Hormonal Regulation of Hepatic Drug Biotransformation and Transport Systems

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ABSTRACT

The human body is constantly exposed to many xenobiotics including environmental pollutants, food additives, therapeutic drugs, etc. The liver is considered the primary site for drug metabolism and elimination pathways, consisting in uptake, phase I and II reactions, and efflux processes, usually acting in this same order. Modulation of biotransformation and disposition of drugs of clinical application has important therapeutic and toxicological implications. We here provide a compilation and analysis of relevant, more recent literature reporting hormonal regulation of hepatic drug biotransformation and transport systems. We provide additional information on the effect of hormones that tentatively explain differences between sexes. A brief discussion on discrepancies between experimental models and species, as well as a link between gender-related differences and the hormonal mechanism explaining such differences, is also presented. Finally, we include a comment on the pathophysiological, toxicological, and pharmacological relevance of these regulations. © 2013 American Physiological Society. *Compr Physiol* 3:1721-1740, 2013.

Introduction

We are daily exposed to a wide variety of xenobiotics including environmental pollutants, food additives, therapeutic drugs, etc. The major routes of exposure to these chemicals are inhalation, dermal absorption, and absorption through the gastrointestinal tract. After their incorporation, xenobiotics are distributed through various compartments in the body according to a number of physicochemical characteristics. Lipid-soluble chemicals readily cross biological membranes and distribute to fluid compartments and particularly to highly perfused tissues, such as the liver, readily entering the parenchymal cells across the sinusoidal membrane. To facilitate their elimination, cellular enzyme systems catalyze the process of biotransformation, producing metabolites that are generally more water-soluble and probably more ionized at physiological pH. The liver is considered the primary site for drug metabolism in the body, which has long been classified into phase I and phase II reactions. Phase I metabolism, consisting of oxidation, reduction, or hydrolysis reactions, introduces minor changes in drug structure or solubility but adds or exposes sites where phase II metabolism can subsequently occur. In contrast, phase II conjugation typically results in a more appreciable change in chemical structure, molecular weight, and water solubility. Because of their increased solubility, metabolized drugs cannot easily diffuse across plasma membranes and are mostly transported into either bile or sinusoidal blood by carrier mediated processes, usually requiring ATP consumption. Figure 1 depicts schematically the complete sequence of uptake, phase I and II reactions, and efflux processes, taking place in hepatocytes.

Because of the implication of these systems in biotransformation and disposition of drugs of clinical application, their modulation has important therapeutic and toxicological implications. According to the FDA, adverse effects of drugs are more common and severe in women than in men (206). Gender-related differences in drug effectiveness and adverse effects are thought to result from differences in their clearance by major metabolizing organs. One of the likely reasons for these differences is the gender-specific differences in the rate of biotransformation and transport of drugs, for example, by the liver. Hormonal regulation of drug metabolism may, in turn, contribute to gender differences. Gender differences in drug receptors may also be of relevance to explain such differences in response. We here provide a compilation and analysis of relevant, more recent literature reporting hormonal regulation of hepatic drug biotransformation and transport systems, with emphasis on hormones explaining differences between sexes. A brief discussion on discrepancies between experimental models and species, as well as a link between gender-related differences and the hormonal mechanism explaining such differences, is also presented. Finally,

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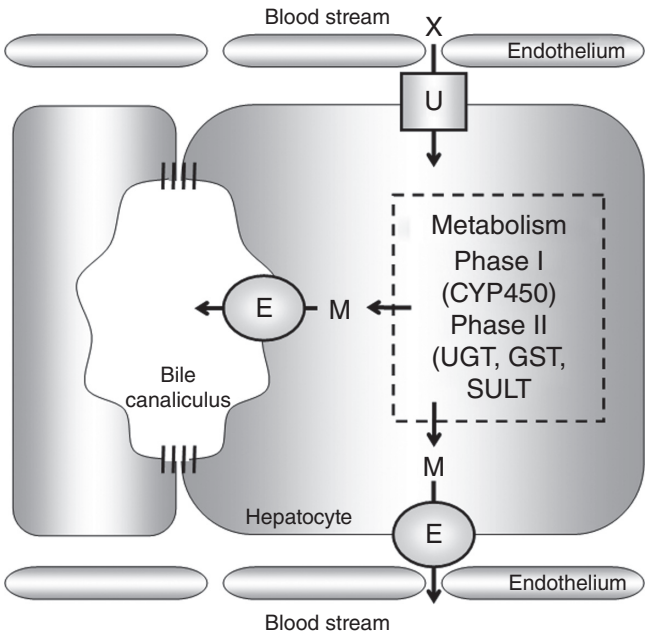

Figure 1 Processing of endo- and xenobiotics in the hepatocytes. X: Endo- or xenobiotic. U: Uptake transporters such as NTCP, OATs, or OATPs. After uptake from the bloodstream, endo- and xenobiotics are metabolized by Phase I (CYP450 family) and/or Phase II (UGT, GST, and/or SULT). M: final metabolite. Metabolites are then eliminated from the cells through secretion into bile canaliculi or into sinusoids by efflux transporters (E) such as MRP2, BSEP, BCRP, P-GP, and ABCG5/8 (canalicular) or MRP3-4-5-6 (basolateral).

we include a comment on the pathophysiological, toxicological, and pharmacological relevance of these regulations.

Liver and Drug Disposition

The liver plays an important role in the uptake, metabolism, and distribution of many endo- and xenobiotics. Hepatocytes are the parenchymal cells of the liver responsible for the biotransformation and transport of these compounds. They are organized into plates that anastomose with one another, separated by vascular channels or sinusoids. This structure is important in directing the excretion of the products of biotransformation out of the hepatocytes into bile or blood. The biotransfomation systems are classified as phase I, phase II, and efflux transport systems. The cytochrome P450 (CYP) system belongs to phase I reactions, while the phase II enzymes are characterized by their ability to conjugate endo- or exogenous molecules using endogenous cofactors. Numerous transporters are responsible for the movement of endo- and xenobiotics across the cell membrane, thus affecting their intracellular concentration and disposition into urine or bile.

Biotransformation Reactions

Cytochrome P450-associated reactions

The CYPs are a superfamily of heme proteins located primarily in the endoplasmic reticulum, involved in oxidative metabolism of endogenous compounds (such as steroid hormones and bile acids) and xenobiotics, including therapeutic agents. The enzymes implicated in xenobiotic metabolism are highly expressed in liver, although lower levels of selected forms are also found in extrahepatic tissues. They are named with the root symbol CYP followed by an Arabic numeral designating a family number, a letter for the subfamily, and a last Arabic numeral indicating the specific CYP isoform. Percentages of amino acid sequence similarities define families and subfamilies. Oxidation mediated by CYPs is the major route of elimination of clinically administered drugs, thus governing their plasma clearance (68, 69). The most representative substrates for CYPs are presented in Table 1, in addition to their function and subcellular localization.

Conjugation reactions

Following phase I-mediated transformation, compounds can undergo Phase II or conjugation reactions. These are biosynthetic reactions in which the xenobiotic is linked to an endogenous group to give a product known as the conjugate. In certain cases, the products of Phase I can be eliminated without subsequent conjugation. However, xenobiotics are generally excreted in a more water soluble, conjugated form (23). Glucuronidation and conjugation with glutathione (GSH) or sulfate are quantitatively the most representative reactions of Phase II (95).

