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Tissue distribution and hormonal regulation of the breast cancer resistance protein (Bcrp/Abcg2) in rats and mice

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Abstract

Breast cancer resistance protein (Bcrp/Abcg2) is a member of the ABC transporter family. The purpose of this study was to quantify Bcrp mRNA in rat and mouse tissues, and to determine whether there are gender differences in Bcrp mRNA expression. Rat Bcrp mRNA levels were high in intestine and male kidney, and intermediate in testes. Mouse Bcrp expression was highest in kidney, followed by liver, ileum, and testes. Male-predominant expression of Bcrp was observed in rat kidney and mouse liver. Furthermore, gonadectomy and hypophysectomy experiments were conducted to determine whether sex steroids and/or growth hormone are responsible for Bcrp gender-divergent expression patterns. Male-predominant expression of Bcrp in rat kidney appears to be due to the suppressive effect of estradiol, and male-predominant expression of Bcrp in mouse liver appears to be due to the inductive effect of testosterone.

Keywords: Tissue distribution; Gender difference; Hormonal regulation; Bcrp; bDNA

Two major groups of cancer multidrug resistance ABC transporters, the multidrug resistance protein (MDR1/P-glycoprotein), as well as members of the multidrug resistance-associated protein (MRP) family, have been characterized in detail. Recently, the breast cancer resistance protein was identified by three independent investigations. It was identified in the atypical multidrug-resistant human breast cancer cell line MCF-7, which was selected in the presence of doxorubicin (DOX) and verapamil [1], from human placenta [2], and from a cell line selected with mitoxantrone [3]. The genes were initially designated as BCRP (ABCG2), ABCP, and MXR, respectively. MXR/BCRP/ABCP, which will be referred to as BCRP in humans and Bcrp in rodents in this paper, confers high levels of resistance to mitoxantrone as well as to the anthracyclines, the camptothecins, topotecan, and SN-38 (the active metabolite of irinotecan) [1,3–6].

The structure of BCRP differs from those of P-glycoprotein and MRP family proteins. BCRP has only one ATP-binding cassette and six-putative-transmembrane domains, and therefore is referred to as a half-ABC transporter, which most likely functions as a homodimer [7]. Although most of the half-ABC transporters are located on intracellular membranes, such as endoplasmatic reticulum, peroxisomes, and mitochondria, immunohistochemical studies reveal that BCRP is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines [8].

In normal human tissues, BCRP mRNA is most highly expressed in placental tissue. Although results between previous studies differ somewhat, low expression of BCRP was reported in human brain, prostate, small intestine, testes, ovary, colon, liver, and kidney [1,9]. In contrast, mouse Bcrp mRNA is highly expressed in kidney, and moderately in liver, colon, heart, spleen,

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and placenta [10]. Tissue distribution of rat Bcrp has not yet been reported. Knowledge of the tissue distribution may be useful for understanding the function of Bcrp.

Gender-associated differences in the membrane transport of endogenous substrates and xenobiotics have been reported in a variety of organs, including organic anion transporting polypeptide 1 (Oatp1) [11], organic anion transporters (Oats) [12–14], organic cation transporters (Octs) [15,16], multidrug resistance 1a (Mdr1a) [17], multidrug resistance 1b (Mdr1b) [17], multidrug resistance 2 (Mdr2) [18], and sodium taurocholate cotransporting polypeptide (Ntcp) [19]. It is also known that sex hormones are responsible for some of these sex-related differences in transporter expression. Testosterone increased Oatp1 and Oct2 expression in rat kidney, whereas estradiol moderately decreased Oatp1 and Oct2 in rat kidney [11,20]. In mouse kidney, the male-predominant expression of Oatp1 is androgen dependent [21]. Castration led to a decrease in Oat3 mRNA in male rat liver, and testosterone treatment restored normal male levels [13]. The gender-specific secretion patterns of growth hormone (GH) are also responsible for some of the gender-specific differences in gene expression. Male and female GH secretion patterns are responsible for gender-specific expression of rat Cyp2c11 and Cyp2c12, respectively [22]. Hypophysectomy (HX) decreased Oat2 mRNA levels in female rat kidney and increased Oat3 mRNA in female liver [13,23].

