

Gender Differences in the Membrane Transport of Endogenous and Exogenous Compounds

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Abstract—Gender differences have been well described in pharmacokinetics and contribute to the interindividual variation in drug disposition, therapeutic response, and drug toxicity. Sex-related differences in the membrane transport of endogenous substrates and xenobiotics have been reported in various organs of the body including kidney, liver, intestine, and brain. These gender-related differences in transport

systems could also contribute to interindividual variability in pharmacokinetics and pharmacodynamics. This review will focus on current knowledge of gender-associated differences in the transport of endogenous and exogenous compounds in a variety of body organs and will discuss the implications and the clinical significance of these observations.

I. Introduction

Gender differences in pharmacokinetics and pharmacodynamics are well documented in animals and humans. Gender is one variable that contributes to differences in pharmacokinetics including absorption, distribution, metabolism, and excretion (Bonate, 1991; Fletcher et al., 1994; Harris et al., 1995). The increased bioavailability of ethanol after oral administration has been reported in women as a result of higher alcohol absorption due to lower gastric alcohol dehydrogenase

activity (Fletcher et al., 1994; Harris et al., 1995), and aspirin is absorbed more slowly in men than in women after oral dosing (Harris et al., 1995). The effect of gender on hepatic metabolism has been extensively examined for a number of drugs (Bonate, 1991; Fletcher et al., 1994; Harris et al., 1995). The enzyme, cytochrome P-450 3A4 (CYP 3A4) is involved in the metabolism of over 50% of drugs in clinical use including erythromycin, lidocaine, and midazolam and is also responsible for the hydroxylation of steroid hormones. The activity of CYP 3A4 in women is 1.4 times greater than that in men (Harris et al., 1995; Gleiter and Gundert-Remy, 1996). Conjugation reactions also demonstrate gender-related differences. The glucuronidation of diflunisal and paracetamol is higher in men than in women due to higher glucuronosyl transferase activity in men, with no sex-associated differences in sulfation (Gleiter and Gundert-Remy, 1996). Gender-based differences in protein bind-

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TABLE 1
Gender-associated differences in transport systems in humans

Compounds	Tissue	Process	Gender Effects	Reference
Amantadine	Kidney	Urinary recovery at 48 h	M > F	Wong et al., 1995
		Renal clearance/weight ^a	M > F	Wong et al., 1995
Uric acid	Kidney	Postsecretory reabsorption	M > F	Anton et al., 1986
Rifamycin SV	Liver	Normal & Gilbert's patients	M < F	Gentile et al., 1985
MDR1 (Pgp)	Liver	Protein expression	M > F	Schuetz et al., 1995
Glucose	Skeletal muscles	Uptake	M < F	Nuutila et al., 1995
Chylomicron	Splanchnic	Uptake	M > F	Nguyen et al., 1996
Triglyceride	tissues			
Palmitate	Splanchnic	Uptake	M > F	Nguyen et al., 1996

^a Renal clearance in males was greater than in females following normalization for body weight, body surface area, or body mass index.

ing have been observed for diazepam, chlordiazepoxide, and imipramine, with nonpregnant women having higher unbound fractions of these drugs compared with men (Harris et al., 1995; Kashuba and Nafziger, 1998). This may be due to the slightly lower concentrations of α -1-acid glycoprotein and lipoprotein reported in women; the plasma concentration of α -1-acid glycoprotein is decreased by estrogen (Beierle et al., 1999). Gender-related differences in drug response have not been extensively studied; however, a gender effect in pharmacodynamics has been well described for psychotropic drugs. The greater improvement and more severe adverse effects in response to antipsychotic drugs such as chlorpromazine and fluspirilene have been reported in women, at least in part, due to differences in estrogen concentrations; estrogen has been shown to act as a dopamine antagonist (Fletcher et al., 1994; Harris et al., 1995). As well, Kaasinen et al. (2001) have reported that women have significantly higher dopamine D2-like receptor binding than men in the frontal cortex, which may contribute to gender-related differences in the incidence, clinical course, or treatment response in neuropsychiatric diseases that are associated with dopaminergic neurotransmission. There is a gender difference in the response to the cholinesterase inhibitors, rivastigmine and physostigmine, in that female rats exhibit a greater inhibition of cholinesterase in the cerebral cortex, hippocampus, and striatum compared with male rats: orchidectomy completely abolished the difference suggesting that a testicular hormone may be suppressing the effect of the cholinesterase inhibitor by affecting its brain uptake or its interaction with cholinesterase (Wang et al., 2000). Women on hemodialysis exhibit lower responses to recombinant erythropoietin than male patients (Ifudu et al., 2001). It is not known if these differences are due to inherent biological differences in response to erythropoietin or due to other factors including differences in endogenous erythropoietin levels (Ifudu et al., 2001). More adverse effects for antihypertensive drugs are reported in women than in men (Harris et al., 1995). The gender-related differences in pharmacokinetics and pharmacodynamics may explain, at least in part, the interindividual variations observed in drug disposition, therapeutic response, and drug toxicity and are of particular concern for those drugs with relatively

narrow therapeutic ranges (Harris et al., 1995; Gleiter and Gundert-Remy, 1996).

Facilitated transport systems in the intestine, liver, and kidney have been known to play important roles in the absorption and elimination of a variety of clinically significant drugs (Zhang et al., 1998). Drugs must traverse across biological membranes via simple diffusion or physiological transporters to produce therapeutic efficacy (Levy, 1998). Gender-associated differences in transport processes for endogenous and exogenous substrates have been reported in various organs of the body, including kidney, liver, intestine, and brain, for rats, mice, and humans (Kleinman et al., 1966; Orzes et al., 1985; Anton et al., 1986; Morissette et al., 1990; Uhland-Smith and DeLuca, 1993; Sibug et al., 1996). Table 1 summarizes the gender-associated differences in transport activities in humans, evaluated predominantly in clearance studies, whereas Table 2 summarizes the literature information regarding gender differences in transporter mRNA and/or protein expression in tissues. This review will focus on recent knowledge of gender-associated differences in the transport of endogenous compounds and xenobiotics in a variety of body organs and will discuss the implications and the clinical significance of these findings.

II. Membrane Transport in Tissues

A. Kidney

There are gender differences in renal handling of both organic and inorganic anions and cations.