Glucuronidation is catalyzed by a family of UDP-glucuronosyltransferases (UGTs) located in the endoplasmic reticulum. Based on a cDNA sequence comparison, mammalian UGTs are divided into four genes families, named *UGT1*, *UGT2* (divided into subfamilies: *2A* and *2B*), *UGT3, and UGT8* (142). The UGT1 family comprises several isoenzymes with different amino-terminal domains (substrate binding domain) but identical carboxyl-terminal domain (cosubstrate binding domain). These isoenzymes result from alternative splicing of transcripts derived from a *UGT1* gene complex (91). *UGT2A1* and *UGT2A2* genes also share exon 6. However, *UGT2A3*, *UGT2B*, *UGT3,* and *UGT8* gene families do not share exons and result from a process of duplication of all exons in the gene. According to the nomenclature, Arabic numerals correspond to the family (e.g., UGT1), a letter represents the subfamily (e.g., UGT1A), and a second Arabic numeral designates the individual isoform (e.g., UGT1A1) (142).

Conjugation of compounds having electrophilic groups with the tripeptide GSH is catalyzed by GSH S-transferases (GSTs). Many other activities are now associated with GSTs, such as prostaglandin and leukotriene biosynthesis, Michael addition, peroxide degradation, ligand binding, and intracellular transport (170). Mammalian GSTs comprise different families, namely cytosolic, mitochondrial, and microsomal GST (now designated MAPEG, membrane-associated proteins in eicosanoid and GSH metabolism) (79). Based on amino acid sequence similarities, seven classes of cytosolic GSTs are recognized (alpha, mu, pi, sigma, theta, omega, and

Table 1 Function, Specificity, and Subcellular Localization of Major Hepatic Phase I and Phase II Biotransformation Systems

Enzyme	Function	Subcellular localization	Substrates	References
CYP1A2	Oxidation	Endoplasmic reticulum	Caffeine, estradiol, lidocaine, tacrine, theophylline, verapamil, (R)-warfarin	(220)
CYP2A6	Oxidation	Endoplasmic reticulum	Nicotine, coumarin, valproic acid, 4-methylnitrosoamino-1-(3-pyridyl)-1-butanone, N-nitrosodiethylamine	(167), (88) (178) (238)
CYP2B1	Hydroxylation	Endoplasmic reticulum	Doxorubicin, androstenedione, hexobarbital	(4,66)
CYP2C9	Oxidation	Endoplasmic reticulum	Glyburide, celecoxib, losartan, diclofenac, phenytoin, piroxicam, (S)-warfarin tetrahydrocannabinol, tolbutamide	(220) (243)
CYP2C11	Oxidation, N-hydroxylation	Endoplasmic reticulum	Desipramine, thalidomide	(8) (78)
CYP2C12	Oxidation	Endoplasmic reticulum and mitochondria	Ethoxyresorufin, steroid sulfates in the position 15-β	(6) (227) (161)
CYP2C19	N-hydroxylation, oxidation	Endoplasmic reticulum	Diazepam, hexobarbital, S-mephenytoin, omeprazole, pentamidine, propranolol, (R)-warfarin, (S)-fluoxetine, thalidomide	(220) (8) (78)
CYP2D6	Oxidation	Endoplasmic reticulum and mitochondria	Codeine, debrisoquine, dextromethorphan, haloperidol, metoprolol, paroxetine, phenothiazines, propanolol, risperidone, sertraline, tricyclic antidepressants	(6) (220)
CYP2E1	8-hydroxylation, oxidation	Endoplasmic reticulum and mitochondria	Acetaminophen, volatile anaesthetics (enflurane, isoflurane, halothane), ethanol, industrial solvents and chemicals (carbon tetrachloride, vinyl chloride, nitrosamines), ketone bodies, glycerol, and different fatty acids	(6) (196) (111)
CYP3A4	8-hydroxylation, oxidation	Endoplasmic reticulum	Amiodarone, clarithromycin, cyclosporine, erythromycin, lovastatin, nifedipine, tamoxifen, terfenadine, verapamil, (R)-warfarin N-desmethyldiltiazem	(196) (220) (78)
UGT1A1	Glucuronidation	Endoplasmic reticulum	Bilirubin, ethynylestradiol (position 3-OH), acetaminophen	(14) (33) (234) (50) (143)
UGT1A5	Glucuronidation	Endoplasmic reticulum	Bilirubin	(221)
UGT1A6	Glucuronidation	Endoplasmic reticulum	Phenols (e.g., acetaminophen)	(221)
UGT2B1	Glucuronidation	Endoplasmic reticulum	Testosterone, ethynylestradiol (position 17-β)	(209) (50) (143)
UGT2B3	Glucuronidation	Endoplasmic reticulum	Testosterone, estradiol (position 17-β)	(209) (50) (143)
UGT2B15	Glucuronidation	Endoplasmic reticulum	(S)-oxazepam and lorazepam, plant derived phenols, anthroquinones, flavonoids	(130)
UGT2B17	Glucuronidation	Endoplasmic reticulum	C19 steroids (dihydrotestosterone, androsterone), androstane-3-alpha	(10)
GSTA	Glutathione conjugation	Cytosol	Leukotriene A4, prostaglandin H2, 1-chloro-2,4-dinitrobenzene, styrene oxide	(80)
GSTM	Glutathione conjugation	Cytosol	Leukotriene A4, prostaglandin H2, 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene	(80)
GSTP	Glutathione conjugation	Cytosol	Leukotriene A4, prostaglandin H2, 1-chloro-2,4-dinitrobenzene, ethacrynic acid	(80)
SULT1A1	Sulfate conjugation	Cytosol	Simple phenols (p-nitrophenol), minoxidil, acetaminophen	(54) (231) (59)
SULT1C1	Sulfate conjugation	Cytosol	N-hydroxy-2-acetylaminofluorene	(81)
SULT1E1	Sulfate conjugation	Cytosol	Estrogens (estradiol, estrone)	(54) (231) (59)
SULT2A1	Sulfate conjugation	Cytosol	Dehydroepiandrosterone sulfate, hydroxymethylpyrene, raloxifene, bile acids	(54) (231) (59)
SULT3A1	Sulfate conjugation	Cytosol	1-Naphthylamine, 1-Naphthol	(214)

zeta). Each enzyme is a dimer of individual subunits designated with a letter and Arabic numerals (e.g., A1; A2, etc. for alpha class) (80).

Cytosolic sulfotransferases (SULTs) are a superfamily of enzymes that catalyze the conjugation of sulfate donated by 3′-phosphoadenosine 5′-phosphosulphate (PAPS) with xenobiotics or endogenous compounds (hormones and neurotransmitters). To date, four human SULT families have been identified: **SULT1,** which is divided into 4 subfamilies, 1A1-4, 1B1, 1C1-3, and 1E1 (equivalent to rat 1E2); **SULT2,** which is divided into 2 subfamilies, 2A and 2B; and **SULT4** and **SULT6** (134). **SULT3** family has only been found in mouse and rabbit (59).

Table 1 presents the most common substrates for each of these conjugation enzymes, in addition to their function and subcellular localization.

Transport Systems

Basolateral transporters

Among the proteins localized to the basolateral membrane of the hepatocyte are found transporters that are responsible for the uptake of several compounds from blood as well as transporters that function as export pumps.

