Neuropharmacology 123 (2017) 395–409

Contents lists available at ScienceDirect

Neuropharmacology

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

The AMPA receptor positive allosteric modulator S 47445 rescues *in vivo* CA3-CA1 long-term potentiation and structural synaptic changes in old mice

Albert Giralt [a, b, c], María Ángeles Gómez-Climent [d], Rafael Alcalá [a, b, c], Sylvie Bretin [e], Daniel Bertrand [f], José María Delgado-García [d], Esther Pérez-Navarro [a, b, c], Jordi Alberch [a, b, c], Agnès Gruart [d, *]

[a] Departament de Biomedicina, Facultat de Medicina, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain
[b] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[c] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain
[d] Division of Neurosciences, Pablo de Olavide University, Seville, Spain
[e] Pôle Innovation Thérapeutique Neuropsychiatrie, Institut de Recherches Internationales Servier, Suresnes, France
[f] HiQScreen, Vésenaz, Geneva, Switzerland

ARTICLE INFO

Article history:
Received 24 January 2017
Received in revised form
27 May 2017
Accepted 5 June 2017
Available online 8 June 2017

Chemical compounds studied in this article:
Hydroxyethylcellulose (PubChem CID: 24846132)
Polysorbate 80 (PubChem CID: 5284448)
S 47445 (PubChem CID: 24857397)

Keywords:
AMPA modulator
LTP
Young and old mice
Paired-pulse facilitation
Spinophilin
VGlut-1

ABSTRACT

Positive allosteric modulators of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are small molecules that decrease deactivation of AMPARs via an allosteric site. These molecules keep the receptor in an active state. Interestingly, this type of modulator has been proposed for treating cognitive decline in ageing, dementias, and Alzheimer's disease (AD). S 47445 (8-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-7,8-dihydro-3H-[1,3]oxazino[6,5-g][1,2,3]benzotriazine-4,9-dione) is a novel AMPAR positive allosteric modulator (AMPA-PAM). Here, the mechanisms by which S 47445 could improve synaptic strength and connectivity were studied and compared between young and old mice. A single oral administration of S 47445 at 10 mg/kg significantly increased long-term potentiation (LTP) in CA3-CA1 hippocampal synapses in alert young mice in comparison to control mice. Moreover, chronic treatment with S 47445 at 10 mg/kg in old alert animals significantly counteracted the deficit of LTP due to age. Accordingly, chronic treatment with S 47445 at 10 mg/kg seems to preserve synaptic cytoarchitecture in old mice as compared with young control mice. It was shown that the significant decreases in number and size of pre-synaptic buttons stained for VGlut1, and post-synaptic dendritic spines stained for spinophilin, observed in old mice were significantly prevented after chronic treatment with 10 mg/kg of S 47445. Altogether, by its different effects on LTP, VGlut1-positive particles, and spinophilin, S 47445 is able to modulate both the structure and function of hippocampal excitatory synapses known to be involved in learning and memory processes. These results open a new window for the treatment of specific age-dependent cognitive decline and dementias such as AD.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is a complex neurodegenerative disorder clinically characterized by a progressive loss of cognitive functions and alteration of different behaviors (Rampa et al., 2013). The currently available symptomatic AD treatments such as acetylcholinesterase inhibitors and memantine have shown efficacy in numerous randomized controlled trials, but the magnitude of effects is limited or restricted to a certain population of patients as observed in comprehensive meta-analysis (Rampa et al., 2013). Moreover, they induced clear secondary effects involving gastrointestinal disorders (Tricco et al., 2013). Thus, pharmacological targeting of other molecular pathways remains necessary in order to have more-effective drugs for AD patients (Partin, 2015; Reuillon et al., 2016; Tayeb et al., 2012).

