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Sex-dependent regulation of hepatic CYP3A by growth hormone: Roles of HNF6, C/EBPα, and RXRα

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ABSTRACT

Sex-based differences in the pharmacological profiles of many drugs are due in part to the female-predominant expression of CYP3A4, which is the most important CYP isoform responsible for drug metabolism. Transcription factors trigger the sexually dimorphic expression of drug-metabolizing enzymes in response to sex-dependent growth hormone (GH) secretion. We investigated the roles of HNF6, C/EBPα, and RXRα in the regulation of human female-predominant CYP3A4, mouse female-specific CYP3A41, and rat male-specific CYP3A2 expression by GH secretion patterns using HepG2 cells, growth hormone receptor (GHR) knockout mice as well as rat models of orchiectomy and hypophysectomy. The constitutive expression of HNF6 and RXRα was GH-dependent, and GHR deficiency decreased HNF6/C/EBPα complex levels and increased HNF6/RXRα complex levels. Feminine GH secretion induced the binding of HNF6 and C/EBPα to the *CYP3A4* and *Cyp3a41* promoters and HNF6/C/EBPα complex levels was more efficiently compared with masculine pattern. Additionally, a greater inhibition of the binding of RXRα to the *CYP3A4* and *Cyp3a41* promoters and HNF6/RXRα complex levels was observed by feminine GH secretion, but less inhibition was observed by masculine pattern. The binding of HNF6, C/EBPα, and RXRα to the *CYP3A2* promoter was not directly regulated by androgens. RXRα completely abolished the synergistic activation of the *CYP3A4*, *Cyp3a41*, and *CYP3A2* promoters by HNF6 and C/EBPα. The results demonstrate that sex-dependent GH secretion patterns affect the expressions and interactions of HNF6, C/EBPα, and RXRα as well as their binding to *CYP3A* genes. RXRα mediates the sex-dependent influence of GH on CYP3A expression as an important signalling molecule.

1. Introduction

The sexually dimorphic expression of drug-metabolizing enzymes is one of the primary causes of sex-based differences in the pharmacological profiles of many drugs and the prevalence of liver diseases. The cytochrome P450 (CYP) superfamily is principally responsible for the biotransformation of clinical drugs, toxins, and endogenous compounds [1]. The more rapid clearance of different drugs in women compared with men has been reported, which is due in part to the female-predominant expression of CYP3A4, the most important CYP isoform responsible for drug metabolism [2–4]. CYP3A4 levels in women vary from approximately 25 to 200% above the observed levels in men [5–8].

Sexually dimorphic growth hormone (GH) secretion is considered to be a major factor in establishing and maintaining sexual dimorphism in hepatic gene transcription, including that of CYP isoforms [3,9–11]. The characteristic GH secretory profiles of all mammals include constant secretion in females and pulsatile secretion in males [3,10,12]. A clinical study revealed that continuous GH administration induces CYP3A4 activity in humans [13]. Both CYP3A4 protein and mRNA levels can be increased by the constant GH treatment in primary human hepatocytes but are suppressed by the pulsatile GH treatment [12,14].

GH signalling is initiated by the binding of GH to the extracellular domain of the growth hormone receptor (GHR), which recruits and/or activates multiple intracellular signalling molecules

Abbreviations: CYP, cytochrome P450; GH, growth hormone; GHR, growth hormone receptor; HNFs, hepatocyte nuclear factors; C/EBP, CCAAT/enhancer-binding protein; RXR, retinoid X receptor; Co-IP, co-Immunoprecipitation; ChIP, chromatin immunoprecipitation.

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[3,15]. Hepatocyte nuclear factors (HNFs) and CCAAT/enhancer-binding protein (C/EBP) α have been considered to be the key transcription factors that collaborate with intracellular signalling molecules to mediate the sex-dependent effects of GH on liver gene expression [16]. Although HNF6 and C/EBPα have been reported to be involved in the regulation of CYP3A4 expression [17,18], the regulatory mechanism controlled by GH secretion is still largely unknown. Retinoid X receptor (RXR) α is an obligate partner for multiple nuclear receptors. RXRα expression was not found to be subject to sexual dimorphism based on microarray-derived data from mouse livers [19]; however, RXRα binding to chromatin was shown to be enriched in 1945 genes and 1214 genes in male and female mouse livers, respectively, including hepatic gender-specific genes [20].

Hypophysectomy and growth hormone replacement indicated that the mRNA level of mouse female-specific CYP3A41 was dependent on the feminine growth hormone profile [21]. However, the rat male-specific CYP3A2 has been shown to be down-regulated by the replacement of the pulsatile GH secretion in the male hypophysectomized rats [22,23]. We investigated the roles of HNF6, C/EBPα, and RXRα in the regulation of human female-predominant CYP3A4, mouse female-specific CYP3A41, and rat male-specific CYP3A2 expression in response to GH secretion patterns. This work adds to current knowledge of the sex-associated roles of individual transcription factors in the regulation of liver gene expression.

