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Emerging role for drug transporters at the blood-testis barrier

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Abstract

Drug transporters are integral membrane proteins that transport a broad range of substrates into and out of cells usually against a concentration gradient. Studies have shown efflux pumps such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) to localize at the blood-testis barrier (BTB), where they protect the testis from drugs and xenobiotics that are detrimental to spermatogenesis. At the same time, efflux pumps might also preclude entry of non-hormonal contraceptives to the testis. In more recent studies, P-gp function was found to correlate with the integrity of the BTB. In this review, we discuss findings that have made a significant impact on our understanding of efflux pumps in the testis. Modulating efflux pump function via specific inhibitors could help to deliver contraceptives to the testis in the future.

Keywords

drug transporter; blood-testis barrier; Sertoli cell; germ cell; testis

Introduction

Drug transporters are integral membrane proteins that facilitate the transport of drugs and xenobiotics into and out of cells usually against a concentration gradient. They have crucial roles in drug and xenobiotic absorption, distribution, clearance and toxicity by acting alone or with drug-metabolizing enzymes. Originally characterized as proteins that brought about multidrug resistance if overexpressed in tumor cells, drug transporters were found to mediate the efflux of chemotherapeutic compounds ¹, thereby reducing efficacy and hampering treatment. Drug transporters are known to translocate a diverse range of drugs and xenobiotics, which also include HIV protease inhibitors, immunosuppressants, antibiotics and steroids ². Several hundred different influx and efflux transporters have been identified so far, and they are expressed at variable levels in many mammalian tissues. For example, the efflux pump P-glycoprotein (P-gp) is highly expressed in many human organs, including the uterus, kidneys, placenta, liver and testis ^{2, 3}. Depending on the driving force behind drug transport, active transporters can be broadly categorized as primary, secondary or tertiary. In primary active transport, substrates are transported unidirectionally using energy generated from ATP hydrolysis. Upon drug binding, one of two ATP-binding domains is activated, resulting in the hydrolysis of one ATP molecule and in the outward

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release of the drug, whereas hydrolysis of a second molecule of ATP restores the efflux pump to its earlier state. Interestingly, mutations in one or both ATP-binding consensus sequences result in the loss of multidrug resistance ⁴. However, secondary and tertiary active transport of substrates is directly coupled to the transport of a co-substrate, and there is no ATP binding ³. Primary transporters belong to the ATP-binding cassette (ABC) superfamily, whereas secondary or tertiary transporters belong to the solute carrier (SLC) superfamily ⁵; the naming scheme was approved and standardized by the Human Gene Nomenclature Committee (<http://www.genenames.org>). To date, the most-well studied, and perhaps the most important, eukaryotic drug transporters are multidrug resistance protein (also known as P-glycoprotein, P-gp, *ABCB1*), multidrug resistance-associated protein (MRP, *ABCC1*) and breast cancer resistance protein (BCRP, *ABCG2*).

