OPINION

Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule?

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Abstract | It is generally thought that many drug molecules are transported across biological membranes via passive diffusion at a rate related to their lipophilicity. However, the types of biophysical forces involved in the interaction of drugs with lipid membranes are no different from those involved in their interaction with proteins, and so arguments based on lipophilicity could also be applied to drug uptake by membrane transporters or carriers. In this article, we discuss the evidence supporting the idea that rather than being an exception, carrier-mediated and active uptake of drugs may be more common than is usually assumed — including a summary of specific cases in which drugs are known to be taken up into cells via defined carriers — and consider the implications for drug discovery and development.

Obtaining a better understanding of the factors that affect drug pharmacokinetics and pharmacodynamics is a key factor in improving the effectiveness of the drug discovery process. Crucial to this understanding is the question of how a drug (or a metabolite thereof), when applied to an organism or tissue, gains access to its target. Putting aside anatomical details, paracellular transport and endocytosis (see BOX 1 for the rationale for these simplifications), and assuming oral administration and a reasonable aqueous solubility, the major issue is then whether or not drugs normally cross cell membranes by diffusion through a lipid bilayer portion of the membrane. Alternatively, drugs may 'hitchhike' on carriers or transporters that act on natural endogenous substrates (albeit that these substrates or carriers are often unknown) (FIG. 1).

In studies of drug absorption and distribution, diffusion through the lipid bilayer is often considered to be the dominant process. For example, Lipinski's influential 'rule of 5' (Ro5)[1] for predicting the likelihood of poor absorption or permeation of orally administered drugs assumes the pre-eminence of diffusion, and classes carrier-mediated uptake of a drug as an exception to which the rule does not apply. The Ro5 predicts that compounds are more likely to have poor absorption or permeation when two or more of the following parameters are exceeded: molecular mass >500 Daltons, calculated octanol–water partition coefficient cLogP >5, number of hydrogen-bond donors >5, and number of hydrogen-bond acceptors >10. These empirical guidelines, which concentrate on lipophilicity and on hydrogen-bond formation, have been of immense importance in our understanding at a phenomenological level of the transfer of drugs across membranes and their disposition within multicellular organisms, as have various related biophysical measures.

Lipinski also noted that: "…orally active therapeutic classes outside the Ro5 are antibiotics, antifungals, vitamins and cardiac glycosides. We suggest that these few therapeutic classes contain orally active drugs that violate the Ro5 because members of these classes have structural features that allow the drugs to act as substrates for naturally occurring transporters." It is also worth noting that most of these compounds are natural products or derivatives thereof (see below).

The types of biophysical forces that determine the interaction of drugs with lipids (especially hydrophobic and hydrogen-bonding interactions) are no different from those involved in their interaction with proteins, especially hydrophobic transport proteins. Therefore, biophysical arguments alone cannot make a mechanistic distinction between the two modes of transport that are outlined in FIG. 1. Indeed, four lines of reasoning together suggest that carrier-mediated cellular uptake of drugs could be more widespread than is assumed at present.

The first and most direct line of evidence is that there are many specific cases in which drugs are known to be taken up into cells via defined carriers. Related to this is the demonstration of the necessity for carriers for certain lipophilic cations that had been assumed to cross membranes solely via diffusion. Furthermore, it is often the case that drugs accumulate in particular tissues (that is, they do not simply leak out of cells down a concentration gradient), and their accumulation is often greater than any possible number of intracellular binding sites. Last, we note the ability to enhance cellular uptake substantially with the prodrug approach using moieties that are known to be substrates for carriers. While we recognize that all scientific evidence may be open to more than one interpretation, we believe that, when taken together over the wide range of systems that we discuss, the argument for a more prominent role of carrier-mediated uptake is compelling. This view has considerable ramifications for future drug discovery, which are summarized in BOX 2.

Transfer of molecules across membranes

A classical means with which to study the transport of molecules across the lipid portion of bilayer membranes is to use membranes made solely of those lipids (often including, importantly, considerable amounts of organic solvent)[2]. Typically, the bilayer lipid membrane (BLM, often referred to as black lipid membrane because its thickness is such as to cause destructive interference of visible light) is formed across a small (~1 mm) orifice in a teflon cup, and separates two aqueous phases. Such BLMs[3,4] have been of immense utility in the study of biophysical

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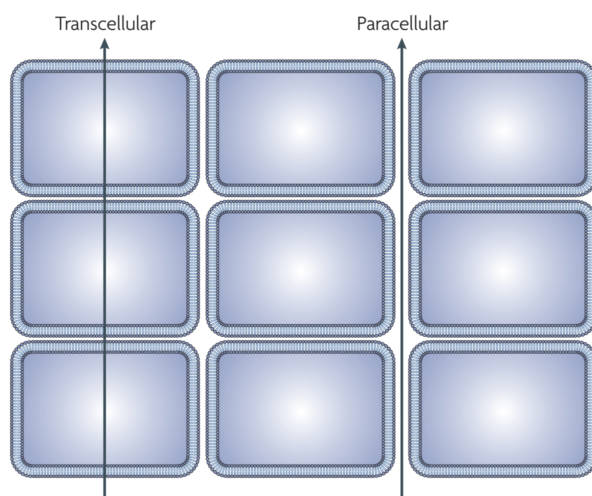
Box 1 | **Molecular transmembrane transport as a focus of this review**

As shown in FIG. 1, the focus of this article begins with the widespread view of biological membranes as a fluid mosaic in which polytopic proteins are embedded in an effectively two-dimensional sea of phospholipid bilayer. Such cells are typically embedded in tissues in which arrays of cells form the actual barrier that must be penetrated. A schematic of such a tissue is shown below. This illustrates two other issues regarding the transfer of molecules across the tissue. The first is that there is the potential for a paracellular route, in which molecules bypass the cell membrane barriers via the extracellular spaces. This does not provide for intracellular access of the drugs of interest. The second is that the ease of passing a tissue from one side to the other might also depend on anatomical factors such as the number of cell membranes that must be crossed. Little is known about the latter, and such effects are in a sense additional to transcellular and paracellular transport. There is also the possibility of intracellular vesiculation (endocytosis), allowing molecules in an external aqueous phase to enter an intracellular aqueous phase without actually crossing a phospholipid bilayer. This, however, cannot serve to effect transfer across a whole tissue.

Last, we largely do not consider other second-order effects, such as membrane curvature and lipid rafts, and simply ask the question: do molecules traverse the barrier that the membrane represents by diffusion through the lipid portion of the membrane, in a manner governed essentially by logP, or by interactions with proteins that mediate their transmembrane transport? This is a very general question that just treats a cell membrane as a closed vesicle separating the inside of a cell from the outside.



phenomena, including transmembrane transport across them, as it is possible to measure directly the rate of passage of molecules from one aqueous compartment to the other that the BLM separates. The dissolution of drugs in aqueous media, and their extraction from them into membranes, are governed by the making and breaking of hydrogen bonds and a general measure of lipophilicity or hydrophobicity (which tends to increase with molecular mass)[5,6]. Leaving aside the question of whether a membrane is truly a solvent, solute partitioning between phases can be reasonably well described by the Abraham model[5,7,8]. This includes as its elements terms for hydrogen-bond donor and acceptor potential (basicity/acidity), for polarizability/dipolarity, for molecular volume, and for the excess molar refraction.

Models such as this are multiple regression models that weight each of the terms differently for different sets of conditions (for example, the pH, the tissue of interest), and are therefore capable of wide applicability. Given the biophysical basis of these elements, we see that the biophysical forces involved in the transfer of molecules across membranes are no different in principle from those involved in ligand binding to protein targets (including carriers)[9]. Therefore, biophysical measures of this type (as described in examples in REFS 10–13) that predict absorption, distribution, metabolism and excretion (ADME) — while assuming they are diffusion-mediated events — could also be applied to transport using a protein or proteins.

It is easy to assume that what is true for a BLM is also true for a biological membrane, but this is by no means logical. First, significant transport across the comparatively unstable BLM is known to occur via pore defects[3,14], a mode of transport that is probably much less significant in biological membranes. Second, the mass, and sometimes area and ratio of protein:lipid in many biological membranes (1:1 to 3:1)[15] is such that it is inevitable that proteins affect the transport properties of lipids or pores that may be used for small-molecule transport in their absence, notwithstanding the effects of specific lipids on the permeability of molecules through BLM or natural membranes. Last, the interactions between lipids and proteins have profound effects on the behaviour and properties of each other, for example, in modulating enzyme activity[16] or in affecting aggregation[17].