Therefore, the purpose of this study was to quantify Bcrp mRNA in rat and mouse tissues, and to determine whether there are gender differences in Bcrp mRNA expression using the branched DNA (bDNA) assay [24]. Because some gender differences in Bcrp mRNA expression were observed, gonadectomy, HX, and sex hormone replacement therapy were conducted to determine whether sex steroids and/or GH are responsible for gender-divergent Bcrp expression patterns in rat kidney and mouse liver.

Materials and methods

Tissue collection. Male and female Sprague–Dawley rats (200–250 g) ($n = 5$/gender) and C57BL/6J mice (7 weeks) ($n = 5$/gender) were purchased from Charles River Laboratories (Wilmington, MA), and housed according to the American Animal Association Laboratory Animal Care guidelines. Rats and mice were euthanized in a CO_2 atmosphere. The following tissues were collected from rats and mice: liver, kidney, stomach, duodenum, jejunum, ileum, large intestine, brain, pituitary, lung, heart, spleen, thymus, muscle, blood vessel, prostate, testes, uterus, ovary, and placenta (pituitary and blood vessel from rats only). Placentas were removed from pregnant rats and mice on gestation day 18 and 17, respectively. The intestine was longitudinally dissected and rinsed in saline, and intestinal epithelial cells were mechanically scraped from the intestine of rats, whereas the entire section of each mouse intestine was collected, snap-frozen in liquid nitrogen, and stored at −80 °C.

Gonadectomy and HX in rats. Rats were gonadectomized at 25 days of age or HX at 30 days of age by Charles River Laboratories. Hypophysectomized rats received 5% glucose water (w/v) ad libitum. Hypophysectomized rats that gained more than 10 g per week before the start of the study were excluded under the assumption that their surgery was incomplete. Gonadectomized rats were terminated at 62 days of age along with age-matched intact controls ($n = 5$/gender). Hypophysectomized rats were terminated at 45–47 days of age, also with age-matched intact controls. After termination, kidneys were snap-frozen in liquid nitrogen and stored at −80 °C.

Replacement treatments with gonadectomized mice. Mice were castrated or ovariectomized at 37 days of age by Charles River Laboratories. At 54 days of age, vehicle, 5α-dihydroxytestosterone (5 mg) or 17β-estradiol (0.5 mg) in 21-day time-release pellets (Innovative Research of America, Sarasota, FL) was subcutaneously implanted in the gonadectomized male and female mice. The mice were separated into six treatment groups ($n = 5$/gender/treatment): (1) castration + vehicle, (2) castration + 5α-dihydroxytestosterone, (3) castration + 17β-estradiol, (4) ovariectomy + vehicle, (5) ovariectomy + 5α-dihydroxytestosterone, and (6) ovariectomy + 17β-estradiol. Intact, untreated, age-matched mice were used as controls. Livers were removed at 64 days of age from gonadectomized and age-matched intact control mice.

RNA isolation. Total RNA was isolated using RNAzol B reagent (Tel Test, Friendswood, TX) according to the manufacturer's protocol. The concentration of total RNA in each sample was quantified spectrophotometrically at 260 nm. The integrity of each RNA sample was evaluated by formaldehyde–agarose gel electrophoresis before analysis.

Branched DNA signal amplification assay. Rat and mouse Bcrp mRNA was measured using the branched signal amplification assay (QuantiGene, High Volume bDNA Signal Amplification Kit; Bayer, Diagnostics Div., Tarrytown, NY) with modifications [24]. Multiple oligonucleotide probe sets (containing capture, label, and blocker probes) specific to rat and mouse Bcrp mRNA transcripts were designed using ProbeDesigner software v1.0 (Bayer, Diagnostics Div.) (Table 1). Probes were designed with an annealing temperature of approximately 63 °C, which enabled the hybridization conditions to be held constant (i.e., 53 °C) during each hybridization step, and for each probe set. Every probe developed in ProbeDesigner was submitted to the National Center for Biotechnological Information for nucleotide comparison by the basic logarithmic alignment search tool (BLASTn), to ensure minimal cross-reactivity with other known rat and mouse sequences, and expressed sequence tags. Oligonucleotides with a high degree of similarity ($\geqslant 80\%$) to other rat and mouse gene transcripts were excluded from the design. Total RNA (1 μg/μl; 10 μl/well) was added to each well of a 96-well plate containing capture hybridization buffer and 50 μl of each diluted probe set. For each gene, total RNA was allowed to hybridize to the probe set overnight at 53 °C. Subsequent hybridization steps were carried out as per the manufacturer's protocol, and luminescence was measured with a Quantiplex 320 bDNA Luminometer interfaced with Quantiplex Data Management Software Version 5.02 for analysis of luminescence from 96-well plates. The luminescence for each well was reported as relative light units (RLU) per 10 μg total RNA.

Statistical analysis. Gender differences in tissue distribution of Bcrp in mice and rats as well as the effect of gonadectomy and HX in rats were determined using Student's t test with significance set at $p \leqslant 0.05$. Replacement treatments of sex hormones to gonadectomized mice were analyzed by analysis of variance, followed by a Duncan's multiple range post hoc test. Significance was set at $p \leqslant 0.05$. Bars represent means ± SEM.

Results

Tissue distribution of rat and mouse Bcrp

To determine the tissue distribution and gender differences of Bcrp in rats, total RNA from five male and

Table 1
Oligonucleotide probes generated for analysis of Bcrp expression by bDNA signal amplification

Gene	GenBank ID	Target[a]	Function[b]	Probe sequence
Rat Bcrp	AB105817	944–963	CE	gccgaagaatctccgttgatTTTTTctcttggaaagaaagt
	AB105817	1012–1034	CE	gcttctctctcttggaaggctctTTTTTctcttggaaagaaagt
	AB105817	1138–1160	CE	atacaggctctctgaaggctgatTTTTTctcttggaaagaaagt
	AB105817	1204–1225	CE	cccgagcaggtttttaaatgaaTTTTTctcttggaaagaaagt
	AB105817	964–987	LE	tgttcccctctgtttaacattacaTTTTTaggcataggacccgtgtct
	AB105817	1035–1060	LE	aaactcggctaaattttctattattgTTTTTaggcataggacccgtgtct
	AB105817	1061–1086	LE	tctccatagatggtggagttgatataTTTTTaggcataggacccgtgtct
	AB105817	1113–1137	LE	gatcctttctttttctgagctactgTTTTTaggcataggacccgtgtct
	AB105817	1161–1184	LE	gctgatgacagaacgaggtaacatTTTTTaggcataggacccgtgtct
	AB105817	1185–1203	LE	cgcctggcaatccatctgaTTTTTaggcataggacccgtgtct
	AB105817	1250–1276	LE	cagtcccagtatgactgtaacaattaaTTTTTaggcataggacccgtgtct
	AB105817	988–1011	BL	Tctgtcttgtttgcttcatggtct
	AB105817	1087–1112	BL	Gaagttgatctaattcagcttttgtt
	AB105817	1226–1249	BL	Ctgagctacagaagcttgaggatt
Mouse Bcrp	NM_011920	218–238	CE	gccaggtttcatgatcccattTTTTTctcttggaaagaaagt
	NM_011920	261–279	CE	gaagacttgcctccgcctgTTTTTctcttggaaagaaagt
	NM_011920	304–327	CE	ccagataatccctttggatctttcTTTTTctcttggaaagaaagt
	NM_011920	491–516	CE	tctttaatgattgtgttaatccgttcTTTTTctcttggaaagaaagt
	NM_011920	188–217	LE	gatatctgatagtatttctttctcaactgtTTTTTaggcataggacccgtgtct
	NM_011920	239–260	LE	tgggtcccagaatagcattaagTTTTTaggcataggacccgtgtct
	NM_011920	328–351	LE	ggtgctccatttatcaaaacatctTTTTTaggcataggacccgtgtct
	NM_011920	464–490	LE	attttttttcatgattcttcatagttgtTTTTTaggcataggacccgtgtct
	NM_011920	517–542	LE	aatctgctacttttttccagacctaacTTTTTaggcataggacccgtgtct
	NM_011920	543–566	LE	ggataaactgagttccgaccttagTTTTTaggcataggacccgtgtct
	NM_011920	280–303	BL	cttgctgctaagacatctagcaac
	NM_011920	352–372	BL	catttgaaatgggcaggttga
	NM_011920	373–396	BL	tcttgaaccacataacctgaacag
	NM_011920	397–415	BL	ggtgcccatcacaacgtca
	NM_011920	416–442	BL	gaactgtaagttttctctcactgtcag
	NM_011920	443–463	BL	tggaagtcgaagagctgctga