Drug transporters are not only important in drug and xenobiotic influx and efflux; they are also involved in other cell processes such as inflammation, differentiation of immune cells, cell detoxification, lipid trafficking, hormone secretion and development of stem cells ⁶⁻¹⁰. As important, drug transporters have also been shown to exert a protective role at the blood-testis barrier (BTB), where they limit drug and xenobiotic entry and accumulation into the testis ^{11, 12}. The functional unit of the testis is the seminiferous epithelium, which is divided into a basal and an adluminal compartment by the BTB ¹³. Each compartment provides a unique environment for germ cell development, and each compartment houses different types of germ cells. Spermatogonia and primary spermatocytes (*i.e.*, preleptotene spermatocytes) reside in the basal compartment, whereas secondary spermatocytes, spermatids and spermatozoa occupy the adluminal compartment. As spermatozoa develop from spermatogonia during a step-wise process known as spermatogenesis, germ cells move upwards in the seminiferous epithelium and towards the tubule lumen so that they can be released upon maturation. These cellular events are orchestrated by ‘nurse-like’ Sertoli cells, which extend from the basement membrane to the luminal edge, and whose role it is to protect and nourish gametes ¹⁴. Unlike most blood-tissue barriers (*i.e.*, blood-brain, blood-retinal barriers), which are constituted by apically-located tight junctions between highly specialized capillary endothelial cells, the BTB is created largely by highly polarized Sertoli cells. Peritubular myoid cells and endothelial capillaries (which are found outside of the seminiferous epithelium) possibly contribute to BTB function as well ¹⁵. Interestingly, the BTB is one of the tightest blood-tissue barriers in the mammalian body (still, it allows entry of essential nutrients such as amino acids, glucose and nucleosides into the adluminal compartment), and it is comprised of at least three different types of co-existing and co-functioning cell junctions, many of which are unique to the testis [Figure 1]. These are tight junctions, desmosome-gap junctions and basal ectoplasmic specializations. During the movement of developing germ cells across the seminiferous epithelium and across the BTB, an enormous amount of restructuring takes place as Sertoli-Sertoli and Sertoli-germ cell junctions are disassembled and reassembled to facilitate cell movement. Regardless, a functional BTB is essential for germ cell development and critical for the extrusion of potentially toxic substances because its dysfunction can result in permanent infertility. In addition to peritubular myoid cells, Leydig cells also reside in the interstitium, and they are known to synthesize and secrete testosterone, which is needed for spermatogenesis ¹⁶. As such, a tightly regulated cascade of cellular events underlies testis function. In this review, we focus on the emerging role of drug transporters at the BTB. This information could have significant impact on our understanding of the pharmacokinetic and pharmacodynamic profiles of new compounds that enter the clinical pipeline in the future. For example, modulating efflux pump function via specific inhibitors might help to deliver non-hormonal contraceptives behind the BTB. This information might also open new ways to overcome drug resistance in other organs.

ABC Transporters

In humans, the ABC transporter family is comprised of approximately 50 members, which are classified into seven subfamilies (A to G) based on their sequence homology to one another. They share a common molecular architecture and contain two distinct domains: (i) the transmembrane domain, which generally consists of six α -helices, and (ii) the cytoplasmic nucleotide-binding domain, which harbors the ATP-binding site. ABC family members are defined as efflux transporters.

P-glycoprotein

P-gp (also known as MDR1) is a well-studied ABC transporter encoded by *Mdr1* in humans, and *Mdr1a* and *Mdr1b* in rodents. First described as an overexpressed protein in tumor cells that were resistant to anticancer drugs, P-gp was subsequently shown to be an ATP-powered efflux pump whose physiological role is to protect organisms from toxic substances by actively transporting compounds out of cells against steep concentration gradients^{2, 17, 18}. In essence, P-gp appears to function as a ‘flippase’ (*i.e.*, transporting substrates from the inner leaflet of the plasma membrane lipid bilayer to the outer leaflet; in this case, the substrate interacts with lipids) and/or as a ‘hydrophobic vacuum cleaner’ (*i.e.*, binding substrates within the plasma membrane and extruding them into the extracellular medium; in this case, the substrate does not interact with lipids)¹⁹. It is believed that P-gp also removes toxic compounds once they have penetrated the plasma membrane and entered into the cytosol, thereby significantly reducing intracellular drug or xenobiotic concentrations. Although P-gp is known to mediate the transport of a wide range of chemotherapeutic compounds out of cells, its substrates are generally lipophilic and amphiphatic compounds ranging from ~300 to 4000 Da in molecular mass. It also participates in the transport of Ca^{2+} channel blockers, HIV protease inhibitors, immunosuppressants, antibiotics, statins and steroids. Interestingly, P-gp expression is widespread and not restricted to tumor cells; expression has also been reported in organs with excretory function (*i.e.*, liver, kidney and small intestine), as well as at blood-tissue barriers (*i.e.*, blood-testis, blood-brain and blood-placenta)^{20, 21}. For example, P-gp localized to Sertoli cells, as well as to peritubular myoid cells and endothelial capillaries, which are also believed to contribute to BTB function¹⁵. P-gp also localized to late spermatids and Leydig cells²²⁻²⁴ [Figure 2]. P-gp’s role at blood-tissue barriers was exemplified best in a study showing that both ivermectin (an antiparasitic drug) and vinblastine (an anticancer drug that inhibits the assembly of microtubules) could penetrate several organs (including the brain and testis) more easily in *mdr1a* $-/-$ versus wild-type mice²⁵. Although *mdr1a* $-/-$ mice were shown to be healthy and fertile (most likely because P-gp is not a structural protein essential for BTB assembly^{25, 26}), these findings reveal that P-gp plays a pivotal role in protecting organisms from harmful substances.

