A Novel Binding Assay for Metabotropic Glutamate Receptors Using [^{3}H] L-Quisqualic Acid and Recombinant Receptors

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mGluR, AMPA Receptor, [^{3}H] Quisqualic Acid Binding Assay

We established a methodology to analyze radioligand binding to the recombinant type 1a metabotropic glutamate receptor (mGluR1a). A full-length cDNA encoding mGluR1a, which was isolated from a λ gt 11 cDNA library of human cerebellar origin, was expressed in a baculovirus/Sf9 insect cell system. Membrane fractions with recombinant receptor expression were analyzed for the binding of [^{3}H]L-quisqualic acid (L-QA), which is known to be a potent agonist of mGluR1a. Efficient binding of the radioligand to the human receptor was observed in a saturable manner, giving an apparent Kd= 0.091 μM. [^{3}H]L-QA bound to the human mGluR1a was displaced by known ligands such as L-QA, L-Glu, t-ACPD ((±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid) with IC_{50}s = 0.056, 0.97 and 4.0 μM, respectively. MCPG (α-methyl-4-carboxyphenylglycine) displaced the radioligand binding with lower potency. Using this binding protocol, we then evaluated the ligand ability of synthetic dipeptides. Among peptides tested, only Glu-containing dipeptides inhibited the radioligand binding, *e.g.* IC_{50} of L-Met-L-Glu was 4.3 μM. When phosphatidyl inositol turnover was assayed in mGluR1a-expressing CHO cells, L-Met-L-Glu was partially agonistic. We further expanded this [^{3}H]L-QA binding protocol to type 5a mGluR, another member of group I mGluRs, as well as to AMPA receptor, a member of ionotropic glutamate receptors, since L-QA is also known to be a potent ligand for these receptors. Data shown here will provide a novel system not only to search for ligands for the glutamate receptors, but also to biochemically analyze the interaction modes between glutamate receptors and their ligands.

Introduction

L-Glu is an excitatory neurotransmitter that plays a pivotal role in the central nervous system as well as peripheral nervous system. Actions of L-Glu are mediated by glutamate receptors that are classified into two major families of ionotropic receptors (NMDA receptor, AMPA receptor and kainate receptor) and G protein-coupled metabotropic receptors (mGluRs) (for reviews, see references Tanabe *et al.*, 1992; Hollman and Heinemann, 1994; Nakanishi and Masu, 1994; Conn and Pin, 1997). The latter is a family of 8 subtypes which are further divided into 3 subgroups, according to the structural relationship and second messenger systems that they are implicated in (Tanabe *et al.*, 1992; Hollman and Heinemann, 1994; Nakanishi and Masu, 1994; Conn and Pin, 1997; Watkins and Collingridge, 1994; Knöpfel *et al.*, 1995; Pin and Duvoisin, 1995; Knöpfel and Gasparini, 1996). Group I receptors (mGluR1 and mGluR5) are coupled to phosphoinositide turnover, while group II receptors (mGluR2 and mGluR3) and group III receptors (mGluR4, mGluR6, mGluR7 and mGluR8) are negatively coupled to cAMP formation.

The mGluR family is implicated in a variety of higher functions and biological processes in the central as well as peripheral nervous systems. These include learning/memory, pain, epileptic sei-

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Abbreviations: ACPD, 1-aminocyclopentane-1,3-dicarboxylic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; mGluR, metabotropic glutamate receptor; MCPG, α-methyl-4-carboxyphenylglycine; NMDA, N-methyl-D-aspartic acid; QA, quisqualic acid.

zure, anxiety and neuronal degeneration, thus providing potentialities of drug development for the treatment of dementic diseases such as Alzheimer disease, ischemia, other neurodegenerative disorders, algesia, epilepsy, neuropsychiatric diseases such as anxiety, depression and so forth (Conn and Pin, 1997; Knöpfel *et al.*, 1995; Knöpfel and Gasparini, 1996; Schoepp *et al.*, 1997).

