(Accepted for publication at ACS Chemical Neuroscience)

Insight into novel treatment for cognitive dysfunctions across disorders

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Abstract

Cognitive dysfunctions, including impaired attention, learning, memory and problem solving, occur in psychiatric diseases, such as depression, and are a hallmark of aging. Altered GABAergic signaling is similarly reported across these conditions, but therapeutic approaches are limited by pan-receptor activities of benzodiazepine-like ligands. α5-GABA-A receptor-preferring ligands uncover novel therapeutics for cognitive dysfunctions.

Cognitive dysfunctions across categorical diagnostics

Cognitive dysfunctions, including impaired attention, learning, memory and problem solving, occur in the majority of patients suffering from psychiatric diseases (major depression, bipolar depression and schizophrenia), are a hallmark of normal aging, and define some age-related disorders (mild cognitive impairment, Alzheimer's disease and other dementias). Cognitive dysfunctions are often maintained in patients in remission from a depressive episode, predict depression relapse and are linked to increased suicidality. Cognitive dysfunctions greatly reduce the quality of life for affected individuals and their family, and significantly impact the health care system and society. The population suffering from cognitive dysfunctions includes 300M+ people

worldwide for depression and at least as many for late-life cognitive disorders. However, there is currently no effective drug treatment targeting cognitive dysfunctions. This unmet therapeutic need and huge burden of cognitive dysfunctions highlight the urgency of developing novel therapeutics targeting cognitive dysfunctions.

Brain GABAergic inhibitory dysfunctions: A shared pathology mediating mood and cognitive symptoms across brain disorders and during aging

Reduced brain inhibitory neurotransmission is well documented in neuropsychiatric and neurodegenerative disorders, and during aging, as measured by magnetic resonance spectroscopy (reduced inhibitory γ-amino butyric acid (GABA) neurotransmitter levels) and transcranial magnetic stimulation (reduced cortical inhibition). At the cellular level, glutamatergic pyramidal neuron (PN) excitatory information is controlled by different subtypes of GABAergic inhibitory interneurons. These interneurons express different neuropeptides, such as somatostatin (SST), parvalbumin (PV) or vasopressin (VIP), and target different PN cellular compartments (**Fig.A**). Studies in human postmortem tissue from our group and others have reported changes affecting GABAergic genes and SST+ interneurons (**Fig.A**) across the same set of brain disorders, and genetic mouse models now show that reducing the function of SST, of SST+ GABAergic neurons, or of the receptor mediating their functions, are sufficient to induce mood-related behaviors and, importantly, cognitive dysfunctions[1,2].

Target identification: α5-GABAA receptors

The inhibitory actions of GABA are mediated via the activation of ionotropic GABAA receptors (GABAA-R) for rapid inhibition through chloride influx, and of metabotropic GABAB-Rs for slower, prolonged inhibition. GABAA-Rs are heteropentameric ligand-gated ion channels assembled from five subunits, most commonly two α, two β, and one γ subunit. They are localized to specific cellular compartments and partly mediate the activity of the distinct GABAergic interneuron subtypes. For instance, α5-GABAA-Rs are expressed exclusively in PN distal dendrites, α1- and α3-GABAA-Rs are expressed in all neuronal compartments, and α2-GABAA-Rs are expressed at the axon hillock. α5- and α2-GABAA-Rs regulate excitatory signal input and output, respectively, and α1- and α3-GABAA-Rs exert a general tuning and toning down of the microcircuit.

SST+ interneurons signal in large part through α5-GABAA-Rs, which are exclusively expressed in brain regions involved in cognitive processes, including high levels in the hippocampus and intermediate levels in cortical regions and ventral striatum. At the synapse, α5-GABAA-Rs are located both extrasynaptically, where they modulate tonic inhibition, and

synaptically, where they mediate up to 60% of GABAergic synaptic functions. In addition to SST, the expression of α5-GABAA-Rs and GABA synthesizing enzymes (GAD65/67) mRNAs are decreased in brain disorders, suggesting reduced signaling from SST+ GABAergic neurons, and reduced input control onto PNs. Consistent with the role of SST+ interneuron signaling in cognitive functions, we hypothesized that activating α5-GABAA-Rs would reduce cognitive dysfunctions in rodent models of psychiatric disorders and of aging. We also hypothesized that facilitating activity of GABA at its α5-GABAA-Rs would prevent dendritic shrinkage and potentially neuronal atrophy in the aging brain. If confirmed, such activity would provide a strong rationale for targeting α5-GABAA-Rs for symptomatic and disease-modifying effects in brain disorders and during aging.

