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Effect of glucocorticoids on the activity, expression and proximal promoter of type II deiodinase in rat brown adipocytes

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ABSTRACT

Triiodothyronine (T3) is important for thermogenesis in brown adipose tissue (BAT). Type II deiodinase (DIO2) produces T3 required for intracellular needs in BAT. Brown adipocytes in culture require T3 for the adrenergic stimulation of DIO2. Glucocorticoids induce adipocyte differentiation (lipogenesis). We investigated the regulation of DIO2 activity, *Dio2* mRNA and *Dio2* promoter activity by glucocorticoids in primary cultures of rat brown adipocytes using dexamethasone (DEX) and hydrocortisone (HC). DEX and HC regulated the adrenergic stimulation of DIO2 activity in a dose- and time-dependent manner, inhibiting DIO2 activity at short treatment times and large doses (1–10 μM) and stimulating DIO2 at low HC doses (1–100 nM) and longer times (DEX). Insulin depletion reduced DIO2 activity but the response to glucocorticoids remained unchanged. DEX and HC inhibited basal DIO2 activity. DEX had no effect on DIO2 half-life, whereas HC stabilized DIO2 activity. DEX and HC inhibited the adrenergic stimulation of *Dio2* mRNA expression (100–10000 nM, 14–96 h), but stabilized *Dio2* mRNA, particularly DEX. DEX increased basal *Dio2* mRNA levels, possibly through stabilization of *Dio2* mRNA. An 807 bp construct of the murine *Dio2* proximal promoter showed maximal reporter activity, with the cAMP response element (CRE) essential for transcriptional activity. DEX caused inhibition in most constructs containing the CRE element whereas HC stimulated reporter activity in the 807 bp construct. Glucocorticoids inhibited the adrenergic stimulation of *Dio2* at the transcriptional level in brown adipocytes, although DIO2 activity increased with HC, possibly due to stabilization of *Dio2* activity and mRNA. The CRE and cEBP elements of the *Dio2* promoter seem involved in the regulation by glucocorticoids.

1. Introduction

Thyroxine (T4) iodothyronine deiodinases regulate triiodothyronine (T3) availability in tissues. Outer ring deiodination is considered the activating pathway that converts T4 into T3, whereas inner ring deiodination results in the formation of inactive compounds. Most of the T3 present in tissues is produced from T4 *via* 5′ deiodination by type I and type II deiodinases (DIO1 and DIO2, respectively). DIO1 and DIO2 differ in their kinetic characteristics, sensitivity to inhibition by 6-propyl-2-thiouracil (PTU), tissue distribution and response to thyroid status. DIO2 prefers T4 as a substrate (Km in the nM range) and is inhibited by T4, but not by PTU. DIO2 is present in brain, pituitary, brown adipose tissue (BAT), pineal gland, maternal side of the placenta and thyroid, and in humans is also present in heart and skeletal muscle. DIO2 increases in hypothyroidism. Deiodinases are seleno-proteins that contain an in-frame TGA codon resulting in the incorporation of a seleno-cysteine in the catalytic center. DIO2 has a complex post-

Abbreviations: ActD, Actinomycin D; BAT, brown adipose tissue; cAMP, cyclic AMP; Br-cAMP, Bromo-cAMP; CBP300, CREB binding protein 300; c/EBP, CCAAT Enhancer Binding Proteins; CHX, Cycloheximide; CRE, cAMP response element; DIO1, type 1 deiodinase; DIO2, *Dio2*, type 2 deiodinase; DIO3, type 3 deiodinase; DEX, Dexamethasone; DMEM, Dulbecco's Modified Eagle's Medium; DTT, dithiothreitol; GATA4, Transcription factor that binds to the DNA sequence GATA; GR, Glucocorticoid receptor; GRE, Glucocorticoid response element; HC, Hydrocortisone; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; I-kB, Regulatory protein that inhibits NF-kB; LPS, lipopolysaccharide; NCS, Newborn calf serum; NE, norepinephrine; NF-kB, Nuclear factor kappa beta; PTU, 6-propyl-2-thiouracil; qRT-PCR, quantitative Real-time PCR; RIA, Radioimmunoassay; T3, triiodothyronine; T4, thyroxine; TCA, trichloroacetic acid; TTF1, Thyroid transcription factor; *Ubc*, ubiquitin; UCP1, uncoupling protein 1.

