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Dihydrotestosterone inhibits hair growth in mice by inhibiting insulin-like growth factor-I production in dermal papillae

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ABSTRACT

We demonstrated that insulin-like growth factor-I (IGF-I) production in dermal papillae was increased and hair growth was promoted after sensory neuron stimulation in mice. Although the androgen metabolite dihydrotestosterone (DHT) inhibits hair growth by negatively modulating growth-regulatory effects of dermal papillae, relationship between androgen metabolism and IGF-I production in dermal papillae is not fully understood. We examined whether DHT inhibits IGF-I production by inhibiting sensory neuron stimulation, thereby preventing hair growth in mice. Effect of DHT on sensory neuron stimulation was examined using cultured dorsal root ganglion (DRG) neurons isolated from mice. DHT inhibits calcitonin gene-related peptide (CGRP) release from cultured DRG neurons. The non-steroidal androgen-receptor antagonist flutamide reversed DHT-induced inhibition of CGRP release. Dermal levels of IGF-I and IGF-I mRNA, and the number of IGF-I-positive fibroblasts around hair follicles were increased at 6 h after CGRP administration. DHT administration for 3 weeks decreased dermal levels of CGRP, IGF-I, and IGF-I mRNA in mice. Immunohistochemical expression of IGF-I and the number of proliferating cells in hair follicles were decreased and hair re-growth was inhibited in animals administered DHT. Co-administration of flutamide and CGRP reversed these changes induced by DHT administration. These observations suggest that DHT may decrease IGF-I production in dermal papillae by inhibiting sensory neuron stimulation through interaction with the androgen receptor, thereby inhibiting hair growth in mice.

1. Introduction

The human skin has been shown to express genes of growth hormone (GH), GH-receptor, and the GH mediator insulin-like growth factor-I (IGF-I) [1,2]. Several lines of evidence have demonstrated that the GH-IGF-I axis functions in the skin [3–5]. IGF-I, a polypeptide composed of 70 amino acids, mediates growth-promoting actions of GH, thereby playing an important role in postnatal and adolescent growth [6]. Furthermore, IGF-I has been shown to have various other important biological activities such as promotion of differentiation of various cell types, potent anti-apoptotic activity, and an anabolic effect [7].

IGF-I is critically involved in the promotion of hair growth by regulating cellular proliferation and migration during the development of hair follicles [8]. Transgenic mice over-expressing IGF-I in the skin have been demonstrated to show earlier hair follicle development than controls [9]. Consistent with these observations, patients with Laron syndrome (primary IGF-I deficiency) show sparse hair growth and frontal recessions [10].

IGF-I is produced by dermal papilla cell, matrix cell, and dermal fibroblast [4,5,11], and IGF-I receptor is detected in keratinocytes and dermal papilla cells in hair follicles [3–5]. These observations suggest that IGF-I produced by dermal papilla cells and matrix cells may act on keratinocytes and dermal papilla cells, thereby promoting hair growth through stimulation of cell proliferation in hair follicles.

Capsaicin-sensitive sensory neurons are nociceptive neurons that can be found in many tissues within the lining epithelia and around blood vessels in many tissues [12]. These sensory neurons release calcitonin gene-related peptide (CGRP) upon activation of the transient receptor potential vanilloid 1 (TRPV1) expressed on them by a wide variety of noxious physical and chemical stimuli [13], thereby showing sensory-efferent functions. CGRP, a 37-amino acid neuropeptide, is produced in dorsal root ganglion (DRG) neurons by tissue-specific alternative processing of the calcitonin gene [14]. CGRP-immunoreactive innervation and the CGRP receptor were demonstrated in hair follicles of rats and humans, respectively [15,16]. CGRP expression was decreased in hair follicles of patients with alopecia areata, and ultraviolet radiation shows therapeutic effect by increasing CGRP expression in hair follicles [17]. These observations suggest that CGRP may play a critical role in hair growth.

We previously demonstrated that IGF-I production in dermal papillae was increased and hair growth was promoted after sensory neuron stimulation in wild-type mice, but not in CGRP-knockout mice [18,19]. We further showed that combined administration of capsaicin and isoflavone improved alopecia in patients with androgenetic

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alopecia by increasing IGF-I production [18]. These observations suggest that sensory neurons may play a critical role in promotion of hair growth by the following mechanisms: exogenous substances such as capsaicin and isoflavone and endogenous substances such as anandamide and prostaglandins activate TRPV1 in sensory neurons, thereby increasing CGRP release from nerve endings [20]. CGRP in turn acts on dermal papilla cells, matrix cells, and keratinocytes to increase IGF-I production, thereby promoting hair growth [4,5,11,19].

Androgenetic alopecia (AGA) occurs in about half of all Caucasian men older than 50 years of age [21]. Androgen is a prerequisite for the development of AGA in genetically predisposed individuals [22]. It is generally accepted that dihydrotestosterone is one of the critical mediators of hair loss in AGA. Dihydrotestosterone (DHT) is derived from the conversion of testosterone by 5-a-reductase, and AGA is associated with increased scalp levels of DHT [23]. Baldness is not seen in men with inherited 5-a-reductase deficiency [24]. Furthermore, activities of 5-a-reductase and DHT levels in hair follicles are higher in AGA patients than those in non-AGA males [25–28]. Individuals who lack the functional AR had no balding [29] and expression of the AR is increased in balding scalp [28,30]. These observations strongly suggest that DHT plays a causal role in the development of AGA by interacting with AR. Wide genomic analysis showed that some chromosomes such as chromosome 20pII in addition to AR gene are associated with AGA development [31]. Thus, androgens and genetic factors play major roles in the pathogenesis of AGA.