1. *Anions.* The renal clearance of *p*-aminohippurate (PAH¹) is decreased in female rats due to decreases in both the filtered and secreted amounts. In females, the maximal uptake (V_{\max}) into kidney basolateral membrane vesicles is decreased by $52 \pm 9\%$ ($p < 0.05$), and

¹Abbreviations: PAH, *p*-aminohippurate; Oatp, organic anion transporter polypeptide; TEA, tetraethylammonium; rOCT, rat organic cation transport protein; hOCT, human organic cation transport protein; BBM, brush-border membrane; BLM, basolateral membrane; BSP, sulfobromophthalein; TBS, tetrabromosulfonephthalein; BSP-GSH, glutathione conjugate of sulfobromophthalein; Ntcp, sodium-dependent taurocholate transporter; cLPM, canalicular liver plasma membrane; MRP, multidrug resistance-associated protein; ; Pgp, P-glycoprotein; DA, dopamine; BBB, blood-brain barrier; 5-HT, 5-hydroxytryptamine; FATP-1, fatty acid transport protein-1.

TABLE 2
Gender differences in transporter expression

Transport Protein	Typical Substrates	Gender Difference (Species/Tissue)	Sex-Hormone Treatment		Reference
			Testosterone	Estradiol	
Oatp mRNA/protein	Bromosulphothalein, taurocholate, ouabain, cortisol, dexamethasone, ajmalinium	Male > female (rat/kidney) male = female (rat/liver)	Strong increase	Decrease	Lu et al., 1996; Simon et al., 1999
rOAT1 protein	PAH, PGE ₂ , urate, salicylate, methotrexate, cAMP, indomethacin, folate	Male > female (rat/kidney)	N.D.	N.D.	Cerrutti et al., 2002
rOCT1 mRNA	Choline, dopamine, epinephrine, serotonin, norepinephrine, MPP, NMN, tyramine	None (rat/kidney)	No effect	No effect	Urakami et al., 1999, 2000
rOCT2 mRNA/protein	Amantadine, TEA, choline, dopamine	Male > female (rat/kidney)	Increase	Moderate decrease	Urakami et al., 1999, 2000
rOCT3 mRNA	Dopamine, guanidine, MPP, TEA	None (rat/kidney)	N.D.	N.D.	Urakami et al., 1999
mdr1a mRNA	Hydrophobic (cationic) compounds, anticancer agents, digoxin, immunosuppressants, steroids	Female > male (rat/liver)	N.D.	N.D.	Piquette-Miller et al., 1998
mdr1b mRNA	Hydrophobic (cationic) compounds, anticancer agents, digoxin, immunosuppressants, steroids	Male > female (rat/liver) female > male (mouse/kidney)	N.D.	N.D.	Schinkel et al., 1994; Piquette-Miller et al., 1998
Mdr2 mRNA	Phospholipids, cholesterol	Female > male (rat/liver)	N.D.	N.D.	Furuya et al., 1994; Salphati and Benet, 1998
MDR total protein	Hydrophobic (cationic) compounds, anticancer agents, digoxin, immunosuppressants, steroids	Female > male (rat/liver) Male > female (human/liver)	N.D.	N.D.	Schuetz et al., 1995; Piquette-Miller et al., 1998
Ntcp mRNA/protein	Bile acids	Male > female (rat/liver)	Decrease	N.D.	Simon et al., 1996, 1999
FATP-1 mRNA	Long chain fatty acids	Female > male (human/skeletal muscle)	N.D.	N.D.	Binnert et al., 2000

N.D., not determined; PGE₂, prostaglandin E₂; MPP, *N*-methyl-4-phenylpyridinium; NMN, *N*-methylnicotinamide.

the Michaelis-Menten constant (K_m) for uptake into kidney brush-border membrane vesicles is increased by $163 \pm 8\%$ ($p < 0.05$), compared with male rats (Cerrutti et al., 2001). Similar results have been noted in older studies using kidney slices. The transport rate of PAH by kidney slices isolated from male rats is higher than that in female rats with higher V_{max} values compared with female rats (Kleinman et al., 1966; Bowman and Hook, 1972) (Table 3). The rate of accumulation of PAH in renal cortical slices of adult male rats is decreased by castration or by blockade of testosterone receptor sites whereas ovariectomy does not increase the transport of PAH in mature female rats. Furthermore, treatment with estradiol in male rats does not reduce renal tubular transport of PAH whereas chronic (repeated) treatment with testosterone stimulates PAH transport in males more than in females. These results indicate the important role of sex hormones in the renal tubular transport of PAH and suggest distinct renal effects of testosterone compared with estradiol (Braunlich et al., 1993). Similar effects have been reported for the renal tubular trans-

port of Diodrast, amino acids, and thiosulfate (as reviewed by Kleinman et al., 1966).

The urinary excretion of zenarestat, an aldose reductase inhibitor, shows remarkable gender differences in rats and mice (Tanaka et al., 1991, 1992), whereas there is no significant difference between male and female dogs and humans (Tanaka et al., 1992) (Table 4). The ratios of the renal clearance of zenarestat to clearance of zenarestat by glomerular filtration are less than one in male rats and substantially greater than one in female rats. After pretreatment of rats and mice with probenecid, an inhibitor of the active secretion of many organic anions, a marked reduction in the urinary excretion of zenarestat is observed in females but not in males. These results suggest that zenarestat is, at least in part, actively secreted in the kidneys of female rats and mice and active renal tubular secretion of this compound is lacking, or negligible, in male rats and mice (Tanaka et

TABLE 4
Sex difference in the excretion of zenarestat in animals and humans^a

Species	Sex	Urine	Feces
Rat	Male	1.5 ± 0.4	96.2 ± 1.4
	Female	46.8 ± 6.2	51.2 ± 6.4
Mouse	Male	3.4 ± 0.7	94.2 ± 0.8
	Female	31.3 ± 5.4	66.8 ± 5.7
Dog	Male	11.9 ± 3.4	84.4 ± 3.2
	Female	9.9 ± 3.4	88.9 ± 2.6
Human	Male	17.7 ± 7.3	
	Female	22.2 ± 8.8	

^a Values are the mean ± S.D. of three animals, 13 male and 12 female humans expressed as percentage of dose. Adapted from Tanaka et al., 1992.

TABLE 3

Maximal transport rate in the kidney for *p*-aminohippuric acid in male and female rats^a

Sex	No. of Animals	Kidney Weight/100 g b.wt.	T_{mPAH} mg/(min × g kidney)
Female	8	0.70 ± 0.01	0.26 ± 0.01*
Male	3	0.71 ± 0.02	0.41 ± 0.04*

* $p < 0.0005$; adapted from Kleinman et al., 1966.

^a Values are the mean ± S.E.