[a] Target refers to the sequence of the mRNA transcript as enumerated in the GenBank file.
[b] Function refers to the utility of the oligonucleotide probe in the bDNA assay (CE, capture extender; LE, label extender; and BL, blocker probe).

five female rats was individually isolated and quantified. Rat Bcrp mRNA levels (Fig. 1) were high in male kidney, small intestine, and large intestine. Moderate expression level of rat bcrp mRNA was observed in testes. Lower levels of rat Bcrp mRNA were observed in other tissues, including placenta, liver, and brain. Bcrp mRNA levels in kidney were much higher in males than in females, whereas expression in female brain was higher than in male brain.

To investigate the tissue distribution and gender differences of Bcrp in mice, total RNA was isolated from the tissues of five male and female mice, and Bcrp mRNA was determined. Mouse Bcrp mRNA levels (Fig. 2) were highest in male and female kidney; moderate in male liver, male and female ileum, and testes; and lower in other tissues, including placenta, ovary, and female liver. Bcrp mRNA levels in liver were significantly higher in males than in females.

Bcrp expression in kidneys of gonadectomized and hypophysectomized rats

To determine the mechanism for the gender difference in Bcrp mRNA levels in rat kidney, the effect of gonadectomy on Bcrp expression was determined. Consistent with the tissue distribution study (Fig. 1), Bcrp mRNA expression in control male kidneys was higher than in control female kidneys (Fig. 3). Castration had no effect on kidney Bcrp mRNA levels, however, ovariectomy increased Bcrp mRNA levels.

In addition to sex steroids, the influence of GH on Bcrp expression was determined by using hypophysectomized rats. As observed previously (Figs. 1 and 3), Bcrp mRNA levels were higher in male kidneys than in females (Fig. 4). Kidney levels of Bcrp mRNA were not significantly altered by HX in either male or female rats.

Effect of sex hormones to gonadectomized mice on liver Bcrp expression

Similarly, the effect of sex steroids on Bcrp mRNA levels in mouse liver was investigated by comparing intact, gonadectomized, and gonadectomized mice that received sex hormone replacement. Bcrp mRNA levels were higher in livers of male mice than those of female mice (Fig. 5). Castration resulted in a decrease in Bcrp mRNA to a level similar to that detected in female liver. 5α-Dihydroxytestosterone replacement increased Bcrp

