Review

The Multifaceted Role of GPCRs in Amyotrophic Lateral Sclerosis: A New Therapeutic Perspective?

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Abstract: Amyotrophic lateral sclerosis (ALS) is a degenerating disease involving the motor neurons, which causes a progressive loss of movement ability, usually leading to death within 2 to 5 years from the diagnosis. Much effort has been put into research for an effective therapy for its eradication, but still, no cure is available. The only two drugs approved for this pathology, Riluzole and Edaravone, are onlyable to slow down the inevitable disease progression. As assessed in the literature, drug targets such as protein kinases have already been extensively examined as potential drug targets for ALS, with some molecules already in clinical trials. Here, we focus on the involvement of another very important and studied class of biological entities, G protein-coupled receptors (GPCRs), in the onset and progression of ALS. This workaimsto give an overview of what has been already discovered on the topic, providing useful information and insights that can be used by scientists all around the world who are putting efforts into the fight against this very important neurodegenerating disease.

Keywords: amyotrophic; sclerosis; ALS; GPCR; adenosine; serotonin; histamine; glutamate; cannabinoid; adrenergic

1. Introduction

Amyotrophic Lateral Sclerosis (ALS, also referred to as “motor neuron disease”) indicates a clinical situation in which the motor neurons of patients undergoa progressive loss in their function and number [1]. This type of neuronal cell, whose cell body is localized in the motor cortex, the brainstem, and the spinal cord, is responsible for the innervation and the control of muscle fibers, essential for voluntary muscle contraction [2]. Their loss has very important consequences on the patient’s life, firstly impairing the ability to chew and walk, then to speak and to move, until even the ability to breath is affected, leading, after 2–5 years, to death due to respiratory failure [3]. ALS can be classified into two main types, “sporadic ALS” (the great majority of all cases), which has no known cause and typically has its onset between the ages of 58 and 63 years, and “familial ALS” (about 5–10% of cases), which is linked to genetic factors, and has its onset between the ages of 47 and 52 years [4].

In both scenarios, the pathology starts with the manifestation of muscle weakness and atrophy, with methods and timing very variable based on the patient and on the parts of the motor neurons that are affected first [5]. Indeed, a classification of the onset of the pathology can be made with reference to the site of its onset. For two-thirds of patients, the limb muscles are affected first (“spinal ALS”), with manifestations mainly in the distal muscles of the dominant hand for the upper limb and in the hamstrings for the lower limb. For the greater part of the remaining patients, the bulbar muscles represent the onset
Another article by Palomo et al. gives an exhaustive panoramic view of the protein kinase important physiological functions such as DNA repair, splicing, and transcriptional regulation.

The next steps of the disease involve the progressive spreading of the neurodegeneration process to the unaffected motor neurons, causing an increasing worsening in the patient’s daily life, making activities such as eating and walking continuously more difficult and leading to their complete loss. The final and worst clinical scenario has its onset when the respiratory function is significantly affected, progressively increasing the risk of respiratory failure, which is the main cause of death due to ALS [7].

Even if much effort has been made among both academic and industrial scientific groups, no cure has yet emerged for ALS. Riluzole [8] and the recently FDA-approved drug Edaravone [9] (both represented in Figure 1) constitute the only two small molecules used for the ALS treatment, and only succeed in slowing down the disease’s progression [10].

The chemical structures of Riluzole (A) and Edaravone (B), which are the only two small-molecule drugs approved currently for ALS treatment.

Such a neurodegenerating process, affecting 1.75–3 people per 100,000 [11], has, in the great majority of cases, no known cause [12], making it even harder to design a therapy for this disease. From a biochemical point of view, the hallmark of ALS is considered to be the presence of inclusion bodies in the cytoplasm of motor neurons. These aggregates are formed by the TAR DNA-binding protein 43 (TDP-43) [13], a protein involved in several important physiological functions such as DNA repair, splicing, and transcriptional regulation. Even if its main localization site is the nucleus, processes such as its hyperphosphorylation or the mutation of its gene (TARDBP) lead to its aggregation in the cytoplasm [14]. This mislocalization directly causes the dysregulation of several cellular events related to RNA metabolism, DNA replication, and oxidative stress management, leading to the loss of the motor neurons affected [15]. Other molecular targets that have been demonstrated to be important for ALS onset and progression are superoxide dismutase (SOD1) [16] and DNA/RNA-binding protein FUS/TLS (Fused in Sarcoma/Translocated in LipoSarcoma, also called “FUS”) [17], which appear to be mutated in the patients.

The knowledge that hyperphosphorylation of TDP-43 is one of the main processes leading to its aggregation has led the scientific community to devote some effort to identifying the protein kinases responsible for such processes, in order to find proper inhibitors for such species [18]. Recent work by Guo et al. goes deep in the examination of the involvement of kinases in ALS progression, enucleating species such as CK1, ERK, GSK3β, and JAK3 as promising targets for the treatment of this neurodegenerating disease [19]. Another article by Palomo et al. gives an exhaustive panoramic view of the protein kinase inhibitors currently in clinical trials for ALS treatment [20]. Riluzole has proven to increase life expectancy by about 2–3 months [21], and even if its main target still remains the NMDA receptor, recent work by Bissaro et al. suggested that this mechanism could be due to its action on the delta isoform of CK1 [22].

Many molecular candidates (both new chemical entities and compounds coming from repurposing strategies) are nowadays in clinical trials for ALS [23], acting on different biological pathways, with the common aim being to restore the neuronal health status in
the affected patients, possibly trying to go in the direction to find a proper cure for this pathology [24]. A comprehensive list of the potential small-molecule drugs now being evaluated by the FDA in clinical phases is reported in Table 1.

