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Characterization of metabotropic glutamate receptors in rat C6 glioma cells

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Abstract

Metabotropic glutamate receptors in rat C6 glioma cells have been characterized by pharmacological and kinetic binding experiments, using both L-[^{3}H]glutamate and [^{3}H](±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid ([^{3}H](±)-*trans*-ACPD) radioligands. Saturation experiments revealed a single binding site with a $K_d = 1250 \pm 101$ nM and $B_{max} = 12.1 \pm 1.8$ pmol/mg protein when the assays were performed with L-[^{3}H]glutamate as radioligand in the presence of AMPA, kainate, NMDA and DL-threo-β-hydroxyaspartic acid. When [^{3}H](±)-*trans*-ACPD was used as radioligand, the kinetic parameters obtained were $K_d = 2605 \pm 1042$ nM and $B_{max} = 13.66 \pm 5.01$ pmol/mg protein. Pharmacological characterization indicated that specific binding of L-[^{3}H]glutamate was sensitive to different agonists of mGlu receptors, showing a rank order of affinity L-glutamate > L-quisqualic acid > (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) > ibotenic acid ⋙ (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I). Specific binding of L-[^{3}H]glutamate to mGlu receptors is regulated by guanine nucleotides. Guanylyl imidodiphosphate (Gpp(NH)p) causes an affinity shift on the L-glutamate dose-response curve, increasing the IC_{50} value. These results support the evidence that metabotropic glutamate receptors are present in rat C6 glioma cells and they are coupled to a G-protein.

Keywords: Metabotropic glutamate receptor; *trans*-ACPD; C6 glioma cell; G-protein

1. Introduction

Glutamate is considered the major excitatory neurotransmitter in the central nervous system (Fonnum, 1984). Glutamate has been implicated in diverse phenomena such as learning, memory formation and neurotoxicity (Collingridge and Singer, 1990; Meldrum and Garthwaite, 1990; Nakanishi, 1992; Bliss and Collingridge, 1993; Schoepp and Conn, 1993). This wide variety of glutamate actions is mediated through multiple glutamate receptors which can be classified into two main groups termed 'ionotropic' and 'metabotropic' receptors. The ionotropic glutamate receptors are ligand-gated ion channels which are permeable to cations and are further divided into subclasses according to their specificity for the agonists (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), *N*-methyl-D-aspartic acid (NMDA) and kainate (Nakanishi, 1992). The metabotropic glutamate receptors, which differ structurally, functionally and pharmacologically from the ionotropic receptors, are coupled to G-proteins and evoke a variety of functions through different effector systems, including the phosphatidyl inositol cascade and inhibition of adenylate cyclase (Pin and Duvoisin, 1995). To date, at least eight different subtypes of mGlu receptor have been identified by cloning ($mGlu_1$ receptor–$mGlu_8$ receptor) (Hollmann and Heinemann, 1994). These can be divided into three groups depending on sequence homology, transduction mechanisms and pharmacology in expression systems (Nakanishi, 1994; Schoepp, 1994). Group I includes $mGlu_1$ receptor (Houamed et al., 1991; Masu et al., 1991) and $mGlu_5$ receptor (Abe et al., 1992) which are coupled to phosphoinositide hydrolysis and are potently activated by L-quisqualic acid, but are also sensitive to 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD). Group II including $mGlu_2$ and $mGlu_3$ receptors (Tanabe et al., 1992) are negatively coupled to adenylate cyclase and are potently activated by 1*S*,3*R*-ACPD and (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I) (Schoepp, 1994). Group III includes $mGlu_4$ (Tanabe et al., 1993), $mGlu_6$

