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Metabotropic glutamate receptor involvement in the pathophysiology of amyotrophic lateral sclerosis: new potential drug targets for therapeutic applications

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Amyotrophic lateral sclerosis (ALS) is a complex genetic, late age-onset, progressive neurodegenerative disorder leading to the death of upper and lower motor neurons. Life expectancy after diagnosis is short due to the ongoing degeneration and to the lack of effective treatments. Axonal alterations, mitochondrial deficits, RNA changes, protein misfolding and turnover, glial dysfunction and hyperexcitability are key players in molecular mechanisms involved in the degeneration of motor neurons. In the context of hyperexcitability, metabotropic glutamate (mGlu) receptors, which are widely distributed throughout the central nervous system and act through many intracellular signaling pathways, are emerging as novel potential drug targets for the therapeutic treatment of ALS, as they are able to counteract excitotoxicity by reducing glutamate release and inducing the production of neurotrophic factors.

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Introduction

Amyotrophic lateral sclerosis (ALS), originally described by Charcot in 1869, has an incidence rate ranging from 0.5 to 3 per 100 000 individuals, according to ancestral origin worldwide, with men being affected almost twice than women in the age >45 years [1•]. ALS is primarily considered as a neuromuscular disease, but very recently, clinical, imaging and neuropathological evidence have emerged indicating the non-motor neuraxis as a new player in ALS pathology. Therefore, ALS is properly described as a neurodegenerative syndrome with a complex and still poorly understood etiology, where genetic factors and environmental factors [2] are interconnected in generating a wide spectrum of clinical phenotype variants [3,4•,5]. ALS can be classified in the sporadic, with an unknown etiology representing more than 90% of cases, or the familial form (~5–10% of cases), where the genetic component is responsible of the pathology, with >30 genes identified and linked to the familial form [6,7•]. Motor symptoms are the primary signs of ALS, but up to 50% of patients can develop cognitive and/or behavioral alterations within the spectrum of frontotemporal dementia, and 13% of patients have a concomitant frontotemporal dementia [3,8–10]. The molecular mechanisms leading to neuronal degeneration are not fully elucidated yet, although many genetic risk factors have been identified and several environmental factors have been hypothesized but not confirmed, such as the cyanobacterial neurotoxin β-methyl-amino-L-alanine, being associated with a high incidence of ALS in Japanese Guam and Kii Peninsula [11], exposure to organic toxic substances and pesticides, and to electromagnetic radiation [2], whereas smoking has been confirmed as a risk factor [12]. Several gene alterations have been identified in ALS patients with a history of familial ALS and, among these genes, four are responsible of about 70% of all cases of familial forms. These genes are C9ORF72, SOD1, TARDBP, and FUS [13]. Among these identified genes involved in regulating neuronal function, the intronic hexanucleotide sequence (GGGCC) expansions, up to hundreds or thousands of times (healthy individuals have a repeat less than 24) in the gene encoding C9ORF72, is considered the most important genetic cause of ALS and frontotemporal dementia [14,15]. The pathogenic mechanisms of the product of C9ORF72 gene suggest a loss of function and gain of a toxic function along the course of the disease. The product of gene C9ORF72, so far, has been involved in many molecular pathways which are altered in the ALS patients, such as actin dynamics, macroautophagy and regulation of membrane trafficking [14,15]. Mutations in the SOD1 gene (encoding superoxide dismutase enzyme) are responsible of familial ALS in about 20% of cases and 3% of all ALS cases. SOD1 gene has been widely studied since its first involvement in ALS [16] and the generation of transgenic mice expressing mutated SOD1 enzyme (SOD1G93A mice) [17] has boosted the research in the field in the last 25 years. This genetic animal model, characterized by a rapid and

