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Hormonal regulation of renal multidrug resistance-associated proteins 3 and 4 (Mrp3 and Mrp4) in mice☆

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Abbreviations:
Mrp, multidrug resistance-associated protein
Cyp, cytochrome P450
ABCC, ATP-binding cassette transporter, subfamily C
DHT, 5α-dihydroxytestosterone
HPX, hypophysectomized
GNX, gonadectomized mice
E2, 17β-estradiol
GH, growth hormone
GHRH, growth hormone-releasing hormone
MP, male-pattern
FP, female-pattern

ABSTRACT

Multidrug resistance-associated proteins 3 and 4 (Mrp3 and Mrp4) are expressed at much higher levels in female than male kidney. Sex steroids and sex-specific growth hormone (GH) secretion patterns often mediate gender-predominant gene expression. Thus, three models were used to investigate potential endocrine regulation of Mrp3 and Mrp4: (1) gonadectomized (GNX) mice with 17β-estradiol (E2) or 5α-dihydroxytestosterone (DHT) replacement; (2) hypophysectomized (HPX) mice receiving E2, DHT, or simulated male-pattern (MP) or female-pattern (FP) GH secretion; (3) *lit/lit* mice, which have a spontaneous mutation in the growth-hormone releasing-hormone (GHRH) receptor, with simulated MP- or FP-GH secretion. GNX and HPX decreased Mrp3 mRNA levels compared with intact females. In both respective models E2 administration increased Mrp3 expression in GNX and HPX mice. DHT markedly repressed Mrp3 from GNX + placebo levels, however, this was not observed in the HPX model. In *lit/lit* mice, Mrp3 expression was lower than in wild-type controls, and MP-GH and FP-GH simulation slightly increased Mrp3 expression. Whereas GNX increased Mrp4 in males to female levels, HPX actually increased Mrp4 expression in both genders +375% and +66%, respectively. In both models DHT markedly repressed Mrp4. Furthermore, Mrp4 was higher in *lit/lit* than wild-type male mice, and simulation of MP-GH secretion suppressed female-predominant Mrp4 expression. In conclusion, these data indicate that E2 contributes to higher Mrp3 mRNA expression in females, yet a role for androgens in Mrp3 repression cannot be discounted. In contrast, Mrp4 mRNA is higher in females due to repression by both DHT and MP-GH secretion in males.

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1. Introduction

Multidrug resistance-associated proteins (Mrp) 3 and Mrp4 are efflux transporters that transport a broad range of conjugated and unconjugated endo- and xenobiotics. Both Mrp3 and Mrp4 are capable of transporting chemotherapeutic drugs and antivirals, as well as several endogeneous ligands, including conjugated bile acids, glucuronidated estrogens, and leukotrienes [1–4]. Because Mrp3 and Mrp4 can transport bile acids, induction of Mrp3 and Mrp4 during cholestasis may serve as a protective response to decrease potentially toxic levels of bile acids in liver and kidney [3,5,6].

The renal functions of Mrp transporters are not well characterized. At the mRNA level, Mrp3 is moderately expressed in mouse kidney, as compared with liver and intestine [7]. Mrp3 is localized to the basolateral membrane in liver and kidney [8]. Contrary to Mrp3, Mrp4 expression is very low in liver, with high expression in kidney. Mrp4 is also localized to the basolateral membrane in hepatocytes, but in kidney, Mrp4 is apically expressed, and is localized primarily in proximal tubules [9]. Previous studies from this laboratory have demonstrated that renal mouse Mrp3 and Mrp4 mRNA expression is markedly female-predominant, with onset of gender-divergent expression occurring at approximately 30 days of age for both transporters [7].

Many genes have gender-specific expression patterns in liver. In rat liver, classic examples of gender-dimorphic expression are Cyp2c11 and 2c12, which are male- and female-specific, respectively [10,11]. Likewise, in mouse liver, Cyp2d9 and 2a4 are male- and female-specific, respectively, and are regulated primarily by gender-specific GH patterns [12,13]. Examples of renal P450s that are regulated by sex hormones or GH patterns are much fewer, but male predominant expression of mouse Cyp2J5 has recently been shown to occur via up-regulation by androgens [14].

