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Enzymatic Metabolic Switches of Astrocyte Responseto Lipotoxicity as Potential Therapeutic Targets for Nervous System Diseases

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A. Article Info

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Abstract: In keeping the brain in a steady state, astrocytes are crucial. Recent studies have shown that palmitic acid (PA) plays a key role in neurotoxicity by activating pro-inflammatory pathways. Also, metabolic switches (MSs) are regulators of metabolic pathways; they may worsen neurotoxicity; this makes them a viable therapeutic target, according to genomic-scale metabolic models and control theory. Using structural characterisation approaches, protein-protein interaction networks, and drug-protein interaction networks, we in silico evaluated these enzymatic MSs as prospective therapeutic targets. Five MSs (P00558, P04406, Q08426, P09110, and O76062) were found to be functionally linked to drug targets in the nervous system. These MSs may be indirectly regulated by certain neurological drugs, including those with polypharmacological potential, such as Haloperidol, Trifluoperazine, Disulfiram, and Trifluperidol. In addition, four microsomes (P00558, P04406, Q08426, and P09110) have druggable potential ligand-binding or allosteric cavities. Based on our findings, it seems that P00558 (phosphoglycerate kinase 1), P04406 (glyceraldehyde-3-phosphate dehydrogenase), Q08426 (peroxisomal bifunctional enzyme, enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase), P09110 (peroxisomal 3-ketoacyl-CoA thiolase), and O76062 (Delta(14)-sterol reductase) are potential targets for the creation or repurposing of pharmacological compounds. These targets could modulate lipotoxic-altered metabolic pathways, opening up new possibilities for the treatment of neurological diseases and other related human conditions.

Keywords: astrocytes; metabolic switches; drug-protein network; druggable cavity; therapeutic targets

1. Introduction

A wide variety of complex brain processes rely on astrocytes, which are specialized glial cells. When it comes to treating and preventing diseases of the central nervous system, they play a key role. Their many roles include, but are not limited to, synaptogenesis, neurotransmitter release, neuroinflammation regulation, and toxic chemical elimination [1–5]. For proper neuronal activity and brain homeostasis, astrocytes carry out a number of critical tasks [6]. The balance of the nervous system is maintained by these cells, which also control the metabolism of cholesterol, sphingolipids, and ions and glutamate, as well as react to environmental variables that have neuroprotective effects.

platform [7]. Because of this, astrocytes play an important role in fatty acid metabolism [8]. Palmitic acid (PA) is a long-chain saturated fatty acid that has recently come to notice due to its importance in inducing proinflammatory effects and reducing astrocyte survival, a phenomenon called lipotoxicity. In this case, neuroinflammation and neuronal injury might occur as a result of reduced astrocyte function and survival caused by the buildup of excess lipids and the subsequent production of lipotoxic chemicals. Specifically, research has shown that PA activates NF-kB and other proinflammatory signaling pathways, leading to an increase in levels of cytokines that promote inflammation, including TNF, IL-1, and IL-6 [9–11]. Inflammation, ceramide production, and oxidative stress are all brought about by these cytokines, which affect cellular metabolism [11–13].

A number of metabolic illnesses, especially neurodegenerative diseases, have focused attention on the complex relationship between lipotoxicity and cellular dysfunction [14]. Dementia, ALS, AD, and PD are all diseases that are linked to saturated fatty acids such palmitic acid (PA) [15]. A significant knowledge vacuum exists on the systemic processes at work at different organizational levels, despite the fact that much recent research on astrocyte metabolism under lipotoxicity has focused on understanding individual components via experimental simulations. A more comprehensive approach is required due to the current state of knowledge about the metabolic connections in diseased diseases. Using control theory and genome-wide metabolic networks (GEM), a more holistic view of the molecular mechanisms underlying brain behavior has been achieved. Even without knowing whether particular cellular targets exist at the in silico level, these novel methods have allowed the discovery of reaction groups that regulate cellular metabolism either directly or indirectly [16]. As a result, we now know how crucial control sites are to the overall operation of a metabolic network thanks to mathematical models of metabolic processes. Our most recent research [8] found that astrocytes treated with PA exhibited sixteen distinct enzymatic metabolic changes. Switches between metabolic states are made possible by enzymatic metabolic switches (MSs), which are crucial regulatory points that enable cells to adapt to different physiological demands and environmental signals. In lipotoxic situations, these switches are essential for regulating and deregulating metabolic pathways; they also increase neurotoxicity and may be useful therapy targets.

Because of their importance, metabolic switches (MSs) should be considered as possible targets for FDA-approved medicines and small compounds with medicinal properties. To suggest these enzymes as therapeutic targets for clinical disorders linked to lipotoxicity, however, we must first identify their pharmacological properties. By translating various structural and physicochemical properties into quantitative measures of druggability, structural-based computational analyses of protein-binding sites have been utilized to forecast the pharmacological potential [17]. Additionally, biological network analyses like the drug-protein interactions network (DPI) and the protein-protein interactions network (PPI) can help organize and analyze biological data [18,19]. They also encourage the analysis of biological information from experimental databases to shed light on new findings regarding polypharmacological activities [19,21] or potential drug-target interactions [19,21].

