and 11βHSD reductive activity. However, a different mechanism must underlie the observed effect of pyruvate on 11βHSD because pyruvate is thought to modulate oxidation-reduction balance via lactate dehydrogenase, changing the intracellular ratio of NAD to NADH, which are not cofactors for 11βHSD-I in Leydig cells. It is possible that intracellular levels of adenosine triphosphate, increased by the metabolism of glucose and pyruvate, influence 11βHSD-I activities. In this way, the regulatory effects of glucose and pyruvate on 11βHSD-I probably were similar to those seen with 17βHSD, which had decreased reductive activity in Leydig cells after the uptake of glucose was blocked (Khanum et al, 1997).

In summary, the present study revealed 2 features of 11βHSD-I not observed in previous studies: 1) 11βHSD oxidative and reductive activities were inversely affected by the composition of the culture medium, cell culture duration, and cell type; 2) the favored direction of 11βHSD-I was not exclusively reductive in intact rat Leydig cells and transfected cell lines and may indeed be predominantly oxidative in vivo in tissues such as the testicular interstitium.

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