TABLE 5
Sex differences in urinary excretion of egualen sodium in rats^a

Parameter	Total Radioactivity after Oral Administration	Unchanged Drug after Oral Administration	Total Metabolites after Oral Administration
Male			
C_{max}	43.2 $\mu\text{g/ml}$	34.2 $\mu\text{g/ml}$	11.3 $\mu\text{g/ml}^c$
AUC	540 $\mu\text{g} \cdot \text{h/ml}$	397 $\mu\text{g} \cdot \text{h/ml}$	143 $\mu\text{g} \cdot \text{h/ml}^d$
Urinary excretion	57.4% ^a	2.1% ^b	52.2% ^e
Female			
C_{max}	38.7 $\mu\text{g/ml}$	36.7 $\mu\text{g/ml}$	2.6 $\mu\text{g/ml}^c$
AUC	353 $\mu\text{g} \cdot \text{h/ml}$	326 $\mu\text{g} \cdot \text{h/ml}$	27 $\mu\text{g} \cdot \text{h/ml}^d$
Urinary excretion	70.4% ^a	39.5% ^b	29.9% ^e

AUC, area under the curve.

^a Oral dose of 20 mg/kg [¹⁴C]egualen sodium. All values are means expressed as micrograms of egualen equivalents (adapted from Sato et al., 2000). Values with the same superscript letter are significantly different, $p < 0.01$.

al., 1991, 1992). In addition, the urinary excretion of zenarestat is decreased in female rats with experimentally induced chronic diabetes mellitus due to a decrease in active secretion, whereas there is an increase in the urinary excretion of the drug in male rats with experimentally induced acute or chronic diabetes, most likely due to a reduction in testosterone levels in diabetic states (Tanaka et al., 1993). Similarly, it has also been reported that there are gender differences in the renal excretion of perfluorooctanoic acid (Hanhijarvi et al., 1982), *S*-pentachlorophenyl-*N*-acetyl-L-cysteine (Smith and Francis, 1983), carnitine (Carter and Stratman, 1982), nilvadipine metabolite (M3) (Terashita et al., 1995) and 1-aminocyclohexanecarboxylic acid (Anton et al., 1986). These compounds are rapidly excreted by an active renal secretion in female rats whereas this secretory mechanism appears to be absent or relatively inactive in male rats (Carter and Stratman, 1982; Hanhijarvi et al., 1982; Smith and Francis, 1983; Anton et al., 1986; Terashita et al., 1995).

Egualen sodium, an antiulcer drug, demonstrates a marked sex-related difference in the urinary excretion of unchanged drug and metabolites in rats. The renal clearance of unchanged drug in male rats is 21 times lower than that in female rats, and the urinary excretion of egualen represented 2.1 and 39.5% of the dose in male and female rats, respectively (Sato et al., 2000) (Table 5). Egualen is secreted in the renal tubules by a probenecid-inhibitable process, which can be inhibited by testosterone. Gonadectomized male rats have a similar renal clearance of egualen as female rats, and treatment of gonadectomized rats with testosterone decreased the renal clearance of egualen (Sato et al., 2000) (Table 6).

Sodium/sulfate cotransport in kidney cortex brush-border (BBM) vesicles and sulfate/anion exchange in basolateral (BLM) vesicles, isolated from female and male guinea pig kidneys, have been studied (Lee et al., 1999a). No statistically significant differences in K_m and V_{max} for uptake were found, although uptake values for female animals tended to be greater; the lack of significance may reflect the small number of animals studied ($n = 4$). Sodium/sulfate cotransport is increased in renal epithelial cells in the presence of estrogen (Lee et al., 1999b). Postmenopausal women demonstrate a de-

creased renal reabsorption of sulfate compared with premenopausal women, although this was not reversed by estrogen supplementation (Benincosa et al., 1995).

A significant gender-related difference occurs in the renal reabsorption of urate in humans, which is of clinical significance. A significant decrease in tubular urate postsecretory reabsorption in the kidneys of adult women leads to a greater urinary excretion and lower serum urate concentrations compared with adult men. Presecretory reabsorption and tubular secretion of urate are similar in women and men. The mechanism underlying this difference is not known but both the renal handling of uric acid and the serum urate levels are not influenced by plasma 17 β -estradiol concentrations (Anton et al., 1986).

Renal organic anion transporting polypeptide (oatp) mRNA expression is higher in male rat kidney than in female kidney and has been shown to be under the control of androgen and to a lesser extent estrogen (Lu et al., 1996). It is speculated that the regulation of kidney oatp expression may be necessary for modulating the renal tubular secretion of conjugated estradiol. Five forms of oatp are expressed in rat kidney. Oatp1 has a wide substrate specificity and substrates include conjugated and unconjugated bile acids, steroid hormones, organic anions such as bromosulphophthalein, and bulky organic cations such as *N*-(4,4-azo-*n*-pentyl)-21-deoxyajmalinium. OATs are multispecific organic anion trans-

TABLE 6
Gender-related differences in renal clearance of egualen sodium in rats^a

	Unchanged Drug <i>ml/min/kg</i>
Male	
Renal clearance	0.009 (0.002)
Probenecid tx	0.031 (0.005)
Gonadectomy	0.158 (0.024)
Probenecid tx after gonadectomy	0.055 (0.004)
Testosterone tx after gonadectomy	0.004 (0.002)
Female	
Renal clearance	0.193 (0.030)**
Probenecid tx	0.081 (0.009)
Gonadectomy	0.272 (0.055)
Testosterone tx after gonadectomy	0.102 (0.033)

** $p < 0.01$. tx, treatment.

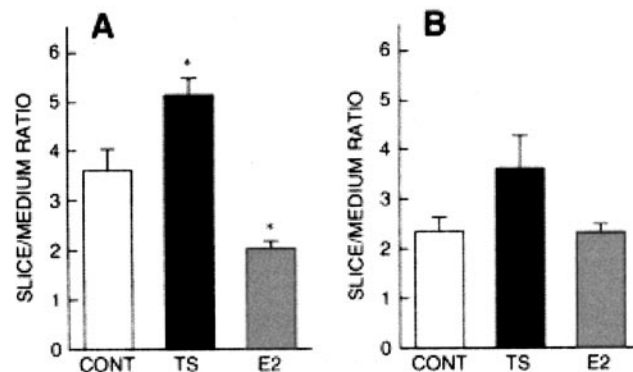
^a IV infusion: rate = 4 $\mu\text{g/min}$. Results expressed as mean (S.E.) (adapted from (Sato et al., 2000).

porters, with all members of the OAT family expressed in the kidney (Sekine et al., 2000). The substrates include endogenous compounds such as prostaglandins, urate and dicarboxylic acids, as well as organic anion drugs including PAH, salicylate, enalapril, and penicillin G (Dresser et al., 2001). Urakami et al. (1999) reported no significant gender-related differences in rat kidney organic anion transporter 1 (rOAT1) mRNA, but Cerrutti et al. (2002) found a significantly lower level of rOAT1 protein expression in rat kidney cortex BLM in females (40% compared with males). The lower expression of rOAT1 in kidney cortex BLM may be responsible, at least in part, for the decreased PAH secretion observed in female rats. Additionally, kidney cortex BBM isolated from female rats exhibit an increased membrane fluidity compared with BBM from male rats (Cerrutti et al., 2002); this may also contribute to the gender differences in membrane transport of substrates.

