Bile acids and neurological disease

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A B S T R A C T
This review will focus on how bile acids are being used in clinical trials to treat neurological diseases due to their central involvement with the gut-liver-brain axis and their physiological and pathophysiological roles in both normal brain function and multiple neurological diseases. The synthesis of primary and secondary bile acid species and how the regulation of the bile acid pool may differ between the gut and brain is discussed. The expression of several bile acid receptors in brain and their currently known functions along with the tools available to manipulate them pharmacologically are examined, together with discussion of the interaction of bile acids with the gut microbiome and their lesser-known effects upon brain glucose and lipid metabolism. How dysregulation of the gut microbiome, aging and sex differences may lead to disruption of bile acid signalling and possible causal roles in a number of neurological disorders are also considered. Finally, we discuss how pharmacological treatments targeting bile acid receptors are currently being tested in an array of clinical trials for several different neurodegenerative diseases.

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Abbreviations: α-MCA, α-muricholic acid; ABCA1, ATP-binding cassette transporter 1; β-MCA, β-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; CCK, cholecystokinin; DCA, deoxycholic acid; FXR, Farnesoid X receptor; GCA, glycocholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GPBA, G-protein-linked bile acid receptor 1; GUDCA, glycoursodeoxycholic acid; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; LXR, liver X receptor; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PXR, pregnane X receptor; S1P2, sphingosine-1-phosphate receptor 2; TCA, taurocholic acid; TCDA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, tauro lithocholic acid; UDCA, ursodeoxycholic acid; VDR, vitamin D receptor.

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1. Introduction

The concept of bi-directional communication between the splanchnic organs and brain dates back to the Hippocratic era. It is only over the last century however, that the neuro-endocrine mechanisms that underpin this connection have been described and more latterly still, their relevance to the pathophysiology of neurodegeneration been appreciated. This review focuses specifically on the role of bile acid species, an important and heterogenous family of biological mediators central to the gut-liver-brain axis and their physiological and pathophysiological roles in neuronal health.

The central role of bile acids is exemplified by the range of disorders, such as non-alcoholic fatty liver disease, obesity, inflammatory bowel disease and diabetes, that arise when their homeostasis is disturbed (Perino, Demagny, Velazquez-Villegas, & Schoonjans, 2021). Originally, bile acids were considered to act only as surfactants to aid absorption of fats, lipid-soluble vitamins and steroids from the intestine into blood for subsequent metabolism by the liver. However, it is now clear that bile acids have a complex biology that influences a wide range of physiological processes and that they are hormone-like signalling molecules, in addition to their emulsifying agent actions (Zangerolamo, Vettorazzi, Rosa, Carneiro, & Barbosa, 2021). However, bile acids can be toxic and their levels require tight control to prevent damage to the liver and other tissues. Remarkably, along with their synthesis in the gut, bile acids can also be synthesized in brain where they regulate cellular lipid and glucose metabolism. Metabolic dysfunction is a significant risk factor for Parkinson’s disease, Alzheimer’s disease and other neurodegenerative diseases and understanding the role of bile acids in their pathophysiology is crucial to developing interventions for their prevention and treatment (Monteiro-Cardoso, Corliano, & Singaraja, 2021).

Here we review the role played by bile acids (Table 1) in normal brain function and in neurological disorders. The interaction of bile acids with the gut microbiome and their effects upon brain glucose and lipid metabolism will also be examined. Cholesterol catabolism, the source of bile acids and the bile acid pool will be briefly discussed. The brain is the most cholesterol rich organ containing approximately a quarter of the total cholesterol found in the body. Cholesterol and related lipid molecules are a crucial constituent of the plasma membrane of neurons and glia, and a major component of myelin (Bjorkhem & Meaney, 2004) (Dietschy, 2009) (Montesinos, Guardia-Laguarda, & Area-Gomez, 2020).

2. Bile acid synthesis

2.1. Primary bile acids

The first step of bile acid synthesis is the generation of primary bile acids, the majority of which occurs in the liver where a sequence of 17 enzymes, including cytochrome p450, modify the steroid ring of cholesterol to remove its short aliphatic side chain and conjugate with glycine (75%) and taurine (25%) ultimately to form conjugated primary bile salts of cholic acid (CA) and chenodeoxycholic acid (CDCA) (Russell, 2009). A detailed description of the synthesis of bile acids can be found elsewhere (Chiang & Ferrell, 2018, 2019a, 2020; Chiang & Ferrell, 2019b). Biosynthesis of bile acids can take place through two major bile acid synthesis pathways termed classic (neutral) and alternate (acidic) (Fig. 1). In the classic pathway the steroid rings of cholesterol are hydroxylated and reduced in the cytosol and endoplasmic reticulum before oxidation of the sidechains in mitochondria and oxidative cleavage in peroxisomes of the reduced sidechains. Whereas, in the alternate pathway the steroid sidechain is first oxidized and then the steroid rings are modified before the steroid sidechain is cleaved by oxidation (Chiang & Ferrell, 2018). In humans the alternative pathway contributes <10% to overall bile acid composition within the gut whereas the alternative pathway may account for as much as 50% of bile acid composition in rodent gut.

2.2. Secondary bile acids

Secondary bile acids are formed by enzymatic modifications of primary bile acids by bacteria present in the colon where they serve as substrates for microbial metabolism (de Aguilar Vallim, Tarling, & Edwards, 2013). The gut microbiome influences the components of the bile acid pool and the bile acids can regulate the gut bacteria composition through direct and indirect antimicrobial effects. Microbial deconjugation of taurine and glycine conjugated primary bile acids via bile salt hydrolase is a highly conserved function across bacterial phylae and archaea and helps diminish the microcidal effect of intraluminal bile acids. Bacteria with bile salt export pumps have a further selective advantage in this environment. The conversion back into unconjugated hydrophobic forms is essential for downstream biotransformation of CA and CDCA to secondary bile acid species deoxycholic acid (DCA) and lithocholic acid (LCA) respectively by 7α-dehydroxylation (Jia, Xie, & Jia, 2018) and is typically a feature of fimbiricute such as clostridiial species (Fig. 1).

3. The bile acid pool

The total amount of bile acids distributed throughout the enterohepatic circulation is termed the bile acid pool and is comprised of bile acids found in the intestines (85–90%), gallbladder (10–15%) and liver (<1%). The majority (~95%) of bile acids are reabsorbed from the ileum and recycled via the gall bladder with those lost in faeces or converted to secondary bile acids in the colon replenished by newly synthesized bile acids (Chiang & Ferrell, 2018). Though renal excretion of hydrophilic bile acid also occurs (Barnes, Gollan, & Billing, 1977). The bile acid pool consists of approximately 40% CA, 40% CDCA and 20% DCA in humans and is overall hydrophobic with a ratio of glycine to taurine conjugated bile acids of approximately 3:1. In mice the bile acid pool is predominantly hydrophilic due to the majority (~95%) of bile acids being conjugated to taurine and the presence of hydrophilic muricholic acids such that the bile acid pool composition is

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Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Bile acids.</th>
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<tbody>
<tr>
<td><strong>Abbreviation</strong></td>
<td><strong>Name</strong></td>
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<tr>
<td></td>
<td>CDCA</td>
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<tr>
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<tr>
<td></td>
<td>LCA</td>
</tr>
<tr>
<td></td>
<td>UDCA</td>
</tr>
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<td>TCA</td>
</tr>
<tr>
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<td>TDCDA</td>
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<td>TDCA</td>
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<td></td>
<td>GLDCA</td>
</tr>
</tbody>
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References:

approximately 60% taurocholic acid (TCA) and 40% taurine-α-muricholic acid (Tα-MCA) and taurine-β-muricholic acid (Tβ-MCA) (Chiang & Ferrell, 2018). Furthermore, in mice the majority of CDCA is immediately converted to α-muricholic acid (α-MCA) and β-muricholic acid (β-MCA) by the enzyme sterol-6β-hydroxylase (CYP2C70) (Y.Yang & Zhang, 2020). The differences in the composition of the bile acid pool and the enzymes that form and regulate them between humans and rodents may be of importance when using data from experimental treatments of rodent with bile acids as a basis for clinical trials to treat human disease, or to understand human bile acid related pathophysiology (de Aguiar Vallim et al., 2013),(Y. Yang & Zhang, 2020).