Table 1. Table reporting the different small molecules currently in FDA clinical trials for ALS treatment (updated 13 April 2022).

| Molecule          | Target/Mechanism                                           | Developer            | Clinical Phase |
|-------------------|------------------------------------------------------------|----------------------|----------------|
| Ibudilast         | Macrophage migration inhibitory factor inhibitor           | MediciNova           | Phase II/III   |
| Prosetin          | Mitogen-activated protein kinase inhibitor                  | ProJenX              | Phase I        |
| Sotuletinib       | Macrophage colony-stimulating factor receptor antagonist   | Novartis             | Phase II       |
| EPI 589           | NAD(P)H dehydrogenase modulator                            | PTC Therapeutics     | Phase II       |
| DNL 343           | Eukaryoticinitiationfactor2b stimulant                     | Denali Therapeutics Inc | Phase I      |
| Celecoxib/ciprofloxacin | Cyclo-oxygenase 2 inhibitors/DNA | NeuroSense Therapeutics | Phase I      |
| Fingolimod        | Apoptosis stimulant and immunosuppressant                  | ALS Therapy Development Institute | Phase II  |
| Trehalose         | Autophagy stimulant and protein aggregation inhibitor       | Massachusetts General Hospital | Phase II/III |
| Sodium cromoglicate | Glial cell modulator and mast cell stabilizer              | AZTherapies          | Phase II       |
| Dexramipexole     | Antioxidant and apoptosis inhibitor                         | Knopp Biosciences    | Phase II       |
| Masitinib         | Tyrosine kinase inhibitor                                  | AB Science           | Phase III      |
| NP 001            | Macrophage modulator                                       | Neuvivo              | Phase II       |
| Fasudil           | Rho-associated kinase inhibitor and vasodilator            | Woolsey Pharmaceuticals | Phase II   |
| Levisimendan      | Calcium-sensitising phosphodiesterase inhibitor and potassium channel agonist | Orion              | Phase III      |
| Apilimodtimesylate | Interleukin 12 inhibitor and interleukin 23 inhibitor       | AI Therapeutics       | Phase II       |
| Verdiperstat      | Peroxidase inhibitor                                       | Biohaven Pharmaceuticals | Phase II/III |
| Pridopidine       | Sigma-1 receptor agonist                                   | Massachusetts General Hospital, Prilenia Therapeutics | Phase II/III |
| Triheptanoin      | Triglyceride replacement agent                             | Ultragenyx Pharmaceutical | Phase I/II   |
| Reldesemtiv       | Troponin stimulant                                         | Cytokinetics         | Phase III      |
| BIIB 100          | Exportin-1 protein inhibitor                               | Biogen               | Phase I        |
| AGX 201           | Histamine receptor modulator                               | AgoneX Biopharmaceuticals | Phase I    |
| Ranolazine extended release | Sodium channel antagonist                                      | Gilead Sciences            | Phase II       |
| GDC 0134          | Mitogen-activated protein kinase 12 inhibitor             | Genentech            | Phase I        |
| NPT520 34         | Phosphatidylinositol 3 kinase modulator                    | Neuropore Therapies   | Phase I        |
Even if some effort has been directed toward trying to highlight the role of protein kinases in ALS progression, this has not been recently or extensively done with respect to G-protein coupled receptors, biological actors which have been demonstrated to be detrimental to neuronal and physiological conditions. It is important to remember that ALS is a non-cell-autonomous disease, which means that the neuronal damage characterizing the pathology is caused by aberrant processes also happening outside the neurons themselves. Indeed, ALS progression has been demonstrated to be strongly related to glial cell dysregulation (mainly microglia and astrocytes) [25]. GPCRs are very widely expressed proteins in the human organism [26], and so a beneficial effect could also be obtained by targeting extraneuronal receptors, which could trigger biological processes that, in the overall scenario, could mitigate if not reverse the disease progression.

GPCRs are membrane receptors and constitute one of the main protein families encoded by human genes, with more than 800 members already identified [27], divided into six different classes (identified alphabetically with letters from “A” to “F”) based on their similarities in sequence and function. They all share a common architecture formed of a seven-α-helix transmembrane domain (usually referred to as “7-TM”), an extracellular N-terminal domain, and an intracellular C-terminal domain. These proteins exert their roles by coupling with an intracellular messenger called “heterotrimeric G protein”, which is formed by α, βγ subunits, and interacts with different intracellular partners based on its type. The α subunit is displaced from the βγ-complex upon GPCR–ligand binding, and its fate depends on its Gα family belonging. Indeed, activated Gαi/0 proteins inhibit adenylyl cyclase (AC), reducing the production of the second messenger cyclic adenosine monophosphate (cAMP); Gsα, conversely, activates adenylyl cyclase, and Gqα subunits activate phospholipase C (PLC), leading to an increase in Ca2+ influx in the cytoplasm [28]. The physiological roles of GPCRs include homeostasis modulation, mood balancing, immune system regulation, neuronal plasticity, and many more [29]. The goal of the present work is to give a panoramic view of the GPCRs which have been linked to ALS onset and progression, presenting what has already been done to modulate their action, and highlighting new potential therapeutical scenarios. Table 2 summarizes the outcomes of our study, listing the GPCR targets that will be discussed and highlighting the new possible paths that can be taken in order to exploit their therapeutic potential for ALS.

Table 2. Table summarizing the evidence about the therapeutic potential of the GPCRs examined in this article for ALS treatment.

| Receptor/Receptor Family | Cellular Expression | Potential for ALS Treatment | References |
|--------------------------|---------------------|-----------------------------|------------|
| Adenosine receptors      | Circulatory, immune, respiratory, and nervous systems | Ambiguous | [30–37] |
| Purinergic receptors P2Y | Almost all human tissues | Antagonism | [38–43] |
| Chemokine receptors      | Predominantly on leukocytes surface | CXCR3, CXCR4, and CCR2 Antagonism | [44–47] |
| Angiotensin II receptors | Adrenal cortex, kidneys, vascular and cardiac muscles, nervous system | AT1 Antagonism | [48–50] |
| Dopamine receptors       | Arteries, heart, kidneys, CNS | D2R Agonism | [51–58] |
| Serotonin receptors      | Almost all human tissues | Ambiguous | [59–63] |
| GPR17 receptor           | CNS, kidneys, heart | Antagonism | [64–71] |
| Adrenergic receptor β2   | GI tract, respiratory system, blood vessels, pancreas, nervous system | Agonism | [72,73] |
| Histamine receptors      | GI tract, circulatory, immune, and nervous systems | Ambiguous | [74–77] |
| Cannabinoid receptors    | CNS and immune system | CB2 agonism | [78–81] |
Table 2. Cont.

| Receptor/Receptor Family                  | Cellular Expression                                                                 | Potential for ALS Treatment                          | References       |
|------------------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------|------------------|
| Prostaglandin E$_2$ receptor             | GI tract, kidneys, reproductive, skeletal, immune, and nervous systems.               | Ambiguous                                            | [82–84]          |
| Vasoactive intestinal peptide receptors  | Almost all human tissues                                                             | Agonism                                              | [85–89]          |
| Metabotropic glutamate receptors         | Nervous system                                                                       | mGluR I antagonism/mGluR II and mGluR III agonism     | [90–94]          |

Our work will be beneficial for all of the scientists who are dedicating their knowledge and efforts to the eradication of ALS.