2. *Cations.* Tetraethylammonium (TEA) accumulation into renal cortical slices from male rats is significantly greater than that from female rats, suggesting a gender difference in the active secretion of hydrophilic organic cations (Bowman and Hook, 1972). TEA uptake into kidney slices from male and female rats is significantly increased with testosterone treatment; estradiol treatment decreased TEA uptake in kidney slices from male rats but not female rats (Urakami et al., 2000) (Fig. 1). The apparent K_m for distal tubular amantadine transport in female rats is significantly higher than that in male rats whereas the value for amantadine transport in isolated proximal tubules is not different in male and female rats. In addition, apparent V_{max} estimates for amantadine uptake in proximal tubules and distal tubules are not significantly different between males and females (Wong et al., 1993). However, a small number of rats were used in this study and significant differences in transport may have been missed.

Rat organic cation transport proteins (rOCT) are present in the kidney and are responsible for the transport of a number of organic cations, including TEA, N^1 -methylnicotinamide, choline, and dopamine. Expression levels of rOCT2 mRNA and protein in the male rat kidney are much higher than in females; there was no difference in rOCT1 or rOCT3 expression (Urakami et al., 1999, 2000). Treatment of male and female rats with testosterone significantly increased the expression of rOCT2 mRNA and protein in kidney and increased the TEA accumulation in kidney slices. Estradiol treatment produced a moderate decrease in kidney rOCT2 and decreased TEA accumulation in kidney slices from male, but not female, rats. Testosterone and estradiol treatment had no effect on rOCT1 mRNA or protein expression (Fig. 1). The authors suggest that OCT2 may have a physiological role in the secretion of endogenous substances. Other transporters may also play a role in the kidney transport of TEA.

Part A



Part B

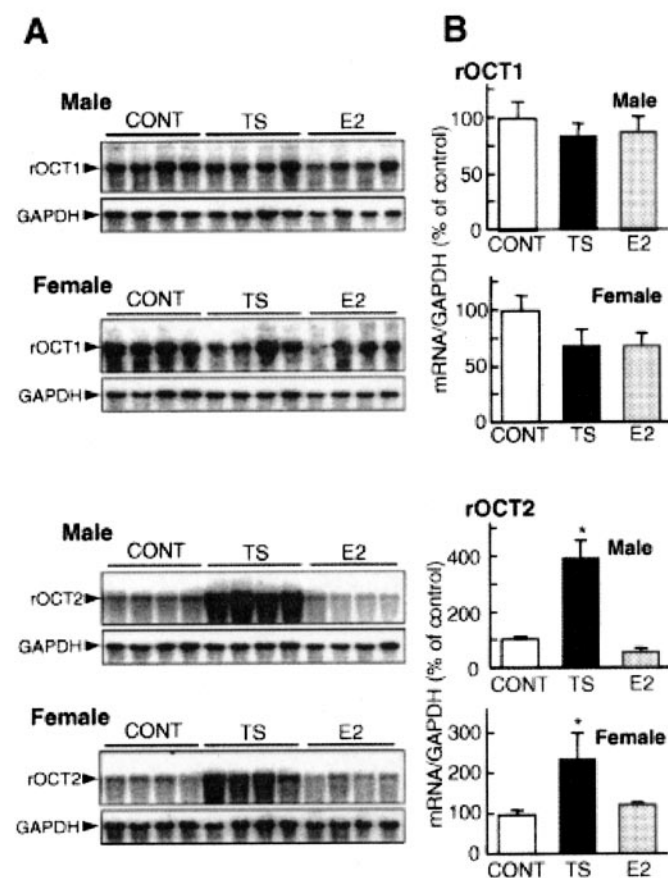


FIG. 1. TEA accumulation by kidney slices from male and female rats treated with testosterone and estradiol. Part A, kidney slices from males (A) and females (B) were incubated at 25°C in buffer containing 50 μM [^{14}C]TEA for 60 min. CONT, rats treated with vehicle; TS, rats treated with testosterone; E2, rats treated with 17 β -estradiol. Each column represents the mean \pm S.E. of three separate experiments. *, $p < 0.05$, significantly different from control. Part B, Northern blot analysis of total RNA of the kidney from male and female rats from CONT, TS, and E2 groups. Densitometric quantitation of rOCT1 and rOCT2 mRNA is corrected for loading using glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Each column represents the mean \pm S.E. of four rats. *, $p < 0.05$. Reprinted with permission (Urakami et al., 2000).

P-glycoprotein (Pgp), present in the brush-border membrane of proximal tubule cells in the kidney, is involved in the renal elimination of a diverse range of lipophilic organic cations. Schinkel et al. (1994) reported a 1-fold higher expression of *mdr1b* in kidney isolated from female mice compared with male mice. The gene products of *mdr1a* and *mdr1b* (in mice) and MDR1 (in humans) are involved in xenobiotic transport and responsible for the multidrug resistance associated with Pgp overexpression in cancer cells. Potential gender differences in the kidney levels of Pgp in humans have not been examined; nor is there information regarding sex hormone effects on Pgp expression in the kidney. However, estrogen and progesterone may be important in the regulation of Pgp function; mRNA and protein expression for Pgp are greatly increased in the secretory luminal and glandular epithelium of the gravid murine uterus, suggesting regulation by the changes in estrogen/progesterone that occur in pregnancy (Arceci et al., 1990).

In clinical studies, the quinidine- and quinine-induced inhibition of renal amantadine clearance occurs only in healthy male subjects (Gaudry et al., 1993) and the urinary recovery at 48 h and the weight normalized renal clearance of amantadine are significantly higher in men than in women (Wong et al., 1995). The human organic cation transporters hOCT1, hOCT2, and hOCT3 have been cloned. hOCT2 is mainly expressed in the kidney but there is no information available regarding gender differences in expression (Dresser et al., 2001).

With regard to inorganic cations, the transepithelial calcium and magnesium reabsorption in the mouse cortical thick ascending limb of Henle's loop is greater in male than female animals, at both 4 and 8 weeks of age; there were no gender-related differences in NaCl transport (Wittner et al., 1997). There are sex differences in the uptake of inorganic mercury into kidney and motor neurons of mice. The uptake of mercury into the female kidney is much lower than that into the male kidney whereas inorganic mercury uptake by female motor neurons is 1.7 times greater than that in males. A smaller accumulation of mercury in the kidney of female mice may result in more circulating mercury which is available to enter muscle and taken up by distal motor axons (Pamphlett et al., 1997).