Although they have been shown to cross BLMs, the small neutral molecules urea[18] and glycerol[19], which have been widely assumed to permeate phospholipid membranes rapidly by diffusion, have been shown to use carriers to penetrate biological membranes. Indeed, glycerol is an osmolyte in yeasts[20], and therefore has to be effectively impermeant across phospholipid bilayers. Even water, which, as judged by osmotic swelling experiments, ostensibly crosses biomembranes extremely rapidly[21,22], can be transported via aquaporin carriers[23]. Moreover, in liposomes the rate of transfer of non-electrolytes depends strongly on molecular mass rather than on logP[24].

Consequently, the true extent to which molecules can cross the BLM by dissolution in a 'bulk' membrane phase could be small. Additionally, models that relate diffusion rates across membranes to specific biophysical properties, such as logP, should be based on large sample numbers and validated with examples that have not been used in the construction of the model. To date, we are not aware of any studies that have succeeded in providing the requisite data.

High-throughput analogues of BLMs, in particular the parallel artificial membrane permeation assay (PAMPA), have been developed and used in the analysis of drug transport. PAMPA[25] involves the study of drug transfer across phospholipid-impregnated filters. However, the flux across them can be poor for some drugs even when their absorption in humans is good (see for example, cephalexin, tiacrilast and others in REF. 25). The correlations of drug uptake, even of established drugs, with both logP and with transport across Caco-2 cells (a widely used cell model of intestinal transport[26,27]) can also be weak (see for example, REFS 28–32). FIGURES 2,3 show some data re-plotted from a recent study[33] comparing drug permeability using PAMPA and Caco-2 cells. Although the selected drugs for analysis are considered to cross membranes by diffusion and also mainly have adequate absorption, it is apparent that several of them with apparently poor PAMPA permeability are in fact absorbed well by cells. Since we are unaware of any other such comparative study (that is, the uptake of drug molecules into biological cells versus that across artificial phospholipid or other hydrophobic membranes lacking carriers), we would recommend that in the future, additional data need to be

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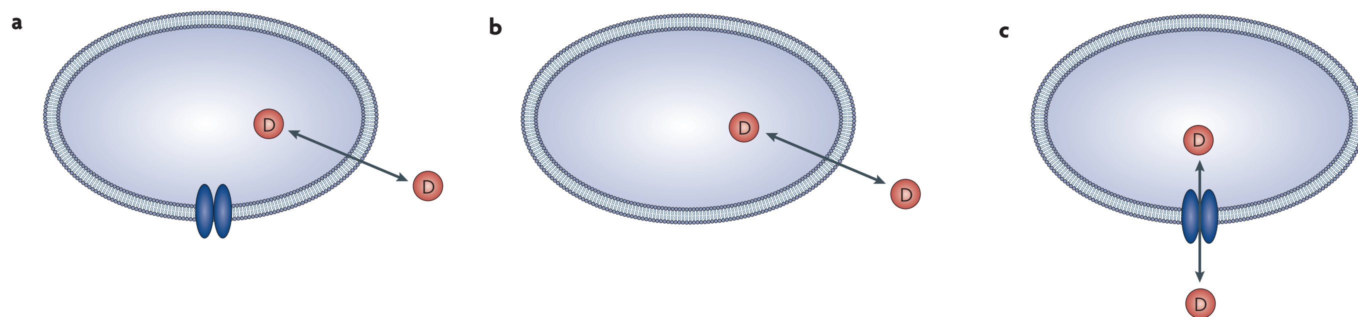

Figure 1 | **Transmembrane transport of drugs. a** | The membrane-bounded compartment is taken to consist of a lipid bilayer in which proteinaceous carriers are embedded. The drug (D) partitions into the lipid bilayer portion of the membrane roughly according to logP (the octanol–water partition coefficient) and redissolves in the intracellular fluid. **b** | In this view, drug transport occurs via transfer across the bilayer membrane exactly as it might do in a phospholipid membrane lacking any proteins (although we note that these may more readily admit passage via aqueous pore defects that do not occur so readily in a protein-containing natural biomembrane). **c** | In an alternative view, which is the focus of this article, most or all of the drug transport occurs via proteinaceous carriers that exist in the membrane and that normally transport natural cellular and extracellular metabolites (that is, those biosynthetically produced by the organsim) but which also show activity in transporting xenobiotics. Models (**b**) and (**c**) are not mutually exclusive and could in principle occur together in the same membrane. Overall, the steady-state, free intracellular concentration of a drug will reflect an interplay between passive uptake and the activities of influx and efflux transporters.

included in the analyses of the permeability of drugs. For example, comparisons of artificial membrane permeability, Caco-2 cell permeability and logP that claim good correlations should give the data in graphical as well as tabular form, and give both the slopes and the correlation coefficients obtained. In addition, they should involve a wide range of chemistries, as a model that describes the behaviour of a homologous series (often via logP) when viewed alone may be inadequate when applied to other moieties. For example, data based on alcohols were poor at predicting the effects of phthalates[34]. Many factors including solubility, formulation, pH and intestinal enzymology can affect drug uptake[35]. The Ro5 favours intermediate values of cLogP[36], reflecting, in part, the need for drugs to exist in both the aqueous phases and in hydrophobic milieux such as membranes (or integral membrane proteins). There are many kinds of structural and biophysical cheminformatic descriptors that can be used to account for the relationships between particular molecular properties and a biological activity such as uptake[37]. However, because optima are often at intermediate values it is not necessarily easy to identify the optimal descriptors.

Unstirred water layer effects describe the fact that the transport of molecules to a surface assumes free diffusion at diffusion-controlled rates; however, layers of water adjacent to membranes can lower this rate[32,38]. Leaving this aside, the rate of uptake of small molecules across BLMs decreases with increasing molecular volume[39,40] but otherwise favours molecules with low polarity or high values of logP[3,33,41,42] (although the number of detailed studies of this matter is small). Again, much of this flux in BLMs is likely to be due to pore defects[43] (or to dissolution in solvents in the membrane-forming mixture) rather than to true dissolution in a biomembrane that mimicks a bilayer type of membrane.

Indeed, for general anaesthetics — the one case in pharmacology in which it is considered that diffusion almost certainly does affect transport (and maybe efficacy) — it was long assumed that the almost non-existent relationship between structure and activity, but the high correlation over many orders of magnitude between activity and logP, meant that both diffusion and their mode of action were controlled by the ability of anaesthetic molecules to partition into biological membranes[44,45]. However, many facts such as the equivalent interactions of these molecules with various proteins[46,47], including direct structural evidence[46], and the correlation between specific receptor binding[48] and potency in specific mutant mice[49], mean that this view is no longer considered tenable (reviewed in REFS 50,51). Indeed, even such a small molecule as ethanol is now being recognized as having relatively specific receptors[52]. As stated above, this reflects the fact that the binding sites of certain carriers for solutes, and the biophysical interactions involved, may be similar to those that are thought to effect their diffusion across membranes via partitioning.

We will now review the evidence demonstrating that drugs can be transported across biological cell membranes into cells via carriers — often of previously unknown specificity — using the main sources highlighted in the introduction. We begin by recognizing that while this does not *per se* say anything about uptake, the existence and importance of many proteins involved in drug efflux — which are of significance, for example, in anti-infective[53,54] and anti-tumour activities[55] — is well established[56]. Not only does this illustrate the widespread existence

Box 2 | **Implications of a more prominent role for carrier-mediated drug uptake**

- A combination of substrate specificity and carrier distribution, additional to target distribution, can account for much of the different tissue distributions of drugs (and hence warn of toxicity issues).
- Drugs or prodrugs may be designed to target specific tissues that express highly the carriers for which they are the substrates.
- Drugs may be designed to avoid specific tissues that lack carriers for them.
- It becomes much easier to understand in principle the tissue distributions of xenobiotics.
- Cross-species sequence homologies may allow better interpretation of tissue distributions in different organisms.
- Uptake carriers may provide novel and rational drug targets.
- Molecular cloning will allow the specificities of individual carries for target drugs to be measured directly.
- Drug–drug interactions may be mediated by competition with or inhibition of influx transporters.

