Neuropharmacology 64 (2013) 145–152

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Selective $GABA_A$ α5 positive allosteric modulators improve cognitive function in aged rats with memory impairment

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ARTICLE INFO

Article history:
Received 16 March 2012
Received in revised form
4 June 2012
Accepted 11 June 2012

Keywords:
GABA agonist
Inverse agonist
Neural hyperactivity
Water maze
Radial arm maze
Mild cognitive impairment

ABSTRACT

A condition of excess activity in the hippocampal formation is observed in the aging brain and in conditions that confer additional risk during aging for Alzheimer's disease. Compounds that act as positive allosteric modulators at $GABA_A$ α5 receptors might be useful in targeting this condition because $GABA_A$ α5 receptors mediate tonic inhibition of principal neurons in the affected network. While agents to improve cognitive function in the past focused on inverse agonists, which are negative allosteric modulators at $GABA_A$ α5 receptors, research supporting that approach used only young animals and predated current evidence for excessive hippocampal activity in age-related conditions of cognitive impairment. Here, we used two compounds, Compound 44 [6,6-dimethyl-3-(3-hydroxypropyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5H)-one] and Compound 6 [methyl 3,5-diphenylpyridazine-4-carboxylate], with functional activity as potentiators of γ-amino-butyric acid at $GABA_A$ α5 receptors, to test their ability to improve hippocampal-dependent memory in aged rats with identified cognitive impairment. Improvement was obtained in aged rats across protocols differing in motivational and performance demands and across varying retention intervals. Significant memory improvement occurred after either intracereboventricular infusion with Compound 44 (100 μg) in a water maze task or systemic administration with Compound 6 (3 mg/kg) in a radial arm maze task. Furthermore, systemic administration improved behavioral performance at dosing shown to provide drug exposure in the brain and in vivo receptor occupancy in the hippocampus. These data suggest a novel approach to improve neural network function in clinical conditions of excess hippocampal activity.

This article is part of a Special Issue entitled 'Cognitive Enhancers'.

1. Introduction

$GABA_A$ receptors are pentameric assemblies of different subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π) forming a chloride-permeable channel that is gated by the neurotransmitter γ-aminobutyric acid (GABA). $GABA_A$ receptors containing the α5 subunit have a restricted localization in rodents and humans (Sur et al., 1998) and mediate tonic inhibition of hippocampal pyramidal neurons (Glykys and Mody, 2006). Efforts to develop drugs with cognitive enhancing properties focused in the past on selective α5 inverse agonists which function as negative allosteric modulators (NAMs) (e.g., Chambers et al., 2003; Atack et al., 2006; Collinson et al., 2006; Dawson et al., 2006; Ballard et al., 2009), but such compounds have yet to prove efficacious in clinical trials designed to treat age-related cognitive impairment (Atack, 2010).

Observations in animal models now indicate that excessive neural activity in the hippocampal formation contributes to cognitive impairment in aging and in conditions that confer increased risk for Alzheimer's disease (AD). In Long-Evans aged rats with cognitive impairment, CA3 pyramidal neurons have elevated firing rates along with a loss in the ability to encode new information (Wilson et al., 2005) and CA3/dentate gyrus network excitability is also elevated in aged Fisher 344 rats (Patrylo et al., 2007). Moreover, aberrant excitability is found in the hippocampal system in transgenic mice overexpressing amyloid precursor protein (Palop et al., 2007) and in mouse models of ApoE4 (Andrews-Zwilling et al., 2010), a genotype that confers greatly elevated risk for sporadic AD. In the latter models, ApoE4 relative to E3 augmented a detrimental effect of aging on hilar interneurons that provide an inhibitory network associated with

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http://dx.doi.org/10.1016/j.neuropharm.2012.06.023

the dentate gyrus-CA3. Consistent with evidence in such animal models, data from functional magnetic resonance imaging (fMRI) has revealed increased activation in the hippocampal system in elderly humans with memory impairment, including those meeting criteria for amnestic mild cognitive impairment (aMCI) and in carriers of an ApoE4 allele (Bookheimer et al., 2000; Dickerson et al., 2005; Putcha et al., 2011; for recent review see Ewers et al., 2011). Moreover, studies using high-resolution fMRI have isolated such excess activation to the dentate-CA3 subregion of the hippocampal formation (Yassa et al., 2010a, 2010b; Bakker et al., 2012).

