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Valérie Rolland, Pierre Fontanel, Fatiha Sebih, Janique Guiramand, Matthieu Rousset, et al.. Activity of gamma-L-glutamyl derivatives on glutamate receptors. 26th annual meeting Ion Channel Society, Sep 2015, SETE, France. hal-04794934

**HAL Id: hal-04794934**

**<https://hal.science/hal-04794934v1>**

Submitted on 21 Nov 2024

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# Activity of gamma-L-glutamyl derivatives on glutamate receptors

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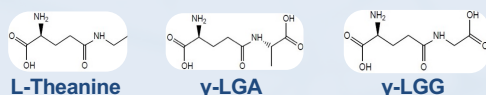
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## Introduction:

**Glutamate** is the major excitatory neurotransmitter in the central nervous system. It has many naturally-occurring derivatives obtained by **modifying the carboxylic moiety in  $\gamma$ -junction**.

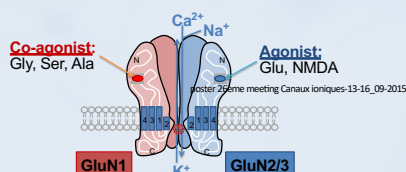
Indeed, **L-theanine** (or  $\gamma$ -glutamyl-N-ethyl) is the major amino acid found in the green tea.

In addition, dipeptides such as  $\gamma$ -L,L-Glutamyl-Alanine ( **$\gamma$ -LGA**) or  $\gamma$ -L-Glutamyl-Glycine ( **$\gamma$ -LGG**), are synthesized during glutathione turnover by the  $\gamma$ -glutamyl-transpeptidases.



## Aim:

As these compounds share a lot of structural similarities with glutamate, we have evaluated their action on glutamate receptors. We have focused the **N-methyl-D-aspartate (NMDA) glutamate receptor subtype**:



## Methods:

### Hippocampal Neuron-Enriched Cultures:

Primary neuronal cultures were established from **18-day-old embryonic rat hippocampi**. After pre-incubation with Versene, hippocampal cells were mechanically dissociated and plated at a density of  $1.5 \times 10^6$  cells/dish, on 8-well plates containing square ( $10 \times 10$  mm<sup>2</sup>) glass coverslips, previously coated with poly(D-lysine) (50  $\mu$ g/mL) and then with DMEM/HAM F12 containing 10% FCS. Cells were grown in a defined medium containing DMEM/HAM F12, supplemented with 33 mM glucose, 2 mM glutamine, 100 U/mL penicillin, 100  $\mu$ g/mL streptomycin, 13 mM sodium bicarbonate, 5.5  $\mu$ g/mL transferrin, 10  $\mu$ g/mL insulin, 1 pM  $\beta$ -estradiol, 3 nM tri-iodothyronine, 20 nM progesterone, 5 ng/mL sodium selenite, and 100  $\mu$ M putrescine.

### Measurements of Cytosolic-Free $Ca^{2+}$ Concentration:

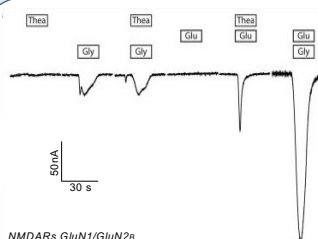
Intracellular calcium concentration ( $[Ca^{2+}]_i$ ) was measured with the fluorescent indicator **Fura-2**. Hippocampal cells grown on glass coverslips were loaded with fura-2 by a 30 min incubation at 37 °C with 5  $\mu$ M fura-2-AM and 0.02% Pluronic in the extracellular solution (in mM, 124 NaCl, 3 KCl, 26 NaHCO<sub>3</sub>, 1.25 KH<sub>2</sub>PO<sub>4</sub>, 1.5 CaCl<sub>2</sub>, 1 MgSO<sub>4</sub>, 10 D-glucose, and 10 HEPES (pH 7.4)).

Fura-2 emission was obtained by exciting alternatively at 340 and 380 nm and by monitoring emissions (F340 and F380) at 510 nm. Drug application was lasted 1 min for each compound tested. Data are expressed as averages ( $\pm$ SEM) of the ratio between the fura-2 fluorescence values of 340/380 nm excitation wavelengths ratios (F340/F380) normalized to the corresponding basal F340/F380 measured prior to any drug application.

### Xenopus Oocyte Injection and Oocyte Current Recording:

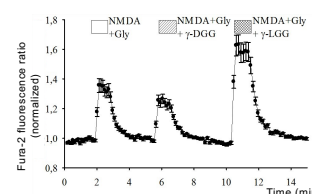
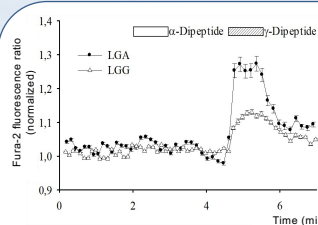
Xenopus oocytes were prepared and injected with in vitro transcribed RNA at 1  $\mu$ g/ $\mu$ L of rat GluN1 and rat GluN2<sub>A</sub> or mouse GluN2<sub>B</sub> with a stoichiometry of 1:1. Macroscopic currents were recorded under **two electrode voltage-clamp** and analyzed in the ND96 HERG recording solution (in mM, 96 NaCl, 3 KCl, 0.5 CaCl<sub>2</sub>, 5 HEPES, pH = 7.2). Current and voltage electrodes (less than 1 M $\Omega$ ) were filled with 3 M KCl.

## Results:



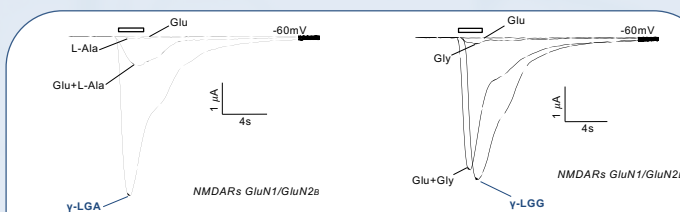
Currents evoked by successive applications or coapplications of 500  $\mu$ M L-theanine, 10  $\mu$ M glycine, and 100  $\mu$ M L-glutamate were measured on xenopus oocyte expressing NMDA receptors.

**L-Theanine displays a weak co-agonist effect in NMDA receptor.**



$[Ca^{2+}]_i$  variation by successive application of 10  $\mu$ M  $\gamma$ -LGA,  $\gamma$ -LGG,  $\gamma$ -DGG and  $\gamma$ -L,L-glutamyl-phenylalanine ( $\gamma$ -LGP) were measured on hippocampal neurons cultured. These recording were performed in an extracellular solution without Mg<sup>2+</sup> + AMPAR/KAR and mGluR antagonists (DNQX et MPEP, respectively).

**Excitatory effect is dependent on the position of the peptidic bond, the amino-acid linked and the chirality of glutamate.**



Currents evoked by successive applications or coapplications of 50  $\mu$ M  $\gamma$ -LGA or  $\gamma$ -LGG, 10  $\mu$ M glycine or L-alanine, and 100  $\mu$ M L-glutamate were measured on xenopus oocyte expressing NMDA receptors.

**$\gamma$ -LGA and  $\gamma$ -LGG are more potent than their composition. They have the ability to activate NMDA receptors alone.**

## Take home:

- **L-Theanine** is a weak co-agonist of NMDA receptor
- **$\gamma$ -LGA and  $\gamma$ -LGG** is agonist/co-agonist of NMDA receptor
- our data suggest that the modification of L-glutamate in gamma-position may lead to a **large variety of glutamate receptor ligands** offering a new approach to modulate neurotransmission.

## Bibliography: