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Application of the Isolated Perfused Rat Kidney Model to Assess Gender Effects on Drug Excretion

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ABSTRACT **Purpose.** To study the effect of gender on the renal disposition of two organic anions, p-aminohippuric acid (PAH) and furosemide (FSM) in the isolated perfused rat kidney (IPK). **Methods.** IPK experiments (3–4 per treatment group) were conducted using kidneys from male and female Sprague Dawley rats. PAH was administered as a continuous infusion (with loading dose, targeted steady-state concentration 10 ug/mL). FSM was added as a bolus dose (2.65 mg, targeted concentration 33 ug/mL). Urine was collected in 10-min. intervals and perfusate was sampled at the midpoint of each collection period. Control (drug naïve) perfusions were performed for both genders. PAH and FSM were measured by HPLC. Kidney viability (GFR [estimated using inulin clearance], sodium reabsorption, glucose reabsorption) was monitored continuously during each perfusion experiment (2-h duration). **Results.** Good kidney function was maintained across all study groups, and lower GFR estimates in female kidneys were due to differences in kidney weight. For PAH, kidney weight corrected renal clearance (0.88 ± 0.37 mL/min/g vs. 0.59 ± 0.19 mL/min/g) and excretion ratio (3.8 ± 1.7 vs. 2.2 ± 0.72) were significantly higher in male kidneys. For FSM, renal clearance was significantly lower in female (0.10 ± 0.05 mL/min/g) compared to male kidneys (0.15 ± 0.07 mL/min/g). Mass balance analysis showed that FSM cumulative urinary excretion was significantly higher and kidney accumulation was significantly lower in experiments with male kidneys. **Conclusions.** The study demonstrates that the IPK is a useful model to assess gender effects on renal drug disposition. The renal excretion of organic anions is reduced in female rats, possibly due to gender differences in expression and/or activity of membrane transporters (both basolateral and luminal) in the kidney.

INTRODUCTION

The kidney is a primary organ of drug clearance from the body. Most medications are eliminated from the body through renal excretion in the form of unchanged drug or metabolites. There are three mechanisms of renal drug handling: glomerular filtration, tubular secretion, and tubular reabsorption (Tucker, 1981). Tubular secretion (and in some cases tubular reabsorption) is an active,

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carrier-mediated process. Membrane-bound transporters are responsible for the translocation of xenobiotics across the basolateral and luminal membranes of the kidney cell (Inui et al., 2000; Kusuhara & Sugiyama, 2002). As a result, active tubular transport contributes to the cellular accumulation and urinary excretion of medications. This can lead to nephrotoxic effects of compounds such as antiviral agents (Lalezari et al., 1997; Kahn et al., 1999) and antibiotics (Tune, 1997). Additionally, these kidney transporters are potential sites for significant drug-drug interactions in vivo.

Over the past decade, significant progress has been made in the cloning, functional expression, and characterization of transporters in the kidney (Fig. 1). Numerous organic cation transporters and organic anion transporters have been cloned (Dresser et al., 2001; Burchardt & Burckhardt 2003; Robertson & Rankin 2005). The organic anion transporter family (OATs) is a group of multispecific organic anion transporters with known substrates that include both endogenous and exogenous compounds. Classes of medications known to be substrates for OATs include diuretics, antibiotics, antivirals, and nonsteroidal anti-inflammatory agents (Sekine et al., 2000; Burckhardt & Burckhardt, 2003).

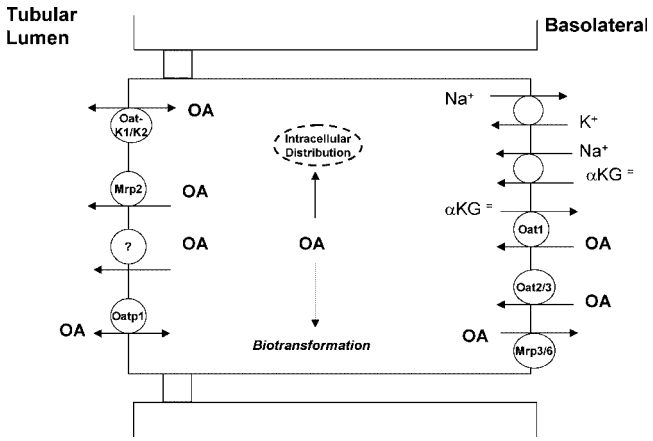

FIGURE 1 **Schematic Representation of Membrane Transport of Organic Anions Across Renal Proximal Tubule Cells (Pritchard & Miller, 1997; Masereeuw & Russel, 2001; Kusuhara & Sugiyama, 2002). Basolateral Transport Mediated by OAT1 and Involves Counter-Transport With α-ketogluturate (α-KG). Other Basolateral Transporters That Have Been Identified Include OAT2, OAT3 and MRP 3/6 (Efflux Transport System). Once Inside the Tubular Cell, There Is a Possibility of Cytoplasmic Binding or Distribution into Intracellular Compartments. Biotransformation Pathways Also Exist Within the Kidney. Luminal Transport of Organic Anions Is Electrogenic and Involves Facilitated Transport. Several Transporters Have Been Identified Including MRP2 OAT-K1/K2 and OATP1 (Bi-directional Transporters). Additional Transport Systems Likely Participate in Organic Anion Luminal Transport (e.g., OAT4), Designated as (?) in the Fig.**

