Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuropharmacology 50 (2006) 245–253
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Among the twenty classical L-amino acids, only glutamate directly activates metabotropic glutamate receptors

Mélanie Frauli [a], Pascal Neuville [a], Claire Vol [b,c,d,e,f], Jean-Philippe Pin [b,c,d,e,f], Laurent Prézeau [b,c,d,e,f,*]

[a] *Faust Pharmaceuticals SA, Illkirch-Graffenstaden, F-67400, France*
[b] *CNRS UMR 5203, Montpellier, F-34094, France*
[c] *INSERM U661, Montpellier, F-34094, France*
[d] *University of Montpellier I, Montpellier, F-34094, France*
[e] *University of Montpellier II, Montpellier, F-34094, France*
[f] *Institut de Génomique Fonctionnelle, Département de Pharmacologie Moléculaire, Montpellier, F-34094, France*

Received 3 March 2005; received in revised form 9 September 2005; accepted 26 September 2005

Abstract

Under pathophysiological conditions, cellular amino acids can be profusely released from cells into the cerebral interstitial space. Because several class-C G protein coupled receptors (GPCRs) display a broad natural ligand spectrum, being sensitive to more than one endogenous ligand, we wondered whether the related metabotropic glutamate (mGlu) receptors could be modulated by various types of L-amino acids, allowing them to sense large increase in extracellular amino acid concentration. Here, the agonist, antagonist and allosteric effects of the twenty classical L-amino acids were evaluated on the eight mGlu receptor subtypes. We show that, in addition to glutamate (Glu), cysteine, aspartate and asparagine also lead to the activation of mGlu3, 4 and 5. Interestingly, our data demonstrate that the effect of these three amino acids did not result from a direct activation of the receptors, but from an indirect action involving Glu-transporters/exchangers. These data first demonstrate that mGlu receptors, unlike other class-C GPCRs, display an extremely high selectivity towards one ligand. Moreover, our results also show that Glu transport systems allow mGlu receptors to sense large increase in the extracellular concentration of some amino acids. Such a system will certainly lead to a large increase in some mGlu receptor activity under pathological conditions, such as seizure, ischemia or other brain injuries.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Metabotropic glutamate receptor; Classical L-amino acid; Functional assay; Glutamate transporter/exchanger; Ligand specificity; Class-C GPCR

1. Introduction

The eight known metabotropic glutamate receptors (mGlu1–8) are G protein coupled receptors (GPCRs) activated by the classical L-amino acid neurotransmitter glutamate (Glu) (Pin and Acher, 2002; Pin et al., 2003). A peculiar feature of mGlu receptors is their large extracellular domain, composed of a cysteine-rich domain and a Venus Flytrap module (VFTM). The X-ray resolution of the crystallized mGlu1 receptor VFTM confirmed the presence of two lobes separated by a cleft in which the Glu molecule binds (Kunishima et al., 2000). Interestingly, the VFTM is structurally similar to some bacterial periplasmic amino acid binding proteins (PBPs) like LIV-BP or LAO-BP, which bind several amino acids, namely leucine, isoleucine and valine, and lysine, arginine and ornithine, respectively (O'Hara et al., 1993), displaying then a very broad natural ligand spectrum. Similarly, the VFTM of the mGlu-related calcium sensing (CaS) receptor binds not only its endogenous ligand Ca^{2+} ions, but is likely the binding site for other natural ligands, like some aromatic L-amino acids (phenylalanine or tryptophan), that have been shown to act as positive allosteric modulators of this receptor (Conigrave et al., 2000; Mun et al., 2004, 2005; Zhang et al., 2002a,b). Moreover, other mGlu-related receptors, some

* Corresponding author. CNRS UMR 5203, Montpellier, F-34094, France. Tel.: +33 04 67 14 29 33; fax: +33 04 67 54 24 32.
E-mail address: laurent.prezeau@igf.cnrs.fr (L. Prézeau).

0028-3908/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2005.09.016

pheromone, taste (sweet and umami taste) and olfactory receptors can bind several related endogenous ligands in their VFTM (Nelson et al., 2001, 2002; Speca et al., 1999; Wellendorph et al., 2005). It is thus puzzling to consider that mGlu receptors bind only one type of L-amino acid, unlike some of their related receptors.

Indeed, the eight mGlu receptors have been shown to be activated by other endogenous molecules that are not classical amino acids, but amino acid derivatives, like L-homocysteine, found to activate native receptors in vivo (Lazarewicz et al., 2003). Moreover, since the discovery of the first specific mGlu receptor agonist, (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) (Palmer et al., 1989), a large panel of synthetic ligands has been identified for G_q coupled group I mGlu receptors (mGlu1 and 5) as well as for $G_{i/o}$ coupled group II (mGlu2 and 3) and group III (mGlu4, 6, 7 and 8) mGlu receptors (Pin and Acher, 2002; Schoepp et al., 1999). The orthosteric ligands (agonists and competitive antagonists) discovered so far are linear or cyclic Glu derivatives. This suggested that mGlu receptors could be sensitive to other classical L-amino acids.

It is noteworthy that intracellular classical L-amino acids are abundantly released under physiopathological conditions involving cell death. An elevation in the interstitial concentration of non-neurotransmitter amino acids was identified as a sign of spreading depression, and has also been described as a consequence of ischemic injury to neuronal tissue (Davies et al., 1995; Molchanova et al., 2004). The potential effect of classical L-amino acids on mGlu receptors is thus a highly interesting parameter to test, as mGlu receptor activity has been involved in both excitotoxicity and neuroprotection (Bruno et al., 2001).

