Supplemental material to this article can be found at:
http://dmd.aspetjournals.org/content/suppl/2022/03/19/dmd.121.000748.DC1

1521-009X/50/6/770–780$35.00
DRUG METABOLISM AND DISPOSITION

dx.doi.org/10.1124/dmd.121.000748
Drug Metab Dispos 50:770–780, June 2022

Localization of Xenobiotic Transporters Expressed at the Human Blood-Testis Barrier[S]

Raymond K. Hau, Robert R. Klein, Stephen H. Wright, and Nathan J. Cherrington

Department of Pharmacology & Toxicology, College of Pharmacy (R.K.H., N.J.C.), and Departments of Pathology (R.R.K.) and Physiology (S.H.W.), College of Medicine, University of Arizona, Tucson, Arizona

Received October 27, 2021; accepted March 7, 2022

ABSTRACT

The blood-testis barrier (BTB) is formed by basal tight junctions between adjacent Sertoli cells (SCs) of the seminiferous tubules and acts as a physical barrier to protect developing germ cells in the adluminal compartment from reproductive toxicants. Xenobiotics, including antivirals, male contraceptives, and cancer chemotherapeutics, are known to cross the BTB, although the mechanisms that permit barrier circumvention are generally unknown. This study used immunohistological staining of human testicular tissue to determine the site of expression for xenobiotic transporters that facilitate transport across the BTB. Organic anion transporter (OAT) 1, OAT2, and organic cation transporter, novel (OCTN) 1 primarily localized to the basal membrane of SCs, whereas OCTN2, multidrug resistance protein (MRP) 3, MRP6, and MRP7 localized to SC basal membranes and peritubular myoid cells (PMCs) surrounding the seminiferous tubules. Concentrative nucleoside transporter (CNT) 2 localized to Leydig cells (LCs), PMCs, and SC apicolateral membranes. Organic cation transporter (OCT) 1, OCT2, and OCT3 mostly localized to PMCs and LCs, although there was minor staining in developing germ cells for OCT3. Organic anion transporting polypeptide (OATP) 1A2, OATP1B1, OATP1B3, OATP2A1, OATP2B1, and OATP3A1-v2 localized to SC basal membranes with diffuse staining for some transporters. Notably, OATP1C1 and OATP4A1 primarily localized to LCs. Positive staining for multidrug and toxin extrusion protein (MATE) 1 was only observed throughout the adluminal compartment. Definitive staining for CNT1, OAT3, MATE2, and OATP6A1 was not observed. The location of these transporters is consistent with their involvement in the movement of xenobiotics across the BTB. Altogether, the localization of these transporters provides insight into the mechanisms of drug disposition across the BTB and will be useful in developing tools to overcome the pharmacokinetic and pharmacodynamic difficulties presented by the BTB.

SIGNIFICANCE STATEMENT

Although the total mRNA and protein expression of drug transporters in the testes has been explored, the localization of many transporters at the blood-testis barrier (BTB) has not been determined. This study applied immunohistological staining in human testicular tissues to identify the cellular localization of drug transporters in the testes. The observations made in this study have implications for the development of drugs that can effectively use transporters expressed at the basal membranes of Sertoli cells to bypass the BTB.

Introduction

The blood-testis barrier (BTB) safeguards developing germ cells from the effects of reproductive toxicants. The physical component of the BTB involves basal junctional complexes formed between adjacent Sertoli cells (SCs) to establish a nearly impermeable network of membranes in the seminiferous tubule (Pelletier, 2011). The expression of membrane transporters that limit chemical flux across the basal membrane of SCs constitutes the physiologic component of the BTB (Mital et al., 2011; Mruk et al., 2011; Mruk and Cheng, 2015). Moreover, the long, thin peritubular myoid cells (PMCs) surrounding the tubules and interstitial Leydig cells (LCs) may also influence drug disposition across the BTB due to their location and transporter expression. Together, this barrier obstructs efficient delivery of some compounds into and across SCs. However, developing germ cells require a steady supply of nutrients to sustain normal reproductive function in the male genital tract (MGT) during spermatogenesis. Consequently, the movement of nutrients, including nucleosides, hormones, sugars, and metabolites that do not readily diffuse across membranes, must involve transporters. Although these transporters play a physiologic role at the BTB, exogenous compounds such as pesticides, cancer chemotherapeutics, male contraceptives, and antivirals can also take advantage of these pathways to circumvent the BTB and ultimately exert pharmacological or toxicological effects in the MGT (Grima et al., 2001; Cheng et al., 2005a; Kato et al., 2005, 2006; Tash et al., 2008a,b; Kato et al., 2009; Klein et al., 2013; Miller et al., 2020; Miller et al., 2021a). Although some compounds are known to cross the BTB, studies assessing the localization and function of the responsible drug transporters have been insufficient for developing a global picture of drug disposition across the BTB.

This work was supported by funding from National Institutes of Health National Institutes of General Medical Sciences [Grant GM123643] and [Grant GM129777], National Institute of Environmental Health Sciences [Grant ES028668] and [Grant ES007091], and National Cancer Institute [Grant CA023074].

The authors declare no conflicts of interest.

dx.doi.org/10.1124/dmd.121.000748.

[S] This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: BCRP, breast cancer resistance protein; BTB, blood-testis barrier; CNT, concentrative nucleoside transporter; ENT, equilibrative nucleoside transporter; LC, Leydig cell; MATE, multidrug and toxin extrusion protein; MGT, male genital tract; MRP, multidrug resistance protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OCTN, organic cation transporter, novel; PBS-T, PBS containing 0.1% Triton X-100; P-gp, P-glycoprotein; PMC, peritubular myoid cell; SC, Sertoli cell.

Some studies have assessed transporter function in SCs; however, fewer have confirmed the presence and location of these transporters in mammalian tissues. Several studies have analyzed the localization of some transporters such as the equilibrative nucleoside transporters (ENTs) and efflux transporters such as multidrug resistance proteins (MRPs), P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP) (Melaine et al., 2002; Bart et al., 2004; Su et al., 2009; Klein et al., 2013; Koraichi et al., 2013; Klein et al., 2014). ENT1 was observed to localize to the basal membrane of human and rodent SCs, whereas ENT2 localized to the apicolateral membranes of SCs (Klein et al., 2013). Moreover, functional transport studies confirmed this polarized distribution of ENT1 and ENT2 in primary rat SCs cultured on Transwell inserts (Klein et al., 2013). Consequently, the ENTs act as a mechanism for endogenous nucleosides and nucleoside analog drugs to circumvent the BTB, facilitating uptake at the basal pole (ENT1) and efflux at the apicolateral pole (ENT2). These transporters may be important for the disposition of antivirals to treat infections in the MGT due to Zika viruses, Ebolaviruses, human immunodeficiency viruses, and other sexually transmitted viruses.

In contrast, studies examining the role of efflux transporters such as P-gp, BCRP, and the MRPs have been more common. These efflux transporters are critically important for maintaining normal endogenous chemical concentrations in the MGT by effluxing toxicants and metabolic wastes back into the blood; however, they do not provide effective pathways for nutrients to enter SCs. Nutrients require unidirectional/bidirectional uptake transporters expressed at the basal membranes of SCs. But these processes will consequently allow some exogenous compounds to bypass the BTB. Unfortunately, fewer studies have surveyed the role of uptake transporters at the BTB, which complicates the drug discovery and development process for compounds designed to specifically target diseases or disorders in the testes. As a result, a more comprehensive drug transporter map of the testes must be generated to better understand drug disposition into the MGT and how these processes may be exploited.

The current study is the first broad investigation of the expression and localization of several drug transporters in human testicular tissue (Hau et al., 2020b,c,d). The expression and localization of each transporter was evaluated by immunohistofluorescence and immunohistochemical staining in several human testicular tissue samples from patients of varying ages and ethnicities. The targeted transporters included pharmacologically relevant processes, such as the organic cation transporters (OCTs), organic cation transporters, novel (OCTNs), organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug and toxin extrusion proteins (MATEs). Many of these transporters were found to be expressed throughout human testicular tissue where they may play important roles in drug disposition to the MGT. Therefore, the localization of the drug transporters observed in this study should facilitate understanding the pharmacokinetics of various classes of compounds across the BTB and into the MGT.

Methods

Reagents. All reagents were purchased from ThermoFisher Scientific (ThermoFisher Scientific, Waltham, MA) unless otherwise noted. Primary and secondary antibodies were purchased from the vendors listed in Table 1 and used at the indicated concentrations. The anti-OATP1A2, OATP1B1, OATP1B3, OATP1C1, OATP3A1_v2, and OATP4A1 antibodies used in this study were a kind gift from Dr. Bruno Stieger of the Department of Clinical Pharmacology and Toxicology at the University of Zurich (Zürich, Switzerland).

Sample Collection. Formalin-fixed, paraffin-embedded human testicular tissue blocks were obtained from eight different patients ranging from age 16 to 65 from the Department of Pathology tissue archive at the Banner-University Medical Center (Tucson, AZ). Each tissue block was examined by a board-certified pathologist (Dr. Robert Klein) who confirmed the tissue was sufficiently healthy and intact for immunohistological experiments. Protocols for obtaining human tissues were approved by the University of Arizona Institutional Review Board under protocol number 1906692571.

Immunohistofluorescence Staining. Sectioning of all formalin-fixed paraffin-embedded tissue samples was accomplished using a microtome with sections sliced 5 μm thick. Tissue slides were deparaffinized by immersion into xylenes three consecutive times for 4 minutes each. Next, tissues were rehydrated with a graded series of ethanol and water as follows: 3X 100% ethanol for 4 minutes, 2X 95% ethanol for 4 minutes, 1X 75% ethanol for 4 minutes, and 2X 100% water for 4 minutes. Antigen retrieval was performed by submerging tissue slides in citrate buffer (pH 6.0) or Tris-EGTA buffer (10 mM Tris, 0.5 mM EGTA, pH 9.0) and boiling in a microwave oven for 6 minutes. After heating the samples, the buffer was replaced with room temperature buffer, placed on ice for 20 minutes, then rinsed under cold tap water for 10 minutes.