B. Liver

1. Anions. Gender-associated differences in hepatic transport have been described for organic anions such as sulfobromophthalein (BSP), thymol blue, bilirubin, indocyanine green, tetrabromosulfonephthalein (TBS), and fatty acids. These organic anions are transported to a greater extent into hepatocytes isolated from the livers of female rats than male rats (Orzes et al., 1985; Sorrentino et al., 1988; Torres, 1996).

Marked differences have been reported for the hepatic uptake of a low concentration of BSP between male and

female rats, both in intact animals and in isolated liver preparations and hepatocytes. The uptake rates of BSP in perfused livers, as well as the fractional plasma BSP disappearance rate, are significantly higher in females than in males. The kinetic constants of the low affinity sites are not different between genders whereas the K_m of the high affinity uptake sites in females is significantly lower than that in males with no difference in V_{max} , suggesting that this may be due to a different structural arrangement of the transporter or to a different membrane environment at the sinusoidal domain (Orzes et al., 1985). TBS liver uptake rate *in vivo*, as well as in sinusoidal liver membrane vesicles, is greater in female rats. V_{max} values for TBS uptake in the membrane vesicles are similar between male and female rats while K_m values in males are significantly higher than that in females (5.5 ± 0.4 versus $17 \pm 4 \mu\text{M}$) (Fig. 2), suggesting that a difference in membrane transport rates may explain the greater accumulation or uptake of TBS in female hepatocytes (Torres, 1996).

Uptake of the glutathione conjugate of sulfobromophthalein (BSP-GSH) at steady state in single-pass liver perfusion studies is increased in female livers compared with male livers. The apparent V_{max} is 48% larger in females whereas the apparent K_m is similar in both sexes. The ratio of influx to efflux, which determines the equilibrium partition of BSP-GSH between the hepatocyte cytosol and plasma compartments, is significantly greater in females with no sex difference in the rate constant of biliary excretion. It has been suggested that these findings indicate that a less negative plasma membrane electrical potential in female livers may provide a more favorable electrochemical driving force for the

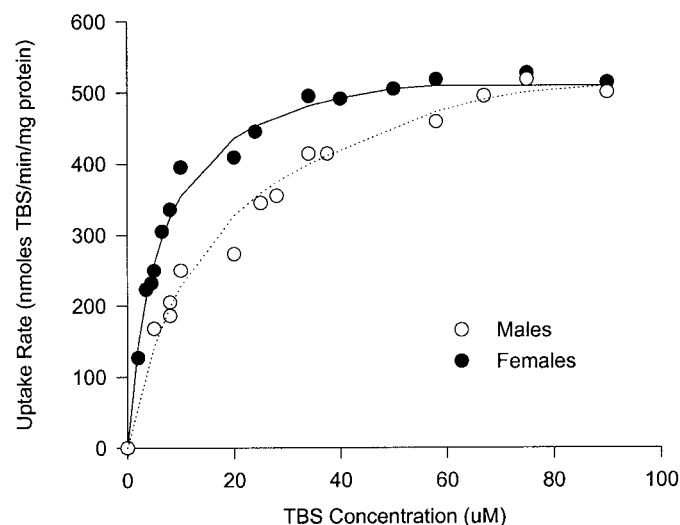


FIG. 2. Kinetics of TBS uptake in sinusoidal liver plasma membrane vesicles from male and female rats. Both curves represent the result of a typical experiment. V_{max} values for TBS uptake are comparable for male and female rats (581 ± 60 versus 544 ± 15 nmol/min/mg of protein, mean \pm S.D., $n = 3$); however, the K_m values for TBS uptake in males are significantly higher than in females (17 ± 4.0 versus $5.5 \pm 0.4 \mu\text{M}$, mean \pm S.D., $n = 3$, $p < 0.05$). Adapted from Torres, 1996 with permission from Elsevier Science.

movement of BSP-GSH into the hepatocytes in females (Sorrentino et al., 1988).

Initial oleate uptake velocity in hepatocytes isolated from female rats is also significantly greater than that from male rats. This may be due to a greater affinity of the transport system for oleate in females since no differences are observed in the V_{max} value for hepatic oleate uptake as well as in the surface expression of plasma membrane fatty acid binding proteins between sexes (Sorrentino et al., 1992). Another fatty acid, palmitate, also exhibits a 2-fold higher steady-state uptake rate in livers of female rats compared with male rats (Luxon et al., 1998). Sex differences in the clearance of palmitate by human hepatocytes have been reported (Pond et al., 1996), with hepatocytes isolated from females exhibiting a 2-fold higher clearance.

Although many organic anions are transported to a greater extent by female hepatocytes, sodium-dependent taurocholate uptake is greater in male rats with a significantly higher V_{max} value reported (Simon et al., 1999). Hepatic uptake of taurocholate, the major bile acid, is mainly mediated by the sodium-dependent taurocholate transporter (Ntcp) and to a lesser extent by Oatp. The initial uptake of sodium-dependent taurocholate uptake is shown over a range of concentrations (Fig. 3A). At every concentration, taurocholate uptake was greater in male hepatocytes. Suggested mechanisms that underlie the increased taurocholate transport in male hepatocytes are the greater expression of Ntcp (2-fold greater for both mRNA and protein levels) and the increased sinusoidal membrane fluidity (Lu et al., 1996). Simon et al. (1999) found that Ntcp, but not Oatp, protein content was significantly greater in males and that the expression of Ntcp was transcriptionally regulated. Hepatic Ntcp mRNA levels from female rats were $54 \pm 4\%$ of the value in males (Simon et al., 1999) (Fig. 3B). Female sinusoidal membranes had decreased fluidity (motional order) compared with male membranes, although bile canaliculi membranes were not different. Liver sinusoidal membranes isolated from female rats exhibited changes in their phospholipid/fatty acid composition, in that they had a significantly increased phosphatidylethanolamine-to-phosphatidylcholine ratio. This decreased membrane fluidity in female hepatocytes may be involved in lower hepatic taurocholate uptake in females (Simon et al., 1999).

The reduction in the hepatic transport of rifamycin SV is more pronounced in male patients than in female patients with Gilbert's syndrome. This more pronounced defect in hepatobiliary transport in male subjects may explain, at least in part, the greater frequency of Gilbert's syndrome, a pathological condition characterized by unconjugated hyperbilirubinemia, in males (Gentile et al., 1985).