There have been reported many mGluR agonists and antagonists synthesized in mimicry of the structure of L-Glu, an endogenous ligand. This strategy, however, has mostly resulted in ligands with only low potency and/or low selectivity over receptor subtypes (Hollman and Heinemann, 1994; Nakanishi and Masu, 1994; Conn and Pin, 1997; Watkins and Collingridge, 1994; Knöpfel *et al.*, 1995; Pin and Duvoisin, 1995; Hayashi *et al.*, 1994), with a few exceptional compounds such as LY354740 which exhibits nM potency at mGluR2 (Schoepp *et al.*, 1997). Furthermore, efforts to develop mGluR ligands have been made largely on the basis of rather laborious protocols to determine the levels of cell-signaling messengers such as inositol phosphates and cAMP in cells expressing mGluR subtypes (Conn and Pin, 1997; Watkins and Collingridge, 1994; Knöpfel *et al.*, 1995).

L-QA has long been known as a potent agonist for group I mGluRs as well as AMPA receptor (Hollman and Heinemann, 1994; Nakanishi and Masu, 1994; Conn and Pin, 1997; Knöpfel *et al.*, 1995; Pin and Duvoisin, 1995; Watkins *et al.* 1990; Kawamoto *et al.*, 1991; Hattori *et al.*, 1994; Hennegriff *et al.*, 1997). In order to provide an efficient methodology that is eligible for ligand screening, we synthesized [^{3}H]-labeled L-QA and developed a protocol to analyze the binding of [^{3}H]L-QA to baculovirally expressed mGluR1a, one of the splicing variants of type 1 mGluR (Masu *et al.*, 1991; Desai *et al.*, 1995). L-Glu and L-QA displaced the radioligand bound to the recombinant receptor with the rank order of potencies well matching with those obtained by a conventional method (analysis of phosphoinositide turnover). On the contrary, ligands known to act at other glutamate receptors exerted only minor or little activity in this assay. Further tests using dipeptides revealed that some L-Glu-containing peptides such as L-Met-L-Glu, L-Glu-L-Trp and L-Glu-L-Tyr inhibited [^{3}H]L-QA binding to mGluR1. These dipeptides were partially agonistic at the same receptor. A similar binding protocol was also shown to be applicable to type 5 mGluR as well as AMPA receptor.

Materials and Methods

Glutamate receptor ligands

All known ligands for glutamate receptors including L-QA, (RS)-AMPA, t-ACPD, 1S,3R-ACPD, (RS)-MCPG, (S)-4CPG, kainic acid and NMDA were the products of Tocris Cookson (Bristol, UK).

*mGluR cDNA cloning, vector construction and trans*fection studies

Rat mGluR1a cDNA was isolated as reported (Masu *et al.*, 1991). In order to clone human mGluR1a cDNA, we first prepared two primer pairs: F6 (5′-ACCAGCGTGGGAACGCGGCT-3′) and R10 (5′-CTCCCGCAATGGGCTTCTTA-3′), and aF2 (5′-CCAAACAGCCGTCAT-CAAACCCCTCACTAA-3′) and aR2 (5′-GTCGCGGAAAGGCGACGGAGGCGT-CAGCGCAGGC-3′). RT-PCR using a F6-R10 primer pair amplified a 0.5 kb DNA fragment from human cerebellar mRNAs (Clontech, Palo Alto, CA), while an aF2-aR2 pair gave a 0.65 kb DNA fragment. Nucleotide sequence analysis revealed that the two fragments corresponded to N-terminal region and C-terminal region, respectively, of the reported human mGluR1a (Desai *et al.*, 1995). Using these DNA fragments as [^{32}P]-labeled probes, a λ gt 11 cDNA library of human cerebellar origin (Clontech) was screened. By combining fragmental cDNA clones, we prepared a 3.9 kb cDNA encoding full-length human mGluR1a, which was then subcloned into pBluescript SK(−) (Stratagene, La Jolla, CA) at EcoR I site. Sequence analysis of the cDNA clone revealed a complete match with the sequence previously reported by another group (Desai *et al.*, 1995), with exceptional replacements at positions Pro993 and Pro1150 by Ser and Thr, respectively.