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translational regulation of its half-life through the ubiquitination pathway, involving WSB-1 and the E3 ubiquitin ligase TEB4 (Bianco et al., 2002; Bianco and Larsen, 2005; Gereben et al., 2008; Zavacki et al., 2009).

In BAT, DIO2 activity is stimulated by hypothyroidism (Leonard et al., 1983); however, the most important stimulatory mechanisms are cold exposure, norepinephrine (NE) and adrenergic stimuli (Silva and Larsen, 1983). DIO2 activity produces most of the T3 found in BAT (Silva and Larsen, 1985) and the T3 produced is necessary for the thermogenic function of BAT, specifically the full expression of uncoupling protein 1 (Ucp1), the specific BAT marker (Bianco and Silva, 1987). DIO2 is considered a good marker of the thermogenic activity of BAT. In addition to adrenergic signaling, DIO2 activity is up-regulated in BAT by insulin (Silva and Larsen, 1986), as shown after insulin injection or by its decrease in diabetic rats. Moreover, insulin stimulates DIO2 activity in floating brown adipocytes (Mills et al., 1987).

In cultured rat brown adipocytes, DIO2 activity is poorly stimulated by NE or cAMP analogs, and T3 is required and amplifies the adrenergic response by 10–20 fold (Hernández and Obregón, 1996). This stimulatory effect of T3 on the DIO2 adrenergic response is observed using NE or β3 adrenergic agonists and is proportional to the pre-exposure period to T3. The effect of T3 requires *de novo* protein synthesis. Insulin increases *Dio2* mRNA levels and is required for the induction of *Dio2* mRNA by T3 in rat brown adipocytes (Martinez-deMena et al., 2002; Martinez-deMena and Obregon, 2005).

Several regulatory elements have been identified in the human *DIO2* promoter: a functional cAMP response element (CRE) that is conserved in several species, two TTF-1 (Nkf-2.1) response elements, several sequences responsive to cardiac transcription factors (GATA4, Nkf-2.5), several NF-kB elements involved in the response to lipopolysaccharide (LPS) in hypothalamus and several CCAAT enhancer binding proteins (c/EBP) elements (Bartha et al., 2000; Canettieri et al., 2000, 2004, 2008, 2012; Dentice et al., 2003; Gereben and Salvatore, 2005; Zeold et al., 2006). A putative CRE element has been identified in the mouse *Dio2* promoter and several c/EBPs elements, GATA binding sites (Song et al., 2000) and putative glucocorticoid response elements (GREs) can be identified *in silico*. Their functional relevance remains unknown.

c/EBPs are transcription factors involved in liver and adipose tissue differentiation. Targeted deletion of α-c/EBP in mice results in low DIO2 activity, low T3 levels and the absence of UCP-1 expression in BAT (Carmona et al., 2002). DIO2 activity therefore seems to be important for the differentiation program of BAT and is dependent on the presence of α-c/EBP.

Glucocorticoids have multiple actions on the immune system and in many other organs, regulating glucose and lipid metabolism and vascular tone. Frequently used as anti-inflammatory agents, their anti-inflammatory activity is ascribed to the inhibition of transcription of interleukins, cytokines and adhesion molecules. Mechanistically, glucocorticoids bind to the glucocorticoid receptor (GR), which belongs to the family of nuclear receptors. GR is bound to several proteins (hsp90, p59, p23) in the cytoplasm. Binding of glucocorticoid to the GR induces conformational changes, resulting in its translocation to the nucleus where it binds as dimers to the GREs leading to trans-activation. Its interaction with coactivators results in increased transcription. In addition to its direct effect on gene regulation, GR also has indirect effects, such as the inhibition of the pro-inflammatory factors NF-kB (increasing the inhibitory protein I-kB) (Almawi and Melemedjian, 2002), AP1 or Stat3, Stat5 or Stat 6.

