Therapeutic potential of alpha 5 subunit containing $GABA_A$ receptors in Alzheimer's disease

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Alzheimer's disease (AD) is a common neurodegenerative disorder and the leading cause of dementia. It has been estimated that over 50 million people worldwide were affected by dementia in 2019, and the prevalence of dementia is expected to double approximately once every 20 years (Govindpani et al., 2017). Most people who die with AD require high levels of care for long periods of time. The underlying causes of AD are not well understood, and there are no current treatments preventing the onset or delaying the progression of the disease. Several decades of intensive research have yielded a range of drugs that alter the metabolism of amyloid-β (Aβ) and tau protein levels, the main components of the aggregates found in the brains of people with AD. Numerous therapeutic approaches that target the production, toxicity, and removal of Aβ and tau protein are being developed, but these have yet to fulfill expectations in clinical trials. A large number of major failed trials are alarming to the AD community of researchers and affected families. Therefore, there is an urgent need to discover ways to improve and restore memory function and find better and more effective ways to care for people with AD.

The balance between excitatory and inhibitory (E/I) neurotransmission is severely disrupted in AD. This imbalance has been associated with the development and progression of AD and it could underlie the cognitive deficits that are characteristic of the condition (Govindpani et al., 2017). Currently, all five drugs approved by the US Food and Drug Administration for the symptomatic treatment of AD are targeted towards the excitatory systems, but these provide only marginal clinical benefits. Neurotoxicity induced by Aβ is mediated via an excitotoxic pathway that involves the accumulation of glutamate and over-activation of the N-methyl-D-aspartate receptors but drugs targeting the inhibitory gamma-aminobutyric acid (GABA) system could reduce neuronal vulnerability to excitotoxic damage by restoring the E/I balance. GABA is the main inhibitory neurotransmitter in the central nervous system and has a key role in maintaining the homeostatic balance between E/I signaling. Restoration of this balance by reducing neuronal hyperexcitability can rescue neuronal dysfunction, preventing cell death, and improve behavioral deficits in AD mice (Busche et al., 2012; Govindpani et al., 2017). GABAergic inhibition of hyperactive neurons is reduced in transgenic APP23xPS45 mice while there is a corresponding increase in the inhibition of hypoactive neurons, suggesting a complex remodeling at the network level (Busche et al., 2012; Calvo-Flores Guzman et al., 2018). Hyperexcitability is observed prior to the formation of plaques and worsen with increasing amyloid plaque load and disease progression in these mice. hAPP transgenic mice also exhibit cortical and hippocampal network hypersynchrony, especially during periods of reduced gamma oscillations. Spontaneous epileptiform activity has also been observed in early-onset familial forms of AD, and less frequently in later-onset sporadic cases (Govindpani et al., 2017). The AD brain is characterized by a decrease in GABA currents, along with remodeling of various GABAR subunits and GABA transporters (Govindpani et al., 2017; Kwakowsky et al., 2018). Besides compensatory alterations, these changes also reflect the reorganization of neuronal circuits and are critical to maintain a stable neuronal network (Govindpani et al., 2017; Kwakowsky et al., 2018). The amyloid cascade hypothesis proposes that Aβ is the main driver of AD pathogenesis, causing neurotoxicity and cell death. Importantly, despite evidence suggesting GABAergic vulnerability to Aβ in AD, the relationship between the GABAergic system and the amyloid cascade hypothesis remains unclear.