4. Brain bile acids

The brain contains numerous conjugated and unconjugated bile acids that enter the brain by either diffusion or active transport through bile acid transporters from the systemic circulation, or by local synthesis in astrocytes and neurons since the brain contains the cytochrome P450 enzymes necessary (CYP8B1, CYP27A1 and CYP7B1) for synthesis of primary bile acids by the alternate pathway (Loera-Valencia et al., 2021) (Nishimura, Yaguti, Yoshitsugu, Naito, & Satoh, 2003). However, the levels of bile acids in brain mirror those found in the circulation suggesting that local synthesis is less important for regulating the level of brain bile acids than entry from systemic circulation (Higashi et al., 2017),(Mano et al., 2004),(Reddy et al., 2018) under normal conditions. Whether this changes in neurological disease is unknown. Secondary bile acids in brain can only be derived from the intestine since enzymes found just in gut bacteria are required for their formation (de Aguiar Vallim et al., 2013).

The mechanisms that underlie bile acid clearance in the brain, in contrast to oxysterol intermediates, are poorly understood. With regards to passive diffusion, the propensity of bile acids to diffuse across the blood brain barrier is influenced by their differential hydrophobicity indices. Furthermore, the hydrophobic unconjugated bile acids have been shown to compromise the integrity of the blood brain barrier. In vivo models of high circulating bile acids including surgical cholestasis and venous administration of unconjugated bile acids have demonstrated increases in blood brain barrier permeability (Quinn et al., 2014).

Virtually all brain cholesterol is formed locally by de novo synthesis with circulating lipoprotein cholesterol effectively excluded from the brain by the blood brain barrier. However, in adult brain synthesis of cholesterol is low because of efficient recycling and the half-life of brain cholesterol is estimated to be at least 5 years (Bjorkhem & Meaney, 2004). The ratio of primary to secondary bile acids is determined by the gut microbiome which is exclusively responsible for the 7α-dehydroxylation of unconjugated primary to secondary bile acids. Since CYP7A1 is not found in brain, the 7α-hydroxycholesterol formed in brain and can only be derived from CA in the circulation or through non-enzymatic oxidation of cholesterol and as the blood-brain barrier is more permeable in patients with neurodegenerative disorders this could result in altered brain levels of bile acids (Grifths et al., 2021).

Conjugated bile acid species which are more hydrophilic are likely to require active transport across the blood brain barrier although the underlying mechanisms are incompletely understood. Organic anion-transporting polypeptide 1A4 (OATP1a4) deficient mice have been shown to have reduced bi-directional transport of TCA across the blood brain barrier suggestive of a role in active transport (Ose et al., 2010). Elimination of [3H]-TCA after cortical microinjection was found to be markedly reduced in the presence of the unconjugated CA species.
Different secondary bile acids throughout the brain (Baloni, et al., 2020). In the liver newly synthesized primary bile acids, CA and CDCA are conjugated with glycine or taurine by an enzyme bile acid-CoA: amino acid N-acetyltransferase (BAAT) before being secreted into the bile. The presence of this enzyme in the brain has so far not been detected, which would make it very unlikely that the locally generated CDCA would be conjugated within the brain tissue. Baloni (2020) and colleagues found measurable levels of multiple different secondary bile acids throughout the brain (Baloni, et al., 2020). Given that secondary bile acids result from bacterial actions in the colon it seems most probable they have entered the brain after being generated in the gut. Although extremely controversial, some groups have proposed that the brain may have its own microbiome mostly consisting of c-proteobacteria (Branton et al., 2013) or of novel brain microbes (Link, 2021). This certainly raises a very interesting notion that a local bacterium could modify bile acids in brain, but these studies have to be approached with caution as they are largely from immunocompromised cases and it is hard to ensure the bacteria were not due to contamination from blood vessels or experimental procedures.

The reported increases in the levels of the secondary bile acids within the brain during neurodegeneration most likely are a result of an altered gut microbiome producing higher levels which subsequently enter the brain. Circulating bile acids can directly induce permeability in the blood brain barrier through a mechanism involving the Rac1-dependent phosphorylation of the tight junction-associated protein Occludin1 (Quinn et al., 2014). Their rate of entry may also be impacted by changes in the expression of several bile acid transporters or direct damage to be blood brain barrier by the bile acids themselves. In liver disease models where serum bile acid levels are increased there is also a detectable increase in brain bile acid levels that have most likely entered by the apical sodium-dependent bile acid transporter (ASBT) transporter whose expression increased in the frontal cortex (McMillin et al., 2016). Several other transporters are expressed in ependyma of the choroid plexus such as ABST, multidrug resistance-associated proteins 2/4 (MRP2/4), bile salt export pump (BSEP), OATP1A2 and sodium-taurocholate co-transporting polypeptide (NTCP). Very little is currently known about the normal physiological function of these transporters or in disease or if bile acid penetration into the brain is a passive or active process. Better understanding of the apical or basolateral expression of these transporters could allow the development of drugs to block or target these transporters and regulate the bilateral flow of bile acids into and out of the brain.

Bile acid synthesis in the liver is chiefly regulated by splanchnic cholesterol status. Biosynthesis of CDCA via the alternative pathway is however influenced by 24-hydroxycholesterol status, an indirect measure of cholesterol status in the brain.

Homeostasis of bile acid levels is crucial to maintain the balance between adequate lipid absorption from the intestine and prevention of accumulation to toxic levels and is governed by Farnesoid X receptor (FXR) and G-protein-linked bile acid receptor 1 (GPBA) receptors in the liver and intestine via a negative feedback mechanism (Makishima et al., 1999), (Maruyama et al., 2002), (Stanimirov, Stankov, & Mikov, 2015). Liver X receptors (LXR) control brain cholesterol homeostasis by regulating transcription of the lipidating transporters ABCA1 and ATP Binding Cassette Subfamily G Member 1 (ABCG1) and hence transport of apolipoprotein E between astrocytes and neurons or oligodendrocytes (Courtney & Landreth, 2016).

5. Bile acid receptor expression

While bile acid receptor expression in the periphery is well documented, there are few studies examining their expression in brain.

5.1. GPBA (G-protein-linked bile acid receptor 1)

The most studied brain bile acid receptor is G-protein-linked bile acid receptor 1 (GPBA, also known as TGR5, GPR131, M-BAR, BC37) (Bonner, Hills, Maguire, & Rosser, 2019). GPBA is a membrane bound receptor that has a widespread distribution in human tissue (kidney, liver, small intestine) but was not detected in brain, colon (without the mucosa) by northern blot analysis (Maruyama et al., 2002). When small intestine and colon were examined with mucosa intact, GPBA was detected in stomach, duodenum, ileoeccum, ileum, jejunum, and ascending colon, transverse colon, descending colon, cecum, and liver, but not in the oesophagus and rectum (Maruyama et al., 2002). Analysis of different intestinal cell lines for GPBA expression revealed expression in cells of enteroendocrine origin, but not epithelial cells (Maruyama et al., 2002). Whereas, in contrast to this finding, Ward and colleagues (Ward, Mroz, & Keely, 2013) detected GPBA mRNA and protein in rat colonic crypt (enterocyte) preparations.

