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Group II metabotropic glutamate receptor-mediated activation of G-proteins in rat hippocampal and striatal membranes

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HIGHLIGHTS

- L-Glutamate stimulates [^{35}S]GTPγS binding to rat hippocampal and striatal membranes.
- The group II mGlu receptors, especially $mGlu_2$ subtypes, are involved.
- LY487379 potentiates the maximum response elicited by L-glutamate.

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ABSTRACT

Stimulation of G-proteins coupled with metabotropic glutamate receptors (mGlu receptors) was investigated by means of guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding assay in rat hippocampal and striatal membranes. The endogenous ligand L-glutamate increased specific [^{35}S]GTPγS binding in a concentration-dependent manner with a mean EC_{50} values of around 10 μM in both brain regions, The maximal % increase over the respective basal binding was highest in cerebral cortex, intermediate in hippocampus, and lowest in striatum. The pharmacological profiles of the responses investigated with a series of glutamatergic agonists and antagonists clearly indicated that they were mediated through group II mGlu receptors, particularly $mGlu_2$ subtype, in both brain regions. The pEC_{50} and relative $\%E_{max}$ values for a series of agonists were essentially identical in both brain regions that were also correlated with those previously reported in cerebral cortical membranes. The selective allosteric potentiator of $mGlu_2$ receptor subtype, LY487379, potentiated the increasing effects of L-glutamate at a maximally effective concentration of 1 mM on specific [^{35}S]GTPγS binding, without altering the basal unstimulated binding. It is concluded that [^{35}S]GTPγS binding assay is applicable to rat hippocampal and striatal membranes to detect functional activation of $G\alpha_{i/o}$ proteins coupled with $mGlu_2$ receptors.

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1. Introduction

L-Glutamate serves as the primary neurotransmitter at the majority of excitatory synapses in the mammalian central nervous system (CNS). In addition to activating the ionotropic glutamate receptors (*N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kinate receptors), it can modulate cell excitability and synaptic transmission via second messenger signaling pathways by activating the neuromodulatory glutamate receptors, called metabotropic glutamate receptors (mGlu receptors). The mGlu receptors are a family of G-protein-coupled receptors (GPCRs) comprising eight subtypes termed $mGlu_1$ to $mGlu_8$, which are classified into three major groups (group I to III) based on their amino acid sequence homology, preferred signal transduction mechanisms and relative pharmacology.

The widespread distribution of mGlu receptors suggests that these neuromodulatory receptors have the ability to participate in numerous functions throughout the CNS and may represent ideal targets for therapeutic intervention in a wide variety of CNS disorders. The group II mGlu receptors are of particular interest as a potential target of novel efficacious psychotropic drugs for the treatment of schizophrenia [2,16,32] and anxiety disorders [25]. The functional coupling of group II mGlu receptors to G-proteins has been successfully detected in rat native cerebral cortical membranes by means of guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding assay [21]. Since mGlu receptor subtypes and G-proteins are heterogeneously expressed in the CNS, it is uncertain whether this method can be applied to other brain regions. In the present study, we performed [^{35}S]GTPγS binding assay in rat

Abbreviations: CNS, central nervous system; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GPCR, G-protein-coupled receptor; mGlu receptor, metabotropic glutamate receptor; [^{35}S]GTPγS, guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; CHO, Chinese hamster ovary.

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Table 1
Agonistic properties of glutamatergic ligands.

Compound	Hippocampus			Striatum		
	N	pEC_{50}	$\%E_{max}$	*N*	pEC_{50}	$\%E_{max}$
LY379268	4	7.86 ± 0.09	69.1 ± 4.5	4	7.52 ± 0.12	68.2 ± 5.3
LY354740	4	7.10 ± 0.09	68.6 ± 3.5	4	6.87 ± 0.04	61.7 ± 7.8
DCG-IV	4	6.29 ± 0.15	58.5 ± 4.3	4	6.70 ± 0.32	56.6 ± 2.3
L-3′-F_2CCG-I	4	6.03 ± 0.17	98.7 ± 3.1	4	6.05 ± 0.11	109.4 ± 17.6
L-CCG-I	4	6.19 ± 0.09	49.6 ± 6.8	4	6.27± 0.04	61.7 ± 7.1
L-Glutamate	4	4.95 ± 0.27	100	4	5.15 ± 0.10	100
(2*R*,4*R*)-APDC	4	5.01 ± 0.10	95.4 ± 2.8	4	4.97 ± 0.09	97.1 ± 7.3
(1*S*,3*R*)-ACPD	4	4.07[a]	92.6[a]	4	4.36[a]	80.1[a]
L-Quisqualic acid	4	3.65[a]	44.1[a]	4	3.68[a]	68.9[a]
α-NAAG	3	<3.5[b]	n.d.[b]	3	<3.5[b]	n.d.[b]
cis-ACPD	4	3.64[a]	63.8[a]	4	3.59[a]	78.8[a]
(*S*)-3C4HPG	3	3.72[a]	65.4[a]	3	<3.5[b]	n.d.[b]
(*S*)-4C3HPG	3	4.47[a]	24.6[a]	3	4.63[a]	22.0[a]
LY341495	3	–[c]	–[c]	3	–[c]	–[c]
(*S*)-4CPG	3	–[c]	–[c]	3	–[c]	–[c]
MAP4	3	–[c]	–[c]	3	–[c]	–[c]
(*RS*)-3,5-DHPG	3	–[c]	–[c]	3	–[c]	–[c]
L-AP4	3	–[c]	–[c]	3	–[c]	–[c]
L-SOP	3	–[c]	–[c]	3	–[c]	–[c]
NMDA	3	–[c]	–[c]	3	–[c]	–[c]
(*RS*)-AMPA HBr	3	<3.5[b]	n.d.[b]	3	<3.5[b]	n.d.[b]
Kainic acid	3	–[c]	–[c]	3	–[c]	–[c]