Multidrug resistance-associated proteins

Thus far, eight different MRPs have been identified. Of these, MRP1 is best studied; it is found in several organs, including the brain, kidney, placenta and testis (specifically in Sertoli and Leydig cells^{22, 27}) where it functions as a transporter of anti-cancer and anti-viral drugs, toxicants, antibiotics and androgens. MRPs are also known to transport cyclic nucleotides²⁸, as well as glutathione (when complexed with a drug), glucuronate or sulphate conjugates (*e.g.*, bilirubin glucuronide) – the end-products of detoxification reactions carried out by phase II conjugating enzymes (*e.g.*, glutathione S-transferases, sulfotransferases and UDP-glucuronosyltransferases) – out of cells. Moreover, MRP1 can also efflux heavy metal oxyanions²⁹, illustrating that it can protect cells from environmental toxicants. Interestingly, MRP1 localized to endocytic vesicles³⁰, illustrating that it is internalized and suggesting that it might contribute to drug resistance by

sequestering drugs within vesicles. In the testis, MRP1 localized to the basolateral region of the Sertoli cell plasma membrane near the BTB, as well as to a similar site in Leydig cells²⁷. These observations are in agreement with the basolateral membrane localization of most MRPs (except MRP2), but they are in contrast to the apical membrane localization of P-gp and BCRP (see below) that was noted in many cell types. Although *mrp1* $-/-$ mice are healthy and fertile, treating these mice with etoposide, a cancer drug known to inhibit topoisomerase II (an enzyme that functions in the cleavage and re-ligation of double-stranded DNA in normal cells) halted spermatogenesis, resulting in transient infertility²⁷. These effects were greatly reduced in wild-type mice treated with the same drug, illustrating that MRP1 precludes drugs from entering the adluminal compartment of the seminiferous epithelium to protect developing germ cells. These findings also suggest that the loss of MRP1 could affect testosterone production and/or transport. This would result in BTB restructuring and germ cell loss from the seminiferous epithelium because testosterone is critical for BTB integrity and germ cell adhesion³¹. Indeed, serum and testicular steroid levels were found to be reduced by several-fold in *Mrp1* knockout mice³². More recent studies have defined transcription factors known as nuclear hormone receptors (*e.g.*, pregnane x and constitutive androstane receptors) as upregulators of drug-metabolizing enzymes and transporters such as MRP and P-gp at blood-tissue barriers^{33, 34}. For example, the blood-brain barrier (BBB) was strengthened in transgenic mice expressing human pregnane X receptor which limited entry of methadone. Similar to P-gp, which is activated by protein kinases³⁵, MRP1 is also phosphorylated³⁶. These results indicate that multiple mechanisms (*i.e.*, transcriptional regulation, protein phosphorylation) underlie drug transport. MRP4 is also known to facilitate the transport of cyclic nucleotides³⁷, which suggests its role in signal transduction events.

BCRP

BCRP was first identified from a doxorubicin-resistant breast cancer cell line as an ABC half-transporter that must oligomerize to confer drug resistance³⁸. Subsequent studies, however, revealed that BCRP is expressed weakly by breast cancer tissue and is present in other organs (*e.g.*, brain, placenta and testis). BCRP has been shown to actively efflux a broad spectrum of hydrophilic and hydrophobic substrates, including anti-cancer, anti-viral and anti-hypertension drugs, antibiotics and organic anions. BCRP can also efflux natural substrates such as phosphatidylserine and protoporphyrin IX. Moreover, BCRP has also been shown to efflux several tyrosine kinase inhibitors, although during clinical development it was anticipated that they would bind specific targets and derange aberrant cancer signaling cascades. Similar to P-gp and MRP1, BCRP localizes predominantly to the plasma membrane^{39, 40}. At the plasma membrane, BCRP was shown to interact and colocalize with Pim-1L, a serine/threonine kinase that mediates BCRP phosphorylation and dimerization⁴¹. In another study, selective inhibition of phosphatidylinositol-3 kinase (PI3K) activity by wortmannin resulted in the translocation of BCRP into the cytoplasm and in the loss of transport activity in gall bladder cells⁴². These findings imply that BCRP-mediated drug resistance might be overcome by targeting the PI3K signaling cascade, which is often activated during cancer and cell movement, or by targeting BCRP-Pim-1 interactions. In addition to kinases, sex hormones such as estrogen, progesterone and testosterone are also known to regulate BCRP^{43, 44, 45}. For example, BCRP transporter activity was shown to decrease in isolated brain capillaries exposed to 17 β -estradiol⁴³. In some cancer cell lines, however, estrogen receptor α activity increased in response to 17 β -estradiol, resulting in an upregulation of BCRP expression⁴⁶. At present, the reason behind this discrepancy is unknown. In the testis, BCRP was shown to limit the entry of phytoestrogens (*e.g.*, genistein), plant compounds that bind to Sertoli and Leydig cell estrogen receptors to produce weak estrogenic or anti-estrogenic effects^{47, 48}. It is worth noting that phyto- and xenoestrogens (*e.g.*, bisphenol A which is known to affect BTB