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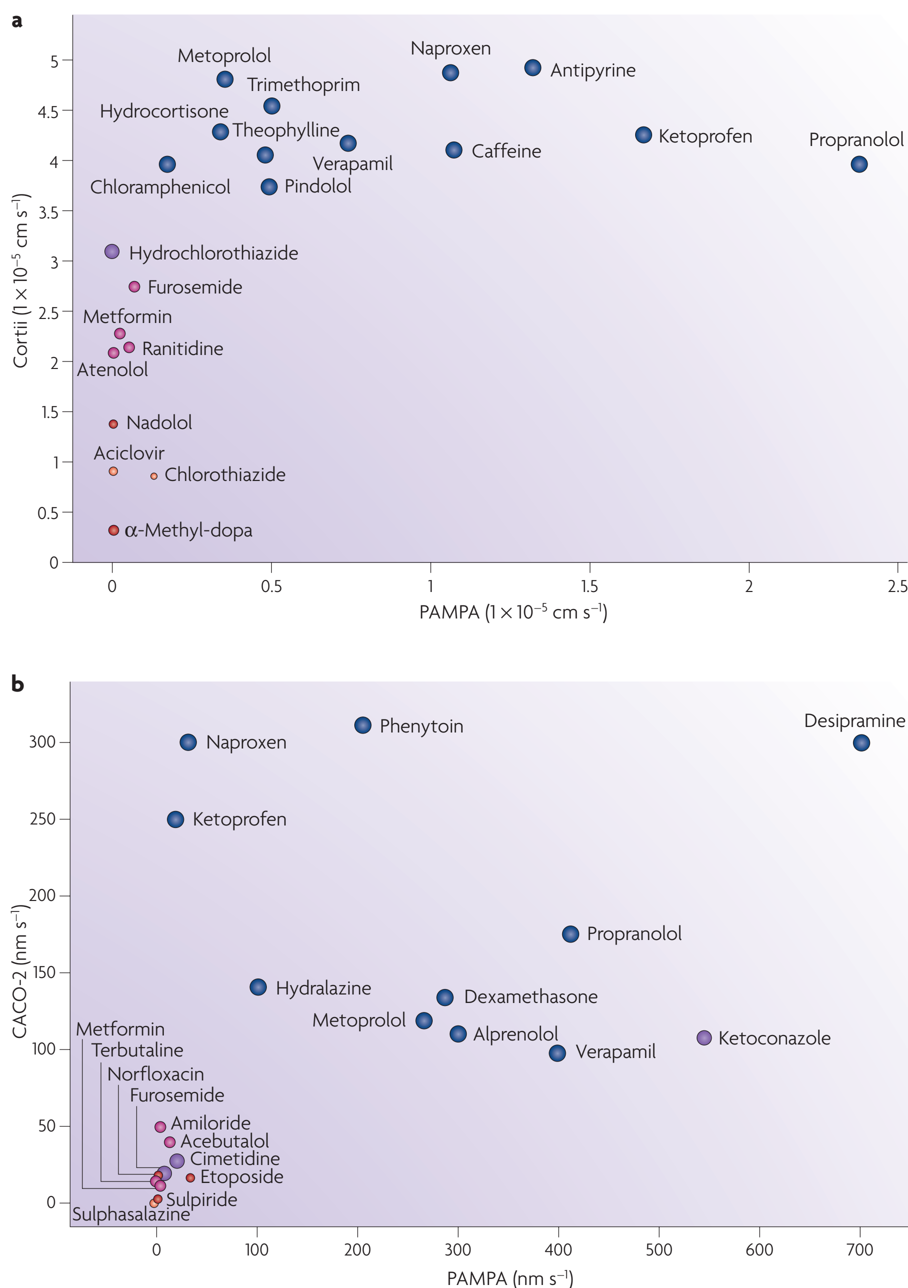

Figure 2 | **Comparisons between drug permeability in natural membranes and artificial systems, and their comparison with oil–water partition coefficients.** First, we show the relationship between the apparent permeability of marketed drugs across artificial membranes, across Caco-2 cells, and the fraction absorbed in humans. **a** | Apparent permeability in the artificial membrane system described in REF. 31 (Corti) versus that in the parallel artificial membrane permeation assay (PAMPA) (note also the numerical differences in the permeability in cm s^{-1}). The size encodes the apparent permeability (small = less absorbed) and colour encodes the fraction absorbed in humans (red = low, blue = high). Data plotted from REF. 31. The ostensibly hyperbolic shape of the graph is best interpreted in terms of two classes of compounds, one of which has a low PAMPA permeability ($<0.4 \times 10^{-5}$ cm s^{-1}) but considerable variation in the permeability across Corti's membrane system, whereas the other set has a high permeability ($>3.5 \times 10^{-5}$ cm s^{-1}) in Corti's system and a variable one in PAMPA. **b** | Caco-2 versus PAMPA (the fraction absorbed in humans in an *in vivo* assay is encoded by both size and colour). In addition we show the absence of any clear linear relationship between permeability and (logarithm of the) oil–water partition coefficient. Data plotted from REF. 33. Overall, it is clear that even when the assays are tuned by varying the type of lipid and solvent, the uptake into human cells cannot be predicted accurately by the uptake across artificial membranes as there is no overall correlation between the two.

of the ability of natural proteins to transport xenobiotic drugs but leads one to recognize that if carriers cause their efflux the same or other carriers might cause their influx too. There could then be a balance between influx and efflux (as well as any passive carrier-independent permeability), and the issue then is how to determine which carriers these are and to assess what might be their natural substrates. We begin by reviewing knowledge of the uptake carriers that are currently known to exist in humans.

What influx carriers are known in humans?

Until recently, the number of identified carriers was modest, but a combination of genomics and post-genomics is rapidly altering this number, and various internet resources act as portals to some of this information (TABLE 1). The approved human gene names for carriers include those that begin with SLC (which stands for solute carrier)[57] or ABC (ATP-binding cassette)[58], and can be found at the HUGO Gene Nomenclature Committee web site (see also TABLES 1–4 and Supplementary information S1 (box)). Based on homology/motif searching and semi-automated curation, the TransportDB web site (in June 2007) lists 758 transporters of all kinds for *Homo sapiens* and 347 for *Saccharomyces cerevisiae* (a number reasonably similar to the 285 manually curated proteins at the Yeast Transport Protein Database). This number for humans exceeds substantially the numbers that appear or are described in most reviews based on 'wet' biological experiments. This therefore suggests (as stated in a recent paper on the reconstruction of the human metabolic network[59]) that we are only scratching the surface of what is there, let alone what might be their specificities for natural molecules and xenobiotic drugs. Similar comments could be made about mitochondria, in which genomic and post-genomic studies now show many hundreds of proteins to be present in these organelles. Many have unknown or novel function[60], with mitochondrial carrier proteins prominent among them[61,62] (many with still unknown substrates[63]). Given, as noted above, that water, glycerol and urea can use carriers, to assume that a molecule is not a substrate for one of these carriers seems risky. In particular, all carriers could potentially contribute to the background permeability of xenobiotics into cells or organelles that are not known to express high levels of any particular carrier of interest.

Proteins of the SLC family are involved in the transport of a broad range of substrates. SLC transporters can be passive (uniporters),

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coupled (symporters) or exchangers (antiporters). Currently, there are more than 40 families containing approximately 340–360 transporters. The state of research into SLCs was reviewed in 2004 (REF. 57). Of the 43 families summarized, an extensive literature search found evidence of non-endogenous uptake activity by 19 SLC families. Among these, SLCO (formerly known as SLC21), the organic anion transporting (OATP) superfamily (reviewed in REF. 64), and SLC22 (reviewed in REF. 65), the organic cation/anion/zwitterion transporter family, are heavily involved in the uptake of many diverse substrates. SLCO and SLC22 exhibit a wide tissue distribution and form part of the major facilitator superfamily.

Among the families for which there is as yet no evidence of non-endogenous substrate transport are many (but not all) of the transporters involved in the transport of metal ions. These include Na^+/Ca^{2+} (SLC8) and Na^+/H^+ (SLC9); transition metal ions (SLC11), Na^+/K^+ and Cl^- (SLC12); Na^+ and inorganic phosphate (SLC20), Na^+-Ca^{2+}/K^+ (SLC24), zinc (SLC30), ferrous iron (SLC40); and divalent metal ions (SLC41). Similarly, the transporters of small ionic species such as bicarbonate (SLC4), and sulphate, oxalate, formate and other similar species (SLC26) do not seem, so far, to exhibit evidence of non-endogenous substrate transport. These small endogenous substrates are markedly dissimilar to most xenobiotics, so it might be expected that they are not so readily involved in xenobiotic transport.

Carrier-mediated uptake: the evidence

We now turn to the four lines of evidence highlighted in the introduction that support a more prominent role for the carrier-mediated uptake of drugs.