2. Materials and methods

2.1. Animals and treatment

Adult GHR knockout mice [24] and their wild-type counterparts (C57/Black) were kindly provided by Professor Michael J. Waters and were bred at the Centre for Animal Experiments Laboratory of Wuhan University. The mice were genotyped using DNA extracted from their tails. Adult male Wistar rats (250–300 g, Certificate No. SCXK2008-0005) were supplied by the Experimental Animal Centre (Hubei, China). The rats were subjected to one of the following operational procedures: bilateral orchiectomy, hypophysectomy, hypophysectomy followed by bilateral orchiectomy, or a sham-operation. After receiving the hypophysectomy–orchiectomy operation, the rats were subcutaneously injected with testosterone (1 mg/kg body weight per day in 2 equally divided doses) (Sigma Chemical, St Louis, MO, USA) or a vehicle (propylene glycol, Cat. #30157018, Sinopharm Chemical Reagent Co. Ltd, China) [25]. All experimental and surgical procedures were approved by the Animal Care Committee of Wuhan University and were conducted in accordance with the Chinese guidelines for the care and use of laboratory animals.

2.2. Surgery

The rats were anesthetized with an intraperitoneal injection of chloral hydrate (Cat. #30037516, Sinopharm Chemical Reagent Co. Ltd, China) (300 mg/kg), and orchiectomy was performed as previously described [26]. The extratesticular rete testis, together with the testicular blood vessels, were ligated as close to the testes as possible and then the testes were removed. Hypophysectomy was performed by removing both the anterior and posterior lobes of the pituitary gland by the peripharyngeal approach as previously described [27]. The anterior and posterior pituitary lobes were removed by suction. In the hypophysectomy–orchiectomy group, the rats received bilateral orchiectomy after the ablations of the anterior and posterior pituitary lobes. The animals in the sham-operation group received the entire surgery without the ablations of the testes and neural lobe. The postoperative conditions of the animals, including food and water ingestion, defecation, and surgical site appearance, were monitored daily. The rats were sacrificed 7 days after the surgery, and blood samples were collected by cardiac puncture.

2.3. Blood analysis

Plasma samples obtained from the orchiectomized rats or the rats received a sham-operation were collected every 30 min from 9:30 AM until 16 PM for the GH assays. Growth hormone levels were subsequently determined using a standard ELISA kit (Reanta Biotechnology Co., Ltd., Beijing, China). Standard curves obtained with this assay were linear up to 240 μg/L, and the least detectable concentration of plasma GH was 1.5 μg/L.

2.4. Cell culture and treatment

To investigate the effects of GH secretion patterns on the levels of HNF6, RXRα, and C/EBPα mRNA, HepG2 cells were incubated with two concentrations (0.2 and 2 ng/mL) of recombinant human growth hormone (Anke Biotechnology, Anhu, China) in Dulbecco's modified Eagle's medium for 24 h as previously described [12,28,29]. For the pulsatile administration, recombinant human GH was added for 30 min, followed by two careful washings with GH-free media that remained in flasks for 11.5 h. The previous study has pointed out that physiologic-like concentration of GH (2 ng/mL) was selected for the in vitro experiment as it was impossible to translate GH secretion pattern into equivalent in vitro doses [12]. To investigate the effects of GH secretion patterns on the binding of HNF6, RXRα, and C/EBPα to *CYP3A4* gene, HepG2 cells were incubated with recombinant human GH on physiologic levels.

2.5. Microsomal and nuclear protein preparation

Livers were manually homogenized on ice using a glass homogenizer. Liver microsomal membranes were prepared as described previously [30]. Nuclear extractions were prepared as previously described [31]. For immunoblotting, the resulting pellets were resuspended in a storage solution containing 100 mM Tris (pH 7.4), 0.1 mM EDTA, 0.1 mM dithiothreitol, 1.15% (w/v) KCl, and 20% (v/v) glycerol.

2.6. Real-time RT-PCR

Total RNA was isolated using TRIzol reagent (Cat. 15596-026, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was synthesized using a cDNA Synthesis Kit (TOYOBO, Osaka, Japan). All real-time PCR reactions with SYBR Green (Cat. K1621, TOYOBO, Osaka, Japan) were performed and analyzed with the ABI Prism 7500 instrument (Applied Biosystems, Foster City, CA) (primers are listed in Table 1). GAPDH was used for the normalization of relative expression levels.

2.7. Immunoblotting

For the detection of the CYP3A2 protein, liver microsomal membranes were serially diluted to establish a linear detection range. In subsequent experiments, 3 μg of microsomal proteins were separated by SDS-polyacrylamide gel electrophoresis (4% stacking and 10% separating gels) and were then transferred overnight onto PVDF membranes. The membranes were immunoblotted with a polyclonal rabbit anti-rat CYP3A2 antibody (Cat. AB1276, Millipore, Billerica, MA, USA) at a 1:3000 dilution, for 2 h. To detect the HNF6, RXRα, and C/EBPα proteins, the nuclear proteins (30 μg) were separated by SDS-polyacrylamide gel