dynamics 49, 50) are endocrine disruptors, *i.e.*, they can adversely affect reproductive function by drastically reducing semen quality and lowering sperm count, ultimately leading to infertility ⁵¹. Taken collectively, these findings show that BCRP helps to maintain spermatogenesis by extruding phyto- and xenoestrogens. In addition, progesterone, (as well as testosterone in combination with 17 β -estradiol) was shown to increase BCRP expression in a human choriocarcinoma cell line ^{52, 53}. These results are interesting because they suggest that BCRP might mediate the efflux of hormones *in vivo* that needed by some types of cancers to survive, and this could help to stunt tumor growth. In Sertoli cells, BCRP might also drive the efflux of hormones that are needed for germ cell development and maturation.

SLC Transporters

The SLC transporter superfamily, which is involved in the transport of a broad range of substrates, includes organic anion (OATs) and organic cation transporters (OCTs), organic anion transporting polypeptides (OATPs), organic cation/carnitine transporters (OCTNs), peptide transporters (PEPTs), concentrative nucleoside (CNTs) and equilibrative nucleoside (ENTs) transporters, multidrug and toxin extrusion transporters (MATEs) and monocarboxylate transporters (MCTs). SLC family members are defined as influx and/or bi-directional (*i.e.*, influx + efflux) transporters. In this section, we focus on OATPs and OCTs that have been studied in the testis.

OATPs

OATPs are six members (*i.e.*, OATP1-6) of the *SLCO* gene family that are known to transport anionic, cationic, neutral and zwitterionic compounds (*e.g.*, anti-cancer and anti-hypertension drugs, HIV protease inhibitors, statins, bile acids, bilirubin, prostaglandins, antibiotics and steroids) into and out of the kidney, liver, brain, intestine, placenta and testis. In the human and rat, OATPs function at blood-tissue barriers to control substrate influx and efflux ^{54, 55}. Their cellular localization is variable depending on cell type; they concentrate either to the apical or the basolateral region of the plasma membrane. The presence of various OATPs (such as OATP1C1, OATP3A1 and OATP4A1) in the testis has been reported ⁵⁶⁻⁵⁸. For example, OATP3A1 is expressed by Sertoli and germ cells ⁵⁸. The polarized expression of OATP3A1 (*SLCO3A1*) in Sertoli cells could account for the influx of substrates such as testosterone across the BTB ⁵⁹.

Organic cation transporters

The organic cation transporter family is comprised of OCTs 1-3 and 6, and OCTNs 1-3, which belong to the *SLC22* gene family. In general, OCTs bi-directionally transport hydrophilic organic cations of low molecular weight (*i.e.*, less than 500 Daltons) and weak bases that are positively charged at physiological pH. However, OCTNs and OCT6 transport cations, as well as carnitine which is important in the transfer of long-chain fatty acids from the cytosol and into the mitochondria for β -oxidation. Other OCT and OCTN substrates include anti-cancer and anti-viral drugs, antibiotics, and Ca²⁺ and Na⁺ channel blockers ⁶⁰. Of these, OCTs 1, 3 and 6, and OCTNs 1-3 have been shown to be expressed by Sertoli cells, in which they regulate the entry of different compounds needed for the maturation of spermatozoa ⁶⁰. Sertoli cells also express CT2, a novel carnitine transporter that is specific to the testis ⁶¹. On a final note, OCT2 expression was upregulated by testosterone in the kidney of the rat ⁶², illustrating that the androgen receptor is involved in the regulation of this influx pump. In light of the importance of sex hormones in the function of certain barriers such as the BTB ^{31, 63}, complementary studies should be performed using other *in vitro* and *in vivo* systems.