[a] Analyzed using the averaged values of replicated experiments collectively.
[b] Apparently agonistic, but not determinable due to the lack of saturability even at the highest concentration (1 mM).
[c] Inactive as an agonist even at the highest concentrations investigated.

hippocampal and striatal membranes, and pharmacological characteristics of the responses in these brain regions were compared with those in rat cerebral cortical membranes.

2. Materials and methods

The experimental protocols were reviewed and approved by the Animal Committee of Saitama Medical University, and the animal care and use procedures conformed to the European Community Guidelines for the use of Experimental Animals (86/609/EEC). The functional coupling between mGlu receptors and G-proteins was determined by [^{35}S]GTPγS binding assay in rat hippocampal and striatal membranes as described in detail previously [21]. In brief, the hippocampal or striatal membranes equivalent to 10–20 μg protein, prepared beforehand from male Sprague-Dawley rats (7-week-old), were incubated in duplicate at 30 °C for 60 min in 500 μl of 50 mM Tris–HCl buffer (pH 7.4) containing 0.2 nM [^{35}S]GTPγS, 20 μM GDP, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.2 mM dithiothreitol, 100 mM NaCl, and various concentrations of glutamatergic ligands. Subsequent to the incubation, the homogenate was filtered under vacuum through glass fiber filters (GF/B; Whatman International) using a Brandel cell harvester with 2 × 5 ml washing with ice-cold 50 mM Tris–HCl buffer (pH 7.4), and the radioactivity content retained on the filter was counted in 8 ml scintillation cocktail Emulsifier-Scintillator Plus (Packard Bioscience) by a liquid scintillation counter. The non-specific binding was measured in the presence of 100 μM unlabeled GTPγS, which was subtracted from the total binding to define the specific [^{35}S]GTPγS binding. The values were represented as the mean ± S.E.M. of the indicated number of independent experiments, each performed in duplicate.

3. Results

3.1. Effects of mGlu receptors agonists

L-Glutamate increased specific [^{35}S]GTPγS binding in hippocampus and striatum in a concentration-dependent manner with a mean EC_{50} value of 11.1 and 7.1 μM, respectively, comparable to the EC_{50} of 6.8 μM determined in cerebral cortex [21]. The maximal % increase over the respective basal binding was different among the three brain regions (one-way ANOVA; $F(2,22) = 13.70$, $P < 0.001$), with the highest in cerebral cortex (51.0 ± 2.0%, $N = 17$), intermediate in hippocampus (38.3 ± 4.3%, $N = 4$), and the lowest in striatum (27.6 ± 4.6%, $N = 4$). The agonistic properties of a series of glutamatergic ligands are summarized in Table 1, wherein the $\%E_{max}$ represents the relative maximal % increase over the basal specific binding, with the value elicited by 1 mM L-glutamate defined as 100%. The most potent agonist was LY379268 in both brain regions, and the rank order of potencies of a series of compounds was quite similar to each other. Most compounds behaved as partial agonists, while L-3′-F_2CCG-I and (2*R*,4*R*)-APDC appeared to be full agonists.

3.2. Effects of mGlu receptors antagonists

The antagonistic effects of several compounds were determined against the increase in specific [^{35}S]GTPγS binding elicited by 100 μM L-glutamate (Table 2). LY341495 was the most potent antagonist (hippocampus: $pIC_{50} = 6.80 \pm 0.09$, Hill slope = −1.16 ± 0.11; striatum: $pIC_{50} = 6.57 \pm 0.27$, Hill slope = −0.84 ± 0.11), followed by MSOP and CPPG. The antagonistic effects of other compounds were insufficient even at the highest concentration examined (1 mM in most cases) to calculate the exact IC_{50} values in both brain regions (Table 2).

3.3. Comparison with the data reported in cerebral cortical membranes

The pharmacological properties of the responses determined in rat hippocampal and striatal membranes were compared with the previously reported data obtained in cerebral cortical membranes [21]. The pEC_{50} values determined for 9 glutamatergic agonists in rat hippocampal and striatal membranes were significantly correlated with those determined in cerebral cortical membranes, with a correlation coefficient of 0.97 ($P < 0.001$) and 0.95 ($P < 0.001$) and a slope of the line of 1.09 and 0.97, respectively (Fig. 1A). With respect

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