We wondered whether, besides Glu, any other classical L-amino acid could bind the VFTM cleft and modulate mGlu receptor activity. In the present study, the eight mGlu subtypes were challenged with these amino acids to determine their potential activities as either agonists or antagonists. Moreover, because few L-amino acids have been identified as positive allosteric modulators of the CaS receptor (Conigrave et al., 2000), we checked whether any of the classical L-amino acids could enhance the Glu response on mGlu receptors.

It turned out that cysteine (Cys), aspartate (Asp) and asparagine (Asn) increase the activity of some mGlu receptors. However, their effect is indirect, involving Glu-transporters and/or -exchangers, which increase the concentration of extracellular Glu, thereby leading to the subsequent activation of the mGlu receptors displaying the highest affinity for Glu. This indirect effect has to be considered when analyzing the possible involvement of mGlu receptors in various physiopathological conditions.

2. Materials and methods

2.1. Materials

Glutamate and the other classical L-amino acids were purchased from Sigma. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP) and methyl-4-carboxyphenylglycine (MCPG) were purchased from Tocris Cookson (Bristol, U.K.). Diphenylacetyl-carbamic acid ethyl ester (Ro01-6128) was synthesized (Wichmann et al., 2002) and provided as a kind gift by Dr Yvo Stray. MCPG and amino acid stocks (100, 50 or 10 mM according to solubility) were prepared in Krebs buffer (note: for tyrosine, HCl 1 N was added to allow dissolution before experiment), aliquoted and stored at −20 °C. Ro01-6128 and MPEP were prepared as 100 mM stock in 100% DMSO, aliquoted and stored at −20 °C. Fresh aliquots from all compounds were used for each experiment.

Culture medium, fetal calf serum (FCS), and other products used for cell culture were purchased from GIBCO BRL Life Technologies (Cergy Pontoise, France). [^{3}H]myo-inositol (23.4 Ci/mol) and L-[^{3}H]glutamic acid (46 Ci/mmol) were purchased from Amersham Pharmacia (Saclay, France). Glutamate–pyruvate transaminase was purchased from Roche (Basel, Switzerland).

2.2. RT-PCR

HEK293 total RNA extraction was performed with Trizol reagent, according to the manufacturer's indications (Invitrogen, Cergy Pontoise, France). RT-PCR was performed using the One-Step RT-PCR Platinum Taq kit from Invitrogen. Human x_c-transporter (forward: 5′ccaccatctccaaaggagg3′; reverse: 5′ctaaaccacctgggtttcttg3′) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward: 5′ccatggagaaggctgggg3′; reverse: 5′caaagttgtcatggatgacc3′) specific primers were designed and ordered from Eurogentec (Angers, France).

2.3. Cell culture and transfection

HEK293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS and transfected by electroporation as previously described (Brabet et al., 1998). Ten million cells were transfected with 1–10 μg plasmid DNA encoding the different mGlu receptors, completed to a total amount of 10 μg of plasmid DNA with carrier vector pRK6. The construction of the plasmids expressing mGlu receptors has been described previously (Gomeza et al., 1996; Joly et al., 1995; Parmentier et al., 1998). Group I mGlu receptors (subtypes mGlu1a and 5a) are naturally coupled to G_q protein pathway. Group II (subtypes mGlu2 and 3) and group III (subtypes mGlu4a, 6, 7a and 8a) receptors were coupled to the phospholipase C (PLC) pathway by co-transfection with plasmid DNA encoding G_{15} or the chimeric G_{qi9} protein (Gomeza et al., 1996). To avoid any influence of Glu naturally released by the cells in the assay medium and to decrease the subsequent high basal activity, mGlu3 and 5 receptors were co-transfected with the high-affinity Glu transporter EAAC-1 (Brabet et al., 1998).

2.4. Intracellular calcium measurements

Receptor subtypes mGlu1 and 5 (which are naturally G_q/PLC coupled), and mGlu2 and 8 (which are efficiently coupled to PLC pathway through G_{qi9}) were tested using intracellular Ca^{2+} measurements (Goudet et al., 2004). After transfection, 10^5 cells per well were seeded in polyornithine-coated, black-walled, clear bottom 96-well plates and cultured for 24 h. Cells were washed with freshly prepared buffer (1× HBSS supplemented with 20 mM HEPES, 1 mM $MgSO_4$, 3.3 mM Na_2CO_3, 1.3 mM $CaCl_2$, 2.5 mM probenecid and 0.5% BSA) and loaded with 1 μM Ca^{2+}-sensitive fluorescent dye Fluo-4 AM (Molecular Probes, Leiden, The Nederlands) for 1 h at 37 °C. After loading, cells were washed twice, and then incubated with 50 μl of buffer. A drug plate was prepared with the various ligands to be tested, and 50 μl of 2×-drug solution (prepared in buffer) was added in each well after 20 s of recording. For basal controls, buffer alone was added after 20 s. Fluorescence signals (excitation 485 nm and emission 525 nm) were measured by using the fluorescence microplate reader FlexStation (Molecular Devices, Sunnyvale, CA, USA) at sampling intervals of 1.5 s for 60 s.

2.5. Inositol phosphate (IP) accumulation

For the receptor subtypes mGlu3, 4, 6 and 7, which respond less efficiently in the Ca^{2+} assay than the other mGlu receptors, the IP accumulation method

Download English Version:

https://daneshyari.com/en/article/2495447

Download Persian Version:

https://daneshyari.com/article/2495447

Daneshyari.com