The ATP-binding cassette proteins ABCC1 and ABCB1 as modulators of glucocorticoid action

Short title: ABC transporters and glucocorticoid transport

Kerri Devine[1,2], Elisa Villalobos[1], Catriona J Kyle[1], Ruth Andrew[1], Rebecca M Reynolds[1], Roland H Stimson[1], Mark Nixon[1], Brian R Walker[1,2*]

[1]BHF Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

[2] Translational & Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK

*corresponding author: Brian.Walker@newcastle.ac.uk

Abstract

Responses to hormones acting through nuclear receptors are controlled by modulation of hormone concentrations not only in the circulation but also within target tissues. In recent decades, the role of enzymes that amplify or reduce local hormone concentrations has become well-established for glucocorticoid and other lipophilic hormones. Moreover, transmembrane transporters have proven critical in determining tissue responses to thyroid hormones, but there has been less consideration of the role of transmembrane transport for steroid hormones. ATP-binding cassette (ABC) proteins were first shown to influence accumulation of glucocorticoids in cells almost three decades ago. More recent observations suggest that differential transport of both exogenous and endogenous glucocorticoids by ABCB1 and ABCC1 transporters provides a mechanism whereby different tissues are preferentially sensitive to different steroids. This Review summarises this evidence, and the new insights that it provides for the physiology and pharmacology of glucocorticoid action, including in new approaches to glucocorticoid replacement.

Introduction

Glucocorticoid hormones are vital for life, with diverse effects on multiple processes and systems. Adverse effects of glucocorticoid excess are well-recognised in Cushing's syndrome, and even subtle dysregulation has implications, for example in causing cardiovascular disease.[1] Over the last thirty years it has been appreciated that the concentration of glucocorticoid in blood does not necessarily reflect that within tissues, with additional pre-receptor control from enzymes (e.g. 11β-hydroxysteroid dehydrogenase) and binding proteins.[2,3]

As lipophilic molecules, glucocorticoids diffuse across cell membranes to interact with intracellular targets, but they can also undergo active transmembrane transport. This was first described for the ABCB1 transporter (of the ATP-Binding Cassette [ABC] protein family), which exports cortisol and a variety of synthetic glucocorticoids from "sanctuary sites" including the brain.[4,5] Intriguingly, corticosterone, the other endogenous human glucocorticoid, is not readily exported by ABCB1, but we have recently discovered that the ABCC1 transporter, found in tissues including adipose, exports corticosterone but not cortisol.[6]

Here we will explore the implications of this tissue-specific glucocorticoid transport in central control of the hypothalamic-pituitary-adrenal (HPA) axis, adipose tissue metabolism, and pregnancy. We will also consider whether the steroid-specificity of ABCB1 and ABCC1 transport offers insights into the different roles of corticosterone and cortisol in humans, and the opportunity for developing glucocorticoid therapies which are better targeted to maximise efficacy and minimise toxicity.

Lipophilic hormone movement

The "free hormone hypothesis" determines that unbound lipophilic hormones move passively down a concentration gradient,[7] and indeed cells without relevant membrane transporters take up steroids freely.[8] Differences in tissue uptake were previously attributed to physicochemical

properties, e.g. lipophilicity, until discovery of specific thyroid hormone transporters challenged traditional assumptions. The level of hormone (triiodothyronine, T3) available to receptors not only depends on hormone synthesis and activation, but also on transport into and out of cells, notably by the monocarboxylate 8 (MCT8) transporter.[9] Neuronal T3 uptake is critically impaired without MCT8, as occurs in the X-linked "Allan-Herndon-Dudley Syndrome" of neurodevelopmental anomalies with abnormal thyroid function.[10]

Cellular uptake of glucocorticoids by membrane transporters has been demonstrated in Drosophila, where loss of the 'Ecdysone Importer' (EcI) produces a steroid-deficient phenotype.[11] Organic anion transporting polypeptide (OATP) transporters mediate the uptake of glucocorticoids in rat liver *ex vivo*, however this has not been reproducible in humans.[12,13] A saturable glucocorticoid uptake mechanism across the blood-brain and blood-CSF (cerebrospinal fluid) barriers in mice was only discernible at supraphysiological concentrations, so may not be physiologically relevant.[14]

Our increasing understanding of the importance of transporters for thyroid hormone function sets a biological precedent for other lipophilic hormones, however, whilst similar active *import* of glucocorticoids in humans has not been shown, there is mounting evidence supporting facilitated *export* of glucocorticoids from cells, particularly by two members of the ABC transporter family.

ABCB1 and ABCC1 are steroid exporters

The ABC protein family

One of the most highly conserved protein superfamilies, ABC proteins shuttle toxins, xenobiotics, and signalling molecules across eukaryotic and prokaryotic cell membranes. Classified into seven subfamilies according to their structural similarity and sequence homology, they have been actively researched for decades, particularly in relation to multidrug resistance. The evolution and relevance of this transporter superfamily in cancer drug efflux has been well-reviewed,[15,16] yet of the

over fifty human ABC proteins that have been identified, only ABCB1 and ABCC1 have recognised roles in glucocorticoid transport.[17]

The typical ABC transporter is a homodimer characterised by two transmembrane domains (TMDs) and two cytoplasmic nucleotide-binding domains (NBDs) (FIG. 1).[18] Each TMD domain has between six and ten transmembrane α-helices depending on the specific transporter, and is involved in substrate recognition. The cytoplasmic NBDs contain conserved motifs for ATP binding and hydrolysis, including the ABC signature motif (or C-loop motif), Walker A motif (P-loop) and Walker B motif.[17] Together, these dimeric NBDs act to hydrolyse ATP and provide energy to drive transport against concentration gradients.

Several models have been proposed to explain the relationship between ATP hydrolysis and TMD-mediated transport,[19] with most purporting that energy from ATP hydrolysis enables switching between inward and outward facing configurations (FIG. 1A). Individual transporters are unidirectional: almost exclusively exporters in eukaryotic cells, but importers (of nutrients) or exporters (of toxins and cell wall substrates) in bacteria.[20] Consistent with this export function, transporters are typically found at luminal surfaces to limit xenobiotic exposure.[17] Substrates range from ions to large proteins and while there is a high degree of overlap between transporters, the molecular basis of this remains poorly documented.

ABCB1 and steroid export

Initially named P-glycoprotein (P-gp) and later MDR-1 (multiple drug resistance protein 1), ABCB1 has been extensively studied as the archetypal multidrug transporter, exporting a broad array of xenobiotics including antineoplastics, antimicrobials and antidepressants (see reviews by Juan-Carlos, Sissung and Hodges).[15,21,22]

Encoded by the human *ABCB1* gene located on chromosome 7q21.12, the resultant protein is 1280 amino acids (141.5 kDa) in size with 12 membrane spanning helices.[23] The polyspecificity of ABC

transporters is often purported to result from plasticity of the drug-binding pocket, both in terms of side chain and backbone arrangements. Numerous attempts have been made over the years to determine the 3D structure of ABC proteins in efforts to understand their transport mechanisms and substrate specificity, however their size and hydrophobicity provide significant challenges.[24] Recent advances in the use of cryo-electron microscopy have enabled structural insights into substrate binding.[25-27] Alam *et al* reconstituted the structure of human ABCB1 in complex to chemotherapeutic drugs and revealed the drug-binding cavity is globular in shape, with interactions from all 12 TMDs (FIG. 1B).[25] Moreover they propose that substrate-induced structural changes in NBD2 confer changes in ATPase activity, which determines transport action.

While a putative steroid-binding site has been identified in human ABCB1, this was based upon a homology model of just the NBDs[28] and is not definitive. However, physiological data does support selective ABCB1-mediated transport of steroids. In the 1960s, murine fibroblasts were observed exporting steroids in an energy- and temperature-dependent manner, consistent with active transport.[29] Cortisol export was later demonstrated in a porcine renal tubular cell line (LLC-PK1) overexpressing human *ABCB1*.[30] In the intervening period, several endogenous and synthetic steroids have been confirmed as ABCB1 substrates. Gruol and Bourgeois stratified steroids into three categories depending on the presence of hydroxyl groups at positions 11 and 17;[31] ABCB1-mediated efflux was highest for steroids with both hydroxyl groups (including dexamethasone, cortisol and prednisolone), lowest for those with neither (deoxycorticosterone and progesterone), and intermediate in those with one group (including corticosterone and aldosterone). Yates further illustrated that A-ring planarity and 6α- and 16α- methyl substitution enhanced transport, in keeping with a critical hydrophobic pocket in the steroid-binding region.[32] Methylprednisolone is the glucocorticoid most effectively exported by ABCB1, followed by prednisolone, betamethasone, prednisone, dexamethasone, cortisol and cortisone.[31-33] Aldosterone appears to be weakly transported, but there is no evidence that sex steroids, 11-deoxycorticosterone and progesterone undergo ABCB1-mediated export,[31] although progesterone does bind avidly to ABCB1 with inhibitory

effect.[34] Corticosterone - the predominant glucocorticoid in rats and mice - was initially shown to be an ABCB1 substrate on the basis of efflux from murine macrophage-like cells.[35] Indeed recent *in vitro* work in murine adrenocortical cells has demonstrated that the ability of these cells to secrete corticosterone is blocked by pharmacological ABCB1 inhibition.[36] This is in contrast to previous *in vitro* work showing no corticosterone export in the murine LMCAT fibroblast line.[31,37-39] Importantly, in studies of murine thymoma cells overexpressing *Abcb1* where corticosterone and cortisol transport was compared, there was lower efflux of corticosterone compared to cortisol,[31] indicating a preference of this transporter for cortisol. Studies of the human transporter have not shown corticosterone to be transported by ABCB1, so affinity may be species specific.[4,40]

ABCC1 and steroid export

First identified and cloned as multidrug resistance protein1 (MRP1), ABCC1 was also discovered in multidrug resistance studies where high levels of expression are poor prognostic indicators in certain malignancies.[41-43] Since then, ABCC1 has been shown to efflux a diverse range of conjugated xenobiotics and physiological organic anions.[44] Like ABCB1, ABCC1 demonstrates polarity in epithelial cells, but is located on the basolateral rather than apical membrane.[45]

Encoded by the human *ABCC1* gene on the short arm of chromosome 16 (16p13.11), strikingly, ABCC1 and ABCB1 share only 23% sequence identity, and differ substantially in their structural and physiological functions. To date, only the structure of bovine ABCC1 has been determined by cryo-electron microscopy.[45] The 190kDa ABCC1 protein has 17 transmembrane α-helices, distributed among three TMDs, rather than the two TMDs observed in ABCB1 (FIG 1C) [45]

The binding site within the transmembrane domain is “bipartite” – having a positively charged “P pocket” which forms hydrogen bonds with glutathione (GSH) residues, and a second “H pocket” which interacts with hydrophobic moieties. This explains why GSH coupling facilitates transport of a

wide range of compounds. ABCC1 substrates tend to be organic anions, whereas those for ABCB1 tend to be weak cations,[45] and whilst ABCB1 is thought to transport substrates partitioning through the bilipid cell membrane (the "hydrophobic vacuum"),[46] ABCC1 extracts them directly from the cytoplasm.[45]

ABCC1 uniquely exhibits affinity for organic anions and phase II hepatic metabolites (endogenous and xenobiotic compounds conjugated with GSH, glucuronide and sulphate to facilitate excretion). There are differences between human and other mammalian isoforms, e.g. the glucuronide conjugate of 17β-oestradiol is a substrate only in humans.[47] It has been shown *in vitro*, both in virally transfected mouse fibroblast LMCAT cells, and subsequently in human adipocytes, that ABCC1 can export corticosterone and 11-deoxycorticosterone, but not cortisol, prednisolone or dexamethasone.[6,39]

ABCB1 and ABCC1 in tissues

The mRNA expression profiles of human ABCB1 and ABCC1 in various tissues are summarised in FIG. 2. Highly expressed in the adrenal gland, ABCB1 is also found at absorptive surfaces (e.g. of the intestines), protective barriers (e.g. testis, blood-brain barrier and placenta) and secretory tissues (e.g. biliary canaliculi and renal tubule).[23] ABCC1 is widely expressed in almost all cell types, with highest levels in thymus, parathyroid and skeletal muscle. It appears poorly expressed in the liver[48] and nervous system, but notably is found in greater quantities than ABCB1 in adipose tissue, and skeletal muscle.[23,49,50]

A model for the consequences of this tissue-specific transporter expression on intracellular concentrations of different glucocorticoids is outlined in FIG. 3. Combining the *in vitro* works of Bourgeois, Webster, and Nixon, glucocorticoids can be separated into three groups depending on relative susceptibility to export by ABCB1 and ABCC1.[6,31,39] This model predicts that intracellular

concentrations of cortisol will be lower in tissues predominantly expressing ABCB1 (including central HPA axis negative feedback sites) and corticosterone will be lower in tissues predominantly expressing ABCC1, such as adipose.