Target engagement: design of novel ligands activating α5-GABAA-Rs

GABA binds at the interface between α and β subunits of GABAA-Rs, whereas benzodiazepines (BZDs), the most common GABAergic drugs, bind to a distinct allosteric site, at the interface between one of four distinct α subunits (α1,2,3,5) and the γ subunit, and positively modulates the action of GABA. Consequently, BZDs have broad sedative, anxiolytic, anticonvulsant and amnesic effects, due to non-specific GABAA-R targeting. This has considerably limited their therapeutic potential. Recent anatomical, genetic and functional characterization of the various α-subunit containing GABAA-Rs has raised hopes that targeting specific α-GABAA-Rs will uncover novel therapeutic opportunities for neuropsychiatric disorders. For instance, existing ligands with activity at α5-GABAA-Rs display pro-cognitive effects, notably in age-related models[3], although none of these ligands are in pharmaceutical development.

Based on the pharmacophore receptor model for $\alpha_5\beta_3\gamma_2$ subtypes (predominant α5-GABAA-Rs), series of ligands were generated and tested for efficacy and potency as positive allosteric modulator (PAM) at α-GABAA-Rs (α5-PAM) and for bioavailability[4]. Specifically, we designed novel imidazobenzodiazepine (IBZD) molecules based on a hybrid diazepam/flumazenil chemical backbone (**Fig.B**), due to binding affinity for the target and proven clinical safety of these molecules. We previously developed ethyl esters of IBZDs; however, esters are quickly metabolized. Amides are commonly used as replacements to improve metabolic stability and bioavailability. We also performed chemical optimization for positive allosteric modulation at the α5-subunit and for drug-like properties[4].

Preclinical characterizations of α5-GABAA-R PAMs

Our group developed a series of amide of IBZD ligands with drug-like properties, acceptable pharmacokinetic and metabolic profiles in rodent and human microsomal assays and preferential

positive allosteric modulation at α5-GABAA-Rs. These ligands have moderate binding affinity (high nM to low μM range) and efficacy at potentiating GABA-induced Cl^- flux, around 10-fold lower affinity and efficacy at α1-GABAA-R compared to α5-GABAA-R and, in some case, >10-fold therapeutic window, predicting safe profile in human, matching available benzodiazepines.

Using rodent behavioral testing, we show that preferential activity at α5-GABAA-Rs with reduced activity at α1-GABAA-Rs uncovers potential antidepressant efficacies, in addition to the expected anxiolytic effects of benzodiazepine-like ligands[5] (**Fig.C-D**). Using the spontaneous alternation task in the Y-Maze apparatus for spatial working memory, we also show that a selection of these ligands can reverse deficits in working memory induced by stress in adult animals (**Fig.E**) or that occur naturally during aging (**Fig.F**). We speculate that the variability in behavioral profiles of the various ligands is due to different activity profiles at the different GABAA-R subtypes, combined with different pharmacokinetic profiles. We also noticed that some ligands maintained their efficacy at reversing working memory impairments after chronic administration, together displaying a drastically different behavioral profile from BZD ligands. Based on these findings, we believe that developing novel therapeutics targeting α5-GABAA-Rs is a promising avenue for the alleviation of cognitive dysfunctions in psychiatric diseases and during aging.

Recent findings from our group also identified potential effect of chronic treatment with an α5-PAM in reversing age-induced dendritic shrinkage in the prefrontal cortex of aging mice (unpublished data), together suggesting both symptomatic and disease-modifying effects.

Summary

The current results indicate that α5-GABAA-R ligands with low affinity and moderate efficacy displayed broader therapeutic profile and reduced side-effects, compared to typical BZD-like ligands with high-potency and pan-activity at multiple α-GABAA-Rs. This *low efficacy-low adversity* is a pharmacological approach that has not been fully exploited by the pharmaceutical industry, although it has great potential, specifically for targeting the GABAergic system. Testing these α5-GABAA-R ligands in mouse models for pro-cognitive and other therapeutic efficacy has now provided critical information on structure-activity relationship, and support the therapeutic potential of augmenting GABAergic functions beyond the known efficacy of BZDs on anxiety. The current results suggest a novel therapeutic avenue for cognitive dysfunctions in depression, during aging, and potentially extending to other brain disorders characterized by reduced SST-positive GABAergic neuron deficits (i.e. bipolar depression and schizophrenia). This novel approach would not only attenuate the symptoms but also limit the underlying pathology responsible in part for the cognitive decline in aging and in brain diseases.

