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Activity of gamma-L-glutamyl derivatives on glutamate receptors

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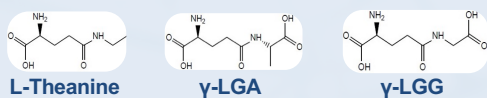
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Introduction:

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

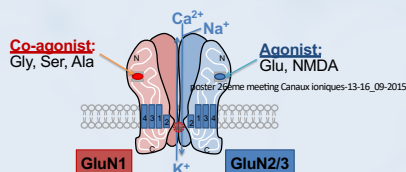
Indeed, **L-theanine** (or γ -glutamyl-N-ethyl) is the major amino acid found in the green tea.

In addition, dipeptides such as γ -L,L-Glutamyl-Alanine (**γ -LGA**) or γ -L-Glutamyl-Glycine (**γ -LGG**), are synthesized during glutathione turnover by the γ -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 1.5×10^6 cells/dish, on 8-well plates containing square (10×10 mm²) glass coverslips, previously coated with poly(D-lysine) (50 μ 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 μ g/mL streptomycin, 13 mM sodium bicarbonate, 5.5 μ g/mL transferrin, 10 μ g/mL insulin, 1 pM β -estradiol, 3 nM tri-iodothyronine, 20 nM progesterone, 5 ng/mL sodium selenite, and 100 μ M putrescine.

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

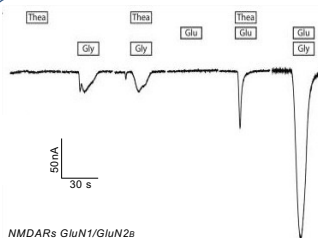
Intracellular calcium concentration ($[Ca^{2+}]_i$) was measured with the fluorescent indicator **Fura-2**. Hippocampal cells grown on glass coverslips were loaded with fura-2 by a 30 min incubation at 37 °C with 5 μ M fura-2-AM and 0.02% Pluronic in the extracellular solution (in mM, 124 NaCl, 3 KCl, 26 NaHCO₃, 1.25 KH₂PO₄, 1.5 CaCl₂, 1 MgSO₄, 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:

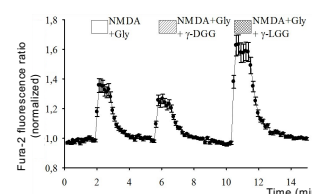
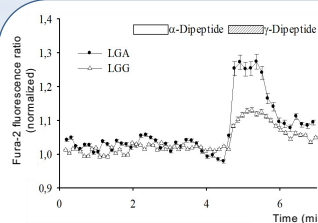
Xenopus oocytes were prepared and injected with in vitro transcribed RNA at 1 μ g/ μ L of rat GluN1 and rat GluN2_A or mouse GluN2_B with a stoichiometry of 1:1. Macroscopic currents were recorded under **two electrode voltage-clamp** and analyzed in the ND96 HERG recording solution (in mM, 96 NaCl, 3 KCl, 0.5 CaCl₂, 5 HEPES, pH = 7.2). Current and voltage electrodes (less than 1 M Ω) were filled with 3 M KCl.

Results:



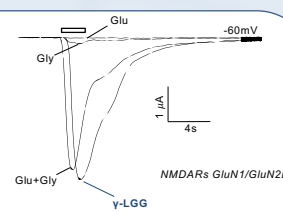
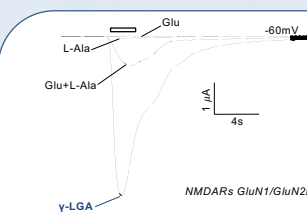
Currents evoked by successive applications or coapplications of 500 μ M L-theanine, 10 μ M glycine, and 100 μ M L-glutamate were measured on xenopus oocyte expressing NMDA receptors.

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



$[Ca^{2+}]_i$ variation by successive application of 10 μ M γ -LGA, γ -LGG, γ -DGG and γ -L,L-glutamyl-phenylalanine (γ -LGP) were measured on hippocampal neurons cultured. These recording were performed in an extracellular solution without Mg^{2+} + 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 μ M γ -LGA or γ -LGG, 10 μ M glycine or L-alanine, and 100 μ M L-glutamate were measured on xenopus oocyte expressing NMDA receptors.

γ -LGA and γ -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
- **γ -LGA and γ -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: