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Metyrapone prevents cortisone-induced preadipocyte differentiation by depleting luminal NADPH of the endoplasmic reticulum

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ABSTRACT

Preadipocyte differentiation is greatly affected by prereceptorial glucocorticoid activation catalyzed by 11β-hydroxysteroid dehydrogenase type 1 in the lumen of the endoplasmic reticulum. The role of the local NADPH pool in this process was investigated using metyrapone as an NADPH-depleting agent. Metyrapone administered at low micromolar concentrations caused the prompt oxidation of the endogenous NADPH, inhibited the reduction of cortisone and enhanced the oxidation of cortisol in native rat liver microsomal vesicles. However, in permeabilized microsomes, it only slightly decreased both NADPH-dependent cortisone reduction and NADP$^+$-dependent cortisol oxidation. Accordingly, metyrapone administration caused a switch in 11β-hydroxysteroid dehydrogenase activity from reductase to dehydrogenase in both 3T3-L1-derived and human stem cell-derived differentiated adipocytes. Metyrapone greatly attenuated the induction of 11β-hydroxysteroid dehydrogenase type 1 and the accumulation of lipid droplets during preadipocyte differentiation when 3T3-L1 cells were stimulated with cortisone, while it was much less effective in case of cortisol or dexamethasone. In conclusion, the positive feedback of glucocorticoid activation during preadipocyte differentiation is interrupted by metyrapone, which depletes NADPH in the endoplasmic reticulum. The results also indicate that the reduced state of luminal pyridine nucleotides in the endoplasmic reticulum is important in the process of adipogenesis.

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Abbreviations: ER, endoplasmic reticulum; G6PT, glucose-6-phosphate translocase; H6PDH, hexose-6-phosphate dehydrogenase; 11βHSD1, 11β-hydroxysteroid dehydrogenase type 1; IBMX, isobutyl-methylxanthine; MOPS, 4-morpholinepropanesulfonic acid; FBS, fetal bovine serum; ADMSC, adipose-derived mesenchymal stem cell; SVF, stromal vascular fraction.

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1. Introduction

Pyridine nucleotides (NAD^+ and its phosphorylated form $NADP^+$) are well known cofactors of cellular metabolism and have been characterized as electron carriers in oxidoreductase reactions. Recently, their regulatory and signaling properties were also revealed and got into the focus of interest [1]. Although the critical role of NADP(H) and its redox state have long been appreciated in cellular antioxidant defense systems and reductive syntheses, there are still considerable uncertainties regarding its cellular dynamics, that is, overall concentration, free and protein-bound state, subcellular compartmentation and the redox state. It seems that NADPH is present in all subcellular compartments, including the lumen of the endoplasmic reticulum (ER) [2–6]. Similarly to cytosol, NADP(H) concentration in this compartment is in the submillimolar range and the reduced form is predominant [5,6]. The maintenance of the reduced state of luminal NADPH is important for the cofactor supply of local reductases [5 and refs therein]. Moreover, the redox state of luminal pyridine nucleotides appears to critically influence the physiological state of the cell and thereby cellular survival [7–10].

Luminal NADPH generation in the ER is primarily attributed to the concerted action of glucose-6-phosphate translocase (G6PT) and hexose-6-phosphate dehydrogenase (H6PDH) [11–13]. The activity of H6PDH allows the functioning of luminal reductases including 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1). 11βHSD1 is responsible for the reduction and prereceptorial activation of glucocorticoids in various tissues [see 14 for a review]. The importance of H6PDH in this process has been demonstrated in H6PDH knockout mice, where the 11βHSD1 reductase activity disappeared, whereas dehydrogenase activity was increased [15]; consequently, glucose output and glucose utilization were abnormal resulting in fasting hypoglycemia [16].

Since active glucocorticoids are required for preadipocyte differentiation, 11βHSD1 plays a decisive role in the process. 11βHSD1 expression is very low in preadipocytes and it shows a strong increase during the late phase of glucocorticoid-dependent differentiation. Inhibition of 11βHSD1 activity by pharmacological agents or shRNA constructs blocked the capability of inactive oxidized glucocorticoid derivatives to promote differentiation [17,18]. These findings are indicative of a positive feedback mechanism: active glucocorticoids induce 11βHSD1 expression, which leads to an enhanced generation of further active glucocorticoids.

It has been reported that in H6PDH knockout mice the direction of 11βHSD1 activity is changed from dehydrogenase to reductase [15]. This strongly suggests that the maintenance of the reduced state of NADPH in the lumen is required for the differentiation. To test this hypothesis preadipocyte differentiation was investigated in the presence of metyrapone, an agent depleting luminal NADPH [5,19]. Our results show that metyrapone decreases cortisone-dependent 11βHSD1 induction and completely prevents the differentiation of the cells.