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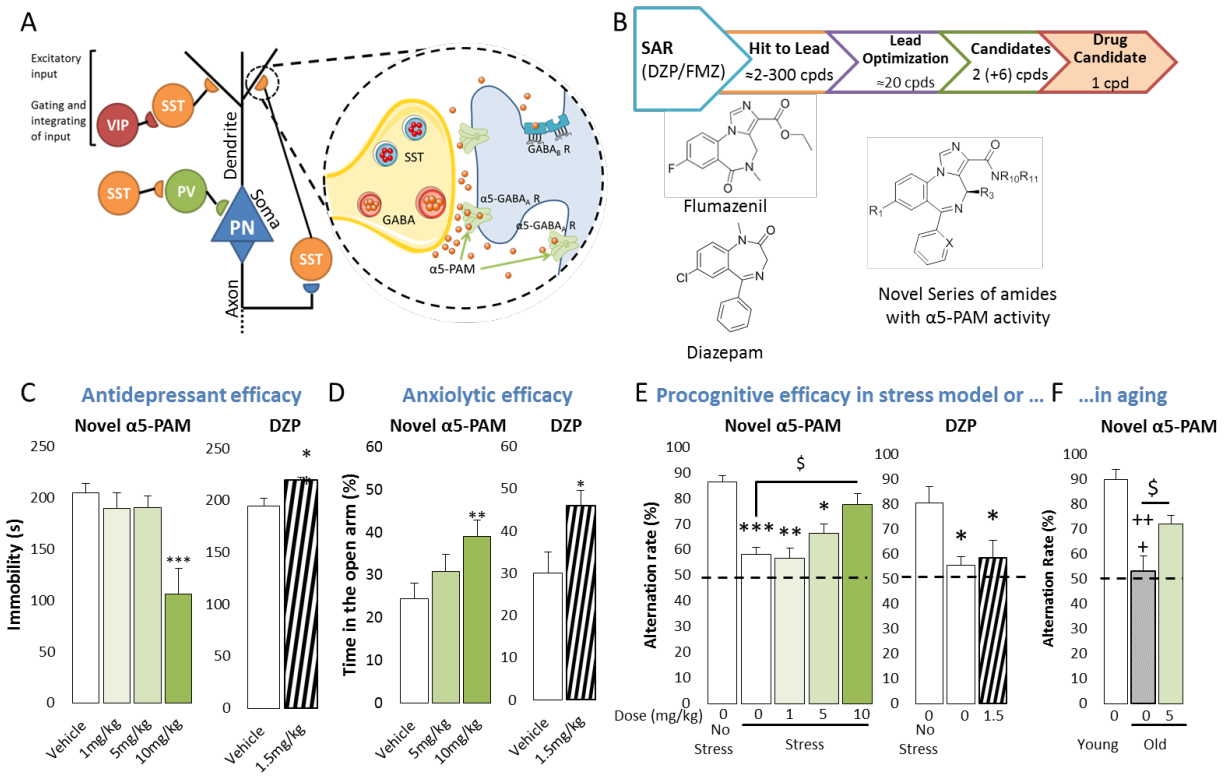

Figure 1: Targeting α5-GABAA-Rs for antidepressant, anxiolytic and pro-cognitive therapeutic efficacies.

Glutamatergic pyramidal neuron (PN) excitatory information is controlled by various interneurons, including somatostatin- (SST), parvalbumin- (PV) and vasopressin- (Vip) expressing cells (A). Based on the pharmacophore of the α5-GABAA receptors, our group has developed a series of ligands using a hybrid backbone formed from diazepam and flumazenil (B). These ligands have been tested in multiple behavioral assays in mice, such as the forced swim test (C), the elevated plus maze (D) or the spontaneous alternation task (E-F). Altogether, results demonstrated antidepressant (C) anxiolytic (D) and pro-cognitive efficacies (E) of the novel α5-PAM in baseline conditions (C-D) or in stress-induced model of cognitive dysfunction (E). The pro-cognitive efficacy was further demonstrated in an age-related model of cognitive dysfunction (F). (C-F) are adapted from Prevot et al[5].

**p<0.05, **<0.01 and ***p<0.001 compared to the "Vehicle" group (C-D) or to the "No Stress" group (E). $p<0.05 compared to the "Stress (0)" group (E) or to the "Old" group. +++p<0.001 compared to the "Young" group (F). Dash-line represents chance level in the spontaneous alternation task for working memory.*