The effect of gender on drug disposition is an important issue for drug development. Sex differences in drug disposition and activity have been the subject of recent reviews (Meibohm et al., 2002; Schwartz, 2003). Differences in pharmacokinetics between males and females are the result of biological differences between genders. These differences include body weight, body composition, and hormones. Additionally, it appears that sex-related differences in membrane transporter expression and activity are an underlying cause of these differences (Morris et al., 2003). In terms of renal drug excretion, differences in renal elimination between males and females may have important clinical consequences.

A number of reports in the literature have addressed gender effects on organic anion disposition by the kidney. The clearance of both p-aminohippuric acid (PAH) and furosemide (FSM) is significantly lower in female rats (Cerutti et al., 2002). Studies comparing orchiectomized and normal rats ascribed gender differences in renal excretion to hormonal regulation of transporter expression in the kidney (Reyes et al., 1998). Recent studies have demonstrated differential expression of rat kidney OATs between genders. Basolateral membrane expression of both OAT1 and OAT3 are increased in male rats, although expression is reduced in castrated and hypophysectomized rats (Ljubojevic et al., 2004). In contrast, female rats (and castrated males) show increased expression of OAT2 in the kidney (Buist et al., 2002).

The isolated perfused rat kidney (IPK) model is an established model to study numerous aspects of renal drug disposition. Among the available ex vivo methods to study renal transport, the IPK allows for elucidation of the overall contributions of renal transport mechanisms on drug excretion (Bekersky, 1983; Taft, 2004). Therefore, IPK studies can provide a bridge between in vitro findings and in vivo disposition. Over the years, the model has been used to elucidate mechanisms of drug excretion, to screen for clinically significant drug interactions, to study renal drug metabolism, and to correlate renal drug disposition and kidney function. Among the other potential applications of the IPK, gender effects on drug disposition are of particular interest. Conducting perfusion experiments with kidneys from both male and female donor rats makes this an ideal technique for studying gender effects on renal disposition.

The overall objective of the research was to assess gender differences in organic anion renal excretion in the IPK model. The renal disposition of organic anions in male and female rats was studied using two

probe compounds: para-aminohippuric acid (PAH) and furosemide (FSM). These compounds are well-established markers of organic anion transport by the kidney, and gender differences in the excretion of these compounds have been reported previously. This study aims to demonstrate the utility of the IPK as a model for studying sex differences in drug excretion.

METHODOLOGY

Chemicals

PAH, FSM, *p*-aminobenzoic (PABA) acid, bovine serum albumin (Fraction V), clinical grade dextran, inulin (from chicory root), potassium chloride, sodium chloride, sodium bicarbonate, magnesium sulfate, calcium chloride, glucose, sodium pentobarbital, sulfadiazine, anthrone, zinc sulfate ($ZnSO_4$*$7H_2O$) and L-amino acids were obtained from Sigma-Aldrich Company (St. Louis, MO). HPLC-grade acetonitrile and water were purchased from EM chemicals (Gibbstown, NJ). Sulfuric acid and monobasic potassium phosphate were purchased from VWR Scientific (West Chester, PA).

Animals

Male and female Sprague Dawley rats (275–350g) were used for perfusion experiments. Rats were purchased from Harlan Sprague Dawley (Indianapolis, IN) and housed in stainless steel cages at the animal housing facility of Long Island University. They were fed a standard diet including water ad libitum. The Institutional Animal Care and Usage Committee (IACUC) of Long Island University approved protocol for this investigation.

Isolated Perfused Rat Kidney Preparation

The surgical technique employed was the Bowman (1975) modification of the Nishiitsutsuji-Uwo procedure (Nishiitsutsuji-Uwo et al., 1967). Anesthesia was induced with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A midline incision was made and the renal segment of the aorta exposed. A ligature was passed under the right renal artery close to the aorta, and distal and proximal ligatures placed around the superior mesenteric artery. The right ureter was catheterized with PE-10 tubing, in order to facilitate urine collection. A cannula was then threaded through the mesenteric artery, across the aorta, and into the right renal artery in situ. The ligatures were tied, securing the cannula in place. The right kidney was then excised from the animal, trimmed of adhering tissue and transferred to the in vitro recirculating perfusion apparatus (Fig. 2).