Gender-related differences in the biliary excretion of the organic anion tartrazine, a food dye, has been reported in the rat (Bertagni et al., 1972). Male and female

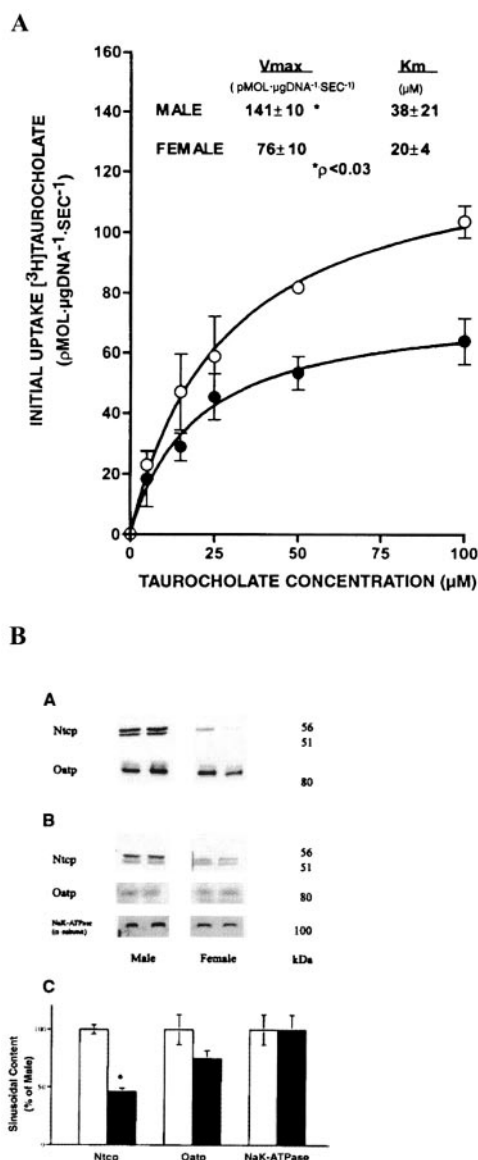


FIG. 3. Saturation kinetics for sodium-dependent taurocholate uptake into isolated rat hepatocytes. A, hepatocytes were isolated from male (\circ) and oophorectomized (ovx) female (\bullet) rats, and initial uptake of [3 H]taurocholate was measured at 60 s in the presence and absence of sodium. These data were used to estimate the maximal uptake (V_{max}) and the Michaelis-Menten constant (K_m) values. Results are means \pm S.E. of three independent experiments in each group. B, immunoblot analysis of sinusoidal membrane proteins from liver homogenates (A) and liver sinusoidal membrane fractions (B), from male and female rats. Liver sinusoidal proteins were identified using specific antibodies. Male (\square) and female (\blacksquare) hepatic steady-state levels of Ntcp, Oatp, and Na $^+$ -K $^+$ -ATPase are shown in panel C. Male levels were set at 100%. Results are mean \pm S.E. of 4 to 12 separate determinations. *, $p < 0.01$. Reprinted with permission (Simon et al., 1999).

rats excrete 13 and 29%, respectively, of an intravenous dose of tartrazine by biliary excretion. Treatment of male rats with estradiol increased the excretion from 14 to 33% of the dose, although treatment of female rats with testosterone decreased the biliary excretion from 31 to 16%. Gender-related differences in the biliary excretion of *S*-ketoprofen have also been reported (Palylyk and Jamali, 1994). In male rats, the major route of elimination is by biliary excretion of the glucuronide

conjugate, whereas in the female rat, the major route of elimination is renal clearance of the conjugate. This results in a marked difference in the amount of *S*-keto-profen glucuronide eliminated in the urine in female and male rats. There are gender differences in the ATP-dependent canalicular transport of dinitrophenyl-glutathione conjugate (Srivastava et al., 1999). Transport is higher in membrane vesicles isolated from male mice compared with female mice. Additionally, whereas only one transport system is present in male mouse cLPM for the transport of dinitrophenyl-glutathione, there is both high and low affinity systems present in cLPM isolated from female mice. The ATP-dependent transport of organic anions, including glucuronide and glutathione conjugates, occurs by multidrug resistance-associated protein 2 (MRP2), also known as the canalicular multi-specific organic anion transporter (cMOAT). MRP2 is the major transporter responsible for secretion of bilirubin glucuronides into bile; gender differences in the expression of MRP2 have not been examined.

2. *Cations.* Pgp is present on the canalicular membrane of hepatocytes and involved in the biliary excretion of phospholipids, cholesterol, and a wide variety of lipophilic organic cations. Hepatic expression of the gene product of *mdr2* in female rats is 7-fold higher than in male rats (Furuya et al., 1994). This isoform of Pgp is mainly involved in phospholipid transport across the canalicular membrane. Piquette-Miller et al. (1998) and Salphati and Benet (1998) reported higher levels of total *mdr* gene products in female rat livers compared with male livers. Gender differences in *mdr* mRNA levels were also seen. Male livers contained more than 2-fold higher levels of *mdr1b* and female livers contained higher levels (approximately 35–50%) of *mdr1a* and *mdr2* (Piquette-Miller et al., 1998; Salphati and Benet, 1998). In humans, hepatic Pgp (total) protein expression is 2-fold higher in men than in women (Schuetz et al., 1995), suggesting that the drug disposition of Pgp substrates could be different between genders, resulting in differences in drug efficacy and toxicity between males and females. Interestingly, there are gender-related differences in Pgp expression and functional activity in peripheral blood samples of subjects with B-type chronic lymphocytic leukemia, with significantly more men (89%) than women (48%) being MDR1 phenotype-positive (Steiner et al., 1998). These findings are consistent with the overall better prognosis for women with chronic lymphocytic leukemia than for men (Steiner et al., 1998).

C. Intestine

Very little is known regarding gender-related differences in intestinal uptake and drug bioavailability. Clinical studies have reported an increased bioavailability of both iron and ethanol in women but these gender-related differences likely do not involve differences in intestinal transporters. For ethanol, the increased bio-

availability is likely due to decreased gastric metabolism of alcohol in women (Lieber et al., 1994). Decreases in both the rate and extent of absorption of acetaminophen occur in late pregnancy; this is likely due to decreases in the rate of gastric emptying (Galinsky and Levy, 1984).

A gender-related difference has been documented in the transport of calcium in the intestine. Kinetic analysis of calcium transport across the rat intestine has shown that there are two transport processes, one of which is saturable and the other nonsaturable. The saturable transport process is regulated by vitamin D and is predominantly located in the proximal intestine whereas the nonsaturable process is not vitamin D-dependent and has similar capacity throughout the intestine (Bronner et al., 1986). Intestinal calcium transport is significantly greater in male rats than in female rats in a vitamin D-sufficient condition, although it is comparable between sexes in the presence of vitamin D deficiency. Vitamin D deficiency produces a markedly lower intestinal transport of calcium in male rats but not in female rats. This observation suggests that calcium transport in the intestine of female rats, unlike male rats, is mediated by a vitamin D-independent mechanism at the calcium intake levels studied in this investigation (Umland-Smith and DeLuca, 1993).