The insert was then constructed into pBacPAK9 transfer vector at Xba I site after blunted by BssH II digestion. The recombinant baculovirus carrying human mGluR1a cDNA was prepared using BacPAK Baculovirus Expression System (Clontech), according to the manufacturer's protocol. Sf9

(*Spodoptera frugiperda*) insect cells ($1-2\times10^6$ per liter) were then infected by recombinant viruses (m.o.i. = 5) at 27 °C in TC-100 insect medium (Gibco, Rockville, MD) containing 10% fetal bovine serum and 0.1% pluronic F68 (Gibco). Four days later, cells were harvested by a low centrifuge ($200\times g$ for 5 min) and were washed with phosphate-buffered saline. For the preparation of membrane fractions on which human mGluR1a was expressed, Sf9 cells were homogenized by Polytron (Kinematika, Littau-Lucerne, Switzerland, power setting at 5 for 15 sec) in 30 ml of 40 mM Tris-HCl (pH 7.4) containing 1 mM EDTA, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml pepstatin, 10 μg/ml futhan and 1 μg/ml aprotinin. Cell homogenates were spun down by a low speed centrifuge ($200\times g$ for 7 min) to remove undisrupted cells, and then were again centrifuged at $40{,}000\times g$ for 20 min. The centrifugal pellet was washed three times with 20 ml of 50 mM Hepes (N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) buffer (pH 7.4). After suspended in 20 ml of the same buffer, membrane fractions were stored in aliquots at −80 °C until use. Membrane fractions were also prepared from mock cells without mGluR1a cDNA insert.

In order to confirm the expression of human mGluR1a in Sf9 cells, membrane proteins separated on sodium dodecylsulfate-polyacrylamide gel electrophoresis (7.5% acrylamide) were immunoblotted using rabbit polyclonal antibody raised against a C-terminal sequence of rat mGluR1a, KPNVTYASVILRDYKQSSSTL (Upstate Biotechnology, Lake Placid, NY), and horseradish peroxidase-conjugated donkey anti-rabbit F(ab')$_2$ antibody (Amersham, Buckinghamshire, UK). A mGluR1a band was detected at its migration rate of ca. 140 kDa by ECL (Enhanced Chemiluminescence) method according to the manufacturer's protocol (Amersham, data not shown). A similar analysis of mock membranes did not show a band at this migration rate.

CHO (Chinese hamster ovary, dhfr$^-$) cells were transfected with human mGluR1a cDNA which was constructed into pdKCR-dhfr vector at EcoR I site. Cell clones with stable expression of the receptor were isolated as previously reported (Hayashi *et al.*, 1994; Aramori and Nakanishi, 1992).

Rat mGluR1a cDNA (Masu *et al.*, 1991) and mouse AMPA receptor (GluR1/α 1) cDNA (a kind gift from Prof. M. Mishina at University of Tokyo; Kawamoto *et al.*, 1991) were similarly constructed into pBacPAK9 transfer vector at BamH I site and Fsp I site, and Xma I site and Xba I site, respectively. Membrane fractions of receptor-expressing Sf9 cells were similarly prepared (see above).

[^{3}H]L-QA binding analysis

Synthesis of racemic [^{3}H]D,L-QA and separation of L-isomer from D-isomer will be described elsewhere. High performance liquid chromatographical analysis estimated the radiochemical purity of [^{3}H]L-QA to be 99.6%. Sf9 cell membranes (10–100 μg protein), on which human mGluR1a was expressed, were incubated for 1 hr at room temperature on a 96-well plate in 200 μl of 50 mM Hepes buffer (pH 7.4) containing 20 nM [^{3}H]L-QA (spec. act. = 323 GBq/mmol) and 10 mM $CaCl_2$. Samples were then transferred onto a 96-well GF/C filter plate and membrane fractions were trapped on the filter using Filtermate Cell Harvester (Packard, Research Parkway Meriden, CT). After washing with Ca^{2+}-containing Hepes buffer (3 ml × 3), the filter was dried and measured for the radioactivity with 25 μl Microscinti-0 (Packard). Specific binding of the radioligand was determined by subtracting, from the total binding, the non-specific binding that was obtained in the presence of a 100-fold molar excess of non-radioactive QA. For displacement studies, other known mGluR ligands such as L-Glu, t-ACPD, etc. were included in the assay system.

This [^{3}H]L-QA binding protocol was applied to screening studies to search for dipeptides that acted at mGluR1a. Monomeric L-Glu impurity in commercially available dipeptides (Kokusan Chemicals, Tokyo) was under the detection limit by an amino acid analysis (Hitachi L-8500, detection limit = 10 pmoles), excluding a possibility for perturbing influence of free L-Glu to the data shown in Fig. 3.

Binding of [^{3}H]L-QA to AMPA receptor (GluR1/α 1) was also tested in 50 mM Hepes buffer (pH 7.4)/10 mM $CaCl_2$/0.1 M KSCN (Hennegriff *et al.*, 1997) using Sf9 membrane fractions with expression of mouse AMPA receptor. For comparison, [^{3}H]L-QA binding was similarly examined using membrane fractions prepared from rat brain cortex (Hennegriff *et al.*, 1997).

All of the kinetic data shown in this paper were those obtained from 5–7 equivalent experiments performed in triplicate. Note that experimental errors are occasionally invisible in computer-assisted figure preparations, when the error ranges were small.

Inositol phosphate analysis

A CHO (dhfr$^-$) cell clone with stable expression of human mGluR1a was established as described above. Phosphoinositide turnover was determined as reported (Aramori and Nakanishi, 1992) with a slight modification. Briefly, cells were plated on a 24-well plate (10^5 cells per well) in 500 μl DMEM (Nissui, Tokyo) containing a reduced concentration (2 mM) of L-glutamine and 10% dialyzed fetal bovine serum. The culture medium was replaced on the next day with fresh, inositol-free DMEM in which 37 kBq/ml [3H] *myo*-inositol (Amersham, spec. act. = 633 GBq/mmol) was included in order to label the cells. On day 2, cells were washed with 500 μl phosphate-buffered saline (20-min soak) and then with the same buffer containing 10 mM LiCl (20-min soak). After a 20-min incubation with mGluR1 agonists in 200 μl LiCl-containing buffer, cells were extracted with 5% trichloroacetic acid for 1 hr at 4 ° C. The acid extract was applied to an AG1 × 8 column (0.6 ml, Bio-Rad 200–400 mesh). [3H]-labeled inositol phosphates were eluted with formic acid-ammonium formate. Radioactivity in the eluate was determined by a liquid scintillation counter.

Other assays

Proteins were determined after Bradford (1976).

Results

Analysis of [3H]L-QA binding to recombinant mGluR1a

Since immunoblot analysis confirmed the expression of the recombinant human and rat mGluR1a in a baculovirus-insect cell system (see Materials and Methods), we addressed to analyze [3H]L-QA binding to the recombinant receptors. More than 90% of the radioligand was detected as a specific binding throughout the range of membrane amounts examined. No significant binding of the radioligand was observed when mock membranes were tested (data not shown). [3H]D-QA, which was originally prepared with [3H]L-QA in a racemic form and then was separated from the sister isoform, did not act as a ligand (data not shown). In Scatchard analysis, [3H]L-QA binding to the human mGluR1a was saturable (Fig. 1A) with a Kd= 0.091 μM (Fig. 1B and Table I).

The bound [3H]L-QA was displaced by known mGluR ligands, such as L-QA, L-Glu, t-ACPD/1S,3R-ACPD (a broad agonist for group I/II

