Journal of Steroid Biochemistry & Molecular Biology 161 (2016) 45–53

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Original article

Characterization of 5α-reductase activity and isoenzymes in human abdominal adipose tissues

Mohamed Fouad Mansour[a,d], Mélissa Pelletier[a,b], André Tchernof[a,b,c,*]

[a] Endocrinology and Nephrology, CHU de Québec Medical Center, Québec, Canada
[b] Québec Heart and Lung Institute Research Center, Laval University, Québec, Canada
[c] School of Nutrition, Laval University, Québec, Canada
[d] Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 10 December 2015
Accepted 4 February 2016
Available online 6 February 2016

Keywords:
5α-reductase
Adipose tissue
Dihydrotestosterone
Testosterone
Androstenedione

ABSTRACT

Introduction: The substrate for the generation of 5α-dihydrotestosterone (DHT) is either androstenedione (4-dione) which is first converted to androstanedione and then to DHT through 17-oxoreductase activity, or testosterone, which is directly converted to DHT. Three 5α-reductase isoenzymes have been characterized and designated as types 1, 2 and 3 (SRD5A1, 2 and 3).
Objective: To define the predominant source of local DHT production in human adipose tissues, identify 5α-reductase isoenzymes and test their impact on preadipocyte differentiation.
Methods: Cultures of omental (OM) and subcutaneous (SC) preadipocytes were treated for 0, 6 or 24 h with 30 nM ^{14}C-4-dione or ^{14}C-testosterone, with and without 500 nM 5α-reductase inhibitors 17-*N*,*N*-diethylcarbamoyl-4-methyl-4-aza-5-androstan-3-one (4-MA) or finasteride. Protein level and mRNA abundance of 5α-reductase isoenzymes/transcripts were examined in whole SC and OM adipose tissue. HEK-293 cells stably transfected with 5α-reductase type 1, 2 or 3 were used to test 5α-reductase inhibitors. We also assessed the impact of 5α-reductase inhibitors on preadipocyte differentiation.
Results: Over 24 h, DHT formation from 4-dione increased gradually ($p < 0.05$) and was significantly higher compared to that generated from testosterone ($p < 0.001$). DHT formation from both 4-dione and testosterone was blocked by both 5α-reductase inhibitors. In whole adipose tissue from both fat compartments, SRD5A3 was the most highly expressed isoenzyme followed by SRD5A1 ($p < 0.001$). SRD5A2 was not expressed. In HEK-293 cells, 4-MA and finasteride inhibited activity of 5α-reductases types 2 and 3 but not type 1. In preadipocyte cultures where differentiation was inhibited by 4-dione ($p < 0.05$, $n = 7$) or testosterone ($p < 0.05$, $n = 5$), the inhibitors 4-MA and finasteride abolished these effects.
Conclusion: Although 4-dione is the main source of DHT in human preadipocytes, production of this steroid by 5α-reductase isoenzymes mediates the inhibitory effect of both 4-dione and testosterone on preadipocyte differentiation.

1. Introduction

5α-reductases are microsomal enzymes responsible for the formation of 5α-dihydrotestosterone (DHT), either through conversion of androstenedione (4-dione) to androstanedione (A-dione) and subsequent 17-oxoreduction, or through direct conversion of testosterone (testo) to DHT [1]. Three isoenzymes have been identified which are the products of three different genes: SRD5A1, SRD5A2, SRD5A3 [2]. The types 1 and 2 isoenzymes of SRD5A have a low homology; they have different chromosomal localizations and kinetic parameters; they also differ in their distribution patterns in androgen target tissues [3]. A third 5α-reductase isoenzyme designated as type 3 was detected in prostate tissue and was reported to be poorly inhibited by dutasteride at high androgen concentrations *in vitro* [4]. SRD5A3 also plays a role in *N*-linked protein glycosylation, and mutations in this gene cause a rare Mendelian disease [5].