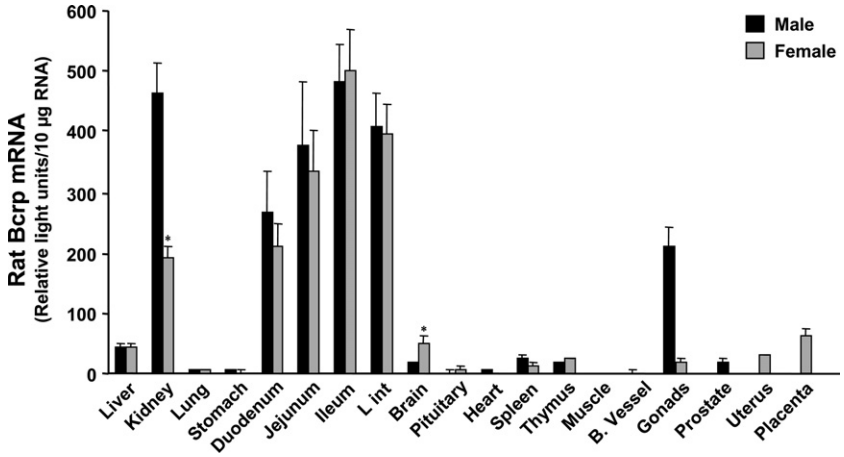

Fig. 1. Tissue distribution of rat Bcrp mRNA. Tissue total RNA was isolated from both male and female rats, and analyzed by the bDNA signal amplification assay for Bcrp mRNA content. The data are presented as mean relative light units (RLU) ± SEM ($n = 5$ animals). Asterisks (*) represent statistically significant differences ($p \leqslant 0.05$) between males and females.

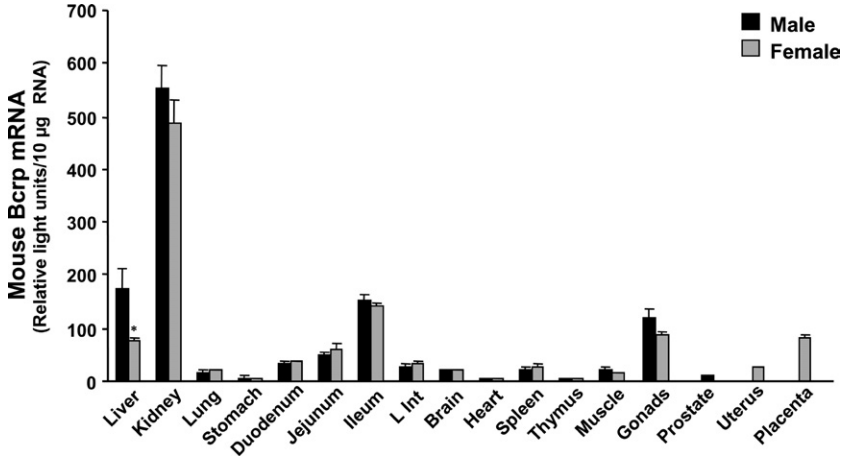

Fig. 2. Tissue distribution of mouse Bcrp mRNA. Tissue total RNA was isolated from both male and female mice, and analyzed by the bDNA signal amplification assay for Bcrp mRNA content. The data are presented as mean RLU ± SEM ($n = 5$ animals). Asterisks (*) represent statistically significant differences ($p \leqslant 0.05$) between males and females.

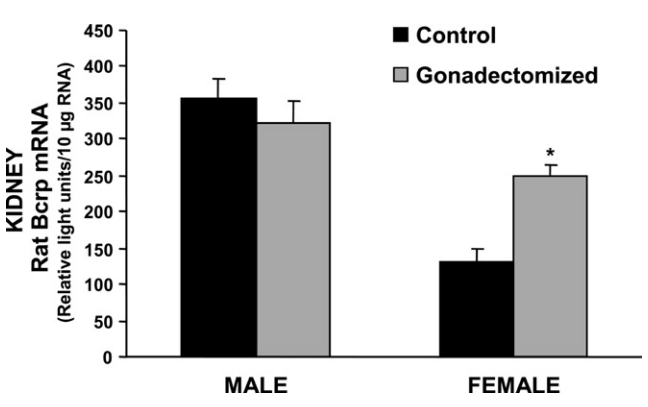

Fig. 3. Rat Bcrp expression in kidney tissue from intact and gonadectomized male and female rats. Total kidney RNA was isolated and analyzed by the bDNA signal amplification assay for Bcrp mRNA content. The data are presented as mean RLU ± SEM ($n = 5$ animals). Asterisks (*) represent statistically significant differences ($p \leqslant 0.05$) between intact and gonadectomized rats.