ABCB1 and ABCC1 modulate the HPA axis

Central control of the HPA axis depends on feedback from circulating glucocorticoids to the hypothalamus and pituitary, but to reach the brain they must traverse the tightly packed endothelium of the "blood-brain barrier", where ABCB1 is found.[51] Murine models have been used extensively to assess ABCB1-dependent modulation of steroid concentrations within tissues, including the brain. Importantly, rodents have two ABCB1 isoforms – ABCB1A (aka MDR1A or MDR3) and ABCB1B (aka MDR1B or MDR1),[52,53] which broadly share the characteristics of the human protein.[53] Indeed, *Abcb1a* knockout mice accumulate 87x more of the ABCB1 substrate ivermectin in brain than wild-type animals,[54] while ABCB1 inhibition with tariquidar increases cerebral uptake of labelled verapamil during positron-emission tomography (PET) imaging and demonstrates the role of ABCB1 at the human blood-brain barrier.[55]

Abcb1a knockout mice exhibit enhanced retention of cortisol and dexamethasone in the brain.[4,5,54,56] As seen *in vitro,* results for corticosterone export *in vivo* are varied, perhaps reflecting redundancy between the murine isoforms. Karssen *et al.* reported no difference in brain corticosterone in adrenalectomised *Abcb1a* knockout versus wild-type mice infused with radio-labelled corticosterone,[4] however the double knockout mouse (*Abcb1ab*$^{-/-}$) retains an excess of both glucocorticoids in the brain.[57] This was greater for cortisol, suggesting that overall ABCB1 activity in mice favours cortisol over corticosterone transport. The opposite was found in studies by the Pariante group where there was retention of both glucocorticoids in *Abcb1a* knockout mice, and cortisol retention alone in *Abcb1ab* double knockouts.[58-60] The authors highlight methodological differences between the studies which limit comparisons; for instance, in one study isotope radioactivity rather

than intact steroid concentration was measured, and use of labelled corticosterone in adrenally intact animals may have resulted in isotope dilution.

From these findings we might predict that the HPA axis would be relatively suppressed by accumulation of glucocorticoids in brain when ABCB1 activity is reduced. *Abcb1a* knockout mice do have evidence of HPA axis suppression, with lower basal and stress-stimulated levels of corticosterone, ACTH and corticotrophin-releasing hormone than controls, with effect localised to hypothalamic level.[61] Furthermore, mice treated with the ABCB1 inhibitor tariquidar have an attenuated corticosterone response to stressful stimulus.[62]

In larger, cortisol-dominant species the ABCB1 protein is well conserved, with a notable exception being in Collie-derived dogs. Like Schinkel's *Abcb1a* knockout mice,[54] these animals are exquisitely sensitive to ivermectin, owing to a 4-bp deletion mutation (termed *Mdr1-1Δ)* for which 40-50% of this breed are homozygotes.[63,64] This mutation results in a severely truncated protein (<10% of normal length) which is predicted to be non-functional. Anecdotally, Collies have been viewed by veterinarians to have a relatively slow illness recovery,[65] and Mealey demonstrated chronic suppression of the HPA axis in animals with the MDR1$^{-/-}$ genotype, with lower basal cortisol levels and greater ACTH suppression in response to dexamethasone than the wild type. It is hypothesised that enhanced brain penetration of cortisol (the dominant canine glucocorticoid) leads to HPA axis suppression, and predisposes the animals to a form of relative corticosteroid insufficiency.[65] This has been supported by a recent metabolomics study demonstrating lower urinary cortisol metabolites in MDR1$^{-/-}$ dogs than controls [reaching significance for Allo-tetrahydro-cortisol (11.2 ± 3.4 ng/L vs 20.7 ± 14.9 ng/L, p=0.006) and β-cortol (105.5 ± 63.3 ng/L vs 221.0 ± 225.5 ng/L, p=0.025)].[66]

In a human study, the corticosterone:cortisol ratio in brain autopsy specimens was 5x greater than the plasma ratio in age- and sex-matched healthy controls.[4] The ratio of corticosterone:cortisol in live subjects is similarly 5-6x higher in CSF than plasma.[67] Many drugs inhibit ABCB1, including

verapamil and cyclosporin A, but their experimental use to test ABCB1 physiology in humans is hampered by toxicity at levels too low for meaningful ABCB1 inhibition.[68]

This is all consistent with the hypothesis that ABCB1 on the blood-brain barrier exports cortisol and thereby modulates HPA axis negative feedback in multiple species. The absence of ABCC1 from the brain and blood-brain barrier is consistent with corticosterone being retained more so than cortisol in brain. One additional complexity, however, is that the pituitary gland (which expresses both transporters)[69] lies outside the blood-brain barrier and yet also contributes to HPA axis control. We have demonstrated that administration of probenecid, an inhibitor of ABCC1, reveals greater tonic negative feedback of the HPA axis in healthy subjects as judged by elevations in ACTH and cortisol during combined mineralocorticoid and glucocorticoid receptor antagonism.[70] This finding is consistent with ABCC1 also contributing to export of corticosterone from the pituitary or other central feedback areas, and warrants further investigation in animal models.

ABCC1 transporter in adipose tissue

In contrast with the blood-brain barrier where ABCB1 is more abundant than ABCC1, the reverse is true in adipose. Glucocorticoids within adipose tissue induce lipogenesis, particularly stimulating central fat accumulation and adipokine production.[71] Global *Abcc1* knockout mice infused with both glucocorticoids showed enhanced corticosterone but not cortisol accumulation in adipose tissue, and upregulation of both glucocorticoid-responsive and adipogenic genes.[6]

We have also demonstrated that human adipocytes preferentially accumulate cortisol over corticosterone, and that this was reversed *in vitro* after treatment with the ABCC1 inhibitors probenecid or MK-571.[6] This was also accompanied by activation of glucocorticoid-responsive and adipogenic genes (*PER1, ADIPOQ, ATGL, HSL*) and resulted in increased fatty acid accumulation in lipid droplets.[6] Moreover, during infusion of cortisol or corticosterone *in vivo* in patients with primary

adrenal insufficiency, there was greater adipose induction of glucocorticoid-responsive gene expression (*PER1, LPL*) in response to cortisol than corticosterone, achieved at plasma glucocorticoid levels which were equipotent for ACTH suppression.[6]

ABCB1 and ABCC1 in the placenta

As the interface between mother and fetus in pregnancy, the placenta functions both as a nutritive source and barrier, including to glucocorticoid transport. The fetus (unable to synthesise cortisol until the third trimester) depends on maternal cortisol, however whilst maternal cortisol levels increase several-fold during pregnancy, this is not transferred to the fetus indiscriminately.[72] The placenta provides a glucocorticoid barrier in early pregnancy when excessive glucocorticoids are detrimental,[73] but has a more facilitative role towards term for fetal organ maturation.[74]

The enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) is viewed as the main component of the placental glucocorticoid barrier, converting active cortisol to inactive cortisone.[75] A study inhibiting the 11β-HSD2 enzyme during *ex vivo* perfusion of recently delivered human placentas suggested that 11β-HSD2 may only contribute part of the glucocorticoid barrier, as cortisol transfer was restricted even at maximal inhibition.[76] The role of other mechanisms at the placental barrier, such as transmembrane transport, warrants further consideration.

ABCB1 is located within syncytiotrophoblasts at the apical border, in direct contact with maternal blood.[77] It is highly expressed in early pregnancy and decreases towards term in keeping with the physiological role suggested above.[78] Glucocorticoids, as in other tissues, have been shown to upregulate *ABCB1* in the first trimester placenta which may enhance the barrier effect.[79] Studies demonstrating low concentrations of ABCB1 substrates (e.g. antiretrovirals) in the fetal circulation both at birth, and in the *ex vivo* perfused placenta indicate this process is active *in vivo*.[80]

ABCC1 is located on the fetal-facing placental surface and has been identified in cytotrophoblasts, syncytiotrophoblasts and fetal endothelium.[81] This may be consistent with a role in transferring substrates (e.g. folic acid) to the fetus and, in contrast to *ABCB1, ABCC1* is upregulated towards term.[81,82] Studies of other ABCC1 substrates with the inhibitors probenecid and MK-571 have not demonstrated a clear effect on cross-placental transfer, so cannot be extrapolated to corticosterone transport.[83] It has been shown that the ratio of cortisol:corticosterone is higher in the maternal circulation (15:1) than in the umbilical vein (7:1) at term,[84] which may be accounted for by fetal adrenal cortisol:corticosterone secretion rates, or by facilitated transport of maternal corticosterone by ABCC1 into the fetal circulation.

Regulation and dysregulation

Regulation of ABCB1

Mechanisms underpinning regulation of *ABCB1* expression are reviewed thoroughly elsewhere.[85-87] The *ABCB1* promoter contains a number of areas of interest, including binding sites for the tumour suppressor p53, heat shock proteins and "adopted orphan receptors" including the Pregnane-X Receptor (PXR) and Constitutive Androstane Receptor (CAR) which bind a number of xenobiotic ligands.[88] Xenobiotics, inflammatory mediators and cellular stress (such as irradiation, heat shock, hypoxia) typically upregulate *ABCB1* expression through common pathways involving nuclear factor kappa B (NF-κB) and Y-box binding protein.[89,90] This appears to be a protective response – polymorphisms in NF-κB are linked with increasing colon cancer risk potentially related to enhanced cellular exposure to toxins.[91]

Glucocorticoids modulate expression of ABCB1 mRNA and protein in rodents and humans. This has been demonstrated across multiple tissues with dexamethasone, prednisolone, cortisol, methylprednisolone and some inhaled glucocorticoids.[33,79,92-97] Glucocorticoids predominantly induce

ABCB1 expression, however this effect may be specific to some species or cell types as there are also instances of *ABCB1* downregulation [98] This glucocorticoid effect is inhibited in the presence of the glucocorticoid receptor (GR) blocker RU486, indicating this is at least partly mediated via the GR, but since no consensus glucocorticoid response element (GRE) has been found in the human *ABCB1* promoter, this is assumed to be an indirect genomic effect. Zhang *et al* showed that dexamethasone-mediated upregulation of *ABCB1* in retinal pigment epithelium was abolished when the PXR receptor was silenced, implying that PXR (which does contain a consensus GRE) is either a co-regulator or target of GR.[97,99,100] This raises concerns about increasing drug efflux when glucocorticoids are used in combination with other ABCB1 substrates (as in chemotherapy protocols), and is theorised to be a cause of glucocorticoid resistance in conditions such as asthma,[33] but this effect has also been exploited clinically e.g. in the treatment of paraquat toxicity with methylprednisolone to increase drug excretion.[101]

Taken together this evidence suggests that in times of increased physiological stress (e.g. in response to illness or injury), ABCB1 is upregulated both by stress-activated glucocorticoids, and by signals released by cellular damage. This upregulation may result in positive feedback on cortisol production by further restricting access to higher negative feedback sites. However, regulation of ABCB1 in inflammation is complex and potentially biphasic: there is evidence from rodent studies that in the very early stages of inflammation ABCB1 is functionally inhibited by lipopolysaccharide and inflammatory cytokines despite maintained mRNA expression, perhaps due to trafficking of ABCB1 away from the cell membrane; later in the evolution of inflammation there is upregulation of mRNA and protein by the cytokines tumour necrosis factor-alpha (TNF-α) and Endothelin 1 (ET-1) converging on the NF-κB pathway.[89] Protein turnover at the cell surface under normal conditions is relatively slow (ABCB1 half-life estimated at just over 24hrs)[102] and there may be a role for post-translational and other mechanisms in modulating this.

Regulation of ABCC1

As with *ABCB1*, most research on factors affecting *ABCC1* expression levels and activity relates to cancer biology and chemotherapeutics, whilst physiological regulation has been poorly studied to date. Basal transcription of *ABCC1* is stimulated by the SP-1 (Specificity protein 1) transcription factor[103] which is in turn inhibited by the tumour suppressor protein p53.[104] It has not been clearly established whether PXR affects *ABCC1* transcription,[105,106] and whilst early mapping of the *ABCC1* promoter in a human leukaemic cell line did reveal a putative GRE site, dexamethasone has not been shown to alter *ABCC1* expression in the human placenta or in lymphocytes.[94,107-109] Furthermore, we cannot clearly conclude whether ABCC1 is affected by acute inflammation as is the case with ABCB1: both unchanged and increased mRNA expression has been reported in response to mediators such as lipopolysaccharide, TNF-α, IL-1 and IL-6.[110-112]

In vitro studies investigating metabolic regulation of *ABCC1* have focused on endothelium, demonstrating downregulation of transcript expression in a hyperglycaemic environment.[113] Metformin, a drug commonly used in treatment of type 2 diabetes, is known to reduce *ABCC1* expression in a human hepatocellular carcinoma cell line through the AMPK (5' AMP-activated protein kinase) - HIF-1α (Hypoxia-inducible factor 1 alpha) pathway.[114]

Whilst limited, overall this evidence suggests that ABCC1 is regulated differently from ABCB1 and is predominantly responsive to metabolic and immunomodulatory signals rather than to mediators of acute stress or inflammation.

Pathological dysregulation

There have been few studies of variations in ABC transporter expression beyond the extensive descriptions in various cancers described above. A recent transcriptomic analysis utilising single-cell RNA sequencing showed upregulation of *ABCB1* in the adrenal cortex of patients with ACTH-

dependent Cushing's disease.[36] This likely reflects the effects of glucocorticoids on *ABCB1* expression, but may contribute to pathogenicity by further enhancing cortisol export from the gland. Expecting that steroid retention in adipocytes would be higher in obese individuals, we found that *ABCC1* mRNA levels are upregulated in adipose tissue (subcutaneous and visceral) of obese versus lean subjects, which may paradoxically reduce glucocorticoid concentrations in adipocytes, although this may only be true for corticosterone.[6]

Lessons from human genetics

Human germline mutations in *ABCB1* and *ABCC1* are rare. To our knowledge, there are just two publications of *ABCB1* mutations: twin girls with toxic encephalopathy during febrile illness,[115] and a thirteen year old boy with ivermectin sensitivity.[116] In both cases the mutations were identified by whole exome sequencing and showed compound heterozygosity. The twin girls were found to have a nonsense mutation (p.Pro1182X) combined with a splicing variant (c.2786 + 1 G>T) and showed markedly enhanced CNS penetration of ^{11}C-verapamil on PET (positron emission tomography) imaging, in comparison to their parents. Their symptoms were suspected to be caused by retention of inflammatory mediators within the brain during intercurrent illness. The investigators estimated from lymphocyte studies that only ~10% of functional ABCB1 protein was expressed. In the other case, the affected boy presented with severe neurological side effects after a single oral dose of ivermectin and was found to have inherited a nonsense mutation in ABCB1 from each parent (c.2380 C>T and c.3053_3056delTTGA), both of which are predicted to result in loss of the C-terminal nucleotide binding domain. The children were otherwise healthy and growing normally in each case.

Similarly, there is only one published mutation of *ABCC1* of clinical significance: a heterozygous missense mutation (c.1769 A>G) recently identified as causing familial sensorineural deafness.[117] ABCC1 has been found within the rodent cochlea where it could be protective against neurotoxins.[118] This mutation is thought to disrupt hydrogen bonds and thus stability between the

helices of the transmembrane domains, and analysis of lymphoblastoid cell lines from affected family members showed loss of around 40-45% of ABCC1 mRNA expression when compared to those unaffected, suggesting additional impairment of mRNA stability. Transport of SNARF-1 (an ABCC1 substrate) from affected cells was subsequently shown to be slower.

With nonsense and frameshift mutations being rare, there have been attempts to correlate common polymorphisms with clinically relevant outcomes, as reviewed by Leschziner and colleagues.[119] Three *ABCB1* variants are common in humans – c.2677 G>A/T, c.3435 C>T and c.1236 C>T. The c.3435 C>T allele is synonymous but may affect mRNA stability;[120] c.1236 C>T is silent; but c.2677 G>A/T does result in amino acid substitution (alanine to serine or threonine), and therefore potentially to substrate changes. Plasma levels of the ABCB1 substrate digoxin have been found to be increased, decreased and unchanged in individuals with these polymorphisms. There is marked variation in frequency across different races, e.g. c.3435 C>T is much less common in African populations (~80% of people from West Africa are homozygous for the C allele versus ~20% of subjects from western Europe).[120,121] However, attempts to correlate polymorphisms with response to chemotherapeutics, drug side effects, and resistance to anti-retroviral and anti-epileptic therapies have all been inconclusive.[122-124]

Studies of the HPA axis in individuals with *ABCB1* variants have been undertaken but have been inadequately powered. Suzuki et al. found no differences in evening cortisol and ACTH in 30 Japanese men with differing c.3435 genotypes; however, Nakamura reported lower levels of 6pm plasma cortisol in those with one or two copies of the T allele (i.e. that associated with potentially reduced transporter mRNA stability) in a study of 51 women, reaching significance only in the follicular menstrual phase.[125,126] The variant c.2677 G>A/T in one candidate gene study of over 5000 Japanese individuals was highly associated with increased body mass index, which could potentially reflect greater HPA axis activity, whilst in a study of 154 depressed individuals, cortisol (but not ACTH) response to corticotrophin-releasing hormone was lower in TT homozygotes, which was taken to

reflect reduced adrenal cortisol release.[36,127] However, neither plasma cortisol levels nor body mass index have been associated with any *ABCB1* polymorphisms in larger cohorts.

Genetic studies have also been undertaken in patients taking exogenous steroids. In a cohort of 171 patients requiring long-term glucocorticoid replacement for adrenal insufficiency, those with the c.3435 TT genotype had lower bone density, suggesting greater steroid absorption or enhanced bone penetration.[128] There have been attempts to correlate glucocorticoid treatment outcomes in patients with rheumatoid arthritis, inflammatory bowel disease, immune thrombocytopenic purpura and nephrotic syndrome with genotype.[129-132] Most, but not all, indicate higher steroid response with the minor allele but are limited by sample size and failure to control for multiple testing.

For *ABCC1*, documented polymorphisms are mostly rare and non-coding, and have not been tested against measures of HPA axis activity or metabolism.[133] Three polymorphisms may predict outcome from acute myeloid leukaemia but any effect on transporter expression or function has not been established.[134]

Implications and research agenda

The observation that two ABC transporters influence tissue glucocorticoid retention allows us to add membrane transporters to the list of factors involved in pre-receptor glucocorticoid metabolism (FIG. 4). These observations provide insights into HPA axis physiology, and how corticosterone and cortisol may serve different functions in species which produce both steroids. They also provide therapeutic opportunities for anti-inflammatory and physiological replacement steroid therapies which might better target tissues mediating efficacy, and avoid those mediating toxicity.

Revised glucocorticoid physiology

In rodents, the lack of steroid 17-hydroxylation means that corticosterone is the sole endogenous glucocorticoid.[135] In humans and other species where both glucocorticoids circulate, it is common to consider them interchangeable. Indeed, cortisol and corticosterone share similar metabolic pathways (e.g. susceptibility to metabolism by 11β-HSD enzymes) and affinities for the glucocorticoid and mineralocorticoid receptors.[136-139] However corticosterone does exhibit differences to cortisol, including more rapid clearance from the circulation, and a greater fold response to ACTH such that the corticosterone/cortisol ratio rises under stress.[140-142]

The findings outlined here further illustrate that cortisol and corticosterone are not interchangeable with respect to glucocorticoid action. Specifically, in tissues where ABCB1 but not ABCC1 is present, such as the brain, cortisol concentrations are constrained by export back into the circulation and corticosterone can play a disproportionate role. Conversely, in tissues such as adipose where ABCC1 but not ABCB1 is expressed, corticosterone is exported and the response to cortisol can be disproportionate (FIG. 5).

This raises the concept of a distinctive role for corticosterone in mediating HPA axis negative feedback. In the stressed state, the ability to restrict high levels of cortisol from higher centres may prevent axis suppression after a stressful event and facilitate recovery, as demonstrated by the *Mdr1-1⊿* Collie dogs.[65] It is recognised in other species that the ratio of cortisol:corticosterone and peak circulating glucocorticoid levels vary seasonally,[143] possibly in response to photoperiod length. If corticosterone is more accessible to negative feedback sites, and less peripherally anabolic than cortisol, then this may both restrain the energy-expending stress response, and improve access to vital adipose energy stores when food is scarce.

Conversely, with slower turnover than corticosterone in the circulation and adipose tissue in comparison to other tissues like brain and liver,[144] cortisol may provide the option for medium term adjustments, in comparison with the acute changes in corticosterone.

Understanding the implications of differential control and actions of cortisol and corticosterone in glucocorticoid physiology will require detailed dissection of the dynamics of ligand availability for receptors within human target tissues *in vivo*. The increasing use of exome-wide sequencing in clinical as well as research settings may well identify further individuals or families with significant *ABCB1* and *ABCC1* mutations, and offer new routes to addressing these key physiological questions.

Novel glucocorticoid treatment approach

A major limitation of glucocorticoid therapies is their narrow therapeutic index. Despite extensive efforts, it has proved difficult to develop selective glucocorticoid receptor modulators with pharmacodynamic interactions which discriminate efficacious and toxic gene transcription.[145] An alternative approach depends on the premise that efficacy and toxicity are often mediated in different tissues, so that the therapeutic index could be improved by modifying the pharmacokinetics of steroid drugs to 'target' them to the tissues where efficacy is mediated while avoiding tissues where toxicity is mediated. Could this be achieved using steroids with different affinity for the ABCB1 and ABCC1 transporters?

When considering physiological replacement in patients with adrenal insufficiency, the challenges of this narrow therapeutic index are well documented, with adverse outcomes including, but not limited to, obesity, osteopenia and insulin resistance attributable to their steroid regime.[146,147] This is particularly difficult in Congenital Adrenal Hyperplasia (CAH), where doses of glucocorticoid which achieve adequate adrenal androgen suppression are invariably associated with morbidity. All glucocorticoids used for hormone replacement (cortisol, i.e. hydrocortisone; prednisolone; dexamethasone; and the active metabolites of pre-drugs cortisone and prednisone) are substrates for ABCB1 and not ABCC1. Although there may be some benefits from pharmacokinetic adjustments,[148,149] this cannot overcome the closeness of the dose-response relationship between efficacy and toxicity.

Corticosterone is not currently available in oral form, but our recent experimental work using intravenous corticosterone has provided proof-of-concept evidence of its potential advantages. As described earlier, there was greater glucocorticoid-responsive gene expression in response to cortisol over corticosterone in adipose of patients with Addison's disease.[6] In a similar study, 14 individuals with CAH also underwent ramped cortisol and corticosterone infusions. Despite higher plasma levels of corticosterone being achieved, there was greater insulin release in response to cortisol – a marker of glucocorticoid effect on adipose to induce insulin resistance.[150]

The potential for glucocorticoid therapies which avoid toxicity in metabolic tissues deserves further investigation, and would require generation of an oral corticosterone preparation.

Conclusions

We have collated evidence from cell, animal and human studies that the ATP-binding cassette transporters ABCB1 and ABCC1 differentially export cortisol, corticosterone and synthetic glucocorticoids from tissues and contribute to pre-receptor glucocorticoid regulation. Differing transporter expression profiles in the brain, placenta and adipose confer different tissue sensitivities to these steroids, which may be important for optimising the responsiveness of the HPA axis, controlling fetal steroid exposure across gestation, and optimising adipose fuel metabolism. Whilst much is known about these transporters when it comes to multidrug resistance, their physiological roles and regulation remain largely unexplored. The prospect of developing steroid therapies with transporter affinities tailored to give improved efficacy, without deleterious peripheral toxicity, gives new avenues for management of inflammatory and endocrine diseases.

References

1 Walker, B. R. *et al.* Increased glucocorticoid activity in men with cardiovascular risk factors. *Hypertension* **31**, 891-895, doi:10.1161/01.hyp.31.4.891 (1998).