In adipose tissue, androgens such as DHT and testo inhibit preadipocyte differentiation [6,7]. They have also been reported to decrease triglyceride synthesis and increase lipolysis in some studies (reviewed in [7]). Testo treatment decreases abdominal fat

* Corresponding author at: Endocrinology and Nephrology & School of Nutrition and CHU de Québec Medical Center, 2705 Laurier Blvd, R–4779, Québec City, QC G1V 4G2, Canada. Fax: +1 418 654 2761.
E-mail address: andre.tchernof@crchudequebec.ulaval.ca (A. Tchernof).

http://dx.doi.org/10.1016/j.jsbmb.2016.02.003
0960-0760/

accumulation in men with the metabolic syndrome [8], whereas DHT has little effects on this parameter [9]. The role of 5α-reductase isoenzymes in adipose tissue androgen homeostasis remains to be determined. Our group reported that DHT formation from 4-dione was significantly decreased by *in vitro* induction of preadipocyte differentiation [10]. The isoforms responsible for this activity have never been clearly identified (reviewed in [11]).

Our objective was to define the predominant source of local DHT production in human adipose tissues, identify 5α-reductase isoenzymes responsible for this reaction and test the impact of 5α-reductase inhibition on preadipocyte differentiation. We tested the hypothesis that adrenal androgen 4-dione is the primary source of DHT in adipose tissue compared to testo. We also postulate that 5α-reductase isoenzymes mediate the effect of testo and 4-dione on preadipocyte differentiation.

2. Subjects and methods

2.1. Subjects

The study sample included obese ($n = 3$, age: 46.4 ± 2.4 years, BMI: 37.7 ± 1.4 kg/m^2) and non-obese ($n = 7$, age: 48.0 ± 7.3 years, BMI: 26.5 ± 1.8 kg/m^2) men recruited through the elective general surgery schedule of the CHU de Québec Medical Center as well as severely obese men undergoing bariatric surgery ($n = 24$, age: 48.8 ± 12.6 years, BMI: 51.6 ± 10.6 kg/m^2). The latter patients were recruited through the Biobank of the *Institut universitaire de cardiologie et de pneumologie de Québec* according to institutionally-approved management modalities. The project was approved by the ethics committees of both institutions. All participants provided written, informed consent.

2.2. Adipose tissue sampling

Subcutaneous (SC) and omental (OM) adipose tissue samples were collected during surgery and immediately carried to the laboratory. A fresh portion of the biopsy sample was used for adipocyte isolation and primary cultures. The remaining tissue was immediately frozen at −80 °C for gene expression measurements.

2.3. Preadipocyte isolation

Adipose tissue samples were digested for 45 min at 37 °C using type I collagenase in Krebs–Ringer–Henseleit (KRH) buffer according to a modified version of the Rodbell method [12]. After tissue digestion, the cell suspension was filtered through nylon mesh and floating adipocytes were washed three times with KRH buffer. The stromal-vascular fraction was isolated using a modification of the Van Harmelen method [13]. The residual buffer containing the stromal-vascular fraction was centrifuged and the pellet was washed in DMEM/F12 supplemented with 10% calf serum. Cells were filtered through 140 μm nylon mesh and placed in culture plates at 37 °C under 5% CO_2 atmosphere. Preadipocytes were maintained in DMEM/F12 supplemented with 2.5% fetal bovine serum, 10 ng/ml endothelial growth factor, 1 ng/ml fibroblast growth factor, 6 μM insulin and antibiotics.