There is abundant evidence for carrier-mediated drug uptake in specific cases where it has been studied. Although some of the later evidence and reasoning we describe may be seen as circumstantial, albeit consistent with our thesis, we start by drawing attention to the increasing evidence from specific cases that particular drugs do enter cells via identified carrier molecules for which they are not the natural ligand. A comprehensive and annotated list of human SLCs and some of their known natural and xenobiotic substrates is given in Supplementary information S1 (box). Listed there are 393 substrate–transporter relationships, covering transporters from 17 SLC families and 203 unique substrates. Evidence

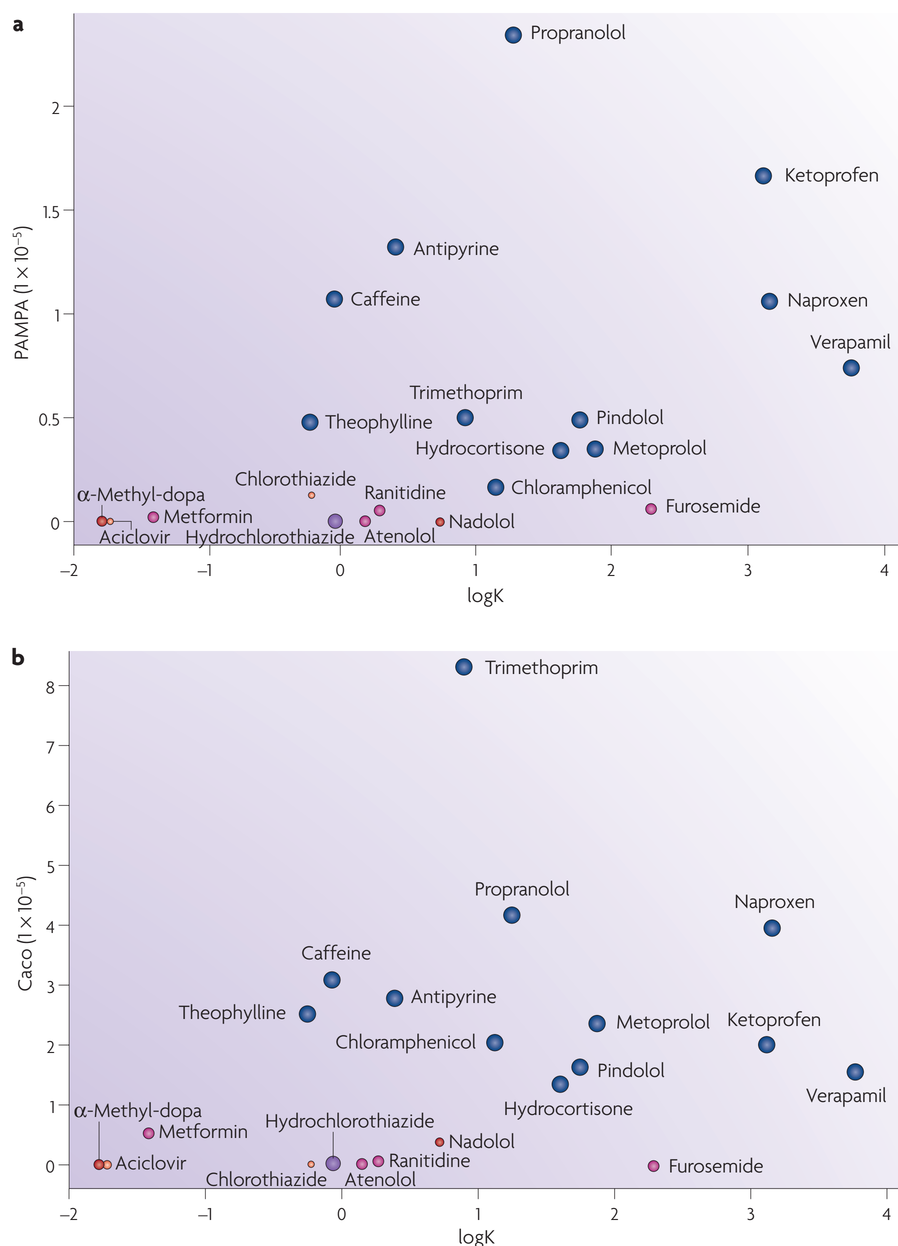

Figure 3 | **Comparisons between drug permeability in natural membranes and artificial systems, and their comparison with oil–water partition coefficients. a** | Lack of correlation between apparent permeability in a parallel artificial membrane permeation assay (PAMPA) and logK (octanol–water coefficient). **b** | Lack of correlation (r^2 = 0.097) between apparent permeability in Caco-2 cells and logK. Data are plotted from those of Corti[31], with size encoding the apparent permeability (small = less absorbed) and colour the fraction absorbed in humans (red = low, blue = high). Overall, it is clear that even when the assays are tuned via the choice of lipid and solvent, the uptake into human cells cannot be predicted accurately by the hydrophobicity encoded in logK.

of transport is based mainly on uptake assays in transfected cells. Some of these SLCs are illustrated diagrammatically in FIG. 4.

The data can be examined in terms of which transporters act on a given drug or conversely, which drugs are the substrates for a given transporter. In this latter vein, TABLE 2 lists the main superfamilies of transporters, whereas TABLES 3,4 provide details of three of the SLC families — SLC15, SLC22 and SLCO — that are considered especially to have a role in xenobiotic drug uptake.

Members of the oligopeptide transporter family (SLC15)[66] mediate proton-coupled co-transport of many diverse peptide and peptidomimetic substrates. Well-characterized family members are PEPT1 (also known as SLC15A1) and

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Table 1 | **Some searchable databases for transporter molecules***

Name	URL	Focus	Refs
Human Membrane Transporter Database	http://lab.digibench.net/transporter/ and for drugs http://lab.digibench.net/transporter/drug.html	Human	200
IUBMB and HUGO Membrane Transport Proteins Nomenclature	http://www.chem.qmul.ac.uk/iubmb/mtp/ http://www.genenames.org/	Human	201
Transport Classification Database using the above names	http://www.tcdb.org/	Various	202
Yeast Transport Protein Database	http://rsat.scmbb.ulb.ac.be/~sylvain/ytpdb/	Yeast	203
SoLute Carrier (SLC) Tables	http://www.bioparadigms.org/slc/menu.asp	Various	57
TP-search	http://www.tp-search.jp/	Mammalian	204
TransportDB	http://www.membranetransport.org/	Multiple and comparative	78,205

*Note that many of these contain large numbers of acronyms, which may be resolved using Acromine (http://www.nactem.ac.uk/software/acromine/)[199]. The behaviour of individual proteins determined by literature analysis can be studied at: http://www.ihop-net.org/UniPub/iHOP/.

PEPT2 (also known as SLC15A2). PEPT1 is highly expressed in the intestine and PEPT2 in the kidney; although expression has also been observed in the bile-duct epithelia, choroid plexus, lung and mammary gland. All 400 dipeptides and 8,000 tripeptides derived from the common protein-forming amino acids are substrates for both, despite large differences in molecular size, net charge and solubility. A detailed characterization of PEPT1 substrates has been performed[67], demonstrating that there is a particular affinity for molecules that have amino and carboxylic acid groups separated by about 6Å, even in non-peptidic substrates. Such information allows a rational approach to prodrug design, for example, in the coupling of valine to acyclovir and ganciclovir to enhance substrate-likeness for PEPT1 (REF. 66). Uptake of prodrug across the apical membrane and rapid hydrolysis by intracellular dipeptidases leads to increased drug availability. Drug substrates of PEPT transporters include many important classes, including antivirals (valacyclovir), antibiotics (β-lactams) and angiotensin-converting enzyme inhibitors[66].

Organic cation/anion/zwitterion transporters (SLC22)[65] are widely distributed, with various family members being expressed in the liver, kidney, skeletal muscle, placenta, heart, lung, spleen and brain (see also REF. 68 for details of expression). Substrates include the endogenous prostaglandins, serotonin, carnitine, adrenaline, 2-oxoglutarate, and the drugs acyclovir, ganciclovir, metformin, memantine, verapamil and zidovudine. There is considerable substrate overlap between group members. We identified 72 substrates for the family, 24 of which are transported by more than one family member. No substrate is identified as a substrate of every SLC22 transporter.

Organic anion transporting polypeptides (SLCO)[64] mediate bidirectional, sodium-independent, pH-dependent substrate–anion exchange. We identified 59 substrates, 34 of which are transported by more than one family member. Known substrates cover a wide range of substrates, including bile salts, steroid hormones and conjugates, thyroid hormones, organic cations, and various drugs such as atorvastatin, bencylpenicillin, enalapril and pravastatin. Generally, substrates are anionic amphipathic molecules with a molecular mass greater than 450 Daltons. Quantitative structure–activity relationship studies defined a pharmacophore with two hydrogen-bond acceptors, one hydrogen-bond donor and two hydrophobic regions[64]. Polyspecific family members tend to have a wide tissue distribution, covering the blood–brain barrier, choroid plexus, lung, heart, intestine, kidney, placenta and testis.

