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Hormonal regulation and characterization of MHG30 gene, a desaturase-like gene of hamster harderian gland

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ABSTRACT

The harderian gland (HG) is an orbital gland of the vast majority of land vertebrates. In the Syrian hamster these glands display a marked sexual dimorphism. Here we present data on a male specific clone named MHG30. The MHG30 cDNA (1470 bp) has significant sequence homologies with human #15μ10#Δ6-desaturase enzymes. The expression of MHG30 has been found in male HG and in the liver of both sexes, no other tissue showing the presence of MHG30 mRNA. Castration brings the MHG30 levels below detectable level in about 7 days. In in vitro cultures of male hamster HG cells, androgens (*A*) determine an enhancement of MHG30 expression in a time-dependent manner. Conversely, a continuous decrement has been observed in control cells and in cells treated with A plus flutamide (*F*) or with *A* and cycloheximide (Cy). Incubation of cells in cultures supplemented with desamethason (Dex) or thyroid hormone (T_3) also increases MHG30 expression while 17β-estradiol prevents the stimulatory effect exerted by *A*, Dex and T_3. Findings strongly suggest that the MHG30 gene could be involved in supporting the sexual dimorphism and its expression is likely triggered by a series of hormonal interactions.

1. Introduction

The Harderian gland (HG), is an orbital gland found in the majority of land vertebrates possessing a nictitating membrane [1–2]. Since it shows a well-developed morphological and biochemical sexual dimorphism in many rodents, such as the golden hamster, it represents a useful model to study genetic control of sexual dimorphism of the secondary characters [1]. In the golden hamster two different cell types are evident in male (types I and II), while the female gland show only Type I cells [1]. In rodents, where it is well developed, the gland secretes lipids and porphyrins. The latter shows often a sex difference, with female glands containing more porphyrins than the male gland; also the lipid composition varies between the male and female gland and the differences are due to the presence or absence of unsaturated fatty acids in ADG branches [1]. These differences are probably due to a different hormonal status between sexes. Castration alters both morphology and biochemistry of the male gland. This effect concerns also the variation of cell types since the morphology of cell type II is converted into type I. All the above changes must be counteract by androgen replacement [testosterone (*T*)] therapy [1]. Similarly, while castrated males show increased levels of porphyrins, androgens administration in females cause a marked decrease of these compounds [1]. There is also an evident sexual dimorphism in protein pattern: the male pattern shows two specific glycoproteins that are significantly reduced after castration, whereas replacement therapy with T restores such a pattern [3].

In male hamsters has been found a cytoplasm androgen receptor (AR) protein [4] and AR mRNA, whose transcription is under androgen control, has also been found in primary cultures of male hamster HG [5].

Subtraction-based differential screening studies of male and female cDNA allowed to isolate and identify several female genes, (FHG, Female Harderian Gland) and male genes (MHG, male harderian gland) [6]. Among FHG genes, FHG22 gene sequence characterization analysis showed a nucleotide and an amino acid sequence similar to those of subunits of prostatein, uteroglobin, major cat allergen F1dI (chain1) and mouse salivary androgen binding protein (subunit a) [7]. The expression of FHG22 has been found only in female gland. Furthermore either in vivo or in vitro experiments have shown that the expression of mFGH22 gene is under 17β-estradiol control [8]. Sequence characterization analysis of a specific male, androgen-controlled gene, MHG07, also revealed a similarity with the sequences of aldehyde oxidase in mouse [9].

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The purpose of the present study was to characterize the MHG30 gene and to investigate among its regulation.

2. Materials and methods

2.1. Animals

Adult male golden hamster (Mesocricetus auratus) were delivered by Nossan (Italy) and housed (3/cage) using a light:dark cycle of 14:10 h at 20 °C. Water and food were given ad libitum. The care and maintenance of animals followed the Ethical Rules drafted by the internal Ethical Committee.

2.2. Chemicals and reagents

All materials were tested for molecular biology grade and tissue culture. [α^{32}P]dCTP (3.000Ci/mmol) was purchased from Amersham International (Amersham, Italy). Testosterone (*T*), 17β-estradiol (*E*), progesterone (*P*), dexamethasone (Dex), tri-iodiotyronine (T_3), 5α-dihydrotestosterone(5α-DHT), cycloheximide (Cy) [inhibitor of protein synthesis], Mifepristone (RU-486) (mf) [glucocorticoid inhibitor] and Amiodarone (am) [anti-thyroid hormone] were purchased from Sigma (St. Louis, MO). ICI 164,384, a pure estrogen inhibitor [10], was generously donated by Imperial Chemical Industries (ICI, Macclesfiel, UK).

Since in pilot experiments the use of T or 5α-DHT gave the same effects, at both biochemical and morphological levels, the term androgen (A) is used indifferently to indicate one of them [5]. This may be due to the present a 5α-reductase activity while aromatase activity in the HG has not been found [1]. The hormone concentration used in the present work was selected at [10^{-8} M] as this concentration have been shown to be the useful concentration in terms of efficacy and reproducibility of results [8,9].