Most of the fast inhibitory neurotransmission in the central nervous system mediated by GABA acts through GABA type A receptors ($GABA_A$Rs) that are pentameric complexes formed by co-assembly from > 20 different subunit types (Sieghart and Savic, 2018). The type A GABA receptor alpha5-subunit ($\alpha5GABA_AR$) containing receptors are preferentially expressed in the hippocampus where they mediate tonic inhibition and are crucial for memory and cognitive processes (Glykys et al., 2008). The extrasynaptic $\alpha5GABA_A$Rs are very sensitive to low concentrations of ambient GABA, and their continuous activation generates a tonic hyperpolarizing conductance and the modulation of tonic activity could be one of the principal targets of GABAergic drugs in the AD brain (Calvo-Flores Guzman et al., 2018). Elevated extrasynaptic GABA levels have been suggested to cause serious pathological changes leading to cell death in the AD brain (Marczynski, 1998; Govindpani et al., 2017). This is in agreement with the observed increased GABA levels in the culture media following $A\beta_{1–42}$ administration (Vinnakota et al., 2020). Due to technical challenges, alterations in extracellular GABA levels have not yet been measured in human AD brains. However, rodent microdialysis studies suggest that ambient GABA concentrations can reach low micromolar levels that have detrimental consequences on neuronal survival *in vitro* (Marczynski, 1998). The chronic presence of ambient GABA paradoxically increases a cell's vulnerability to glutamate toxicity, resulting in neuronal dystrophy and cell death. *In vitro* kainate death is enhanced by 1–10 M GABA acting on $GABA_A$Rs. GABA seems to promote kainate toxicity, because in cortical cell cultures at 3 h after incubation with 1 mM kainate and 1 mM GABA the initial toxic effects of GABA decline at 6 hours, and disappears by 24 hours (Marczynski, 1998). The GABA synthesizing enzymes and GABA transporters, play an important role in the regulation of synaptic and extracellular GABA levels, and thus in the modulation of GABAergic tone and E/I balance (Govindpani et al., 2017). They exhibit altered expression profiles in AD and might contribute to and/or compensate for the disrupted E/I balance and anomalous neuronal excitability observed in the disease (Kwakowsky et al., 2018).

In CA1 pyramidal cells, tonic $GABA_A$R-mediated inhibition exhibits marked outward rectification. The degree of outward rectification depends inversely on the extracellular GABA concentration (Pavlov et al., 2009). This outward rectification means that tonic $GABA_A$R-mediated conductance has a minimal effect upon subthreshold membrane potential variations, but predominantly affects neurons at the spiking threshold. Therefore contrary to expectation, tonic inhibition in hippocampal pyramidal cells acts predominantly as an offset and has minimal effect on neuronal gain (Pavlov et al., 2009). Changes in offset alter the input detection threshold without changing the neuron's sensitivity to changes in input. Modulation of tonically active $GABA_A$Rs by fluctuations in extracellular GABA concentrations potentially provides a powerful mechanism to alter neuronal offset independently of neuronal gain. An increase in tonic inhibition could increase the threshold of the input before the pyramidal cell starts firing, but would maintain the sensitivity of the input-output relationship to a change in input (Pavlov et al., 2009).

Several studies suggest that the maintenance of $\alpha5GABA_AR$ expression levels is critical for learning and memory processes. $\alpha5GABA_AR$ knock-out or point mutant mice have enhanced cognitive performance. α5 subunit knockout mice perform significantly better in spatial memory tasks, whilst mice with selective reduction of the α5 subunit in hippocampal pyramidal cells showed improvements in associative learning. On the other hand, the upregulation of $\alpha5GABA_A$Rs in the dentate gyrus of 5×FAD mice leads to enhanced tonic inhibition and impairments in spatial memory. We have also found increased levels of $\alpha5GABA_AR$ in the CA1 hippocampal subregion and subiculum of the human AD brain (Kwakowsky et al., 2018). We demonstrated that $A\beta_{1–42}$ is able to upregulate $\alpha5GABA_AR$ expression in hippocampal primary neuronal cultures (Vinnakota et al., 2020) and increases tonic GABAergic current in the CA1 pyramidal neurons in mice (Calvo-Flores Guzman et al., 2020). Hyperexcitability in AD might lead to the upregulation of $\alpha5GABA_AR$ expression that could be compensatory for the altered GABAergic tone, a mechanism to enhance tonic inhibition. However, increased tonic inhibition also suppresses long-term potentiation (LTP) and results in memory deficits in 5×FAD mice, while reducing tonic inhibition rescues cognitive function (Calvo-Flores Guzman et al., 2018). Thus, compounds that can enhance LTP through blocking $\alpha5GABA_A$Rs and decreasing tonic inhibition could be a potential target in the amelioration of GABAergic deficits in AD.