2 Hammond, G. L. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. *J. Endocrinol.* **230**, R13-25, doi:10.1530/joe-16-0070 (2016).

3 Seckl, J. R. & Walker, B. R. Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology* **142**, 1371-1376, doi:10.1210/endo.142.4.8114 (2001).

4 Karssen, A. M. *et al.* Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. *Endocrinology* **142**, 2686-2694, doi:10.1210/endo.142.6.8213 (2001).

5 Meijer, O. C. *et al.* Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology* **139**, 1789-1793, doi:10.1210/endo.139.4.5917 (1998).

6 Nixon, M. *et al.* ABCC1 confers tissue-specific sensitivity to cortisol versus corticosterone: A rationale for safer glucocorticoid replacement therapy. *Sci. Transl. Med.* **8**, 352ra109, doi:10.1126/scitranslmed.aaf9074 (2016).

7 Mendel, C. M. The Free Hormone Hypothesis: A physiologically based mathematical model. *Endocr. Rev.* **10**, 232-274, doi:10.1210/edrv-10-3-232 (1989).

8 Ponec, M. & Kempenaar, J. A. Biphasic entry of glucocorticoids into cultured human skin keratinocytes and fibroblasts. *Arch. Dermatol. Res.* **275**, 334-344, doi:10.1007/BF00417208 (1983).

9 Friesema, E. C. *et al.* Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J. Biol. Chem.* **278**, 40128-40135, doi:10.1074/jbc.M300909200 (2003).

10 Friesema, E. C. *et al.* Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* **364**, 1435-1437, doi:10.1016/s0140-6736(04)17226-7 (2004).

11 Okamoto, N. *et al.* A membrane transporter Is required for steroid hormone uptake in Drosophila. *Dev. Cell* **47**, 294-305.e297, doi:10.1016/j.devcel.2018.09.012 (2018).

12 Allera, A. & Wildt, L. Glucocorticoid-recognizing and -effector sites in rat liver plasma membrane. Kinetics of corticosterone uptake by isolated membrane vesicles--II. Comparative influx and efflux. *J. Steroid Biochem. Mol. Biol.* **42**, 757-771 (1992).

13 Bossuyt, X., Muller, M., Hagenbuch, B. & Meier, P. J. Polyspecific drug and steroid clearance by an organic anion transporter of mammalian liver. *J. Pharmacol. Exp. Ther.* **276**, 891-896 (1996).

14 Mason, B. L., Pariante, C. M., Jamel, S. & Thomas, S. A. Central nervous system (CNS) delivery of glucocorticoids is fine-tuned by saturable transporters at the blood-CNS barriers and nonbarrier regions. *Endocrinology* **151**, 5294-5305, doi:10.1210/en.2010-0554 (2010).

15 Juan-Carlos, P. M., Perla-Lidia, P. P., Stephanie-Talia, M. M., Mónica-Griselda, A. M. & Luz-María, T. E. ABC transporter superfamily. An updated overview, relevance in cancer multidrug resistance and perspectives with personalized medicine. *Mol. Biol. Rep.* **48**, 1883-1901, doi:10.1007/s11033-021-06155-w (2021).

16 Silverton, L., Dean, M. & Moitra, K. Variation and evolution of the ABC transporter genes ABCB1, ABCC1, ABCG2, ABCG5 and ABCG8: implication for pharmacogenetics and disease. *Drug Metabol. Drug Interact.* **26**, 169-179, doi:10.1515/dmdi.2011.027 (2011).

17 Vasiliou, V., Vasiliou, K. & Nebert, D. W. Human ATP-binding cassette (ABC) transporter family. *Hum Genomics* **3**, 281-290 (2009).

18 Wilkens, S. Structure and mechanism of ABC transporters. *F1000prime reports* **7**, 14-14, doi:10.12703/P7-14 (2015).

19 Seeger, M. A. & van Veen, H. W. Molecular basis of multidrug transport by ABC transporters. *Biochim. Biophys. Acta* **1794**, 725-737, doi:10.1016/j.bbapap.2008.12.004 (2009).
20 Kang, J. *et al.* PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 2355-2360, doi:10.1073/pnas.0909222107 (2010).
21 Hodges, L. M. *et al.* Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). *Pharmacogenet. Genomics* **21**, 152-161, doi:10.1097/FPC.0b013e3283385a1c (2011).
22 Sissung, T. M. *et al.* Pharmacogenetics of membrane transporters: an update on current approaches. *Mol. Biotechnol.* **44**, 152-167, doi:10.1007/s12033-009-9220-6 (2010).
23 Uhlén, M. *et al.* Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419, doi:10.1126/science.1260419 (2015).
24 Esser, L. *et al.* Structures of the multidrug transporter P-glycoprotein reveal asymmetric ATP binding and the mechanism of polyspecificity. *J. Biol. Chem.* **292**, 446-461, doi:10.1074/jbc.M116.755884 (2017).
25 Alam, A., Kowal, J., Broude, E., Roninson, I. & Locher, K. P. Structural insight into substrate and inhibitor discrimination by human P-glycoprotein. *Science* **363**, 753-756, doi:10.1126/science.aav7102 (2019).
26 Lusvarghi, S., Robey, R. W., Gottesman, M. M. & Ambudkar, S. V. Multidrug transporters: recent insights from cryo-electron microscopy-derived atomic structures and animal models. *F1000Res* **9**, doi:10.12688/f1000research.21295.1 (2020).
27 Nosol, K. *et al.* Cryo-EM structures reveal distinct mechanisms of inhibition of the human multidrug transporter ABCB1. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 26245-26253, doi:10.1073/pnas.2010264117 (2020).
28 Mares-Sámano, S., Badhan, R. & Penny, J. Identification of putative steroid-binding sites in human ABCB1 and ABCG2. *Eur. J. Med. Chem.* **44**, 3601-3611, doi:10.1016/j.ejmech.2009.02.027 (2009).
29 Gross, S. R., Aronow, L. & Pratt, W. B. The active transport of cortisol by mouse fibroblasts growing in vitro. *Biochem. Biophys. Res. Commun.* **32**, 66-72 (1968).
30 Ueda, K. *et al.* Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.* **267**, 24248-24252 (1992).
31 Bourgeois, S., Gruol, D. J., Newby, R. F. & Rajah, F. M. Expression of an mdr gene is associated with a new form of resistance to dexamethasone-induced apoptosis. *Mol. Endocrinol.* **7**, 840-851, doi:10.1210/mend.7.7.8105374 (1993).
32 Yates, C. R. *et al.* Structural determinants of P-glycoprotein-mediated transport of glucocorticoids. *Pharm. Res.* **20**, 1794-1803 (2003).
33 Crowe, A. & Tan, A. M. Oral and inhaled corticosteroids: differences in P-glycoprotein (ABCB1) mediated efflux. *Toxicol. Appl. Pharmacol.* **260**, 294-302, doi:10.1016/j.taap.2012.03.008 (2012).
34 Yang, C. P., DePinho, S. G., Greenberger, L. M., Arceci, R. J. & Horwitz, S. B. Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of gravid uterus. *J. Biol. Chem.* **264**, 782-788 (1989).
35 Wolf, D. C. & Horwitz, S. B. P-glycoprotein transports corticosterone and is photoaffinity-labeled by the steroid. *Int. J. Cancer* **52**, 141-146 (1992).
36 Lopez, J. P. *et al.* Single-cell molecular profiling of all three components of the HPA axis reveals adrenal ABCB1 as a regulator of stress adaptation. *Sci Adv* **7**, doi:10.1126/sciadv.abe4497 (2021).
37 Medh, R. D., Lay, R. H. & Schmidt, T. J. Agonist-specific modulation of glucocorticoid receptor-mediated transcription by immunosuppressants. *Mol. Cell. Endocrinol.* **138**, 11-23 (1998).

38 Pariante, C. M. *et al.* Antidepressants enhance glucocorticoid receptor function in vitro by modulating the membrane steroid transporters. *Br. J. Pharmacol.* **134**, 1335-1343, doi:10.1038/sj.bjp.0704368 (2001).

39 Webster, J. I. & Carlstedt-Duke, J. Involvement of multidrug resistance proteins (MDR) in the modulation of glucocorticoid response. *J. Steroid Biochem. Mol. Biol.* **82**, 277-288 (2002).

40 Peng, R., Zhang, H., Zhang, Y. & Wei, D. Y. Impacts of ABCB1 (G1199A) polymorphism on resistance, uptake, and efflux to steroid drugs. *Xenobiotica* **46**, 948-952, doi:10.3109/00498254.2016.1138249 (2016).

41 Pajic, M., Norris, M. D., Cohn, S. L. & Haber, M. The role of the multidrug resistance-associated protein 1 gene in neuroblastoma biology and clinical outcome. *Cancer Lett.* **228**, 241-246, doi:10.1016/j.canlet.2005.01.060 (2005).

42 Paprocka, M. *et al.* MRP1 protein expression in leukemic stem cells as a negative prognostic marker in acute myeloid leukemia patients. *Eur. J. Haematol.* **99**, 415-422, doi:10.1111/ejh.12938 (2017).

43 Cole, S. P. *et al.* Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258**, 1650-1654 (1992).

44 Cole, S. P. Multidrug resistance protein 1 (MRP1, ABCC1), a "multitasking" ATP-binding cassette (ABC) transporter. *J. Biol. Chem.* **289**, 30880-30888, doi:10.1074/jbc.R114.609248 (2014).

45 Johnson, Z. L. & Chen, J. Structural basis of substrate recognition by the multidrug resistance protein MRP1. *Cell* **168**, 1075-1085.e1079, doi:10.1016/j.cell.2017.01.041 (2017).

46 Higgins, C. F. & Gottesman, M. M. Is the multidrug transporter a flippase? *Trends Biochem. Sci.* **17**, 18-21, doi:https://doi.org/10.1016/0968-0004(92)90419-A (1992).

47 Stride, B. D. *et al.* Pharmacological characterization of the murine and human orthologs of multidrug-resistance protein in transfected human embryonic kidney cells. *Mol. Pharmacol.* **52**, 344-353, doi:10.1124/mol.52.3.344 (1997).

48 Nishimura, M. & Naito, S. Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab. Pharmacokinet.* **20**, 452-477, doi:10.2133/dmpk.20.452 (2005).

49 Flens, M. J. *et al.* Tissue distribution of the multidrug resistance protein. *Am. J. Pathol.* **148**, 1237-1247 (1996).

50 Cole, S. P. Targeting multidrug resistance protein 1 (MRP1, ABCC1): past, present, and future. *Annu. Rev. Pharmacol. Toxicol.* **54**, 95-117, doi:10.1146/annurev-pharmtox-011613-135959 (2014).

51 Cordon-Cardo, C. *et al.* Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc. Natl. Acad. Sci. U. S. A.* **86**, 695-698, doi:10.1073/pnas.86.2.695 (1989).

52 Gruol, D. J., Vo, Q. D. & Zee, M. C. Profound differences in the transport of steroids by two mouse P-glycoproteins. *Biochem. Pharmacol.* **58**, 1191-1199 (1999).

53 Su, L. *et al.* Abcb1a and Abcb1b genes function differentially in blood–testis barrier dynamics in the rat. *Cell Death Dis.* **8**, e3038-e3038, doi:10.1038/cddis.2017.435 (2017).

54 Schinkel, A. H. *et al.* Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**, 491-502 (1994).

55 Wagner, C. C. *et al.* A pilot study to assess the efficacy of tariquidar to inhibit P-glycoprotein at the human blood-brain barrier with (R)-11C-verapamil and PET. *J. Nucl. Med.* **50**, 1954-1961, doi:10.2967/jnumed.109.063289 (2009).

56 Schinkel, A. H., Wagenaar, E., van Deemter, L., Mol, C. A. & Borst, P. Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J. Clin. Invest.* **96**, 1698-1705, doi:10.1172/jci118214 (1995).

57 Uhr, M., Holsboer, F. & Muller, M. B. Penetration of endogenous steroid hormones corticosterone, cortisol, aldosterone and progesterone into the brain is enhanced in mice deficient for both mdr1a and mdr1b P-glycoproteins. *J. Neuroendocrinol.* **14**, 753-759 (2002).

58 Mason, B. L., Pariante, C. M. & Thomas, S. A. Changes in the brain accumulation of glucocorticoids in abcb1a-deficient CF-1 mice. *J. Neuroendocrinol.* **24**, 1440-1446, doi:10.1111/j.1365-2826.2012.02353.x (2012).