2. GPCRs Involved in ALS

2.1. Purinergic Receptors P2Y and Adenosine Receptor A$_{2A}$AR

Purinergic receptors are a peculiar class of membrane receptors, sensitive to a wide series of purinergic ligands such as ATP, ADP, UTP, UDP, UDP-glucose, and adenosine. The kind of molecules interacting with them defines their classification into one of the three subfamilies forming this class. The first group, called “P1 receptors”, is formed of GPCRs activated upon adenosine binding (and for these reasons are also known as “adenosine receptors”), while “P2Y receptors” are GPCRs that can bind to ATP, UDP, and their diphosphate analogs ADP and UDP (with the addition of UDP-glucose). The last subfamily, named “P2X receptors”, are ligand-gated ion channels exclusively sensitive to ATP [95]. Being the P2X family not formed by GPCRs, our evaluations will focus on the first two families of receptors.

The “P1” subfamily, more commonly referred to as adenosine receptors (ARs), is a group of purinergic class AGPCRs divided into four subtypes, A$_1$AR, A$_{2A}$AR, A$_{2B}$AR, and A$_3$AR, each involved in many different physiological processes. While the functions of A$_{2B}$AR and A$_3$AR are mainly related to the circulatory, immune and respiratory systems, the A$_1$AR and A$_{2A}$AR proteins are importantly present in the central nervous system (CNS) [96]. Moreover, the A$_1$AR and the A$_{2A}$AR receptors have been demonstrated to play a crucial role in neuroprotection, neuronal survival, and neuroinflammation [30]. A study from Vincenzi et al. reported an upregulation of A$_{2A}$AR receptors in the lymphocytes of people affected by ALS [31], while Yoshida et al. measured adenosine levels in the cerebrospinal fluid of ALS patients, finding out that these were significantly higher with respect to the control subjects [32]. Unexpectedly, treatment with the A$_{2A}$AR antagonist caffeine (Figure 2, panel A), which is usually referred to as a protective agent against Alzheimer’s Disease (AD) and Parkinson’s Disease (PD), was demonstrated to shorten the survival of ALS-affected SOD1$^{G93A}$ mice, a well-known experimental model for ALS (Potenza et al.) [33], even if some explanation for this phenomenon can be provided by the non-selectivity of caffeine [34]. Indeed, Ng et al. showed that suppression of A$_{2A}$AR signaling delays the progression of ALS in the same SOD1$^{G93A}$ mouse model [35]. This is in accordance with the study of Mojsilovic-Petrovic et al., which demonstrated that A$_{2A}$AR inhibitors (such as the non-selective enprofylline [Figure 2, panel B], and the A$_{2A}$AR-selective KW-6002 [Figure 2, panel C], also called Istradefylline) protect motor neurons from toxic insult, highlighting the beneficial effects of such activity for ALS patients [36].

Despite all this evidence, a study from Liu et al. showed that A$_{2A}$AR activation, suppressing AMPK activation, suppressed TDP-43 mislocalization [37]. The multifactorial nature of ALS makes it very difficult to define sharply whether agonism or antagonism of the A$_{2A}$AR receptor has the best risk/benefit ratio, but the literature clearly defines this GPCR (represented in Figure 3) as one of the promising targets for ALS treatment.
Metabotropic glutamate receptors

The second subfamily of purinergic receptors, “P2Y”, is present in a great variety of human tissues, but their main biological roles are identifiable in blood clotting, vasodilatation, and immune response [100]. The P2Y family comprises eight different isoforms, among which P2Y12 has gained the interest of the scientific community for its role in neuroinflammation, as addressed by Morillas et al. [38] and Amadio et al. [39].

Jacobson et al. recently highlighted the proinflammatory effect of P2Y agonists, reporting that antagonizing this class of GPCRs could be considered a way of treating inflammatory conditions [40]. Even if inhibitors of the P2Y12 isoform are already marketed as antiplatelet drugs (e.g., Clopidogrel, Prasugrel, Ticagrelor, all represented in Figure 4), Jacobson et al. pointed out that there is a lack of selective and versatile P2Y ligands for each subtype, meaning that the drug discovery process is still very active in this specific field.

**Figure 2.** The chemical structures of the non-selective adenosine receptors antagonists caffeine (A) and Enprofylline (B), and the selective A2AAR antagonist Istradefylline (C).

**Figure 3.** Representation of the structure of the A2AAR receptor (sourced from the Protein Data Bank [97], PDB code: 5IU4 [98], method: X-ray diffraction, resolution: 1.72 Å). The image was created and rendered with the Molecular Operating Environment (MOE) suite [99].

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**Figure 4.** The chemical structures of the selective and irreversible P2Y12 receptor antagonists Clopidogrel (A) and Prasugrel (B). Ticagrelor, a selective, reversible, allosteric P2Y12 receptor antagonist, is also reported (C).
Specifically, D’Ambrosi et al. reported an upregulation of P2Y$_6$ receptors in the microglia SOD1 mutant models of ALS, also remembering that this phenomenon is associated with brain damage [41]. Moreover, P2Y$_{12}$ (represented in Figure 5) is upregulated in spinal cord microglia upon nerve injury, as pointed out by Kobayashi et al. [42]. Converging information is provided by a study from Moore et al. [43], further confirming P2Y$_{12}$ as a potential target for modulating neuroinflammation and neuronal damage. The data currently available help in suggesting the practical possibility of ALS regulation through purinergic receptor modulation, and this will be realizable as soon as proper inhibitors can be designed, potentially avoiding the non-desired antiplatelet effect of these molecules.