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(Nakajima et al., 1993), $mGlu_7$ (Okamoto et al., 1994; Saugstad et al., 1994) and $mGlu_8$ receptors (Duvoisin et al., 1995). They are also negatively coupled to adenylate cyclase but are selectively activated by L-2-amino-4-phosphonobutyric acid (L-AP4) (Schoepp, 1994). $mGlu_1$, $mGlu_2$, $mGlu_3$, $mGlu_4$, $mGlu_5$ and $mGlu_7$ receptors are primarily expressed in brain, $mGlu_6$ receptor is expressed in retina and $mGlu_8$ receptor is primarily expressed in retina and olfactory bulb. The diversity within the mGlu receptor family is further increased by the existence of alternative spliced variants of $mGlu_1$, $mGlu_4$ and $mGlu_5$ receptors with regard to the C-terminal domain leading to proteins termed $mGlu_{1A,1B,1C}$, $mGlu_{4A,4B}$ and $mGlu_{5A,5B}$ receptors (Pin et al., 1992; Iversen et al., 1994; Minakami et al., 1994).

In the nervous system mGlu receptors are coupled to a variety of effector systems as well, including stimulation of phospholipase D (Boss and Conn, 1992), inhibition of Ca^{2+} currents (Sayer et al., 1992), modulation of K^+ currents (Pin and Duvoisin, 1995) and increased release of arachidonic acid via phospholipase A_2 activation (Dumuis et al., 1990). In addition, activation of mGlu receptor by the selective agonist 1*S*,3*R*-ACPD increases cAMP accumulation in hippocampal slices by potentiating cAMP responses to other neurotransmitter receptors positively coupled to adenylate cyclase, notably adenosine A_2 receptors (Winder and Conn, 1992, 1993).

The last few years, metabotropic glutamate receptors have been the focus of intensive investigation into both their function and possible involvement in several diseases (Meldrum and Garthwaite, 1990; Nakanishi, 1992; Hayashi et al., 1993; Schoepp and Conn, 1993; Nakanishi and Masu, 1994; Pin and Duvoisin, 1995). However, studies of mGlu receptor functions are greatly hampered for lack of potent and selective radioligands. Recently, a mGlu receptor-specific agonist, [^{3}H](±)-*trans*-ACPD, has been reported by Tocris Cookson (London, UK). In the present study we show the presence of metabotropic glutamate receptors in rat C6 glioma cells, where they have been characterized by kinetic and pharmacological binding assays, using both L-[^{3}H]glutamate and [^{3}H](±)-*trans*-ACPD. The effect of guanylyl imidodiphosphate (Gpp(NH)p) on the mGlu receptor-specific binding has also been studied using L-[^{3}H]glutamate.

2. Materials and methods

2.1. Materials

L-[^{3}H]Glutamic acid (48.1 Ci/mmol) was obtained from Dupont-NEN (Boston, MA, USA). [^{3}H](±)-*trans*-ACPD (40 Ci/mmol), *trans*-ACPD, L-quisqualic acid, ibotenic acid and L-CCG-I were purchased from Tocris Cookson (London, UK). L-Glutamate, NMDA, AMPA and DL-threo-β-hydroxyaspartic acid were from Sigma (St. Louis, MO, USA). Gpp(NH)p was from Boehringer-Mannheim (Mannheim, Germany). All other reagents were of analytical grade.

2.2. Cell culture

Rat C6 glioma cells were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% foetal calf serum, 2 mM L-glutamine, 1% nonessential amino acids and antibiotics in a humidified atmosphere of 95% air, 5% CO_2 at 37°C.

2.3. Membrane isolation

C6 cell plasma membranes were isolated as described by Kessler et al. (1989). After removal of the medium and washing with isolation buffer (50 mM Tris, 10 mM $MgCl_2$), cells were homogenized in the same buffer and centrifuged at $800 \times g$ for 5 min. The supernatant was then centrifuged at $12\,000 \times g$ for 30 min. The pellet was resuspended in isolation buffer and stored at −70°C until assays were performed.