In addition to biotransformation genes, there are several examples of gender-dimorphic transporter expression. Male-predominant expression of organic anion transporter 3 (Oat3) is upregulated by androgens, and suppressed by female-pattern GH secretion [15]. Likewise, male-predominant renal expression of organic anion transporting polypeptide 1a1 (Oatp1a1) in rats and mice is due to stimulation by androgens [16,17]. Furthermore, multidrug resistance protein 1b (Mdr1b), an efflux transporter similar to Mrps, has female-predominant expression in mice [18]. In rats, Mrp4 is male-predominant, but the mechanism is not known [19]. It is important to note that exposure times and amount of protein loaded were optimized for quantification between genders, and does not reflect absolute quantification between Mrp3 and Mrp4; under homeostatic conditions, Mrp4 is much higher than Mrp3 in kidney, and vice versa in liver (data not shown).

Gender-divergent transporter expression can manifest in differential disposition of endogenous substrates, toxicants, and therapeutics. For instance, the lack of luminal expression of Oatp1a1 on the brush-border membrane in female kidney may be responsible for the 250-fold higher rate of urinary excretion of exogenously administered estradiol-17β-D-glucuronide in female compared to male rats [20]. Additionally, marked female-predominant Oat2 mRNA expression in rat kidney correlates with a 70-fold higher urinary excretion rate of perfluorooctanoic acid in females compared to males [21].

The mechanisms of gender divergent gene expression may occur by direct action of androgens or estrogens, by gender-specific GH-secretion patterns, or by a combination of these systems. Androgens and estrogens may alter gene expression by directly stimulating gene transcription through nuclear hormone receptors [22–24]. Furthermore, differential male and female patterns of GH are also known to mediate sex-dependent gene expression. In rats, males secrete GH in high-amplitude pulses with a regular frequency every three to four hrs. Between pulses, serum GH levels are non-detectable [25]. In contrast, female rats secrete GH in low-amplitude pulses with greater frequency and higher trough levels than males, resulting in a continuously detectable serum GH concentrations [26,27]. These secretion patterns are responsible for masculinization and feminization of livers, respectively. Growth-hormone-secretion patterns in male mice are similar to those in male rats [28]. In female mice, GH is secreted at regular intervals with a non-detectable baseline between pulses; however, the pulses are more frequent (1–1.5 h) than those in male mice [28]. The male-GH-secretion pattern is responsible for induction of male-predominant Cyp2d9 and repression of female-predominant Cyp2a4 in mouse liver [29,30].

Hypophysectomy (HPX) and the *little* (*lit*/*lit*) mouse are models for examining the influence of gender-specific GH-secretion patterns on gene expression. Total hypophysectomy involves ablation of the pituitary gland, resulting in loss of several hormones such as (1) luteinizing hormone, (2) follicle-stimulating hormone, and (3) adrenocorticotropic hormone, which are critical mediators of sex steroid production, as well as growth hormone, which can also masculinize or feminize expression of some genes. Although the HPX model is complicated by the complete removal of all pituitary-derived hormones, each hormone can be administered individually to assess the contribution of each hormone to gender-specific expression. The *lit*/*lit* mouse has a spontaneous mutation in the growth-hormone releasing-hormone (GHRH) receptor, resulting in a mutated, non-functional GHRH receptor, thus preventing GH secretion [31–34]. The *lit*/*lit* mice are known to respond to GH therapy in a manner comparable to the HPX model with the benefit of other endocrine factors not being disrupted [30,35].

Gender-divergent expression of Mrp3 and Mrp4 exists in mouse kidney, although the mechanism of this expression pattern is unknown. Therefore, the purpose of this study is to determine whether the gender differences in renal expression of Mrp3 and Mrp4 are due to sex hormones, GH-secretion patterns, or a combination of these two factors.

2. Materials and methods

2.1. Materials and reagents

Rat growth hormone (GH; Lot #AFP611) was purchased through the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (Torrance, CA). Pellets for subcutaneous (sc) release of the hormones used in this study [GH (1 mg; 21-day release), 5α-dihydroxytestosterone (DHT; 5 mg; 21-day release), and