Among glutamatergic pathways, the positive modulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

* Corresponding author. Division of Neurosciences, Pablo de Olavide University, Seville 41013, Spain.
E-mail address: agrumas@upo.es (A. Gruart).

http://dx.doi.org/10.1016/j.neuropharm.2017.06.009

receptors is one interesting target, since AMPA receptors are key actors of long-term potentiation (LTP), a form of synaptic plasticity that mediates learning and memory (Bliss and Collingridge, 1993; Brown and Banks, 2015; Chater and Goda, 2014; Gruart et al., 2015; Huganir and Nicoll, 2013; Whitlock et al., 2006). Moreover, a clear dysfunction of central glutamatergic pathways occurs during ageing and dementias such as AD (Henley and Wilkinson, 2013; Robbins and Murphy, 2006; Rudy et al., 2015). N-methyl-D-aspartate (NMDA) and AMPA receptors are reported to be dramatically reduced at synapses from human AD postmortem brain tissues (Gong et al., 2009) and Aβ impairs synaptic plasticity by several mechanisms impacting glutamate receptors—notably, by inducing endocytosis of glutamate receptors and activating signaling pathways involved in long-term depression and spine loss (Gasparini and Dityatev, 2008; Parameshwaran et al., 2008).

Herein, we report the *in vivo* characterization of S 47445 (8-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-7,8-dihydro-3H-[1,3]oxazino[6,5-g][1,2,3]benzotriazine-4,9-dione), a novel selective positive allosteric modulator of the AMPA receptors (AMPA-PAM) discovered through collaboration between Cortex Pharmaceuticals and Servier (Fig. 1). In a preliminary study, the mechanism of action of S 47445 towards AMPA receptors and its selectivity were tested on AMPA, NMDA, and kainate receptors expressed in *Xenopus laevis* oocytes (Danober et al., 2016). Subsequently, and based on the glutamatergic dysfunctions observed in AD, this study was focused on hippocampal CA3-CA1 synaptic plasticity and connectivity in young (3-months-old) and old (14-months-old) freely moving mice, and assessed whether S 47445 corrects the aged deficit after either acute or chronic treatment. The micro-cytoarchitecture in hippocampal and cortical tissue was also evaluated in order to have a wider perspective of the drug's effects since preclinical and clinical evidence had shown that a functional coupling exists between hippocampal formation and prefrontal cortex and has an important role in cognition and emotional regulation (Godsil et al., 2013). In particular, we evaluated the drug's effect on the presynaptic vesicular glutamate transporter VGlut1, which maintains the level of glutamate stored in vesicles (Balschung et al., 2010; Cheng et al., 2011; Fremeau et al., 2001; Wojcik et al., 2004), and on the postsynaptic protein spinophilin, which participates in functional plasticity in dendritic spines (Stafstrom-Davis et al., 2001), in both young and old mice.

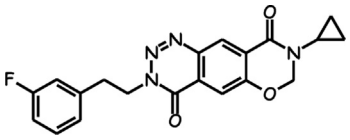

Fig. 1. Chemical structure of S 47445. Chemical name of S 47455 is 8-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-7,8-dihydro-3H-[1,3]oxazino[6,5-g][1,2,3]benzotriazine-4,9-dione.

2. Material and methods

2.1. Electrophysiological recordings in Xenopus laevis oocytes

Following deep anesthesia in 0.15% tricaine methanesulfonate (MS222), a small incision was made in the lower part of the abdomen of *Xenopus laevis* and the ovary was removed. Animals were euthanatized according to the Swiss animal welfare rules under the authorization No 27479 GE/15/16. Following standard mechanical and enzymatic dissociation using collagenase, oocytes were placed at 17 °C in a sterile Barth solution containing (in mM) NaCl 88, KCl 1, $NaHCO_3$ 2.4, HEPES 10, $MgSO_4 \cdot 7H2O$ 0.82, $Ca(NO_3)2$ 0.33, and $CaCl_2$ 0.41, at pH 7.4, and supplemented with 20 μg/mL of kanamycin, 100 unit/mL penicillin, and 100 μg/mL streptomycin. On the second day after dissociation, oocytes were placed in a 96-well microtiter plate with conical bottom, and injected with 10 nL solution containing the cDNA encoding for the desired receptors (human NR1a/NR2B, human GluA1flop/GluA2flip, or human GluK2) at a concentration of 0.2 μg/μL using the Roboinjected (Multichannel Systems, Reutlingen, Germany). They were then stored at 17 °C until use (typically 1–2 weeks).