Considering the characteristics of those drugs that have been identified as a substrate for an uptake transporter, there is a rather blurred distinction between which are natural products (for example, erythromycin), semi-synthetic molecules that are typically modified natural products (for example, benzylpenicillin), completely synthetic products that are nevertheless an analogue of a natural metabolite (for example, propranolol, nominally an analogue of histamine) or completely synthetic products that are not considered to be an analogue of any human metabolite (for example, atorvastatin). Scrutiny of TABLE 4 indicates that almost all of the compounds fall into the first three categories identified as a substrate for an uptake transporter. Indeed, perhaps they could be seen to be analogues of natural metabolites for which one could reasonably imagine the existence in evolution of transporter molecules, which have been selected implicitly via the experience of medicinal chemists or simply for reasons of efficacy.

Additionally, it is well known that natural products, that is, bioactive 'secondary' metabolites[69,70], do not obey the Ro5 (for example, most antibacterials[71]), and it is certainly known in some cases that they are the substrates of active transporters in the producing organisms[72]. Given that bioactive microbial products are necessarily secreted, evolution must have produced carriers that are capable of binding the relevant chemical structures[73]. The fact that these bioactive secondary metabolites are often active on other cells of the producer organism[74], as well as the higher organism, reinforces the view that suitable protein binding motifs must exist widely throughout evolution[75,76]. This suggests that it is to be expected that there are likely to be transporters for these kinds of bioactive secondary metabolites in higher organisms, which has indeed been found to be the case[58,67,77–79]. Note too that a high proportion of drugs that we have noted as having transporters are in some sense analogues of natural products (TABLE 4). A standard principle in cheminformatics and in medicinal chemistry is the idea that molecules that are similar structurally will also tend to have similar activities. It is consequently reasonable to surmise that such activities will include the ability to act as substrates for transporters if the molecules are like natural molecules endogenous to the target organism. Such a quantitative survey has yet to be done.

An interesting example related to this issue from TABLE 4 is provided by the statins, a family of drugs that all inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Statins include agents that could be considered as natural products or derivatives thereof (for example, lovastatin, simvastatin), and also what would appear to be totally synthetic agents (for example, atorvastatin). Several studies have demonstrated that a major route of transport

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Table 2 | **Overview of the human transporter superfamilies and families with possible/known roles in drug uptake**

Superfamily	Family	TCDB code	General topology	Transport	Substrates
Amino acid/ polyamine/organic cation superfamily	Amino acid/auxin permease (SLC36)	2.A.18	10–11 TM α-helices	Symport/ antiport	Amino acids, auxin (indole-3-acetic acid)
	Amino acid/ polyamine/organic cation (SLC7)	2.A.3	14 TM α-helices	Symport/ antiport	Amino acids, choline, polyamines
Anion transporter superfamily	Bile acid:Na^+ symporter (SLC10)	2.A.28	7–10 TM spanners	Symport	Bile acids and other organic acids
Major facilitator superfamily	Major facilitator (SLC2, 16–18, 22, 33, 37, 43)	2.A.1	Mostly 12, 14 or 24 α-helical TM α-helices	Uniport/ symport/ antiport	Sugars, drugs, neurotransmitters, metabolites, amino acids, peptides, nucleosides, organic and inorganic anions
	Proton-dependent oligopeptide transporter (SLC15)	2.A.17	12 TM α-helices	Symport	Peptides, histidine, antibiotics
	Organic anion transporter (SLCO/21)	2.A.60	12 TM α-helices	Uniport/ antiport	Organic anions, organic cations, bromosulphophthalein, prostaglandins, bile acids, steroid conjugates, oligopeptides, drugs, toxins, and many others
Resistance-nodulation-cell division superfamily	Eukaryotic (putative) sterol transporter	2.A.6.6	N-TM-extracytoplasmic domain-5 TM-extracytoplasmic domain-6 TM-C	Antiport	Sterols, lipids
Drug/metabolite transporter superfamily	Nucleotide–sugar transporters	2.A.17.10 2.A.17.11 2.A.17.12	8–12 TM α-helices	Antiport	Exchange nucleotides for nucleotide–sugars
ATP-gated cation channel		1.A.7	2 TM spans + extracellular receptor domains	Facilitated diffusion	Prolonged exposure of certain forms to ATP leads to pore dilation; pore permeable to solutes up to 1 kDa
Solute:sodium symporter (SLC5)		2.A.21	13–15 TM α-helices	Symport	Sugars, amino acids, organic cations, nucleosides, inositols, vitamins, urea, anions
Neurotransmitter: sodium symporter (SLC6)		2.A.22	12 TM α-helices	Symport	Neurotransmitters, amino acids
Dicarboxylate/amino acid:cation (Na^+ or H^+) symporter (SLC1)		2.A.23	8 TM spanners and 1 or 2 pore loop structures (putative)	Symport	Malate, succinate, fumarate, glutamate, aspartate, neutral and acidic amino acids, zwitterionic and dibasic amino acids
Mitochondrial carrier (SLC25)		2.A.29	6 TM α-helices	Antiport	Citrate, malate, phosphoenolpyruvate, lysine, arginine, aspartate, glutamate, others
Nucleobase:cation symporter 2 (SLC23)		2.A.40	12 TM α-helices	Symport	Nucleobases, ascorbate
Concentrative nucleoside transporter (SLC28)		2.A.41	10–14 TM α-helices	Symport	Nucleosides
Reduced folate carrier (SLC19)		2.A.48	12 TM α-helices	Symport/ antiport	Folate, reduced folate and derivatives, methotrexate, thiamine
Equilibrative nucleoside transporter (SLC29)		2.A.57	11 TM α-helices	Symport	Nucleosides and analogues
Bilirubin transporter		2.A.65	Uncertain	Symport	Bilirubin, organic anions, rifamycin, nicotinic acid
Organic solute transporter		2.A.82	Chain α: 7 TM spanners Chain β: 1 TM spanner	Facilitated diffusion	Bile acids, prostaglandin E1, digoxin, steroids

This is largely based on material from the Transport Classification Database (TCDB) at http://www.tcdb.org/ (see also REF. 202). TM, transmembrane.

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is via the various OATPs[79–91], many of which naturally transport bile acids. For instance[79], OATP1B1 and OATP1B3 are both highly expressed in the human liver and are able to transport atorvastatin, cerivastatin, fluvastatin, pitavastatin, pravastatin and rosuvastatin. Multiple versions of these transporters are present, and even individual variants can account for 35%[91] to 90%[92] of the uptake. Statins such as simvastatin, lovastatin and pravastatin are also substrates for monocarboxylate transporters[93]. OATP2 transports pravastatin, lovastatin, simvastatin and atorvastatin[84]. They can also be transported for instance by the bile acid transporter SLC10A1 (REF. 91) and the monocarboxylate transporter SLC16A1 (REF. 94) (see Supplementary information S1 (box)).

Lipophilic cations need carriers to transfer them across cell membranes. The assumption that highly lipophilic molecules can partition straightforwardly into membranes and thereby transfer across them is both common and implicit in the view of the importance of logP in determining uptake. These considerations are taken to apply to neutral rather than to charged molecules,

Table 3 | **Examples of drug uptake by three of the most significant families of transporter**