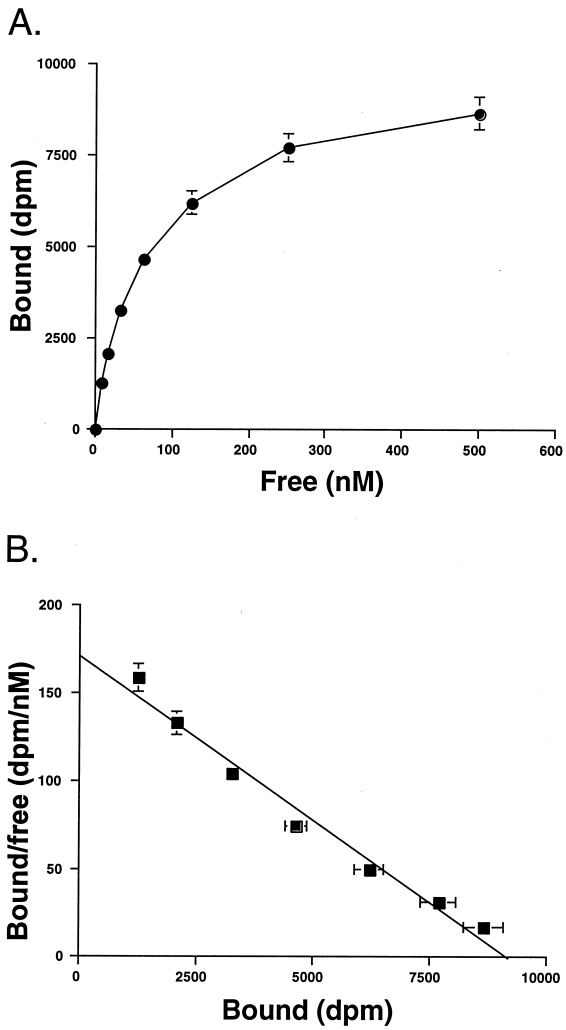

Fig. 1. [3H]L-QA binding to human mGluR1a expressed in a baculovirus-Sf9 cell system. The 'bound'-'free' relationship was schematized in panel A, revealing a saturable manner of [3H]L-QA binding to the receptor. Kd was 0.091 μM as determined by Scatchard plot (panel B, also see Table I).

Table I. Kinetic aspects of [3H]L-QA binding to group I mGluRs.

	Kd	IC_{50}						
		L-Glu	L-QA	t-ACPD	1S,3R-ACPD	MCPG	4-CPG	AMPA
[3H]L-QA binding								
Human mGluR1a/Sf9	0.091	0.97	0.056	4.0	9.2	60	n.t.	> 1 mM
Rat mGluR1a/Sf9	0.069	2.0	0.048	n.t.	n.t.	n.t.	12.5	> 1 mM
Rat mGluR1a/CHO	0.068	0.45	0.024	n.t.	n.t.	n.t.	7.9	n.t.
Human mGluR5a/Sf9	0.20	3.9	0.052	n.t.	59	n.t.	n.t.	> 1 mM

Note. Values are expressed in μM except for data on AMPA. n.t. denotes not tested. All of the kinetic data shown in this table were obtained from 5–7 different batches of experiments in triplicate, mostly within 10% error ranges.

mGluRs) and (RS)-MCPG (a non-selective antagonist for group I/II mGluRs)/(S)-4CPG (competitive group I mGluR antagonist with selectivity for mGluR1a over mGluR5a/5b) . As summarized in Table I, the displacing potency of L-QA was IC_{50} ~ 0.05 μM for human and rat mGluR1a expressed in Sf9 cells, while L-Glu was of weaker potency (IC_{50} = 1.0–2.0 μM) and the other mGluR ligands were far less potent displacers, i.e., IC_{50}s = 4.0, 9.2 and 60 μM for t-ACPD, 1S, 3R-ACPD and MCPG, respectively, at human mGluR1a. Because of a high sequence homology (93%) between human and rat mGluR1a (Desai *et al.*, 1995), it seems reasonable that the kinetic parameters of [3H]L-QA binding to mGluR1a of the two species were essentially similar. Table I also shows only minor dependence of such parameters on the difference in host cells employed for ectopic receptor expression (see data on rat mGluR1a expressed in Sf9 cells and CHO cells).

In a similar experiment, an affinity of [3H]L-QA for the recombinant mGluR5a was a Kd= 0.2 μM. L-Glu and 1S,3R-ACPD were somewhat less potent in displacing the mGluR5a-bound radioligand, when compared to their potency obtained for mGluR1a. Representative ligands for ionotropic glutamate receptors such as (RS)-AMPA, kainic acid and NMDA were of no effect up to 1 mM in displacing the [3H]L-QA binding to group I mGluRs (Table I).

We then tested effects of known mGluR1 agonists on phosphoinositide turnover in CHO cells expressing recombinant human mGluR1a. L-QA was the most potent, compared to L-Glu and 1S,3R-ACPD (Fig. 2).