2. Materials and methods

2.1. Materials

Cortisone, cortisol, dexamethasone, metyrapone, isobutylmethylxanthine (IBMX), glucose-6-phosphate, $NADP^+$, NADPH, triton X-100, collagenase (type IA), and 4-morpholinepropanesulfonic acid (MOPS) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Cell culture media, and fetal bovine serum (FBS) were from Cambrex Profarmaco Milano S.r.l., Milan, Italy. All other reagents and solvents were of analytical grade.

2.2. Preparation of rat liver microsomes

Microsomal fractions were prepared from livers of overnight fasted male Sprague Dawley rats (180–230 g), as reported [12]. Microsomes were washed and resuspended in KCl/MOPS buffer (100 mM KCl, 20 mM NaCl, 1 mM $MgCl_2$, 20 mM MOPS, pH 7.2) and kept in liquid nitrogen until use. The protein concentration in microsomal suspensions was determined using the method of Lowry [20] with BSA as a standard.

2.3. Fluorimetric detection of reduced pyridine nucleotides

Detection of reduced pyridine nucleotides in microsomes was based on their characteristic fluorescent spectrum [5]. Fluorescence was monitored at a 350-nm excitation and a 460-nm emission wavelength by using a Cary Eclipse fluorescence spectrophotometer (Varian, Inc., Palo Alto, CA, USA).

2.4. 11βHSD1 activity in rat liver microsomes

11βHSD1 activity was evaluated in rat liver microsomes (1 mg protein/ml) permeabilized with alamethicin (0.1 mg/mg protein) to allow the free access of the cofactor to the intraluminal active site of 11βHSD1. Reductase (as cortisone to cortisol conversion) and dehydrogenase (as cortisol to cortisone conversion) activities were measured upon the addition of 5 μM cortisone and 1 mM NADPH or 5 μM cortisol and 1 mM $NADP^+$, respectively. Reductase activity of 11βHSD1 was also evaluated upon the addition of 5 μM cortisone and 50 μM G6P to intact microsomes. Reactions were conducted in KCl/MOPS buffer (pH 7.2) for 15 min at 37 °C in the presence or absence of metyrapone. The reaction was terminated by the addition of equal volume of ice-cold methanol and the samples were kept at −20 °C until analysis. After sedimentation of the precipitates by centrifugation (20,000 × *g* for 10 min at 4 °C), the cortisol and cortisone content of the supernatants was measured by HPLC (Alliance 2690; Waters Corp., Milford, MA, USA) using a Nucleosil 100 C18 column (5 μm 25 × 0.46) (Teknokroma). The gradient was composed of water (solvent A) and acetonitrile (solvent B) at a constant flow rate of 1.3 ml/min. Initial 74% A − 26% B for 0.5 min was followed by a linear change to 70% A − 30% B in 12 min, which was maintained for 0.5 min before a linear change back to 74% A − 26% B in 0.5 min. Samples were eluted for 16 min and the absorbance was detected at 245 nm wavelength (Dual λ Absorbance Detector 2487; Waters Corp., Milford, MA, USA).

The retention times of cortisol (approx. 13.2 min) and cortisone (approx. 14.1 min) were determined by injecting standards.

2.5. Culturing and differentiation of 3T3-L1 fibroblasts

3T3-L1 fibroblasts (American Type Culture Collection, Rockville, MD, USA) were cultured and differentiated as described in [21]. Briefly, 2 days after confluence – referred to as day 0 – adipogenesis was induced by the addition of DMEM containing 10% FBS, 5 μg/ml insulin, 0.5 mM IBMX, and 0.5 μM dexamethasone. Two days later, the medium was removed and cells were further cultured for 2 days in DMEM containing 10% FBS and 5 μg/ml insulin. Cells were then maintained in DMEM containing 10% FBS until use, normally at the 7th day after starting adipogenesis.

2.6. Preparation, culturing and differentiation of adipose-derived mesenchymal stem cells

Adipose-derived mesenchymal stem cells (ADMSCs) were prepared from human adipose tissue obtained from patients undergoing elective liposuction or lipectomy procedures. Cells were isolated using a protocol previously described [22] with some modifications. Adipose tissue was minced into small pieces and washed with phosphate buffered saline (PBS), containing antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin). To isolate the stromal vascular fraction (SVF), the tissue fragments were treated with 0.075% collagenase and 0.25 mM $CaCl_2$, in PBS (4 ml of solution for g of tissue), for 1 h, at 37 °C, under gentle agitation. Digestion was stopped by adding an equal volume of α-MEM containing 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine and the cell suspension was centrifuged at 600 × *g*, for 10 min, at 22 °C. The pellet was resuspended in culture medium, filtered through nylon sheet (100 μm mesh) and centrifuged again as above. The SVF pellet was resuspended in culture medium and filtered again through 70 μm nylon mesh. The stromal cells were counted and plated at 100,000 cells/cm^2 onto tissue culture plastic dishes for 24 h at 37 °C in a humidified atmosphere of 5% CO_2, 95% air. Contaminating erythrocytes and non-adherent cells were then removed by washing dishes with PBS. Cells were cultured for 7–10 days to reach a 70–80% confluence, detached with 0.25% Trypsin/0.53 mM EDTA in PBS and seeded at 4000 cells/cm^2 and subcultured after 7 days. Cells were used between passages 2 and 5. For differentiation into adipocytes, cells were plated at 20,000 cells/cm^2 in MesenCult® Basal Medium supplemented with 10% FBS, antibiotic and 1% L-glutamine. After 24–48 h the same medium containing 1 μM dexamethasone, 10 μM insulin, 0.5 mM IBMX, and 200 μM indomethacin, was added. At the 15th day, cells were used to evaluate reductase and dehydrogenase activity of 11βHSD1.

2.7. Oil Red O staining

3T3-L1 adipocytes were washed with PBS and fixed with 4% formaldehyde in PBS for 30 min at 4 °C. After washed in PBS, cells were rinsed with 60% isopropanol and were stained for 1 h in freshly diluted Oil Red O solution (three parts Oil Red O stock solution and two parts H_2O; Oil Red O stock solution is 0.25% Oil Red O in isopropanol). The stain was then removed, and the cells were washed with 60% isopropanol and finally with water.

2.8. Real-time RT-PCR assay

Total RNA from 3T3-L1 cells was isolated with RNeasy Plus Mini kit (Qiagen S.p.A., Milan, Italy), according to manufacturer's instructions. One microgram of RNA was reverse transcribed in a final volume of 20 μl, by using the SuperScript first-strand synthesis System III (Invitrogen S.r.l., Milan, Italy) and Oligo(dT)12–18.

Expression levels of 11βHSD1 and a reference gene were quantified by fluorescent real-time PCR with an Opticon Monitor 3.1 (MJ Research, Inc., Waltham, MA, USA). Analyses were performed in triplicate in a 25 μl reaction mixture. cDNA (1 μl) was amplified with Platinum SYBR Green qPCR SuperMix UDG (Invitrogen S.r.l., Milan, Italy) and 50 nM of the sense and antisense primers. The primers were: sense, 5′-CCA GCA AAG GGA TTG GAA GAG A-3′; antisense, 5′-GTA GTG AGC AGA GGC TGC TCC-3′. Amplification protocol was: 95 °C (15 min), 45 cycles of 95 °C (20 s), 55.2 °C (20 s), 72 °C (20 s). The PCR amplification efficiency was 93%. Since neither β-actin nor glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was expressed stably during cell differentiation (data not shown), acidic ribosomal phosphoprotein PO (36B4; NM_007475) was used as reference gene due to its resistance to hormonal regulation [23]. The primers were: sense, 5′-AAG CGC GTC CTG GCA TTG TCT-3′; antisense, 5′-CCG CAG GGG CAG CAG TGG T-3′. Amplification protocol was: 95 °C (15 min), 45 cycles of 95 °C (20 s), 58 °C (20 s), 72 °C (20 s). The PCR amplification efficiency was 99%. Every assay was run in triplicate and negative controls (no template, template produced with no reverse transcriptase enzyme) were always included. In the negative controls, no signal was detected in the investigated amplification range (45 cycles).

3. Results

3.1. Metyrapone oxidizes the intraluminal NADPH pool of the ER

The NADPH depleting effect of metyrapone was elucidated in rat liver microsomal vesicles due to practical reasons. Liver microsomes, similarly to microsomes from adipose tissue, contain G6PT, H6PDH and 11βHSD1 [5,24]. Moreover, ER-derived liver microsomes maintain luminal pyridine nucleotides and their redox state during a prolonged incubation [5]. Addition of metyrapone caused a quick oxidation of pyridine nucleotides (Fig. 1). The effect was concentration-dependent and reached its maximum at 10 μM metyrapone concentration (data not shown). Cortisol that has been shown to reduce luminal $NADP^+$ [5] counteracted the effect of metyrapone. Sequential administration of metyrapone and cortisone resulted in compensatory effects (Fig. 1).