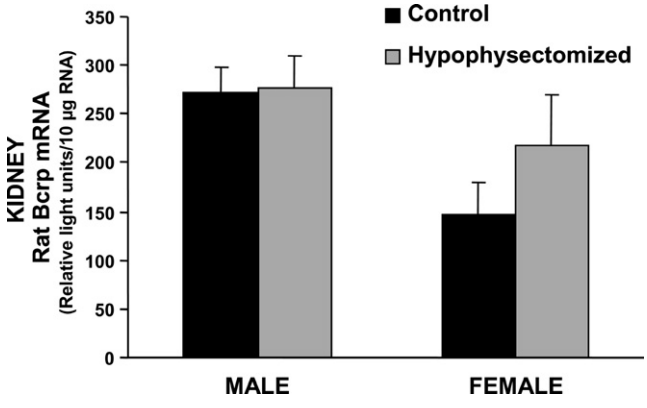

Fig. 4. Rat Bcrp expression in kidney tissue from intact and hypophysectomized male and female rats. Total kidney RNA was isolated and analyzed by the bDNA signal amplification assay for Bcrp mRNA content. The data are presented as mean RLU ± SEM ($n = 5$ animals). Asterisks (*) represent statistically significant differences ($p \leqslant 0.05$) between intact and hypophysectomized rats.

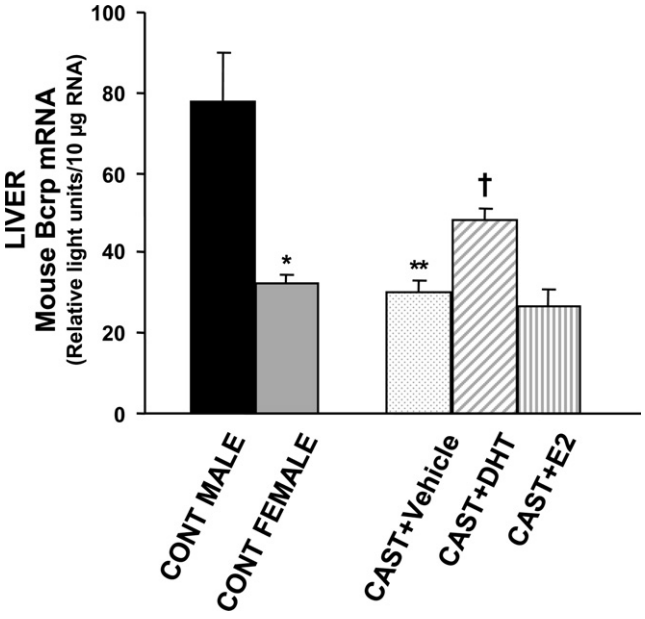

Fig. 5. Effects of castration and sex hormone treatments on mouse Bcrp mRNA in liver. Total liver RNA was isolated and analyzed by the bDNA signal amplification assay for Bcrp mRNA content. The data are presented as mean RLU ± SEM (each group, $n = 5$ animals). CAST + Vehicle, vehicle administered to castrated mice; CAST + DHT, 5α-dihydroxytestosterone administered to castrated mice; and CAST + E2, 17β-estradiol administered to castrated mice. Asterisks (*) represent statistically significant differences ($p \leqslant 0.05$) between intact males and intact females; double asterisks (**) represent statistically significant differences ($p \leqslant 0.05$) between intact males and untreated castrated males; and single dagger (†) represents statistically significant differences ($p \leqslant 0.05$) between untreated castrated males and castrated males administered 5α-dihydroxytestosterone.