Nonspecific epitopes in each tissue sample were blocked using a solution of 4% fish skin gelatin and 5% goat/donkey serum in PBS containing 0.1% Triton X-100 (PBS-T) for 30 minutes. Tissue samples were washed once with PBS-T and then probed overnight at 4°C with the primary antibody diluted in 1% fish skin gelatin and 2% goat/donkey serum in PBS-T. The following day, each sample was washed once more with PBS-T. An appropriate Alexa Fluor 488 secondary antibody diluted in 1% fish skin gelatin and 2% goat/donkey serum in PBS-T was applied for 1 hour at room temperature and washed again with PBS-T. Nuclei were counterstained using 1 μg/mL DAPI. Tissue autofluorescence was blocked using TrueBlack Lipofuscin Autofluorescence Quencher (Biotium, Fremont, CA, Catalog #23007) according to the manufacturer's protocol. Coverslips were mounted using ProLong Diamond Antifade Mountant (Invitrogen, Carlsbad, CA, Catalog #P36961) and allowed to dry in the dark overnight before imaging. Slides were imaged using a Leica SP5-II confocal microscope (Leica Camera AG, Wetzlar, Germany) with a HC PL APO 40x/1.25 GLYC CORR CS2 objective. Images were further processed by linear histogram stretching for the blue and green color channels independently to clearly visualize positive staining using Adobe Photoshop CC 2019 (Adobe, San Jose, CA). The image representing the green channel for Alexa Fluor 488–stained proteins and the image representing the blue channel for DAPI-stained nuclei were superimposed to generate the final representative, merged image for each figure. Bright-field images were captured for each sample to illustrate tissue structure. Each experiment included two negative control slides in which one slide was not exposed to any primary antibodies but was treated with a secondary antibody, and the second was not exposed to any primary or secondary antibodies. The negative control slides were treated the same as the other tissue samples and exhibited no positive or background staining. Images were cropped from the original image to illustrate clearer staining patterns. At least two to three different tissue samples were used to test each antibody in immunohistofluorescence or immunohistochemical experiments, and the images shown are representative of at least two similar observations.

Immunohistochemical Staining. Immunohistochemical staining of tissues were performed with similar steps as described in the *Immunohistofluorescence Staining* methods section with some differences. Briefly, 5 μm tissue sections were deparaffinized with xylenes and rehydrated with graded ethanol and water. Antigen retrieval was performed for each tissue sample, and endogenous peroxidases were quenched using 3% H_2O_2 in methanol for 20 minutes. Tissues were

TABLE 1

List of primary antibodies used in this study at the indicated concentrations and references assessing their selectivity

Antibody	Concentration	Provider	Catalog Number	References
OAT1	1:300	Abcam	ab135924	(Basit et al., 2019)
OAT2	1:100	Abcam	ab58683	(Lundquist et al., 2014)
OAT3[a]	Varied	Biorbyt	orb11178	(Ohara et al., 2015)
OCT1	1:400	Sigma-Aldrich	AV41516	(Segal et al., 2011; Han et al., 2013; Shao et al., 2014)
OCT2	1:400	Sigma-Aldrich/Atlas Antibodies	HPA008567	(Shao et al., 2014; Hubeny et al., 2016; Chen et al., 2019; Chen et al., 2020)
OCT3	1:400	Abcam	ab124826	(Patel et al., 2013; Gai et al., 2016; Le Roy et al., 2016; Cervenkova et al., 2019)
OCTN1	1:300	Abcam	ab107727	(Dolberg and Reichl, 2018)
OCTN2	1:400	Abcam	ab180757	(Sekhar et al., 2019)
OATP1A2	1:500	Bruno Stieger	N/A	(Huber et al., 2007)
OATP1B1	1:500	Bruno Stieger	N/A	(Huber et al., 2007)
OATP1B3	1:500	Bruno Stieger	N/A	(Huber et al., 2007)
OATP1C1	1:500	Bruno Stieger	N/A	(Huber et al., 2007)
OATP2A1	1:1000	Sigma-Aldrich/Atlas Antibodies	HPA013742	(Patik et al., 2015; Umeno et al., 2015; Jimbo et al., 2020)
OATP2B1	1:500	Bruno Stieger	N/A	(Huber et al., 2007)
OATP3A1_v2	1:250	Bruno Stieger	N/A	(Huber et al., 2007)
OATP4A1	1:500	Bruno Stieger	N/A	(Huber et al., 2007)
OATP6A1[a]	Varied	Sigma-Aldrich/Atlas Antibodies	HPA054126	(Djureinovic et al., 2014; Patik et al., 2015)
CNT1[a]	Varied	Abcam	ab192438	(Gandhi et al., 2019; Yamamura et al., 2021)
CNT2	1:500	Abcam	ab199631	(Klein et al., 2017)
MRP3	1:200	Abcam	ab3375	(Hardwick et al., 2011; Canet et al., 2015)
MRP6	1:600	Santa Cruz Biotechnology	sc-59618	(Le Saux et al., 2011; Xue et al., 2014; Pomozi et al., 2017)
MRP7 (ABCC10)	1:200	Invitrogen	PA5-23652	(Zhang et al., 2014)
MATE1	1:200	Santa Cruz Biotechnology	sc-138983	(Fujita et al., 2019; Zhang et al., 2019)
MATE2[a]	Varied	Aviva Systems Biology	ARP51904_P050	None
V5	1:1000	Invitrogen	R960	(Pelis et al., 2006; Zhang and Wright, 2009)
Flag	1:10	Developmental Studies Hybridoma Bank	12C6c	None

[a]Antibodies were tested at several concentrations in different testicular tissue samples with inconclusive staining.

washed once with PBS-T before incubating with Background Sniper (Biocare Medical, Pacheco, CA, Catalog #BS966) for 10 minutes. Tissues were washed once more with PBS-T and probed overnight at 4°C with a primary antibody diluted in 1% fish skin gelatin and 5% bovine serum albumin in PBS-T. The following day, each sample was washed once more with PBS-T and then probed with MACH 4 Universal HRP-Polymer (Biocare Medical, Catalog #M4U534) for 15 minutes. Tissue slides were further developed using the Betazoid DAB Chromogen Kit (Biocare Medical, Catalog #BDB2004) and rinsed several times with water. Nuclei were counterstained with Hematoxylin Solution, Gill No. 3 (Sigma-Aldrich, St. Louis, MO, Catalog #GHS332-1L) for 15 seconds. Tissues were then dehydrated in graded ethanol and xylenes. Coverslips were mounted with Cytoseal Mountant XYL (ThermoFisher Scientific, Catalog #22050262) and allowed to dry overnight before imaging. All slides were imaged using a Leica DM3000 microscope (Leica Camera AG) with a HC PL APO 40x/0.85 CORR objective. Representative images were further processed by linear histogram stretching to clearly visualize positive staining using Adobe Photoshop CC 2019 (Adobe). Each experiment included a negative control slide that was not exposed to any primary antibodies but was treated the same as the other tissue samples. There was no positive staining observed in the negative control slides. Some images were cropped from the original image to illustrate clearer staining patterns.

Results

Immunohistofluorescent Staining of Concentrative Nucleoside Transporters. The distribution of the concentrative nucleoside transporters in human testicular tissues was evaluated by immunohistofluorescence. Positive staining for concentrative nucleoside transporter 1 (CNT1) was not observed at varying antibody concentrations in different tissue samples (data not shown). Further analysis of CNT1 antibody specificity was performed with liver tissue due to its high expression (Pennycooke et al., 2001). Intense punctate staining for CNT1 was observed throughout the cytosol of hepatocytes (Supplemental Fig. 1E), which is consistent with a previous report on the canalicular and intracellular localization in rat liver tissues (Duflot et al., 2002). This intracellular staining was attributed to a transcytotic process that relocalizes CNT1 from the sinusoidal membrane to intracellular endosomes and the canalicular membrane. No observable staining was observed in liver tissue that was only probed with a secondary antibody or neither antibody (Supplemental Fig. 1, A and C). Although positive CNT1 staining was absent in the testes, CNT2 was observed to be primarily localized to the apicolateral membranes of SCs in the ectoplasmic specialization between germ cells and in vascular endothelial cells with minor staining in PMCs surrounding the tubules and in LCs (Fig. 1E).

Immunohistofluorescent Staining of Organic Cation Transporters. Immunohistofluorescent staining was also performed for the OCTs and OCTNs in human testes. OCT1, OCT2, and OCT3 localized to PMCs and LCs, although there was minor staining in the developing germ cells within the seminiferous tubules for OCT3 (Fig. 2, A, C, and E). Interestingly, moderate staining of OCT1 and OCT2 was also detected along the basal membrane of SCs, whereas staining for OCT3 at this location was less pronounced. Similarly, positive staining for OCTN2 was observed at PMCs and LCs with more distinct staining along the basal membranes of SCs (Fig. 2I). On the other hand, OCTN1 only localized to the basal membrane of SCs with a lack of staining throughout the rest of the tissue (Fig. 2G). These staining patterns

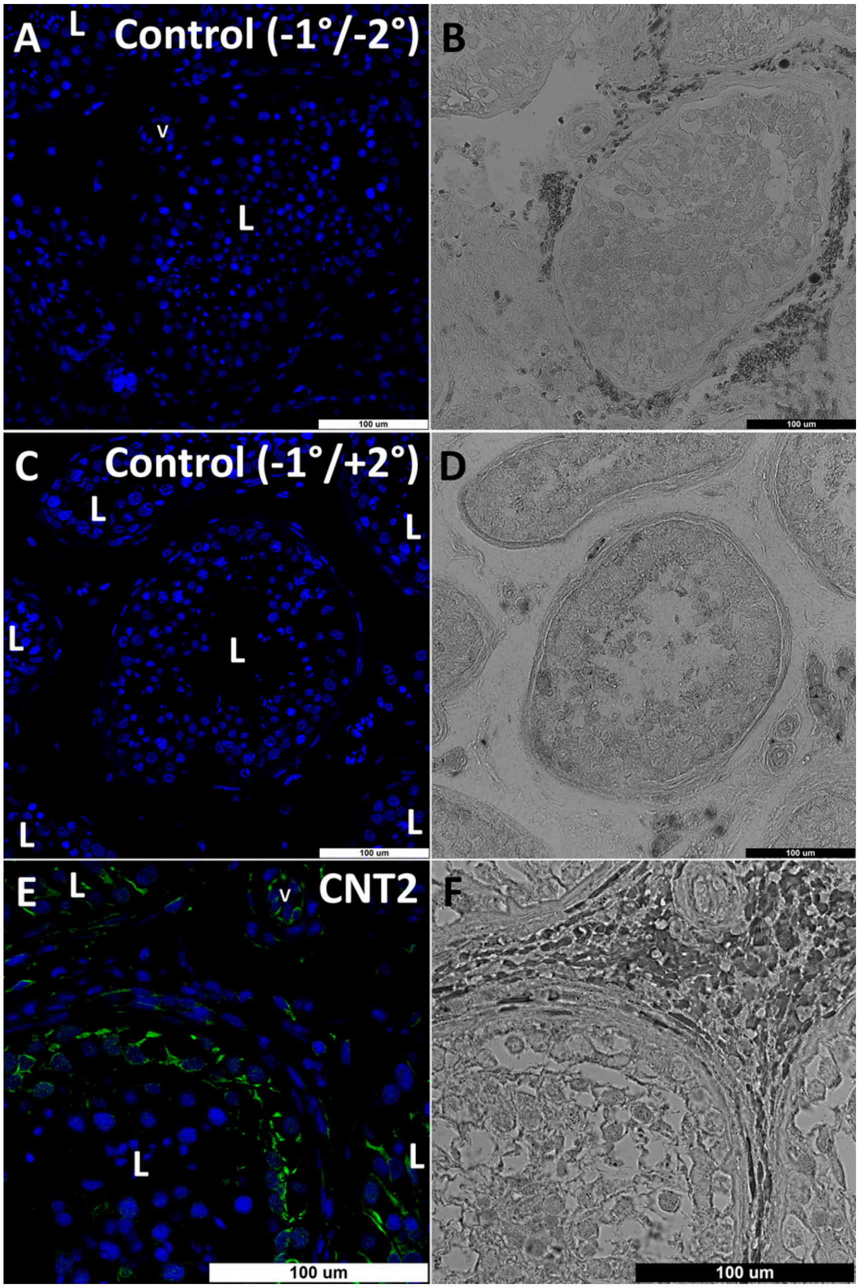

Fig. 1. Immunohistofluorescence analysis for CNT2 in formalin-fixed paraffin-embedded human testicular tissue. Representative control images for all the following immunohistofluorescence experiments that were negative for a fluorescent signal when probed with (A) no primary or secondary antibodies or (C) only a secondary antibody. (E) Positive staining in green for CNT2 primarily observed at the apicolateral membranes of SCs and vascular endothelial cells, with minor staining in LCs, PMCs, and germ cells. Nuclei of all cells were counterstained with DAPI in blue. (B, D, F) Bright-field images of each tissue sample illustrates intact tissue structure. Images were captured at 40× magnification with a laser confocal microscope. Scale bar, 100 μm. L, lumen of the seminiferous tubules; v, vascular endothelial cells.

were consistent with their role as uptake transporters in other tissues such as the kidneys, intestines, and lungs.

Immunohistofluorescent Staining of Organic Anion Transporters. In addition to the OCTs, the expression and localization of the OATs in human testicular tissue was examined. Both OAT1 and OAT2 were primarily localized along the basal membrane of SCs, consistent with their role in compound uptake, with minor staining throughout the adluminal compartment of the seminiferous tubules for OAT2 (Fig. 3, A and C). Some positive staining was also observed for OAT1 in LCs and vascular endothelial cells, although it was primarily expressed by SCs (Fig. 3A). Definitive staining for OAT3 in several human testicular tissue samples was not observed for any antibody concentrations employed (data not shown). Immunocytofluorescence staining for CHO cells overexpressing human OAT3 with a C-terminal Flag epitope tag was performed with the same antibody at a 1:100 dilution and an anti-Flag epitope antibody at a 1:10 dilution. CHO-OAT3 cells probed with the anti-Flag antibody exhibited positive staining along the membranes with minor staining in the cytosol (Supplemental Fig. 2B). The anti-OAT3 antibody produced intense positive staining throughout the cytosol of CHO-OAT3 cells (Supplemental Fig. 2D), indicating greater nonspecificity and that OAT3 may still be expressed in the testes. Both control cell lines did not exhibit positive staining when probed with either antibody (Supplemental Fig. 2, A and C).

Immunohistochemical Staining of Organic Anion Transporting Polypeptides. Standard immunohistochemical staining for the OATPs was performed on human testicular tissue due to unreliable staining with immunohistofluorescent methods. Intense positive staining in brown for OATP1A2, OATP1B1, OATP1B3, OATP2A1, OATP2B1, and OATP3A1-v2 was primarily observed along the basal membrane of SCs; however, diffuse staining throughout the adluminal compartment of the seminiferous tubules was also noted (Fig. 4, B–D and F–H). Positive basal membrane staining of OATP1B3 and OATP2A1 was less

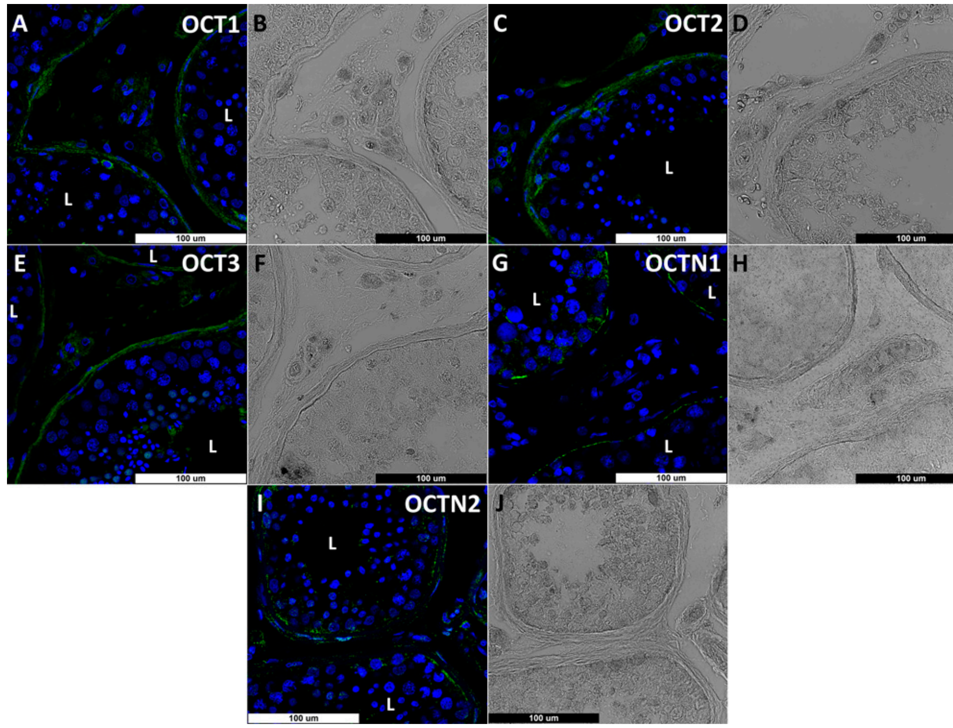

Fig. 2. Immunohistofluorescence analysis for OCTs and OCTNs in formalin-fixed paraffin-embedded human testicular tissue. (A) OCT1, (C) OCT2, and (E) OCT3 were predominantly localized to PMCs and LCs with minor staining around developing germ cells for OCT3. Distinct staining along the basal membranes of SCs for (G) OCTN1 and (I) OCTN2 was observed. Additional staining for (I) OCTN2 was also noted at PMCs and LCs. Nuclei of all cells were counterstained with DAPI in blue. (B, D, F, H, J) Bright-field images of each tissue sample illustrates intact tissue structure. Images were captured at 40× magnification with a laser confocal microscope. Scale bar, 100 μm. L, lumen of the seminiferous tubules.

intense than other transporters but was distinctly different than in the control tissue (Fig. 4, A, D, and F). Diffuse staining in LCs was also noted for OATP1A2, OATP1B1, and OATP1B3 (Fig. 4, B–D). OATP1C1 and OATP4A1 were predominantly localized to LCs with some diffuse staining throughout the rest of the tissue. (Fig. 4, E and I). Additionally, the location of OATP1C1 observed in this study is consistent with a previous report of its localization in LCs in human testes (Pizzagalli et al., 2002).

Positive staining for OATP6A1 was not observed with the antibody or tissues tested with immunohistochemical- or -fluorescence-based detection methods (data not shown). Immunocytofluorescence staining for CHO cells overexpressing human OATP6A1 with a C-terminal V5 epitope tag was performed with the same antibody at a 1:100 dilution and an anti-V5 epitope antibody at a 1:1000 dilution. Both antibodies produced an intense positive signal throughout the cytosol of CHO-OATP6A1 cells (Supplemental Figs. 3B and 4D), which is consistent with the lack of functional membrane expression from previous studies (data not shown). These observations indicate antibody specificity for OATP6A1, although its binding epitope may not be appropriate for immunohistology. Control cells probed with either antibody did not produce an observable signal (Supplemental Fig. 3, A and C).

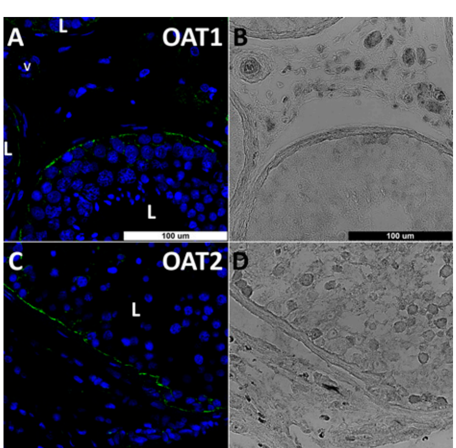

Fig. 3. Immunohistofluorescence analysis for OATs in formalin-fixed paraffin-embedded human testicular tissue. Intense positive staining in green for (A) OAT1 and (C) OAT2 along the basal membranes of SCs with minor staining in the adluminal compartment for OAT2. Some staining for OAT1 was also detected in LCs and vascular endothelial cells, with no additional noteworthy staining for OAT2. Nuclei of all cells were counterstained with DAPI in blue. (B, D) Bright-field images of each tissue sample illustrates intact tissue structure. Images were captured at 40× magnification with a laser confocal microscope. Scale bar, 100 μm. L, lumen of the seminiferous tubules; v, vascular endothelial cells.