HUGO symbol (synonyms)	Description	Substrate	Code
SLC family 15*			
SLC15A1 (PEPT1)	Oligopeptide transporter	Amoxicillin[209], Cefaclor[209]	DADC
		Cefalexin[206], Bestatin[207,208], Amoxicillin[207], Ampicillin[207], Cefadroxil[207,210], Cefixime[210], Temocapril[116], Temocaprilate[116], Enalapril[116], Midodrine[212], Valacyclovir[213], Valganciclovir[214]	DATC
		Ceftibuten[211]	DCA
SLC15A2 (PEPT2)	H+/peptide transporter	Amoxicillin[209], Cefaclor[209]	DADC
		Cefadroxil[215]	DADT
		Bestatin[208], Valganciclovir[214]	DATC
SLC family 22‡			
SLC22A1 (OCT1)	Organic cation transporter	Zidovudine[216], Acyclovir[217], Ganciclovir[217], Metformin[218], Cimetidine[218]	DATC
SLC22A2 (OCT2)	Organic cation transporter	Memantine[219], Metformin[220], Propranolol[221], Cimetidine[222], Zidovudine[223],	DATC
		Pancuronium[224], Cyanine863 (REF. 224), Quinine[224]	TOATC
SLC22A3 (OCT3; EMT)	Extraneuronal monoamine transporter	Cimetidine[222], Tyramine[225]	DATC
SLC22A4 (OCTN1)	Organic cation transporter	Quinidine[226], Pyrilamine[226], Verapamil[226]	DATC
SLC22A5 (OCTN2)	Organic cation transporter	Quinidine[227], Pyrilamine[227], Verapamil[227], Valproate[227], Cephaloridine[228]	DATC
SLC22A6 (OAT1)	Organic anion transporter	Adefovir[229], Didofovir[229], Acyclovir[230], Zalcitabine[230], Didanosine[230], Stavudine[230], Trifluridine[230], Ganciclovir[217], Lamivudine[230], Zidovudine[230], Methotrexate[231], Ketoprofen (low uptake)[232], Ibuprofen (low uptake)[232], Cimetidine[233], Tetracycline[234]	DATC
		Cephaloridine[235]	IATC
SLC22A7 (OAT2)	Organic anion transporter	Zidovudine[217], Tetracycline[234], Salicylate[236], Methotrexate[237], Erythromycin[238], Theophyline[238]	DATC
SLC22A8 (OAT3)	Organic anion transporter	Valacyclovir[217], Zidovudine[217], Methotrexate[239], Salicylate[239], Cimetidine[239]	DATC
		Cephaloridine[235]	IATC
SLC22A11 (OAT4)	Organic anion/cation transporter	Zidovudine[217]	DATC
		Cephaloridine[235]	IATC
SLCO family§			
SLCO1A2 (OATP; OATP-A; OATP1A2)	Organic anion transporter	Fexofenadine[240]	TOATC
		Rocuronium[241], Enalapril[242], Temocaprilat[243], Rosuvastatin[91]	DATC
SLCO1B1 (OATP-C; LST1; OATP1B1; OATP2)	Organic anion transporter	Benzylpenicillin[244], Pravastatin[84], Rifampicin[245], Atorvastatin[81], Capsofungin[246], Cerivastatin[81], Fexofenadine[240], Fluvastatin[248], Pitavastatin[92]	DATC
		Methotrexate[247]	IATC
SLCO1B3 (LST-2; OATP1B3; OATP8)	Organic anion transporter	Digoxin[249], Rifampicin[245], Fexofenadine[250], Fluvastatin[248], Pitavastatin[92], Rosuvastatin[91]	DATC
		Methotrexate[247]	IATC
SLCO2B1 (OATP2B1; OATP-B)	Organic anion transporter	Pravastatin[83], Glibenclamide[251], Atorvastatin[87], Benzylpenicillin[244], Fluvastatin[248], Rosuvastatin[91]	DATC
SLCO4C1 (OATP4C1)	Organic anion transporter	Methotrexate[252], Digoxin[252]	DATC

*See REF. 66 for more information. ‡See REF. 65 for more information. §See REF. 64 for more information.. DADC, direct assay in differentially expressing cell lines; DADT, direct assay in differentially expressing tissues; DATC, direct assay in transfected/mutant cells (a direct assay of transport in cells in which transporter expression has been induced; also includes mutant forms of cells where the mutation knocks out transporter function); DCA, direct competition assay (refers to a competition assay in which the presence of the query substrate is demonstrated on the opposite side of the membrane in a manner that is altered by competition with a known transporter substrate); IATC, indirect assay in transfected/mutant cells; TOATC, transporter operation assay linked to substrate in transfected cells.

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Table 4 | **Some common drug substrates of the most prolific SLC transporter families***

Substrate	SLC15A1	SLC15A2	SLC22A1	SLC22A2	SLC22A3	SLC22A4	SLC22A5	SLC22A6	SLC22A7	SLC22A8	SLC22A11	SLCO1A2	SLCO1B1	SLCO1B3	SLCO1C1	SLCO2A1	SLCO2B1	SLCO3A1	SLCO4A1	SLCO4C1
Acyclovir			•					•												
Amoxicillin	•	•																		
Atorvastatin													•				•			
Benzylpenicillin													•				•	•	•	
Bestatin	•	•																		
Caspofungin													•							
Cefaclor	•	•																		
Cefalexin	•																			
Ceftibuten	•																			
Cephaloridine							•	•		•	•									
Cidofovir								•												
Cimetidine			•	•	•			•		•										
Didanosine								•												
Enalapril	•											•								
Erythromycin									•											
Fexofenadine												•	•	•						
Fluvastatin													•	•			•			
Ganciclovir			•					•												
Glibenclamide																	•			
Ibuprofen								•												
Lamivudine								•												
Metformin			•	•																
Methotrexate								•	•	•			•	•						•
Midodrine	•																			
Pitavastatin													•	•						
Pravastatin													•				•			
Propranolol				•																
Pyrilamine						•	•													
Quinidine						•	•													
Rifampicin													•	•						
Rosuvastatin												•		•			•			
Salicylate									•	•										
Stavudine								•												
Temocaprilat	•											•								
Tetracycline								•	•	•	•									
Trifluridine								•												
Valacyclovir	•									•										
Valganciclovir	•	•																		
Valproate							•													
Verapamil						•	•													
Zalcitabine								•												
Zidovudine				•				•	•	•	•									

*As judged by the number of substrates referenced in TABLE 3 and Supplementary information S1 (table). SLC, solute carrier (organic cation transporter); SLCO, solute carrier organic anion transporter.

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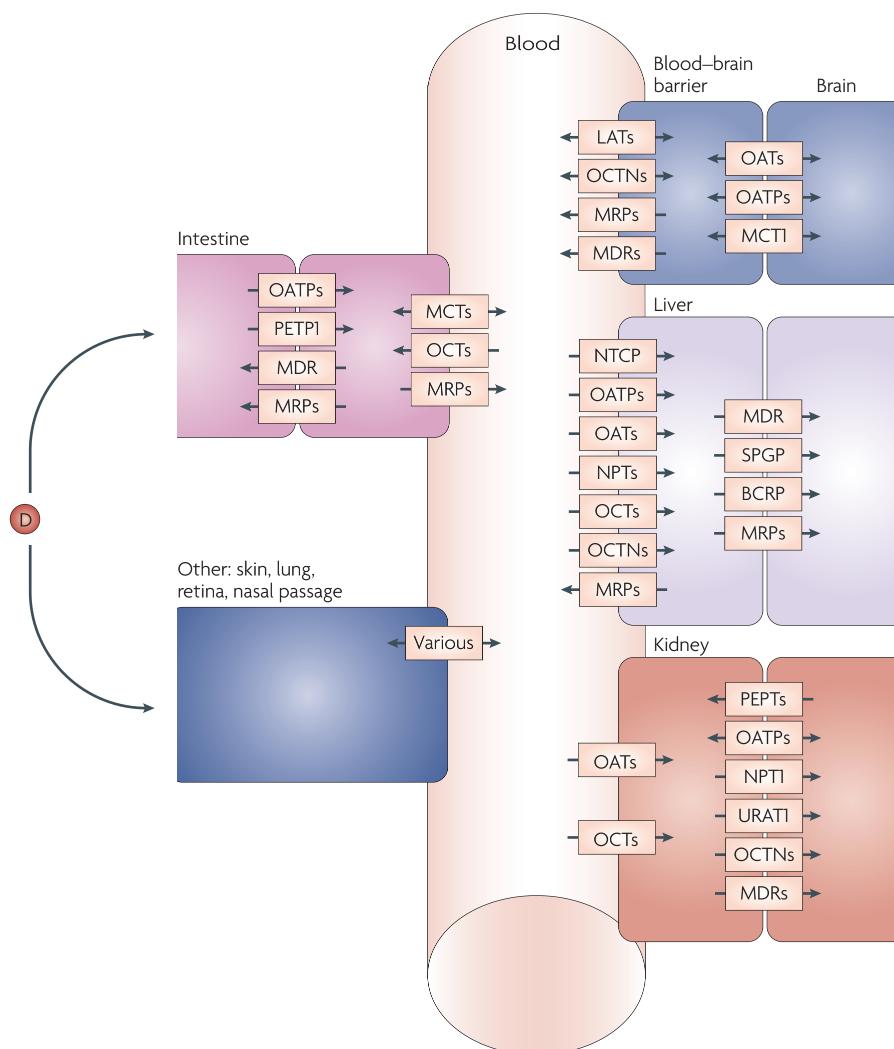

Figure 4 | **Multiple drug carriers in different tissues, all of which may need to be permeated.** BCRP, breast cancer-resistant protein (also known as ABCG2); LATs, L-type amino-acid transporters; MCT1, monocarboxylate transporter 1 (also known as SLC16A1); MDR, multidrug-resistant; MRPs, multidrug-resistance-related proteins; NPT1, sodium phosphate transporter 1 (also known as SLC17A1), NTCP, sodium-dependent taurocholate co-transporter (also known as SLC10A1); OATs, ornithine aminotransferases; OATPs, organic anion transporting polypeptides; OCTs/OCTNs, organic cation transporters; PETP1, peptide transporter 1 (also known as SLC15A1); SPGP, sister P-glycoprotein (also known as ABCB11); URAT1, urate anion exchanger 1 (also known as SLC22A12). Figure modified with permission from REF. 198 © (2004) Elsevier Science.