Kawamata and colleagues (Kawamata et al., 2003) used qPCR to examine the tissue distribution of GPBA mRNA in human and rabbit tissue. High levels of GPBA mRNA were detected in lung, spleen and monocytes/macrophages, with moderate amounts found in liver, stomach, small intestine. Low levels were seen in spinal cord, pituitary and thyroid glands, whereas whole brain and some brain regions (hippocampus, cerebellum, hypothalamus) contained only very low levels of GPBA mRNA (Kawamata et al., 2003). Another study also using qPCR confirmed the low expression of GPBA mRNA in mouse brain (Vassileva et al., 2006).

More recently, Keitel and colleagues (Keitel et al., 2010) used immunofluorescence to show that GPBA was expressed by neurons and astrocytes throughout rat brain but with no enrichment in any brain region and they also reported that it was expressed by human neural tissue grown in vitro. However, the expression of GPBA throughout human brain has not been studied in detail. Endogenous neurosteroids (e.g., pregnanolone) can activate the GPBA receptor leading to an increase in intracellular cAMP and calcium levels, which could lead to oxidative stress in neurons through activation of voltage-gated calcium channels (Thomas et al., 2009). Another study similarly demonstrated the presence of GPBA in neurons and microglia in mouse cerebral cortex and found that stimulation of GPBA with betulinic acid suppressed microglia activation in an in vitro mouse model of hepatic encephalopathy (McMillin et al., 2015). Together these studies indicate that GPBA could be a target to control neuroinflammation in brain. This is supported by a recent study that detected increased GPBA immunoreactivity and mRNA in astrocytes and blood vessels in white matter lesions of patients with multiple sclerosis compared to tissue from control brains (Bhargava et al., 2020). Using a mouse reporter model with GPBA and green fluorescent protein under control of the Gpbar1 regulatory gene locus, Perino and colleagues (Perino et al., 2021) demonstrated high levels of GPBA in hypothalamic neurons and glial cells.

5.2. Farnesoid X receptor

Farnesoid X receptor (FXR, also known as NR1H4 (McDonnell, 2019), mRNA was undetectable in mouse (Huang et al., 2015), (Y. Zhang, Kast-Woelbern, & Edwards, 2003) and rat (Akanuma, Hori, Ohtsuki, Fujiyoshi, & Terasaki, 2008) brain. Yet FXR knockout mice show impaired memory and reduced motor coordination and altered glutamatergic, GABAergic, serotonergic, and norepinephrinergic neurotransmission in either hippocampus or cerebellum suggesting that FXR contributes to homeostasis of multiple neurotransmitter systems in brain, most likely by increasing circulating systemic bile acid levels leading to raised relative concentrations of bile acids in brain (Huang et al., 2015). In addition, bile acids have been previously shown to modulate muscarinic acetylcholine and sphingosine-1-phosphate receptor 2 (S1P2) receptors and GABA_A and NMDA ion channels (Raifman et al., 2002), (Schubring, Fleischer, Lin, Haas, & Sergeeva, 2012), (Seyedsadr et al., 2019) which could explain...
the link between bile acids and neurological function as discussed later in the review. In contrast another more recent study detected FXR mRNA and protein in rat hippocampus and medial prefrontal cortex by qPCR and western blot (W. G. Chen, Zheng, Xu, Hu, & Ma, 2018). McMillin and colleagues (McMillin et al., 2016) detected FXR mRNA in mouse frontal cortex by qPCR and also found that FXR protein immunoreactivity co-localized with a neuronal marker (neuN) suggesting FXR expression in brain was neuronal. Furthermore, FXR immunoreactivity has been detected in white matter lesions of patients with multiple sclerosis in nuclei of CD68+ macrophages (Bhargava et al., 2020), (Hucke et al., 2016). What proportion of these cells were infiltrating macrophages as opposed to resident microglia is unknown.

5.3. Pregnane X receptor

Initial studies showed that the pregnane X receptor (PXR, also known as NR1I2) (Christakos, 2019) has high expression in human liver, colon and small intestines but was not detectable in brain or other tissue by northern blot (Bertilsson et al., 1998), (Blumberg et al., 1998), (Jones et al., 2000), (Kliwer et al., 1998). Similar results were obtained from studies of rodent tissue, except low levels of PXR mRNA were also found in mouse brain (Lehmann et al., 1998) and rat (H. Zhang et al., 1999) kidney and stomach. However, another study did not detect PXR mRNA in rat or rabbit kidney and stomach (Jones et al., 2000). Using qPCR Nishimura and colleagues (Nishimura, Naito, & Yoku, 2004) confirmed the northern analysis tissue expression data and showed that PXR mRNA was present in human brain, albeit at levels 10,000-fold lower than those occurring in liver. In contrast other studies have failed to detect PXR in brain by qPCR (Miki, Suzuki, Tazawa, Blumberg, & Sasano, 2005). Marini and colleagues (Marini et al., 2007) examined the regional distribution of PXR in rabbit brain by semi-quantitative reverse transcription PCR and found high levels in cortex, a faint signal in midbrain and cerebellum and no signal in striatum, hypothalamus or hippocampus. PXR mRNA and protein have been detected in porcine brain endothelial cells and primary hippocampal neuron cell cultures (Lemmen, Tozakidis, & Galla, 2013), (Litwa et al., 2016).

5.4. Liver X receptor

Liver X receptor α and β (LXRα, also known as NR1H3; LXRβ, also known as NR1H2) (McDonnell, 2019) are both expressed in liver and gut tissue, but each has a distinct pattern of expression in other tissues with LXRβ having ubiquitous expression including being detectable in mouse brain by northern blot whereas LXRα was not found in mouse brain (Lu, Repa, & Mangelsdorf, 2001), (Song, Kokontis, Hiipakka, & Liao, 1994), (Willy et al., 1995). However, LXRα is expressed by macrophages and therefore could be in brain since microglia are macrophages (Ricote, Valledor, & Glass, 2004). This is supported by a study that showed that the LXR agonist T0901317 reduced microglial activation in an animal model of Parkinson’s disease (Paterniti et al., 2017). In mice during early development (embryonic day 13–16) LXR mRNA was observed widely distributed throughout the brain, but the distribution became more limited to specific neuronal populations (reduced in the brainstem) during later ontogeny (embryonic day 17–21) before reverting to a ubiquitous distribution in adult brain that was predominantly neuronal, although astrocytes were also found to express LXR mRNA (Kainu, Kononen, Enmark, Gustafsson, & Pelto-Huikko, 1996). A widespread expression of functional LXR in brain is confirmed by a study in LXR knockout mice that found altered brain cholesterol homeostasis and neurodegeneration in the substantia nigra and globus pallidus of knockout mice compared to wildtype animals. (Wang et al., 2002).

5.5. Sphingosine-1-phosphate receptor 2

Sphingosine-1-phosphate receptor 2 (S1P2) is expressed in hippocampus, cerebellum and motor cortex of adult brain (Kempf et al., 2014). Conjugated bile acids have been shown to activate the receptor to regulate barrier function of endothelial cells in hepatobiliary and intestinal systems (Studer et al., 2012). Similarly, in brain S1P2 activation can cause disruption of the blood brain barrier leading to neutrophil infiltration and promote activation of microglia resulting in neuroinflammation (Xiang et al., 2021). Disruption of the blood brain barrier could potentially allow more bile acids to enter the brain. As such, S1P2 antagonists might be of use in the treatment of neurodegenerative diseases where the integrity of the blood brain barrier is compromised. S1P2 activation also affects synaptic plasticity in brain through interaction with NOGOA to decrease long-term potentiation (Kempf et al., 2014).

5.6. Vitamin D receptor

Nuclear expression of vitamin D receptor (VDR) in human brain has been demonstrated by proteomics, western blot and immunohistochemistry (Eyles, Liu, Josl, & Cui, 2014; Eyles, Smith, Kinobe, Hewison, & McGrath, 2005). VDR was expressed by neurons and astrocytes throughout the brain with highest levels in the hypothalamus and large neurons (presumed to be dopaminergic due to their morphology) within the substantia nigra (Eyles et al., 2005).

It is evident that there is a lack of detailed knowledge about regional and cellular expression of bile acid receptors in the normal human central nervous system (Table 2) and even less is known about their status under pathological conditions during disease. The application of current mRNA detection techniques and immunohistochemistry utilizing the specific antibodies to bile acid receptors that are now available should help resolve this problem and may point towards novel drug targets for the treatment of neurological disorders.

6. Bile acid receptor pharmacology

A detailed account of the pharmacology of bile acids and their receptors and their use in the treatment of liver and systemic diseases is outside the scope of this review but can be found elsewhere (e.g., (De Marino, Festa, Sepe, & Zampella, 2019), (Fiorucci & Distrutti, 2019), (Fiorucci, Distrutti, Carino, Zampella, & Biagioli, 2021). A summary of key bile acid receptor pharmacologically active ligands relevant to brain function is presented in Table 3. Compounds that have been tested in animal models of neurological disorders or that are being used in clinical trials or are discussed in the next section. Details of the numerous other ligands that act on bile acid receptors can be found in the IUPHAR/BPS Guide to Pharmacology database (Home | IUPHAR/BPS Guide to PHARMACOLOGY). Though some formulations of endogenous bile acids and synthetic bile acid receptor ligands are licensed for treatment of systemic metabolic disease (Table 1) there are currently no drugs acting on bile acid receptors that are specifically licensed for treatment of neurological disorders.

7. Neurological disorders

Despite the characteristics making them distinct disorders, many neurological diseases share common pathological features such as deposits of misfolded proteins and neuroinflammation in addition to neuronal loss and disturbed cellular function such as mitochondrial dysfunction and oxidative stress. Recently, bile acids and their receptors have been associated with pathological processes in many neurological diseases and bile acids have shown promise as putative treatments for a number of these disorders. Aging, which is the greatest risk for neurodegenerative disorders, can also cause several changes in the gut that can impact on the gut microbiome-brain axis including reduced stability and diversity of microbial communities, increased levels of inflammation and thinning of the mucosal lining and reduced bioavailability of microbial metabolites with immunoregulatory actions such as secondary bile acids and short-chain fatty acids (Conway & N, 2021).

Table 2
Brain bile acid receptor expression.

<table>
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<tr>
<th>Nomenclature</th>
<th>gene</th>
<th>neurons</th>
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<th>microglia</th>
<th>endothelial</th>
<th>oligodendrocytes</th>
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<td>GPBAR1</td>
<td>4+5 9.6</td>
<td>4.3+ 9.6</td>
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Regional brain bile acid receptor expression

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<tr>
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<tr>
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<tr>
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<td>nd</td>
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</tr>
</tbody>
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+ = present, 0 = no signal, 1 = very low, 2 = low, 3 = moderate, 4 = high, nd = not determined.
Bold indicates detected in human tissue.

7.1. Parkinson’s disease

Parkinson’s disease is a neurodegenerative disorder characterised by progressive loss of catecholamine and cholinergic neurons in the brainstem and midbrain together with a caudal to rostral spread of aggregated α-synuclein deposits that form Lewy bodies and neurites (Braak et al., 2003). The consequent reduction in striatal dopamine levels results in the classic motor symptoms (bradykinesia, rigidity, tremor, posture and gait impairment), while changes in the levels of other neurotransmitters in brain, together with pathological changes outside of the CNS, may explain the non-motor symptoms of the disorder (Hornykiewicz, 1966).

Table 3
Bile acid receptor pharmacology.

<table>
<thead>
<tr>
<th>Endogenous ligands</th>
<th>GPBA</th>
<th>FXR</th>
<th>PXR</th>
<th>LXR</th>
<th>S1P2</th>
<th>VDR</th>
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<td>4.0</td>
<td>5.0</td>
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</tr>
<tr>
<td>CDCA</td>
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<td>5.3</td>
<td>5.3</td>
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<tr>
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<tr>
<td>LCA</td>
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<td>5.3</td>
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<tr>
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<tr>
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<td></td>
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<td>5.3</td>
<td></td>
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<tr>
<td>24(25)-cholesterol</td>
<td></td>
<td>5.4</td>
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<tr>
<td>27-cholesterol</td>
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<td>7.1</td>
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<tr>
<td>Sphingosine-1-phosphate</td>
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<td>8.1</td>
<td>8.5</td>
<td></td>
<td></td>
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<tr>
<td>Guggulsterone</td>
<td>5.7-6.0</td>
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<tr>
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A number of preclinical studies in rodents have provided evidence that bile acids might be involved in the pathogenesis of Parkinson’s disease. A metabolome analysis of brain from mice receiving intracerebral injections of preformed α-synuclein fibrils into the olfactory bulb showed alterations in pathways for tyrosine metabolism which could affect tyrosine conjugation of tyroursoxycholic acid (TUDCA) and ursoxycholic acid (UDCA) (Graham et al., 2018; Graham et al., 2018). The primary bile acids (CA, total MCA and β-MCA) and secondary bile acids (TUDCA, taurohyodeoxycholic acid (THCA) and DCA) were increased in faecal samples analysed by ultraperformance liquid chromatography mass spectrometry and the 16S rRNA faecal microbiome was altered in rats receiving a bilateral injection of α-synuclein into the substantia nigra (O’Donovan et al., 2020). Treatment of rotenone treated rats with UDCA restored striatal dopamine levels and normalised markers of inflammation and apoptosis (Abdelkader, Safar, & Salem, 2016) and TUDCA treatment reduced 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced motor deficits and pro-inflammatory effects in mice (Rosa et al., 2018). Two clinical trials are currently underway (NCT02967250 and NCT03840005) to assess the efficacy of UDCA as a treatment for patients with Parkinson’s disease (see below).

A recent study found elevated levels of alternative pathway cholesterol metabolites (oxysterols) in CSF from Parkinson’s disease patients compared to controls that correlated with symptoms of depression in Parkinson’s disease but not other clinical measures of the disorder (Griffiths et al., 2021). Oxysterols can contribute to inflammation and depression in Parkinson’s disease and has been linked to markers of systemic inflammation (Duc, Vigne, & Pot, 2019) (Lindqvist et al., 2013). Interestingly, pro-inflammatory oxidative cholesterol metabolites have been shown to accelerate α-synuclein aggregation in vitro suggesting that abnormal cholesterol metabolism in combination with inflammation and oxidative stress may contribute to the pathogenesis of α-synucleinopathies (Bosco et al., 2006). However, the role of cholesterol levels in the pathogenesis of Parkinson’s disease is far from clear. Most published studies found that high cholesterol was associated with a reduced prevalence of Parkinson’s disease and that treatment with statins to reduce cholesterol increases the risk of Parkinson’s disease (Liu et al., 2017). This counters the prevailing view that statins...
are neuroprotective agents and recent studies in patients with Parkinson’s disease have produced conflicting results. Jeong and colleagues found that patients with Parkinson’s disease who were being treated with statins have lower striatal dopamine transporter levels (indicating more nigrostriatal degeneration) and an increased likelihood of dementia (Jeong et al., 2021). Whereas Palermo and co-workers found statin treatment slowed motor deterioration in patients with early Parkinson’s disease (Palermo et al., 2021). The reason for the differing outcomes remains unknown but could relate to different hydrophobicity of the statins, with hydrophilic but not lipophilic statins being associated with faster Parkinson’s disease progression (Lewis et al., 2022). A recent trial (NCT02787590) of simvastatin to slow progression in Parkinson’s disease showed no benefit (https://eureparkinsons.org.uk/2020/09/simvastatin-results/). In summary, the relationship of peripheral cholesterol levels and the use of statins to Parkinson’s disease is uncertain.

