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Stimulation of group I metabotropic glutamate receptors evokes calcium signals and *c-jun* and *c-fos* gene expression in human T cells

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Abstract

To study if the activation of group I mGlu receptors in human T cells modifies intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and cell function, we measured $[Ca^{2+}]_i$ on cell suspensions (spectrofluorimetric method) or single cell (digital Ca^{2+} imaging system) using fura-2 as indicator. Early-inducible gene (*c-jun* and *c-fos*) expression was studied by reverse transcriptase-polymerase chain reaction assay as representative of Ca^{2+}-sensitive gene expression. (1*S*,3*R*)-ACPD (100 μM), the selective mGlu receptor agonist, evoked a significant increase (34.1 ± 4.9%) of $[Ca^{2+}]_i$, pharmacologically characterized as mediated by group I mGlu receptors, since both (*S*)-3,5-DHPG (100 μM), a selective group I mGlu receptor agonist and CHPG (1 mM), the specific $mGlu_5$ receptor agonist, reproduced the effects, that were abolished by AIDA (1 mM), a selective group I mGlu receptor antagonist. (*S*)-3,5-DHPG-induced a rapid $[Ca^{2+}]_i$ rise (initial phase) followed by a slow decrease (second phase) to the baseline. Both extracellular Ca^{2+} and Ca^{2+} released from intracellular stores contribute to the $[Ca^{2+}]_i$ increase which depend on PLC activation. In a Ca^{2+}-free buffer, the second phase rapidly return to the baseline; $LaCl_3$ (1–10 μM), an inhibitor of extracellular Ca^{2+} influx, significantly reduced the second phase only; thapsigargin (1 μM), by discharging intracellular Ca^{2+} stores, U 73122 (10 μM) and D609 (300 μM), by inhibiting PLC activity, prevented both phases. In our system, PTX pre-treatment increased (*S*)-3,5-DHPG effects, demonstrating that PXT-sensitive $G_{i/o}$ proteins are involved. Finally, specific stimulation of these receptors in Jurkat cells upregulates *c-jun* and *c-fos* gene expression, thus activating multiple downstream signalling regulating important T cell functions.

1. Introduction

L-Glutamate is both a ubiquitous amino acid and a chemical transmitter not only in the mammalian CNS, but also in the peripheral tissues. In the CNS, L-glutamate is the principal excitatory neurotransmitter, which binds to and activates a variety of receptors. These receptors have been fully characterized and classified as ionotropic (iGlu) and metabotropic (mGlu) receptors [1]. iGlu receptors are oligomeric, ligand-gated ion channels that mediate fast

Abbreviations: iGlu, ionotropic glutamate; mGlu, metabotropic glutamate; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; PLC, phospholipase C; AC, adenylate cyclase; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; mAb, monoclonal antibodies; PHA, phytohemagglutinin; ERK, extracellular signal-regulated kinase; PBMC, peripheral blood mononuclear cells; fura-2/AM, fura-2 acetoxymethyl ester; PTX, pertussis toxin; TG, thapsigargin; (1*S*,3*R*)-ACPD, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; AIDA, (*R*,*S*)-1-aminoindan-1,5-dicarboxylic acid; CHPG, (RS)-2-chloro-5-hydroxyphenylglycine; (*S*)-3,5-DHPG, (*S*)-3,5-dihydroxyphenylglycine; LY 367385, (S)-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; U 73122, 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione; U 73343, 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-pyrrolidine-2,5-dione; D609, tricyclodecan-9-yl xanthogenate; RT-PCR, reverse transcriptase-polymerase chain reaction; *GAPDH*, glyceraldehydes-3-phosphate dehydrogenase; VGCC, voltage-gated Ca^{2+} channels; TRPC, canonical transient receptor potential; HBSS, Hank's balanced salts solution; EAAT, excitatory amino acid transporters; APC, antigen-presenting cells

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synaptic responses [2]. They are pharmacologically defined as *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors. mGlu receptors are members of the family 3 (or C) of G protein-coupled receptors, including Ca^{2+} and Mg^{2+} receptors, $GABA_B$ receptors, a number of receptors found in the vomeronasal organ and receptors for sweet molecules [3]. They mediate a wide variety of functions, such as learning and memory processes, motor coordination and pain. Eight mammalian genes coding for mGlu receptors has been identified so far. These receptors are divided into three groups according to their primary sequence identity, namely group I: $mGlu_1$ and $mGlu_5$ receptors, group II: $mGlu_2$ and $mGlu_3$ receptors, and group III: $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ receptors. Group I mGlu receptors are preferentially coupled to the $G_{q/11}$/phospholipase C (PLC) transduction pathway, leading to intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase, whereas the others are coupled to the $G_{i/o}$/adenylate cyclase (AC) pathway, leading to changes in intracellular cAMP accumulation [4].