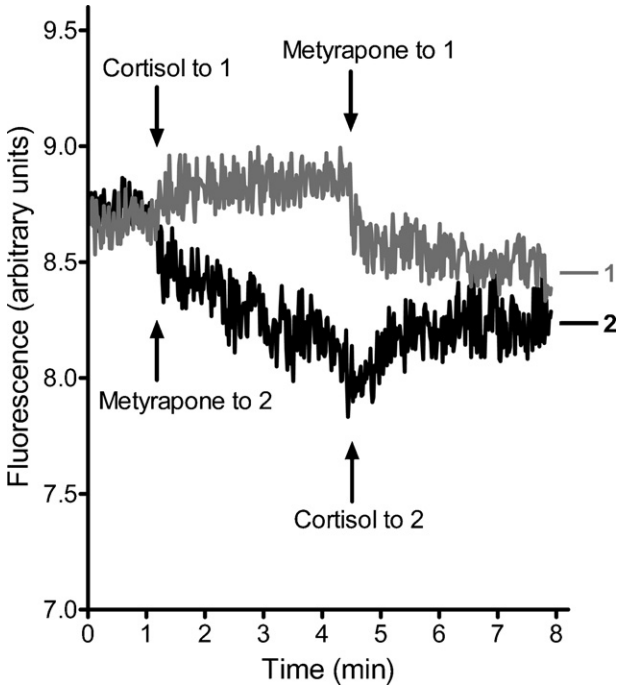

Fig. 1 – Oxidation of luminal pyridine nucleotides by metyrapone in rat liver microsomes. Microsomes (2 mg of protein/ml) were incubated in a fluorimeter cuvette, and the characteristic fluorescence of reduced pyridine nucleotides was monitored. Decreasing fluorescence indicates oxidation of pyridine nucleotides. Trace 1: subsequent addition of 10 μM cortisol and 10 μM metyrapone. Trace 2: subsequent addition of 10 μM metyrapone and 10 μM cortisol. Representative traces from four experiments are shown.

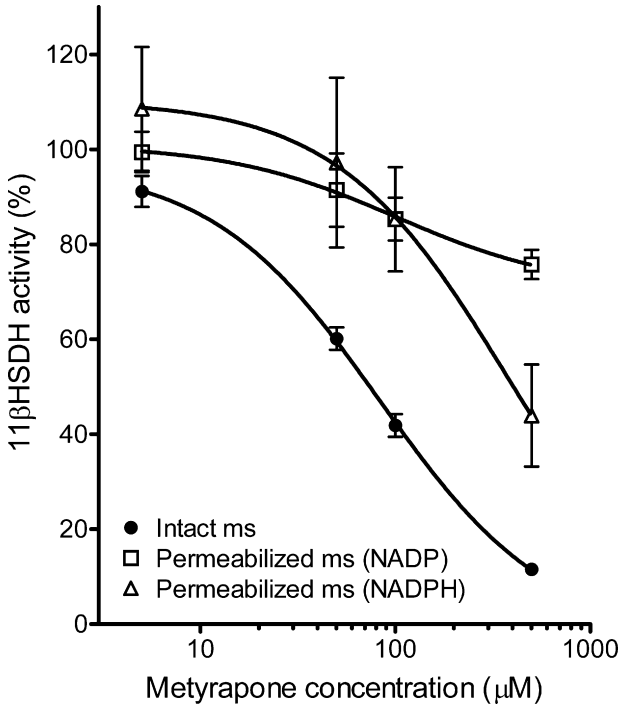

Fig. 2 – Effect of metyrapone on 11βHSDH1 activity in intact and permeabilized rat liver microsomes. Intact (solid symbols) or alamethicin-permeabilized (empty symbols) rat liver microsomes (1 mg protein/ml) were incubated in MOPS-KCl buffer at 37 °C for 15 min in the presence of cortisol or cortisone (5 μM each) and metyrapone at the indicated concentrations (horizontal axis, logarithmic scale). The enzyme activity was calculated after the measurement of final cortisone and cortisol concentrations by HPLC. Reduction of cortisone to cortisol was fuelled by glucose-6-phosphate (50 μM) without exogenous pyridine nucleotides in intact microsomes (●) and by exogenous NADPH (1 mM) in permeabilized microsomes (△). $NADP^+$-dependent (1 mM) oxidation of cortisol to cortisone (□) was also measured in permeabilized microsomes. Data are means ± S.D. of four separate experiments and shown as percents of control. Control activities: reductase activity in intact vesicles: 106.8 ± 7.4 pmol/min/mg protein, reductase activity in permeabilized vesicles: 24.7 ± 1.9 pmol/min/mg protein, dehydrogenase activity in permeabilized vesicles: 199.7 ± 23.2 pmol/min/mg protein.