Although DHT has been shown to be a negative growth-regulatory effect on hair follicles by increasing transforming growth factor-beta 1 (TGF-b1) expression *in vitro*[32], the precise mechanism by which DHT causes alopecia *in vivo* is not fully understood.

Finasteride, a type II 5-a-reductase inhibitor, has been proven to be efficacious in the treatment of AGA with a hair growth rate of about 65% in men aged 18 to 41 years after 5 years of therapy [33]. Finasteride has been shown to increase the expression level of IGF-I in dermal papilla cells of patients with AGA [22]. Androgen receptor is shown to be present in sensory neurons [34], and DHT has been shown to interact with the androgen receptor [35].

Taken together, these observations raise the possibility that DHT inhibits IGF-I production in dermal papilla cells by inhibiting sensory neuron stimulation through interaction with the androgen receptor. We examined this possibility using mice in the present study.

2. Methods

2.1. Animals and reagents

Male C57BL/6 mice (7–8 week old; Nihon SLC, Hamamatsu, Japan) were used in this study. The care and handing of the animals were in accordance with the National Institute of Heath guidelines. All the experimental procedures described below were approved by Nagoya City University Animal Care Committee. Dihydrotestosterone and flutamide were purchased from Sigma Chemical Co. (St. Louis, MO). Rat αCGRP was purchased from Peptide Institute (Osaka, Japan). All other reagents were of analytical grade.

2.2. Hair shaving treatment and androgen-dependent alopecia model

Studies started when animals were at 8–9 weeks of age, as their hair was in the telogen phase. Under anesthesia with intraperitoneal (i.p.) injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), the dorsal areas (2 cm in width and 4 cm in length) of mice were shaved with clippers.

Dihydrotestosterone (DHT) was suspended in sesame oil. Following hair shaving, mice were given subcutaneous injection of sesame oil (vehicle control), DHT 50 mg/kg, DHT 5 mg/kg, and DHT 5 mg/kg in combination with the non-steroidal androgen-receptor antagonist flutamide (200 mg/kg) in a total volume of 0.2 mL once in a day for 3 weeks. Flutamide (200 mg/kg) alone was administered by subcutaneous injection once in a day for 3 weeks. CGRP (10 μg/kg) was dissolved in sterile distilled water and administered by intraperitoneal injection once in a day for 3 weeks as described previously [36].

Changes in hair coat were recorded by a photography using a digital camera once per week for 3 weeks, until a significant difference was observed from each group. Then the skin from the dorsal areas was removed under anesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg) for determination of dermal CGRP, IGF-I, IGF-I mRNA levels and histological analysis as described below.

2.3. Isolation and culture of dorsal root ganglion (DRG) neurons

The lumbar, cervical, and thoracic DRG neurons were dissected from both sides of the spinal cord of mice as previously described [37]. After 5 days in culture, the medium was aspirated gently and washed with serum-free Ham's F-12 medium. Cells were incubated with DHT (100 nM), flutamide (10 μM), DHT (100 nM) + flutamide (10 μM), alone or in combination with capsaicin (1 μM) for 30 min in Ham's F-12 medium containing 1% supplemented calf serum without nerve growth factor. Capsaicin has long been known to be a stimulant of CGRP release from dorsal spinal cord neurons and was used here as a positive control. After incubation, supernatants were sampled and stored at −20 °C for CGRP measurements. CGRP levels were determined using a specific enzyme immunoassay kit (SPI-BIO, Massy, France).

2.4. Determination of dermal CGRP level

Skin dermal CGRP levels were determined in mice by a modification of the methods described previously [38]. Skins were weighed and homogenized in 2 mL of 2 N acetic acid. Homogenates were bathed in 90 °C water for 20 min and then centrifuged at 4500 *g* for 10 min (4 °C). CGRP was extracted from the supernatant using reverse-phase C18 columns (Amersham, Little Chalfont, UK). Columns were prepared by washing with 5 mL methanol onto the column followed by a wash with 20 mL of 0.1% trifluoroacetic acid, and the solvent was evaporated under a stream of nitrogen gas. The concentration of CGRP was assayed by using a specific enzyme immunoassay kit (SPI-BIO, Massy, France). The sensitively of the CGRP assay was 10 pg/mL. The antiserum cross-reacted 100% of rodent α- and β-CGRP according to the manufacturer's data sheet. Results are expressed as micrograms of CGRP per gram of tissue.

2.5. Determination of dermal IGF-I level

Dermal levels of IGF-I were determined in animals by modification of the methods as described previously [39]. The skins were minced and homogenized in 2 mL of 1 N acetic acid according to the manufacturer's instruction (Diagnostic Systems Laboratories Inc., Webster, TX). The homogenates were then centrifuged at 3000 *g* for 10 min. The supernatants were kept at −80 °C until assayed for IGF-I concentration by using a specific enzyme immunoassay kit (Diagnostic Systems Laboratories Inc., Webster, TX).

2.6. Quantitative mRNA analysis

Quantitative mRNA analysis was performed as previously described [40]. Total RNAs were extracted from skins using TRIzol Reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instruction. Sample cDNAs were amplified in the Model 7700 Sequence detector (Applied Biosystems, Perkin Elmer Japan, Chiba, Japan) with IGF-I or CGRP primers, dual-labeled fluogenic probes, and a Taqman PCR Kit (Applied Biosystems, Branchburgh, NJ). Thermal cycler conditions were 10 min at 95 °C for denaturation preceding 40 cycles for 15 s at 95 °C for denaturation and 1 min at 60 °C for bath annealing and extension. IGF-I mRNA levels were standardized to those of β-actin from same sample.