Role of efflux pumps at the BTB

It is now well accepted that efflux pumps are also key components of physiological barriers, including the BTB ²² [Figures 1· 2]. For example, *Mdr1a*, *Mrp1* and *Bcrp*, as well as occludin, were found to increase in the rat brain following chronic administration of morphine ⁶⁴, suggesting that occludin might tighten the BBB to morphine via the upregulation of drug transporters. In this section, we discuss how efflux pumps interact with the cytoskeleton and cell junctions to protect the testis from unwanted drugs and xenobiotics.

Several studies have reported drug transporters such as P-gp and MRP1 to be regulated by cytoskeletal changes that manifest downstream of cell junction and barrier dynamics. For example, P-gp was shown to co-localize and to co-immunoprecipitate with actin [Figure 1], as well as with ezrin, radixin and moesin (ERM) ⁶⁵, a small family of widely distributed adaptor proteins that links transmembrane proteins to actin filaments at adherens junctions ⁶⁶. Similarly, a pull-down assay demonstrated that MRP1 (and possibly MRP2) interacts with radixin ⁶⁰. When antisense oligonucleotides were used to inhibit ERM function in CEM-VBL100 cells, a change in P-gp localization was observed, P-gp–actin interactions were lost, and P-gp-mediated drug efflux was affected ⁶⁵, illustrating that P-gp–actin and P-gp–ERM binding are critical for drug transport. Current data reveal that ERM proteins are also important in the establishment of cell polarity which is crucial for barrier function ⁶⁷. This suggests that spatial cues from the polarity complex could be essential for P-gp and MRP1 localization and function in Sertoli cells. For example, deregulation of Scribble, a scaffolding protein whose primary function is to correctly position junctions within epithelial cells, was shown to perturb cell polarity, block three-dimensional morphogenesis, promote uncontrolled proliferation and inhibit apoptosis ⁶⁸, processes that might directly contribute to multidrug resistance. P-gp was also shown to be regulated by RhoA, an important regulator of F-actin and microtubule dynamics, cell polarity and junction dynamics. Silencing of this GTPase in HT29 cells (human colon adenocarcinoma) resulted in decreased P-gp ATPase activity and increased sensitivity to doxorubicin ⁶⁹. Furthermore, actin-perturbing compounds such as cytochalasin D have been shown to down-regulate P-gp expression in hepatocytes ⁷⁰ and to enhance drug accumulation in leukemia cells ⁷¹, indicating that cytoskeletal integrity, as well as cell polarity, contribute to P-gp overexpression and drug efflux.

Interestingly, P-gp can also link to the cytoskeleton via caveolin ⁷², a scaffolding protein best known for its role in forming 50-100 nm invaginations of the plasma membrane [Figure 1]. These invaginations or caveolae are rich in proteins and lipids, and they function in signal transduction and endocytosis ⁷³. When P-gp associated with the caveola protein caveolin-1, its transport activity was shown to decrease ⁷² [Figure 1]. In support of these data, P-gp was also shown to transit between the plasma membrane and the early endosome ^{74, 75}, revealing that P-gp function can be regulated by discrete changes in cellular localization. It is also plausible that P-gp sequesters drugs to intracellular vesicles that are subsequently eliminated by exocytosis. Other studies have demonstrated ubiquitination, a process that targets proteins for proteolytic degradation, to modulate P-gp function as well by controlling its stability and turnover ⁷⁶. Specifically, proteasome inhibitors were found to slow down the aging of P-gp, thereby perpetuating its presence at the plasma membrane where it contributed to multidrug resistance ⁷⁷. These results establish that the multidrug phenotype can be partially reversed, at least at the *in vitro* level, by altering the cellular localization of P-gp. However, it is presently unknown if this multifactorial mechanism also applies to the regulation of other efflux pumps such as MRP or BCRP. These findings have also raised many other important questions. For example, which signaling events underlie P-gp internalization? Previous studies using different cell types have demonstrated that certain

cytokines can up- or downregulate the expression of various drug transporters such as P-gp⁷⁸⁻⁸⁰. Surprisingly, the converse was also true: P-gp was shown to inhibit the secretion of cytokines. This information is important because many cytokines can disrupt the BTB by compromising tight and anchoring junctions^{81, 82}, and this can permit entry of harmful compounds into the adluminal compartment of the seminiferous epithelium [Figure 1]. For example, the proinflammatory cytokine tumor necrosis factor α is directly involved in the endocytosis and degradation of integral membrane proteins, thereby resulting in junction disassembly⁸³. Taken collectively, these findings illustrate that P-gp function is dependent on barrier integrity.