![Figure 5. Representation of the structure of the P2Y$_{12}$ receptor (sourced from the Protein Data Bank, PDB code: 4NTJ [101], method: X-ray diffraction, resolution: 2.62 Å). The image was created and rendered with MOE.](image)

### 2.2. Chemokine Receptors

Chemokine receptors constitute a group of about 20 classes of GPCRs found mainly on the surface of leukocytes, which respond to specific ligands to control chemotaxis. The ligands for these proteins are called chemokines and are a peculiar kind of cytokine used for inducing a directional movement of certain types of cells, such as epithelial and immune ones. The chemokines, as well as the receptors they act on, can be divided into four families, namely CC (e.g., chemokine CCL4), CXC (e.g., chemokine CXCL8, also known as IL-8), XC (e.g., XCL1), and CX3C (of which the only member today is CX3CL1, also called neurotactin) [102]. The activation of chemokine receptors leads to Ca$^{2+}$ influx and cell mobilization [103]. Several of these receptors are important in the progression of motor neuron damage. La Cognata et al. highlighted an upregulation of CXCR2 in both sporadic ALS patients and SOD1$^{G93A}$ mice, showing that treating the mouse models with the CXCR2 allosteric inhibitor Reparixin (Figure 6, panel B), the neuromuscular function of the subjects was improved [44]. Another interesting paper published by Rabinovich-Nikitin et al. highlighted the benefits in terms of lifespan and motor function obtained on SOD1$^{G93A}$ mouse models through the administration of the CXCR4 antagonist AMD3100 (also known as “Plerixafor”, Figure 6, panel A) [45].

Several scientific works have reported an increase in circulating chemokines and cytokines in ALS patients, as recently detailed by Liu et al. [46], and the upregulation of chemokine receptors CXCR3, CXCR4, and CCR2 was also highlighted in the pathology of interest by Perner et al. [47], who also proposed CXCR3 and its ligands as possible therapeutic targets for ALS. These scientific works converge in addressing chemokine receptor modulation as a possibility for ALS treatment, focusing on the antagonism of certain isoforms. The three-dimensional structures of CXCR2, CXCR3, and CXCR4 are represented in Figure 7.
To treat hypertension, many efforts have been directed towards the creation of drugs that target the peptide hormone angiotensinogen, which is secreted by the liver and cleaved by renin to form angiotensin I, which is then converted to angiotensin II by the angiotensin-converting enzyme (ACE), produced by the lungs. Angiotensin II acts on its receptors and modulates several physiological processes, such as aldosterone secretion (in the adrenal glands), water and sodium retention (in the kidneys), sanguine pressure, and vasopressin production (in the CNS). Briefly, the pathways for blood pressure regulation and fluid and electrolyte balance have been underlined by Iwasa et al., who reported evidence of the neurotrophic effects on spinal motor neurons. La Cognata et al. highlighted an upregulation of CXCR2 in both sporadic and familial ALS patients and SOD1 transgenic ALS patients, suggesting a potential target for modulating neuroinflammation and protecting against motor neuron damage. CXCR2 and CXCR3 receptors are more concentrated in the fetus and neonate, and their functions are more strongly related to neuronal development and excitability.

2.3. Angiotensin II Receptors (ATRs)

Angiotensin II receptors (ATRs) are a group of GPCRs that have gained fame for their importance in the therapy of hypertension. Indeed, their main physiological role is related to the renin–angiotensin–aldosterone system, one of the main physiological pathways for blood pressure regulation and fluid and electrolyte balance. Briefly, the peptide hormone angiotensinogen is secreted by the liver and cleaved by renin to form angiotensin I, which is then converted to angiotensin II by the angiotensin-converting enzyme (ACE), produced by the lungs. Angiotensin II acts on its receptors and modulates several processes, such as aldosterone secretion (in the adrenal glands), water and sodium retention (in the kidneys), sanguine pressure, and vasopressin production (in the CNS). To treat hypertension, many efforts have been directed towards the creation of drugs acting as ATRs inhibitors. The most famous drug family designed for this purpose is represented by the “Sartans”, which selectively bind to the first isoform of angiotensin receptors. Indeed, ATRs can be divided into four isoforms, AT1, AT2, AT3, and AT4. While the latter two are still in the early stages of research, the first two isoforms have been more deeply characterized. AT1 is mainly found in blood vessels, heart, kidney, brain, and adrenal cortex, mediating vasoconstrictive effects. AT2 receptors are more concentrated in the fetus and neonate, and their functions are more strongly related to...
neuronal development and excitability [110]. A study by Kawajiri et al. highlighted a reduction in angiotensin II levels in the CSF coming from ALS patients, reporting two opposite consequences: the reduction of protection and repair mediated by AT$_1$, on the one hand, and the reduction of oxidative stress due to AT$_2$ on the other. Indeed, Kawajiri et al. hypothesized that angiotensin II could be downregulated in CSF of ALS patients as a protective reaction, avoiding excessive activation of AT$_1$ [48]. The benefits of AT$_1$ antagonism have also been underlined by Iwasaki et al., who reported evidence of the neurotrophic effects on spinal motor neurons of the drug Olmesartan (Figure 8, panel A), specifically referring to its potential application in ALS [49]. Furthermore, an article by Mammana et al. highlighted the AT$_1$ antagonism-mediated neuroprotective effects of Telmisartan (Figure 8, panel B), also outlining the decrease in neuronal injury and microglial activation caused by it [50].

![Figure 8](image-url)

**Figure 8.** The chemical structures of the AT$_1$ antagonists Olmesartan (A) and Telmisartan (B).

Summing up the information obtainable from the literature, AT$_1$ inhibition could be examined as a potential new therapeutic method of fighting ALS conditions. Both AT$_1$ and AT$_2$ receptors are represented in Figure 9 below.

![Figure 9](image-url)

**Figure 9.** Representation of the three-dimensional structures of the angiotensin II receptors that could be considered for ALS treatment. AT$_1$ receptor (A) (sourced from the Protein Data Bank, PDB code: 4ZUD [111], method: X-ray diffraction, resolution: 2.80 Å) and AT$_2$ receptor (B) (sourced from the Protein Data Bank, PDB code: 7JN [112], method: X-ray diffraction, resolution: 3.00 Å). The images were created and rendered with MOE.