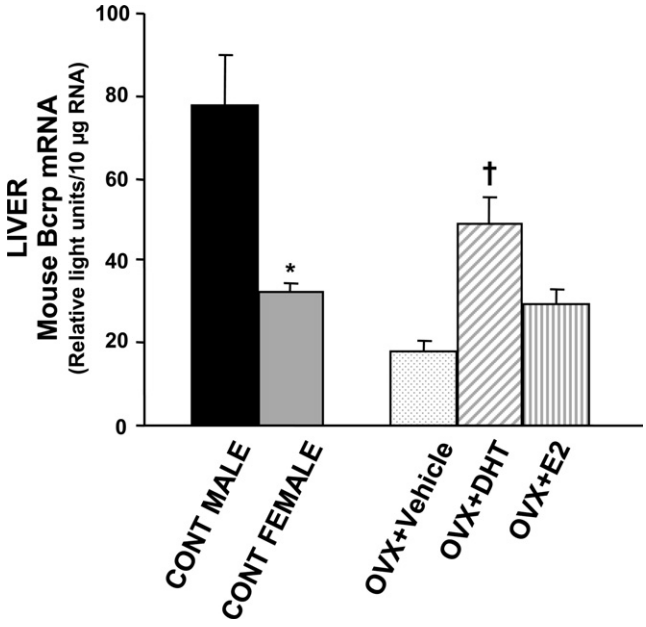

Fig. 6. Effects of ovariectomy and sex hormones treatments on mouse Bcrp mRNA in liver. Total liver RNA was isolated and analyzed by the bDNA signal amplification assay for Bcrp mRNA content. The data are presented as mean RLU ± SEM (each group, $n = 5$ animals). OVX + Vehicle, vehicle administered to ovariectomized mice; OVX + DHT, 5α-dihydroxytestosterone administered to ovariectomized mice; and OVX + E2, 17β-estradiol administered to ovariectomized mice. Asterisks (*) represent statistically significant differences ($p \leqslant 0.05$) between intact males and intact females; single dagger (†) represents statistically significant differences ($p \leqslant 0.05$) between untreated ovariectomized females and ovariectomized females administered 5α-dihydroxytestosterone.

mRNA levels in castrated mice. However, 17β-estradiol replacement did not alter Bcrp mRNA levels in castrated mice.

Ovariectomy did not significantly alter the level of Bcrp mRNA in female livers (Fig. 6). Administration of 5α-dihydroxytestosterone increased Bcrp mRNA levels of the ovariectomized mice, however, administration of 17β-estradiol did not.

Discussion

Bcrp has been cloned from human, mouse, and rat [1,4,25,26]. Studies have examined the tissue distribution of BCRP in human tissues [1,9]. Only one study on the tissue expression of Bcrp in mice has been reported [10], and the tissue distribution of Bcrp in rats has not been evaluated. To determine the expression pattern of rat and mouse Bcrp at the mRNA level, approximately 20 different tissues from male and female rats and mice were assessed using the bDNA signal amplification assay. The bDNA assay is a highly sensitive method that detects gene-specific transcripts in a quantitative manner. This assay has been described in detail for the detection of several cytochrome P450 isoforms [24].

The present study demonstrates that Bcrp mRNA in rats is predominantly expressed in male kidney and intestine, with moderate expression in testes, and low expression in the other tissues. Mouse Bcrp mRNA is also high in kidney, with moderate expression in male liver, ileum, and testes, and lower expression in the other tissues. The present study on the tissue distribution of Bcrp in mice agrees with a previous report that detected the highest Bcrp mRNA expression in kidney and moderate expression in liver [10]. Although high expression of BCRP mRNA was found in human placenta [1,9], the present study indicates that Bcrp mRNA levels in both rat and mouse placenta are low.

One study on the subcellular localization of BCRP in normal human tissues has reported that BCRP is localized to the apical membrane of the hepatocyte, the epithelium of the small intestine and colon, and the placental syncytiotrophoblasts [9]. Taken together, the presence of BCRP in human placenta suggests that BCRP may protect the fetus from xenobiotics [10], but the low expression in rat and mouse placenta suggests it is less important in these species for this proposed

function. Conversely, the expression of Bcrp in rat and mouse kidney, and small intestine, especially ileum, is relatively high, but is low in these tissues of humans [1,9]. The high level of Bcrp mRNA in kidney suggests that Bcrp may play an important pharmacologic role in the renal excretion of drugs in rodents. It has been reported that Bcrp-null mice are extremely sensitive to the dietary chlorophyll-breakdown product pheophorbide A, and exposure results in severe phototoxicity [27]. This suggests that intestinal Bcrp may limit the uptake of the pheophorbide A. Bcrp expression is relatively high in ileum of rats and mice, and may be involved in the excretion of numerous xenobiotics, including topotecan, into the lumen of the gastrointestinal tract [10]. Interestingly, there is a species difference in the expression of Bcrp in large intestine of rats and mice. Bcrp expression is high in large intestine of rats, but much lower in mice.