Our results have also cast new light on the role of P-gp at the BTB. As anticipated, P-gp was found to be present at the BTB and to display a ring-like localization pattern in seminiferous tubules at all stages of the epithelial cycle²⁴, revealing that P-gp protects germ cells residing in the adluminal compartment from harmful drugs and xenobiotics [Figure 2]. P-gp was also shown to associate with germ cells, in particular elongated spermatids lining the luminal edge of the seminiferous epithelium [Figure 2]. Equally important, this efflux pump was found to interact (albeit weakly) with several tight junction proteins, namely occludin, claudin-11 and junctional adhesion molecule-A (JAM-A) if testis lysates were used for co-immunoprecipitation experiments performed under stringent conditions²⁴. Interestingly, these protein-protein interactions increased several-fold shortly after adult rats were orally treated with adjuvin, a compound that affects adhesion of germ cells to Sertoli cells, thereby resulting in transient infertility^{84, 85} [Figure 1]. These findings are important for several reasons. First, they illustrate that P-gp is a constituent of the junctional complex. Second, they suggest that P-gp may control the cellular distribution of tight junction proteins at the plasma membrane and finally, that an increase in P-gp-occludin/claudin-11/JAM-A interactions may result in the tightening of the barrier, possibly to impede entry of additional adjuvin or other substrates into the adluminal compartment. Although there is ample room for additional experiments, these data show that a unique mechanism exists in the testis to protect germ cell development.

Concluding remarks

In this review, we have highlighted recent findings to support the emerging role of drug transporters at the BTB. We have stressed that efflux pumps are highly expressed at the BTB to protect the testis from unwanted drugs and xenobiotics. Although the extrusion of drugs and xenobiotics is a positive attribute of efflux pumps, in some cases it can be a negative one, especially if the target of the therapeutic agent is located behind the blood-tissue barrier. In essence, modulating efflux pump function via specific inhibitors, (if they can be delivered specifically to the testis) might help to deliver non-hormonal contraceptives behind the BTB. Still, there are too few studies of this nature in the testis, and additional research is needed to better understand how drugs and xenobiotics gain or fail to gain entry into the adluminal compartment of the seminiferous epithelium.

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Glossary

ABC transporter	a transporter that functions as an influx pump. It can transport a broad range of substrates, including anti-cancer drugs, HIV
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	protease inhibitors, Ca ²⁺ channel blockers, immunosuppressants, antibiotics, statins and steroids.
Adherens junction	zonula adherens, a type of adhesive junction found between epithelial cells. Adherens junction proteins are anchored to the actin cytoskeleton via adaptor proteins.
Adjudin	1-(2,4-dichlorobenzyl)-1 <i>H</i> -indazole-3-carbohydrazide, a contraceptive compound that selectively affects adhesion between Sertoli and germ cells, thereby resulting in transient infertility.
Blood-testis barrier	an immunological barrier formed largely by Sertoli cells that separates the seminiferous epithelium into two compartments: basal and adluminal. The BTB is found near the basement membrane but above preleptotene spermatocytes, and it is comprised of co-existing and co-functioning tight junctions, desmosome-gap junctions and basal ectoplasmic specializations. Peritubular myoid cells which encircle seminiferous tubules and unfenestrated endothelial capillaries which reside in the interstitium may also contribute to BTB function.
Desmosome-gap junction	a unique hybrid type of adhesive junction found between Sertoli cells at the blood-testis barrier and between Sertoli cells and non-elongating/elongated germ cells (<i>i.e.</i> , those up to, but not including, step 8 spermatids in the rat) in the testis. The desmosome-gap junction shares characteristics of both the desmosome and the gap junction. Desmosome proteins are anchored to the intermediate filament cytoskeleton via adaptor proteins.
Drug transporter	an integral membrane protein that utilizes ATP hydrolysis or ion exchange/co-transport to carry drugs, xenobiotics and other compounds into or out of cells.
Ectoplasmic specialization	a unique hybrid type of junction found between Sertoli cells at the blood-testis barrier (<i>i.e.</i> , basal ectoplasmic specialization) and between Sertoli cells and elongating/elongated spermatids (<i>i.e.</i> , apical ectoplasmic specialization) in the testis. It has roles in germ cell adhesion, spermatid orientation and positioning within the seminiferous epithelium and shaping of the spermatid head. Ectoplasmic specialization proteins are anchored to the actin cytoskeleton via adaptor proteins.
Leydig cell	a cell found in the interstitium between seminiferous tubules in the testis. It produces testosterone (an androgen) in the presence of luteinizing hormone (a hormone produced by the pituitary gland).
Peritubular myoid cell	a cell that surrounds seminiferous tubules and contributes to the making of the basement membrane by secreting extracellular matrix components and growth factors. Peritubular myoid cells interact intimately with Sertoli cells. They may also have a role in BTB function.
Seminiferous epithelium	the functional unit of the testis where spermatogenesis occurs.