2.4. Radioligand binding assays

Metabotropic glutamate receptors were determined using the radioligand L-[^{3}H]glutamate, as described previously (Martín et al., 1991, 1993). 30–50 μg membranes were incubated in 10 mM potassium phosphate buffer, pH 7.4, for 60 min at 25°C in the presence of 100 nM L-[^{3}H]glutamate and 100 μM AMPA, 100 μM NMDA and 100 μM kainate, in order to eliminate the binding to these receptor types, 100 μM of the L-glutamate uptake inhibitor DL-threo-β-hydroxyaspartic acid also was present in the binding assays. 1 mM of different mGlu receptor agonists were used to determinate nonspecific binding. For competition experiments, different concentrations of unlabeled L-glutamate were used under the above conditions. Saturation analyses were performed using different concentrations of L-[^{3}H]glutamate (100–800 nM). L-Glutamate to a concentration of 10^5 times of the radioligand was used to obtain nonspecific binding. K_d and B_{max} values were determined by Scatchard analysis. IC_{50} values obtained in pharmacological experiments were determined graphically.

The binding to metabotropic receptors using [^{3}H](±)-*trans*-ACPD was determined by incubation of 40 μg of protein with 1000 nM radioligand for 60 min at 25°C in 10 mM potassium phosphate buffer pH 7.4. For competition experiments, different concentrations of the indicated specific agonist were used under the above-mentioned conditions. Saturation analyses were performed using different concentrations of [^{3}H](±)-*trans*-ACPD (25–1750 nM) and in the presence of unlabeled glutamate to a concentration of 10^5 times of the radioligand to obtain nonspecific binding. All these experiments were performed in the

absence and in the presence of 100 μM Gpp(NH)p in order to investigate the effect of this nonhydrolyzable nucleotide.

After incubation, bound and free ligands were separated by rapid centrifugation (12 000 rpm for 10 min in a Hettich Mikroliter bench centrifuge) at 4°C. The supernatant was aspirated and the pellet washed once with 1 ml of ice-cold buffer and dissolved in 0.1 ml of 0.01% sodium dodecyl sulfate (SDS) overnight. Bound radioactivity was counted in 3 ml of Ready-Safe liquid (Beckman).

2.5. Protein determination

Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

2.6. Statistical analysis

Data statistical analysis was performed using the Student *t*-test.

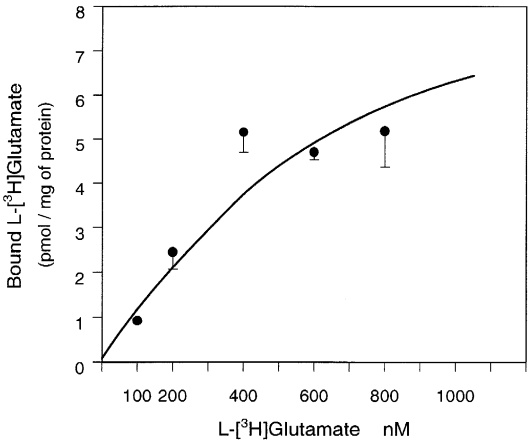

Fig. 1. Saturation curve of L-[^{3}H]glutamate binding to metabotropic glutamate receptors in C6 plasma membranes. Binding assays were performed, as described in Section 2, using L-[^{3}H]glutamate in a concentration range from 100 nM to 800 nM and unlabeled L-glutamate in a concentration of 10^5 times of the radioligand to obtain nonspecific binding. Scatchard analysis of equilibrium binding data shows a $K_d = 1250 \pm 101$ nM and $B_{max} = 12.1 \pm 1.8$ pmol/mg protein. These results are mean ± S.E.M. values of four individual experiments performed in duplicate, each using different plasma membranes preparations.