Immunohistofluorescent Staining of Multidrug Resistance Proteins. Previous work has determined the localization of several MRPs in rodent, human, and nonhuman primate testicular tissue. However, definitive staining for MRP3, MRP6, and MRP7 in testicular tissues h not been explored. Consequently, immunohistofluorescence staining for MRP3, MRP6, and MRP7 was performed on human testicular tissues to determine the localization of these proteins. MRP3, MRP6, and MRP7 were primarily localized to the basal membranes of SCs with minor staining at the PMCs (Fig. 5, A, C, and E), where they may serve as one of many essential efflux transport processes to protect SCs and developing germ cells from metabolites or toxicants. In addition to these locations, minor positive staining was observed for each of the three transporters in LCs as well as in vascular endothelial cells for MRP7 only.

Immunohistofluorescent Staining of Multidrug and Toxin Extrusion Proteins. The localization of MATE1 was identified by immunohistofluorescence staining in human testicular tissue. Positive staining for MATE1 was primarily observed throughout the adluminal compartment of the seminiferous tubules with minor staining was observed at the PMCs and LCs (Fig. 6A). Conclusive staining for MATE2 was not observed with the antibody or testicular tissues tested (data not shown). Immunocytofluorescence staining for CHO cells overexpressing human MATE2-K with a C-terminal V5 epitope tag was performed with the same antibody at a 1:100 dilution and an anti-V5 epitope antibody at a 1:1000 dilution. Although MATE2-K is a kidney-specific

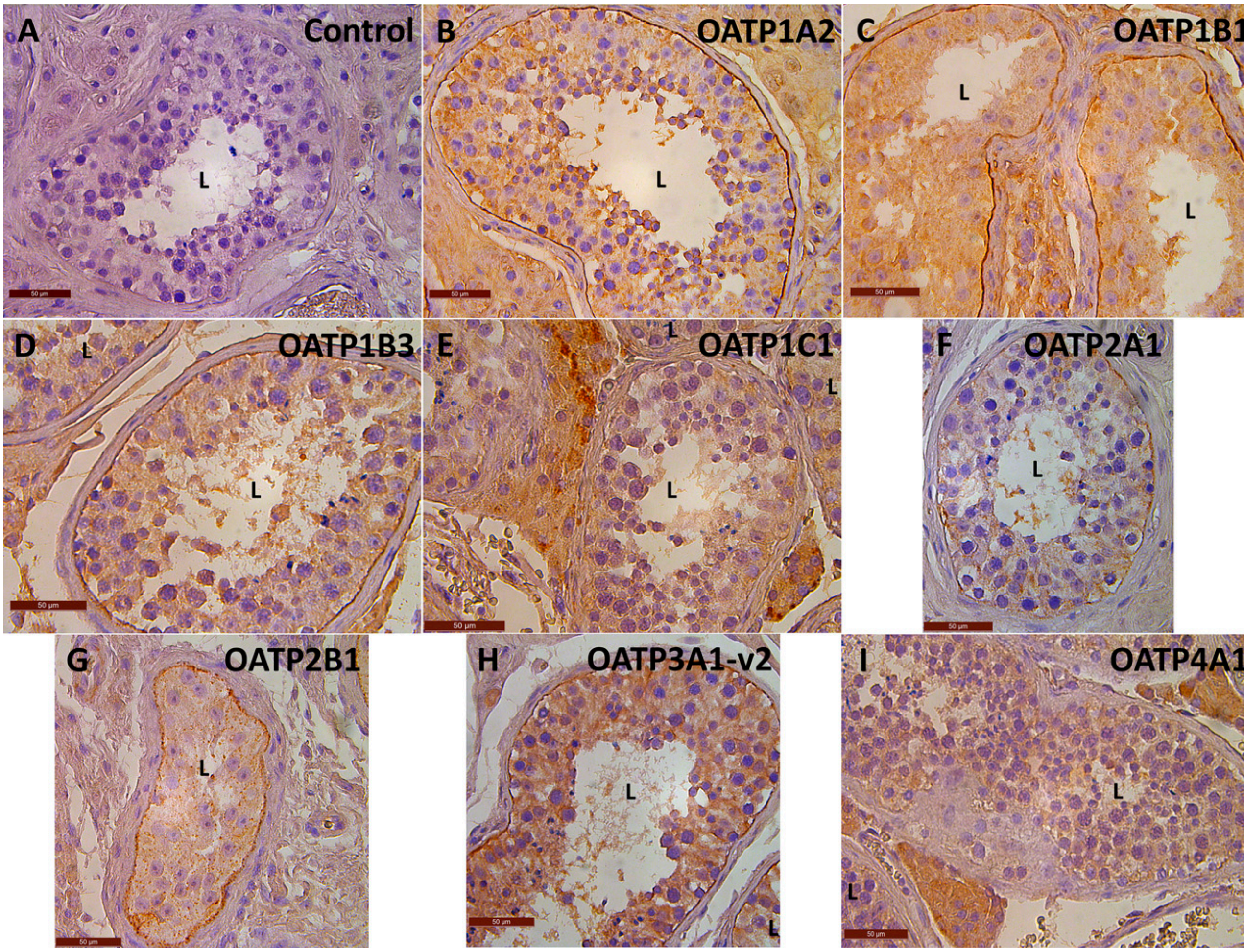

Fig. 4. Immunohistochemical analysis for OATPs in formalin-fixed paraffin-embedded human testicular tissue. (A) Representative control image for the following immunohistochemical experiments that indicated a lack of positive staining when tissues were not exposed to a primary antibody. Positive staining in brown was observed for (B) OATP1A2, (C) OATP1B1, (D) OATP1B3, (F) OATP2A1, (G) OATP2B1, and (H) OATP3A1-v2 along the basal membranes of SCs with diffuse staining within the tubules. Diffuse staining for (A) OATP1A2, (B) OATP1B1, and (C) OATP1B3 was also noted in LCs. (E) OATP1C1 and (I) OATP4A1 were primarily detected in LCs. Nuclei of all cells were counterstained with hematoxylin. Images were captured at 40× magnification with a standard light microscope. Scale bar, 50 μm. L, lumen of the seminiferous tubules.

isoform, the anti-MATE2 antibody used in this study recognizes an extracellular C-terminal epitope that is present in the canonical and kidney-specific isoforms. Both antibodies produced a positive signal in the cytosol of CHO-MATE2-K cells with some plasma membrane staining, indicating specific staining for MATE2 (Supplemental Fig. 4, B and D), with no observable staining in control cells (Supplemental Fig. 4, A and C).

Discussion

Among the various transporters expressed at the BTB, the expression, localization, and function of the ENTs has been rigorously studied (Kato et al., 2005, 2006, 2009; Klein et al., 2013; Hau et al., 2020a; Miller et al., 2020; Miller et al., 2021a,b,c). Unfortunately, the physiologic and pharmacological roles of the CNTs in the testes have been understudied. A recent study noted the mRNA expression of CNT1 and CNT2 in human SCs (Hau et al., 2020a). However, CNT1 was not detected in human testicular tissue but was evident in human liver tissue (Supplemental Fig. 1E) in the present study. Moreover, the expression and function of this transporter has been observed in rat SCs (Kato et al., 2005, 2009). Here, CNT2 predominantly localized to the apicolateral membranes of SCs in human testes (Fig. 1E), but the role for this transporter at this location is unknown. Previous reports identifying the localization of the structural protein vimentin in rat testes show that it adopts a similar staining pattern as CNT2 along the apicolateral membranes of SCs (Kopecky et al., 2005). It was recently reported that uptake of uridine in a human SC line was primarily ENT1-mediated, with negligible contribution from ENT2 or the CNTs (Hau et al., 2020a). Nevertheless, it is possible that CNT2 delivers nucleosides from the cytoplasm of SCs to the space between SCs and developing germ cells (Gray et al., 2004). Free nucleosides that accumulate in this space can then be taken up by other nucleoside transporters expressed by germ cells. Another possibility is that CNT2 salvages free nucleosides from the adluminal compartment, although further work is required to define the role of CNT2 in the testes. Altogether, CNT2 could play a significant role in the disposition of nucleosides and nucleoside analog antivirals between SCs and the adluminal compartment.

The OCTs are primarily expressed in the liver and kidneys and are involved in the uptake of cationic compounds such as choline, histamine, and metformin from the blood. This is the first study to explore OCT localization in human testicular tissue. Expression of OCT mRNA has been reported in the testes and SCs (Augustine et al., 2005; Maeda et al., 2007; Hau et al., 2020a), and here, OCT1, OCT2, and OCT3 localized to PMCs and LCs with minor positive staining at the basal membrane of