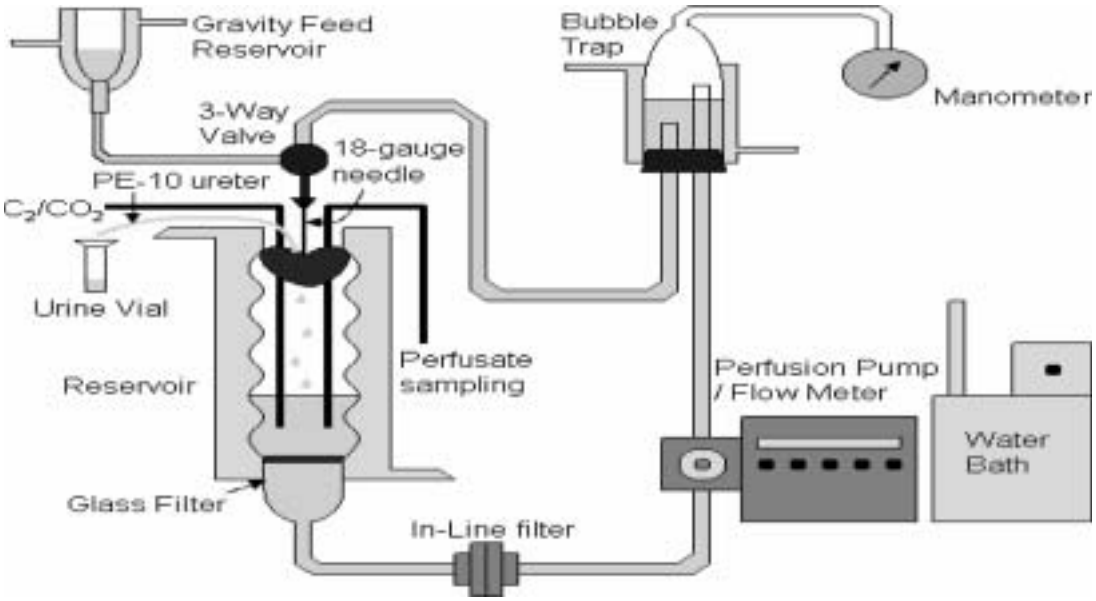

FIGURE 2 **Schematic Representation of a Recirculating IPK Apparatus (Reprinted from Taft, 2004, With Permission From Bentham Science Publishers).**

Perfusion of the kidney proceeded according to the method described by Bowman (1978). Perfusate consisted of the following: Krebs-Henseleit buffer (pH 7.4), 4.00 % BSA, 1.67% clinical-grade dextran, 100 mg/dL glucose, 60 mg/dL inulin and amino acids. The volume of the recirculating perfusate was 80 mL.

Study Groups

Perfusion experiments (n=3-4/group) were conducted with kidneys obtained from both male and female donor rats. A total of six study groups were utilized in this research. Control perfusions (drug naïve) were conducted using kidneys obtained from both sexes to determine the effect of gender and/or drug administration on kidney function. Additionally, the renal excretion of PAH and FSM were evaluated separately in both males and females.

Following administration of a bolus dose (800 ug), PAH was continuously infused throughout the perfusion experiment, targeting a steady-state plasma concentration of 10 ug/mL. The infusion rates (15 ug/min in females, 23 ug/min in males) were optimized through a series of preliminary IPK studies (bolus dose only) using kidneys from both sexes (data not shown). For FSM, studies were conducted using a bolus dose (2650 ug) targeting an initial perfusate concentration of 33.13 ug/mL (100 uM). This concentration has been used in previous IPK studies with this compound (Lee et al., 1986).

Study Design

Following kidney excision and transfer to the recirculating perfusion system, a stabilization period (10 min.) preceded any pharmacokinetic experimentation. Drug was then added, and perfusate (0.8 mL) sampled initially at 5 min. and every 10 min. thereafter. Urine was collected in 10-min. intervals for a total of 90 min. post-dose. Volume lost due to sampling or urine excretion was replaced as needed with a 50:50 mixture of perfusate and deionized water. All samples were stored frozen at –20°C prior to analysis.

Aliquots of both perfusate and urine were analyzed for sodium and glucose using a Beckman Synchron CX-3 Autoanalyzer (Brea, CA). Inulin was measured using a non-radioactive colorimetric method (Poola et al., 2002). Parameters monitored to assess kidney function throughout an IPK perfusion included glomerular filtration rate (GFR, estimated as inulin clearance), fractional reabsorption of glucose (FR_{GLU}), fractional reabsorption of sodium (FR_{Na}), urine flow rate, and urine pH. Perfusion pressure was maintained at 100 ± 10 mm Hg by adjusting perfusion flow rate as necessary. Perfusion experiments that did not meet minimum viability criteria (Taft 2004) were repeated.