Supporting the view that excess hippocampal activity is a dysfunctional condition, beneficial effects in the animal models are being reported using treatments to control it, including transfection of an inhibitory neuropeptide into CA3 and systemic use of certain antiepileptic drugs (AEDs) in both aged rats (Koh et al., 2010; Rose and Corbin, 2010) and in mouse models (Sanchez et al., 2011). Pentobarbital was also reported to rescue impairment in ApoE4 knock-in mice with deficits in the function of hippocampal inhibitory interneurons (Andrews-Zwilling et al., 2010). A translational clinical study using low doses of the AED levetiracetam in aMCI patients recently demonstrated significant reduction of dentate-CA3 overactivity as measured by fMRI and improved memory performance in the scanning task (Bakker et al., 2012).

In this context, $GABA_A$ α5 receptors could be an important therapeutic target for age-related cognitive impairment. Principal neurons in the hippocampus, including CA3 pyramidal neurons, express high levels of α5 mRNA and protein (Wisden et al., 1992; Fritschy and Mohler, 1995; Haberman et al., 2011), and receptor binding follows that same topography in rodents and humans (Sur et al., 1998, 1999). In conditions of excessive neural activity, therefore, the use of positive allosteric modulators (PAMs), rather than NAMs, might be indicated. Here, we tested that concept in a well-characterized model of age-related memory impairment using Long-Evans rats. Individual variability in the degree of hippocampal-dependent memory impairment in this model has shown strong test-retest reliability (Colombo et al., 1997) and is closely related to changes in hippocampal integrity and function (Gallagher and Rapp, 1997; Wilson et al., 2006).

Behavioral improvement was obtained with two different $GABA_A$ α5 PAMs in aged rats across protocols differing in motivational and performance demands and across varying retention intervals. Significant improvement occurred after either intracereboventricular (ICV) infusion or systemic administration. Finally, systemic administration with one of the test compounds improved behavioral performance at dosing shown to provide drug exposure in the brain and in vivo receptor occupancy in the hippocampus.

2. Materials and methods

2.1. Subjects

Pathogen-free aged, male Long-Evans rats were obtained at 8–9 mo of age from Charles River Laboratories (Raleigh, NC) and housed in a vivarium at Johns Hopkins University until 24–26 mo of age. Young rats obtained from the same source were tested at 6 mo of age. Receptor occupancy studies were conducted at Covance Inc. (Indianapolis, IA) using adult, male Long-Evans rats (Charles River Laboratories, Portage, MI). All procedures were approved by Institutional Animal Care and Use Committees in accordance with the National Institutes of Health directive.

Young and aged rats were screened in a standardized assessment of hippocampal-dependent spatial cognition prior to behavioral studies with experimental treatments. Background characterization used a Morris water maze protocol as described in detail elsewhere (Gallagher et al., 1993). Briefly, rats were trained for eight days (3 trials per day) to locate a camouflaged escape platform that remained at the same location throughout training. Every sixth trial consisted of a probe (no escape platform for 30 s) that served to assess the development of a spatially localized search. A learning index was generated from the proximity of the rat to the escape platform during probe trials and was used to define impairment in the aged rats. The learning index is the sum of weighted proximity scores obtained during probe trials, with low scores reflecting a more accurate search. Cue training (visible escape platform; 6 trials) occurred on the last day of training to test for sensorimotor and motivational factors independent of spatial learning. Aged rats with impaired performance (i.e., those with learning index scores outside the young "normative" range) but successful cued training were used for the studies below.