The greatest risk factor for Parkinson’s disease is increasing age. Liver function deteriorates with age and hence bile acid homeostasis changes, with reduced bile acid synthesis and increased peripheral cholesterol accumulation (Bertolotti et al., 2007). However, brain cholesterol decreases with age in rats which indicates that cholesterol metabolism is different in brain compared to peripheral organs (Smiljanic et al., 2013). The influence and ramifications of aging on cholesterol metabolism and bile acid levels are outside the scope of this review, but excellent discussion of this topic can be found elsewhere (V. S. Nunes, da Silva Ferreira, & Quintao, 2022) (Perino, Demagny, et al., 2021).

Another way that bile acids may impact on Parkinson’s disease is through the expression of gut hormones by stimulation of receptors in specific brain regions, such as cholecystokinin (CCK) a gastrin-like peptide released in the gastrointestinal tract and brain and in particular a C-terminal sulphated octapeptide fragment of CCK called CCK-8. The maintenance of adequate levels of dopamine neurotransmission in the brain depends on signalling of CCK through its G-protein coupled receptors CCKR-1 and CCKR-2 that enhance cAMP levels and activate the PLC–PKC cell signalling pathway to modulate neurotransmitter expression. CCK receptors are expressed at high densities in the main dopamine neuron clusters in the brain: the ventral tegmental area which is the origin of the mesocorticocollucbic dopamine pathways and the substantia nigra pars compacta which communicates with the striatum via the nigrostriatal dopamine neuron pathway (Fallon, 1988). A recent study found that a CCK analogue demonstrated neuroprotective effects in the MPTP mouse model of Parkinson’s disease by decreasing glial activation and inflammation and reducing MPTP-induced autophagy dysfunction, mitochondrial damage and ER stress (Z. Zhang et al., 2022). In addition, through activation of the cAMP/PKA/CREB pathway the CCK analogue promoted the survival of dopaminergic neurons by inhibiting apoptosis. One primary target of circulating CCK are CCK type A receptors on vagal afferents and nodose ganglia neurons. GPBA receptors colocalise with CCK type A receptors where DCA and CCK-8 increase neuronal firing rates synergistically (Wu et al., 2020). Whilst this synergy has not yet been identified in the brain this suggests that bile acids may have a physiological role in regulating neurotransmitter levels in combination with CCK peptides.

7.2. Alzheimer’s disease

Alzheimer’s disease is a progressive form of dementia characterised pathologically by the presence of amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau, together with neuronal loss of cholinergic neurons in the forebrain (DeTure & Dickson, 2019), (Hampel et al., 2018). Altered ratios of 23 serum bile acids have been associated with cerebrospinal fluid biomarkers of Alzheimer’s disease (Nho, et al., 2019). In addition, levels of plasma LCA (lithocholic acid) increased in Alzheimer’s disease patients versus controls and plasma levels of glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDC) and glycolithocholic acid (GLCA) were significantly elevated in Alzheimer’s disease patients compared to mild cognitive impairment patients. Baloni and colleagues (Baloni, et al., 2020) reported similar increases of bile acids in Alzheimer’s disease post-mortem brain samples along with an increased ratio of GCDCA:CA and increased secondary bile acids DCA, LCA, TDCA and GCDCA in Alzheimer’s disease samples. This was accompanied by higher serum taurine levels. As secondary bile acids synthesis does not occur in brain, this suggests the serum and brain tissue changes may reflect alterations in the gut microbiome of Alzheimer’s disease patients. A longitudinal analysis of MRI images from Alzheimer’s disease patients showed that lower serum concentrations of CA and CDCA were associated with a more rapid accumulation of white matter lesions, more rapid brain atrophy and higher deposition of brain amyloid (Varma et al., 2021). CDCA is derived from oxysterols produced via a non-specific 7 α-hydroxylase (CYP7B1). Several studies have shown decreased levels of CYP7B1 in dentate neurons (Yau et al., 2003), (Shafaati et al., 2011), which may reduce hippocampal CDCA production.

Changes in bile acid synthesis and cholesterol catabolism were greater in males than females (Varma et al., 2021) suggesting that during dementia there may be sex-specific effects on bile acid signalling in the brain. Several possible mechanisms for this have been proposed including T3 thyroid hormone levels (Drover & Agellon, 2004), sex specific expression of retinoid X receptor alpha (RXRα) and liver X receptor (LXR) receptors driven through oestrogen induced changes in transcription (Cai et al., 2003) and a sexual dimorphism of the bile acid pool. One study has demonstrated that faecal microbiome complexity and richness can be strongly associated with oestrogens levels (Flores et al., 2012) where the gut microbiota regulates oestrogens through secretion of β-glucuronidase. Changes in androgen metabolism have been reported in germ free mice compared to mice with a normal gut microbiome where a healthy gut maintains high levels of dihydrotestosterone (Collden et al., 2019). Decreased levels of dihydrotestosterone are reported as a risk factor for Alzheimer’s disease (Rosario & Pike, 2008) and may regulate the accumulation of β-amyloid. This raises the possibility that dysbiosis of gut-microbiome leads to changes in sex-hormones that can alter bile acid regulation in the brain in sex specific ways. Much more research is needed to understand the complex interplay between the gut microbiome, sex hormones levels and the effects of changes of these on bile acid synthesis but does raise the question of whether this will impact the way men and woman respond to drugs that alter bile acid levels.

Intraventricular administration of the GPBA agonist INT-777 (60-ethyl-23(5)-methylcholeic acid) improved cognitive impairment and reduced markers of neuroinflammation and apoptosis in hippocampus and frontal cortex of mice that had received an intraventricular injection of aggregated Aβ1-42 suggesting that activation of GPBA receptors might be a promising strategy for the treatment of Alzheimer’s disease (Wu et al., 2018). In contrast, the FXR agonist INT-747 (60-ethyl-chenoxycholic acid) potentiated Aβ1-42-induced apoptosis in SH-SY5Y cells via activation of the cAMP-response element-binding protein-brain-derived neurotrophic factor pathway (Q, Chen et al., 2019). Another study found that ALIC44, induced cognitive and spatial deficits in rats were reduced by treatment with the potent FXR agonist CDCA (Bazzari, Abdallah, & El-Abhar, 2019). Furthermore, feeding mice with 0.4% (wt/wt) TUDCA reduced amyloid oligomer accumulation and improved cognition in the APP/PS1 mouse model of Alzheimer’s disease (A. F. Nunes et al., 2012), (Zangerolamo et al., 2021). Also, treatment with UDCA exerted a neuroprotective effect on mitochondrial membrane potential and morphology in primary fibroblasts through Drp1 actions on fission and fusion (Bell et al., 2018). Together these studies suggest dysregulated bile acid homeostasis may have a role in the pathogenesis of Alzheimer’s disease and that treatment with bile acid or synthetic molecules acting on bile acid receptors may be useful as Alzheimer’s disease therapies.