3. Results

3.1. Glutamate binding activity in rat C6 glioma cells

L-[^{3}H]Glutamate was used for binding studies in order to establish the possible presence of glutamate receptors in rat C6 glioma cells. First, glutamate binding activity to total glutamate receptors (ionotropic and metabotropic) was measured using a single concentration of L-[^{3}H]glutamate (100 nM) and 1 mM of unlabeled L-glutamate in order to determine nonspecific binding. As shown in Table 1, in these conditions a specific total glutamate receptor binding activity of 3.20 ± 0.47 pmol/mg protein was obtained. To determine the presence of metabotropic glutamate binding, we performed assays in conditions in which the binding to ionotropic receptors was inhibited, adding 100 μM NMDA, 100 μM kainate and 100 μM AMPA. In this case, 1 mM of different unlabeled mGlu receptor-specific agonists was used in order to determine nonspecific binding. Table 1 shows that 70–80% of total L-[^{3}H]glutamate binding sites in plasma membranes of rat C6 glioma cells correspond to metabotropic glutamate receptors.

To further characterize mGlu receptor present in C6 cells, we performed kinetic and pharmacological assays. As shown in Fig. 1, L-[^{3}H]glutamate binds to C6 plasma membranes in a saturable manner. Scatchard plot of the binding data showed a single population of binding sites for L-[^{3}H]glutamate with $K_d = 1250 \pm 101$ nM and $B_{max} = 12.1 \pm 1.8$ pmol/mg protein. L-Glutamate binding sites present in plasma membranes were further studied by determining the inhibitory potencies of several compounds

Table 1
Specific binding activity of L-[^{3}H]glutamate to metabotropic glutamate receptors in rat C6 glioma cells plasma membranes

	Specific binding (pmol/mg protein)		%	NMDA, kainate, AMPA (100 μM)
Total glutamate binding	3.206 ± 0.479	(5)	100	−
Metabotropic glutamate binding				
L-Glutamate	2.731 ± 0.370	(6)	85.20	+
L-Quisqualic acid	2.506 ± 0.571	(4)	78.17	+
trans-ACPD	1.192 ± 0.181	(4)	37.19	+
Ibotenic acid	2.707 ± 0.416	(4)	84.42	+
L-CCG-I	2.216 ± 0.476	(5)	69.13	+

40 μg plasma membrane was incubated with 100 nM L-[^{3}H]glutamate in potassium phosphate buffer, in the absence (total glutamate binding) and in the presence (metabotropic glutamate binding) of 100 μM kainate, NMDA and AMPA. Nonspecific binding was determined using 1 mM of unlabeled L-glutamate, in the case of total glutamate binding, and 1 mM of the indicated agonist, in the case of metabotropic glutamate binding. Total glutamate binding represents the binding to all glutamate receptor subtypes (ionotropic and metabotropic). Data are mean ± S.E.M. of a number of experiments, expressed in parentheses, each performed in duplicate using different plasma membrane preparations.

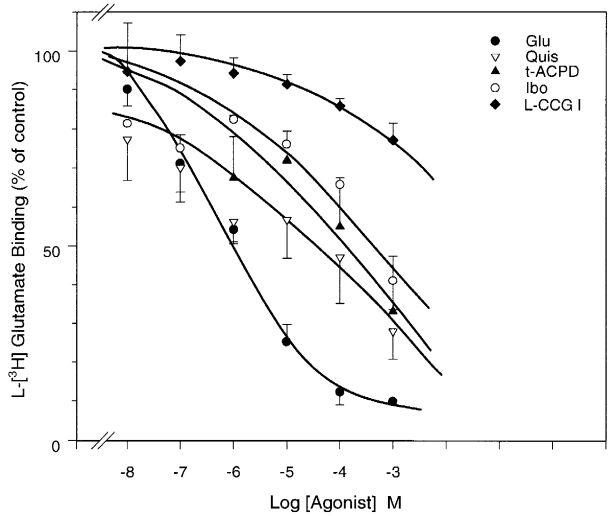

Fig. 2. Competition curves of specific L-[^{3}H]glutamate binding in C6 plasma membranes. 40 μg plasma membranes were incubated with 100 nM L-[^{3}H]glutamate, as described in Section 2, with or without increasing concentrations of different agonists in a concentration range from 10^{-8} to 10^{-2} M. Data points represent mean ± S.E.M. of at least three individual experiments performed in duplicate, each using different plasma membrane preparations. IC_{50} values of these curves are 1.14 ± 0.19 μM for L-glutamate (●), 34.93 ± 6.32 μM for L-quisqualic acid (▽), 161.98 ± 41.8 μM for *trans*-ACPD (▲), 340.33 ± 82.83 μM for ibotenic acid (○) and more than 1 mM for L-CCG-I (◆).