2.2. Surgery and drug infusion

Aged behaviorally characterized rats selected for the drug study involving ICV infusion were implanted unilaterally with a guide cannula (26 gauge, Plastics One, Roanoke, VA) into the lateral ventricle under isoflurane anesthesia. Stereotaxic coordinates were 1.0 mm posterior to bregma, 1.5 mm lateral to midline, and 3.5 mm ventral to skull surface. The cannula was anchored to the skull with stainless steel screws and dental cement. A 33-gauge dummy cannula was inserted into each guide cannula to prevent clogging. Rats were given a week to recover before the start of behavioral training and drug treatment. Drug infusion was done through a 33-gauge injector cannula connected to a Hamilton microsyringe (Reno, NV). The injector extended 1.0 mm beyond the guide cannula. During the infusion, rats were held in the experimenter's lap while the drug was slowly infused. The injector cannula was left in place for an additional minute to allow diffusion of the drug away from the cannula tip.

2.3. Behavioral assessment of drug treatments

Rats with ICV cannula were trained and tested in a novel water maze environment to assess the effect of drug treatment. The water maze used was housed in a different building and was surrounded by curtains with a novel set of patterns relative to the maze used for initial assessment of cognitive status. In order to eliminate any response tendencies associated with cue training at the end of background water maze characterization, the training protocol here consisted of two days of pre-training (6 trials per day) in which the hidden escape platform location was varied from day to day. No drug treatment was given during pre-training. Rats were then given two days of training (8 trials per day in 2 training blocks) in which the hidden escape platform remained in the same location. Each trial was 60 s, with a 60 s inter-trial interval. On the third day, the rats were given a probe test (120 s) in which the platform was removed. To assess memory for the target location, the time spent and the number of passes in an annulus (1.5 times the size of the platform) where the platform was located during training was compared to those registered in a control annulus in the opposite quadrant.

A hippocampal-dependent radial arm maze task was also used to assess the effect of drug treatment as described in detail elsewhere (Chappell et al., 1998; Koh et al., 2010). The protocol allowed repeated within-subject assessment using systemic administration of drugs at different doses and in combinations. Prior research using this model has shown that reliable individual differences in hippocampal-dependent memory among aged rats translate across the water maze used for initial characterization and the radial maze task (Koh et al., 2010).

Pre-training consisted of habituation, standard win-shift training, and win-shift training with delays interposed between information and memory test phases on the eight-arm maze. Drug treatments began a day after the completion of pre-training. Three arms were blocked at the beginning of each trial (information phase). The identity and configuration of the blocked arms were varied across trials. Food-deprived rats were allowed to retrieve food reward (Kellogg's Froot Loops cereal) from the five unblocked arms. The rat was then removed from the maze for 2 h (retention interval for aged rats) or 5 h (retention interval for young rats to approximate the number of errors committed by memory-impaired aged rats under drug-free conditions), during which time the barriers on the blocked arms were removed allowing access to all eight arms. Rats were then placed back onto the center platform and allowed to retrieve the remaining food rewards (memory test phase). An error consisted of returning to an arm (all four paws on the arm) from which food had already been obtained. Note that young rats rarely commit errors in the post-delay retention test at the delay used for the aged rats in the current study (Chappell et al., 1998). The number of errors made in the retention phase was used to assess memory performance. Rats were tested with a series of drug doses in ascending/descending order; each dose, including vehicle alone, was thus tested twice.

2.4. Compounds

For the proof of concept study involving the water maze task, 6,6-dimethyl-3-(3-hydroxypropyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5H)-one, hereafter referred to as Compound 44 (a $GABA_A$ α5 receptor PAM, synthesized by DavosPharma, Upper Saddle River, NJ; Chambers et al., 2003), was dissolved in dimethyl sulfoxide. This compound has activity as a PAM at α5 receptors and high selective affinity to those binding sites (+25 ± 10% potentiation of GABA EC_{20} concentration, with a Ki of 4.7 nM at α5 relative to other $GABA_A$ binding sites, Ki > 48 nM, as reported in Chambers et al., 2003). Compound 44 (or vehicle of equal