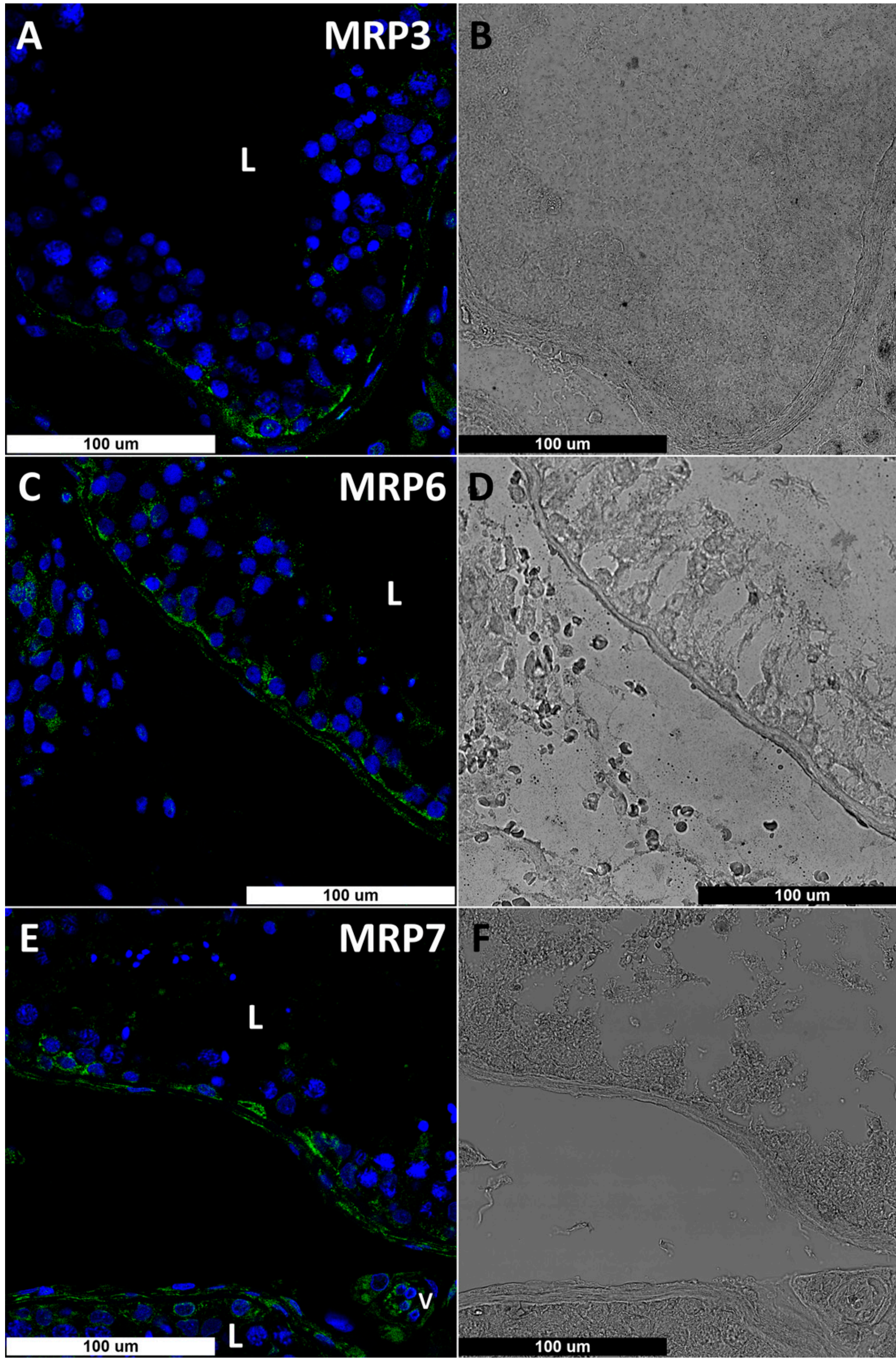

Fig. 5. Immunohistofluorescence analysis for MRPs in formalin-fixed paraffin-embedded human testicular tissue. (A) MRP3, (C) MRP6, and (E) MRP7 localized to PMCs, LCs, along the basal membrane of SCs, moderately in the adluminal compartment of the seminiferous tubules, and in vascular endothelial cells for MRP7. Nuclei of all cells were counterstained with DAPI in blue. (B, D, F) Bright-field images of each tissue sample illustrates intact tissue structure. Images were captured at 40× magnification with a laser confocal microscope. Scale bar, 100 μm. L, lumen of the seminiferous tubules; v, vascular endothelial cells.

SCs or throughout the adluminal compartment for OCT3 (Fig. 2, A, C, and E). One study assessed the OCTs in cultured rat SCs and noted the lack of expression of OCT2, although OCT1 and OCT3 were shown to be functionally expressed by measuring [^{14}C]TEA uptake (Maeda et al., 2007). Based on transepithelial transport experiments with rat SCs cultured on Transwell inserts, the authors also concluded OCT1 was expressed on the basal membrane and OCT3 was expressed on the apical membrane of SCs (Maeda et al., 2007), but the present results cannot confirm that in human SCs. It must be noted that cultured SCs do not form a tight, impermeable barrier compared with MDCK or Caco-2

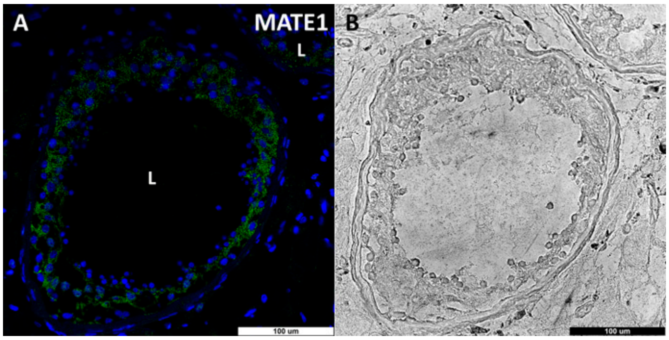

Fig. 6. Immunohistofluorescence analysis for MATE1 in formalin-fixed paraffin-embedded human testicular tissue. (A) Immunohistofluorescent detection of MATE1 in human testicular tissues revealed intense positive staining throughout the adluminal compartment of seminiferous tubules. Minor staining was also noted at PMCs and LCs. Nuclei of all cells were counterstained with DAPI in blue. (B) Bright-field image of the tissue sample illustrates intact tissue structure. Images were captured at 40× magnification with a laser confocal microscope. Scale bar, 100 μm. L, lumen of the seminiferous tubules.

cells. Typical transepithelial electrical resistance values for SCs cultured on Transwell inserts reach a peak value of ∼10–70 Ohms•cm^2 depending on species, cell type, and culture conditions, which are a stark contrast to the 1000+ Ohms•cm^2 of MDCK and Caco-2 cells (Prozialeck and Lamar, 1997; Chui et al., 2011; Mruk and Cheng, 2011; Srinivasan et al., 2015; Papadopoulos et al., 2016; Siemann et al., 2017; Wu et al., 2019; Gerber et al., 2020; Hau et al., 2020a; Tsetsarkin et al., 2020). Consequently, the barrier physiology of these SCs in culture are not completely representative of the in vivo BTB, and conclusions that rely on protein localization of the OCTs in culture models are controversial.

In addition to the OCTs, OCTN1 and OCTN2 were observed to localize to the basal membrane of SCs, although OCTN2 also localized to PMCs (Fig. 2, G and I). OCTN1 and OCTN2 are known to transport L-carnitine, which is important in maintaining normal male reproductive function, although ergothioneine is the preferred substrate for OCTN1 (Tamai et al., 1997; Wang et al., 1999; Palmero et al., 2000; Agarwal and Said, 2004; Grundemann et al., 2005; Kobayashi et al., 2005). Previous studies suggested that OCTN2 localizes to the basal membrane of SCs by assessing [^{3}H]L-carnitine and [^{14}C]TEA uptake across rat SC monolayers cultured on Transwell inserts, where rat OCTN1 is a low affinity transporter for TEA and rat OCTN2 has a significantly higher affinity (Kobayashi et al., 2005; Maeda et al., 2007). The functional data for OCTN2 illustrated in those studies is consistent with the immunohistological observations made here. However, one of the aforementioned studies also speculated that OCTN1 localizes to the apicolateral membrane of rat SCs (Maeda et al., 2007), which is contrary to the basal membrane localization in the present study. These conclusions were also made based on transepithelial transport studies that demonstrated 200 μM and 20 mM unlabeled TEA inhibited basal to apicolateral flux of [^{14}C]TEA, whereas only 20 mM unlabeled TEA inhibited apicolateral to basal flux. Due to these differences in TEA flux through each membrane, the authors concluded that the low affinity TEA transporter, OCTN1, was localized to the apicolateral membrane of SCs. However, this study did not directly assess the function or localization of OCTN1 in cultured cells or tissue. As a result, additional studies that evaluate the expression, localization, and function of the OCTNs are required to provide a comprehensive understanding of disposition across the BTB.

The expression of some OATs and OATPs has been observed in SCs, although there is a lack of information regarding their localization and function in drug disposition in the testes (Kullak-Ublick et al., 1995; Suzuki et al., 2003; Augustine et al., 2005; Cheng et al., 2005b; Bleasby et al., 2006; Huber et al., 2007; Schnabolk et al., 2010; Hau et al., 2020a). These transporters are typically studied in the context of drug disposition to the kidneys or liver; however, they may play a major role in the delivery of some compounds across the BTB. The OATs and OATPs transport many endogenous molecules, including prostaglandins, thyroid hormones, and steroids as well as drugs (e.g., statins, antivirals, and nonsteroidal anti-inflammatory drugs) with varying specificity and selectivity. SCs require these endogenous chemicals to maintain normal spermatogenic processes, which suggests that OATs and OATPs may be involved (Walker and Cheng, 2005; Wagner et al., 2008; Frungieri et al., 2015; Rey-Ares et al., 2018). The basal membrane localization of OAT1, OAT2, OATP1A2, OATP1B1, OATP1B3, OATP2A1, OATP2B1, and OATP3A1-v2 (Fig. 3, A and B; Fig 4, B–D and F–H) may play a role in the uptake of these compounds into SCs, although bidirectional transport is possible for the OATPs. Furthermore, the diffuse staining observed in the adluminal compartment for OATP1A2, OATP1B1, OATP1B3, OATP2B1, and OATP3A1-v2 introduces an interesting mechanism for bidirectional transport across the apicolateral membranes of SCs to supply nutrients for developing germ cells or to recycle compounds in the lumen (Fig. 4, B–D, G, and H). Expression of transporters at basal and apical membranes is not a novel discovery as it has been observed for some transporters such as OATP1A2 or P-gp in the brain (Gao et al., 1999; Ose et al., 2010; Ashraf et al., 2014; Sano et al., 2018); however, expression at one membrane may be higher than the other. It is also possible that the custom OATP antibodies (provided by Dr. Bruno Stieger) used in this study also targeted nonspecific epitopes in human testicular tissues, although there is a large body of evidence supporting their specificity (Gao et al., 2000; Kullak-Ublick et al., 2001; Pizzagalli et al., 2002; St-Pierre et al., 2002; Gao et al., 2005; Huber et al., 2007; Patik et al., 2015; Zhang et al., 2017; Patik et al., 2018; Zhang et al., 2020). Nevertheless, further studies will have to assess the functional expression of these transporters at the basal and/or apicolateral membranes of SCs.

OATP2A1 localization to the basal membrane of SCs is consistent with its role as the prototypical prostaglandin transporter because SCs produce prostaglandins and respond to extracellular prostaglandins, which would be permitted entry or efflux into SCs via OATP2A1 (Cooper and Carpenter, 1987; Samy et al., 2000; Wilhelm et al., 2005). Additionally, diffuse staining of OATP3A1-v2 in seminiferous tubules with the same antibody has been shown in another study, although basal membrane staining was less evident (Huber et al., 2007). Intense staining for OATP1C1 was observed in LCs (Fig. 4E), which is consistent with its primary role as a thyroid hormone transporter and a previous report showing its localization in LCs in human testicular tissue (Pizzagalli et al., 2002). OATP4A1 was also observed in LCs (Fig. 4I), where it may also function as a thyroid hormone transporter (Fujiwara et al., 2001). However, additional studies are required to verify the physiologic function of these transporters in the testes.