2.4. Dopamine Receptors

Dopamine receptors are among the most important and widely studied G-protein coupled receptors, mainly for their important physiological roles in neurotransmission. This
class of GPCRs is divided into five isoforms, which are separated into two classes. The first, also called the “D1-like family”, comprises the D1R and D5R receptors, which are coupled to a Gα protein responsible for adenylyl cyclase activation upon binding. The second family, also known as the “D2-like” family, comprises the D2R, D3R, and D4R proteins, all coupled with a Gi protein with inhibitory activity on adenylyl cyclase. Dopamine receptors are localized in different peripheral parts of the organism, such as arteries, heart, and kidneys, but their activities much more determinant within the CNS. Indeed, dopamine is the main neurotransmitter involved in the reward system, and its signaling is of crucial importance for processes such as cognition, memory, and motor control [113]. Dysregulation of the dopaminergic system in the brain represents the main cause of very important diseases such as schizophrenia, attention-deficit hyperactivity disorder (ADHD), and Parkinson’s disease [114]. Several articles highlight a correlation between dopamine signaling and ALS development [51]. We have previously reported what was assessed by Liu et al. regarding the protective effect of the A2A receptor on TDP-43 mislocalization [37]. A recent study by Lai et al. details how this beneficial activity can be blocked by D2R activation [52]. Despite this, D2R was also identified as important for the modulation of motor neuron excitability by Huang et al. [53].

Fujimori et al. showed how the treatment with Ropinirole (Figure 10, panel A), an agonist for receptors D2R, D3R, and D4R mainly used for Parkinson’s disease, has neuroprotective effects in ALS models [54]. Additionally, D2R agonists such as Bromocriptine and Sumanire (both represented in Figure 10, panels B and C, respectively) were tested by Huang et al., who reported that the final effect of such activity on ALS models was an increase in motor neuron survival [53]. Another agonist for the “D2-like family” of dopamine receptors is the R(+) enantiomer of the Parkinson’s disease drug Pramipexole, known as Dexpramipexole (Figure 10, panel D).

![Chemical Structures](image-url)

**Figure 10.** The chemical structures of the dopamine receptor agonists Ropinirole (A), which has an affinity for D2R, D3R, and D4R, Bromocriptine (B), also non-selective with an affinity for D2R, D3R, and D4R, and Sumanire (C), selective for D2R. (D) Chemical structure of Dexpramipexole (its neuroprotective effects are attributed to dopaminergic-independent activities).

Even if Pramipexole is a powerful agonist of D2R, D3R, and D4R (all depicted in Figure 11) [55], its R(+) enantiomer has a very low affinity for dopamine receptors, so its neuroprotective effects have to deduce to a non-dopaminergic action [56]. This molecule has been considered a promising candidate for ALS conditions [57]. After the phase III clinical trial, however, its development in Europe was discontinued [58].

D2R is still a very relevant target for Parkinson’s disease treatment, but the presented literature concords in considering it also a protein of high therapeutic potential for treating ALS.
Dysregulation of the dopaminergic system in the brain... and a cation channel (5-HT₂₅₅₂₅). All of the images were created and rendered with MOE.

2.5. Serotonin (5-HT) Receptors

Serotonin (also called 5-hydroxytryptamine, or 5-HT) receptors represent one of the most populated subfamilies of class AGPCRs, consisting of 13 G-protein coupled receptor isoforms (5-HT₁₆, 5-HT₂₅, 5-HT₃₆, 5-HT₄₆, 5-HT₅₆, 5-HT₆₆, 5-HT₂₅₆, 5-HT₇₆, and 5-HT₈₆) distributed throughout the entire human organism, and a cation channel (5-HT₃₆), mainly involved in gastrointestinal motility [118]. It is also interesting to note that almost all of these isoforms are present in the CNS [119]. Concerning ALS, the serotonin receptor which has gained the greatest popularity is 5-HT₂₅. An article from Oussini et al., reported that the activity of this biological entity could limit the degeneration of spinal cord mononuclear phagocytes, which is a process typical of neurodegenerative diseases. This article highlighted that the ablation of the 5-HT₂₅ gene resulted in an acceleration of ALS progression in mutant SOD1 mouse models. Indeed, they showed that the administration of a 5-HT₂₅ selective antagonist (SB204741, Figure 12, panel A) caused an important reduction in microglia viability, while treatment with the agonist BW723C86 (Figure 12, panel B) induced an increase in viability [59]. Another work by Dentel et al., reported that the spasticity associated with ALS progression could be strongly alleviated by the administration of inverse agonists of 5-HT₂₅/C such as SB206553 and Cyproheptadine (both depicted in Figure 12, panels C and D, respectively) [60]. A recent article by Arnoux et al., on the other hand, highlighted the lack of beneficial effects when ALS-affected SOD1G93R mutants were treated with the 5-HT₂₅ agonist BW723C86 [61].

Despite the promising outcomes of the prior experiments [63], no effective slowing down in the progression of the pathology was evidenced. Even if some limitations have been encountered in serotonergic modulation for motor neuron disease, the 5-HT receptorshave been demonstrated to be targets of relevance in the ALS scenario. The three-dimensional structures of 5-HT₁₆, 5-HT₂₅, and 5-HT₂₅ are represented in Figure 13.
Despite the promising outcomes of the prior experiments, some limitations have been reported that its involvement is still debated (as observed in a recent study by Raffaele et al., who also observed some limitations have been reported). What is known is that GPR17, (also called 5-HTHT) distributed throughout the human organism, and a cation is a process typical of neurodegenerative diseases. This article highlighted that the ablation of serotonin receptor which has gained the greatest popularity is 5-HT2B. D2R is still a very relevant target for Parkinson’s disease. This receptor is activated by uracil nucleotides such as UDP, UDP-glucose, and UDP-galactose, but is also sensitive to CysLTs, like Leukotriene D4 and C4 [124]. GPR17 is mainly expressed in the CNS (but also in kidneys, heart, and generally

2.6. GPR17 Receptor

GPR17 (also known as “uracil nucleotide/cysteinyl leukotriene receptor”) is a protein belonging to the 15th subfamily of class A GPCRs. One of its peculiarities is that its structure is phylogenetically related to both cysteinyI leukotriene (CysLT) receptors and to purinergic P2Y receptors [123]. This receptor is activated by uracil nucleotides such as UDP, UDP-glucose, and UDP-galactose, but is also sensitive to CysLTs, like Leukotriene D4 and C4 [124]. GPR17 is mainly expressed in the CNS (but also in kidneys, heart, and generally
in organs that can experience ischemic damage), and a more pronounced presence of this protein has been highlighted in oligodendrocyte precursor cells (OPCs). Upregulation of GPR17 can be observed in neuronal cells surrounding an ischemic-injured area, making this protein a marker for cellular stress and death. It has been reported in the literature that in the case of a demyelinating event, GPR17 is involved in the remyelination process, but the mechanism of its involvement is still debated [125]. What is known is that GPR17 is deputed to accompany the OPCs in the early stages of their differentiation process, and so its downregulation is necessary for these cells to complete their maturation. As a result of this, overexpression of this protein leads to incomplete OPC development, impairing myelination and promoting inflammatory responses [64].