59 Mason, B. L., Pariante, C. M. & Thomas, S. A. A revised role for P-glycoprotein in the brain distribution of dexamethasone, cortisol, and corticosterone in wild-type and ABCB1A/B-deficient mice. *Endocrinology* **149**, 5244-5253, doi:10.1210/en.2008-0041 (2008).

60 Pariante, C. M. The role of multi-drug resistance p-glycoprotein in glucocorticoid function: studies in animals and relevance in humans. *Eur. J. Pharmacol.* **583**, 263-271, doi:10.1016/j.ejphar.2007.11.067 (2008).

61 Muller, M. B. *et al.* ABCB1 (MDR1)-type P-glycoproteins at the blood-brain barrier modulate the activity of the hypothalamic-pituitary-adrenocortical system: implications for affective disorder. *Neuropsychopharmacology* **28**, 1991-1999, doi:10.1038/sj.npp.1300257 (2003).

62 Thoeringer, C. K., Wultsch, T., Shahbazian, A., Painsipp, E. & Holzer, P. Multidrug-resistance gene 1-type p-glycoprotein (MDR1 p-gp) inhibition by tariquidar impacts on neuroendocrine and behavioral processing of stress. *Psychoneuroendocrinology* **32**, 1028-1040, doi:10.1016/j.psyneuen.2007.08.001 (2007).

63 Mealey, K. L., Bentjen, S. A., Gay, J. M. & Cantor, G. H. Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics* **11**, 727-733 (2001).

64 Tappin, S. W. *et al.* Frequency of the mutant MDR1 allele in dogs in the UK. *Vet. Rec.* **171**, 72, doi:10.1136/vr.100633 (2012).

65 Mealey, K. L., Gay, J. M., Martin, L. G. & Waiting, D. K. Comparison of the hypothalamic–pituitary–adrenal axis in MDR1-1Δ and MDR1 wildtype dogs. *J. Vet. Emerg. Crit. Care (San Antonio)* **17**, 61-66, doi:doi:10.1111/j.1476-4431.2006.00196.x (2007).

66 Gramer, I. *et al.* Urinary cortisol metabolites are reduced in MDR1 mutant dogs in a pilot targeted GC-MS urinary steroid hormone metabolome analysis. *J. Vet. Pharmacol. Ther.* **45**, 265-272, doi:10.1111/jvp.13050 (2022).

67 Raubenheimer, P. J., Young, E. A., Andrew, R. & Seckl, J. R. The role of corticosterone in human hypothalamic-pituitary-adrenal axis feedback. *Clin. Endocrinol. (Oxf.)* **65**, 22-26, doi:10.1111/j.1365-2265.2006.02540.x (2006).

68 Thomas, H. & Coley, H. M. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. *Cancer Control* **10**, 159-165, doi:10.1177/107327480301000207 (2003).

69 Bernstein, H. G. *et al.* Vascular and extravascular distribution of the ATP-binding cassette transporters ABCB1 and ABCC1 in aged human brain and pituitary. *Mech. Ageing Dev.* **141-142**, 12-21, doi:10.1016/j.mad.2014.08.003 (2014).

70 Kyle, C. J. *et al.* ABCC1 modulates negative feedback control of the hypothalamic-pituitary-adrenal axis in vivo in humans. *Metabolism* **128**, 155118, doi:10.1016/j.metabol.2021.155118 (2022).

71 Lee, M. J., Gong, D. W., Burkey, B. F. & Fried, S. K. Pathways regulated by glucocorticoids in omental and subcutaneous human adipose tissues: a microarray study. *Am. J. Physiol. Endocrinol. Metab.* **300**, E571-580, doi:10.1152/ajpendo.00231.2010 (2011).

72 Rainey, W. E., Rehman, K. S. & Carr, B. R. Fetal and maternal adrenals in human pregnancy. *Obstet. Gynecol. Clin. North Am.* **31**, 817-835, x, doi:10.1016/j.ogc.2004.08.006 (2004).

73 Michael, A. E. & Papageorghiou, A. T. Potential significance of physiological and pharmacological glucocorticoids in early pregnancy. *Hum. Reprod. Update* **14**, 497-517, doi:10.1093/humupd/dmn021 (2008).

74 Larry, C. G. Effect of corticosteroids for fetal maturation on perinatal outcomes. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. *JAMA* **273**, 413-418, doi:10.1001/jama.1995.03520290065031 (1995).

75 Brown, R. W., Chapman, K. E., Edwards, C. R. & Seckl, J. R. Human placental 11 beta-hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology* **132**, 2614-2621, doi:10.1210/endo.132.6.8504762 (1993).

76 Stirrat, L. I. *et al.* Transfer andmetabolism of cortisol by the isolated perfused human placenta. *J. Clin. Endocrinol. Metab.* **103**, 640-648, doi:10.1210/jc.2017-02140 (2018).

77 Iqbal, M., Audette, M. C., Petropoulos, S., Gibb, W. & Matthews, S. G. Placental drug transporters and their role in fetal protection. *Placenta* **33**, 137-142, doi:10.1016/j.placenta.2012.01.008 (2012).

78 Sun, M. *et al.* Expression of the multidrug resistance P-glycoprotein, (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. *Placenta* **27**, 602-609, doi:10.1016/j.placenta.2005.05.007 (2006).

79 Lye, P. *et al.* Glucocorticoids modulate multidrug resistance transporters in the first trimester human placenta. *J. Cell. Mol. Med.* **22**, 3652-3660, doi:10.1111/jcmm.13646 (2018).

80 Nanovskaya, T. *et al.* Role of P-glycoprotein in transplacental transfer of methadone. *Biochem. Pharmacol.* **69**, 1869-1878, doi:10.1016/j.bcp.2005.03.030 (2005).

81 Nagashige, M. *et al.* Basal membrane localization of MRP1 in human placental trophoblast. *Placenta* **24**, 951-958, doi:10.1016/s0143-4004(03)00170-x (2003).

82 Pascolo, L. *et al.* Effects of maturation on RNA transcription and protein expression of four MRP genes in human placenta and in BeWo cells. *Biochem. Biophys. Res. Commun.* **303**, 259-265, doi:https://doi.org/10.1016/S0006-291X(03)00327-9 (2003).

83 Rahi, M. M., Heikkinen, T. M., Hakala, K. E. & Laine, K. P. The effect of probenecid and MK-571 on the feto-maternal transfer of saquinavir in dually perfused human term placenta. *Eur. J. Pharm. Sci.* **37**, 588-592, doi:https://doi.org/10.1016/j.ejps.2009.05.005 (2009).

84 Sippell, W. G., Becker, H., Versmold, H. T., Bidlingmaier, F. & Knorr, D. Longitudinal studies of plasma aldosterone, corticosterone, deoxycorticosterone, progesterone, 17-hydroxyprogesterone, cortisol, and cortisone determined simultaneously in mother and child at birth and during the early neonatal period. I. Spontaneous delivery. *J. Clin. Endocrinol. Metab.* **46**, 971-985, doi:10.1210/jcem-46-6-971 (1978).

85 Johnson, R. A., Ince, T. A. & Scotto, K. W. Transcriptional repression by p53 through direct binding to a novel DNA element. *J. Biol. Chem.* **276**, 27716-27720, doi:10.1074/jbc.C100121200 (2001).

86 Labialle, S., Gayet, L., Marthinet, E., Rigal, D. & Baggetto, L. G. Transcriptional regulators of the human multidrug resistance 1 gene: recent views. *Biochem. Pharmacol.* **64**, 943-948, doi:10.1016/s0006-2952(02)01156-5 (2002).

87 Scotto, K. W. Transcriptional regulation of ABC drug transporters. *Oncogene* **22**, 7496-7511, doi:10.1038/sj.onc.1206950 (2003).

88 Yan, J. & Xie, W. A brief history of the discovery of PXR and CAR as xenobiotic receptors. *Acta Pharmaceutica Sinica B* **6**, 450-452, doi:https://doi.org/10.1016/j.apsb.2016.06.011 (2016).

89 Miller, D. S. Regulation of P-glycoprotein and other ABC drug transporters at the blood-brain barrier. *Trends Pharmacol. Sci.* **31**, 246-254, doi:10.1016/j.tips.2010.03.003 (2010).

90 Zhou, S. F. Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. *Xenobiotica* **38**, 802-832, doi:10.1080/00498250701867889 (2008).

91 Andersen, V., Christensen, J., Overvad, K., Tjønneland, A. & Vogel, U. Polymorphisms in NFkB, PXR, LXR and risk of colorectal cancer in a prospective study of Danes. *BMC Cancer* **10**, 484, doi:10.1186/1471-2407-10-484 (2010).

92 Demeule, M., Jodoin, J., Beaulieu, E., Brossard, M. & Béliveau, R. Dexamethasone modulation of multidrug transporters in normal tissues. *FEBS Lett.* **442**, 208-214, doi:10.1016/s0014-5793(98)01663-9 (1999).
93 Iqbal, M., Gibb, W. & Matthews, S. G. Corticosteroid regulation of P-glycoprotein in the developing blood-brain barrier. *Endocrinology* **152**, 1067-1079, doi:10.1210/en.2010-1227 (2011).
94 Manceau, S. *et al.* Expression and induction by dexamethasone of ABC transporters and nuclear receptors in a human T-lymphocyte cell line. *J. Chemother.* **24**, 48-55, doi:10.1179/1120009x12z.00000000010 (2012).
95 Narang, V. S. *et al.* Dexamethasone increases expression and activity of multidrug resistance transporters at the rat blood-brain barrier. *Am. J. Physiol. Cell Physiol.* **295**, C440-450, doi:10.1152/ajpcell.00491.2007 (2008).
96 Perloff, M. D., von Moltke, L. L. & Greenblatt, D. J. Ritonavir and dexamethasone induce expression of CYP3A and P-glycoprotein in rats. *Xenobiotica* **34**, 133-150, doi:10.1080/00498250310001630215 (2004).
97 Zhang, Y. *et al.* Expression and activity of p-glycoprotein elevated by dexamethasone in cultured retinal pigment epithelium involve glucocorticoid receptor and pregnane X receptor. *Investig Ophthalmol Vis Sci* **53**, 3508-3515, doi:10.1167/iovs.11-9337 (2012).
98 Nishimura, M. *et al.* Comparison of inducibility of multidrug resistance (MDR)1, multidrug resistance-associated protein (MRP)1, and MRP2 mRNAs by prototypical microsomal enzyme inducers in primary cultures of human and cynomolgus monkey hepatocytes. *Biol. Pharm. Bull.* **31**, 2068-2072, doi:10.1248/bpb.31.2068 (2008).
99 Pascussi, J.-M., Drocourt, L., Fabre, J.-M., Maurel, P. & Vilarem, M.-J. Dexamethasone induces pregnane X receptor and retinoid X receptor-α expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol. Pharmacol.* **58**, 361-372, doi:10.1124/mol.58.2.361 (2000).
100 Pascussi, J. M., Busson-Le Coniat, M., Maurel, P. & Vilarem, M. J. Transcriptional analysis of the orphan nuclear receptor constitutive androstane receptor (NR1I3) gene promoter: identification of a distal glucocorticoid response element. *Mol. Endocrinol.* **17**, 42-55, doi:10.1210/me.2002-0244 (2003).
101 Lin, J. L., Lin-Tan, D. T., Chen, K. H. & Huang, W. H. Repeated pulse of methylprednisolone and cyclophosphamide with continuous dexamethasone therapy for patients with severe paraquat poisoning. *Crit. Care Med.* **34**, 368-373 (2006).
102 Katayama, K. *et al.* Revealing the fate of cell surface human P-glycoprotein (ABCB1): The lysosomal degradation pathway. *Biochim. Biophys. Acta* **1853**, 2361-2370, doi:10.1016/j.bbamcr.2015.06.001 (2015).
103 Zhu, Q. & Center, M. S. Evidence that SP1 modulates transcriptional activity of the multidrug resistance-associated protein gene. *DNA Cell Biol.* **15**, 105-111, doi:10.1089/dna.1996.15.105 (1996).
104 Wang, Q. & Beck, W. T. Transcriptional suppression of multidrug resistance-associated protein (MRP) gene expression by wild-type p53. *Cancer Res.* **58**, 5762-5769 (1998).
105 Kauffmann, H. M. *et al.* Influence of redox-active compounds and PXR-activators on human MRP1 and MRP2 gene expression. *Toxicology* **171**, 137-146, doi:10.1016/s0300-483x(01)00570-4 (2002).
106 Magnarin, M. *et al.* Induction of proteins involved in multidrug resistance (P-glycoprotein, MRP1, MRP2, LRP) and of CYP 3A4 by rifampicin in LLC-PK1 cells. *Eur. J. Pharmacol.* **483**, 19-28, doi:10.1016/j.ejphar.2003.10.010 (2004).
107 Gu, X. & Manautou, J. E. Regulation of hepatic ABCC transporters by xenobiotics and in disease states. *Drug Metab. Rev.* **42**, 482-538, doi:10.3109/03602531003654915 (2010).