The basolateral uptake transporters can be classified as Na^+-dependent and Na^+-independent (175). Na^+-taurocholate cotransporting polypeptide (rodents Ntcp, Solute carrier family: Slc10a1; human NTCP, SLC10a1) is exclusively expressed in hepatocytes and is the major transporter responsible for the uptake of bile salts from plasma (76). The organic anion transporting polypeptides (rodents, Oatps; human, OATPs, and SLC gene family SLC21/SLCO) constitute an important superfamily of proteins that are responsible for facilitating the hepatocellular uptake of substrates from the portal circulation. A function of some of its members consists of exchanging anions with reduced GSH or bicarbonate (153). Moreover, some Oatps/OATPs, may mediate a bidirectional transport when the substrate reaches a sufficiently high intracellular concentration, functioning as an extrusion system when necessary (131). Although most Oatps/OATPs are expressed in a wide variety of tissues, including the brain, heart, intestine, kidney, lung, placenta, and testis, some Oatps/OATPs are selectively expressed in rodent and human liver (217). These liver-specific transporters are involved in the Na^+-independent hepatic transport of bile salts and nonbile salt organic anions from blood to bile. Included in the liver-specific subfamily of Oatps/OATPs are human OATP1B3 [previously named OATP-8 (SLC21A8)], and its rat and mouse ortholog Oatp1b2 [previously called Oatp-4 (Slc21a10)] (75). Oatp1b2 seems to be the most abundantly expressed organic anion transporting polypeptide in rat liver (132). A complete list of Oatp/OATP substrates is presented in Hagenbuch and Meier (74, 75) and Tirona and Kim (222). Among the Oatps expressed in rodent liver are Oatp1 (Oatp1a1, Slc21a1), Oatp2 (Oatp1a4, Slc21a5), and Oatp4 (Oatp1b2, Slc21a10), involved in the uptake of a wide variety of structurally unrelated amphipathic organic compounds from sinusoidal blood and representing multispecific transport systems with distinct but partially overlapping transport specificities (62). Organic Anion Transporters (OATs) are members of the Solute Carrier Family 22 (SLC22) (93, 114). Their primary localization is in kidney, but selected OATs are also present in liver, where OAT2/SLC22A7 is the predominant form, and OAT3/SLC22A8 is expressed to a lesser extent (21). Similar to other organic anion transporters, OATs have broad substrate specificity and the ability to exchange extracellular for intracellular organic anions.

The basolateral eflux transporters belong to the ATP-binding cassette (ABC) superfamily, exhibit similar structural and functional characteristics (105, 116) and comprise Multidrug resistance-asociated proteins 3, 4, 5, and 6 (Mrp3/MRP3, Abcc3/ABCC3; Mrp4/MRP4, Abcc4/ABCC4; Mrp5/MRP5, Abcc5/ABCC5; and Mrp6/MRP6, Abcc6/ABCC6). These transporters act coordinately with Phase II conjugating enzymes to increase the excretion of a wide range of modified drugs and other conjugated metabolites from cells (106, 116, 182).

Aquaporin-9 (AQP9) is a broad-selectivity neutral solute channel that facilitates the hepatic uptake of glycerol (126). In the liver, AQP9 protein expression is predominantly confined to the basolateral plasma membrane domain in perivenous hepatocytes (126).

Table 2 shows relevant substrates of major hepatic basolateral transporters, as well as their function.

Canalicular transporters

Transporters localized to the canalicular/apical membrane of hepatocytes participate in bile formation. Bile is formed by an osmotic process driven by active secretion into the bile canaliculus of solutes across the hepatocyte apical membrane, followed by passive in-flow of water and electrolytes (152). The secretion of different compounds across the canalicular membrane is also essential for the elimination of endo- and xenobiotics from the body, including drugs. The key proteins which are involved in this process are members of the ABC superfamily and function as ATP-dependent unidirectional export pumps (13). Among these transporters are: the bile salt export pump (Bsep/BSEP, Abcb11/ABCB11) which mediates the efflux of bile salts (122), the Multidrug resistance-associated protein 2 (Mrp2/MRP2, Abcc2/ABCC2) with substrates including numerous anions conjugated with GSH, sulfate, and glucuronic acid, as well as oxidized and reduced GSH (96), P-glycoprotein (P-gp/P-GP, Abcb1/ABCB1), which transports a wide range of xenobiotics (210), the breast cancer resistance protein (Bcrp/BCRP, Abcg2/ABCG2) preferentially recognizing sulfate conjugates as substrates (212), and Abcg5/8/ABCG5/8 that mediates the secretion of sterols (240).

In 2005, mammalian multidrug and toxin extrusion (MATE) was identified as an orthologue of the bacterial

Table 2 Function, Specificity, and Membrane Domain Localization of Major Hepatic Transporters

Transporter	Function	Subcellular localization	Substrates		References
			Endogenous	Exogenous	
NTCP (SLC10A1)	Uptake	Basolateral	Conjugated and unconjugated bile salts	Rosuvastatin, micafungin	(73) (84) (239)
OATP1 (OATP1A1)	Uptake	Basolateral	Estrone-3-sulfate dehydroepiandrosterone sulfate, estradiol-17 β glucuronide	Statins, antidiabetic drugs, Chemotherapeutic agents	(51) (16) (121) (67)
OATP2 (OATP1A4)	Uptake	Basolateral	estradiol-17 β glucuronide	Pitavastatin, dehydroepiandrosterone sulfate	(171)
OATP4 (OATP1B2)	Uptake	Basolateral	estradiol-17 β glucuronide dehydryepiandrosterone-3-sulfate	Pravastatin, rifampin bromosulfophthalein, microcystin, phalloidin	(237) (241)
OATP1B3 (SLC21A8)	Uptake	Basolateral	estradiol-17 β glucuronide, dehydroepiandrosterone	Rifampin, cefadroxil, methotrexate, bosentan, digoxin, rosuvastatin, paclitaxel and the gastrointestinal peptide hormone cholecystokinin, octapeptide, fexofenadine, hydrochloride, microcystin, and phalloidin	(102) (67) (199) (74) (75) (222)
OAT2 (SLC22A7)	Uptake	Basolateral	Nucleobases, nucleosides and nucleotides, L-ascorbate, ES, glutamate, orotate, prostaglandins E2 and F2α, urate	Bumetanide, pravastatin, benzylpenicillin, cimetidine, erythromycin, 2′,3′-dideoxycytidine, methotrexate, zidovudine	(211) (53) (113) (191) (55) (43)
OAT3 (SLC22A8)	Uptake	Basolateral	Second messengers cAMP and cGMP, cholate, taurocholate, cortisol, dehydroepiandrosterone, and estrone-3-sulfate and the prostaglandins E2 and F2α	OAT3 exhibits a preference for compounds with a higher number of hydrogen bond donors	(21)
MRP1 (ABCC1)	Efflux	Basolateral	Glutathione, estradiol-17 beta-glucuronide, bilirubin glucuronides, leukotriene C4	Methotrexate, doxorubicin, vincristine, chlorambucil, cyclophosphamide, flutamide, etoposide, aflatoxin B1	(205) (138)
MRP3 (ABCC3)	Efflux	Basolateral	Di and monovalent bile salts, conjugated bile acids, bilirubin glucuronides, estradiol-17 beta-glucuronide, leukotriene C4	Methotrexate, paracetamol	(205)
MRP4 (ABCC4)	Efflux	Basolateral	Conjugated bile acids; estradiol-17 beta-glucuronide, leukotrienes, cyclic nucleotides, prostanoides, bile salts	Zidovudine monophosphate, para-aminohippurate, antimetabolites	(205)
MRP5 (ABCC5)	Efflux	Basolateral	Cyclic nucleotides	Methotrexate, antimetabolites	(205)
MRP6 (ABCC6)	Efflux	Basolateral	Unidentified	Unidentified	(172) (117)
AQP9	Water channel	Basolateral	Water, glycerol	Unknown	(147)
BCRP (ABCG2)	Efflux	Canalicular	Estrone 3-sulfate, estradiol 3-sulfate	2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine, genistein, nitrofurantoin, cimetidine, topotecan	(212) (92) (183) (127) (155)
P-GP (ABCB1)	Efflux	Canalicular	Steroids, hydrophobic cationic compounds	Paclitaxel, doxorubicin, vincristine, antracyclines, epipodophyllotoxins, topotecan	(210) (195) (205)

(*Continued*)

Table 2 (*Continued*)