Protein Binding Determination

The perfusate binding of PAH and FSM was determined by ultrafiltration. Aliquots of perfusate containing varying concentrations of drug were incubated at 37°C under constant stirring for 30 min to ensure binding equilibrium. The drug concentrations chosen for PAH (1–25 ug/mL) and FSM (1–33 ug/mL) encompassed the range of values expected from IPK studies. After incubation, a 500-ul aliquot of sample was collected for determination of total drug perfusate concentration. A second aliquot (1 mL) was transferred to an Amicon Centrifree™ Micropartition system (Millipore Corporation, Billerica, MA) and centrifuged at 1500 × g for 10 min. The resultant ultrafiltrate containing free drug was stored at –20°C prior to analysis. The fraction of drug unbound in perfusate (f_u) was estimated as the ratio of unbound and total drug concentrations.

Analytical Method for PAH and FSM

PAH and FSM was measured in all samples using a validated HPLC method. The HPLC system consisted of a system controller (SCL-6B, Shimadzu Scientific Instruments, Columbia, MD), an auto-injector (SIL-6B, Shimadzu Scientific Instruments), and a scanning ultraviolet detector (SPD-10AV UV-Vis, Shimadzu Scientific Instruments). Output was processed using a Hewlett-Packard personal computer with Turbochrome? integration software (version 4.0, Perkin Elmer Instruments, Norwalk, CT) and a PE Nelson 900 series interface. For detection of FSM, a fluorescence detector (RF-10AXL, Shimadzu Scientific Instruments) was used.

HPLC Assay for PAH

The HPLC method used has been previously described (Savant et al., 2001). PABA was used as an internal standard. Separation was accomplished using

a Waters μBondapak Phenyl Column (3.9 × 300 mm, 10 um particle size). The mobile phase consisted of 0.1 M acetic acid and acetonitrile (99:1). The detection wavelength and mobile phase flow rate were 254 nm and 1 mL/min, respectively. Reference standards were prepared in perfusate and KHS buffer (for urine analysis) over a concentration range of PAH from 1 to 25 ug/mL.

The sample preparation procedure is described as follows: 25 uL of internal standard solution (PABA 0.5 mg/mL) was added to a 500 μL aliquot of sample (perfusate, urine). 500 μL of zinc sulfate (1% w/v) was added, and the mixture was vortexed for 1 min. and centrifuged at 8000 × g for 10 min. 100 uL of the resultant supernatant was injected into the HPLC system.

HPLC Assay for FSM

Samples (perfusate, urine) collected from perfusion experiments were analyzed for FSM using a previously published assay (Smith et al., 1980). The mobile phase consisted of 0.015M phosphoric acid (H_3PO_4) and acetonitrile (65:35). Separation was achieved using Waters μBondapak-C18 reversed phase column (30-cm × 3.9-mm i.d). FSM was analyzed by fluorescence. The excitation and emission wavelengths for FSM were set at 345 and 405 nm, respectively. The mobile phase flow rate was 2 mL/min.

Reference standards were prepared in perfusate and KHS buffer over a range of concentrations from 50 to 5000 ng/mL. Since the expected concentration range for FSM in IPK experiments was roughly 10 to 33 μg/mL, IPK samples were diluted 30-fold prior to analysis. To each perfusate or urine sample (100 uL) 200 ul of acetonitrile was added (for precipitation of protein). The mixture was then vortexed for 30 s, and centrifuged at 10,000 rpm for 10 min. One hundred microliters of the resultant supernatant were then diluted ten times with water, and 50 uL of the diluted mixture was injected into the HPLC system.

Data Analysis

For each urine collection period, renal clearance (Cl_R) was calculated as the ratio of the rate of urinary excretion and perfusate concentration (sampled at the midpoint of urine collection). Filtration clearance (Cl_{FILT}) was estimated as the product of fraction of drug unbound in perfusate (f_u) and GFR. Secretory clearance (Cl_{SEC}) was calculated as the difference between Cl_R and Cl_{FILT} (assuming no drug reabsorption by the kidney). For PAH studies, clearance was also estimated as the ratio of infusion rate and perfusate concentration at steady state (denoted Cl_{PERF}). Given the differences in body weight between genders, these parameters were normalized for kidney weight.

In addition to the above calculations, excretion ratio (XR), an indicator of the net mechanism of excretion, was calculated as a ratio of Cl_R and CL_{FILT}. An XR value of less than one indicates a mechanism of filtration with net reabsorption; a value greater than one indicates filtration with net secretion. Renal accumulation of drug was estimated via mass balance analysis.

Statistical Analysis

Mean parameter estimates of IPK viability criteria for control and drug treatment groups were compared using analysis of variance (ANOVA). Dunnett's test was utilized to identify those study groups that differed from control perfusions in terms of viability criteria. Consequently, gender differences in kidney function were assessed in addition to alterations induced by drug administration. Mean values for drug excretion parameters between genders were compared using Student's t-test.

RESULTS

Table 1 summarizes the parameters used to assess kidney function in the IPK. Overall, good kidney function was maintained across all treatment groups. The data presented in the table are consistent with IPK viability indices reported in the literature (Statkevich et al., 1993; Sweeney et al., 1995; Poola et al., 2002). Higher GFR values in male compared to female kidneys were attributed to differences in organ weight. When GFR data were normalized by kidney weight, no significant differences were noted among treatment groups. The significant increase in urine flow rate in kidneys treated with FSM was consistent with the diuretic effects of the compound. This effect was more pronounced in perfusion experiments with female kidneys.

Plots of perfusate disposition and cumulative urinary excretion of PAH are provided in Fig. 3. As demonstrated in the figure, steady state was maintained

TABLE 1 **Estimates of IPK Viability Parameters: Comparison of Male and Female Rat Kidney Donors**

	Male Kidney Donors			Female Kidney Donors		
Parameter[a]	Control[b]	FSM[c]	PAH[c]	Control[b]	FSM[c]	PAH[c]
Perfusion Flow Rate(mL/min)	14 ± 1.2	20 ± 3.2*,**	15 ± 2.8	12 ± 0.73	10 ± 1.3**	12 ± 2.3
Urine Flow Rate (mL/min)	0.12 ± 0.03	0.15 ± 0.05*	0.12 ± 0.04	0.13 ± 0.05	0.19 ± 0.05*,**,***	0.15 ± 0.06*
Urine pH	7.4 ± 0.16	6.7 ± 0.28*	6.9 ± 0.16*	6.8 ± 0.12*	6.8 ± 0.23*	6.8 ± 0.12*
GFR (mL/min)	0.66 ± 0.23	0.84 ± 0.26*	0.78 ± 0.39	0.58 ± 0.17	0.61 ± 0.17***	0.71 ± 0.25**
GFR/KW[d] (mL/min/g)	0.40 ± 0.13	0.50 ± 0.19*	0.47 ± 0.28	0.43 ± 0.13	0.49 ± 0.17	0.47 ± 0.17
FR_{glu}[e]	0.96 ± 0.03	0.97 ± 0.03	0.96 ± 0.05	0.98 ± 0.01	0.96 ± 0.09	0.97 ± 0.04
FR_{Na}[f]	0.86 ± 0.07	0.84 ± 0.07	0.87 ± 0.09	0.80 ± 0.09	0.81 ± 0.10	0.83 ± 0.08

[a]data reported as mean (standard deviation) representing three or four perfusions per treatment group.
[b]represents control group (drug naïve perfusions).
[c]drug-treated perfusions with FSM (2650 ug, target concentration 33 ug/mL) or PAH (800 ug bolus dose & continuous infusion, target concentration 10 ug/mL).
[d]represents GFR is normalized per kidney weight.
[e]represents fraction of glucose reabsorbed by kidney.
[f]represents fraction of sodium reabsorbed by kidney.
*significantly different (p< 0.05) from the male control group.
**significantly different (p< 0.05) from the female control group.
***significantly different (p< 0.05) from the male FSM-treated group.

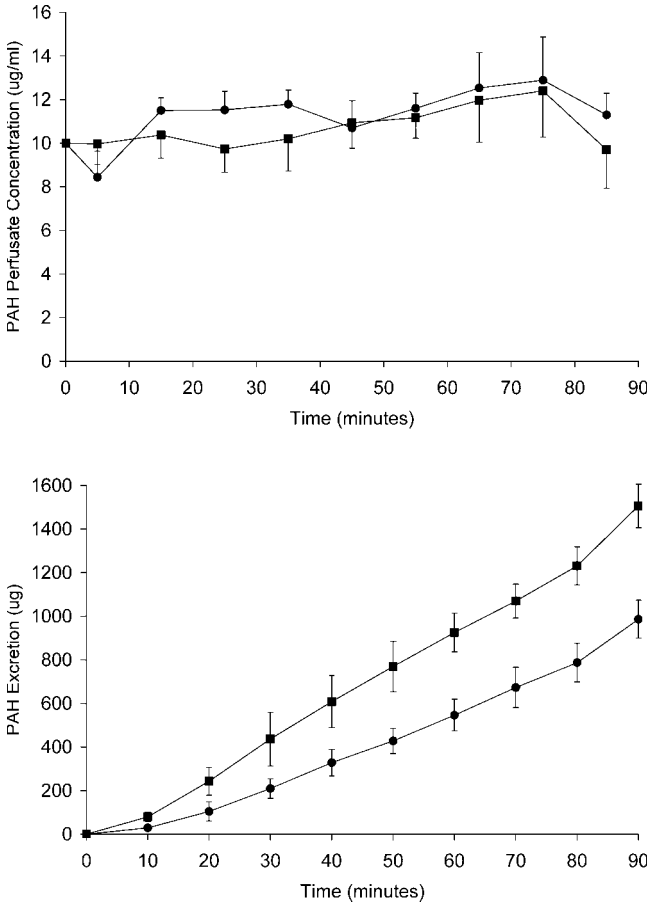

FIGURE 3 **Gender Differences in PAH Renal Excretion in the IPK. Upper Panel: Mean (SD) Perfusate Concentrations of PAH as a Function of Time. Lower Panel: Mean (SD) Cumulative Excretion of PAH as a Function of Time. PAH was Administered as a Continuous Infusion with a Loading Dose, Targeting a Steady-State Plasma Concentration of 10 ug/mL (● = data From Perfusion Experiments With Kidneys From Female Rat Donors; ■ = Data From Perfusion Experiments With Kidneys From Male Rat Donors).**