Moreover, GPR17 upregulation in neurons was linked to increased cell damage by Zhao et al., who also observed that its knockdown attenuated neuronal injury and microgliosis [65]. In the field of ALS, GPR17 was demonstrated by Bonfanti et al., to be upregulated in the spinal cord of SOD1G93A mouse models [66]. As reported in a recent study by Raffaele et al., while the application of non-selective GPR17 antagonists such as HAMI3379 (Figure 14, panel A) or Montelukast (a marketed CysLT receptor inhibitor, which is represented in Figure 14, panel B) has been shown to improve remyelination processes (as also demonstrated by Merten et al. for the first of these two molecules [67]), the implementation of agonists has also been demonstrated to be beneficial in pushing OPCs to start differentiating [68]. Jin et al., asserted that the inhibition of GPR17 by Cangrelor (Figure 14, panel C) results in the amelioration of cognitive deficits through the inhibition of oxidative stress and neuroinflammation in Alzheimer’s Disease mouse models [69].

Figure 14. The chemical structures of the GPR17 inhibitors. The non-selective inhibitors HAMI3379, Montelukast (sold as a CysLT receptors inhibitor for asthma), and Cangrelor (an antiplatelet drug, reversible inhibitor of P2Y12 receptor), respectively (A–C).

Marschallinger et al., in a recent paper, highlighted a restoration in cognitive function and a reduction in neuroinflammation in rats treated with Montelukast [70]. Another study by Burnstock et al., indicated that the in vivo knockdown of GPR17 markedly reduced brain damage [71].

The information available nowadays converges in indicating GPR17 (which threedimensional structure is provided in Figure 15) as a promising target for neuroinflammation and neurodegeneration diseases. Even if, at the present moment, these efforts are more focused on multiple sclerosis treatment, GPR17 regulation for ALS is also attracting increasing interest from the scientific community.
very important physiological functions such as smooth muscle contraction and relaxation, these studies open new possibilities in drug discovery for ALS, focusing special attention on β and the amelioration of mitochondrial function. Another study by Teng et al. reported a effects attributed by the authors to this class of ligands are the increase in muscle strength PI3K-Akt-mTOR pathway, and the PKA/SIRT1 pathway. Other than neuroprotection, other biologically important for cell homeostasis, such as the cAMP/PKA/CREB pathway, the

2.7. Adrenergic Receptor β2

Adrenergic receptors are part of the 17th subfamily of class A GPCRs and are divided into nine different isoforms (α1A, α1B, α1D, α2A, α2B, α2C, β1, β2, β3), which are involved in very important physiological functions such as smooth muscle contraction and relaxation, heart muscle contraction (mainly receptors β1 and β2) [126], and glycogenolysis [127]. The research to find a link between adrenergic transmission and ALS has been focused on the β2 isoforms of this GPCR. Historically, β2 agonists represent one of the main solutions for asthma therapy [128], and drugs such as Salbutamol, Clenbuterol, and Formoterol (all depicted in Figure 16) are an example of this. A recent work by Bartus et al. highlighted the potentialities of β2 agonists for ALS, reporting that the downstream effects of these molecules can be useful for protecting spinal cord neurons, both preserving and/or restoring their function [72].

Figure 15. Structure of the GPR17 receptors (with no experimentally resolved structure available, the model sourced from the AlphaFold database is presented). The image was created and rendered with MOE.

Figure 16. The chemical structures of the adrenergic β2 receptor agonists Salbutamol (A), Clenbuterol (B), and Formoterol (C).

The pathways responsible for such outcomes presented by Bartus et al. are very biologically important for cell homeostasis, such as the cAMP/PKA/CREB pathway, the PI3K-Akt-mTOR pathway, and the PKA/SIRT1 pathway. Other than neuroprotection, other effects attributed by the authors to this class of ligands are the increase in muscle strength and the amelioration of mitochondrial function. Another study by Teng et al. reported a favorable effect of the β2 agonist Clenbuterol on SOD1G93A mice [73]. The outcomes of these studies open new possibilities in drug discovery for ALS, focusing special attention on adrenergic β2 receptor modulation. A three-dimensional representation of the adrenergic β2 receptor is provided in Figure 17.
Figure 17. Representation of the structure of the adrenergic β2 receptor (sourced from the Protein Data Bank, PDB code: 7DHI [129], method: cryo-EM, resolution: 3.26 Å). The image was created and rendered with MOE.

2.8. Histamine Receptors

Histamine receptors represent a group of class AGPCRs that has attracted a lot of interest in the pharmaceutical world in recent decades. This family is composed of four different members (H1, H2, H3, and H4), each with a specific localization in the organism. Their functions, vary from one isoform to another, ranging from vasoconstriction (H1) to gastric acid secretion (H2), to neurotransmitter release (H3), to immunoregulation (mainly H2 and H4) [130]. For each histamine receptor, the research has mainly focused on the mechanism of antagonism, of which several marketed drugs are still now relevant examples (e.g., Cetirizine, Figure 18, panel A, for H1 antagonism; or Famotidine, represented in Figure 18, panel B, for H2 blockage) [131].

![Chemical structures of histamine receptor antagonists](image_url)

**Figure 18.** The chemical structures of the H1 receptor antagonists Cetirizine (A) and Clemastine (B). The H2 receptor antagonist Famotidine is also reported (C).