Although OATP6A1 is a known testes-specific transporter (Suzuki et al., 2003; Fietz et al., 2013), the antibody tested in this study did not exhibit positive staining in several testicular tissue samples. However, OATP6A1 expression was observed in a heterologous expressing CHO cell line using the same antibody, suggesting the experimental applicability of this antibody in these tissues may not be entirely appropriate due to issues with specimen handling during collection or tissue fixation. OATP6A1 function has been poorly characterized; however, its upregulated expression in certain cancers implies an important physiologic role (Lee et al., 2004). Altogether, the distribution of unidirectional/bidirectional uptake transporters observed here unveils potential mechanisms to effectively deliver pharmaceutics across the BTB and into the MGT but will require functional transport experiments with appropriate models before proceeding with targeted studies.

Several efflux transporters such as P-gp, BCRP, and some MRPs have been shown to be expressed at the basal or apicolateral membranes of SCs (Melaine et al., 2002; Bart et al., 2004; Su et al., 2009; Koraichi et al., 2013; Klein et al., 2014). However, the localization of MRP3, MRP6, and MRP7 to the basal membrane of SCs and the PMCs are the first to be identified here (Fig. 5, A, C, and E). The MRPs that localize to PMCs may act as a first-line defense mechanism to prevent toxicants from reaching the SCs, although SCs can also efflux the toxicants that get through PMCs. Interestingly, MATE1 localized throughout the adluminal compartment of SCs, where apicolateral membrane expression would permit efflux into the lumen and may be coupled with OCT and OCTN uptake at the basal membranes of SCs (Fig. 6A). Cytosolic staining for MATE1 in SCs is likely attributed to expression along the membranes of intracellular vesicles involved in the endosomal-lysosomal pathway as observed in a previous study (Martinez-Guerrero et al., 2016). In the same study, MATE1 was shown to sequester organic cations in these intracellular vesicles (Martinez-Guerrero et al., 2016), which may serve a physiologically important role in SCs for storing OCT/OCTN substrates in reservoirs to maintain normal spermatogenic processes. However, the physiologic and pharmacological roles of these efflux transporters in the testes compared with well-studied efflux transporters such as P-gp, BCRP, MRP1, and MRP2 has been understudied. Consequently, these transporters may adversely influence the disposition of drugs to the MGT to achieve their intended therapeutic effect, and these observations must be taken into consideration.

In conclusion, this study is the first to broadly identify the localization of several pharmacologically relevant drug transporters at the BTB (Hau et al., 2020b,c,d). These data will require additional validation in appropriate in vitro and in vivo models to further understand drug disposition in the testes. However, these findings provide insight into the mechanisms by which chemical contraceptives, antivirals, cancer chemotherapeutics, environmental toxicants, and other relevant compounds can circumvent the BTB to provide therapy to MGT-related disorders.

Acknowledgments

The authors thank Dr. Bruno Stieger of the Department of Clinical Pharmacology and Toxicology at the University of Zurich (Zürich, Switzerland) for providing several anti-OATP antibodies for this study. Additionally, the authors thank Doug Cromey of the Department of SWEHSC Cellular Imaging Facility Core at the University of Arizona (Tucson, AZ) for his advice regarding microscopy and imaging. The authors also thank Dr. John Kanady of the Department of Physiology at the University of Arizona (Tucson, AZ) for his advice on immunohistology and fluorescence microscopy.

Authorship Contributions

Participated in research design: Hau, Klein, Wright, Cherrington.

Conducted experiments: Hau.

Performed data analysis: Hau, Klein, Wright, Cherrington.

Wrote or contributed to the writing of the manuscript: Hau, Klein, Wright, Cherrington.

Citation of Meeting Abstracts

Hau, R., Wright, S., Cherrington, N. (2020b). Expression and Immunolocalization of Xenobiotic Transporters at the Human Blood-Testis Barrier. In: 2020 Mountain West Society of Toxicology Annual Meeting, 2020.

Hau, R., Wright, S., & Cherrington, N. (2020c). Expression and Immunolocalization of Xenobiotic Transporters at the Human Blood-Testis Barrier. The FASEB Journal, 34, 1-1. https://doi.org/10.1096/fasebj.2020.34.s1.08992

Hau, R., Wright, S., & Cherrington, N. (2020d). Expression and Immunolocalization of Xenobiotic Transporters at the Human Blood-Testis Barrier. In: 2020 Annual Meeting Abstract Supplement, Society of Toxicology, 2020. Abstract no. 2875.

References

Agarwal A and Said TM (2004) Carnitines and male infertility. *Reprod Biomed Online* **8**:376–384.

Ashraf T, Kao A, and Bendayan R (2014) Functional expression of drug transporters in glial cells: potential role on drug delivery to the CNS. *Adv Pharmacol* **71**:45–111.

Augustine LM, Markelewicz Jr RJ, Boekelheide K, and Cherrington NJ (2005) Xenobiotic and endobiotic transporter mRNA expression in the blood-testis barrier. *Drug Metab Dispos* **33**:182–189.

Bart J, Hollema H, Groen HJ, de Vries EG, Hendrikse NH, Sleijfer DT, Wegman TD, Vaalburg W, and van der Graaf WT (2004) The distribution of drug-efflux pumps, P-gp, BCRP, MRP1 and MRP2, in the normal blood-testis barrier and in primary testicular tumours. *Eur J Cancer* **40**:2064–2070.

Basit A, Radi Z, Vaidya VS, Karasu M, and Prasad B (2019) Kidney Cortical Transporter Expression across Species Using Quantitative Proteomics. *Drug Metab Dispos* **47**:802–808.

Bleasby K, Castle JC, Roberts CJ, Cheng C, Bailey WJ, Sina JF, Kulkarni AV, Hafey MJ, Evers R, Johnson JM et al. (2006) Expression profiles of 50 xenobiotic transporter genes in humans and pre-clinical species: a resource for investigations into drug disposition. *Xenobiotica* **36**:963–988.

Canet MJ, Merrell MD, Hardwick RN, Bataille AM, Campion SN, Ferreira DW, Xanthakos SA, Manautou JE, A-Kader HH, Erickson RP et al. (2015) Altered regulation of hepatic efflux transporters disrupts acetaminophen disposition in pediatric nonalcoholic steatohepatitis. *Drug Metab Dispos* **43**:829–835.

Cervenkova L, Vycital O, Bruha J, Rosendorf J, Palek R, Liska V, Daum O, Mohelnikova-Duchonova B, and Soucek P (2019) Protein expression of ABCC2 and SLC22A3 associates with prognosis of pancreatic adenocarcinoma. *Sci Rep* **9**:19782.

Chen L, Chen L, Qin Z, Lei J, Ye S, Zeng K, Wang H, Ying M, Gao J, Zeng S et al. (2019) Upregulation of miR-489-3p and miR-630 inhibits oxaliplatin uptake in renal cell carcinoma by targeting OCT2. *Acta Pharm Sin B* **9**:1008–1020.

Chen L, Wang Z, Xu Q, Liu Y, Chen L, Guo S, Wang H, Zeng K, Liu J, Zeng S et al. (2020) The failure of DAC to induce OCT2 expression and its remission by hemoglobin-based nanocarriers under hypoxia in renal cell carcinoma. *Theranostics* **10**:3562–3578.

Cheng CY, Mruk D, Silvestrini B, Bonanomi M, Wong CH, Siu MK, Lee NP, Lui WY, and Mo MY (2005a) AF-2364 [1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide] is a potential male contraceptive: a review of recent data. *Contraception* **72**:251–261.

Cheng X, Maher J, Chen C, and Klaassen CD (2005b) Tissue distribution and ontogeny of mouse organic anion transporting polypeptides (Oatps). *Drug Metab Dispos* **33**:1062–1073.

Chui K, Trivedi A, Cheng CY, Cherbavaz DB, Dazin PF, Huynh AL, Mitchell JB, Rabinovich GA, Noble-Haeusslein LJ, and John CM (2011) Characterization and functionality of proliferative human Sertoli cells. *Cell Transplant* **20**:619–635.

Cooper DR and Carpenter MP (1987) Sertoli-cell prostaglandin synthesis. Effects of (follitropin) differentiation and dietary vitamin E. *Biochem J* **241**:847–855.

Djureinovic D, Fagerberg L, Hallström B, Danielsson A, Lindskog C, Uhlén M, and Pontén F (2014) The human testis-specific proteome defined by transcriptomics and antibody-based profiling. *Mol Hum Reprod* **20**:476–488.

Dolberg AM and Reichl S (2018) Expression analysis of human solute carrier (SLC) family transporters in nasal mucosa and RPMI 2650 cells. *Eur J Pharm Sci* **123**:277–294.

Duflot S, Calvo M, Casado FJ, Enrich C, and Pastor-Anglada M (2002) Concentrative nucleoside transporter (rCNT1) is targeted to the apical membrane through the hepatic transcytotic pathway. *Exp Cell Res* **281**:77–85.

Fietz D, Bakhaus K, Wapelhorst B, Grosser G, Günther S, Alber J, Döring B, Kliesch S, Weidner W, Galuska CE et al. (2013) Membrane transporters for sulfated steroids in the human testis–cellular localization, expression pattern and functional analysis. *PLoS One* **8**:e62638.

Frungieri MB, Calandra RS, Mayerhofer A, and Matzkin ME (2015) Cyclooxygenase and prostaglandins in somatic cell populations of the testis. *Reproduction* **149**:R169–R180.

Fujita S, Hirota T, Sakiyama R, Baba M, and Ieiri I (2019) Identification of drug transporters contributing to oxaliplatin-induced peripheral neuropathy. *J Neurochem* **148**:373–385.

Fujiwara K, Adachi H, Nishio T, Unno M, Tokui T, Okabe M, Onogawa T, Suzuki T, Asano N, Tanemoto M et al. (2001) Identification of thyroid hormone transporters in humans: different molecules are involved in a tissue-specific manner. *Endocrinology* **142**:2005–2012.

Gai Z, Visentin M, Hiller C, Krajnc E, Li T, Zhen J, and Kullak-Ublick GA (2016) Organic Cation Transporter 2 Overexpression May Confer an Increased Risk of Gentamicin-Induced Nephrotoxicity. *Antimicrob Agents Chemother* **60**:5573–5580.