Several studies have reported gender differences in the expression of xenobiotic transporters in laboratory animals [11–19]. The present study indicates that in rats there are gender differences in the expression of Bcrp in kidney, and in mice expression of Bcrp in liver is also gender-dependent. In rats, higher Bcrp expression was observed in the kidney of males than females, and in mice, higher expression of Bcrp was found in liver of males than females.

The sex hormones, testosterone and estradiol, appear to be involved in gender-specific expression of transporters [11,13,20,21]. The secretion patterns of GH have also been considered a regulator of gender-related differences. Secretion patterns of GH are regulated by the pituitary gland. Although the removal of the pituitary eliminates other hormones, HX provides a method of GH removal from the physiology of an in vivo system. Therefore in the present study, experiments were performed to determine whether the gender differences in expression of Bcrp in rats and mice are under control of sex steroids and/or GH [13,22].

Castration had no effect on Bcrp mRNA levels in kidneys of rats. However, renal Bcrp levels in ovariectomized female rats were higher than those present in kidneys from control females. In contrast, HX had no remarkable effect on either male or female rat kidney levels of Bcrp mRNA. These data suggest that male-predominant expression of Bcrp in rat kidney is due to the suppressive effect of female steroid sex hormones, and that this gender difference in rat kidneys is not due to gender-specific secretion patterns of GH by the pituitary gland. Furthermore, these findings agree with a previous study that estrone and 17β-estradiol reverse BCRP-mediated multidrug resistance by the inhibition of the drug efflux in BCRP-transduced K562 cells [28]. Although it is unclear why rat Bcrp is regulated by estrogens in the kidney, one might speculate that Bcrp expression may be important in modulating the renal tubular secretion of endogenous substrates of Bcrp, such as sulfated estrogens [29,30], estradiol [31] or 17β-estradiol-17β-D-glucuronide [32].

Mouse Bcrp expression is male-predominant in liver. Thererfore, studies were performed to determine whether sex steroids mediate this gender difference. To determine the effects of sex hormones on mouse Bcrp expression in liver, 5α-dihydroxytestosterone and 17β-estradiol were replaced in gonadectomized mice, allowing subsequent comparisons between untreated and treated gonadectomized mice, as well as to intact controls. After castration, Bcrp mRNA levels, in male mouse livers, decreased to a similar level to that in female livers. Furthermore, replacement of 5α-dihydroxytestosterone to castrated males and ovariectomized females increased Bcrp mRNA levels. However, ovariectomy only slightly decreased the level of Bcrp mRNA in female livers, and replacement of 17β-estradiol to castrated and ovariectomized mice did not remarkably change Bcrp mRNA levels. These data suggest that male-predominant expression of Bcrp in mouse liver appears to be regulated by the inductive effect of testosterone. Similar to rat renal Bcrp expression, this gender difference in hepatic mouse Bcrp expression may play a role in regulating the excretion of endogenous substrates into bile for maintaining hormonal balance.

In conclusion, similarities exist between mice and rats in the tissue expression of Bcrp mRNA, with high expression in the kidney of both species. However, species differences were observed in the tissue distribution of Bcrp, in that rats express Bcrp at higher levels in intestine than do mice. Gender differences in the expression of Bcrp were observed in both species. Male-predominant expression of Bcrp in rat kidney seems to be due to the suppressive effect of estradiol, whereas male-predominant expression of Bcrp in mouse liver appears to be due to the inductive effect of testosterone. Furthermore, these data suggest that gender differences of Bcrp in rat kidney and mouse liver may play a role in regulating hepatic and renal excretion of endogenous substrates for keeping hormonal balance.

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