Transporter	Function	Subcellular localization	Substrates		References
			Endogenous	Exogenous	
MRP2 (ABCC2)	Efflux	Canalicular	Divalent bile salts, glutathione, glutathione conjugates, bilirubin mono and diglucuronides, estradiol-17 beta-glucuronide, leukotriene C4	Methotrexate, vinblastine, etoposide, vincristine, cisplatin, doxorubicin, chlorambucil, cyclophosphamide, arsenic-glutathione complexes	(96) (205)
BSEP (ABCB11)	Efflux	Canalicular	Taurine and glycine conjugates of cholic and chenodeoxycholic acids. deoxycholic and ursodeoxycholic acids and its conjugates	Vinblastine, taxol, calcein	(13) (44) (120)
AQP8	Water channel	Canalicular membrane and mitochondria	Water, ammonia, hydrogen peroxide	Unknown	(147)
ABCG5/8	Efflux	Canalicular	Sterols	Unknown	(240) (154) (109)
MATE1	Efflux	Canalicular	Estrone sulfate	Cephalexin, cephradine, tetraethylammonium, 1-methyl-4-phenylpyridinium, cimetidine, metformin, guanidine, procainamide, topotecan, acyclovir, ganciclovir	(163)

MATE family (173). Human MATE1, encoded by the *SLC47A1* gene, is primarily expressed in the kidney and liver, where it is localized to the luminal membranes of the renal tubules and hepatocytes. MATE1 mediates the H+-coupled electroneutral exchange of tetraethylammonium and 1-methyl-4-phenylpyridinium. These compounds are typical substrates of renal and hepatic H+-coupled organic cation antiporters (163).

Aquaporin-8 (AQP8), localized to canalicular membranes, modulates membrane water permeability providing a molecular mechanism for the osmotically coupled transport of solute and water during bile formation. There is experimental evidence suggesting that defective hepatocyte AQP8 expression leads to alterations in normal bile physiology (147).

Table 2 shows relevant substrates of major hepatic canalicular transporters, as well as their function.

Gender-related Differences and Hormonal Regulation of Biotransformation Systems

Observed sex differences in CYPs can be attributed to changes in the regulation of their expression and activity, most likely through endogenous hormonal influences. In humans, pharmacokinetic analyses reveal sex-based differences in drug plasma concentration that are associated with gender differences in hepatic enzyme-specific expression. In rats, hepatic *P450* genes are differentially activated during development. This process is regulated by the physiological stimulus of growth hormone (GH). The secretory pattern of GH is in turn determined by steroid hormones. Male and female rats differ in their GH secretory patterns. Males have a pulsatile secretion with high peaks and low troughs, while females have a constant and higher level of secretion than males (242). GH sexually dimorphic secretion occurs in most species, including mouse and man. However, differences are not as marked as in the rat (94, 144, 235).

Interspecies and experimental model variations

Table 3 summarizes the relevant more recent information found in the literature regarding sex differences in major CYP isoforms. It can be seen that expression and activity of major hepatic isoforms of CYP differ between humans and other animal species, particularly from rodents. Moreover, for a single species, differences are also evident between activity and expression measures, and even between *in vitro* assessment of activity and *in vivo* determination of drug clearance. For example, human CYP3A4 activity determined in isolated hepatocytes is higher in females (177), and does not correlate with expression studies, where no differences were reported (232). Also, CYP2C19-mediated metabolism determined *in vivo* shows discrepancies since, depending on the substrate, women present higher (148), lower (87), or similar (101, 124, 181) activities when compared to men. For some specific isoforms,

Table 3 Gender-Related Differences in Major Hepatic Phase I and Phase II Biotransformation Systems

Isoform	Experimental system	Species	Gender related differences	References
CYP1A2	Activity	Human	♀ < ♂	(180)
	Clearance of model substrates	Human	♀ < ♂	(99) (12) (174) (179)
	Theophylline pharmacokinetic	Human	♀ > ♂	(166)
	Activity	Human	♀ < ♂	(177)
	Activity	Pig and minipig	♀ > ♂	(204)
	mRNA expression	Mouse	♀ > ♂	(56)
CYP2A6	Activity and protein expression	Human	♀ > ♂	(203)
CYP2B1	Activity, protein and mRNA expression	Rat	♀ < ♂	(4)
CYP2B6	Protein expression	Human	♀ > ♂	(125)
CYP2C9	Specific substrates pharmacokinetics/serum concentration	Human	♀ = ♂	(101) (215) (17) (151)
CYP2C11	Protein expression	Rat (male specific)	♀ < ♂	(176) (229)
CYP2C12	Protein expression	Rat (female specific)	♀ > ♂	(176) (52, 229) (49)
CYP2C19	Clearance of model substrates	Human	♀ = ♂	(124) (101) (181) (77)
	Clearance of model substrates	Human	♀ > ♂	(148)
	Clearance of model substrates	Human	♀ < ♂	(87)
CYP2D6	Activity	Human	♀ = ♂	(148) (12)
	Oral desipramine clearance	Human	♀ < ♂	(3)
	Clomipramine metabolism	Human	♀ < ♂	(64)
	Racemic propranolol metabolism	Human	♀ < ♂	(65) (224)
	Serum concentrations of sertraline	Human	♀ < ♂	(123)
	Activity	Human	♀ > ♂	(123) (77)
CYP2E1	Chlorzoxazone metabolism	Human	♀ ≤ ♂	(107) (169) (141)
	Activity	Pigs and minipig	♀ > ♂	(204)
CYP3A4	Clearance/pharmacokinetics of model substrates	Human	♀ > ♂	(119) (118) (70) (129) (192) (89)
	Clearance/pharmacokinetics of model substrates	Human	♀ = ♂	(86)
	Activity (testosterone 6β-hydroxylation)	Human (hepatocytes)	♀ > ♂	(177)
	Activity (testosterone 6β-hydroxylation)	Human (microsomes)	♀ = ♂	(177)
	Erythromycin breath test	Human	♀ > ♂	(226) (89)
	Activity and protein expression	Hamster	♀ > ♂	(159)
	Activity and protein expression	Human	♀ = ♂	(193) (198) (63)
	mRNA and protein expression	Human	♀ > ♂	(218) (236)
	mRNA expression	Human	♀ = ♂	(232)
UGT1A1	Activity	Rat	♀ > ♂	(164)
	mRNA expression	Rat	♀ = ♂	(197)
	mRNA expression	Mouse	♀ > ♂	(56)
UGT1A5	mRNA expression	Mouse and rat	♀ > ♂	(56) (197)
UGT1A6	mRNA expression	Mouse	♀ < ♂	(56)
	Activity and protein expression	Rat	♀ < ♂	(29) (30)
	mRNA expression	Rat	♀ = ♂	(197)
	Activity and protein expression	Human	♀ < ♂	(33)
	Paracetamol metabolism	Human	♀ ≤ ♂	(156) (12) (1)
UGT2B1	mRNA expression	Rat	♀ > ♂	(216) (209) (197)
	mRNA expression	Mouse	♀ < ♂	(19) (56)
UGT2B2	mRNA expression	Rat	♀ > ♂	(197)
UGT2B3	mRNA expression	Rat	♀ = ♂	(197)
UGT2B15	Activity	Human	♀ < ♂	(34) (32)
UGT2B17	Activity and mRNA	Human	♀ < ♂	(58)

(*Continued*)

Table 3 (*Continued*)