Therapeutic targets for associated disorders, including as nervous system ailments, may lie in the enzymatic MSs of the human astrocyte response to lipotoxicity, which were the focus of this work. By combining various in silico methods, such as structural bioinformatics and network analysis, this research aims to find new targets in the lipotoxic cascade that could be used to slow down the neurodegenerative processes caused by astrocytes. The ultimate goal is to find new therapeutic molecules that can help people with neurodegenerative diseases.

2. Results and Discussion

2.1. Enzymatic Metabolic Switches (MSs) Are Functionally Related to Metabolic Pathways That Are Altered under Neurodegenerative Conditions

2.2.

We tested the hypothesis that these metabolic switches could be therapeutic targets for neurological illnesses by creating a functional link between these MSs and known medication targets for these conditions. Figure 1a shows the initial results of our analysis, which show that 16 MSs are linked to 175 proteins. The majority of these proteins are listed as targets for diseases affecting the central nervous system in the Open Target database. The various interaction sources used by STRING, including text mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence, provide strong evidence for these functional interactions. Figure 1b (Table S1 in the Supplementary Materials) shows that these proteins are present in mitochondria and peroxisomes, and they engage in enhanced metabolic pathways such fatty acid catabolism and the one-carbon folate cycle. Because these MSs regulate various metabolic pathways, it is well-known that they are (de)regulated in human astrocytes when exposed to lipotoxic circumstances [8]. In addition, prior research has shown a connection between neuroinflammation and neurodegeneration and changes in mitochondrial and peroxisomal fatty acid oxidation, among other mitochondrial and peroxisomal processes [22-25].

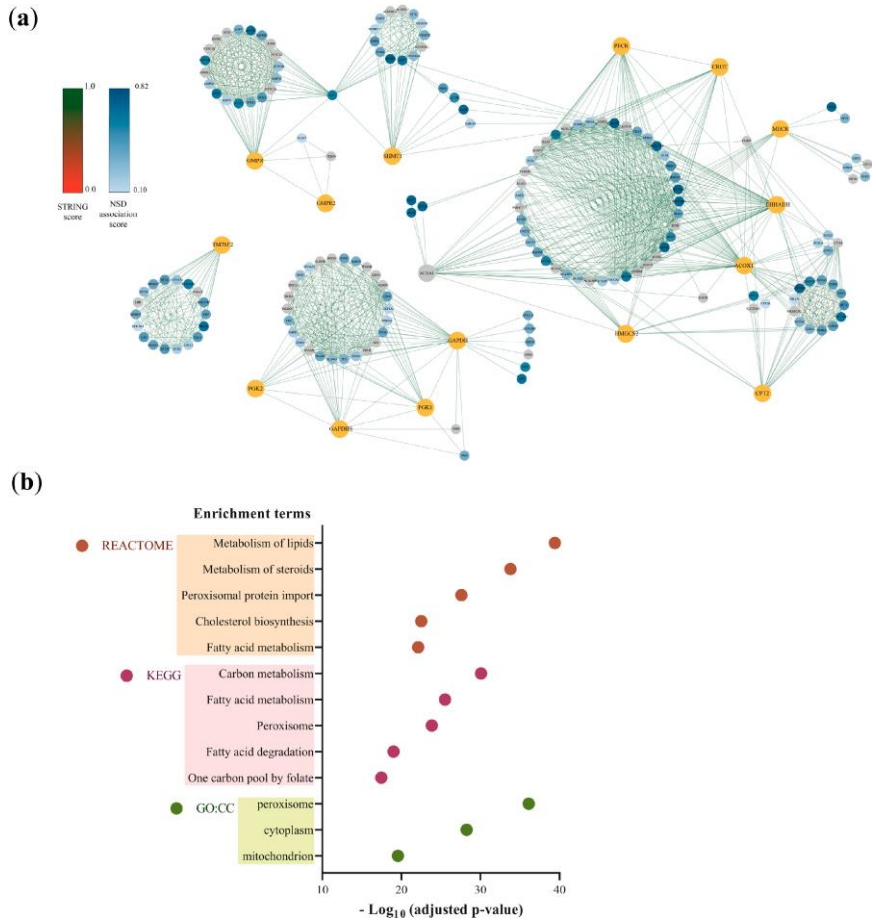


Figure 1. (**a**) Protein–protein interaction (PPI) network between metabolic switches and their STRING first neighbors. Metabolic switches are shown in orange, and STRING first neighbors (N1) are shown on a blue scale according to their nervous system disease (NSD) association score (score from 0.1 to 1.0) obtained from the Open Target Database. Those N1 with little or no association (<0.1) are shown in gray. (**b**) Enriched terms in proteins functionally related to metabolic switches, obtained from Gene Ontology Cellular Component (GO:CC), KEGG, and REACTOME databases. The adjusted *p*-value reflects the statistical significance of each term's enrichment within the set of proteins associated with MSs.

2.3. Trifluperidol, Trifluoperazine, Disulfiram, and Haloperidol Would Significantly Affect MSs

After establishing the proteins functionally associated with MSs, we generated a drug–target interaction network (DPI), integrating the approved neurological drugs previ- ously reported for proteins in the PPI network. Interestingly, none of the drugs were found to directly target a metabolic switch, which supports the novelty of MSs as therapeutic targets of nervous system diseases. In the DPI, we identified 37 drugs that interact with proteins functionally related to five metabolic switches (Figure 2), which may be modulat- ing these MSs indirectly during their action. A search in OpenTargetsDB showed that two of these five proteins (PGK1: P00558, and GAPDH: P04406) are highly associated with neurodegenerative diseases according to the association score for this neurological condition, supported primarily by Pathways System Biology data (Table S2). By contrast, EHHADH (Q08426), ACAA1 (P09110), and TM7SF2 (O76062) presented little or no association with these diseases (Table S2), which argues for their novelty as proteins potentially related to neurodegenerative diseases.