7.3. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (also known as motor neuron disease) is characterised by degeneration of upper motor neurons that project
from the motor cortex to the brainstem and spinal cord and lower motor neurons that project from the brainstem and spinal cord to muscles that leads to muscle weakness and paralysis (Hardiman et al., 2017). Cholesterol was elevated in cerebrospinal fluid from amyotrophic lateral sclerosis patients and (25R)-26-hydroxycholesterol (also known as 27-hydroxycholesterol) from the acidic branch of bile acid biosynthesis was reduced leading to failure in the brain to clear excess cholesterol (Abdel-Khalik et al., 2017). In the main mouse model of amyotrophic lateral sclerosis in which SOD1<sup>G93A</sup> mice exhibit a phenotype similar to amyotrophic lateral sclerosis patients, significant elevations of CDCA, UDCA, β-MCA, TCA, TCDDA (taurochenodeoxycholic acid), TDCA, TUDCA, glycocholic acid (GCA) and GDCA were identified in the spinal cord of end-stage SOD1<sup>G93A</sup> mice compared to wild type along with increased expression of CYP7B1 (Dodge, Yu, Sardi, & Shihabuddin, 2021). These authors proposed this could indicate an attempt by the body to mitigate toxicity from excess cholesterol by its conversion into bile acids. However, at higher concentrations bile acids function as membrane solubilisers and can induce cell death and provide neuroprotection (Mignarri et al., 2016). Reduced levels of the neural tissue cholesterol clearing enzyme CYP46A1 were found in putamen from patients dying with Huntington's disease, a rodent model of Huntington's disease (R6/2 mice) and a striatal cell line (SThdhQ111) expressing the HTT gene. Adenovirus-mediated restoration of CYP46A1 and hence lanosterol and desmosterol levels, protected striatal neurons from cell death in R6/2 mice and SThdhQ111 cells (Boussicault et al., 2020). While systemically administered TUDCA was shown to improve locomotor and sensorimotor deficits in toxin (3-nitropropionic acid) and genetic (R6/2 mice) models of Huntington's disease (Keene et al., 2001; Keene et al., 2002). These data suggest that bile acids may be useful in slowing progression of Huntington's disease.

Recent evidence has suggested that gut dysbiosis may occur in Huntington's patients and some genera of microbiota have been correlated with specific clinical scores, namely Intestinimonas with total functional capacity scores and Lactobacillus negatively correlated with mini-mental state examination scores (Wasser et al., 2020). Clostridium XVIII was significantly decreased in Huntington's disease patients. Gut dysbiosis has been reported in both R6/1 (Kong et al., 2020) and R6/2 (Stan et al., 2020) Huntington's disease mouse models with differential α-diversity. It is proposed that increased diversity may be associated with heightened gut microglial volatility and perturbed gut microbiome function (Kong et al., 2021). Indeed, the levels of Intestinimonas also correlated with plasma IL-4 levels. No studies have yet examined changes in bile acids in Huntington's disease patients, but given the dysfunctions reported in cholesterol signalling in the brain and gut dysbiosis it seems probable that they occur and changes in the bile acid pool could alter this regulation. A phase I clinical trial with UDCA (NCT00514774) in patients with Huntington's disease was started in 2007, but the results of the trial were never published.

### 7.6. Prion diseases

In contrast to other neurodegenerative diseases, studies investigating whether bile acids could ameliorate toxicity or slow protein aggregation caused by prion protein strains in vitro have had differing outcomes. Cortez and colleagues (Cortez et al., 2015) found that TUDCA and UDCA can interfere with the seeding efficiency of prions and provide neuroprotection in prion-infected brain slice cultures. But Ladner-Keay and colleagues (Ladner-Keay et al., 2018) found that TUDAC treatment preserved rod and cone structure and function in toxin (3-nitropropionic acid) and provide neuroprotection in prion-infected brain slice cultures. While systemically administered TUDCA was shown to improve locomotor and sensorimotor deficits in toxin (3-nitropropionic acid) and genetic (R6/2 mice) models of Huntington's disease (Keene et al., 2001; Keene et al., 2002). These data suggest that bile acids may be useful in slowing progression of Huntington's disease.

### 7.7. Degenerative retinal diseases

The eye is a part of the central nervous system and subject to a number of neurodegenerative diseases (retinitis pigmentosa, diabetic retinopathy, age-related macular degeneration, glaucoma) that can ultimately lead to blindness. Bile acids have been shown to reduce retinal damage in animal models of these diseases which indicates that despite differing causes these diseases appear to share a common mechanism (e.g., oxidative stress) of cell death. TUDCA treatment preserved rod and cone structure and function and synaptic connections in the P23H rat model of retinitis pigmentosa...
(Fernandez-Sanchez, Lax, Pinilla, Martin-Nieto, & Cuenca, 2011), While treatment with TUDCA of two strains of mice with rapid retinal degeneration from the β-Pde6 (rd1) mouse model found the treatment partially preserved function and structure in B6-C3-Pde6b (rd1) Hps4 (le)/J mice but only partially preserved structure in C57BL/6 J-Pde6b (rd1-2)/J mice (Lawson et al., 2016). Tao and colleagues (Tao et al., 2019) found that TUDCA treatment modulated apoptosis and reduced oxidative stress to reduce retinal damage induced by systemic administration of the toxin N-methyl-N-nitrosourea and Zhang and co-workers (X. Zhang, Shahani, Reilly, & Shu, 2019) showed that TUDCA treatment reduced microglia activation and prevented photoreceptor damage in retinitis pigmentosa GTPase regulator knockout mice. Together these data indicate that TUDCA may be of use in patients with neurodegenerative eye diseases. A clue to the mechanism comes from work by Lobysheva and colleagues (Lobysheva, Taylor, Marshall, & Kisselev, 2018) who showed that TUDCA interacts specifically with rhodopsin, which may contribute to its wide-ranging effects on retinal physiology and explains its potential therapeutic effect on retinal degenerative diseases. Furthermore, treatment of HRPEpIC primary retinal pigment epithelial cells with TCA reduced paraquat toxicity and vascular endothelial growth factor induced angiogenesis, suggesting that TCA may have protective effects against both degenerative and neovascular forms of age-related macular degeneration (Warden, Barnett, & Brantley Jr., 2020). Similarly, the glycine conjugated bile acids GCA, GDCA and glycursoxycholic acid (GUDCA) reduced paraquat toxicity in HRPEpIC primary retinal pigment epithelial cells. GCA and GUDCA also reduced angiogenesis in vascular endothelial growth factor treated RF/6A macaque choroidal endothelial cells, whereas GCA had no effect (Warden & Brantley Jr., 2021).