Gandhi R, Cawthorne C, Craggs LJL, Wright JD, Domarkas J, He P, Koch-Paszkowski J, Shires M, Scarsbrook AF, Archibald SJ et al. (2021) Cell proliferation detected using [^{18}F]FLT PET/CT as an early marker of abdominal aortic aneurysm. *J Nucl Cardiol* **28**:1961–1971.

Gao B, Hagenbuch B, Kullak-Ublick GA, Benke D, Aguzzi A, and Meier PJ (2000) Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J Pharmacol Exp Ther* **294**:73–79.

Gao B, Huber RD, Wenzel A, Vavricka SR, Ismair MG, Remé C, and Meier PJ (2005) Localization of organic anion transporting polypeptides in the rat and human ciliary body epithelium. *Exp Eye Res* **80**:61–72.

Gao B, Stieger B, Noé B, Fritschy JM, and Meier PJ (1999) Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. *J Histochem Cytochem* **47**:1255–1264.

Gerber J, Rode K, Hambruch N, Langeheine M, Schnepel N, and Brehm R (2020) Establishment and functional characterization of a murine primary Sertoli cell line deficient of connexin43. *Cell Tissue Res* **381**:309–326.

Gray JH, Owen RP, and Giacomini KM (2004) The concentrative nucleoside transporter family, SLC28. *Pflugers Arch* **447**:728–734.

Grima J, Silvestrini B, and Cheng CY (2001) Reversible inhibition of spermatogenesis in rats using a new male contraceptive, 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide. *Biol Reprod* **64**:1500–1508.

Gründemann D, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, and Schömig E (2005) Discovery of the ergothioneine transporter. *Proc Natl Acad Sci USA* **102**:5256–5261.

Han TK, Everett RS, Proctor WR, Ng CM, Costales CL, Brouwer KL, and Thakker DR (2013) Organic cation transporter 1 (OCT1/mOct1) is localized in the apical membrane of Caco-2 cell monolayers and enterocytes. *Mol Pharmacol* **84**:182–189.

Hardwick RN, Fisher CD, Canet MJ, Scheffer GL, and Cherrington NJ (2011) Variations in ATP-binding cassette transporter regulation during the progression of human nonalcoholic fatty liver disease. *Drug Metab Dispos* **39**:2395–2402.

Hau RK, Miller SR, Wright SH, and Cherrington NJ (2020a) Generation of a hTERT-Immortalized Human Sertoli Cell Model to Study Transporter Dynamics at the Blood-Testis Barrier. *Pharmaceutics* **12**:1005.

Hubeny A, Keiser M, Oswald S, Jedlitschky G, Kroemer HK, Siegmund W, and Grube M (2016) Expression of Organic Anion Transporting Polypeptide 1A2 in Red Blood Cells and Its Potential Impact on Antimalarial Therapy. *Drug Metab Dispos* **44**:1562–1568.

Huber RD, Gao B, Sidler Pfändler MA, Zhang-Fu W, Leuthold S, Hagenbuch B, Folkers G, Meier PJ, and Stieger B (2007) Characterization of two splice variants of human organic anion transporting polypeptide 3A1 isolated from human brain. *Am J Physiol Cell Physiol* **292**:C795–C806.

Jimbo K, Okuno T, Ohgaki R, Nishikubo K, Kitamura Y, Sakurai Y, Quan L, Shoji H, Kanai Y, Shimizu T et al. (2020) A novel mutation in the SLCO2A1 gene, encoding a prostaglandin transporter, induces chronic enteropathy. *PLoS One* **15**:e0241869.

Kato R, Maeda T, Akaike T, and Tamai I (2005) Nucleoside transport at the blood-testis barrier studied with primary-cultured sertoli cells. *J Pharmacol Exp Ther* **312**:601–608.

Kato R, Maeda T, Akaike T, and Tamai I (2006) Characterization of novel Na+-dependent nucleobase transport systems at the blood-testis barrier. *Am J Physiol Endocrinol Metab* **290**:E968–E975.

Kato R, Maeda T, Akaike T, and Tamai I (2009) Characterization of nucleobase transport by mouse Sertoli cell line TM4. *Biol Pharm Bull* **32**:450–455.

Klein DM, Evans KK, Hardwick RN, Dantzler WH, Wright SH, and Cherrington NJ (2013) Basolateral uptake of nucleosides by Sertoli cells is mediated primarily by equilibrative nucleoside transporter 1. *J Pharmacol Exp Ther* **346**:121–129.

Klein DM, Harding MC, Crowther MK, and Cherrington NJ (2017) Localization of nucleoside transporters in rat epididymis. *J Biochem Mol Toxicol* **31**:e21911.

Klein DM, Wright SH, and Cherrington NJ (2014) Localization of multidrug resistance-associated proteins along the blood-testis barrier in rat, macaque, and human testis. *Drug Metab Dispos* **42**:89–93.

Kobayashi D, Goto A, Maeda T, Nezu J, Tsuji A, and Tamai I (2005) OCTN2-mediated transport of carnitine in isolated Sertoli cells. *Reproduction* **129**:729–736.

Kopecky M, Semecky V, and Nachtigal P (2005) Vimentin expression during altered spermatogenesis in rats. *Acta Histochem* **107**:279–289.

Koraichi F, Inoubli L, Lakhdari N, Meunier L, Vega A, Mauduit C, Benahmed M, Prouillac C, and Lecoeur S (2013) Neonatal exposure to zearalenone induces long term modulation of ABC transporter expression in testis. *Toxicology* **310**:29–38.

Kullak-Ublick GA, Hagenbuch B, Stieger B, Schteingart CD, Hofmann AF, Wolkoff AW, and Meier PJ (1995) Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* **109**:1274–1282.

Kullak-Ublick GA, Ismair MG, Stieger B, Landmann L, Huber R, Pizzagalli F, Fattinger K, Meier PJ, and Hagenbuch B (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120**:525–533.

Le Roy B, Tixier L, Pereira B, Sauvanet P, Buc E, Pétorin C, Déchelotte P, Pezet D, and Balayssac D (2016) Assessment of the Relation between the Expression of Oxaliplatin Transporters in Colorectal Cancer and Response to FOLFOX-4 Adjuvant Chemotherapy: A Case Control Study. *PLoS One* **11**:e0148739.

Le Saux O, Fülöp K, Yamaguchi Y, Iliás A, Szabó Z, Brampton CN, Pomozi V, Huszár K, Arányi T, and Váradi A (2011) Expression and in vivo rescue of human ABCC6 disease-causing mutants in mouse liver. *PLoS One* **6**:e24738.

Lee SY, Williamson B, Caballero OL, Chen YT, Scanlan MJ, Ritter G, Jongeneel CV, Simpson AJ, and Old LJ (2004) Identification of the gonad-specific anion transporter SLCO6A1 as a cancer/testis (CT) antigen expressed in human lung cancer. *Cancer Immun* **4**:13.

Lundquist P, Lööf J, Sohlenius-Sternbeck AK, Floby E, Johansson J, Bylund J, Hoogstraate J, Afzelius L, and Andersson TB (2014) The impact of solute carrier (SLC) drug uptake transporter loss in human and rat cryopreserved hepatocytes on clearance predictions. *Drug Metab Dispos* **42**:469–480.

Maeda T, Goto A, Kobayashi D, and Tamai I (2007) Transport of organic cations across the blood-testis barrier. *Mol Pharm* **4**:600–607.

Martinez-Guerrero LJ, Evans KK, Dantzler WH, and Wright SH (2016) The multidrug transporter MATE1 sequesters OCs within an intracellular compartment that has no influence on OC secretion in renal proximal tubules. *Am J Physiol Renal Physiol* **310**:F57–F67.

Melaine N, Liénard MO, Dorval I, Le Goascogne C, Lejeune H, and Jégou B (2002) Multidrug resistance genes and p-glycoprotein in the testis of the rat, mouse, Guinea pig, and human. *Biol Reprod* **67**:1699–1707.

Miller SR, Hau RK, Jilek JL, Morales MN, Wright SH, and Cherrington NJ (2020) Nucleoside Reverse Transcriptase Inhibitor Interaction with Human Equilibrative Nucleoside Transporters 1 and 2. *Drug Metab Dispos* **48**:603–612.

Miller SR, Jilek JL, McGrath ME, Hau RK, Jennings EQ, Galligan JJ, Wright SH, and Cherrington NJ (2021a) Testicular disposition of clofarabine in rats is dependent on equilibrative nucleoside transporters. *Pharmacol Res Perspect* **9**:e00831.

Miller SR, Lane TR, Zorn KM, Ekins S, Wright SH, and Cherrington NJ (2021b) Multiple Computational Approaches for Predicting Drug Interactions with Human Equilibrative Nucleoside Transporter 1. *Drug Metab Dispos* **49**:479–489.

Miller SR, Zhang X, Hau RK, Jilek JL, Jennings EQ, Galligan JJ, Foil DH, Zorn KM, Ekins S, Wright SH et al. (2021c) Predicting Drug Interactions with Human Equilibrative Nucleoside Transporters 1 and 2 Using Functional Knockout Cell Lines and Bayesian Modeling. *Mol Pharmacol* **99**:147–162.

Mital P, Hinton BT, and Dufour JM (2011) The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol Reprod* **84**:851–858.

Mruk DD and Cheng CY (2011) An in vitro system to study Sertoli cell blood-testis barrier dynamics. *Methods Mol Biol* **763**:237–252.

Mruk DD and Cheng CY (2015) The Mammalian Blood-Testis Barrier: Its Biology and Regulation. *Endocr Rev* **36**:564–591.

Mruk DD, Su L, and Cheng CY (2011) Emerging role for drug transporters at the blood-testis barrier. *Trends Pharmacol Sci* **32**:99–106.

Ohara K, Oshima S, Fukuda N, Ochiai Y, Maruyama A, Kanamuro A, Negishi A, Honma S, Ohshima S, Akimoto M et al. (2015) The inhibitory effect of kakkonto, Japanese traditional (kampo) medicine, on brain penetration of oseltamivir carboxylate in mice with reduced blood-brain barrier function. *Evid Based Complement Alternat Med* **2015**:917670.

Ose A, Kusuhara H, Endo C, Tohyama K, Miyajima M, Kitamura S, and Sugiyama Y (2010) Functional characterization of mouse organic anion transporting peptide 1a4 in the uptake and efflux of drugs across the blood-brain barrier. *Drug Metab Dispos* **38**:168–176.

