Functional characterization of the potent AMPA positive allosteric modulator S 47445

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INTRODUCTION

The findings that positive allosteric modulators of the AMPA receptors display procognitive and antidepressant-like effects that would be valuable for the treatment of neurological or psychiatric diseases such as Alzheimer or major depression [1], prompted the search of new molecules showing enough efficacy and selectivity at these glutamate receptors receptor subtypes.

The compound S 47445 showed efficacy in different in vivo animal models assessing cognition or antidepressant/anxiolytic-like effects [2; 3]. Calling for further characterization of the mode of action of S 47445, functional experiments on recombinant human AMPA receptors expressed in *Xenopus* oocytes or mammalian cells were conducted.

[1] Lynch G (2006) Glutamate-based therapeutic approaches: ampakines. Curr Opin Pharmacol 6:82-88

[2] Bretin (2017) Pharmacological characterisation of S 47445, a novel positive allosteric modulator of AMPA receptors. PLoS One. Sep 8;12(9).

[3] David (2017) S 47445 produces antidepressant and anxiolytic-like effects through neurogenesis dependent and independent mechanisms. Front Pharmacol. 8:462.

MATERIALS AND METHODS

- **Electrophysiological recordings in *Xenopus* laevis oocytes on human AMPA subunits:**

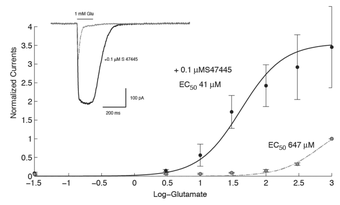
Oocytes were injected with 10 nl solution containing the cDNA encoding for the desired subunits at a concentration of 0.1 µg/µl. For experiments conducted on AMPA/Kainate chimera, oocytes were injected with 20 nl solution of mRNA at 0.2 µg/µl. Injected oocytes were incubated at 16-18° C for a minimum 3 days in Barth's solution before electrophysiological testing. They were then stored at 4° C until use (typically 1-2 weeks). For recordings, application of either 300 µM or 1 mM glutamate was first performed for 20 s. S 47445 was then bath-applied on the same oocyte for 45 s before and 20 s during the application of glutamate in a concentration dependant manner with 2.5 min interval. Amplitudes of evoked currents were evaluated at the peak of the inward currents. The amplitude of agonist-evoked currents in the presence of AMPA-PAMs was normalized to unity versus the initial control response to the agonist alone evoked on the same oocyte (taken as unity). Data were filtered at 10 Hz, captured at 100 Hz and analysed . For GluK2 receptors data were filtered at 100 Hz and captured at 300 Hz.

- **Patch clamp recordings in transiently transfected HEK-293 cells for expression of GluR1o/GluR2i:**

Whole cell patch clamp recording was done at holding potential of –80 mV in a medium containing 150mM NaCl, 4mM KCl, 8mM HEPES, 1.8mM $CaCl_2$, 1mM $MgCl_2$ and 20 mg/ml bovine serum albumin adjusted to pH 7.4 at room temperature using borosilicate glass pipettes filled with a medium containing 135 mM CsCl, 1 mM $MgCl_2$ and 10 mM HEPES, pH adjusted to 7.2 with CsOH and presenting about 3 MΩ connected to an AxonClamp 200A amplifier. Data were captured with an analog to digital converter, recordings were done at 2 KHz and analysis was conducted off line.

RESULTS

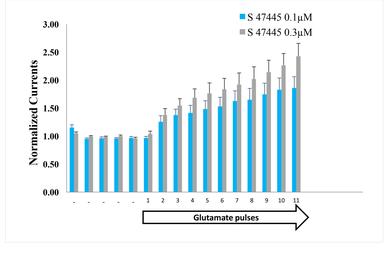
Potentiation of the glutamate-evoked current in GluA1flop/GluA2flip AMPA receptors expressed in HEK-293 cells



Typical current recorded in the whole cell configuration in response to a brief pulse of glutamate in control (grey line) and during exposure to S 47445 (black line). Cell was held at -80 mV. Plot of the concentration activation curve recorded in control and in presence of 0.1 µM S 47445 illustrates that this compound causes a left shift of the curve and increase of the maximal amplitude (n = 4).

Exposure to a low concentration of S 47445 (0.1 µM) increased the sensitivity to glutamate (from 647 ± 21.9 to 41 ± 10.9 µM) and the amplitude of the response (from 94.7 ± 32.8 to 247 ± 70 pA).

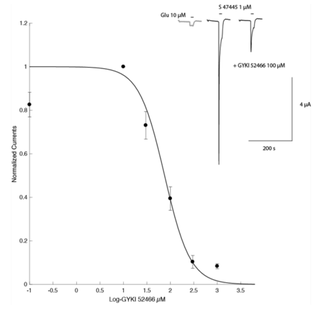
Potentiation of the glutamate-evoked current in GluA1flop/GluA2flip AMPA receptors expressed in *Xenopus* oocytes



Normalised currents in response to repetitive pulses of 10 µM glutamate applied for 20 s, 1/min and during exposure to low concentrations of S47445 (0.1 and 0.3 µM). Currents were normalized to the mean of the first 5 recordings in vehicle conditions illustrated by the symbol (-)

Exposure to low concentrations of S 47445 causes a rapid increase of potency of the AMPA receptors currents with no development of desensitization following several pulses of glutamate.

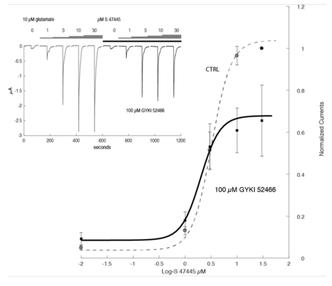
Mean concentration-response to GYKI52466 in presence of 1 µM of S 47445



Typical currents in a cell expressing are shown in the upper panel and concentration-response curve in the lower panel.

The potentiation effect of S 47445 observed at the concentration of 1 µM on the amplitude of the glutamate-evoked (10 µM) was reduced by the selective AMPA receptor antagonist GYKI52466 applied from the 10 to 1000 µM µM concentration.

The inhibitor GYKI52466 attenuates the potentiation caused by S 47445 but not the sensitivity



Response evoked by 10 µM glutamate conducted in absence or presence of GYKI52466. Typical currents evoked by a 10 µM glutamate test pulse recorded in the same cell are illustrated in the inset. Currents were normalized to unity for the maximal value recorded in presence of 30 µM glutamate alone on the same oocytes (n = 3).

Exposure to GYKI52466 caused a significant reduction of the magnitude of the potentiation but no significant shift in sensitivity for S 47445. EC_{50} values are comparable (3.23 ± 0.5 and 1.99 ± 0.3 µM in absence and presence of 100 µM of GYKI52466.

CONCLUSION

Collectively, following several pulses of glutamate, the fast-acting effect of S 47445 on AMPA receptors currents was not accompanied by the development of desensitization. The potentiation effect of S 47445 was concentration-dependently reversed by GYKI52466 and confirmed the positive modulatory effect of this drug on AMPA receptors. The absence of a detectable shift in sensitivity further indicated that GYKI52466 and S 47445 are interacting at independent binding sites.