over the duration of the perfusion experiment. As described previously (in the Study Design section), the infusion rate of PAH was optimized in a series of preliminary studies (target steady-state level was 10 ug/mL), and higher infusion rates were utilized in perfusion experiments using male kidneys.

Estimates of PAH renal excretion parameters are presented in Table 2. Significant differences in PAH disposition were noted between male and female study groups. Total renal clearance was calculated two ways: 1) Cl_R, the ratio of urinary excretion rate and perfusate concentration 2) Cl_{PERF}, the ratio of infusion rate and perfusate concentration (assuming steady state). Using both approaches, PAH clearance was significantly higher in male kidneys. No differences in Cl_{FILT} were observed. However, XR and Cl_{SEC} were significantly greater in male perfusion experiments.

The results for IPK experiments with FSM are presented in Fig. 4 and Table 3. FSM is a highly protein-bound compound. Perfusate binding was estimated to be $> 95\%$ ($f_u = 0.038$). In perfusion experiments, the dose of FSM was the same in both treatment groups (2.65 mg). As seen in Fig. 4, perfusate levels of FSM appeared to decline faster in perfusion experiments with female kidneys. However, urinary excretion of FSM was enhanced in male kidneys. This gender difference is reflected in Table 3, where higher estimates of excretion parameters were obtained in perfusions with male kidneys (Cl_R, Cl_{SEC}, XR, and urinary recovery of FSM). Mass balance analysis indicated that FSM kidney accumulation was higher in female kidneys.

TABLE 2 **PAH Renal Excretion Parameters in the IPK: Effect of Gender[a]**

Parameter	Male	Female	P-Value
Cl_R (mL/min/g)[b]	0.88 ± 0.37	0.59 ± 0.19	0.00007
Cl_{FILT} (mL/min/g)[c]	0.27 ± 0.17	0.28 ± 0.19	0.91
Cl_{SEC} (mL/min/g)[d]	0.65 ± 0.33	0.30 ± 0.16	0.0001
Cl_{PERF} (mL/min/g)[e]	1.2 ± 0.04	0.89 ± 0.07	0.001
XR[f]	3.8 ± 1.7	2.2 ± 0.72	0.0001
% Kidney Accumulation[g]	16 ± 0.75	14 ± 0.54	0.57

[a]data reported as mean ± standard deviation representing three perfusions per treatment group.
[b]renal clearance calculated as the ratio of urinary excretion rate and perfusate concentration. The data was normalized by kidney weight.
[c]filtration clearance = GFR * f_u (fraction of PAH unbound in perfusate), normalized to kidney weight. The estimate of f_u (0.38) was experimentally measured.
[d]secretion clearance ($Cl_{SEC} = Cl_T - Cl_{FILT}$) normalized to kidney weight.
[e]perfusate clearance, the ratio of infusion rate and steady state perfusate concentration.
[f]excretion ratio = Cl_R/Cl_{FILT}.
[g]kidney accumulation (% dose) estimated by mass balance.

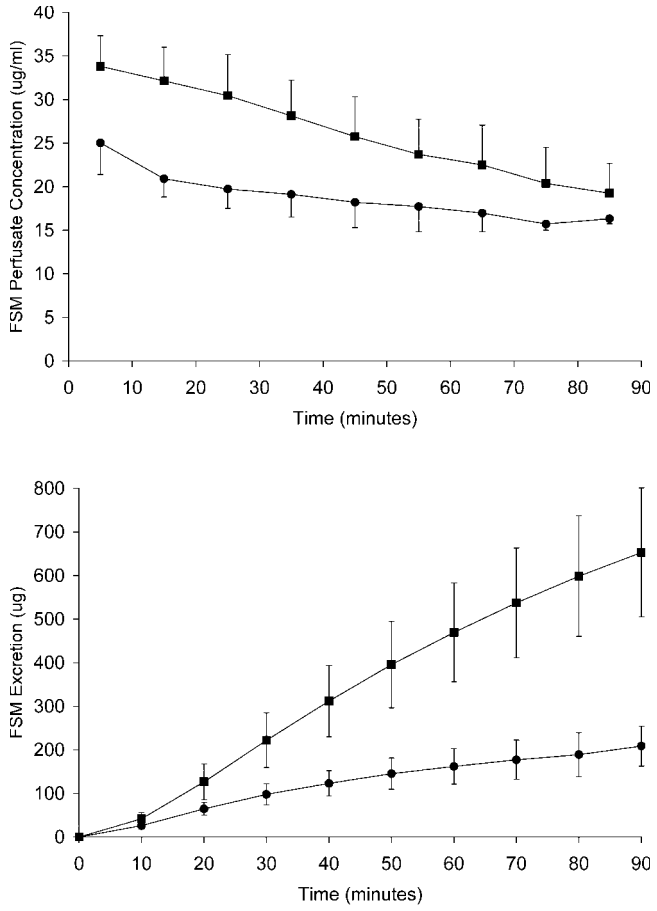

FIGURE 4 **Gender Differences in FSM Renal Excretion in the IPK. Upper Panel: Mean (SD) Perfusate Concentrations of FSM as a Function of Time. Lower Panel: Mean (SD) Cumulative Excretion of FSM as a Function of Time. FSM Was Added as a Single Bolus Dose (2650 ug) (● = Data From Perfusion Experiments With Kidneys From Female Rat Donors; ■ = Data From Perfusion Experiments With Kidneys From Male Rat Donors).**

DISCUSSION

The proximal tubule of the kidney contains several organic anion transport systems. Overall, tubular transport of organic anions proceeds against an electrochemical gradient at the basolateral membrane, with facilitated transport across the luminal membrane into the urine (Burchhardt & Burckhardt, 2003). The primary transport system in the basolateral membrane is the organic anion transporter, OAT1 (Fig. 1). While OAT1 is the principle anion basolateral transporter in the kidney, other members of the OAT family may also be involved, including OAT2, OAT3, and OAT4 (Sekine et al., 2000, Dresser et al., 2001).

Likewise, a number of transport pathways have been proposed for the luminal exit of acidic compounds. The organic anion transporting polypeptide OATP1 and the multidrug resistance protein MRP2 are expressed in the brush border membrane of the kidney (Masereeuw & Russel, 2001). Additionally, OAT-K1 and OAT-K2 are kidney-specific transporters structurally similar to OATP1 (van Aubel et al., 2000). While it is assumed that these are efflux transport systems (lumen → urine), several of these systems (including OATP1) may be involved in luminal reabsorption (urine → kidney). Overall, identification and characterization of membrane transport systems in the kidney continues to evolve.

In this investigation, the IPK model was utilized to study the effect of gender on the renal excretion organic anions. The probes selected for this study, PAH and FSM, are compounds with varying degrees of renal extraction efficiency. The renal excretion of

TABLE 3 **FSM Renal Excretion Parameters in the IPK: Effect of Gender[a]**

Parameter[a]	Male	Female	P-Value
Cl_R (mL/min/g)[b]	0.15 ± 0.07	0.10 ± 0.05	0.004
Cl_{FILT} (mL/min/g)[c]	0.02 ± 0.008	0.02 ± 0.008	0.69
Cl_{SEC} (mL/min/g)[d]	0.13 ± 0.07	0.08 ± 0.05	0.002
XR[e]	6.7 ± 2.4	4.2 ± 1.3	0.005
% Dose Excreted[f]	26 ± 0.13	7.9 ± 0.02	<0.0001
% Kidney Accumulation[g]	29 ± 0.21	46 ± 0.03	0.003

[a]data reported as mean ± standard deviation representing three perfusions per treatment group.
[b]renal clearance calculated as the ratio of urinary excretion rate and perfusate concentration. The data was normalized by kidney weight.
[c]filtration clearance = GFR * f_u (fraction of FSM unbound in perfusate), normalized to kidney weight. The estimate of f_u (0.038) was experimentally measured.
[d]secretion clearance ($Cl_{SEC} = Cl_T - Cl_{FILT}$) normalized to kidney weight.
[e]excretion ratio = Cl_R/Cl_{FILT}.
[f]represents cumulative mass excreted in the urine (ratioed to administered dose).
[g]kidney accumulation (% dose) estimated by mass balance.

both compounds was significantly greater in perfusion experiments with kidneys from male donors. Moreover, these differences could not be explained on the basis of differences in organ weight. Kidney weight-corrected estimates of GFR and Cl_{FILT} were similar for male and female kidneys. However, significant gender differences existed in Cl_R for both PAH and FSM despite a correction for weight. Collectively, the findings indicate the renal tubular secretion of organic anions is greater in males compared to females.

The results of the present study are consistent with reports in the literature that demonstrate gender effects on organic anion disposition. Cerutti et al. (2002) studied the renal excretion of PAH and FSM in vivo. The systemic clearance of both compounds was significantly lower in female rats. Studying the renal excretion of PAH in rats, Reyes et al. (1998) demonstrated that the renal excretion of PAH was faster in male compared to female rats. In studies with castrated males, however, PAH excretion was reduced to levels observed in females. Likewise, testosterone pretreatment increased PAH excretion in female rats. These results were ascribed to a role of testosterone on transporter expression in the kidney.