Displacement of [3H]L-QA bound to mGluR1a by L-Glu-containing dipeptides

We screened synthetic dipeptides for their ability to displace [3H]L-QA bound to human

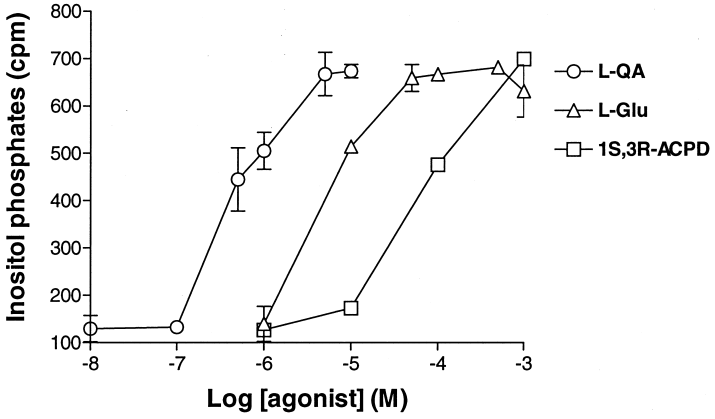

Fig. 2. Agonist-induced phosphoinositide turnover in mGluR1a-expressing cells. CHO cells expressing human mGluR1a were preloaded with [3H] *myo*-inositol. Cells were then stimulated by mGluR1 agonists, L-QA, L-Glu or 1S,3R-ACPD. Formation of radiolabeled inositol phosphates was determined as described in Materials and Methods. Note that the cell signaling was activated by these agonists in the rank order of potency which was in good accordance with that previously reported.

mGluR1a. Among nearly 250 distinct dipeptides tested, only L-Glu-containing ones exerted such activity as revealed in Fig. 3. L-Met-L-Glu was the best peptide in displacing [3H]L-QA with IC_{50}= 4.3 μM, while the other L-Glu-containing peptides were less active, *e.g.* IC_{50}s = 25, 37, 80, 120 and 65 μM for L-Glu-L-Trp, L-Glu-L-Tyr, L-Glu-L-Lys, L-Glu-L-Ser and L-Val-L-Glu, respectively. L-Glu-L-Glu was also less potent (IC_{50}= 72 μM, not shown in the figure). The position of L-Glu residue (either L-Glu-X or X-L-Glu, X denotes any amino acids) did not seem critical for the displacing ability of dipeptides. Dipeptides without L-Glu residue were of no significant activity up to 1 mM. L-Aspartic acid did not substitute for L-Glu as a residue of active dipeptides (data not shown).

As mentioned in Materials and Methods, amino acid analysis of commercially available dipeptides revealed no detectable level of monomeric L-Glu impurity. This in turn indicates the concentration of free L-Glu impurity was $<10^{-9}$ M with no significant perturbing influence in the data shown in Fig. 3.

Two dipeptides shown in Fig. 3 were further tested for their ability to induce inositol phosphate formation in mGluR1a-expressing CHO cells. Free L-Glu dose-dependently enhanced phosphoinositide turnover, i.e., *ca.* 3-fold and 5–6-fold increase vs. control by 10^{-5} and 10^{-4} M L-Glu, respectively, while to a lesser extent (2–3-fold) by 10^{-4} M L-Met-L-Glu or L-Glu-L-Trp (data not shown).

[3H]L-QA binding to AMPA receptor

Since L-QA is also known to be an potent ligand for ionotropic AMPA receptor family (Hollman and Heinemann, 1994; Nakanishi and Masu, 1994; Watkins *et al.*, 1990; Kawamoto *et al.*, 1991; Hattori *et al.*, 1994), we constructed a baculovirus-Sf9 cell system for the expression of mouse AMPA receptor (GluR1/α 1) (see Materials and Methods). Membrane fractions (100 μg) prepared from the recombinant insect cells were revealed to efficiently bind [3H]L-QA with a low (<5%) non-specific binding, as observed in mGluR1a. No significant binding of the radioligand was observed in membranes prepared from the mock cells. Scatchard analysis estimated a Kd= 0.042 μM for AMPA receptor (data not shown). When membrane fractions (100 μg) of rat brain cortex were examined, the specific binding of [3H]L-QA was still observable, but less evident compared to that obtained in the recombinant receptor system (data not shown).