The $\alpha5GABA_A$Rs are targets for GABAergic drugs that improve cognitive function by regulating tonic inhibitory currents (Calvo-Flores Guzman et al., 2018). An inverse agonist of $\alpha5GABA_AR$, 3-(5-methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methyloxy]-1,2,4-triazolo[3,4–a] phthalazine (α5IA) has cognition-enhancing properties (Dawson et al., 2006; Atack, 2010). α5IA is an $\alpha5GABA_AR$-selective inverse agonist with well-characterized *in vitro* and *in vivo* pharmacological properties (Dawson et al., 2006; Atack, 2010). The compound was shown to significantly enhance LTP and cognition in wild-type rodents without inducing convulsions, seizures or anxiety, or altering motor function. In young and elderly human subjects α5IA is well tolerated up to a dose of 6 mg in multiple-dose studies. Although α5IA does not improve performance in elderly subjects in a paired-associate learning task, it is able to reverse the ethanol-induced impairment in the performance of healthy young volunteers. However, the number of studies assessing the drug's effects on cognitive performance in humans is very limited. Therefore, more studies are required to draw conclusions about its effectiveness in different age groups and cognitive impairment types. Recent mice studies demonstrate that α5IA restores learning and memory functions of Ts65Dn mice, a genetic model for Down syndrome, in the novel-object recognition and in the Morris water maze tasks. Furthermore, following behavioral stimulation, α5IA enhances learning-evoked immediate early gene products in specific brain regions involved in cognition. These data demonstrated that in humans α5IA may be effective at increasing

performance at least under certain conditions (Dawson et al., 2006; Calvo-Flores Guzman et al., 2018). However, despite all the positive indications for potential, no studies have yet investigated the ability of the drug to ameliorate cognitive impairment in chronic conditions, such as AD.

Our recent study characterized the effects of α5IA on amyloid beta ($A\beta_{1–42}$)-induced molecular and cellular changes (Vinnakota et al., 2020). Mouse primary hippocampal cultures were exposed to either $A\beta_{1–42}$ alone, or α5IA alone, α5IA with $A\beta_{1–42}$ or vehicle alone, and we found that $A\beta_{1–42}$-induced cell death was significantly reduced by α5IA. Treatment with 100 nM of α5IA reduced $A\beta_{1–42}$-induced cell loss after 6 hours, 24 hours, and 5 days of treatment. We found that levels of GABA were altered following $A\beta_{1–42}$ treatment, with an increase in released GABA and a decrease in intracellular GABA in primary hippocampal neuronal cultures. Furthermore, we detected the upregulation of $\alpha5GABA_AR$ expression, a key pathological feature of the AD hippocampus (Kwakowsky et al., 2018; Vinnakota et al., 2020). We have also demonstrated that tonic GABAergic current is increased in the CA1 pyramidal neurons of $A\beta_{1–42}$-injected mice (Calvo-Flores Guzman et al., 2020). Therefore, these data suggest that $A\beta_{1–42}$-induced increase in ambient GABA and upregulation of $\alpha5GABA_AR$ expression could result in increased activation of $5GABA_ARs$, leading to increased tonic inhibition, ultimately disrupting the E/I balance and causing cell death which potentially contributes to the observed cognitive decline in AD (**Figure 1**) (Kwakowsky et al., 2018; Calvo-Flores Guzman et al., 2020; Vinnakota et al., 2020). We also found that α5IA decreased the $A\beta_{1–42}$-induced increase in ambient GABA levels and restored $5GABA_AR$ expression. Hence, it is possible that α5IA, which binds to $\alpha5GABA_ARs$ and acts as an inverse agonist, works to restore the E/I balance, thus preventing cell death. The modulation of tonic activity could be a promising target of GABAergic drugs in the AD brain but more research is required to explore their potential (Calvo-Flores Guzman et al., 2018). These novel findings and the observation that $\alpha5GABA_ARs$ are relatively preserved in the AD hippocampus, led us to suggest for the first time that these receptors might be an important drug target in AD. Several negative modulators of $\alpha5GABA_ARs$ have been demonstrated to enhance hippocampal-dependent learning and memory with minimal off-target effects. Treatment with these compounds was shown to restore learning and memory function in various animal models as well as in healthy humans (Sieghart and Savic, 2018). $GABA_AR$ negative allosteric modulators act by decreasing the effect of GABA at its receptor, decreasing the excessive tonic conductance caused by the elevated extrasynaptic GABA levels in the AD brain. However, the exact mechanisms behind the memory-enhancing effects are unknown. Evidence also suggests beneficial effects of positive allosteric modulators at $\alpha5GABA_ARs$ on cognition in the aging brain and neuropsychiatric disorders characterized by cognitive deficits with memory impairments but more research is required to explore if they might hold potential as future AD therapeutics (Calvo-Flores Guzman et al., 2018; Sieghart and Savic, 2018).