3.2. *Cortisone and metyrapone compete for the luminal NADPH of the ER*

The cortisone–cortisol conversion was measured in the presence and in the absence of metyrapone in rat liver microsomes. In the first set of experiments, the reduced state of endogenous NADPH in intact vesicles was maintained by the addition of glucose-6-phosphate, taking advantage of the concerted action of G6PT and H6PDH. Metyrapone showed a strong inhibitory effect on the reduction of cortisone in these conditions (Fig. 2). However, cortisol formation was much less hindered when the effect of metyrapone was investigated in permeabilized vesicles in the presence of exogenous NADPH (1 mM) (Fig. 2). Metyrapone was also minimally inhibitory on cortisol-to-cortisone conversion driven by $NADP^+$ (Fig. 2). On the whole, metyrapone at low concentration inhibited 11βHSD1 activity only in native microsomal vesicles. These findings suggested that cortisone and metyrapone compete for the luminal NADPH of the ER rather than for 11βHSD1. To confirm this assumption, intact microsomes were incubated with cortisol in the presence of metyrapone at various concentrations. It was found that metyrapone greatly stimulated the oxidation of cortisol to cortisone (Fig. 3), which is inconsistent with the behavior of a competitive inhibitor of 11βHSD1.

3.3. *Metyrapone inhibits the cortisone–cortisol conversion in adipocytes*

3T3-L1 murine preadipocytes and human stem cells were differentiated to adipocytes and the cortisone–cortisol conversion was measured at different stages of the differentiation. The conversion was not detectable in the undifferentiated cells, and was gradually increasing during the differentiation process, in accordance with previous

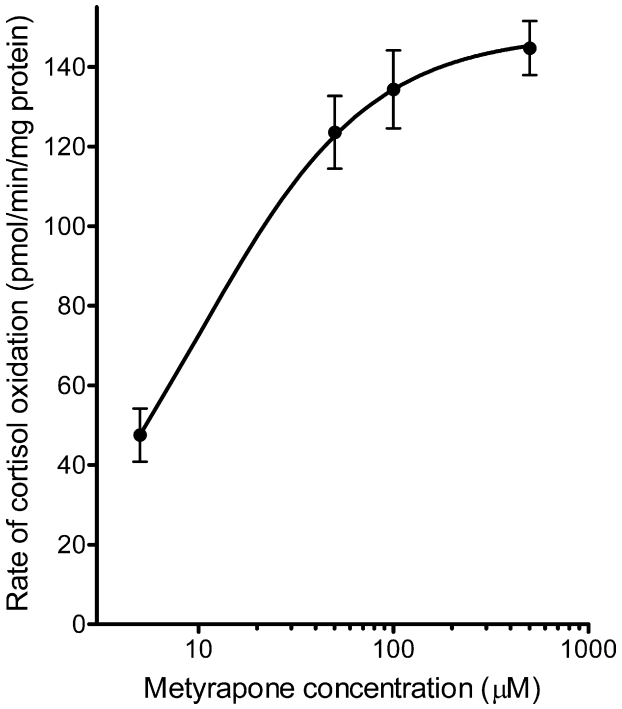

Fig. 3 – Metyrapone-dependent oxidation of cortisol to cortisone in intact rat liver microsomes. The reaction mixture in the MOPS-KCl medium (pH 7.2) contained rat liver microsomes (1 mg protein/ml), 5 μM cortisol and metyrapone at the indicated concentrations (horizontal axis, logarithmic scale). Cortisol oxidation was calculated from the level of cortisone and cortisol measured by HPLC after 15 min incubation at 37 °C. No measurable cortisol oxidation was found in the absence of metyrapone. Data are means ± S.D. of three separate experiments.

findings (data not shown). Metyrapone (50 μM) greatly inhibited cortisone reduction in both cells (Fig. 4). The inhibition was about 30% and 60% in 3T3-L1-derived adipocytes and adipocytes differentiated from human stem cells, respectively. On the other hand, in agreement with the microsomal results, metyrapone did not inhibit, rather downright stimulated the (otherwise negligible) cortisol–cortisone conversion (Fig. 4).

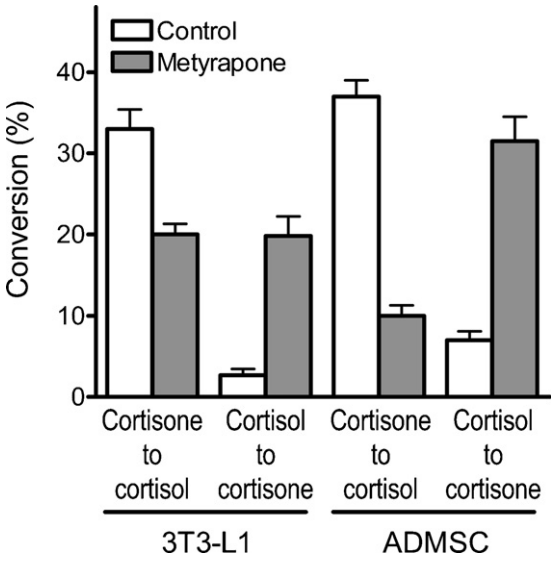

Fig. 4 – Effect of metyrapone on cortisone reduction and cortisol oxidation in differentiated adipocytes. 3T3-L1 fibroblasts and ADSMCs were cultured and differentiated to adipocytes as detailed under Section 2. Cells ((0.2–1) × 10^6 per ml) were incubated in DMEM containing 1 μM cortisone or cortisol for 2 h at 37 °C, in the absence or presence of 50 μM metyrapone. The level of cortisone and cortisol was measured by HPLC. Conversion refers to the relative amount of the product as the percentage of the initial steroid. Data are means ± S.D. of four separate experiments.

3.4. *Metyrapone decreases the induction of 11βHSD1 during adipocyte differentiation*

3T3-L1 murine preadipocytes were differentiated to adipocytes with the administration of dexamethasone, cortisol or cortisone. The expression of 11βHSD1 was detected at the level of mRNA by real-time RT-PCR. As expected, dexamethasone treatment resulted in the highest induction, while cortisol was slightly more effective than cortisone. Co-administration of metyrapone dramatically inhibited the cortisone-dependent induction of 11βHSD1, while it was less effective in case of dexamethasone or cortisol treatment (Table 1).

Table 1 – Effect of metyrapone on the glucocorticoid-induced expression of 11βHSD1 mRNA in 3T3-L1 cells

Treatment	Threshold cycle numbers				Fold decrease in 11βHSD1 expression caused by metyrapone
	36B4		11βHSD1		
	Control	With metyrapone	Control	With metyrapone	
Dexamethasone	14.89	14.81	21.88 ± 1.07	22.75 ± 0.88	1.70
Cortisol	14.94	14.71	22.92 ± 1.62	24.78 ± 1.17	3.40
Cortisone	14.79	14.99	23.07 ± 0.65	26.32 ± 0.78*	8.47

Adipogenesis was induced in 3T3-L1 cells by the administration of a medium containing 0.5 μM dexamethasone, cortisol or cortisone without (control) or with metyrapone (50 μM). Total RNA was extracted from the cells after 7 days. Real-time RT-PCR was performed using primers specific to 36B4 (reference gene) and to 11βHSD1. Relative expression levels were calculated from the threshold cycle numbers. Values are means ± S.D. of four independent experiments; *$p < 0.0001$.

3.5. *Metyrapone prevents cortisone-induced adipocyte differentiation*

3T3-L1 murine preadipocytes were differentiated to adipocytes with the administration of dexamethasone, cortisol or cortisone. Differentiation was examined by Oil Red O staining at day 7 of differentiation. As expected, dexamethasone treatment resulted in the highest mean droplet size and percent lipid area, while cortisol was slightly more effective than cortisone. Co-administration of metyrapone completely