Electrophysiological recordings were performed at 18–20 °C by using two-electrode voltage clamp recordings with the HiClamp automated system (Multichannel Systems, Reutlingen, Germany). Oocytes were superfused with OR2 medium containing (in mM) NaCl 88.5, KCl 2.5, HEPES 5, $MgCl_2$ 1, $CaCl_2$ 1.8, and $Na2HPO_4$ 1, pH 7.4. For NMDA recordings, $MgCl_2$ was omitted from the medium. Unless indicated, cells were held at −80 mV. Compounds were tested by bath application. For recordings on oocytes expressing subunits of human AMPA receptors or GluK2 or NR1a/NR2B, application of 300 μM of glutamate or 100 μM of glutamate + 10 μM of glycine was first performed for 20 s, respectively. S 47445 (micronized form) was then bath-applied at 100 μM on the same oocyte for 45 s before and 20 s during the application of glutamate. S 47445 was dissolved in DMSO. The DMSO concentration was below 1%, which is known to have no significant effects in the experimental models tested (see Fig. 2).

2.2. Experimental animals

Male C57Bl6 mice were used in this study. Animals were

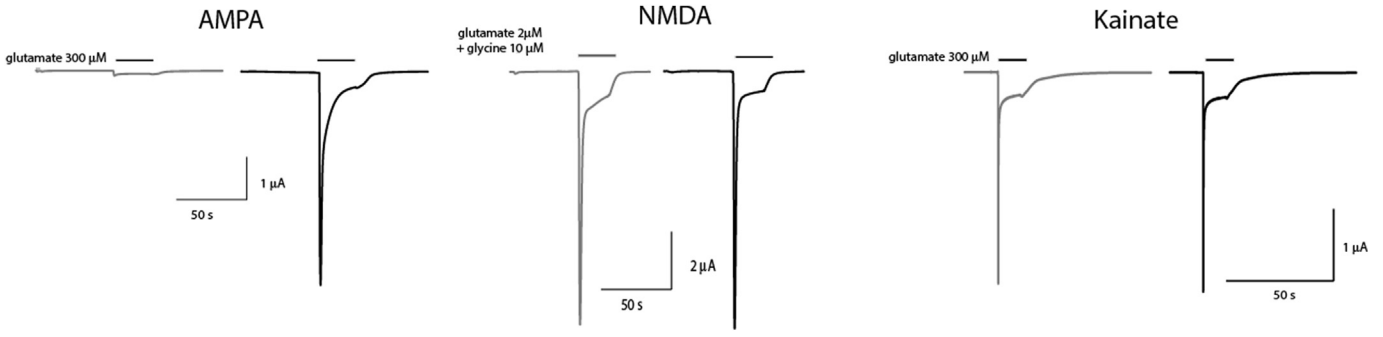

Fig. 2. S 47445 is a powerful and selective allosteric modulator of AMPA receptors. Effects of S 47445 tested at human AMPA, NMDA (NR2B-containing), and kainate receptors expressed in Xenopus oocytes. Exposure to 100 μM S 47445 causes a very large potentiation of the glutamate-evoked current at AMPA receptors (GluA1flop/GluA2flip AMPA receptors) compared with the response evoked in the same cell recorded in controls. Recordings obtained in comparable conditions at NMDA receptors (NR1a-NR2B) revealed that S 47445 causes no potentiation of the glutamate-evoked current. Similar results were observed at the kainate receptors. Gray traces were recorded in control conditions while black traces were recorded during exposure to 100 μM S 47445.