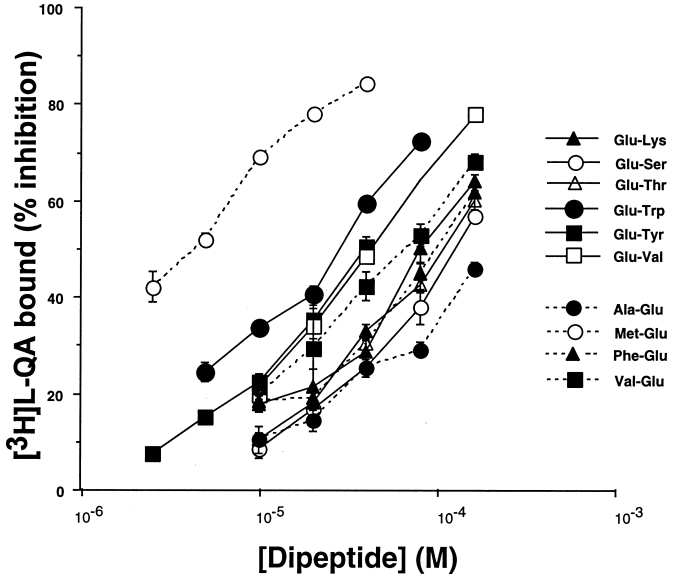

Fig. 3. Displacement of [3H]L-QA bound to mGluR1a by L-Glu-containing dipeptides. The interaction between L-QA and Sf9-expressed human mGluR1a was inhibited by L-Glu-containing dipeptides in a dose-dependent manner. L-Met-L-Glu was of the highest potency (IC_{50}= 4.3 μM), which was comparable to IC_{50} ~ 1 μM of L-Glu (see Table I).

Discussion

Applicability of [3H]L-QA binding protocol

[3H]L-QA binding assay clearly showed a high affinity of the radioligand for human mGluR1a (Kd= 0.091 μM, Fig. 1 and Table I). Both [3H]L-QA displacement analysis (Table I) and inositol phosphate analysis (Fig. 2) showed the rank order of potencies of the ligands in good agreement within our data and with the reported ones (Tanabe *et al.*, 1992; Hollman and Heinemann, 1994; Nakanishi and Masu, 1994; Conn and Pin, 1997; Masu *et al.*, 1991; Desai *et al.*, 1995). These data thus confirm the validity of the [3H]L-QA binding protocol described here as a selective and sensitive one that is eligible for the search of type 1 mGluR ligands. Since L-QA is also known as a potent ligand for mGluR5 and AMPA receptor (Hollman and Heinemann, 1994; Nakanishi and Masu, 1994; Conn and Pin, 1997; Watkins and Collingridge, 1994; Knöpfel *et al.*, 1995; Pin and Duvoisin, 1995; Kawamoto *et al.*, 1991; Hattori *et al.*, 1994; Hennegriff *et al.*, 1997), it is reasonable that similar [3H]L-QA binding protocols are applicable to these receptors (Table I and see Results).

Furthermore, the [3H]L-QA binding protocol is eligible to characterize the interaction mode between mGluR1 (as well as mGluR5 and AMPA receptor) and their ligands. Indeed, Okamoto *et al.* employed this protocol to characterize the aspects of the soluble form of mGluR1 composed of only N-terminal extracellular domain of the receptor molecule (Okamoto *et al.*, 1998).

Partially agonistic L-Glu-containing dipeptides

Using the [3H]L-QA binding protocol, we tested various synthetic dipeptides for their ligand ability. As revealed in Fig. 3, only L-Glu-containing dipeptides caused displacement of the radioligand bound to mGluR1a. Particularly, L-Met-L-Glu was the best peptide whose IC_{50} was 4.3 μM, comparable to *ca.* μM range of IC_{50} for free L-Glu (see Table I). The other L-Glu-containing dipeptides were less potent (*e.g.* IC_{50}= 25 μM for L-Glu-L-Trp). These data indicate the essentiality of L-Glu residue for being a mGluR1 ligand, but an extra residue of the individual dipeptide is probably interfering with the intact interaction between L-Glu and mGluR1. Both L-Met-L-Glu and L-Glu-L-Trp triggered inositol phosphate signaling, though with a lesser extent compared to the effect of L-Glu (see Results), suggesting that these dipeptides were only partially agonistic to mGluR1a. Such data still appear to support the essentiality of L-Glu as an endogenous ligand.

The discovery of L-Glu-containing dipeptides through a combination of [3H]L-QA binding assay and cell signaling assay further ensures the validity of our [3H]L-QA binding protocol as a useful methodology for screening of ligands that potentially act at mGluR1.

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