with demonstrated activity on metabotropic glutamate receptors. Fig. 2 shows that L-glutamate is the most potent inhibitor of L-[^{3}H]glutamate binding, with an IC_{50} value of 1.14 ± 0.19 μM, similar to the K_d value obtained in the above-mentioned kinetic experiments (1250 nM). After L-glutamate, the rank order of affinity was L-quisqualic acid > *trans*-ACPD (racemic mixture) > ibotenic acid with respective IC_{50} values of 34.93 ± 6.32, 161.98 ± 41.8 and

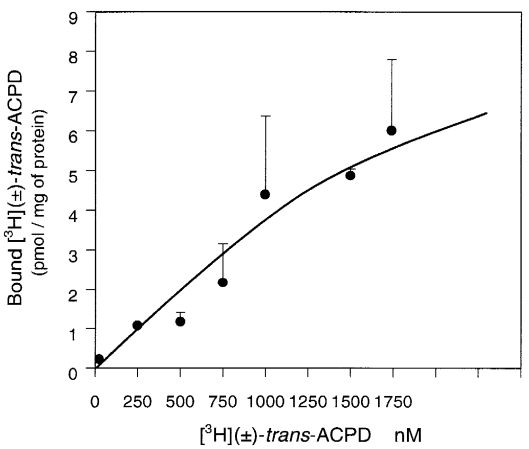

Fig. 3. Saturation curve of [^{3}H](±)-*trans*-ACPD binding to metabotropic glutamate receptor in C6 plasma membranes. Binding assays were performed as described in Section 2 and in the legend of Fig. 1, using *trans*-ACPD as radioligand, in a concentration range from 25 nM to 1750 nM. Scatchard analysis of equilibrium binding data showed a $K_d = 2605 \pm 1042$ nM and $B_{max} = 13.66 \pm 5.01$ pmol/mg protein. These results are mean ± S.E.M. values of four individual experiments performed in duplicate, each using different plasma membranes preparations.

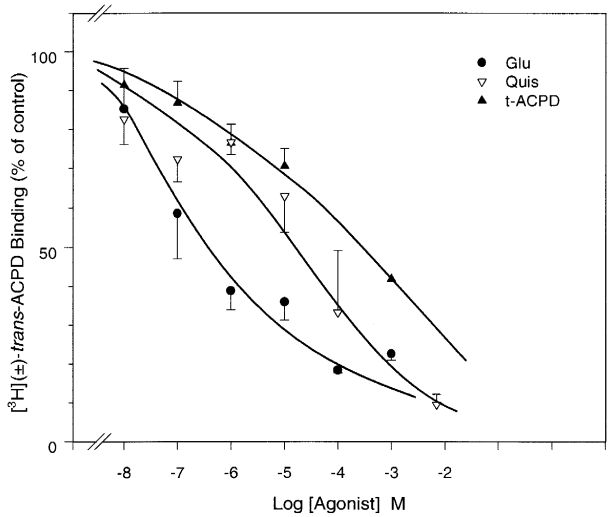

Fig. 4. Pharmacology of specific [^{3}H](±)-*trans*-ACPD binding activity in C6 plasma membranes. 1000 nM of [^{3}H](±)-*trans*-ACPD was incubated in the presence of increasing concentrations of the indicated agonists, from 10^{-8} M to 10^{-2} M. Results represent mean ± S.E.M. values of, at least, three individual experiments performed in duplicate, using different plasma membranes preparations. IC_{50} values of these agonists were 313.67 ± 71.2 nM for L-glutamate (●), 16.55 ± 1.98 μM for L-quisqualic acid (▽) and 242.58 ± 42.6 μM for *trans*-ACPD (▲).