These in vivo drug disposition studies are supported by accumulating evidence of differential expression of renal OATs between genders. Basolateral membrane expression of both OAT1 and OAT3 are increased in male rats, although expression is reduced in castrated and hypophysectomized rats (Ljubojevic et al., 2004). In contrast, females (and castrated males) show increased expression of OAT2 in the kidney (Buist et al., 2002).

The present study in the IPK provides supportive evidence that gender-dependent transport differences exist at both the basolateral and luminal membrane. It is widely accepted that PAH is a substrate for OAT1 (Burckhardt et al., 2001), and differences in PAH excretion are consistent with reduced expression and/or activity of OAT1 in female kidneys. Like PAH, FSM is also a substrate for OAT1 (Uwai et al., 2000). Since perfusate exposure of FSM in the IPK appeared to be reduced in females (suggesting enhanced renal uptake), it appears that a compensatory basolateral transport mechanism exists for FSM in female kidneys. Based on studies of OAT1 transport in *Xenpous laevis* oocytes, Uwai et al. (2000) suggested that the efficient tubular secretion of loop diuretics might be due to an additional transport mechanism. This mechanism may involve OAT2, which shows increased expression in females. OAT2-mediated transport has been shown for the loop diuretic bumetanide, a compound with a similar structure to FSM (Burckhardt & Burckhardt 2003). Alternatively, data may reflect gender differences in a drug efflux mechanism in the basolateral membrane. Although such mechanisms have yet to be identified, FSM efflux may be reduced in females. In this scenario, reduced uptake by OAT1 in females would be balanced by lower drug efflux (kidney → perfusate), making basolateral uptake of FSM comparable in female and male kidneys.

The lower urinary excretion of FSM in female kidneys suggests differences in luminal membrane transport between genders. For example, reduced transporter expression may have resulted in capacity-limited luminal efflux of FSM. To date, there are no

published data regarding the affinity of FSM for any of the luminal transporters that have been identified. It is likely that additional transport systems are present in the kidney, and these may participate in the secretion of compounds such as FSM. For example, studies have demonstrated luminal expression of OAT transporters including OAT2 and OAT4 (Sekine et al., 2000; Komiya et al., 2002). Interestingly, the diuretic effect of FSM was more pronounced in perfusions with female kidneys. This finding is consistent with a recent in vivo investigation of gender-related differences in the pharmacodynamics of FSM in rats (Brandoni et al., 2004). In that study, the diuretic effect of FSM was higher in females. The authors correlated these findings with reduced expression of the Na-K-2Cl cotransporter, the therapeutic target for loop diuretics, in female kidneys. The results of the present IPK study suggest that differences in luminal membrane transport of FSM may also contribute to differences in diuretic response between male and female kidneys. Further studies are needed to test this hypothesis.

Gender differences in drug disposition are an emerging issue for drug development. In the case of renal excretion, differences in the renal secretion of organic anions exist between males and females. Considering the wide array of compounds that undergo secretion by the kidney, disparities in transporter expression and function between males and females may result in gender differences in systemic exposure of narrow therapeutic range medications and risks to nephrotoxic agents. More studies are needed to clarify the clinical importance of gender differences in renal drug transport. Likewise, models to study sex differences in drug disposition must be developed and validated. To this end, the results of this study suggest that the perfused rat kidney model is a potentially useful model to study gender differences in renal drug excretion.

Like any experimental technique, the IPK model has some limitations. Although glomerular and proximal tubule function is well-preserved in the IPK, abnormalities in renal hemodynamics, urinary concentration and dilution ability, and excretion of fluid and electrolytes are noted defects of the preparation. High perfusate flow rates and diminished distal tubular function are responsible for these abnormalities (Taft 2004). Moreover, it is not known whether OAT expression and functionality are retained in the IPK model. A limit of the investigation was that drug excretion in the IPK was not correlated with transporter expression. To date, there are no published IPK studies addressing transporter expression and functionality. This is an area that requires further study both in terms of identification of membrane transporters (particularly in the luminal membrane) and clarification of their role in drug excretion. In the case of FSM disposition, luminal transport is likely mediated by an unknown mechanism. Given the accumulating evidence of gender-dependent transporter expression, reduced urinary excretion in females likely reflects differences in membrane transport.

In conclusion, the renal clearance of PAH and FSM in the IPK was higher in perfusion experiments using kidneys from male rat donors. The observed gender differences in organic anion excretion were attributed to differences in renal tubular transport both on the basolateral and luminal membrane. While the data endorse the IPK as a useful experimental tool to investigate gender effects on renal drug disposition, additional studies are needed to correlate drug excretion in the IPK with transporter expression in the kidney.

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