Total intestinal absorption of calcium is enhanced during pregnancy and lactation in vitamin D-deficient rats. Intestinal calcium absorption during the rat estrous cycle is highest during estrus and lowest during diestrus following the administration of both high and low calcium diets. Since the highest serum levels of estradiol, progesterone, prolactin, follicle-stimulating hormone, and luteinizing hormone are present during estrus (Butcher et al., 1974; Brommage et al., 1990), the greatest intestinal absorption of calcium observed during estrus may be related, either directly or indirectly, to any one of several sex hormones (Brommage et al., 1993). Intestinal mucosal cells contain estrogen receptors, and calcium uptake in duodenal cells is significantly enhanced by about 60% by 17β -estradiol at a concentration of 10 nM (Arjmandi et al., 1993). Administration of 17β -estradiol at a dose of 40 $\mu\text{g}/\text{kg}$ b.wt./day for 21 days significantly elevated intestinal absorption of calcium in female rats whereas serum levels of 1,25-dihydroxyvitamin D were unaltered (Arjmandi et al., 1994). These findings suggest that transluminal calcium uptake is promoted by a direct action of 17β -estradiol on the intestinal tract with no increase in the circulating levels of 1,25-dihydroxyvitamin D (Arjmandi et al., 1993, 1994).

The implications of these observations are as follows. First, estrogen may play an important physiological role in regulation of intestinal calcium absorption. High estrogen levels during pregnancy and estrus may promote calcium absorption, and estrogen deficiency in menopause may result in calcium malabsorption by a direct action on the intestine. The malabsorption of calcium in

the intestine as a result of ovarian hormone deficiency in postmenopausal women is often associated with osteoporosis characterized by bone loss (Heaney et al., 1978; Gallagher et al., 1979, 1980; Gallagher, 1990). Second, the rate and extent of intestinal calcium absorption may be modulated by compounds that block or mimic estrogen action.

Aluminum, at a concentration of 2 μM , significantly decreases mucosa-to-serosa calcium influx in duodenal everted sacs both of male and female rats compared with aluminum-free controls; however, the percentage of reduction in females (31.2%) is greater than that in males (17.8%). The sensitivity to the inhibitory effect of aluminum on duodenal calcium flux is raised with increasing serum levels of 17 β -estradiol in ovariectomized female rats with no alterations in the maximal response, whereas the effect of aluminum on calcium flux in duodenal sacs is not dependent of serum testosterone levels in castrated male rats injected with testosterone. These results demonstrate that there are gender-associated differences in the inhibitory effect of aluminum on trans-luminal calcium transport in the duodenum of the rat (Orihuela et al., 1996).

D. Brain

There have been few studies that have examined the potential for gender-related differences in transport across the blood-brain barrier (BBB). 17 β -Estradiol treatment of ovariectomized rats increases 2-deoxyglucose uptake into brain, which is likely due to the increase in the mRNA and protein expression of glucose transporter 1 (GLUT-1) in the BBB epithelium (Shi and Simpkins, 1997). These results support a modulatory role for estrogens in the brain transport of glucose.

Both Pgp and MRP1 are present in the BBB epithelium and are responsible for the active efflux of drugs from the brain, minimizing brain exposure to many organic anions and cations. Although Pgp exhibits gender differences in expression in liver, this has not been examined for the BBB. Gender differences in the BBB uptake of verapamil have been reported in mice where female mice have increased functional Pgp activity, resulting in decreased verapamil influx into the brain (Dagenais et al., 2001). However, gender differences in uptake were not observed for two other Pgp substrates, morphine or quinidine (Dagenais et al., 2001), so the significance of these findings is unknown.

The effect of gender on the reuptake of dopamine (DA) by the sodium-dependent DA transporter into nerve terminals, the primary mechanism for inactivation of DA following its release into the synapse, has been examined. An increased synaptosomal DA reuptake in the anterior hypothalamus is observed in ovariectomized rats treated with estradiol due to an increase in the number of DA uptake binding sites (Cardinali and Gomez, 1977). The maximal binding density (B_{max}) of striatal DA uptake sites is significantly elevated 15 and 30

min after an injection of a physiological dose of 17 β -estradiol in ovariectomized rats with no change in the binding affinity (K_d) of the DA uptake sites. There is no effect of progesterone on striatal DA uptake after progesterone treatment of ovariectomized rats. The increase of DA uptake binding sites by the administration of 17 β -estradiol is rapid and short-lasting and is associated with peak 17 β -estradiol plasma levels, suggesting most likely a membrane-linked nongenomic effect of 17 β -estradiol (Morissette et al., 1990). When ovariectomized rats are chronically treated with 17 β -estradiol and/or progesterone at pharmacological doses, DA uptake site density in the striatum is significantly increased by 16 to 23% without an alteration in the binding affinity, most likely due to an increased synthesis of the DA transporter by a genomic effect of these female sex hormones. In addition, chronic exposure to 17 β -estradiol and/or progesterone up-regulates the DA uptake sites in the nigrostriatal dopaminergic pathway whereas the nucleus accumbens and the substantia nigra pars reticula are not affected (Morissette and Di Paolo, 1993a). Striatal DA uptake site density is significantly lower in normal male rats, gonadectomized male rats, and ovariectomized female rats compared with normal female rats and fluctuates during the female estrous cycle with a peak occurring in the morning of proestrus when estradiol is elevated and progesterone is low, suggesting an up-regulation of striatal DA uptake sites by estradiol (Morissette and Di Paolo, 1993b). This agrees with the findings of an investigation examining the effect of acute treatment with 17 β -estradiol (Morissette et al., 1990). It has also been shown that 17 β -estradiol increases DA uptake in mesencephalic neurons isolated from females but not in male neurons, and male sex hormones, testosterone and dihydrotestosterone, have no effect (Engele et al., 1989). In humans, DA and serotonin (5-hydroxytryptamine; 5-HT) transporter availability is greater in females compared with males, as determined by single photon emission computed tomography imaging using an analog of cocaine (CIT) that labels DA and 5-HT transporters (Staley et al., 2001). Therefore, gonadal hormones may play an important role in the effects of psychoactive drugs acting on neuronal DA uptake sites and modulation of the DA transporter by these hormones will represent a source of interindividual variability in the treatment of neuropsychiatric disorders and neurologic diseases such as Parkinson's disease (Cardinali and Gomez, 1977; Engele et al., 1989; Morissette et al., 1990; Morissette and Di Paolo, 1993a,b).