7.8. Multiple sclerosis

Multiple sclerosis is an autoimmune demyelinating inflammatory disorder of the central nervous system resulting in motor, sensory and cognitive dysfunction that presents in three main forms: relapsing-remitting multiple sclerosis, primary progressive multiple sclerosis, and secondary progressive multiple sclerosis (Grzegorski & Losy, 2019). Bhargava and colleagues (Bhargava et al., 2020) conducted a metabolic analysis of patients with different types of multiple sclerosis and found lower levels of primary bile acid metabolites in primary progressive multiple sclerosis patients compared to healthy controls and lower levels of secondary bile acid metabolites in primary progressive multiple sclerosis and relapsing-remitting multiple sclerosis groups, indicating alterations in bile acid metabolism in multiple sclerosis that was greatest in patients with primary progressive multiple sclerosis. The primary progressive multiple sclerosis patients were older than the relapsing-remitting multiple sclerosis and healthy controls. However, similar findings were measured in patients with paediatric-onset multiple sclerosis compared to age-matched controls, which suggests that age was not a factor in the altered bile acid metabolism occurring in multiple sclerosis (Bhargava et al., 2020). Experimentally, the same authors found that treatment with TUDCA and the GBP agonist INT-577 prevented neuroinflammation (reactive astrocytes and proinflammatory polarization of microglia) in a dose-dependent manner in cultured astrocytes and microglia. Furthermore, TUDCA reduced behavioural deficits and neuropathological changes in mice with experimental autoimmune encephalomyelitis through its effects on GBP. Together these data identify dysregulated bile acid metabolism as a potential therapeutic target in multiple sclerosis (Bhargava et al., 2020). Another study found reduced plasma and elevated cerebrospinal fluid levels of 25-hydroxycholesterol in patients with relapsing-remitting multiple sclerosis (Crick et al., 2017). The elevated cerebrospinal fluid level may reflect metabolism of cholesterol released from dying neurons via the alternate pathway and similar increases have been observed in cerebrospinal fluid from patients with Parkinson’s disease and Alzheimer’s disease who also show reduced plasma levels of 25-hydroxycholesterol (Crick et al., 2017).

Hucke and colleagues (Hucke et al., 2016) found decreased levels of FXR in peripheral immune cells from relapsing-remitting multiple sclerosis and primary progressive multiple sclerosis patients and showed that treatment of experimental autoimmune encephalomyelitis mice with the FXR agonist GW400264 promoted generation of anti-inflammatory cytokines and reduced the behavioural phenotype. Furthermore, they showed that activation of FXR in monocytes from healthy controls and multiple sclerosis patients resulted in an anti-inflammatory phenotype that was interpreted as showing FXR was important in control of T cell-mediated autoimmunity by promoting anti-inflammatory macrophage responses (Hucke et al., 2016). Another study found that FXR knockout mice had more severe experimental autoimmune encephalomyelitis-induced deficits than wildtype animals and that treatment with the synthetic FXR agonist INT-747 or the FXR ligand CDCA ameliorated symptoms in mice with established experimental autoimmune encephalomyelitis (Ho & Steinman, 2016).

7.9. Hepatic encephalopathy

Brain dysfunction ranging from subclinical neurological alterations to coma resulting from liver disease (irrespective of the cause) is termed hepatic encephalopathy and while it is considered a metabolic disorder that is curable if the cause of the liver problems is successfully treated, the neurological damage resulting from neuroinflammation and neuronal cell death persists (Rose et al., 2020).

While elevated neurotoxic levels of ammonia in brain due to the impaired urea cycle found in an inflamed liver are thought to cause much of the neuroinflammation in brain, bile acids are also elevated in patients with severe liver disease. This is likely in part due to high circulating concentrations of toxic primary bile acids in the context of cholestasis which, together with systemic inflammation compromises the integrity of the blood brain barrier with resultant high bile acid concentrations (Rose et al., 2020). This is supported by studies in patients with hepatic encephalopathy and animal models of the disorder. A metabolomic analysis of cerebrospinal fluid from patients with hepatic encephalopathy found elevated levels of bile acids, amino acids, acylcarnitines and xenobiotics compared to control individuals (Weiss et al., 2016). Experimentally, Quinn and colleagues (Quinn et al., 2014) showed that elevated bile acids occurring after bile duct ligation in rats resulted in increased blood brain barrier permeability. In other studies, lowering serum bile acid levels by chemical or genetic means reduced behavioural deficits in models of hepatic encephalopathy (McMillin et al., 2016),(Xie et al., 2018). A role for microglia-mediated neuroinflammation in these effects is suggested by the findings of McMillin and colleagues (McMillin et al., 2017) who reported that elevated bile acids (predominantly TCA) activate STP in brain leading to microglia activation via induction of chemokine ligand 2. Furthermore, in an animal model of hepatic encephalopathy, inhibition of FXR signalling in azoxymethane-treated mice attenuated accumulation of cholesterol in brain and reduced cognitive and neuromuscular deficits (McMillin et al., 2018).

7.10. X-linked adrenoleukodystrophy

X-linked adrenoleukodystrophy arises from mutations in the ABCD1 gene that encodes the adrenoleukodystrophy-protein that transports unbranched very long chain fatty acids (> 22 carbon atoms) into peroxisomes for enzymatic β-oxidation degradation. It comprises two main phenotypes: adrenomyeloneuropathy and a cerebral demyelinating form (Kemp, Berger, & Aubourg, 2012). Adrenomyeloneuropathy is characterised by a noninflammatory, slowly progressive neuropathy within the spinal cord and peripheral nerves whereas, the cerebral form has inflammatory involvement and is more severe (Kemp et al., 2012). Platek and co-workers (Platek et al., 2018) found dysregulation
of the primary bile acid synthesis pathway in a Polish family affected by the disease that led to a significant reduction in total plasma bile acids with unconjugated CA and CDCA the most affected. The level of plasma lathosterol was also reduced compared to controls. In a preclinical mouse model of X-linked adrenoleukodystrophy (Abcd1−/− mice), feeding mice TUDCA (0.4% wt/wt) in their diet for three months normalised the markers of the unfolded protein response that are chronically activated in these animals, reversed behavioural motor deficits and reduced the amount of activated microglia and reactive astrocytes in spinal cord (Launay et al., 2017).

8. Clinical trials using bile acids

Based on the success of treatment with TUDCA or UDCA in animal models of neurodegenerative disease, a number of clinical trials are in progress (Table 4) to evaluate the efficacy and/or safety of these bile acid receptor agonists as treatments for Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and multiple sclerosis (Khalaf, Torrese, Cocco, & Albanese, 2022).

8.1. Parkinson's disease

Two clinical trials are currently ongoing to study the effects of UDCA in Parkinson's disease. The first is a phase 1 study (NCT02967250) that seeks to understand the bioenergetic improvements that underlie Parkinson's disease by investigating the UDCA levels in patients at baseline and following four weeks of repeated high oral doses of UDCA (50 mg/kg/day) and to simultaneously measure the cortical bioenergetic profile and ATPase in patients at baseline and after four weeks of treatment. Secondary aims are to characterize oral UDCA pharmacokinetics model the pharmacokinetic/pharmacodynamic relationship between peripheral measurements of UDCA (and associated conjugates) and peripheral measures and/or central (brain) bioenergetic measurements measured by magnetic resonance spectroscopy. To date no results have been posted.

The other trial (NCT03840005) aims to explore the potential of UDCA to slow Parkinson's disease progression in a randomized, double-blind, placebo-controlled, proof of concept study. The primary objective is to ascertain safety and tolerability of UDCA in patients. Study participants consist of individuals diagnosed with Parkinson's disease within three years of the start date. Treatment consists of 30 mg/kg/day for 48 weeks. The primary outcome is based on reporting of adverse effects and completion of the study. The secondary outcomes are: 1) measurement of the change from baseline to week 48 of scores on the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part 3 motor subsection in the OFF-medication state. 2) Estimates of ATP, phosphocreatine and inorganic phosphate levels by 31P-Magnetic Resonance Spectroscopy of the basal ganglia and related motor regions and 3) quantification of motor impairment using motion sensors. To date no results have been posted.