340.33 ± 82.83 μM. L-CCG-I was almost inactive in displacing L-[^{3}H]glutamate-specific binding with an IC_{50} value of more than 1 mM.

3.2. *trans*-ACPD binding activity in rat C6 glioma cells

In order to confirm the presence of metabotropic glutamate receptors in the plasma membranes of rat C6 glioma

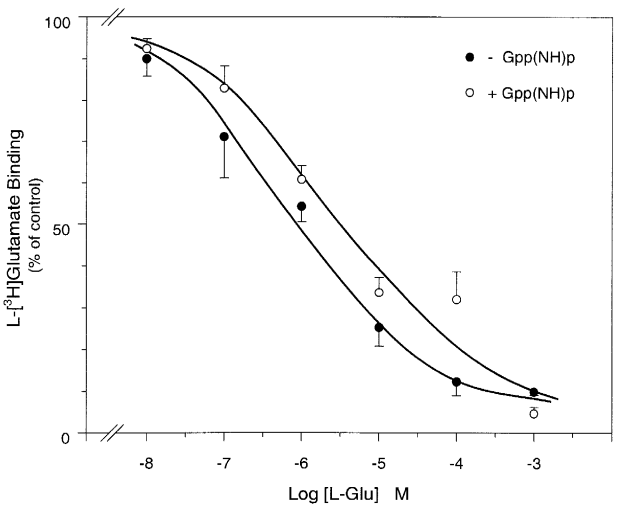

Fig. 5. Effect of 100 μM Gpp(NH)p on L-[^{3}H]glutamate competition curves in C6 plasma membranes. Specific binding of L-[^{3}H]glutamate in the absence (●) and in the presence (○) of 100 μM Gpp(NH)p was determined at various concentrations of L-glutamate. Results are expressed as percentage of maximal specific binding in each case. IC_{50} values were 1.14 ± 0.19 μM and 3.92 ± 0.43 μM in the absence and in the presence of Gpp(NH)p, respectively. Data points are mean ± S.E.M. values of six experiments performed in duplicate, each using different plasma membranes preparations.

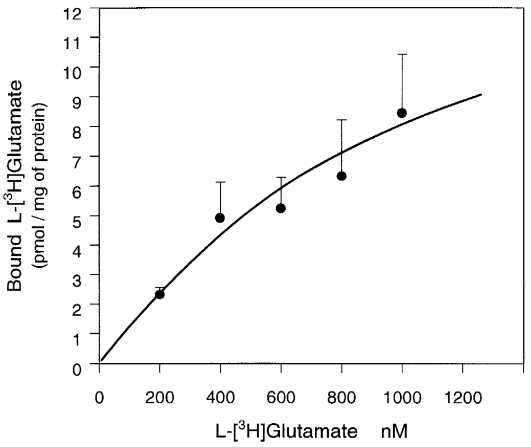

Fig. 6. Effect of 100 μM Gpp(NH)p on L-[^{3}H]glutamate saturation curve in C6 plasma membranes. Binding assays were performed as described in the legend of Fig. 1, in the presence of 100 μM Gpp(NH)p and using a concentration range of L-glutamate from 200 nM to 1000 nM. Scatchard analysis of equilibrium binding data shows a $K_d = 1598 \pm 434$ nM and a $B_{max} = 21.05 \pm 2.42$ pmol/mg protein. Data are mean ± S.E.M. of four separate experiments performed in duplicate, each with different plasma membranes preparations.