Glucocorticoids increase lipid storage (Strack et al., 1995) as observed in glucocorticoid overexposure situations such as in Cushing's syndrome where increased visceral fat depots lead to insulin resistance and the metabolic syndrome. Glucocorticoids decrease non-shivering thermogenesis and *UCP1* mRNA expression (Galpin et al., 1983; Moriscot et al., 1993; Poggioli et al., 2013; Rothwell and Stock, 1986; Soumano et al., 2000; Strack et al., 1995; Viengchareun et al., 2001). They also modulate β-adrenergic receptors and adenyl cyclase in brown fat (Bakopanos and Silva, 2002; Scarpace et al., 1988).

Glucocorticoids are widely used as a culture supplement to induce adipogenesis in 3T3-L1 cells (mouse fibroblast-like cells that differentiate into preadipocytes), in human preadipocytes and cell lines in culture (1 μM dexamethasone (DEX) is commonly used). However, chronic use of DEX in large doses inhibits cell proliferation and the phenotype of brown adipocytes is transformed into white-like adipocytes with a large unilocular fat droplet.

Glucocorticoids are known to affect DIO2 activity; indeed, they have been used to induce DIO2 activity (100 nM hydrocortisone (HC)) in primary astroglial cell cultures (Courtin et al., 1989; Farwell and Leonard, 1989). A similar up-regulation is observed in chicken brain using DEX (Reyns et al., 2005). Our previous work showed that the combination of HC plus diBu-cAMP stimulates DIO2 activity in cultured brown adipocytes (Hernández and Obregón, 1996), whereas the combination with other similar compounds (e.g. Br-cAMP) provokes inhibitory effects. Two reports published in 2003 described opposing effects of glucocorticoids on DIO2 activity and mRNA expression in murine tumor cell lines, inhibition in a mammary tumor and stimulation in a cell line producing corticotropin (Araki et al., 2003; Song and Oka, 2003), leading to the conclusion that glucocorticoid effects are tissue-specific.

The aims of the present study were to first examine the effects of two glucocorticoids on DIO2 activity in primary cultures of rat brown adipocytes: DEX, a synthetic glucocorticoid with an extended half-life and higher affinity for nuclear receptors, and HC, which is frequently used to increase DIO2 activity in glial cell cultures. DEX and HC were used alone (basal) and combined with adrenergic stimulation (NE) with/without insulin. We also studied their effects on *Dio2* mRNA and on the half-life of DIO2 activity and *Dio2* mRNA. Furthermore, we examined their effects on the murine *Dio2* proximal promoter, both under basal conditions and also after adrenergic stimulation using NE + T3. We found regulation of DIO2 at multiple levels, including inhibition of transcription and stabilization of *Dio2* expression.

2. Materials and methods

2.1. Materials

The source of most of the reagents used has been previously described (Hernández and Obregón, 1996). Newborn calf serum (NCS) was purchased from Flow (Irvine, Scotland) or from Gibco Life Technologies (Uxbridge, UK). NE, HC and DEX were obtained from Sigma Chemical (St. Louis, MO). Ion exchange resins AG1-X8 and AG50W-X2 were obtained from BioRad (Richmond, CA).

2.2. Brown adipocyte culture

Precursor cells were obtained from the interscapular BAT of 20-day-old Sprague–Dawley rats as described (Néchad et al., 1983) with modifications (Martinez-deMena and Obregon, 2005) using collagenase digestion (0.2%) and filtration through 250-μm silk filters. Mature adipocytes were allowed to float and the infranatant was filtered through 25-μm silk filters and centrifuged. Precursor cells were seeded in 25 cm^2 culture flasks (1500–2000 cells/cm^2) on day one and grown in DMEM

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