and it is well recognized that charged molecules cannot easily cross the interior of BLMs. This is because of the enormously unfavourable Born charging energy required to transfer them across a low dielectric[43,95,96]. However, it is reasonable to assume that the addition of sufficient lipophilic groups to an ion, thus delocalizing the ionic charge, would decrease the Born charging energy and thereby confer membrane-permeating ability to such ions. In this vein, an early series of studies, motivated by questions of bioenergetics following the chemiosmotic proposals of Mitchell[97], showed that even ionically charged lipophilic molecules could cross both BLM and cellular membranes. Although this activity could be strongly promoted by the presence of 'catalytic' amounts of lipophilic ions of opposite charge such that the membrane-permeating species was then probably neutral[98]. This then led to assumptions being made (see for example, REFS 99,100) that such molecules could penetrate biological membranes in the absence of any carriers being necessary. However, there is clear evidence that proteinaceous carriers are required for at least some of these lipophilic cationic molecules, which had been assumed on such biophysical grounds to cross biological membranes without them. For example, the requirement of a functional thiamine carrier to effect transfer of the dibenzyldimethylammonium cation[101] (and see also REFS 102,103). Other experiments by these authors showed that dibenzyldimethylammonium uptake is inhibited completely by thiamine disulphide, a competitive inhibitor of thiamine transport. These findings of carrier-mediated uptake of such molecules (as in the case of thallous ion transport[104]) also possibly calls into question the use of such lipophilic cations in the estimation of transmembrane potentials in such systems.

Drugs can concentrate in specific tissues. The steady-state concentration of a drug in a particular cell, cellular compartment or tissue is determined in large measure by the activity of the relative rates of influx and efflux, and their binding to targets (whether functional and specific or gratuitous and nonspecific). Binding is probably not the major issue as intracellular concentrations can be significantly larger than any plausible stoicheiometric concentration of binding sites. Therefore, the fact that some drugs can concentrate in specific tissues[105–112] suggests that these drugs do not leak out so as to equilibrate with extracellular concentrations as they would if transmembrane diffusion on the basis of logP alone was the whole (or even most of) the story. Additionally, the fact that they are concentrated then necessarily (on thermodynamic grounds) suggests some kind of active uptake. Some of these examples are based on specific tissues (for example, REFS 105,106), while others concentrate on specific organisms (for example, the mouse[109]), on the drug discovery pipeline[107,110], on pharmacokinetics and pharmacodynamics[108,111,112], and on drug–drug interactions[113]. Although we have largely avoided focusing above on specific tissues (see REFS 68,105,112,113; but see BOX 3 for a discussion of the blood–brain barrier), there are clear cases in which rational modifications can beneficially affect efflux[114] as well as influx (see prodrugs, below). However, we note in particular that selective tissue-concentrating mechanisms may also be a cause of toxicity[115], and that there are other problems, such as drug–drug interactions[116–119], both in general[116,118] and in specific tissues such as the liver[117] and the kidney[120], which are not our primary focus. Similarly, if drugs compete with nutrients or intermediary metabolites for carrier sites, one might suppose that this could be a significant mechanism for drug–nutrient interactions[121].

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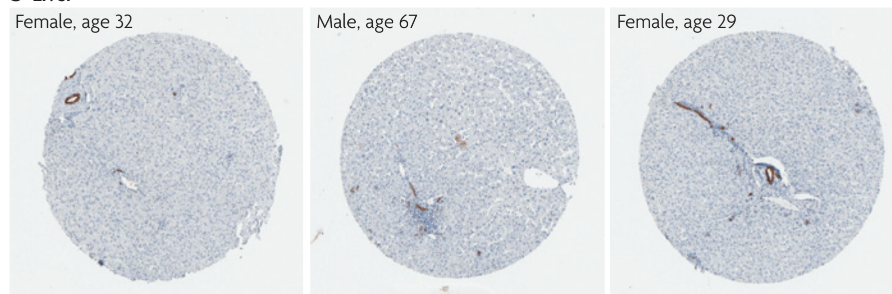
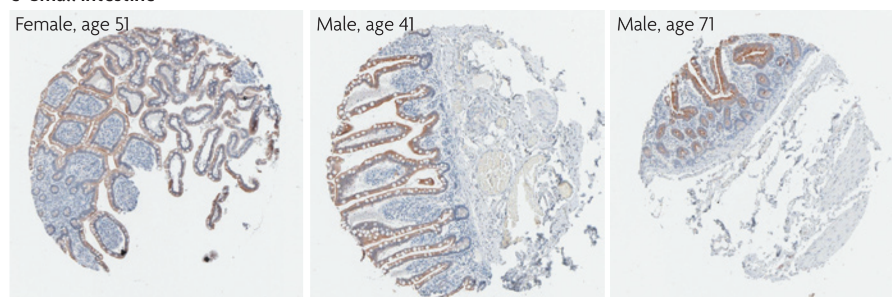
Impermeable drugs can be made permeable by creating prodrugs that hitchhike on carriers. Many nominally drug-like compounds are recognized as being membrane-impermeable. However, it has been shown in many cases that it is possible to enhance permeability substantially by modifying the drug chemically to form a prodrug that can act as a substrate for known drug carriers and thereby enter cells[122–130]. The case of peptide transporters is particularly clear[67,129,131–133] and hitchhiking on peptide transporters can demonstrably improve the activity of certain anti-bacterials[134]. Coupling of drugs such as chlorambucil[135], cisplatin[136] and acyclovir[137] to bile-acid derivatives or of carindacillin to monocarboxylates[138] can also be highly effective. Such couplings often lower the lipohilicity of the drugs while enhancing their uptake, a phenomenon that is hard to explain in terms of logP alone. However, in other cases, permeability is enhanced by making drugs more lipophilic, for example, by esterifying carboxylic acids. The assumption then is that these can diffuse in, although whether such influx is by diffusion, by carrier mediation (given that any change in the structure of a substrate can often have large effects on the activity of an enzyme for which it is a substrate) or even by endocytosis is not in fact known, given that we have little knowledge of the extent to which existing carriers are responsible for the baseline uptake of molecules that is observed.

Implications

The above analysis has implications for both drug design and for present chem-informatic concepts of lead-likeness[139,140] and drug-likeness[141–143] (and even CNS-likeness[144]) in drug design and discovery. This is because many of the recent trends in molecular drug design and development have been towards increased lipophilicity, leading to a greater likelihood of both a lack of selectivity and of attrition[145]. There is, therefore, the clear need to bring together the (moderately limited) bioinformatics knowledge of transporter specificity with the more common and largely biophysical cheminformatics descriptors. If drugs are mainly transported by carriers, this gives a ready explanation as to why general descriptors are not normally effective in individual cases. It also promotes the view that we need to understand much better than we do now at a mechanistic level the specificities for existing and candidate drugs of known drug transporters.





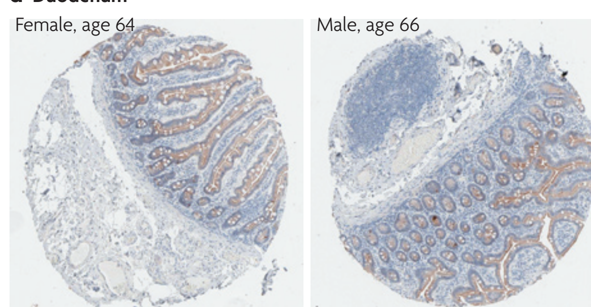


Figure 5 | **Tissue-selective expression of solute carrier molecules, where brown colouration indicates presence of protein.** Expression levels of SLC7A3 (cationic amino-acid transporter, y+ system) are high in oesophagus epithelial cells (**a**) and liver bile duct cells (**b**), low in hepatocytes themselves (**b**), moderate in glandular cells of the small intestine (**c**), and low in glandular cells of the duodenum (**d**). Antibody-based histochemical staining pictures are reproduced with permission from the Human Protein Atlas (http://www.proteinatlas.org/tissue_profile.php?antibody_id=3629).