Isoform	Experimental system	Species	Gender related differences	References
GSTA	mRNA expression	Mouse (A1 subunit)	♀ < ♂	(56)
	Protein and mRNA expression	Rat (A1 subunit)	♀ < ♂	(207) (31)
	Protein and mRNA expression	Rat	♀ > ♂	(184)
	Protein and mRNA expression	Mouse	♀ > ♂	(158)
	Protein expression	Mouse (A1, A2 subunits)	♀ = ♂	(37)
GSTM	Protein and mRNA expression	Rat (M1, M2 subunits)	♀ < ♂	(90) (207) (31)
	Activity and protein expression	Mouse	♀ = ♂	(184) (158)
	mRNA expression	Mouse (M1 subunit)	♀ < ♂	(56)
	mRNA expression	Mouse (M2 subunit)	♀ > ♂	(56)
	Protein expression	Mouse (M1, M2 subunits)	♀ = ♂	(37)
GSTP	Activity and/or mRNA and protein expression	Mouse	♀ < ♂	(150) (72) (158) (37) (56) (110)
SULT1A1	mRNA expression	Mouse	♀ > ♂	(56) (5)
	mRNA expression	Rat	♀ < ♂	(108)
SULT1C1	mRNA expression	Mouse and rat	♀ < ♂	(108) (5)
SULT1E2	mRNA expression	Rat	♀ < ♂	(108)
SULT3A1	mRNA expression	Mouse	♀ > ♂	(56) (5)

only partial studies are available, for example, *in vivo* clearance assessment or *in vitro* activity determination are available, with no data on isoform expression, or *vice versa.*

Link between gender differences and hormone effects

As shown in Table 3, CYP2A6 and CYP3A4 exhibit an overall association between activity and expression, with higher values for women. Gender-associated differences in these two specific isoforms could be associated with a positive effect of estradiol on the respective mRNAs (see Table 4). CYP1A2 presents higher activity in women, whereas the opposite occurs with CYP2D6, and no differences are apparent for CYP2C9 or CYP2C19. Consistent with these findings, oral contraceptives exert a positive effect on clearance of substrates of CYP1A2, whereas oral contraceptives negatively regulate CYP2C19, and no conclusive data are found for CYP2D6 (Table 4). It is not possible to generalize regarding the association between gender-dependent differences in CYP expression/activity and hormonal regulation for most of CYP isoforms because of the scarce information and because parallel studies are not usually performed by the same authors. Additionally, different substrates are used in gender versus hormonal studies by different authors, precluding confident correlations.

Among all CYPs found in liver and presented in Table 3, CYP3A4 is the most abundant isoform and is responsible for metabolizing more than 50% of known therapeutic drugs. As mentioned above, clearance of drugs biotransformed by CYP3A4 is consistently higher in women than men. Initially, it was assumed that this difference was a consequence of higher CYP3A4 protein expression in women compared to men. However, controversial reports are found in the literature. In some cases, the evaluations of CYP3A4 protein content and activity from human hepatic microsomes report no significant sex-related differences between women and men (63,193,198). Only one study clearly reports higher CYP3A4 activity in microsomes from women versus men, using erythromycin as substrate (89). Cummins et al. (35) hypothesized on the observed discrepancy in CYP3A4 activity evaluated *in vitro* versus *in vivo*. For drugs that are substrates of both CYP3A4 and P-gp, the different level of P-gp activity between sexes could tentatively explain this discrepancy, since women express a lower level of hepatic P-gp than men (194). When a drug (administered i.v. or i.m.) reaches the liver, the chances of being metabolized by CYP3A4 increases as P-gp activity decreases, which is the case in women. A greater extent of metabolism, even though there is no difference in enzyme expression/activity, then results in greater elimination of the metabolite via an alternative transporter. Interestingly, for drugs such as midazolam that are substrates for CYP3A4 but not for P-gp, no significant sex differences in clearance following intramuscular administration were reported (86). The controversy remains unsolved, as a more recent study reports twofold higher values for CYP3A4 mRNA and protein expression in female versus male donors with no differences in the expression of P-gp (236). No conclusive studies are available on whether this isoform is regulated by sex hormones, except for a very recent report demonstrating that estradiol positively modulates CYP3A4 mRNA expression (46), consistent with higher activities in women. CYP3A4 is just one example that illustrates how difficult it is to predict whether a specific drug is metabolized at different rates in women versus men

Table 4 Hormonal Regulation of Major Hepatic Phase I and Phase II Biotransformation Systems

Enzyme	Experimental system	Species	Hormones	Regulation	References
CYP1A2	Clinical pharmacokinetics	Human	Oral contraceptives	Positive	(156) (99) (104)
	mRNA and protein expression and activity	Rat (female)	Estradiol	Positive	(45)
CYP2A6	mRNA expression	Human (ER-positive MCF-7 and HepG2 cells)	Estrogens	Positive	(83)
	mRNA expression	Human (primary human hepatocytes)	Estradiol, progesterone	Positive	(46)
CYP2B6	mRNA expression and activity	Human (primary human hepatocytes)	Estradiol	Positive	(115)
CYP2C9	mRNA expression	Human	Oral contraceptives, estradiol, ethynylestradiol	Negative	(190) (165)
	Activity	Primary human hepatocytes	Estradiol	Positive	(46)
CYP2C11 (male specific)	Protein expression	Rat and mouse	GH (male pattern)	Positive	(160) (103) (229)
	Protein expression	Rat (male and female)	Testosterone	Positive	(22) (9)
	Protein expression	Rat (male and female)	Estradiol	Negative	(22) (9)
	Protein expression	Rat (female)	Oxandrolone	Positive	(225)
	Activity and protein expression	Rat (adult male castration)	Lack of testosterone	Negative	(128) (228)
CYP2C12 (female specific)	Protein expression	Rat and mouse	GH (female pattern)	Positive	(103)
	mRNA, protein expression and activity	Rat (female)	Estradiol	Positive	(45)
	mRNA expression	Rat	Estradiol	Positive	(40)
CYP2C19	Activity	Human	Oral contraceptives	Negative	(124) (77)
CYP2D6	Activity	Human	Oral contraceptives	Uncertain	(123)
CYP2E1	Activity	Human (primary human hepatocytes)	Estradiol	Positive	(46)
CYP3A4	mRNA expression	Human (primary human hepatocytes)	Estradiol, progesterone	Positive	(46)
	Model substrate pharmacokinetic	Human	Oral contraceptives (ethynylestradiol and norgestrel, 10 days)	No effect	(11)
UGT1A1	Activity and protein expression	Rat	Testosterone	No effect	(208)
	mRNA expression	Mouse (hypophysectomized)	Testosterone	No effect	(18)
	mRNA expression	Mouse (hypophysectomized)	Estradiol	No effect	(18)
	mRNA expression	Mouse (male and female, gonadectomized)	Testosterone	Negative	(18)
	mRNA expression	Mouse (male and female, gonadectomized)	Estradiol	Negative	(18)
UGT1A5	mRNA expression	Mouse (hypophysectomized)	Testosterone	Positive	(18)
	mRNA expression	Mouse (hypophysectomized)	Estradiol	Positive	(18)
	mRNA expression	Mouse (gonadectomized male)	Testosterone	No effect	(18)
	mRNA expression	Mouse (gonadectomized female)	Testosterone	Neative	(18)
	mRNA expression	Mouse (gonadectomized male and female)	Estradiol	No effect	(18)
UGT1A6	Activity	Rat	Testosterone	Positive	(29) (30)
	Paracetamol metabolic clearance	Human	Oral contraceptives	Positive	(156) (2)
UGT2B1	mRNA and protein expression	Rat	Testosterone	Positive	(209) (133)
	mRNA expression	Rat	Testosterone	Positive	(18)
	mRNA expression	Mouse (hypophysectomized)	Testosterone	No effect	(18)
	mRNA expression	Mouse (hypophysectomized)	Estradiol	No effect	(18)
	mRNA expression	Mouse (gonadectomized male and female)	Testosterone	Positive	(18)
	mRNA expression	Mouse (gonadectomized male and female)	Estradiol	No effect	(18)
UGT2B3	mRNA and protein expression	Rat	Testosterone	Positive	(209)
	mRNA and protein expression	Rat	GH	Negative	(209)
	mRNA expression	Rat	Testosterone	Positive	(133)
	mRNA expression	Rat	GH	Negative	(133)