Sertoli cell	a somatic cell within the seminiferous epithelium of the testis whose function is to support developing germ cells throughout spermatogenesis.
SLC transporter	a transporter that functions as an influx and/or bi-directional pump. It can transport a broad range of substrates, including anti-cancer and anti-hypertension drugs, HIV protease inhibitors, antibiotics, statins and steroids.
Spermatid	a haploid germ cell whose cell precursor is the spermatocyte. Spermatids undergo spermiogenesis to form spermatozoa.
Spermatocyte	a germ cell whose cell precursor is the spermatogonium. Primary spermatocytes (diploid) undergo meiosis I and II to become secondary spermatocytes (haploid) and spermatids, respectively.
Spermatogenesis	a complex process in which spermatozoa are formed from spermatogonia.
Spermatogonium	a diploid germ cell residing at the basement membrane of the seminiferous epithelium. Type A spermatogonia are capable of self-renewal, whereas type B spermatogonia differentiate into primary spermatocytes.
Spermatozoon	a mature germ cell capable of fertilizing the ovum.
Spermiation	the release of spermatozoa from the seminiferous epithelium at the completion of spermatogenesis.
Tight junction	zonula occludens, a type of junction found between epithelial and endothelial cells whose function is to seal the intercellular space and to create an impermeable barrier. Tight junction proteins are anchored to the actin cytoskeleton via adaptor proteins.
Xenobiotic	a compound not normally found in the body.

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Figure 1. Role of drug transporters at the BTB

(a) Cross-section of the seminiferous epithelium in the adult rat testis. Two Sertoli cells situated atop the tunica propria, which is composed of the basement membrane, type I collagen, peritubular myoid cells, lymph and the lymphatic endothelium, are depicted. Leydig cells and developing germ cells are also shown. All of these cell types are known to express drug transporters (see text). (b) A magnified view of tight junctions at the BTB in the presence of drug or xenobiotic but *without* the multidrug resistance (MDR) phenotype. Here, P-gp and MRP1 are present at the plasma membrane, and also within caveolae and lysosomes where they are inactive. At the plasma membrane, P-gp interacts with occludin, claudin-11 and JAM-A, as well as with actin. Protein kinases such as s-Src, which is known to function at the BTB, may play a role in P-gp phosphorylation, thereby shuttling P-gp away from the plasma membrane. (c) A magnified view of tight junctions at the BTB in the presence of drug or xenobiotic and *with* the multidrug resistance phenotype. Here, P-gp, and possibly MRP1, was overexpressed at the plasma membrane where likewise it associated with occludin, claudin-11, JAM-A and actin. This increase in P-gp–occludin/claudin-11/JAM-A interactions resulted in the tightening of the barrier, thereby impeding entry of additional adjuvant or other substrates into the adluminal compartment.

Figure 2. Localization of P-gp in the adult rat testis

(a-h) Frozen sections were immunostained for P-gp using a monospecific antibody (Santa Cruz Biotechnology, Cat. # sc-55510, Lot # B1108). P-gp immunoreactivity (*i.e.*, brown precipitate) was found at the BTB [see arrowheads in (a)], as well as at the apical ectoplasmic specialization [see asterisks in (e)]. Leydig cells and endothelial cells stained for P-gp. Cell nuclei were stained with hematoxylin. Stages of the seminiferous epithelial cycle are noted as roman numerals. bv, blood vessel. Bar in (a) = 220 μm ; (b) = 100 μm ; (c), also applies to (d)-(h) = 50 μm .