If carriers are heavily involved in drug uptake, they will have natural substrates and we may expect not only to find them (in the same way that opioid and other receptors, and their endogenous substrates, were found by pharmacological means) but to use this knowledge to exploit them via the design of prodrugs or the redesign of drugs to allow their transport by such carriers. There will also be cases in which simply affecting the carriers themselves will have profound pharmacological effects. Thus, glycocholic acid and polyamine conjugates are able to inhibit transporters involved in hepatic and

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Box 3 | The blood–brain barrier

The blood–brain barrier is of special interest as CNS-active drugs have to permeate it, and in many ways (but given experimental difficulties perhaps unsurprisingly) it is still poorly understood. Certainly, a major feature is the limited possibility for paracellular transport[174,175] (see discussion in BOX 2). While there are clearly influx carriers[93,176–183] there is also considerable evidence that the activity of efflux carriers is effective in removing xenobiotics from the CNS[184–186] such that both influx and efflux activities as well as binding need to be understood if selective blood–brain barrier penetration is to be achieved[187–189]. Known influx carriers include those for large neutral amino acids (LAT1), glucose (GLUT1), monocarboxylates (MCT1), choline (CHT) and nucleobases such as adenosine (CNT2), but most remain unknown[130,190]. Thus system L transports large neutral amino acids, L-glutamine, L-asparagine, D-amino acids, and the drug melphalan[93]. High expression of LAT1 mRNA is detected in brain tissue by Northern blot analysis[191], whereas system y+ transports cationic amino acids. Its CAT1 RNA is enriched 38-fold in rat cerebral microvessels and choroid plexus compared with whole brain[192]. Certain organic cation transporters, such as OCT3 (REF. 193), are known to be expressed in the brain. OCT3 mediates the uptake of the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) and the neurotransmitter dopamine when expressed in mammalian cells. Organic anion transporters are also reported in the brain, and OATP-A is present at the human blood–brain barrier[194]. Brain expression of the peptide/histidine transporter (PHT1) was confirmed by *in situ* hybridization. PHT1 substrates include histidine and carnosine, with many di- and tri-peptides inhibiting histidine uptake[195]. Lee[177] has reviewed drug transporters in the CNS. Evidence for the importance of efflux carriers come from the large increases in brain concentration of various drugs such as amprenavir[196] and SB-487946 (REF. 197) when, for instance, the P-glycoprotein carrier is inhibited pharmacologically or knocked out at the genetic level. There is a clear role for *in silico* studies here, as well as 'wet' experimental approaches.

intestinal bile acid uptake[146], and as secretion and reuptake are common in chemical neurotransmission it is reasonable that such molecules may prove to be useful targets. Indeed this is the known mode of action of some important kinds of CNS-active substances, including those targeting the uptake of glutamate and dopamine[147] and serotonin[148].

The way forward

Towards a systems biology that includes human drug carriers. In a post-genomic era we can begin to move towards and beyond a knowledge of what transporters exist, and useful starting points are the web-accessible databases (TABLE 1). Armed with the knowledge of the existence of these carriers, we can seek to study them as targeted entities using the methods of molecular biology, and this is already providing important new knowledge on their distribution, activities and specificities[54,105,149–153]. Such methods based on expression cloning are likely to be more powerful and persuasive than the more traditional methods for implicating carriers based on criteria such as saturability, which is a poor criterion as non-saturability can be caused by multiple carriers of which some may have very weak affinity constants. As web-accessible data on tissue-selective expression profiles become available at both the transcriptomic[154,155] (for example, the Gene Expression Atlas) and proteomic[156–158] (for example, the Human Protein Atlas) levels, this will begin to allow us to understand which transporters are likely to be expressed and thus functionally active in which tissues (an example is given in FIG. 5). This would thereby provide the means with which to integrate the available knowledge[159]. All else being equal, one may expect straightforward correlations between the extent of accumulation of drugs in a tissue and the tissue expression of the carriers responsible for their import, thereby allowing one to infer the relevant carriers by rank-comparing the tissue distributions of drugs and of the various carriers. Expression-cloning studies will then easily establish the specificities of the proposed carriers for existing and candidate drugs, just as is now done routinely for the cytochrome P450 enzymes[160]. Cassette dosing and mass spectrometric assays will be particularly useful here. Specifically, it is stressed that if a chief determinant of drug uptake into cells is represented by the amount and activities of individual carriers for which these drugs are the substrates, then tissues that express active drug carrier proteins in high concentrations are likely to take such molecules up in greater amounts, with concomitant risks of toxicity.

As more studies on cloned transporters are performed, we may also expect significant improvements in our knowledge of the molecular enzymology of these processes, including details of binding and structure–activity relationships, as per the 'bottom-up' systems biology agenda[161] (FIG. 6). There is also a significant role for model organism studies here[162,163], as many of the carriers known to be active in humans have homologues in experimentally more tractable organisms (see above). For example, existing data regarding the interaction of yeast cells with drugs have revealed several cases in which changes in the activity of specific carriers increase or decrease the sensitivity of cells to xenobiotics[164–167], with the clear implication that such carriers effect the entry of these drugs into cells or their exit from them. Evidently, similar studies in genetically tractable higher organisms will be of value. In addition, chemical genetics strategies for determining the mode of action

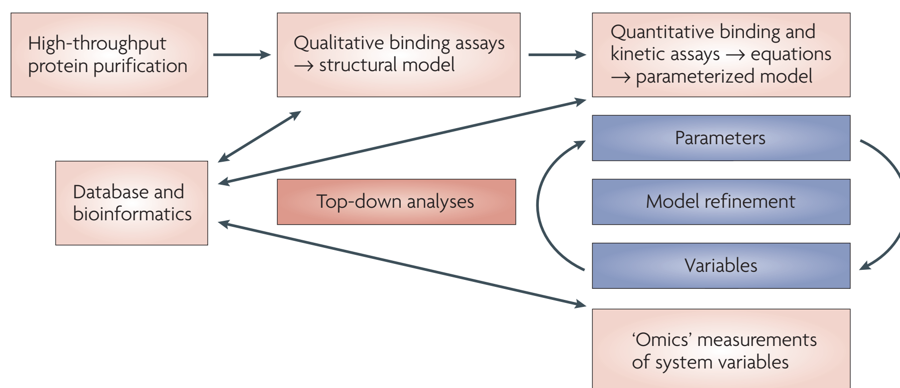

Figure 6 | **The 'bottom-up' systems biology agenda.** This begins with the purification (or at least concentration) of (usually recombinant) proteins, then assays of which molecules act as substrates or effectors of these proteins. Titrations of these proteins are then performed to acquire kinetic parameters and the equations that describe the activity of the individual steps. Such assays may be carried out *in vitro* (that is, in liposomes), but will more likely be done in cells (lacking an excessive background) *in situ* by expression cloning, noting that the exact membrane composition can affect the kinetic parameters that are estimated. These kinetic data may be used to populate metabolic models, typically described using ordinary differential equations. The models make predictions of the system variables such as metabolic fluxes, and these can be compared with experiment.

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of small-molecule inhibitors on their cellular targets[168,169] apply equally to their interactions with the drug transporters that may be required to get them there. As mentioned above, the issue is that we do not know which carriers these are, although a reasonable starting strategy in some cases is to use the methods of cheminformatics and molecular similarity analysis to assess which natural metabolites they most clearly resemble according to appropriate criteria. Although the type of such transport (uniport, antiport, symport, group transfer) is not part of the focus of this article, we recognize that once a particular influx is seen to be going via a specific kind of transporter then it will be of considerable interest to determine the mode of transport and role of any co-substrates.

Systems biology involves an iterative interplay between wet experiments, modelling and technology development, and to take forward the role of carriers in human drug transport a systems biology strategy is desirable. An essentially bottom-up strategy (FIG. 6) seems appropriate, as we are at such an early stage, and reflects the primary necessity for establishing which carriers transport which molecules. At the moment the quantitative pharmacological evidence for drug uptake by carriers is sparse, as this has not been a focus of most studies. This will lead to what we essentially desire: the eventual availability of a digital human in which we can simulate far more effectively than we can now the entire metabolism and control in human biochemical networks, including the spatially differentiated metabolism of drugs. This can and should be done as a community effort, preferably in a loosely coupled or distributed way. The availability of the first major versions of the entire human metabolic network[59,170] in a machine readable form (that is, the Systems Biology Markup Language[171]) provides an outstanding starting point for this endeavour[172,173].

Concluding remarks

What we have sought to do here is to bring together a rather scattered but, we believe, ultimately persuasive literature on the role of membrane transporters in cellular drug uptake. What we hope we have therefore achieved is a more coherent view that leads one to focus on the mechanistic significance of membrane transporters in all aspects of drug ADME and toxicity, including the effects of polymorphisms, adverse drug reactions and drug–drug and drug–nutrient interactions. If one accepts that most of this transport may indeed occur via carriers, the next stage is to begin to understand their specificity and energy coupling mechanisms and put together the relevant transporters into the rest of the metabolic network, using the standard bottom-up methods of systems biology[161]. Only when this is done may we hope to have a predictive biology of human drug disposition.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

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