Palmero S, Bottazzi C, Costa M, Leone M, and Fugassa E (2000) Metabolic effects of L-carnitine on prepubertal rat Sertoli cells. *Horm Metab Res* **32**:87–90.

Papadopoulos D, Dietze R, Shihan M, Kirch U, and Scheiner-Bobis G (2016) Dehydroepiandrosterone Sulfate Stimulates Expression of Blood-Testis-Barrier Proteins Claudin-3 and -5 and Tight Junction Formation via a Gnα11-Coupled Receptor in Sertoli Cells. *PLoS One* **11**:e0150143.

Patel H, Younis RH, Ord RA, Basile JR, and Schneider A (2013) Differential expression of organic cation transporter OCT-3 in oral premalignant and malignant lesions: potential implications in the antineoplastic effects of metformin. *J Oral Pathol Med* **42**:250–256.

Patik I, Kovacsics D, Német O, Gera M, Várady G, Stieger B, Hagenbuch B, Szakács G, and Özvegy-Laczka C (2015) Functional expression of the 11 human Organic Anion Transporting Polypeptides in insect cells reveals that sodium fluorescein is a general OATP substrate. *Biochem Pharmacol* **98**:649–658.

Patik I, Székely V, Német O, Szepesi Á, Kucsma N, Várady G, Szakács G, Bakos É, and Özvegy-Laczka C (2018) Identification of novel cell-impermeant fluorescent substrates for testing the function and drug interaction of Organic Anion-Transporting Polypeptides, OATP1B1/1B3 and 2B1. *Sci Rep* **8**:2630.

Pelis RM, Suhre WM, and Wright SH (2006) Functional influence of N-glycosylation in OCT2-mediated tetraethylammonium transport. *Am J Physiol Renal Physiol* **290**:F1118–F1126.

Pelletier RM (2011) The blood-testis barrier: the junctional permeability, the proteins and the lipids. *Prog Histochem Cytochem* **46**:49–127.

Pennycooke M, Chaudary N, Shuralyova I, Zhang Y, and Coe IR (2001) Differential expression of human nucleoside transporters in normal and tumor tissue. *Biochem Biophys Res Commun* **280**:951–959.

Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, and Meier PJ (2002) Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol Endocrinol* **16**:2283–2296.

Pomozi V, Brampton C, Szeri F, Dedinszki D, Kozák E, van de Wetering K, Hopkins H, Martin L, Váradi A, and Le Saux O (2017) Functional Rescue of ABCC6 Deficiency by 4-Phenylbutyrate Therapy Reduces Dystrophic Calcification in Abcc6$^{-/-}$ Mice. *J Invest Dermatol* **137**:595–602.

Prozialeck WC and Lamar PC (1997) Cadmium (Cd2+) disrupts E-cadherin-dependent cell-cell junctions in MDCK cells. *In Vitro Cell Dev Biol Anim* **33**:516–526.

Rey-Ares V, Rossi SP, Dietrich KG, Köhn FM, Schwarzer JU, Welter H, Frungieri MB, and Mayerhofer A (2018) Prostaglandin E_2 (PGE_2) is a testicular peritubular cell-derived factor involved in human testicular homeostasis. *Mol Cell Endocrinol* **473**:217–224.

Samy ET, Li JC, Grima J, Lee WM, Silvestrini B, and Cheng CY (2000) Sertoli cell prostaglandin D2 synthetase is a multifunctional molecule: its expression and regulation. *Endocrinology* **141**:710–721.

Sano Y, Mizuno T, Mochizuki T, Uchida Y, Umetsu M, Terasaki T, and Kusuhara H (2018) Evaluation of Organic Anion Transporter 1A2-knock-in Mice as a Model of Human Blood-brain Barrier. *Drug Metab Dispos* **46**:1767–1775.

Schnabolk GW, Gupta B, Mulgaonkar A, Kulkarni M, and Sweet DH (2010) Organic anion transporter 6 (Slc22a20) specificity and Sertoli cell-specific expression provide new insight on potential endogenous roles. *J Pharmacol Exp Ther* **334**:927–935.

Segal ED, Yasmeen A, Beauchamp MC, Rosenblatt J, Pollak M, and Gotlieb WH (2011) Relevance of the OCT1 transporter to the antineoplastic effect of biguanides. *Biochem Biophys Res Commun* **414**:694–699.

Sekhar GN, Fleckney AL, Boyanova ST, Rupawala H, Lo R, Wang H, Farag DB, Rahman KM, Broadstock M, Reeves S et al. (2019) Region-specific blood-brain barrier transporter changes leads to increased sensitivity to amisulpride in Alzheimer's disease. *Fluids Barriers CNS* **16**:38.

Shao R, Li X, Feng Y, Lin JF, and Billig H (2014) Direct effects of metformin in the endometrium: a hypothetical mechanism for the treatment of women with PCOS and endometrial carcinoma. *J Exp Clin Cancer Res* **33**:41.

Siemann DN, Strange DP, Maharaj PN, Shi PY, and Verma S (2017) Zika Virus Infects Human Sertoli Cells and Modulates the Integrity of the *In Vitro* Blood-Testis Barrier Model. *J Virol* **91**:e00623–17.

Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, and Hickman JJ (2015) TEER measurement techniques for in vitro barrier model systems. *J Lab Autom* **20**:107–126.

St-Pierre MV, Hagenbuch B, Ugele B, Meier PJ, and Stallmach T (2002) Characterization of an organic anion-transporting polypeptide (OATP-B) in human placenta. *J Clin Endocrinol Metab* **87**:1856–1863.

Su L, Cheng CY, and Mruk DD (2009) Drug transporter, P-glycoprotein (MDR1), is an integrated component of the mammalian blood-testis barrier. *Int J Biochem Cell Biol* **41**:2578–2587.

Suzuki T, Onogawa T, Asano N, Mizutamari H, Mikkaichi T, Tanemoto M, Abe M, Satoh F, Unno M, Nunoki K et al. (2003) Identification and characterization of novel rat and human gonad-specific organic anion transporters. *Mol Endocrinol* **17**:1203–1215.

Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, and Tsuji A (1997) Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. *FEBS Lett* **419**:107–111.

Tash JS, Attardi B, Hild SA, Chakrasali R, Jakkaraj SR, and Georg GI (2008a) A novel potent indazole carboxylic acid derivative blocks spermatogenesis and is contraceptive in rats after a single oral dose. *Biol Reprod* **78**:1127–1138.

Tash JS, Chakrasali R, Jakkaraj SR, Hughes J, Smith SK, Hornbaker K, Heckert LL, Ozturk SB, Hadden MK, Kinzy TG et al. (2008b) Gamendazole, an orally active indazole carboxylic acid male contraceptive agent, targets HSP90AB1 (HSP90BETA) and EEF1A1 (eEF1A), and stimulates Il1a transcription in rat Sertoli cells. *Biol Reprod* **78**:1139–1152.

Tsetsarkin KA, Acklin JA, Liu G, Kenney H, Teterina NL, Pletnev AG, and Lim JK (2020) Zika virus tropism during early infection of the testicular interstitium and its role in viral pathogenesis in the testes. *PLoS Pathog* **16**:e1008601.

Umeno J, Hisamatsu T, Esaki M, Hirano A, Kubokura N, Asano K, Kochi S, Yanai S, Fuyuno Y, Shimamura K et al. (2015) A Hereditary Enteropathy Caused by Mutations in the SLCO2A1 Gene, Encoding a Prostaglandin Transporter. *PLoS Genet* **11**:e1005581.

Wagner MS, Wajner SM, and Maia AL (2008) The role of thyroid hormone in testicular development and function. *J Endocrinol* **199**:351–365.

Walker WH and Cheng J (2005) FSH and testosterone signaling in Sertoli cells. *Reproduction* **130**:15–28.

Wang Y, Ye J, Ganapathy V, and Longo N (1999) Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. *Proc Natl Acad Sci USA* **96**:2356–2360.

Wilhelm D, Martinson F, Bradford S, Wilson MJ, Combes AN, Beverdam A, Bowles J, Mizusaki H, and Koopman P (2005) Sertoli cell differentiation is induced both cell-autonomously and through prostaglandin signaling during mammalian sex determination. *Dev Biol* **287**:111–124.
Wu H, Jiang X, Gao Y, Liu W, Wang F, Gong M, Chen R, Yu X, Zhang W, Gao B et al. (2019) Mumps virus infection disrupts blood-testis barrier through the induction of TNF-α in Sertoli cells. *FASEB J* **33**:12528–12540.
Xue P, Crum CM, and Thibodeau PH (2014) Regulation of ABCC6 trafficking and stability by a conserved C-terminal PDZ-like sequence. *PLoS One* **9**:e97360.
Yamamura T, Narumi K, Ohata T, Satoh H, Mori T, Furugen A, Kobayashi M, and Iseki K (2021) Characterization of deoxyribonucleoside transport mediated by concentrative nucleoside transporters. *Biochem Biophys Res Commun* **558**:120–125.
Zhang G, Ma Y, Xi D, Rao Z, Sun X, and Wu X (2019) Effect of high uric acid on the disposition of metformin: in vivo and in vitro studies. *Biopharm Drug Dispos* **40**:3–11.
Zhang H, Kathawala RJ, Wang YJ, Zhang YK, Patel A, Shukla S, Robey RW, Talele TT, Ashby Jr CR, Ambudkar SV et al. (2014) Linsitinib (OSI-906) antagonizes ATP-binding cassette subfamily G member 2 and subfamily C member 10-mediated drug resistance. *Int J Biochem Cell Biol* **51**:111–119.
Zhang X and Wright SH (2009) MATE1 has an external COOH terminus, consistent with a 13-helix topology. *Am J Physiol Renal Physiol* **297**:F263–F271.
Zhang Y, Boxberger KH, and Hagenbuch B (2017) Organic anion transporting polypeptide 1B3 can form homo- and hetero-oligomers. *PLoS One* **12**:e0180257.
Zhang Y, Ruggiero M, and Hagenbuch B (2020) OATP1B3 Expression and Function is Modulated by Coexpression with OCT1, OATP1B1, and NTCP. *Drug Metab Dispos* **48**: 622–630.

Address correspondence to: Nathan J. Cherrington, Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, 1703 E. Mabel Street, Tucson, AZ 85721. E-mail: cherring@pharmacy.arizona.edu