2.3. RNA extraction

The extraction of total cellular RNA, its yield and quality was performed as described by Esposito et al. [9].

2.4. MHG30 sequencing

MHG30 cDNA sequencing and analysis were carried out as previously described [9].

2.5. RNA analyses

Northern blots and slot blot analysis of RNA was performed as previously described [9]. Hybridizations were performed as described by Esposito et al. [9] using a 0.35 kb BamHI-XbaI fragment from the male hamster MHG30 cDNA (pcDNAII) or with a 2.1 kb BamHI fragment from the human cDNA of β-actin (pHFbA-1) [11]. For androgen receptor analysis a 1.7 kb SmaI-EcoRi fragment of rat AR cDNA was used as previously described [12]. Labeling, hybrizations and exposure were performed as previously described [9].

2.6. Primary cultures

Primary HG cultures were prepared as previously described [13] with material deriving from 20 male hamster/experiment. Before implementing any kind of treatment, cells were maintained in the culture medium for up to 24 h after plating to allow recovery from culture shock.

This procedure was important to assure good reproducibility.

2.7. In vivo experiments

2.7.1. Castration

Male hamsters (24 animals/experiment) was either submitted to bilateral gonadectomy or sham-surgery (controls), under anesthesia. Three castrated males were sacrificed under anesthesia after 1, 2, 3, 7, and 13 days after gonadectomy. The HGs were withdraw and RNA was extracted as above and used for Northern blots.

2.7.2. Tissue specificity of MHG30 expression

Animals were sacrificed while under anesthesia and total cellular RNA was extracted from HGs, testis, liver, kidney, seminal vesicle, prostate, spleen, brain, submaxillary gland, and epididymis male hamster and from HGs, ovary, liver, submaxillary gland and brain of female hamster to study the tissue distribution of MHG30 gene expression by Northern blotting.

2.8. In vitro experiments

The experiments described below were conducted in triplicate at least, using three different cell preparations.

2.8.1. (a) androgens (A) and MHG30 expression

HG cells were treated as follows: cells were exposed either to *A* (10^{-8} M) [b], *F* (10^{-6} M) [c], Cy (1 mg/ml) [d], *A* (10^{-8} M) + *F* (10^{-6} M) [e] or *A* (10^{-8} M) + Cy (1 μg/ml) [f]; unexposed cells [a] were used as control. Incubation was carried out for periods of 0, 24, 48, 72 h. The medium and various additives were replaced each 24 h. At the end of every interval, cells were washed and RNA extracted and bound to nylon membrane in a slot-blot apparatus, as previously described [9]. Duplicate filters were hybridized with either MHG30 or β-actin probes. The blot analysis was carried out by scanning the slot-blot hybridization signals of samples exposed for the same length of time (i.e. at 24 h); [a], [b], [c], [d], [e], [f] were loaded in succession. The values obtained by scanning the signals at every time interval were expressed as fold of induction of MHG30 mRNA with respect to the control.

2.8.2. (b) 17β-Estradiol and MHG30 expression

HG cells were treated as follows: cells were exposed to *A* (10^{-8} M) [b], *A* (10^{-8} M) + 17β-estradiol (10^{-8} M) [c] or *A* (10^{-8} M) + 17β-estradiol (10^{-8} M) + ICI (10^{-6} M) [d]; unexposed cells [a] were used as control. Incubation and analyses were as above.

2.8.3. (c) dexamethasone and MHG30 expression

HG cells were treated as follows: cells were exposed to *A* (10^{-8} M) [b], *A* (10^{-8} M) + Dex (10^{-8} M) [c] or *A* (10^{-8} M) + Dex (10^{-8} M) + mf (10^{-6} M); unexposed cells [a] were used as control. Incubation and analyses were as above.

2.8.4. (d) Thyroid hormone T_3 and MHG30 expression

HG cells were treated as follows: cells exposed to *A* (10^{-8} M) [b], *A* (10^{-8} M) + T_3 (10^{-8} M) [c] or *A* (10^{-8} M) + T_3 (10^{-8} M) + am (10^{-6} M); unexposed cells [a] were used as control. Incubation and analyses were carried out as above.

2.8.5. (e) progesterone and MHG30 expression

HG cells were treated as follows: unexposed cells served as control [a]; cells were exposed to *A* (10^{-8} M) [b], *P* (10^{-8} M) *A* (10^{-8} M) + *P* (10^{-8} M)[c] or to *A* (10^{-8} M) + *P* (10^{-8} M) [d]. Incubation and analyses were carried out as above.

2.8.6. (f) Dose–response effect of A on MHG30 expression

Cultured cells were exposed to different concentrations of *A*, ranging from 10^{-12} M to 10^{-6} M, for up to 48 h using unexposed