In non-neuronal peripheral tissues, the existence of a glutamate-mediated transmission has been demonstrated in several systems [5,6], including immune system. Here specific high affinity L-glutamate binding sites are found on the surface of human T cells [7], while the expression of both iGlu and mGlu receptors is demonstrated in cells of the T lineage (thymocytes and lymphocytes) [8–12]. Through these receptors L-glutamate modulates lymphocyte functions: (i) it potentiates T cell responses ($[Ca^{2+}]_i$ rise) to specific stimuli [anti-CD3 monoclonal antibodies (mAb) and phytohemagglutinin (PHA)] by acting on NMDA and non-NMDA iGlu receptors [13]; (ii) it increases lymphocyte adhesion to extracellular matrix proteins and cell motility by acting on AMPA iGlu receptors [10]; (iii) it induces reactive oxygen species formation by acting on NMDA iGlu receptors [12,14].

Concerning the transduction pathways linked to the activation of mGlu receptors in lymphocytes no conclusive data have been published yet. In fact, Storto et al. [8] reported that mGlu receptor stimulation significantly increases phosphoinositide hydrolysis, enhances Ins(1,4,5)P_3 formation, and reduces forskolin-induced cAMP formation in mouse thymocytes. Pacheco et al. [11] have since shown that group I mGlu receptor stimulation does not increase $[Ca^{2+}]_i$ but significantly raised cAMP intracellular levels, and activates extracellular signal-regulated kinases (ERK)1/2 in human lymphocytes and Jurkat leukemic T cells. Therefore, further studies are needed to fully characterize these signals.

The aim of this study was to further examine the transduction pathways activated by mGlu receptor stimulation in human T cells, specifically regarding Ca^{2+} signalling.

Our results clearly show that group I mGlu receptor stimulation evokes calcium signals and immediate early gene (*c-jun* and *c-fos*) expression in human peripheral blood mononuclear cells (PBMC) and Jurkat cells.

2. Materials and methods

2.1. Materials and chemicals

Ficoll-Paque PLUS was obtained from Amersham Bioscience (Uppsala, Sweden). BSA, EDTA, EGTA, fura-2 acetoxymethyl ester (fura-2/AM), HEPES, penicillin, pertussis toxin (PTX), poly-L-lysine, probenecid, streptomycin and thapsigargin (TG), were obtained from Sigma–Aldrich (Milan, Italy). (1*S*,3*R*)-1-Aminocyclopentane-1,3-dicarboxylic acid [(1*S*,3*R*)-ACPD], (*R*,*S*)-1-aminoindan-1,5-dicarboxylic acid (AIDA), (*RS*)-2-chloro-5-hydroxyphenylglycine (CHPG), (*S*)-3,5-dihydroxyphenylglycine [(*S*)-3,5-DHPG], (*S*)-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid (LY 367385), 2-methyl-6-(phenylethynyl)-pyridine (MPEP), 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U 73122), 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]-hexyl]-pyrrolidine-2,5-dione (U 73343), tricyclodecan-9-yl xanthogenate (D609), verapamil, diltiazem and nifedipine were from Tocris Cookson (Bristol, UK). RPMI 1640, and foetal bovine serum were purchased from Gibco (Milan, Italy). All other reagents were analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Cell culture

Heparinized peripheral blood (15–20 ml) was collected from healthy donors after informed consent. PBMC were separated by centrifugation at 450 × *g* for 30 min at room temperature over a Ficoll-Paque PLUS gradient as described by Boyum [15]. After washing, the cells were resuspended in RPMI 1640 supplemented with heat inactivated foetal bovine serum (10%, v/v), L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and incubated on dishes for 1 h in a 37 °C, humidified 5% CO_2–95% air incubator. Non-adherent cells were collected and maintained in supplemented RPMI 1640 medium in a 37 °C, humidified 5% CO_2–95% air incubator. Cell viability, evaluated at the end of the cell isolation by Trypan blue dye exclusion test, was always >98%.

Jurkat cells (clone E6-1) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA), and cultured in supplemented RPMI 1640 medium in a 37 °C, humidified 5% CO_2–95% air incubator.

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from Jurkat cells with the GeneElute Mammalian Total RNA Kit (Sigma–Aldrich) and treated (1 h, 25 °C) with 5 U DNase I, RNase-free (Roche Diagnostics, Penzberg, Germany). DNase was then inactivated by heating for 5 min at 95 °C. Resulting RNA was reverse-transcribed with oligo(dT) primers