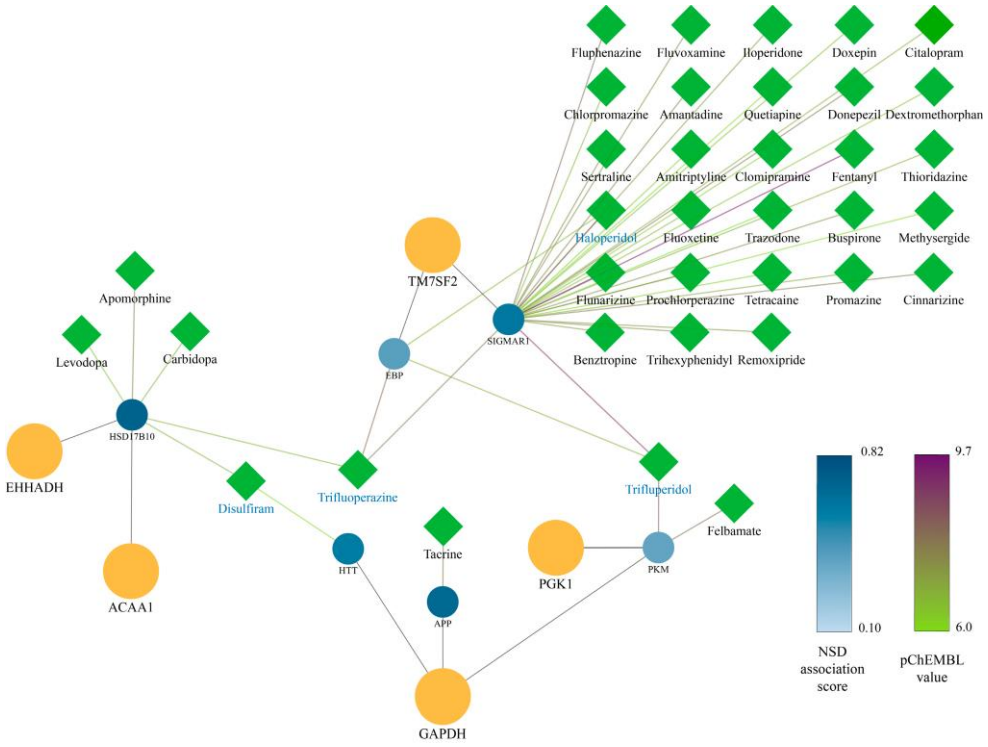


Figure 2. DPI network. Metabolic switches are shown as orange circles and their first neighbors as circles on a blue scale according to their association score for nervous system diseases (NSD) obtained from the Open Target Database. Edges between nervous system phase 4 drugs (green diamonds) with first neighbors are colored on a green–violet scale according to their pChEMBL interaction score. Drug names colored in blue correspond to molecules with more than one target in the DPI network.

Finally, to identify the drugs with the greatest impact on the MSs, we determine the drugs with multiple PPI targets, finding that four of these drugs (CHEMBL15023: Trifluperidol, CHEMBL422: Trifluoperazine, CHEMBL964: Disulfiram, CHEMBL54: Haloperidol) have polypharmacological potential over MS-related proteins (Table 1). The 33 drugs with a single target in the DPI network are described in Table S3 in the Supplementary Materials.

Trifluperidol (CHEMBL15023) has had three targets (PKM, EBP, and SIGMAR1) that are functionally related to the three MSs (PGK1, GAPDH, and TM7SF2) (Table 1). Thus, the pharmacological activity of trifluperidol may be associated with the modulation of glycolysis and cholesterol biosynthesis [26,27]. This drug has been widely used for NR1a/2B receptors and is indicated for treating schizophrenia, behavioral disorders, motor disorders, and edema in psychotic illnesses [28]. Trifluperidol has preclinical evidence of neuropro-

tective properties by promoting the reduction of tumor necrosis factor-alpha (TNF-α) and nitric oxide secretion in microglial cells [29] and modulating proinflammatory cytokines (IL- 1-beta and IL-2) in glial cells [30]. Furthermore, the modulatory nature of trifluperidol contains inhibitory characteristics. For example, the modulation of TNF-α can have an impact on neurodegenerative processes associated with demyelination and degeneration, as it induces the production of growth-promoting cytokines and neurotrophins, as well as nitric oxide and free radicals, among others that favor apoptotic processes [31–33]. Although most studies have described that the energy demand in patients with processes involving the CNS increases, there is a lack of studies that describe the metabolic effect of drugs such as trifluperidol. However, it has been described that antipsychotic drugs managed to improve the enzymatic activity associated with the glycolysis pathway [34].

Table 1. Nervous-system drugs identified in the DPI network with potential polypharmacological potential against MSs.

Drug Name (CHEMBL ID)	Drug Group	Pharmacological Activity	Number of Targets	ID UniProt Target	Targets	Metabolic Switch Related
Trifluperidol (CHEMBL15023)	Butyrophenone derivatives	Modulation of glycolysis and cholesterol biosynthesis	3	P14618 Q15125 Q99720	PKM EBP SIGMAR1	PGK1, GAPDH TM7SF2 TM7SF2
Trifluoperazine (CHEMBL422)	Phenothiazines with piperazine structure	Modulation of fatty acid oxidation, cholesterol biosynthesis, and amino-acid catabolism	3	Q15125 Q99714 Q99720	EBP HSD17B10 SIGMAR1	TM7SF2 ACAA1, EHHADH TM7SF2
Disulfiram (CHEMBL964)	Drugs used in alcohol dependence	Glycolysis and fatty acid oxidation modulation	2	Q99714 P42858	HSD17B10 HTT	ACAA1, EHHADH GAPDH
Haloperidol (CHEMBL54)	Butyrophenone derivatives	Modulation of lipid metabolism	2	Q15125 Q99720	EBP SIGMAR1	TM7SF2 TM7SF2

PKM: Pyruvate kinase; EBP: 3-Beta-hydroxysteroid-Delta(8), Delta(7)-isomerase; SIGMAR1: Sigma non-opioid intracellular receptor 1; HSD17B10: 3-hydroxyacyl-CoA dehydrogenase type-2; HTT: Huntingtin; PGK1: phosphoglycerate kinase 1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; TM7SF2: Delta(14)-sterol reductase; ACAA1: 3-ketoacyl-CoA thiolase; EHHADH: peroxisomal bifunctional enzyme (enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase).

Table 1 shows that trifluoperazine (CHEMBL422) contains three MSs (TM7SF2, ACAA1, and EHHADH) that are functionally connected to three targets (ESGMAR1, HSD17B10, and EBP). Trifluoperazine modifies fatty acid oxidation, cholesterol production, and amino acid catabolism, which may explain its pharmacological effect [26,35,36]. Dopamine receptor type 2 (DR2) inhibition is the mechanism of action of this antipsychotic medication [37]. The anticancer effects of trifluoperazine are due to its ability to suppress cytokine secretion [38]. Trifluoperazine has recently been shown to lower levels of interleukin-1-beta and tumor necrosis factor-alpha, which in turn reduces the generation of mediators that promote inflammation [39,40]. Trifluoperazine inhibits the protein calmodulin, which binds to Ca+2 in edema brain processes [39,41], and so lowers acute hypothalamus inflammation (Huang et al., 2021). It does this by reducing gliosis caused by saturated fatty acids. There is evidence that this medicine improves glucose route homeostasis after delivery, suggesting that it affects metabolic dys-function [39,42,43]. Similarly, trifluoperazine may alleviate PD by reducing a specific kind of alpha-synuclein and rescuing dopaminergic cells [37]. Trifluoperazine treatment ameliorates demyelination and motor coordination deficits in multiple sclerosis [44].

Table 1 shows that the three MSs (ACAA1, EHHADH, and GAPDH) that disulfiram (CHEMBL964) functionally relates to have two targets (HSD17B10 and HTT). Glycolysis and fatty acid oxidation regulation may therefore be connected with disulfiram's pharmacological effects [35,45]. Its anti-alcoholic properties have made it a popular choice for relapse prevention [46]. The anti-inflammatory and neuroprotective properties of disulfiram have been shown [47]. It raises ADAM10 activity and lowers BACE-1 promoter activity, for instance [47, 49]. Therefore, disulfiram could be a therapeutic strategy by increasing the amount of sAAP-alpha protein, which has neurotrophic and neuroprotective properties. This protein protects primary neurons in the hippocampus from apoptosis via the AKT survival pathway, as demonstrated by authors like Reinhardt et al. (2018). Furthermore, disulfiram raises the

behaviour that is functional and impacts the pathophysiology of AD [47,50]. Considering the small number of clinical trials, it is still challenging to anticipate the favorable effects of disulfiram. There is a functional relationship between a single MS (TM7SF2) and two targets (EBP and SIGMAR1) that are associated with haloperidol (CHEMBL54) (Table 1). Therefore, it is possible that haloperidol's pharmacological action involves regulating lipid metabolism [26]. If you or someone you know is experiencing signs of schizophrenia, including but not limited to, behavioral changes brought on by Alzheimer's disease, or hallucinations, this antipsychotic medication may help [51]. Extrapyramidal effects, such as greater memory loss in Alzheimer's disease and Parkinson's disease caused by dopamine-receptor blockage, which generates reactive oxygen species (ROS) [52,53], restrict the usage of haloperidol. So, haloperidol increases oxidative stress and lipid peroxidation, which leads to a decline in antioxidant enzyme storage and ultimately neurodegeneration [51,53]. We showed that five MSs (TM7SF2: Delta(14)-sterol reductase; PGK1: phosphoglycerate kinase 1; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; ACAA1: peroxisomal 3-ketoacyl-CoA thiolase; and EHHADH: peroxisomal bifunctional enzyme) have functional relationships to neuropathological drug targets, indicating that these MSs could be indirectly modulated as part of drug action. Furthermore, it has been shown that several of these medications have polypharmacological action by acting on many MS-related sites. Given the complex nature of most neurodegenerative disorders, the traditional "one drug, one target, one disease" paradigm has come under scrutiny in the field of drug discovery [54,55]. Nevertheless, by taking a more comprehensive view, we have been able to identify the many of pathways that contribute to neurodegenerative diseases [56,57]. The fact that an FDA-approved medicine may have a therapeutic impact on several targets associated with distinct disease pathways is what has brought polypharmacology into the spotlight [56,58]. Yet, there is concern about their neuroprotective effects and the hazards of using them over an extended period of time.
The link between these drugs and neurodegeneration needs more investigation. Inflammation, oxidative stress, and dysfunctions in neural transmission are basic molecular processes shared by both psychiatric and neurodegenerative illnesses, even though the majority of drugs discovered are associated with mental disorders [31]. Several research have looked at the connection between these two fields of medicine, and the results show that there are genetic and epidemiological risk factors, as well as different and common causal proteins [59]. Dopaminergic and synaptic dysfunction as well as neuroplasticity are shared molecular pathways between schizophrenia and Parkinson's disease, for instance [60]. Additionally, it seems that excitotoxicity is the last common mechanism across several neurodegenerative and neuropsychiatric diseases [59]. Additionally, there is conflicting evidence on the potential benefits and risks of antipsychotics in individuals suffering from neurodegenerative diseases. The mental symptoms of Alzheimer's disease, for instance, have been treated with atypical antipsychotics like risperidone. Some worry, nonetheless, that this group may have unintended consequences, such as a higher risk of stroke and a decrease in cognitive function [61]. Another antipsychotic drug that has shown promise as a treatment for multiple sclerosis (MS) and other neurodegenerative diseases is trifluoperazine [39]. It does this by lowering inflammation in the brain caused by a high-fat diet and by reducing demyelination in MS. It may have cytotoxic consequences, however, as it may cause mitochondrial cell death. When it comes to treating neurodegenerative illnesses and other ailments, more study is necessary to fully grasp its advantages and hazards.
One example of a pharmaceutical that is converted to an active metabolite is disulfiram, which is used for chronic alcoholism therapy. This metabolite, diethyldithiocarbamate, may modulate secretase activity and have neuroprotective effects [47]. In addition, disulfiram inhibits neuroinflammation by reducing NLRP3 inflamm-masome activity [62]. Last but not least, a putative therapeutic target against neurodegenerative illnesses is DJ-1, and a new research found that disulfiram is a strong activator of this protein [63]. Although the literature indicates that

We may need further study or topic-specific studies to fully comprehend the possible therapeutic benefits of disulfiram on neurodegeneration, although preliminary evidence suggests that it may have some.
The trifluperidol, trifluoperazine, disulfiram, and haloperidol targets that have been identified interact with the MSs in a functional way. Our research leads us to believe that the four medications' pharmacological action may include indirect modulation of the MSs. Since these processes may be regulated by medications for neurological disorders, this concept emphasizes the interconnections between MSs and pharmacological targets. Diseases of the neurological system, such as those induced by these medications (psychiatric disorders) or those associated with multiple sclerosis (neurodegenerative diseases), may include metabolic pathways that need more investigation.

2.4. Enzymatic metabolic switches associated with drugs for the nervous system include intriguing structural features that hold promise as drugs.
Over the last several decades, structural geometry-based approaches, protein cavity surface and

volume analyses, surface area calculations, and hydrogen bonding abilities have helped identify the structural features of proteins that facilitate ligand recognition [64–66]. In light of these findings, we used ligandability and druggability scores for each enzyme and its natural ligand to conduct an in silico characterization of the druggable cavities of the five MSs linked to neurological drugs (see Supplementary Materials, Table S4).

Table 2 shows the five switches that were used for druggable cavity analysis. These switches had ligand and orthosteric binding sites identified. According to Table 2, four out of the fifteen MSs that were evaluated showed druggable cavities in their active sites. These MSs exhibited adequate ligandability, with Pred. Max pKd values ranging from 9.48 to 11.39, and druggability scores, ranging from 656 to 3753. As a result, their pharmacological potential was assessed. We excluded Delta(14)-sterol reductase TM7SF2 as a potential directed target since it is the only one of the MSs we examined that has druggable holes. We were able to anticipate possible binding sites of covalent ligands (drugs) by the detection, characterisation, and analysis of cavities, which promoted the investigation and creation of medications with the ability to interact with metabolic switches [67,68].

Table 2. Ligand-binding and allosteric druggable cavities characterized in MSs associated with neurological drug targets.