2.4. Preadipocyte differentiation

For gene expression, differentiation of fully confluent preadipocyte cultures was induced in 12-well plates for 14 days using a commercial differentiation medium from Zen-Bio (Durham, NC, USA). This medium consisted of DMEM/F12 supplemented with a PPAR-γ agonist, insulin, dexamethasone and 3-isobutyl-1-methylxanthine. For glycerol-3-phosphate dehydrogenase (G3PDH) activity measurements, subcutaneous preadipocyte cultures in 96-well plates were pre-incubated 2 h with 500 nM of 5α-reductase inhibitors 17-*N*,*N*-diethylcarbamoyl-4-methyl-4-aza-5-androstan-3-one (4-MA) or finasteride (Sigma, Oakville, ON, Canada). 4-Dione or testo (0, 10 nM and 30 nM) was added for a 24 h incubation period at 37 °C. The next day, preadipocyte differentiation was induced for 14 days using serum-free Zen-Bio medium (Durham, NC, USA) supplemented with charcoal-treated serum. Inhibitors and 4-dione or testo were added to the medium prior to preadipocyte differentiation induction. G3PDH activity was measured as described previously [14]. Briefly, 14 days differentiated cells from two separate wells of 96-well plates were washed with PBS and homogenized in cold homogenization solution (100 μl/well; 20 mM Tris, 1 mM EDTA, 1 mM β-mercaptoethanol, pH 7.3). Activity was assessed in reaction buffer (90 μl/well; 100 mM triethanolamine, 2.5 mM EDTA, 0.1 mM β-mercaptoethanol and 353 μM NADH, pH 7.7) for 10 min at 37 °C and the assay was initiated by the addition of 8 mM dihydroxyacetone phosphate (DHAP). Optical density at 340 nm was measured at repeated intervals for 3–5 min with a Spectramax340pc (Molecular Device, Sunnyvale, CA, USA) and milliunits of G3PDH activity was calculated using a standard curve of purified G3PDH enzyme (Sigma, Oakville, ON, Canada). DNA content per well was quantified with a NanoVue spectrophotometer (GE Healthcare, Baie D'Urfe, QC, Canada) and used to normalize. G3PDH activity was expressed as percentage of control wells (% of control). A total of 7 cultures showed significant response to 4-dione out of 10 cultures, and 5 cultures responded to testo out of 9.

2.5. 5α-reductase activity in preadipocytes

OM and SC preadipocyte cultures were seeded in 24-well plates and incubated in a time course experiment (0, 6 and 24 h) with 30 nM of either ^{14}C-4-dione or ^{14}C-testo (American Radiolabeled Chemicals, St. Louis, MO, USA). For enzyme inhibition, preadipocytes were pre-incubated 2 h with 500 nM of 4-MA or finasteride (Sigma, Oakville, ON, Canada). After the pre-incubation period, 30 nM of ^{14}C-4-dione or ^{14}C-testo was added for 24 h incubation at 37 °C under 5% CO2 atmosphere. Steroids from OM and SC preadipocyte culture media were extracted twice with diethyl ether. The dried steroids were dissolved with dichloromethane and migrated on thin layer chromatograms (TLC) with toluene-acetone (4:1). TLC plates were scanned on a Storm 860 PhosphorImager (GE Healthcare, Baie D'Urfe, QC, Canada). Densitometric analyses were performed using ImageJ software (NIH, Bethesda, MD, USA). The conversion rates of androgen substrates (4-dione or testo) were expressed as percentage of metabolite formation divided by protein amounts in the culture well (% formation/μg protein).

2.6. 5α-reductase activity in stably transfected HEK-293 cells

Human embryonic kidney cells (HEK-293) expressing each of the 3 subtypes of the human SRD5A genes were kindly provided by Dr. Van Luu-The from the CHU de Québec Research Center, Québec, Canada. Briefly, the cDNA fragments for the 3 enzyme isoforms were subcloned into a pCMV-Neo expression vector to generate stably transfected HEK-293 cells (Table 1). Transfected cells were maintained in DMEM high glucose medium, supplemented with 10% fetal bovine serum and 400 μg/ml of G418. Untransfected HEK-293 and SRD5A-transfected cells were pretreated for 2 h with 500 nM of 4-MA or finasteride. After the pre-incubation period, 30 nM of ^{14}C-4-dione was added for 24 h at 37 °C. Steroids from the culture media were extracted with diethyl ether and resolved on TLC as described above. The results were expressed as percentage of total radioactive density of each steroid on protein concentration (% of total density/μg protein).