108 Manceau, S. *et al.* ABC drug transporter and nuclear receptor expression in human cytotrophoblasts: influence of spontaneous syncytialization and induction by glucocorticoids. *Placenta* **33**, 927-932, doi:10.1016/j.placenta.2012.07.016 (2012).

109 Zhu, Q. & Center, M. S. Cloning and sequence analysis of the promoter region of the MRP gene of HL60 cells isolated for resistance to adriamycin. *Cancer Res.* **54**, 4488-4492 (1994).

110 Cherrington, N. J., Slitt, A. L., Li, N. & Klaassen, C. D. Lipopolysaccharide-mediated regulation of hepatic transporter mRNA levels in rats. *Drug Metab. Dispos.* **32**, 734-741, doi:10.1124/dmd.32.7.734 (2004).

111 Evseenko, D. A., Paxton, J. W. & Keelan, J. A. Independent regulation of apical and basolateral drug transporter expression and function in placental trophoblasts by cytokines, steroids, and growth factors. *Drug Metab. Dispos.* **35**, 595-601, doi:10.1124/dmd.106.011478 (2007).

112 von Wedel-Parlow, M., Wölte, P. & Galla, H. J. Regulation of major efflux transporters under inflammatory conditions at the blood-brain barrier in vitro. *J. Neurochem.* **111**, 111-118, doi:10.1111/j.1471-4159.2009.06305.x (2009).

113 Neuser, J., Fraccarollo, D., Wick, M., Bauersachs, J. & Widder, J. D. Multidrug resistance associated protein-1 (MRP1) deficiency attenuates endothelial dysfunction in diabetes. *J Diabetes Complicat* **30**, 623-627, doi:10.1016/j.jdiacomp.2016.02.002 (2016).

114 Ling, S. *et al.* Metformin reverses multidrug resistance in human hepatocellular carcinoma Bel-7402/5-fluorouracil cells. *Mol Med Rep* **10**, 2891-2897, doi:10.3892/mmr.2014.2614 (2014).

115 Seo, J. *et al.* Biallelic mutations in ABCB1 display recurrent reversible encephalopathy. *Ann Clin Transl Neurol* **7**, 1443-1449, doi:10.1002/acn3.51125 (2020).

116 Baudou, E. *et al.* Serious Ivermectin Toxicity and Human ABCB1 Nonsense Mutations. *N. Engl. J. Med.* **383**, 787-789, doi:10.1056/NEJMc1917344 (2020).

117 Li, M. *et al.* Extrusion pump ABCC1 was first linked with nonsyndromic hearing loss in humans by stepwise genetic analysis. *Genet. Med.* **21**, 2744-2754, doi:10.1038/s41436-019-0594-y (2019).

118 Saito, T. *et al.* Expression of multidrug resistance protein 1 (MRP1) in the rat cochlea with special reference to the blood-inner ear barrier. *Brain Res.* **895**, 253-257, doi:10.1016/s0006-8993(01)02020-0 (2001).

119 Leschziner, G. D., Andrew, T., Pirmohamed, M. & Johnson, M. R. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.* **7**, 154-179, doi:10.1038/sj.tpj.6500413 (2007).

120 Hoffmeyer, S. *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 3473-3478, doi:10.1073/pnas.050585397 (2000).

121 Cascorbi, I. *et al.* Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin. Pharmacol. Ther.* **69**, 169-174, doi:https://doi.org/10.1067/mcp.2001.114164 (2001).

122 Dumond, J. B. *et al.* A phenotype-genotype approach to predicting CYP450 and P-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir. *Clin. Pharmacol. Ther.* **87**, 735-742, doi:10.1038/clpt.2009.253 (2010).

123 Hu, W. *et al.* MDR1 gene polymorphism correlated with pathological characteristics and prognosis in patients with primary hepatocellular carcinoma receiving interventional therapy. *Anticancer Drugs* **30**, 233-240, doi:10.1097/cad.0000000000000680 (2019).

124 Skalski, D. *et al.* Associations between MDR1 C3435T polymorphism and drug-resistant epilepsy in the Polish population. *Acta Neurol. Belg.* **117**, 153-158, doi:10.1007/s13760-016-0690-6 (2017).

125 Suzuki, A. *et al.* C3435T polymorphism of the MDR1 gene is not associated with blood levels of hypothalamus-pituitary-adrenal axis hormones in healthy male subjects. *Genet. Mol. Res.* **16**, doi:10.4238/gmr16019447 (2017).
126 Nakamura, T. *et al.* Effects of ABCB1 3435C>T genotype on serum levels of cortisol and aldosterone in women with normal menstrual cycles. *Genet. Mol. Res.* **8**, 397-403 (2009).
127 Ichihara, S. *et al.* Association of a polymorphism of ABCB1 with obesity in Japanese individuals. *Genomics* **91**, 512-516, doi:10.1016/j.ygeno.2008.03.004 (2008).
128 Lovas, K. *et al.* Glucocorticoid replacement therapy and pharmacogenetics in Addison's disease: effects on bone. *Eur. J. Endocrinol.* **160**, 993-1002, doi:10.1530/eje-08-0880 (2009).
129 Cuppen, B. V. *et al.* Polymorphisms in the multidrug-resistance 1 gene related to glucocorticoid response in rheumatoid arthritis treatment. *Rheumatol. Int.* **37**, 531-536, doi:10.1007/s00296-017-3653-1 (2017).
130 Pawlik, A., Wrzesniewska, J., Fiedorowicz-Fabrycy, I. & Gawronska-Szklarz, B. The MDR1 3435 polymorphism in patients with rheumatoid arthritis. *Int. J. Clin. Pharmacol. Ther.* **42**, 496-503 (2004).
131 Yang, Q. F. *et al.* Contribution of MDR1 gene polymorphisms on IBD predisposition and response to glucocorticoids in IBD in a Chinese population. *J. Dig. Dis.* **16**, 22-30, doi:10.1111/1751-2980.12205 (2015).
132 Han, S. S., Xu, Y. Q., Lu, Y., Gu, X. C. & Wang, Y. A PRISMA-compliant meta-analysis of MDR1 polymorphisms and idiopathic nephrotic syndrome: Susceptibility and steroid responsiveness. *Medicine (Baltimore)* **96**, e7191, doi:10.1097/md.0000000000007191 (2017).
133 Conseil, G., Deeley, R. G. & Cole, S. P. Polymorphisms of MRP1 (ABCC1) and related ATP-dependent drug transporters. *Pharmacogenet. Genomics* **15**, 523-533, doi:10.1097/01.fpc.0000167333.38528.ec (2005).
134 Kunadt, D. *et al.* Multidrug-related protein 1 (MRP1) polymorphisms rs129081, rs212090, and rs212091 predict survival in normal karyotype acute myeloid leukemia. *Ann. Hematol.* **99**, 2173-2180, doi:10.1007/s00277-020-04163-7 (2020).
135 Koren, L. *et al.* Cortisol and corticosterone independence in cortisol-dominant wildlife. *Gen. Comp. Endocrinol.* **177**, 113-119, doi:10.1016/j.ygcen.2012.02.020 (2012).
136 Arriza, J. L. *et al.* Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* **237**, 268-275, doi:10.1126/science.3037703 (1987).
137 Dunn, J. F., Nisula, B. C. & Rodbard, D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J. Clin. Endocrinol. Metab.* **53**, 58-68, doi:10.1210/jcem-53-1-58 (1981).
138 Giannopoulos, G. & Keichline, D. Species-related differences in steroid-binding specificity of glucocorticoid receptors in lung. *Endocrinology* **108**, 1414-1419, doi:10.1210/endo-108-4-1414 (1981).
139 Mani, O., Nashev, L. G., Livelo, C., Baker, M. E. & Odermatt, A. Role of Pro-637 and Gln-642 in human glucocorticoid receptors and Ser-843 and Leu-848 in mineralocorticoid receptors in their differential responses to cortisol and aldosterone. *J. Steroid Biochem. Mol. Biol.* **159**, 31-40, doi:10.1016/j.jsbmb.2016.02.017 (2016).
140 Nishida, S., Matsumura, S., Horino, M., Oyama, H. & Tenku, A. The variations of plasma corticosterone/cortisol ratios following ACTH stimulation or dexamethasone administration in normal men. *J. Clin. Endocrinol. Metab.* **45**, 585-588, doi:10.1210/jcem-45-3-585 (1977).
141 Peterson, R. E. Plasma corticosterone and hydrocortisone levels in man. *J. Clin. Endocrinol. Metab.* **17**, 1150-1157, doi:10.1210/jcem-17-10-1150 (1957).
142 Peterson, R. E. & Pierce, C. E. The metabolism of corticosterone in man. *J. Clin. Invest.* **39**, 741-757, doi:10.1172/jci104091 (1960).

143 Vera, F., Antenucci, C. D. & Zenuto, R. R. Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (Ctenomys talarum). *Gen. Comp. Endocrinol.* **170**, 550-557, doi:10.1016/j.ygcen.2010.11.012 (2011).
144 Hughes, K. A., Reynolds, R. M., Andrew, R., Critchley, H. O. & Walker, B. R. Glucocorticoids turn over slowly in human adipose tissue in vivo. *J. Clin. Endocrinol. Metab.* **95**, 4696-4702, doi:10.1210/jc.2010-0384 (2010).
145 Sundahl, N., Bridelance, J., Libert, C., De Bosscher, K. & Beck, I. M. Selective glucocorticoid receptor modulation: New directions with non-steroidal scaffolds. *Pharmacol. Ther.* **152**, 28-41, doi:https://doi.org/10.1016/j.pharmthera.2015.05.001 (2015).
146 Arlt, W. *et al.* Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J. Clin. Endocrinol. Metab.* **95**, 5110-5121, doi:10.1210/jc.2010-0917 (2010).
147 Bergthorsdottir, R., Leonsson-Zachrisson, M., Oden, A. & Johannsson, G. Premature mortality in patients with Addison's disease: a population-based study. *J. Clin. Endocrinol. Metab.* **91**, 4849-4853, doi:10.1210/jc.2006-0076 (2006).
148 Johannsson, G. *et al.* Improving glucocorticoid replacement therapy using a novel modified-release hydrocortisone tablet: a pharmacokinetic study. *Eur. J. Endocrinol.* **161**, 119-130, doi:10.1530/eje-09-0170 (2009).
149 Mallappa, A. *et al.* A phase 2 study of Chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* **100**, 1137-1145, doi:10.1210/jc.2014-3809 (2015).
150 Kyle, C. *et al.* Proof of concept that corticosterone has a higher therapeutic index than hydrocortisone in patients with Congenital Adrenal Hyperplasia. *JES* **3(1)**, doi:10.1210/js.2019-SAT-009 ((Abstract SAT 009) 2019.).

Highlighted References

Alam, A., Kowal, J., Broude, E., Roninson, I. & Locher, K. P. Structural insight into substrate and inhibitor discrimination by human P-glycoprotein. *Science* **363**, 753-756, doi:10.1126/science.aav7102 (2019).
This recent paper provides novel insights into the structure and mechanism of human ABCB1.

Johnson ZL, Chen J. Structural basis of substrate recognition by the multidrug resistance protein MRP1. *Cell.* 2017;168(6):1075-1085.e1079.
This paper on bovine ABCC1 provides the best insight into the structure and function of the protein.

Leschziner, G. D., Andrew, T., Pirmohamed, M. & Johnson, M. R. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.* **7**, 154-179, doi:10.1038/sj.tpj.6500413 (2007).
A detailed overview of the significance of *ABCB1* polymorphisms in humans and their clinical applications.