A sexual dimorphism in the density of norepinephrine transporters has been demonstrated in the frontal cortex of rats, with males having significantly fewer binding sites than females, whereas the binding affinity of the uptake sites was not different between genders (Vathy et al., 1997). 5-HT uptake in the anterior and middle hypothalamus of intact female rats exceeds sig-

nificantly that in intact male rats (by about 30–40%) and is similar to that in neonatally castrated adult rats (Fig. 4), suggesting that androgens may play a key role in the development of the hypothalamic serotonergic system over the neonatal period by inhibiting either the serotonergic axon ingrowth to the hypothalamus or the ramifications of the axonal terminal portions (Borisova et al., 1996). Estradiol treatment stimulates a significant increase in the density of 5-HT_{2A} binding sites in the anterior frontal, anterior cingulate and piriform cortex, the olfactory tubercle, the nucleus accumbens and the lateral dorsal raphe nucleus, areas of brain concerned with cognition, emotion, and motor control, suggesting that the antidepressant action of estrogen may be mediated by a serotonergic mechanism (Fink et al., 1996).

E. Other Tissues

The rate of glucose uptake in skeletal muscle, under hyperinsulinemic and normoglycemic conditions, is significantly greater in women than in men (Fig. 5), suggesting an increased sensitivity to insulin in women (Nuutila et al., 1995). Basal and maximal insulin-stimulated glucose transport is also significantly higher in adipocytes isolated from female rats and human female subjects compared with males (Foley et al., 1984). In skeletal muscle, fatty acid transport protein-1 (FATP-1) mRNA levels are higher in lean women than in lean men (2.2 ± 0.1 versus 0.6 ± 0.2 attomoles/ μg of total RNA, $p < 0.01$). FATP-1 mRNA was significantly decreased in skeletal muscle of obese women, but no change in FATP-1 expression was seen in men. Additionally, insulin infusion reduced FATP-1 mRNA in muscle of lean women, but not in men (Binnert et al., 2000). This study indicates that lean women may be able to utilize lipids to a greater extent than men, although whether differences

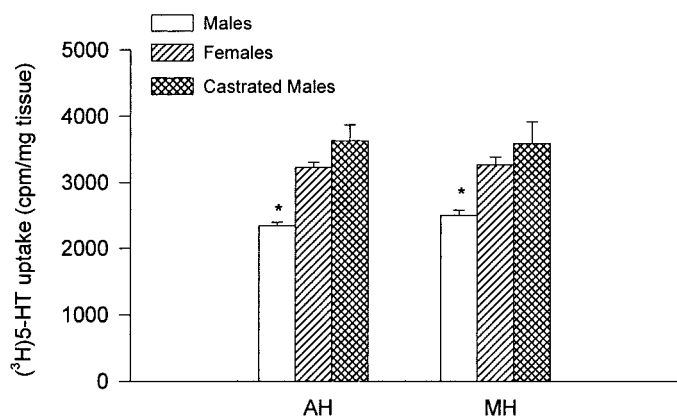


FIG. 4. Specific uptake of radioactively labeled serotonin ($[^3\text{H}]5\text{-HT}$) by the anterior (AH) and middle hypothalamus (MH) in adult male, adult female, and neonatally castrated males. Specific uptake is the difference of $[^3\text{H}]5\text{-HT}$ uptake in the absence and presence of citalopram 10^{-5} M. The columns represent the values for 10 to 25 rats. *, $p < 0.001$ compared with the levels in females and castrated males. Adapted with permission from Borisova et al., 1996.

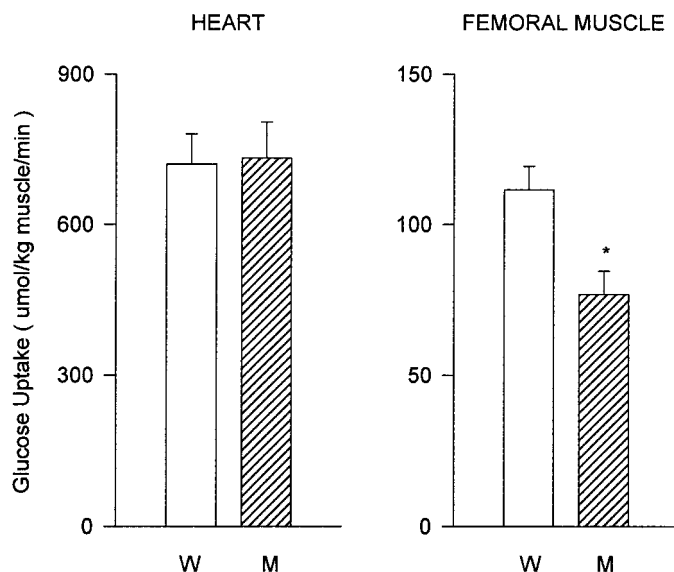


FIG. 5. Rates of glucose uptake in the heart and femoral muscles (micromoles per kilogram of muscle per minute) in normal women (W) and men (M). Insulin sensitivity of glucose uptake was determined in heart and muscle tissues using positron emission tomography under hyperinsulinemic and normoglycemic conditions. *, $p < 0.01$ compared with women. Adapted with permission from Nuutila et al., 1995.

in FATP-1 mRNA result in corresponding differences in FATP-1 protein expression is not known.

A marked difference in the splanchnic uptake of chylomicron triglyceride is observed between men and women. Chylomicron uptake in the splanchnic tissues in men and women accounts for 71% and 20% of meal triglyceride disposal, respectively, indicating greater meal fatty acid storage in visceral adipose tissue in men and gender-specific differences in body fat distribution (Nguyen et al., 1996).

III. Conclusions

Gender differences in the transport of numerous drugs and endogenous substrates exist in animals and humans. Sex-associated differences are described for renal tubular secretion of organic anions and cations, hepatic uptake of taurocholate and organic anions including endogenous compounds, intestinal calcium transport, and Pgp-mediated and neurotransmitter transport in the brain. 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. In addition, gonadal hormones can be used to treat neurologic diseases and neuropsychiatric disorders by modulating the DA uptake sites in the brain.

Gender differences in membrane transport in humans are not always consistent with differences reported in animal studies. For example, Pgp, an ATP-dependent efflux pump present in cancer cells and excretory or-

gans, demonstrates a higher hepatic expression in men than in women (Schuetz et al., 1995), but opposite changes have been reported in rats (Furuya et al., 1994; Piquette-Miller et al., 1998). In addition, gender differences in the urinary excretion of zenarestat are observed in mice and rats but not in dogs and humans (Tanaka et al., 1992). This emphasizes the importance of performing studies in humans to evaluate the effect of gender.

Gender-associated differences in the nature and prevalence of many diseases may be explained, at least in part, by the differences in the transport processes of substrates between male and female subjects. In addition, these gender-related differences in transport systems may be responsible, at least in part, for interindividual variability in drug disposition, therapeutic response, and drug toxicity. Research is needed to evaluate potential gender differences in regulation, expression, and activity of known transport proteins involved in the uptake or secretion of both endogenous and exogenous compounds.

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