(*Continued*)

Table 4 (*Continued*)

Enzyme	Experimental system	Species	Hormones	Regulation	References
UGT2B15	mRNA expression	Human (primary human hepatocytes)	Estradiol	Positive	(115)
GSTA	mRNA expression	Rat (male; A1 subunit)	GH (female pattern)	Negative	(207)
	Activity	Rat (co-culture of hepatocytes and biliary cells)	Estradiol	Negative	(31)
	Activity	Rat (co-culture of hepatocytes and biliary cells)	Testosterone and 5α-dihydrotestosterone	No effect	(31)
	Protein expression	Rat (A1, A2 subunits)	Testosterone	Positive	(31)
	Protein expression	Rat (A2 subunit)	Estradiol	Positive	(31)
	mRNA expression	Human (primary human hepatocytes, A3 subunit)	Estradiol	Positive	(115)
GSTM	mRNA expression	Rat (male; M1, M2 subunits)	GH (female pattern)	Negative	(207)
	Activity	Rat	Estradiol	Negative	(31)
	Protein expression	Rat (co-culture of hepatocytes and biliary cells, M1, M2 subunits)	Testosterone and 5α-dihydrotestosterone	No effect	(31)
GSTP	Protein expression	Rat (P1 subunit)	Testosterone and 5α-dihydrotestosterone	No effect	(31)
	Protein expression	Rat (P1 subunit)	Estradiol	Negative	(31)
SULT1A1	mRNA expression	Rat (hypophysectomized male)	GH (male and female pattern)	No effect	(108)
	mRNA expression	Rat (hypophysectomized female)	Androgen	Negative	(5)
	mRNA expression	Rat (hypophysectomized female)	GH (male pattern)	Negative	(5)
	mRNA expression	Mouse	Estrogens	Positive	(5)
	mRNA expression	Mouse	GH (female pattern)	Positive	(5)
SULT1C1	mRNA expression	Rat (hypophysectomized male and female)	GH (male pattern)	Positive	(108)
	mRNA expression	Rat (hypophysectomized male and female)	GH (female pattern)	No effect	(108)
	mRNA expression	Mouse	Androgens	Positive	(5)
SULT1E2	mRNA expression	Rat (hypophysectomized male)	GH (male pattern)	Negative	(108)
SULT2A1	mRNA expression	Human (primary human hepatocytes)	Estradiol	Positive	(115)
SULT3A1	mRNA expression	Mouse	Androgens	Negative	(5)
	mRNA expression	Mouse	Estrogens	Positive	(5)
	mRNA expression	Mouse	GH (female pattern)	Positive	(5)

GH: growth hormone.

and the underlying association with hormonal regulation. The preliminary assumption that women are more susceptible to drug effects than men should not be generalized.

Numerous factors are known to modify human UGT activity *in vivo*, including age, diet, genetic polymorphisms, disease states, and hormonal effects, among others (48, 157). Gender differences in drug conjugation are generally associated with gender-divergent expression of UGT isoenzymes. For example, higher bisphenol A glucuronidation in female versus male rats was correlated with the female predominant mRNA expression of one of the isoforms primarily responsible for its conjugation, UGT2B1 (216). Additionally, some studies suggest that the lipid composition of microsomal membrane modulates UGT activity. Thus, gender-dependent differences in UGT activity could be attributed to differences in both the amount and functional state of the enzyme (28). Table 3 compiles the sex differences observed in content or activity of most representative isoforms of UGT. Data in Table 4 also illustrate the complexity of making associations between gender-dependent differences and hormonal regulation, since in most of the cases, testosterone and estrogens exert similar effects, either positive, negative or absent. Predictions regarding hormonal effects and gender differences on glucuronic acid conjugation of a specific drug should be cautiously done, with the data in Tables 3 and 4 being only illustrative of the issues.

The expression of GSTs is also subject to sex-specific regulation. Limited studies have also demonstrated a role for GH in sex-dependent expression in the rat (207, 230). Sex hormones and GH secretory pattern (male or female) are similarly responsible for the sex differences in SULTs activities observed in rats (135, 136). The study of sex differences and regulation of rodent SULTs has expanded our understanding of the transcriptional regulation of this family of enzymes. Tables 3 and 4 summarize the most relevant information about sex differences and hormonal regulation of GST classes and

SULTs. Unfortunately, information available on these two enzyme systems is mostly restricted to rodents.

Gender-related Differences and Hormonal Regulation of Transporters

Hepatic transport proteins of the SLC and ABC family are expressed and regulated in a gender-specific manner. These findings may partly explain the well-known sex differences in hepatic disposition of their substrates, for example, organic anions (185). Table 5 summarizes the gender-associated differences in the expression of basolateral and canalicular transporters. Gender-related differences in transporter mRNA and protein expression represent an important mechanism for the regulation of hepatic transport processes. Furthermore, female sex hormones, mainly estradiol, and male sex hormones, primarily testosterone, appear to be involved in these gender-related differences in transport either directly or indirectly (162). Table 6 summarizes the effect of sex hormones on expression and activity of hepatic transporters.

Interspecies and experimental model variations

With respect to drug transporters, differences in dimorphic gene expression among species are frequently observed

Table 5 Gender-Related Differences in Major Hepatic Transport Systems

Transporter	Parameter	Species	Gender-related differences	References
NTCP (SLC10A1)	Protein and mRNA expression	Rat	♀ < ♂	(200) (201)
	Protein and mRNA expression	Mouse	♀ > ♂	(41)
	Protein and mRNA expression	Human	♀ = ♂	(41)
OATP1 (OATP1A1)	Protein and mRNA expression	Rat	♀ = ♂	(132) (185) (42)
	mRNA expression	Mouse	♀ < ♂	(56)
OATP2 (OATP1A4)	Protein expression	Rat	♀ < ♂	(185) (42)
	mRNA expression	Mouse	♀ > ♂	(56)
	mRNA expression	Rat	♀ = ♂	(132)
	Protein and mRNA expression	Rat (40-45 days)	♀ > ♂	(71)
OATP4 (OATP1B2)	Protein expression	Rat	♀ = ♂	(185) (132)
	mRNA expression	Rat	♀ = ♂	(132)
	mRNA expression	Rat	♀ > ♂	(185)
	mRNA expression	Mouse	♀ = ♂	(56)
	mRNA expression	Mouse	♀ > ♂	(241)
OAT2 (SLC22A7)	mRNA expression	Rat	♀ = ♂	(112)
OAT3 (SLC22A8)	mRNA expression	Rat	♀ < ♂	(112) (20)
MRP1 (ABCC1)	mRNA expression	Mouse	♀ < ♂	(146)
MRP3 (ABCC3)	Protein and mRNA expression	Rat	♀ > ♂	(185)
	Protein and mRNA expression	Rat	♀ = ♂	(201)
	mRNA expression	Mouse	♀ > ♂	(146) (56)
MRP4 (ABCC4)	mRNA expression	Mouse	♀ > ♂	(146) (56)
	mRNA expression	Rat	♀ = ♂	(140) (38)
MRP5 (ABCC5)	mRNA expression	Mouse and rat	♀ = ♂	(140) (146)
MRP6 (ABCC6)	mRNA expression	Mouse and rat	♀ = ♂	(140) (146)
AQP9	Protein and mRNA expression	Rat	♀ < ♂	(168) (126)
BCRP (ABCG2)	Protein, mRNA and pharmacokinetic studies	Human and mouse	♀ < ♂	(155)
	mRNA expression	Mouse	♀ > ♂	(56) (219)
	mRNA expression	Rat	♀ = ♂	(219)
P-GP (ABCB1)	Protein and mRNA expression	Rat	♀ > ♂	(57) (189)
	mRNA expression	Rat	♀ = ♂	(140)
	Protein expression	Human	♀ < ♂	(194)
MRP2 (ABCC2)	Protein and mRNA expression	Rat	♀ > ♂	(185) (202) (98)
	mRNA expression	Mouse	♀ = ♂	(146)
BSEP (ABCB11)	Protein and mRNA	Rat and mouse	♀ = ♂	(201) (41)
	mRNA expression	Human	♀ = ♂	(41)
ABCG5/8	mRNA expression	Rat and mouse	♀ = ♂	(82) (140) (56)