Protein	Cavity Number	Pred. Max pKd	Drug Score	Druggability	Residues	Allosteric Cavities	Z-Score (>0.5)
P00558	1	11.01	1424	Strong	LEU:63, GLY:64, ARG:65, PRO:66, ASP:67, LYS:74, TYR:75, ARG:122, GLU:128, LY:166, THR:167, ALA:168, HIS:169, ARG:170, HIS:172, GLY:212, GLY:213, ALA:214, LYS:215, VAL:216, ALA:217, ASP:218, LYS:219, GLY:236, GLY:237, GLY:238, MET:239, PHE:241, SER:255, LEU:256, ASP:258, PHE:285, VAL:286, PHE:291, ASP:292, GLU:293, MET:311, GLY:312, LEU:313, ASP:314, CYS:315, ASN:336, GLY:337, PRO:338, VAL:339, GLY:340, VAL:341, PHE:342, GLU:343, TRP:344, PHE:347, THR:351, GLY:371, GLY:372, GLY:373, ASP:374, THR:375, ALA:376, THR:377, CYS:378, LYS:381, THR:393, GLY:394, GLY:395, GLY:396, ALA:397, SER:398, GLU:400	1 2 3 4 5	1.57 1.26 0.89 0.79 0.62
P04406	1	11.39	1477	Strong	GLY:82, PHE:83, GLY:84, ARG:85, ILE:86, GLY:87, ARG:88, ASP:121, THR:123, HIS:124, GLU:168, SER:169, THR:170, GLY:171, VAL:172, TYR:173, LEU:174, ILE:192, SER:193, ALA:194, PRO:195, SER:196, PRO:197, MET:201, ALA:222, SER:223, CYS:224, THR:225, THR:226, ASN:227, MET:247, THR:249, VAL:250, HIS:251, SER:252, TYR:253, THR:254, ALA:255, THR:256, GLN:257, LYS:258, PRO:263, SER:264, ARG:265, LYS:266, ALA:267, ASP:270, GLY:271, ILE:279, PRO:280, ALA:281, SER:282, THR:283, GLY:284, ALA:285, ALA:286, LYS:287, ALA:288, VAL:289, LYS:291, GLY:302, MET:303, ALA:304, PHE:305, ARG:306, THR:309, PRO:310, ASP:311, SER:313, TYR:386, ASN:388, GLU:389, TYR:392, SER:393, VAL:396	1 2	2.89 0.94
Q08426	1	10.53	656	Strong	ASP:62, ILE:63, ARG:64, GLY:65, PHE:66, SER:67, ALA:68, LEU:129, LEU:259, LEU:260, GLN:261, SER:262, GLY:263, ALA:265, ARG:266, ALA:267, LEU:268, GLN:269, TYR:270, ALA:271, PHE:272, PHE:273, ALA:274, GLU:275, ARG:276, LYS:277, ALA:278, ASN:279, LYS:280, SER:642, GLU:644, ASP:647, PHE:665, LEU:709, LYS:710	1	1.84
P09110	1	9.48	3753	Strong	ARG:50, ALA:51, GLY:52, ASN:90, VAL:91, LEU:92, GLN:93, PRO:94, GLY:95, ASN:120, ARG:121, GLN:122, CYS:123, SER:124, SER:125, GLU:151, SER:152, MET:153, SER:154, LEU:155, ALA:156, ASP:157, ARG:158, GLY:159, ASN:163, ILE:164, THR:165, SER:166, LEU:168, ASP:176, CYS:177, LEU:178, ILE:179, PRO:180, MET:181, GLY:182, ILE:183, THR:184, ALA:263, PHE:264, THR:270, THR:271, ALA:272, GLY:273, SER:275, SER:276, GLN:277, VAL:278, SER:279, ASP:280, PRO:313, PRO:314, ASP:315, ILE:316, MET:317, ASN:345, GLU:346, ALA:347, PHE:348, VAL:373, HIS:377, PRO:378, LEU:379, CYS:408, ILE:409, GLY:410, THR:411, GLY:412, MET:413	-	-

It is important to emphasize the role of MSs in processes such as glycolysis/gluconeo- genesis, folate metabolism, fatty acid oxidation, purine catabolism, peroxisomal trans- port, and extracellular transport (Table S5 in the Supplementary Materials). The capacity to inhibit or activate enzymes associated with fatty acid oxidation has been studied in neurodegenerative contexts. The enzyme 3-ketoacyl-CoA thiolase (ACAA1, EC:2.3. 1.9; Uniprot ID: P09110) has been extensively studied for treatment with trimetazidine to im- prove insulin resistance in obese mice experimentally [69,70]. In addition, recent studies have shown the positive effect of Trimetazidine and Progesterone on brain injury [71]. Furthermore, multiple interactions (alkyl, pi-sigma, and pi-cation) have been described between Trimetazidine/Progesterone and the amino acids Ser, Val, and Phe that are present in the active site of the molecular targets 3-ketoacyl-CoA thiolase (UniProt ID: P09110) and enoyl reductase (UniProt ID: Q9BV79) [69]. Interestingly, ligand-binding pockets predicted in 3-ketoacyl-CoA thiolase (Figure 3a,b) showed similar interactions with the predicted ligand. The interaction of this enzyme with these compounds is an example of the ligand- binding ability of the druggable cavities identified in 3-ketoacyl-CoA thiolase, confirming its potential as a drug receptor.

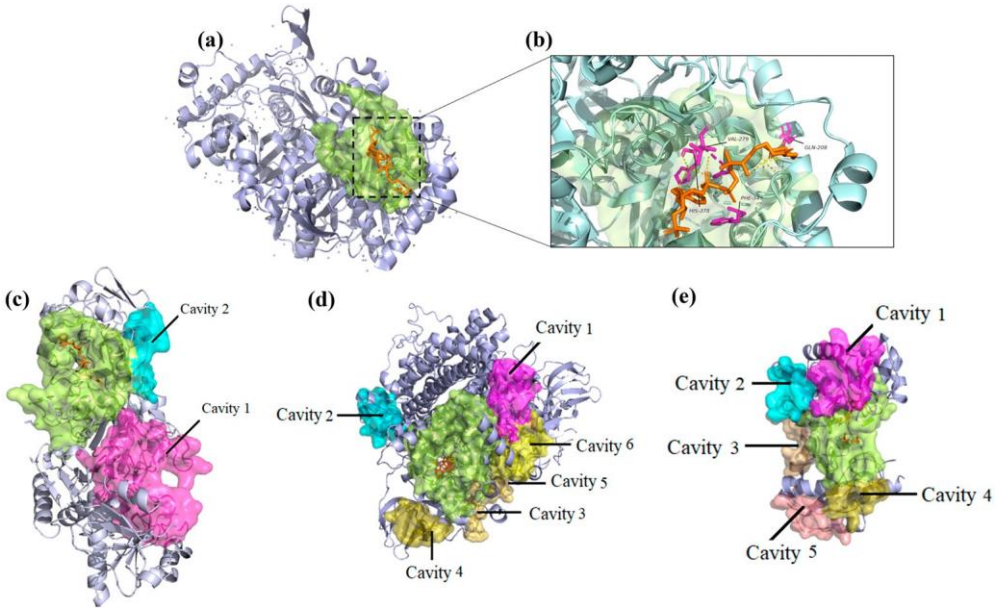


Figure 3. Four out of five MSs with potential druggable cavities and tertiary structures were associated with targets in the neurological system. (a) In the presence of the ligand Acetyl coenzyme A, the 3-ketoacyl-CoA thiolase catalyzes the condensation reaction of acyl-CoA or acyl-acyl ACP with malonyl-CoA to generate 3-ketoacyl-CoA. The druggable cavity in this enzyme is shown in the green region of the ligand-binding site. This is the 3-ketoacyl-CoA thiolase (Uniprot ID: P09110) enzyme's monomeric structure, as seen on the surface. (b) The binding amino acid interactions with the ligand (Gln208, Ser277, Val279, Phe349, and His378) visible under a microscope. c–e) Predicted druggable cavities in glyceraldehyde-3-phosphate dehydrogenase, peroxisomal bifunctional enzyme, and phosphoglycerate kinase 1, respectively, according to Uniprot IDs: O14556, Q08426, and O14556. The green regions represent the predicted druggable cavities in the binding site for the ligand. Allosteric cavities that are not green have a druggable potential at the place where the ligand binds.

In the case of the glyceraldehyde 3-phosphate dehydrogenase enzyme (GAPDH, EC 0.1.2.1.12; UniProt ID P04406), we discover a cavity that has a high potential for druggability and may be controlled by two allosteric druggable cavities that have been previously found (Table 2, Figure 3c,d). Research has shown that this enzyme controls alpha-synuclein aggregation in PD via its interactions with alpha-synuclein in Lewy bodies and the GAPDH active site, particularly with cysteine residues [72,73]. But research shows that this enzyme's active site substrate channeling is regulated allosterically via

both the oligomerization process and the NAD binding site integration [72,73]. Furthermore, prior research has shown that Ser 50 and Tyr 41 are crucial for enzyme stabilization and, by extension, for metabolic regulation through substrate-GAPDH interactions mediated by allosteric regulation due to substrate-substrate rate differences in deactivation [72].

The multi-omic computational model developed by [8] indicates that reactions involving

phosphoglycerate kinase, such as the catalysis of the reversible transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP-producing 3-phosphoglycerate and ATP (PGK, EC. 2.7.2.3; UniProt ID: P00558), are partially inhibited when PA is present [8]. This enzyme might be a therapeutic target since it has a cavity at the active site that is highly amenable to druggability. Here, new research has shown that pharmacologically regulating PGK, which is involved in cell cycle regulatory kinase activity, can reduce protein phosphorylation, including that of pyruvate dehydrogenase kinase 1, which in turn can inhibit glioma proliferation [74,75]. To the best of our knowledge, no neuropathological setting has yet proposed peroxisomal bifunctional enzymes (EHHADH, EC. 1.3.3.6; Uniprot ID: Q08426) as potential pharmaceutical targets. Then it may provide a brand-new therapeutic target for diseases exacerbated by lipotoxicity. See Figure 3 for a representation of the ligand-binding and allosteric druggable cavities found in PGK and EHHADH. Validation using experimental data is essential to guarantee accuracy and biological relevance, boosting the value of in silico studies in scientific research. In silico studies are beneficial for examining factors, drug discovery, and minimizing dependence on animal experiments. As an example, more recent studies on ACAA1, which is one of the MSs that might be exploited as a target for disorders affecting the nervous system, have focused on how it interacts with the β-oxidation pathway and how it could be involved in neurodegeneration. Fatty acid metabolism in the brain and central nervous system relies on ACAA1. Neurodegenerative diseases may develop from deficiencies [76]. Evidence from experiments [76] suggests that ACAA1 interacts with IP3R and SERCA to regulate intracellular Ca2+ levels, which may have an effect on neurodegenerative illnesses such as Alzheimer's and Parkinson's.
This work delves into the possibility of targeting pro-inflammatory pathways initiated by palmitic acid as a treatment for neurological illnesses linked to astrocyte-mediated neurotoxicity. Despite these promising results, the study does not identify any disease type since it relies on in vitro characterizations of MSs linked to the human astrocyte lipotoxicity response. In order to find out whether computational predictions may be used to treat problems of the nervous system, we suggest doing experiments in the future to validate our theories and overcome the difficulties.

Materials and Methods

Section 2.4: The Metabolic Switches Examined Here

An earlier research has already identified the enzymatic metabolic switches that were considered in this investigation [8]. In Table S5 of the Supplementary Materials, you may find a description of all the MSs that have been reported before.

Protein-Protein Interactions (PPI) Network, 2.5

Human PPI for each enzymatic metabolic switch (MS) was obtained from the STRING database [78] and used in conjunction with the Knime analytics platform v4.3.0 [77] to build a complete map of MS-associated protein interactions. utilizing Cytoscape v3.8.2 [79], an MS-PPI network was constructed utilizing just the first shell (N1) that had a medium confidence interaction score (>0.400) according to all STRING interaction sources. Next, we used the g:Profile server to conduct an enrichment analysis of the KEGG and REACTOME pathways, together with gene ontology keywords for cellular components (GO:CC) [80]. It was then applied to N1 proteins. The STRING database was also used to extract MS-MS interactions.

The Open Targets database was searched for N1 proteins [81] to find the greatest mean association score for any condition affecting the nervous system.

Drug-Protein Interactions (DPI) Network, Section 2.6

Using a predefined pChEMBL value threshold, we built a pipeline using Knime to find chemicals that inhibit protein-protein interactions (PPIs) [82]. The data used in this analysis came from the ChEMBL database, specifically the release #CHEMBL31, which was last updated on November 21, 2022 [83]. We only included medications that met certain requirements, such as being "small molecules" and having data on their "max phase" (0, 1, 2, 3, and 4). Where a medicine had more than one pChEMBL value against a particular biological target, we used the highest one. After that, we narrowed the data down to authorized neurological medications that were in Phase 4 and interacted with the targets that were found. We finished by building a drug-protein interaction (DPI) network using Cytoscape and the improved dataset. All disorders related to the neurological system

were retrieved by searching the DPI-network MSs in the Open Targets database [81].

3.7.1 Aims at Three-Dimensional Structures and Related Ligands

The Protein Data Bank (PDB) database was queried to get the three-dimensional structures of MSs that were functionally associated with neurological therapeutic targets [84]. We evaluated any experimental setup that fulfilled these requirements: (1) complete coverage, (2) resolution above 2.6 Å, and (3) absence of fragmentation. For proteins that do not have a PDB structure, we used the AlphaFold database [85].

Reviewing each protein's enzymatic reaction in the UniProt and Mechanism and Catalytic Site Atlas databases helped identify its natural ligands [86]. Any molecule that undergoes modification as a result of protein activity is considered a natural ligand; in the absence of such a ligand, a cofactor is chosen. The PDB reports crystallographic complexes from which the natural ligands and binding sites were extracted. Utilizing the COACH-D server, proteins devoid of co-crystallized ligands were examined [87]. To improve the coupling between the ligand and the projected binding site, AutoDock Vina was used on the COACH-D server to forecast the binding sites of probable ligands based on crystallographic complexes of related proteins. The number 88.

Table S4 in the Supplementary Material summarizes the protein and ligand data used in this investigation.

Classification of Cavities Possessing Therapeutic Potential 2.8

Each MS and its natural ligand were analyzed on the CavityPlus server using the "With Ligand" option [67] to define cavities associated with the ligand-binding sites of MSs. We also used the same server's Cavity and CorrSite2.0 tools to forecast possible allosteric binding sites with the "No Ligand" option [67]. Any pocket that had a CorrSite value greater than 0.5, showing a connection with the ligand-binding site pocket, was considered allosterically controlled in this context. The detected cavities were then evaluated for their druggability (DrugScore) and ligandability (Pred. Max pKd) using the CavPharmer program from the CavityPlus service. This allowed us to determine their druggable potential. Cavities were deemed to have druggable potential if they had a Pred. Max pKd more than 6.0 and a DrugScore of medium or strong. A molecular graphics system called PyMOL, version 2.5 (Schrödinger, 2015), was then used to show the druggable cavities.

3. Conclusions

Various cellular and molecular mechanisms contribute to the complexity of neurodegenerative disorders. Pathological circumstances underlying these disorders must be studied holistically. For example, the effect of palmitic acid (PA) on astrocytes, which is linked to lipotoxicity, must be considered. In order to create successful therapies, this thorough knowledge is crucial. We focused on the identification in our research discover and characterize enzymatic metabolic switches (MSs) as prospective pharmaceutical targets. In the past, these MSs were associated with changes in astrocyte metabolism brought on by PA. Using this method, we were able to identify five enzymatic MSs (P00558: phosphoglycerate kinase 1, P04406: glyceraldehyde-3-phosphate dehydrogenase, Q08426: peroxisomal bifunctional enzyme—enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase, P09110: peroxisomal 3-ketoacyl-CoA thiolase, and O76062: Delta(14)-sterol reduc-tase) that were functionally related to the drug targets linked to neurological disorders. In addition, the ligand-binding sites of the first four MSs were connected to cavities that may be druggable. We conclude that peroxisomal 3-ketoacyl-CoA thiolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase 1, and peroxisomal bifunctional enzyme—enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase—are potential therapeutic targets for neurological disorders.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ph17050648/s1: Table S1: biological processes, Cellular components and Metabolic pathways terms enriched in proteins functionally related to Metabolic Switches; Table S2: OpenTargetDB association scores of neurodegenerative diseases to five MS indirectly regulated by ChEMBL nervous system drugs; Table S3: description of the 32 nervous system drugs associated with a single target in the PPI network; Table S4: metabolic Switches and natural ligands analyzed in the identification of druggable cavities; Table S5: enzymatic metabolic switches under PA (palmitic acid)-mediated cellular regulation.

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