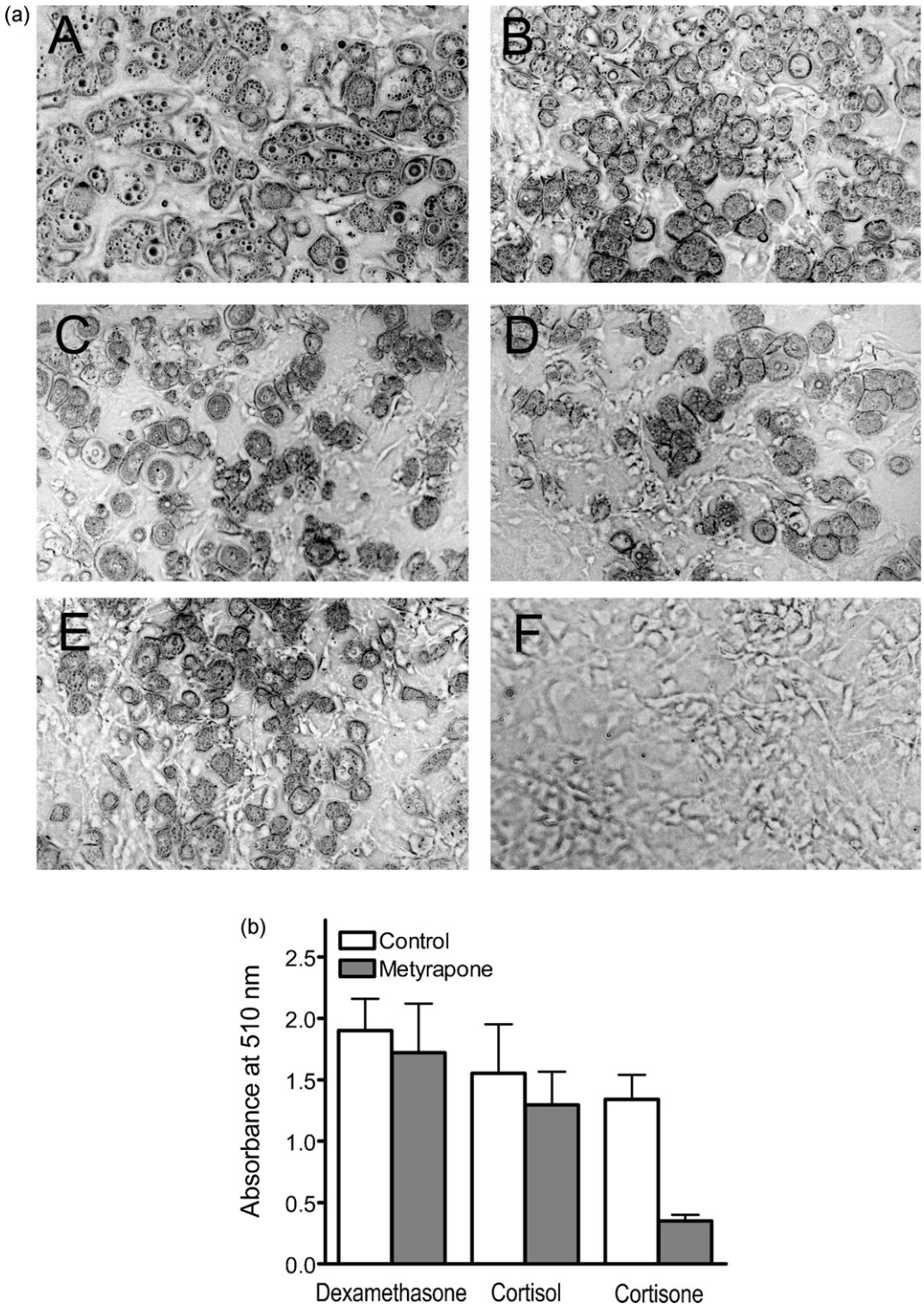

Fig. 5 – Effect of metyrapone on cortisone-induced adipogenic differentiation of 3T3-L1 cells. Adipogenesis was induced in 3T3-L1 cells with a medium containing 0.5 μM dexamethasone (pictures A and B), cortisol (pictures C and D) or cortisone (pictures E and F). Metyrapone (50 μM) was added together with the steroids to cells shown in pictures B, D and F. Cells were stained with Red Oil O and examined by phase contrast microscopy after 7 days. The diagram shows the absorbance at 510 nm of the Red Oil O dye extracted with isopropanol from the cells treated as indicated. Data are means ± S.D. of three separate experiments.

abolished the cortisone-induced differentiation of the cells, while it was again less effective in case of dexamethasone or cortisol treatment (Fig. 5).

4. Discussion

The present results demonstrate that metyrapone effectively depletes NADPH in the lumen of the ER and prevents cortisone-induced preadipocyte differentiation.

Metyrapone has been known as an adrenal and extra-adrenal inhibitor of cortisol formation. The latter effect is thought to be achieved through the inhibition of prereceptorial glucocorticoid activation. In fact, 11βHSD1, the enzyme responsible for the interconversion of 11-keto and 11-hydroxy glucocorticoids, is capable of acting as carbonyl reductase in the detoxification of various xenobiotics including metyrapone [25]. Metyrapone was postulated as a competitive inhibitor of the enzyme [26]. However, the inhibition was observed at relatively high metyrapone concentrations (with an apparent K_i of 30 μM), and exclusively in the cortisone reductase direction [26]. Accordingly, another study reported an approximately 0.3 mM IC_{50} value with 11-dehydrodexamethasone as substrate [27]. Moreover, it was found that metyrapone did not inhibit cortisol production during the first linear phase of the reaction in rat liver microsomes; only became effective when the reaction was allowed to proceed for longer time [28]. These observations suggest that – although metyrapone is a substrate for purified 11βHSD1 [25] and can interact with 11βHSD1 at high concentration – it does not appear to have the characteristics of a conventional competitive enzyme inhibitor in pharmacologically relevant concentrations.