Table 6 Hormonal Regulation of Major Hepatic Transporters

Transporter	Parameter	Species/experimental system	Hormones	Regulation	References
NTCP (SLC10A1)	mRNA expression	Rat (ovariectomized)	Estradiol	No effect (but negative on ntcp induced by Prolactin)	(26)
	mRNA expression	Rat (male)	Estradiol	Negative	(201)
	mRNA expression	Rat (female)	Estradiol	No effect	(201)
	mRNA and protein expression	Rat	Prolactin	Positive	(26) (25) (60) (61)
	mRNA, protein expression and Na+-dependent taurocholate uptake in basolateral vesicles	Rat	Ethynylestradiol	Negative	(15) (62)
	mRNA, protein expression and Na+-dependent taurocholate uptake in basolateral vesicles	Rat (hypophysectomized)	Ethynylestradiol	No effect	(201)
	mRNA expression	Rat (In vivo and primary culture)	GH	Positive	(25) (201)
OATP1 (OATP1A1)	Protein expression	Rat	DHEA	Negative	(185)
	mRNA and protein expression	Rat	Ethynylestradiol	Negative	(62)
	mRNA expression	Mouse (gonadectomized)	Testosterone	Positive	(42)
	mRNA expression	Mouse (hypophysectomized)	Testosterone	No effect	(42)
	mRNA expression	Mouse (hypophysectomized)	GH (male pattern)	Positive	(42)
OATP2 (OATP1A4)	Protein expression	Rat	DHEA	Negative	(185)
	mRNA and protein expression	Rat	Ethynylestradiol	Negative	(62)
	mRNA expression	Mouse (gonadectomized)	Testosterone	Negative	(42)
	mRNA expression	Rat (hypophysectomized)	Testosterone	No effect	(42)
	mRNA expression	Mouse (hypophysectomized)	GH (male pattern)	Negative	(42)
OATP4 (OATP1B2)	Protein and mRNA expression	Rat	DHEA	Negative	(185)
	mRNA and protein expression	Rat	Ethynylestradiol	Negative	(62)
	mRNA expression	Rat (primary culture)	Prolactin	Positive	(237)
	mRNA expression	Rat (primary culture)	GH	Positive	(237)
OATP1B3 (SLC21A8)	HepG2 promoter assay	Human	Prolactin	Positive	(237)
	HepG2 promoter assay	Human	GH	Positive	(237)
OAT3 (SLC22A8)	mRNA expression	Rat	Testosterone	Positive	(112)
	mRNA expression	Rat	GH (female pattern)	Negative	(20)
MRP3 (ABCC3)	Protein and mRNA expression	Rat	DHEA	Positive	(185)
	Protein and mRNA expression	Rat	Ethynylestradiol	Positive	(100) (188) (187)
MRP4 (ABCC4)	Activity	HEK293 cells (human)	Progesterone	Negative	(233)
	mRNA expression	Mouse	GH (male pattern)	Negative	(145)
AQP9	mRNA and protein expression	WIF B (rat hepatoma and human fibroblast hybrid cell line)	Estradiol	Negative	(126)
BCRP (ABCG2)	mRNA expression	Mouse	Testosterone	Positive	(219)
P-GP (ABCB1)	Protein expression and activity	Rat (female)	Testosterone	Negative	(213)
	Protein and mRNA expression	Rat	Ethynylestradiol	No effect	(223)
MRP2 (ABCC2)	Protein expression	Rat (female)	DHEA	No effect	(185)
	Protein expression	Rat (male)	DHEA	Slightly decreased	(185)
	Protein and mRNA expression	Rat	Testosterone	Negative	(213) (202)
	mRNA expression	Rat (male)	Estradiol	Positive	(202)
	mRNA and protein expression	Rat	Ethynylestradiol	Negative	(223)
	mRNA and protein expression	Rat	GH (female pattern)	Positive	(202)
BSEP (ABCB11)	mRNA and protein expression	Rat	Ethynylestradiol	Negative	(223)
	mRNA and protein expression	Rat (ovariectomized)	Prolactin	Positive	(25)
AQP8	Protein and mRNA expression	Rat	Ethynylestradiol	Negative	(27)
ABCG5/8	mRNA expression	Rat	Ethynylestradiol	Negative	(100)

DHEA: Dehydroepiandrosterone.

(20, 219). P-gp demonstrates a higher hepatic expression in men than in women (194) but the opposite has been reported in male versus female rats (57, 189). More recently, Wolbold et al. (236) reported no differences in hepatic expression of P-gp between women and men. In humans and mice, BCRP/Bcrp is predominant in the male, whereas there are no gender differences in rats (56,155,219). Ntcp expression does not show a significant difference between sexes in humans, whereas female rats present lower expression than males, and the opposite occurs in mice (41, 200, 201). There are also differences among species in Oatps, Mrp2, and Mrp4 protein expression. These differences are not consistent among the different species and depend on the particular transporter, with the relevant more recent data summarized in Table 5. In contrast, the responses of Mrp3, Bsep, and Abcg5/8, are more consistent among species, with no differences between sexes for Bsep and Abcg5/8, and a trend toward higher expression in females for Mrp3. It is important to note that there are no reports regarding the existence of gender differences in humans for these three specific transporters. As mentioned above for biotransformation enzymes, this emphasizes the importance of selecting appropriate models to evaluate the effect of gender, and any extrapolation to humans should be done with caution.

Link between gender differences and hormone effects

For some transporters, sexual dimorphic expression is not due to the direct effect of sex steroid hormones, but is controlled by pituitary hormones. For example, Simon et al. (202) found that expression of Mrp2, which is predominantly expressed in female versus male rat livers, is induced by estradiol and decreased by testosterone, and that these effects are prevented after hypophysectomy. The authors propose that the female pattern of GH and thyroxine are indeed responsible for the preferential expression of Mrp2 in females. In mice, Cheng et al. (42) found that the positive effect of testosterone on Oatp1 expression and the negative effect on Oatp2 expression are also indirect effects, with GH being responsible for these effects. This is in agreement with the higher levels of Oatp1 and the lower levels of Oatp2, respectively, found in male versus female mice (56). Mrp4 is another transporter that is expressed predominantly in female mouse liver (56,140,146), with the male specific pattern of GH secretion being the underlying mechanism (145). 17 α-Ethynylestradiol administration to hypophysectomized male or female rats did not decrease Ntcp expression, indicating that estrogens require pituitary hormones to decrease the liver Ntcp mRNA levels (201).