An article from Apolloni et al. reported the involvement of histaminergic signals in ALS progression, highlighting that histamine receptors are dysregulated in the cortex, spinal cord, and hypothalamus of SOD1G93A ALS-affected mice [74]. This study reported that histamine could counteract the pro-inflammatory phenotype of microglia, mainly through its H1 and H4 receptor isoforms. This would be mediated by both the reduction of NOX-2 and NF-kB expression and the increase in production of other species, such as IL-6 and IL-10. Another work by Volontè et al. highlighted the neuroprotective effects of histamine signaling in ALS, again giving higher relevance to H1R (represented in Figure 19, panel A) and H4R [75]. On the other hand, Zhang et al. described H1 and H4 receptors as being responsible for pro-inflammatory cytokine release in microglia, while H2 and H3 were considered to be the main actors of anti-inflammation in that environment (the H2 receptor is depicted in its three-dimensional structure in Figure 19, panel B) [76]. Another article by Apolloni et al. reported an amelioration in ALS progression of SOD1G93A mice...
A group of class AGPCRs that are of high interest at the present date is certainly the cannabinoid receptors. These biological entities are the main actors in the endocannabinoid system, and play relevant roles in several physiological processes. Indeed, the first of its two main isoforms, called CB1, is mainly located in both the central and peripheral nervous system, acting as a neurotransmitter release modulator in response to the binding of its agonists (mainly anandamide, but also 2-arachidonoylglycerol, both represented in Figure 20, panels A and B, respectively). In the majority of cases, CB1 is coupled with G\textsubscript{i/o} protein, leading to adenylyl cyclase inhibition and consequent decrease in cAMP upon activation. The final effect of such an action is the reduction of neurotransmitter release in the synapse. On the other hand, the CB2 receptor is mainly localized on the surface of the immune system cells. Its main agonist is 2-arachidonoylglycerol, binding of which leads to the inhibition of adenylyl cyclase through G\textsubscript{i/o} subunit action [134]. The final main effect is immunosuppression [135]. As reported by an article from Giacoppo and Mazzon, several studies have shown how the application of cannabinoid receptor agonists in SOD1\textsuperscript{G93A} mouse models of ALS could be beneficial for the neuroprotective effects mediated by them [78]. Similarly, in 2019, Urbi et al. performed meta-analysis on the studies regarding the application of cannabinoids in ALS murine models, highlighting the effective concordance in assessing that their application leads to a delay in disease progression [79]. A study by Shoemaker et al. highlighted that the increase in survival could be more addressable to the CB2 isoform, showing that the administration of the CB2 selective agonist AM-1241 (Figure 20, panel C) increased survival by 56% [80]. This molecule was also examined for cannabinoid-mediated ALS treatment by Kim et al., with similar results [81].
In addition to this, Bilsland et al. reported that the knock-out of CB1 receptors in SOD1<sup>G93A</sup> ALS-affected mice had no appreciable effect on disease onset [136], and regarding this, Shoemaker et al. reported that the activation of CB1 could exacerbate disease progression [80]. The literature available today regarding the application of molecules acting on the endocannabinoid system for ALS treatment converges in the possible evaluation of a therapy based on CB2 selective agonists. A three-dimensional representation of both CB1 and CB2 receptors is provided in Figure 21.

![Figure 20](image-url) Figure 20. The chemical structures of the endogenous CB1 and CB2 receptors agonists anandamide (A) and 2-arachidonoylglycerol (B). The selective CB2 receptor agonist AM-1241 is also reported (C).

![Figure 21](image-url) Figure 21. The structures of (A) the CB1 receptor (sourced from the Protein Data Bank, PDB code: 6KPG [137], method: cryo-EM, resolution: 3.00 Å) and (B) the CB2 receptor (sourced from the Protein Data Bank, PDB code: 6PT0 [138], method: cryo-EM, resolution: 3.20 Å). The images were created and rendered with MOE.

2.10. Prostaglandin E<sub>2</sub> Receptor (PGE2R)

Prostaglandin E<sub>2</sub> receptors (PGE<sub>2</sub>) are a series of class A GPCRs that selectively bind to prostaglandin E<sub>2</sub> (also known as dinoprostone), an endogenous arachidonic acid derivative of high importance for several physiological functions. PGE<sub>2</sub> can be divided into four isoforms, named E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, and E<sub>4</sub> (all represented in their three-dimensional structure in Figure 22). With the exception of the first, which stimulates phospholipase C if agonized, the other isoforms act on adenylyl cyclase and, specifically, the EP<sub>2</sub> and EP<sub>4</sub> isoforms (coupled with a G<sub>a</sub> subunit) stimulate its function when agonized, while EP<sub>3</sub> inhibits AC through its action (being coupled to a G<sub>i/o</sub> subunit) [139]. EP<sub>1</sub> function has been correlated with hyperalgesia [140], immunoregulation [141], and colon cancer progression [142]. EP<sub>2</sub>, which is active in the reproductive, visual, cardiovascular, skeletal, and nervous systems, has also been strictly related to tumor promotion, as highlighted in a 2018 study by Sun and Li [143]. Minor correlations with cancer have been reported for EP<sub>3</sub>,...
which is also important for a large variety of functions, ranging from digestion [144] to blood pressure [145] and clotting [146], in addition to pain management [147]. The spectrum of systems in which the fourth isoform of PGE₂ receptors, EP₄, is involved is also very wide. Additionally, in this case, EP₄ has been reported to be hyper-expressed in various types of cancer, mainly prostate cancer [148]. Talking about ALS onset and progression, Ilzecz found increased levels of PGE₂ in the cerebrospinal fluid of ALS patients, and therefore concluded that this mediator could play a role in disease progression, suggesting that its inhibition could be beneficial [82]. Additionally, Kosuge et al., highlighted the role of PGE₂ in the ROS generation pathway, focusing on its impact on ALS conditions. The same conclusion was reported in 2008 by Liang et al., who suggested EP₂ receptors were proposed to have an unexpected neuroprotective effect on motor neurons by Bilak et al., who reported that the neuroinflammatory process typical of ALS was mainly due to COX-2-mediated, prostaglandin-independent processes [84]. Taken together, all of these studies converge in evaluating PGE₂ receptors as interesting pharmacological targets for ALS, being strongly correlated with the significant neuroinflammation characterizing the pathology.

Figure 22. Structure of the PGE₂ receptors (A) EP₁ (with no experimentally resolved structure available, the model sourced from the AlphaFold database is presented), (B) EP₂ (sourced from the Protein Data Bank, PDB code: 7CX2 [149], method: cryo-EM, resolution: 2.80 Å), (C) EP₃ (sourced from the Protein Data Bank, PDB code: 6AK3 [150], method: X-ray diffraction, resolution: 2.90 Å), and (D) EP₄ (sourced from the Protein Data Bank, PDB code: 7D7M [151], method: cryo-EM, resolution: 3.30 Å). All of the images were created and rendered with MOE.