8.1. Parkinson's disease

The phase 2 trial of AMX0035 (NCT03533257) which is a combination therapeutic consisting of 3 g of phenylbutyrate and 1 g TUDCA in patients with late mild cognitive impairment or early Alzheimer's disease dementia sought to evaluate the safety, tolerability, drug target engagement and neurobiological effects of AMX0035 (6 g + 2 g/day) treatment over 24 weeks. AMX0035 is a combination therapy designed to reduce neuronal death through blockade of key cellular death pathways originating in the mitochondria and endoplasmic reticulum. The primary outcome was to quantify adverse effects compared to placebo treated patients. Secondary outcomes consisted of MRI measurements of whole brain and hippocampal atrophy, clinical assessment of cognition and neuropsychiatric measures and functional MRI imaging. CSF and plasma biomarkers were also measured. The study met its primary endpoint of safety and tolerability and there was a significant reduction of Alzheimer’s biomarkers (tau protein, phosphorylated tau protein, modulation of the amyloid beta 42/40 ratio and an increase of 8-hydroxy-2′-deoxyguanosine). However, no differences between groups were seen in cognitive or imaging measures, but the study did not have sufficient power to evaluate such differences (https://www.amylyx.com/2021/11/09/amylyx-pharmaceuticals-announces-results-from-pegasus-trial-of-amx0035-in-alzheimers-disease-at-the-clinical-trials-on-alzheimers-disease-ctad-conference/).

8.3. Amyotrophic lateral sclerosis

Early trials in patients with amyotrophic lateral sclerosis that showed that TUDAC was well tolerated in patients, though without significant clinical benefit (Min et al., 2012), (Parry et al., 2010). But, as mentioned above, when combined with riluzole, TUDCA did slow progression of symptoms (Elia et al., 2016). A recent trial (CENTAUR, NCT03127514) found that AMX0035 slowed functional decline compared to the placebo by a mechanism thought to involve a reduction in endoplasmic reticulum stress and mitochondrial dysfunction (Pagnoni et al., 2020). A subsequent follow up analysis of the long-term (3 year) survival of the participants in the CENTAUR trial found those receiving the drugs had a 44% lower risk of death that translated into 6.5 months longer survival time (Pagnoni et al., 2021).

Four trials are currently ongoing investigating the effects of bile acids in the treatment of amyotrophic lateral sclerosis. The first trial (NCT03800524) is a Phase 3, multi-centre, randomized, double-blind, placebo-controlled, parallel-group study to evaluate safety and efficacy of TUDCA as an add-on treatment in patients with amyotrophic lateral sclerosis. Patients will receive TUDCA 1 g twice a day for 18 months in combination with riluzole 50 mg twice a day. The primary outcome was the number of patients who improved by at least 20% on the ALSFRS-R slope. Secondary measures were survival time, amyotrophic lateral sclerosis disease functional and assessment scale rating, forced vital capacity, EuroQol 5-Dimension-5 Levels (EQ-5D-5L) scale, Medical Research Council Scale, neurofilament levels and MMP-9 levels. To date no results have been posted.

The other three trials are all testing AMX0035. The Centaur Open Label Extension Study (CENTAUR-OLE) (NCT03488524) is designed to provide longer term access to AMX0035 for patients with amyotrophic

### Table 4

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose per day</th>
<th>Duration</th>
<th>Phase</th>
<th>Trial</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parkinson's disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UDCA</td>
<td>50 mg/kg</td>
<td>1.5 months</td>
<td>1</td>
<td>NCT02967250</td>
<td>Recruitment completed</td>
</tr>
<tr>
<td>UDCA</td>
<td>30 mg/kg</td>
<td>12 months</td>
<td>2</td>
<td>NCT03840005</td>
<td>Recruitment completed</td>
</tr>
<tr>
<td><strong>Alzheimer's disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMX0035*</td>
<td>6 g + 2 g</td>
<td>6 months</td>
<td>2</td>
<td>NCT03533257</td>
<td>Recruitment completed</td>
</tr>
<tr>
<td><strong>Amyotrophic Lateral Sclerosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUDCA + riluzole</td>
<td>2 + 100 mg</td>
<td>18 months</td>
<td>3</td>
<td>NCT03800524</td>
<td>Recruiting</td>
</tr>
<tr>
<td>AMX0035*</td>
<td>6 g + 2 g</td>
<td>≤ 30 months</td>
<td>2</td>
<td>NCT03488524</td>
<td>Recruitment completed</td>
</tr>
<tr>
<td>AMX0035*</td>
<td>6 g + 2 g</td>
<td>6 months</td>
<td>2/3</td>
<td>NCT04516096</td>
<td>Enrolling by invitation</td>
</tr>
<tr>
<td>AMX0035*</td>
<td>6 g + 2 g</td>
<td>12 months</td>
<td>3</td>
<td>NCT05621536</td>
<td>Recruiting</td>
</tr>
<tr>
<td><strong>Multiple sclerosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUDCA</td>
<td>1 g</td>
<td>4 months</td>
<td>1/2</td>
<td>NCT03423121</td>
<td>Active, not recruiting</td>
</tr>
</tbody>
</table>

* AMX0035 is a combination therapeutic consisting of 3 g of phenylbutyrate and 1 g TUDCA.
lateral sclerosis who participated in the CENTAUR study. The study will assess longer term safety and therapeutic potential of AMX0035. The primary outcome is the quantity of adverse events and serious adverse events observed in the study. Secondary outcomes are hospitalizations, rate of progression on the amyotrophic lateral sclerosis rating scale, rate of progression in strength measurements, rate of change in breathing capabilities by slow vital capacity, gastric tube frequency and number of patients needing permanent invasive ventilation.

The Phoenix Trial (NCT05021536) is a randomized double-blind placebo controlled Phase III trial to evaluate the safety and efficacy of AMX0035 for treatment of amyotrophic lateral sclerosis. Primary outcome measures are change in slope of Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) over treatment duration, number of participants with adverse events and a comparison of the number of participants in each group able to remain on study drug until planned discontinuation between groups. Secondary outcome measures are rate of decline in slow vital capacity, participant quality of life, decline in King's and MiToStages and ventilation free survival time. The composite outcome is defined as death, a death-equivalent event (tracheostomy), or hospitalization, whichever occurs first. Participant health status will be measured using the EQ-5D descriptive system and the EQ visual analogue scale patient reported outcomes questionnaire. Long-Term Survival will be obtained by monitoring of all-cause mortality.

Trial NCT04516096 is an open-label,compassion extended use study for patients who have completed The Phoenix Trial. The primary outcome being treatment of emergent adverse effects. To date no results have been posted for any of the ongoing amyotrophic lateral sclerosis trials. But should they achieve any the primary or secondary outcomes they are likely to be of benefit to the patients.

8.4. Multiple sclerosis

There is currently one trial (NCT03423121) investigating the safety and tolerability of supplementation with 1 g TUDCA per day for 16 weeks in patients with progressive multiple sclerosis. The primary outcome is the incidence of treatment-related adverse effects. Secondary outcomes include metabolomics of fasting plasma bile acid levels, shotgun metagenomic sequencing of gut microbiota in patient stool, and quality of life assessments. To date no results have been posted for this trial.

9. Looking forward

It is increasingly evident that bile acid signalling is bidirectional in the gut-brain axis, signalling metabolic status and contributing to cholesterol homeostasis in the brain. Disturbances in these pathways have been implicated in several neurodegenerative diseases suggestive that they may represent an important therapeutic target. Translational progress has been made with UDCA and TUDCA based on the premise of an improved mitochondrial function, however pathways such as FXR and GPBA which do not classically mediate the functions of UDCA. The primary outcome measures have been posted for any of the ongoing amyotrophic lateral sclerosis trials. But should they achieve any the primary or secondary outcomes they are likely to be of benefit to the patients.

Data availability

No data was used for the research described in this manuscript.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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