Lopez, J. P. *et al.* Single-cell molecular profiling of all three components of the HPA axis reveals adrenal ABCB1 as a regulator of stress adaptation. *Sci Adv* **7**, doi:10.1126/sciadv.abe4497 (2021).
The authors use a variety of approaches to show the role of ABCB1 in humans and rodents in modulating steroid secretion and the HPA axis. Single cell RNA profiling details expression of Abcb1a and Abcb1b across the axis.

Mealey KL, Gay JM, Martin LG, Waiting DK. Comparison of the hypothalamic–pituitary–adrenal axis in MDR1-1Δ and MDR1 wildtype dogs. *J Vet Emerg Crit Care (San Antonio).* 2007;17(1):61-66.

The importance of ABCB1 in modulating the HPA axis in a cortisol-dominant species is highlighted by these affected canines.

Nixon M, Mackenzie SD, Taylor AI, Homer NZ, Livingstone DE, Mouras R, Morgan RA, Mole DJ, Stimson RH, Reynolds RM, Elfick AP, Andrew R, Walker BR. ABCC1 confers tissue-specific sensitivity to cortisol versus corticosterone: A rationale for safer glucocorticoid replacement therapy. *Sci Transl Med.* 2016;8(352):352ra109.
The specificity of ABCC1 for transport of corticosterone over cortisol in humans and rodents adipose is shown here.

Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T, Hori R. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem.* 1992;267(34):24248-24252.
The original study showing that ABCB1 is a steroid transporter.

Webster JI, Carlstedt-Duke J. Involvement of multidrug resistance proteins (MDR) in the modulation of glucocorticoid response. *J Steroid Biochem Mol Biol.* 2002;82(4-5):277-288.
This study in a murine line shows the specificity of Abcb1 for transport of cortisol over corticosterone, and of Abcc1 for transport of corticosterone over cortisol *in vitro*.

Acknowledgements: The authors' research on ABC transporters has been funded by the Wellcome Trust and British Heart Foundation. Figures created with BioRender.com.

Author contributions: KD, EV, MN and BRW drafted the manuscript. All other authors reviewed and edited the manuscript and made intellectual contributions to its content.

Competing interests: The authors declare no competing interests.

Figure legends

Figure 1: Action and structure of ABCB1 and ABCC1. (A) In general, most ABC transporters are comprised of 2 transmembrane domains (TMD) and 2 nucleotide binding domains (NBD). In this proposed model of action, binding of ATP dimerises the NBDs and induces conformational change within the TMDs, resulting in the switch between "inward" and "outward" facing configurations.[17,18] Subsequent hydrolysis of ATP returns the transporter to baseline. (B) Ribbon diagram of human ABCB1 (Protein Data Bank ID 6QEX) and (C) Ribbon diagram of bovine ABCC1 (Protein Data Bank ID 5UJA). The N- and C- terminal halves are coloured magenta and blue respectively. NBD1 and NBD2 are coloured green and yellow respectively, with drug-binding pocket highlighted.

Figure 2: Tissue-specific expression of ABCB1 and ABCC1. Human expression of *ABCB1* and *ABCC1* is shown as derived from data from the Human Protein Atlas. Expression is normalised to an Nx (Normalised expression) value based on outputs from the Human Protein Atlas, GTEX and FANTOM5 transcriptomic analyses (data available online from v21.proteinatlas.org).[23] Tissues are ranked in order of *ABCB1:ABCC1* ratio, such that those towards the top of the y axis have greater *ABCB1* expression, and those at the bottom higher *ABCC1*.

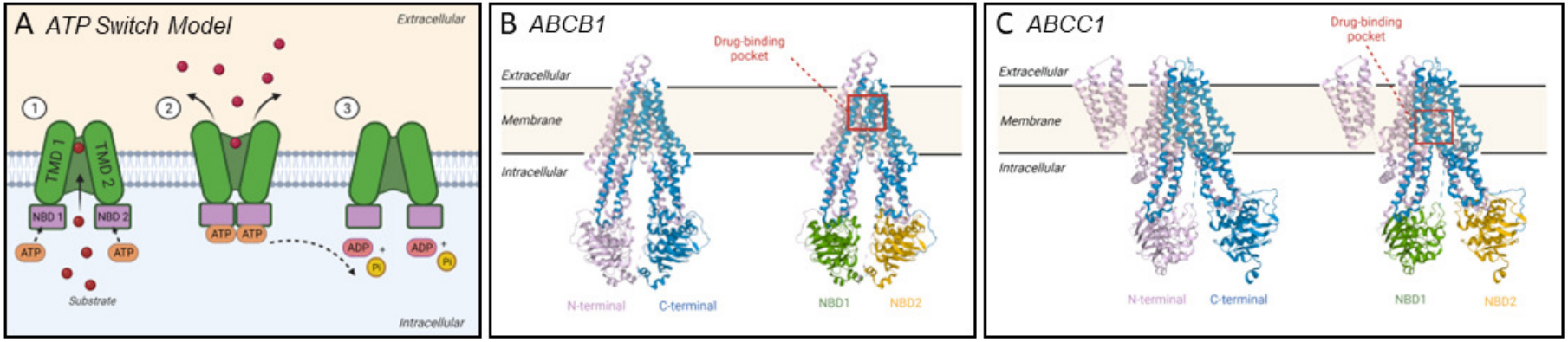
Figure 3: Tissue ABC transporter expression determines glucocorticoid sensitivity. The influence of ABCB1 and ABCC1 on retention of common glucocorticoids within human target tissues depending on transporter affinity is depicted. Steroids in red are predominantly substrates for ABCB1, those in dark blue predominantly substrates for ABCC1 and those in light green for neither transporter. Diffusion indicated by double-headed arrow.

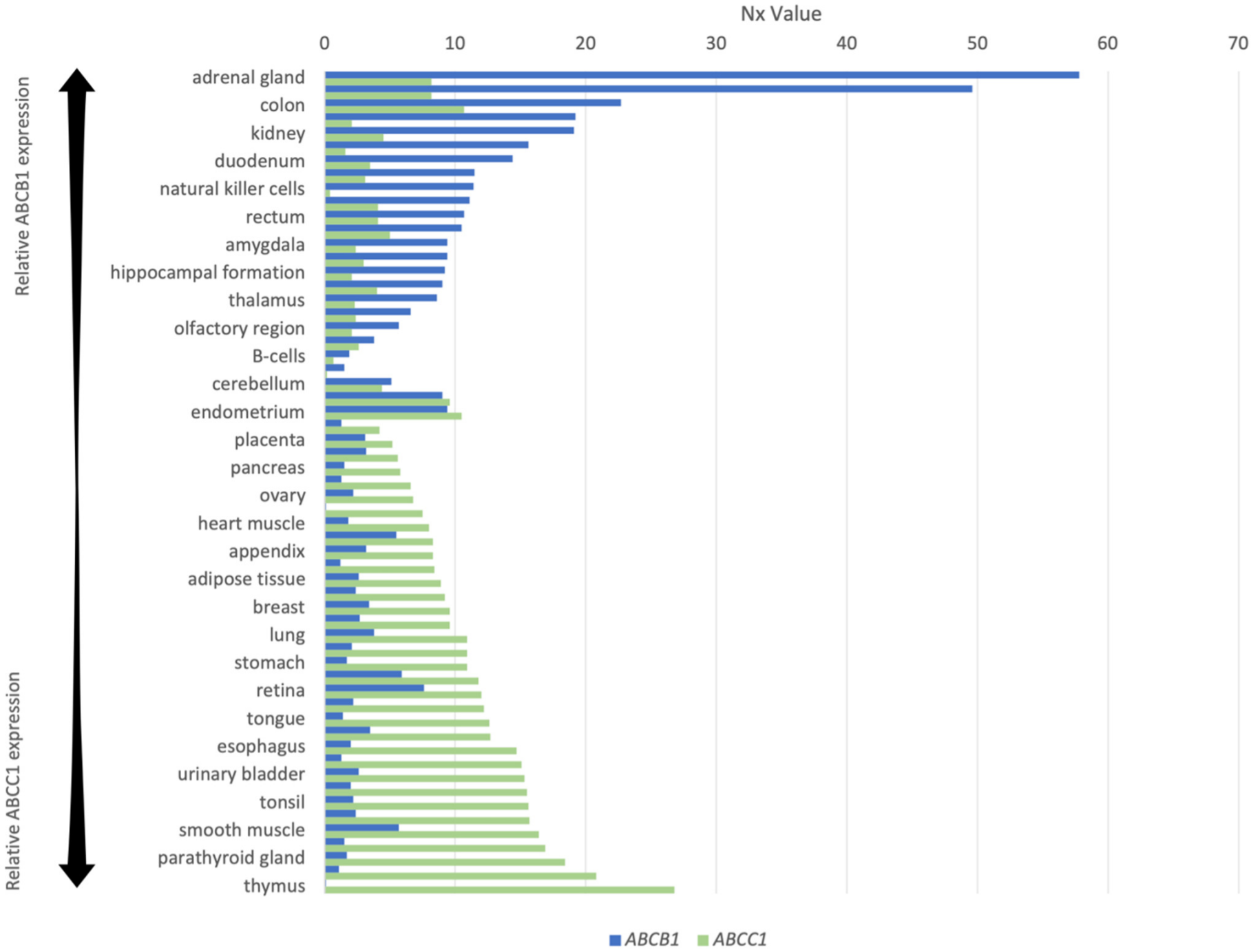
Figure 4: Intracellular glucocorticoid regulatory pathways. After diffusing into cells (double-headed arrow), glucocorticoids cortisol and corticosterone: may be exported by membrane-bound ATP transporters ABCB1 and ABCC1 (1); may undergo enzymatic metabolism by 11β-HSD (11β-hydroxysteroid dehydrogenase), 5α reductase or carbonyl reductase enzymes (2,3) or may become incorporated in the intracellular lipid pool (4). These processes restrict access to the nuclear glucocorticoid +/– mineralocorticoid receptors (GR and MR), which mediate the cellular response (5).

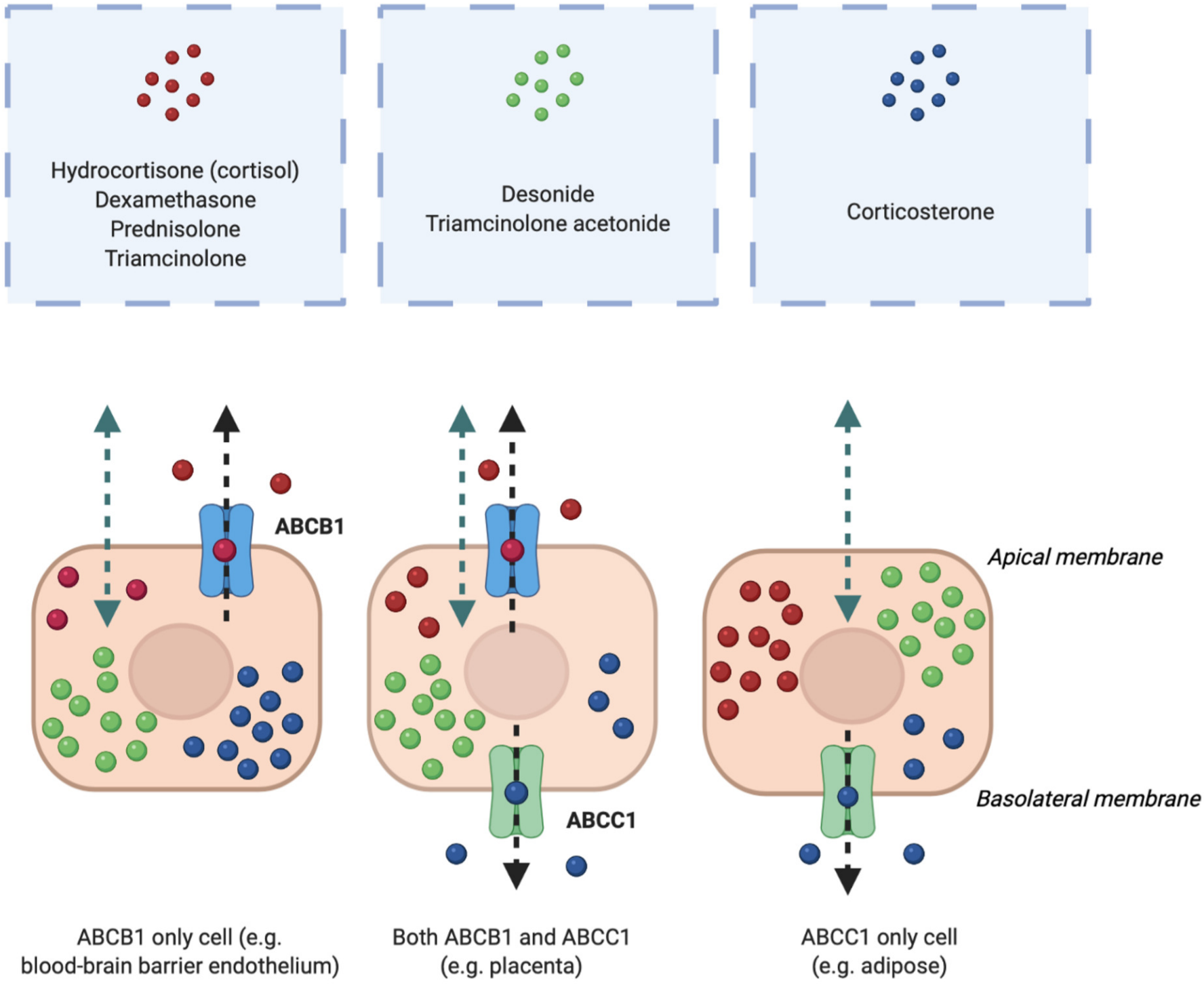
Figure 5: Modulation of the HPA axis by ABCB1 and ABCC1. Glucocorticoids are secreted from the adrenal cortex upon stimulation from hypothalamic and pituitary signals. They act peripherally on sites throughout the body, and feed back to hypothalamus, pituitary and higher centres to maintain homeostasis. ABCB1 present at the blood–brain barrier may act to restrict access of cortisol to feedback sites. Conversely ABCC1, which is found without ABCB1 in adipose and skeletal muscle, exports corticosterone but not cortisol. Activity of the adrenal enzyme CYP17 determines the secreted ratio of cortisol:corticosterone.