Other pituitary hormones are also linked indirectly to differential modulation of transport systems between sexes. Prolactin associated with lactation has been shown to increase expression of Ntcp in rat liver via activation of Stat5 and two Stat5 response elements in the Ntcp promoter (61). These findings were confirmed in cultured primary rat hepatocytes, and further showed that placental lactogen is also effective in activating Stat5a and 5b in this cell model (24). Intravenous infusion of ovariectomized rats with ovine prolactin increased expression of both Ntcp and Bsep (25), and increased ATP-dependent bile acid transport in canalicular membranes (137), consistent with increased expression of Bsep. Subsequent studies (26) demonstrated that estradiol acts via ER-α to repress the actions of prolactin by blocking tyrosine phosphorylation of Stat5a and its subsequent binding to response elements in the promoter. These studies further demonstrated that treatment of rats with estradiol at physiological concentrations blocked the ability of prolactin to increase Ntcp expression *in vivo*. Thus, cross-talk between ER-α and Stat5a in liver can block the actions of prolactin, or placental lactogen, which is elevated in pregnancy, to increase Ntcp expression during this period. Stat5 response elements are also present in the promoters of rat Oatp1b2 and human OATP1B3, so that prolactin and GH were shown to increase mRNA expression of Oatp1b2 in primary rat hepatocytes, and luciferase activity in OATP1B3 promoter assays in HepG2 cells (237). Simon et al. (201) proposed that the sexually dimorphic differences in Ntcp expression, as well as the downregulating effect of estrogens on Ntcp, results from the female specific pattern of GH secretion. Because estrogens also increase prolactin secretion, and as noted above, can inhibit activation of Stat5, it is difficult to define precisely the mechanisms by which estrogens, prolactin, GH and placental lactogens regulate gene expression. The existence of putative Stat5b response elements may explain why gender differences exist in the expression of mouse Ntcp, but not in mouse Bsep. In the human *NTCP* promoter, there is one putative DNA binding site of Stat5b (–2270 bp), which is absent in the human *BSEP* promoter (20,41). Buist et al. (20) demonstrated that androgens increase the level of Oat3 mRNA in male rat liver. The female GH secretion pattern suppresses Oat3 mRNA in female liver, thus accentuating the gender difference in Oat3 mRNA expression.

To our knowledge, there is no additional information regarding involvement of pituitary hormones in regulating the expression of other hepatic transporters. However, there are reports implicating sex hormones in their sexual dimorphic expression. Suzuki et al (213) demonstrated that testosterone decreased P-gp expression in rats. This is in accordance with the lower expression of this transporter in male rats compared with female rats either at protein (189) or mRNA levels (57). After castration, Bcrp mRNA levels in livers from male mice decreased to a similar level found in female livers. Furthermore, replacement of 5α-dihydroxytestosterone in castrated males and ovariectomized females increased Bcrp mRNA levels. However, ovariectomy only slightly decreases the level of Bcrp mRNA in female livers, and replacement of 17 β-estradiol in castrated and ovariectomized mice does not remarkably change Bcrp mRNA levels. Taken together, these data suggest that male-predominant expression of Bcrp in mouse liver is regulated by the inductive effect of testosterone (56, 155, 219). AQP9 is expressed at higher levels in male respect to female rat livers. This fact is in accordance

with a negative effect of estradiol on AQP9 expression in WIFB cells (126). Regarding AQP8, it was demonstrated that the synthetic estrogen ethynylestradiol decreases its protein levels (27), but there are no reports about the existence of sex-related differences in its expression. Less known is about the existence of gender differences in MATE1 or if it is regulated by sex hormones.

Controversy remains regarding gender-specific Oatp1b2 expression. While Li et al. 2002 were unable to find differences in Oatp1b2 mRNA expression between male and female rats, Rost et al. (185) found higher levels in female compared to male rats, with no differences at protein levels. These differences cannot readily be explained and may result from the different RNA-quantification techniques used in these studies. Finally, there is also controversy regarding gender differences in expression of Oatp1b2 in mice. Zhaer et al. (241) reported higher levels of Oatp1b2 (Oatp4) mRNA in female mice versus male mice, whereas Fu et al. (56) reported comparable levels of Oatp1b2 (Oatp4) mRNA in females and males.

Pathophysiological, Toxicological, and Pharmacological Relevance of Hormonal Regulation

Major factors implicated in drug disposition are drug-metabolizing enzymes and drug transporters. Sex-related differences in pharmacokinetics and pharmacodynamics have usually been considered as potentially significant determinants for the clinical effectiveness/toxicity of drug therapy. In rats, the activity of UGT1A6 towards p-nitrophenol is higher in male than in females. The administration of spironolactone (a diuretic with inducer properties) was able to induce hepatic UGT1A6 activity selectively in females (29). Thus, if a drug substrate of UGT1A6 were coadministered with spironolactone, then a possible occurrence of drug-drug interaction in females, but not in males, should be considered.

It is known that 17β-estradiol and progesterone plasma levels rise during pregnancy (36), altering the rate and extent of hepatic drug metabolism (7, 85). The antiepileptic drug lamotrigine is commonly used in pregnant women and is extensively metabolized by UGT1A4 (186). Its increased oral clearance observed in pregnancy (47) was attributed to UGT1A4 upregulation produced by 17β-estradiol (39). Similarly, an increase in oral clearance of the antihypertensive drug labetalol was reported in pregnant women. A potential role of progesterone in modulating labetalol elimination was proposed by Jeong et al. (97), since they observed a concentration dependent increased activity of the promoter of UGT1A1, the main isoform of UGT involved in labetalol glucuronidation.

Doxorubicin has a delay in its disappearance from plasma in male rats with respect to female rats (213), though its major metabolizing enzyme, CYP2B1 (66), is expressed at higher levels in male rats (4). However, because biotransformation is only a minor component in doxorubicin clearance, Suzuki et al. (213) proposed that the lower levels of Mrp2 and Pg-P in male rats compared to female rats are rather responsible for the lower rate in plasma disappearance. These latter examples illustrate the complex interplay between biotransformation and transport systems and the influence of gender that could affect many other drugs.

Female mice are relatively resistant to acetaminophen-induced hepatotoxicity compared to male mice. One of the factors implicated in this phenomenon may be the slightly higher glutamate cysteine ligase activity and Mrp3 levels expressed in female over male mice (56, 146, 149). Similarly, Merino et al. (155) demonstrated that sexual dimorphism in hepatic expression of Bcrp causes the sex difference observed in the pharmacokinetics of several known Bcrp substrates, such as the therapeutic drugs nitrofurantoin, cimetidine, and topotecan, and the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, resulting in higher plasma levels after their oral and i.v. administration, and lower biliary excretion in female versus male mice. These represent predictable and consistent examples of gender differences in rodents, with mechanisms apparently elucidated. Similarly, clearance of topotecan was found to be faster in men than in women, as a consequence of higher expression of BCRP in liver of men (155). This occurs in spite of the fact that topotecan is also a substrate for P-gp and that P-gp activity does not differ between women and men (195). Metabolism of topotecan, that could also affect its clearance, is considered a minor route of inactivation (139). Clearly, knowledge of gender-specific differences in expression and/or activity of only one of the enzymes or transporters involved in drug disposition is likely not sufficient to predict *in vivo* clearance.

Conclusions

The gender-related differences described in biotransformation and transport systems may be responsible, at least in part, for interindividual variability in drug disposition, therapeutic response, and drug toxicity. Although factors affecting biotransformation and transport systems in response to hormones or in different sexes are complex, it is worth evaluating potential sex-specific differences during the clinical drug developmental process of new compounds. This should help to identify drugs displaying sex-dependent or hormonal-sensitive pharmacokinetics.

It is clear from the literature compiled in this review that the mechanisms responsible for the gender dependence of hepatic biotransformation enzymes and transport function are far from being completely understood. Whether sex hormones operate directly on these systems or are mediated through gender-dependent patterns of GH and/or prolactin secretion is less understood. Differential participation of sex steroid receptors and regulatory proteins (e.g., coactivators, corepressors, etc.) are additional factors, not considered in this review, but of similar relevance.

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