aggressive disease course mimicking the clinical and pathological features of human ALS, has provided new insights in the pathophysiology at molecular, cellular and systems levels and has been an extremely valid tool for the identification, development and validation of novel therapeutic strategies. Recently, the generation of the C9ORF72 mouse model [18•], characterized by molecular, cellular, behavioral, and neurodegenerative alterations resembling human ALS, in association with other mouse models, will allow a deeper understanding of the molecular pathophysiological mechanisms and identification of novel targets for the development of new drugs in the treatment of ALS. Although the molecular mechanisms involved in neurodegeneration in ALS are not clear, several cellular and molecular processes have been identified. The proposed mechanisms range from excitotoxicity, mitochondrial alterations, formation of toxic protein aggregates which do not undergo to a proper degradation through the proteasome and/or autophagic systems, spreading of altered protein in a prion-like fashion, disarrangement of cytoskeletal structures, reduced production of neurotrophic factors from glial cells, increased oxidative stress overcoming the physiological buffering mechanisms, increased metabolic activity, neuroinflammation, and increased production of toxic RNA species leading to toxicity [1,19]. No drug is available to slow down or arrest the ongoing neurodegeneration, and the only drug that improves survival of ALS patients, albeit to a modest extent is riluzole [20]. Very recently (May 2017), the US Food and Drug Administration (FDA) approved the use of edaravone, a free radical scavenger (already approved in Japan in 2015) for the treatment of ALS in the United States based on a positive clinical trial showing a slower disease progression. This positive effect was limited to a subgroup of ALS patients with particular clinical characteristics [21•]. Thus, there is an urgent need for treatments that slow the progression of ALS. In the context of excitotoxicity, glutamate acting on ionotropic (iGlu) and metabotropic glutamate (mGlu) receptors, plays an important role. mGlu receptors have been involved in the pathophysiology of several neurodegenerative diseases [22] and are considered potential drug targets for the treatment of these diseases by counteracting excitotoxicity and inducing the production of neurotrophic factors. Data are still in progress and much remains to be understood. Here, we will focus on the latest developments on mGlu receptors and their involvement in ALS in light of novel genetic approaches and mGlu receptor ligands tested in experimental models of ALS.

Metabotropic glutamate (mGlu) receptors

Glutamate is the most important excitatory neurotransmitter of the central nervous system acting on ionotropic glutamate (iGlu) and metabotropic glutamate (mGlu) receptors, and is involved in almost all physiological brain functions. Extracellular glutamate neurotransmitter concentrations are kept low by glial and neuronal uptake through five subtypes of sodium-dependent high-affinity glutamate transporters (GLT). GLT1 (or excitatory amino acid transporter 2 (EAAT2)) is the most expressed during the adult life and accounts for more than 90% of glutamate clearance by glial cells [23]. Therefore, glial cells are key players in buffering extracellular glutamate levels which are responsible of excitotoxicity when they reach and exceed a threshold level. mGlu receptors are seven-domain transmembrane proteins coupled to G proteins and are subdivided into three groups according to their sequence homology, intracellular signaling pathways and pharmacological profile of activation. These eight receptor subtypes show presynaptic, perisynaptic and postsynaptic neuronal localization where modulate fast excitatory synaptic transmission, but are also expressed by glial cells where induce the synthesis and release of neurotrophic factors [22]. They are considered potential targets for drugs aimed at the treatment of neurodegenerative diseases by counteracting excitotoxicity and inducing the production of neurotrophic factors.

Group I mGlu receptors in ALS

Group I mGlu receptors (mGlu1 and mGlu5) are coupled to membrane polyphosphoinositide hydrolysis, negatively modulate K^+ channels, are expressed in the postsynaptic and perisynaptic zone of the nerve terminal close to iGlu receptors and amplify fast excitatory synaptic transmission [24]. mGlu5 receptors are also found in astrocytes, oligodendrocytes, and microglia where their function can be modified in pathological conditions [25,26]. Glial cells operate to reduce extracellular glutamate levels avoiding excitotoxicity when glutamate reaches and exceeds the threshold level. Glutamate uptake is reduced in the motor cortex and spinal cord of ALS patients due to a functional reduction of glial EAAT in these regions suggesting that glutamate excitotoxicity could be increased [27]. Under physiological conditions, mGlu1 and mGlu5 receptors are expressed in human spinal cord neurons [28,29]. mGlu1 receptors are highly expressed in the ventral horn neurons, whereas mGlu5 receptors are expressed in the dorsal horn neurons [29]. Astrocytes express low levels of mGlu receptors, whereas high levels are observed in reactive glial cells in gray and white matter of ALS spinal cord [29]. *In vivo* studies of positron emission tomography imaging of mGlu5 receptors and inflammatory response in the brain and spinal cord of SOD1G93A mice have showed an increased expression of these receptors in the striatum, hippocampus, and frontal cortex during the progression of disease and a significant increased inflammatory state in the spinal cord and lungs [30]. Thus, upregulation of group I mGlu receptors in reactive astrocytes may be considered a mechanism in the modulation of glial function in the context of glial-neuronal communication during the degeneration of motor neurons in ALS. Moreover, it has also been shown an abnormal glutamate release in