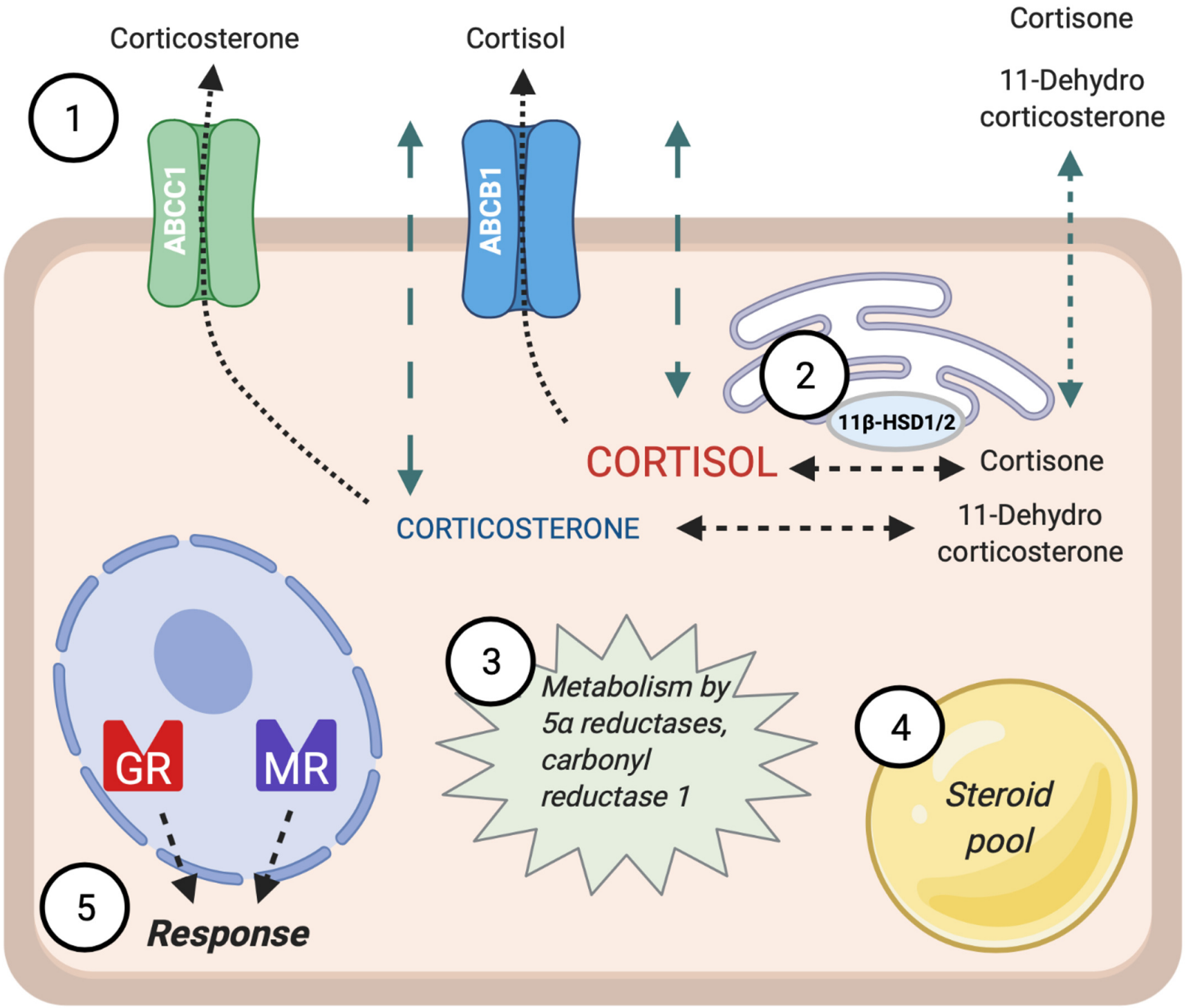
Key points

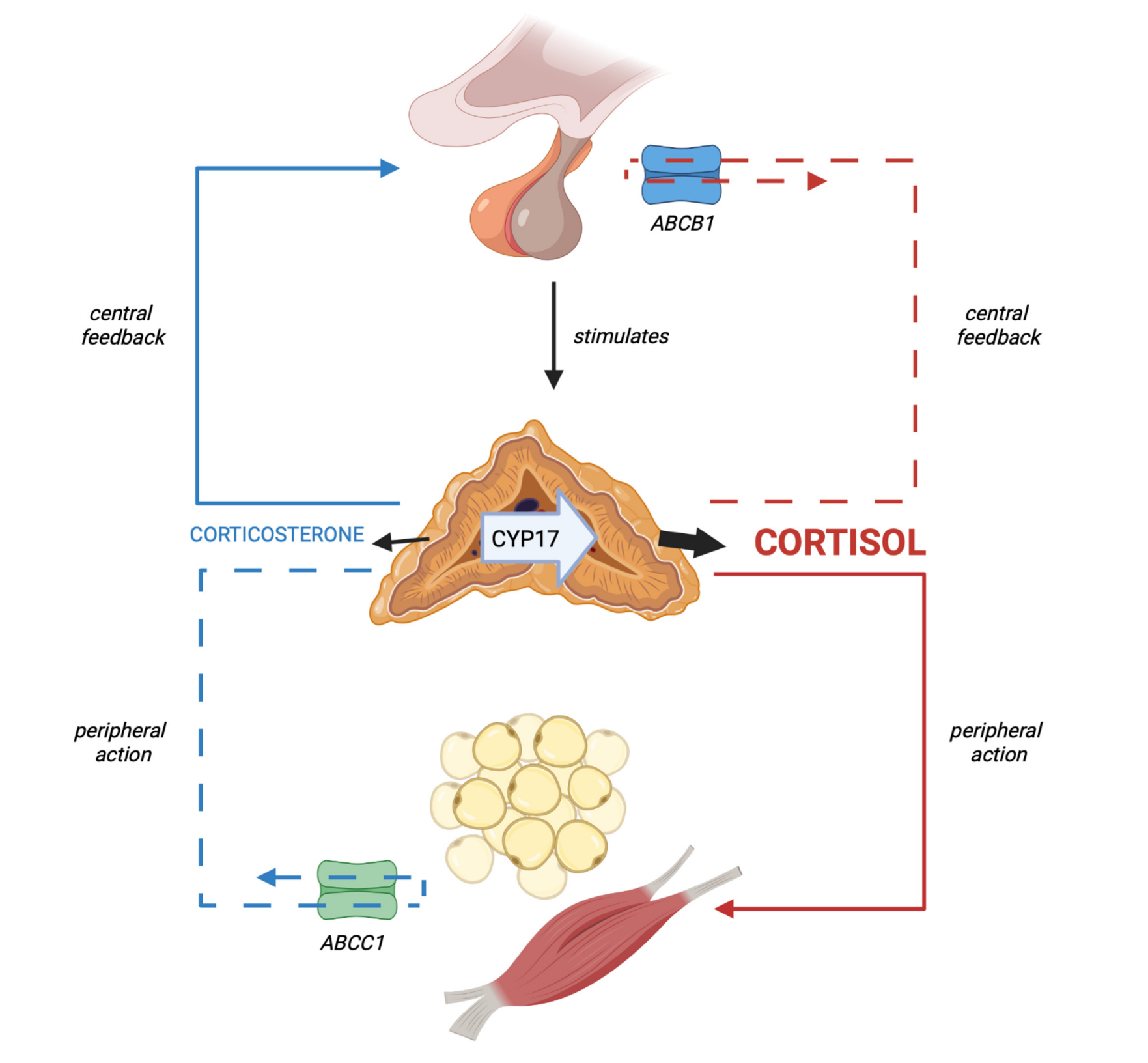
- Humans have two circulating glucocorticoid hormones - cortisol and corticosterone – which diffuse into cells to become transcription factors when bound to their intracellular receptors.
- The availability of glucocorticoids to interact with their receptors depends not only on their plasma concentration but also on their intracellular concentration which is modulated by intracellular enzymes and by transmembrane transporters.
- Glucocorticoids are susceptible to cellular export by membrane transporters from the ABC (ATP-binding cassette) transporter family: cortisol is a substrate for the ABCB1 transporter, and corticosterone for ABCC1.
- Tissues expressing ABCB1 (such as the brain) may be relatively sensitive to corticosterone over cortisol; those expressing ABCC1, such as adipose, may be more sensitive to cortisol.
- In future, therapeutic glucocorticoids may be selected on the basis of lower susceptibility to transport from sites of efficacy and higher transport from sites where harmful side effects occur.


A ATP Switch Model
Extracellular
1
2
3
TMD 1
TMD 2
NBD 1
NBD 2
ATP
ATP
ATP
ATP
ADP
Pi
ADP
Pi
Substrate
Intracellular
B ABCB1
Drug-binding pocket
Extracellular
Membrane
Intracellular
N-terminal
C-terminal
NBD1
NBD2
C ABCC1
Drug-binding pocket
Extracellular
Membrane
Intracellular
N-terminal
C-terminal
NBD1
NBD2


Nx Value
0
10
20
30
40
50
60
70
Relative ABCB1 expression
Relative ABCC1 expression
adrenal gland
colon
kidney
duodenum
natural killer cells
rectum
amygdala
hippocampal formation
thalamus
olfactory region
B-cells
cerebellum
endometrium
placenta
pancreas
ovary
heart muscle
appendix
adipose tissue
breast
lung
stomach
retina
tongue
esophagus
urinary bladder
tonsil
smooth muscle
parathyroid gland
thymus
ABCB1
ABCC1


Hydrocortisone (cortisol)
Dexamethasone
Prednisolone
Triamcinolone
Desonide
Triamcinolone acetonide
Corticosterone
ABCB1
ABCC1
Apical membrane
Basolateral membrane
ABCB1 only cell (e.g. blood-brain barrier endothelium)
Both ABCB1 and ABCC1 (e.g. placenta)
ABCC1 only cell (e.g. adipose)


Corticosterone
Cortisol
Cortisone
11-Dehydro corticosterone
1
ABCC1
ABCB1
2
11β-HSD1/2
CORTISOL
Cortisone
CORTICOSTERONE
11-Dehydro corticosterone
GR
MR
3
Metabolism by 5α reductases, carbonyl reductase 1
4
Steroid pool
5
Response


ABCB1
central feedback
stimulates
central feedback
CORTICOSTERONE
CYP17
CORTISOL
peripheral action
peripheral action
ABCC1