2.11. Vasoactive Intestinal Peptide Receptors

The receptors for the vasoactive intestinal peptide are part of the first subfamily of class B GPCRs. As part of this group of proteins, these receptors are responsive to signals mediated by the peptide hormone VIP (vasoactive intestinal polypeptide), formed by 28 amino acids and belonging to the glucagon/secretin superfamily [152]. After being produced by organs such as the gut, pancreas, and brain, VIP act in different physiological functions depending on the target tissue and the receptor isoform interacting with it. Indeed, two vasoactive intestinal polypeptide receptor isoforms are known, namely VPAC₁ and VPAC₂ (both represented in Figure 23). Both of these proteins are highly expressed throughout the human body, from the smooth muscle of the GI tract and blood vessels to the reproductive system, lungs, spleen, and brain [153]. Their activity is mediated by a Gα protein, and involves the activation of adenylyl cyclase upon binding to VIP, consequently activating the protein kinase A (PKA) [154].
ALS onset and progression is more than possible. Anneser et al. found a strong upregulation of mGluR1 and mGluR5, predominantly postsynaptic, which, once activated, cause the stimulation of protein kinase A (PKA) and activates the protein kinase A (PKA), resulting in the activation of adenylyl cyclase and the production of cAMP.

The functions of this family of proteins are majorly related to signals throughout the human body, from the smooth muscle of the reproductive system, lungs, spleen, and brain. The interaction with receptors of vasoactive peptides has been demonstrated to be highly physiological. Two of their best-known members are the vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP), which are involved in the modulation of neuroinflammation and degenerative effects of ALS.

Figure 23. Structure of the receptors (A) VPAC1 (sourced from the Protein Data Bank, PDB code: 6VN7 [155], method: cryo-EM, resolution: 3.20 Å), and (B) VPAC2 (with no experimentally resolved structure available, the model sourced from the AlphaFold database is presented). The images were created and rendered with MOE.

The implication of VIP in the CNS has been noticed when studying both the circadian rhythm and schizophrenia [153], but its importance as a potential target for ALS therapy is of more recent discovery. In 2008, Staines considered the possibility of studying vasoactive neuropeptides for degenerating pathologies such as MS and ALS [85], and a previous article by Iwasaki et al. specifically referred to the neurotrophic properties exerted by VIP in the degenerating diseases of motor neurons [86]. Solés-Tarrés et al. analyzed the neuroprotective effects of both VIP and PACAP (pituitary adenylate cyclase-activating polypeptide, a peptide hormone binding to both VPACs and PACAP receptors), also evaluating the synthetic derivatives available to mimic their action, with a special focus on the intrinsic pharmacokinetic problems of these species [87]. Waschek already identified both VIP and PACAP as promising targets for neuroinflammation in the CNS [88], as pointed out again in recent work by Martinez et al. [89].

The interaction with receptors of vasoactive peptides has been demonstrated to be a promising way to counteract the neuroinflammatory and degenerative effects of ALS, mainly through biological and/or chemical species mimicking the functions of the endogenous peptides. Further research on this topic will define the best way to accomplish this task.

2.12. Metabotropic Glutamate Receptors (mGluRs)

Metabotropic glutamate receptors (mGluRs) are GPCRs belonging to class C of this family of proteins. As the name suggests, these entities bind to the neurotransmitter glutamate, exerting different functions in both the central and peripheral nervous systems. They can be divided into three groups, with the first being composed of mGluR1 and mGluR5, predominantly postsynaptic, which, once activated, cause the stimulation of phospholipase C (PLC) through Gq-mediated signaling. Group II (formed by mGluRs 2 and 3) and III (of which mGluRs 4, 6, 7, and 8 are a part) receptors are mainly presynaptic and are all coupled with a Gq/11 subunit, which inhibits the activation of adenylyl cyclase, causing presynaptic inhibition. The functions of this family of proteins are majorly related to the nervous system, from the modulation of neurotransmission (e.g., gabaergic and dopaminergic) and of other proteins’ signaling (e.g., NMDA receptors), to synaptic plasticity regulation [156]. This being said, it appears clear that the possibility of their involvement in ALS onset and progression is more than possible. Anneser et al. found a strong upregulation of mGluRs in the spinal cord with ALS, leading to the propagation of glial proliferation [90]. Hyperactivity of group I mGluRs has been correlated with neuroinflammation. Indeed, as demonstrated by Milanese et al., SOD1G93A ALS-affected mice with mGluR1 knockdown experience a reduction in microglia and astrocyte activation, decreasing mitochondrial...
damage and improving survival [91], and this phenomenon was also highlighted by Rossi et al. [92].

Anneser et al. showed the beneficial and protective effects of both agonism and antagonism of group I mGluRs for motor neuron disease, while less promising effects were derived from modulation of other mGluRs [93]. Crupi et al. recently pointed out that the beneficial therapeutic modulation of mGluRs is usually achieved through the reduction of the excitotoxicity drive via mGluR I inhibition or mGluR II and III agonism [94]. In conclusion, the literature supports the possibility of investing resources in the treatment of motor neuron diseases via mGluR modulation. A three-dimensional representation of mGluR1, mGluR2, and mGluR4 receptors is provided in Figure 24.

**Figure 24.** One example of each group of the metabotropic glutamate receptors. (A) mGluR1, a member of the first group of mGluRs (sourced from the Protein Data Bank, PDB code: 7DGE [157], method: cryo-EM, resolution: 3.65 Å), (B) mGluR2 (owing to mGluRs group II, sourced from the Protein Data Bank, PDB code: 7MTS [158], method: cryo-EM, resolution: 3.20 Å), and (C) mGluR4, part of group III of the mGluRs (sourced from the Protein Data Bank, PDB code: 7E9H [159], method: cryo-EM, resolution: 4.00 Å). All of the images were created and rendered with MOE.

3. Conclusions

In this review, we provided a panoramic view of the involvement of different G-protein-coupled receptors in the onset and progression of ALS, evaluating what has already been discovered on these biological entities, and highlighting what the next steps in research could be, always on the basis of the present literature on the topic. Our analysis shows that a GPCR-based therapy for ALS could be considered a practical possibility for the eradication of the ALS condition, and we encourage scientific groups all around the world in directing efforts towards this field.

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