Previous results demonstrated that metyrapone is an effective NADPH-depleting agent in microsomal vesicles [5,19]. Since the NADPH-depleting effect reaches the maximum at low micromolar metyrapone concentration, it is unlikely that 11βHSD1-driven metyrapone reduction would be solely responsible for the phenomenon. It is more probable that other powerful NADPH-dependent metyrapone reductase activities also present in the ER lumen are underlying the effect. The present results show that the NADPH-depleting effect of metyrapone can be reverted by the addition of cortisol, and *vice versa*: cortisol-dependent $NADP^+$ reduction can be turned back with metyrapone in rat liver microsomes (Fig. 1). This observation suggests that cortisol oxidation and metyrapone reduction are coupled to one another through the commonly used luminal pyridine nucleotide pool. This assumption was directly verified in an experiment where metyrapone addition resulted in a concentration-dependent stimulation of cortisol oxidation (Fig. 3).

In agreement with the results gained in rat liver microsomes, metyrapone influenced similarly the cortisone–cortisol conversion in differentiated 3T3-L1 adipocytes and in human stem cells differentiated to adipocytes. In both cell types, high cortisone reductase and minimal cortisol dehydrogenase activities were observed (Fig. 4). Metyrapone addition caused a switch in the activity from reductase to dehydrogenase, definitely because of its NADPH-depleting effect. It should be noted that similar switch but in the opposite direction was reported by other groups upon the induction of the NADPH-producing H6PDH [13,29,30]. The reduced state of the ER luminal pyridine nucleotides maintained principally by H6PDH seems to play a role in the antioxidant defense of the organelle [7,31] and potentially in the function of other NADPH-dependent steroid metabolizing enzymes in the compartment. Although the exact topology of the membrane bound steroid metabolizing dehydrogenase enzymes has not been extensively studied, it can be assumed that the preferential direction of their activity depends on the localization of their active site as well as on their relative affinities to pyridine nucleotides, such as NAD(H) and NADP(H) [32,33]. Therefore, the NADPH-depletion caused by metyrapone may have additional consequences, which need further investigations.

Active glucocorticoids are known to be indispensable in preadipocyte differentiation. The hormone activation by 11βHSD1, which regulates the local level of glucocorticoids, has been suggested to be involved in the development of obesity. A definitive functional role for 11βHSD1 in adipogenesis, however, remains to be established. Silencing of 11βHSD1 by siRNA [34] or by shRNA [18] attenuated the accumulation of lipid droplets and the expression of adipogenesis marker genes, which was induced by corticosterone or dexamethasone in 3T3-L1 preadipocytes. 11βHSD1 shRNA delivered by lentiviral vectors after the induction of differentiation, however, did not affect the progression of adipogenesis. These observations show that the prereceptorial glucocorticoid activation (or reactivation) plays a significant functional role in the initiation of adipogenesis. Since 11βHSD1 is indispensable in preadipocyte differentiation and its reductase activity requires continuous NADPH supply, agents depleting luminal NADPH must prevent the differentiation. In fact, metyrapone (50 μM) attenuated the accumulation of lipid droplets and the induction of 11βHSD1 provoked by cortisone in 3T3-L1 preadipocytes. Metyrapone was much less effective in case of cortisol or dexamethasone treatment. The applied metyrapone concentration was fairly below the in vitro inhibitory concentration on 11βHSD1, but higher than the effective NADPH-depleting concentration.

In conclusion, the following scenario can be outlined: metyrapone depletes the ER luminal NADPH pool in 3T3-L1 preadipocytes. NADPH depletion switches the activity of 11βHSD1 from reductase to dehydrogenase, which prevents the activation of cortisone (and promotes the inactivation of cortisol). The prevention of the prereceptorial glucocorticoid activation attenuates the differentiation and the enhanced expression of 11βHSD1. Attenuated 11βHSD1 induction, in turn, further decreases the adipogenesis. Metyrapone, therefore, refracts the positive feedback mechanism operative during preadipocyte differentiation. The results expose a new function of the luminal pyridine nucleotide pool of the ER; i.e. its permissive role in the process of adipogenesis.

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