In summary, our findings provided evidence that targeting $\alpha5GABA_ARs$ with α5IA reverses the Aβ-induced increase in ambient GABA levels, α5 subunit upregulation, and reduces cell death. These findings suggest for the first time that the $\alpha5GABA_ARs$ might be important drug targets in AD. Targeting these receptors might enable us to ameliorate the imbalance in E/I neurotransmission, prevent cell death, and potentially have a disease-modifying effect in AD. $\alpha5GABA_AR$ inverse agonists could offer promising avenues in the development of these therapeutic strategies. Importantly, more research is necessary to determine whether α5IA or other inverse agonists targeting $\alpha5GABA_AR$ have efficacy to improve cognitive deficits of the kind found in AD. Furthermore, the exact mechanism of action behind the beneficial effects of α5IA treatment on cognitive performance, the molecular, cellular, and physiological changes involved have yet to be determined.

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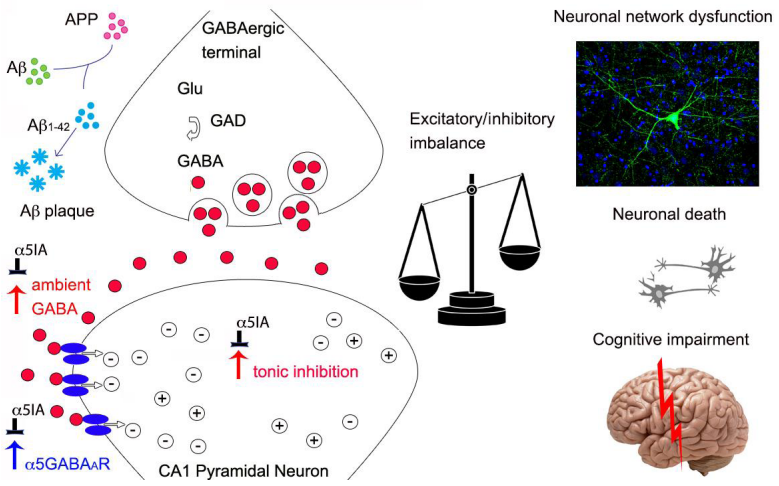

Figure 1 | α5IA restores $A\beta_{1–42}$-induced increase in ambient GABA levels, upregulation of $\alpha5GABA_AR$ expression, and increase in tonic inhibitory conductance.
The extra-synaptic high affinity $\alpha5GABA_ARs$ can be persistently activated by low concentrations of ambient GABA to generate tonic inhibition, which is important for learning and memory. $A\beta_{1–42}$-induced increase in ambient GABA and upregulation of $\alpha5GABA_AR$ expression could result in increased activation of $\alpha5GABA_ARs$, leading to increased tonic inhibition, ultimately disrupting the excitatory/inhibitory balance, leading to network dysfunction and causing cell death which potentially contribute to the observed cognitive decline in Alzheimer's disease. $\alpha5GABA_ARs$ are potential drug targets for the treatment of the disease. The inverse agonist α5IA acting through these receptors decreases $A\beta_{1–42}$-induced increase in tonic inhibition, restores ambient GABA levels, and expression of α5GABAARs. Aβ: Amyloid-β; APP: amyloid precursor protein; α5IA: 3-(5-methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methyloxy]-1,2,4-triazolo[3,4–a]phthalazine; GABA: gamma-aminobutyric acid; $GABA_AR$: GABA type A receptor; GAD: glut amic acid decarboxylase; Glu: glutamate.

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