cells, we performed binding assays using the specific radiolabeled agonist *trans*-ACPD, recently available from Tocris. [^{3}H](±)-*trans*-ACPD binds to plasma membranes in a saturable manner. Scatchard plot of binding data showed a $K_d = 2605 \pm 1042$ nM and a $B_{max} = 13.66 \pm 5.01$ pmol/mg protein (Fig. 3). These results were very similar to those obtained when L-[^{3}H]glutamate was used. Binding of *trans*-ACPD in C6 plasma membranes was also explored by a pharmacological approach. As shown in Fig. 4, the affinity order was similar to that obtained when L-[^{3}H]glutamate was used as radioligand, with L-glutamate as the most potent inhibitor with an IC_{50} value of 0.31 ± 0.071 μM, followed by L-quisqualic acid with an IC_{50} of 16.55 ± 1.98 μM and *trans*-ACPD (racemic mixture) with a 242.58 ± 42.6 μM IC_{50} value.

3.3. Guanine nucleotide effect on L-[^{3}H]glutamate-specific binding in rat C6 glioma cell plasma membranes

Guanine nucleotides modulate the binding of numerous hormone and neurotransmitter agonists to their receptors (Gilman, 1987; Birnbaumer, 1990; Milligan, 1993). To further characterize the mGlu receptors present in C6 plasma membranes and considering that these receptors are coupled to G-proteins (Sugiyama et al., 1987; Schoepp et al., 1990), the kinetic and pharmacological characteristics in the absence and in the presence of Gpp(NH)p, a non-hydrolyzable analogue of GTP, were studied. As shown in Fig. 5, Gpp(NH)p brought about a shift to the right in the competition curve, increasing the IC_{50} value for L-glutamate from 1.14 μM to 3.92 μM. An increase of the kinetic parameters (K_d and B_{max}) was also obtained when saturation assays were performed in the presence of this nucleotide (Fig. 6).

4. Discussion

In the present study we report the presence of metabotropic glutamate receptors in rat C6 glioma cells. The evidence for this is presented by binding experiments using both L-[^{3}H]glutamate and [^{3}H](±)-*trans*-ACPD as specific mGlu receptor radioligands. Binding assays were performed in the presence of NMDA, kainate and AMPA, in order to prevent the binding to these receptor types, as described in Section 2. In these conditions L-[^{3}H]glutamate binds to C6 plasma membranes and 70–80% of the receptors seem to correspond to the metabotropic type (Table 1) as compared with the binding in the absence of those ionotropic receptor agonists.

The binding assays performed with L-[^{3}H]glutamate presented herein showed a single population of L-glutamate binding sites in C6 plasma membranes (Fig. 1) whose kinetic parameters are in agreement with previous results found in rat brain (Catania et al., 1994); bovine brain coated vesicles (Martín et al., 1991, 1993) and olfactory plasma membrane-rich fraction (Pang et al., 1994).

Pharmacological studies have shown the existence of different subtypes of excitatory amino acid receptors in the neuron system (Monaghan et al., 1989) one of which is the metabotropic receptor (Hollmann and Heinemann, 1994; Pin and Duvoisin, 1995). As it has been pointed out above, the existence of several forms of mGlu receptors ($mGlu_1$ receptor–$mGlu_8$ receptor) by pharmacological (Schoepp et al., 1992; Manzoni et al., 1990), electrophysiological (Sugiyama et al., 1987; Izumi et al., 1991) and cloning approaches (Masu et al., 1991; Tanabe et al., 1992; Abe et al., 1992) has been shown. The experiments described herein indicate that L-glutamate caused a maximal displacement of L-[^{3}H]glutamate binding (Fig. 2). This result agrees with other reports which show that L-glutamate is a more potent agonist than L-quisqualic acid and *trans*-ACPD and ibotenic acid in stimulating phosphoinositide metabolism in different brain regions for $mGlu_{1A}$ receptor (Houamed et al., 1991; Masu et al., 1991; Tanabe et al., 1992; Aramori and Nakanishi, 1992) and $mGlu_5$ receptor (Abe et al., 1992). Nevertheless, our data contrast with those reported by Palmer et al. (1989), who found that *trans*-ACPD was a more potent agonist than L-quisqualic acid in stimulating phosphoinositide metabolism in hippocampal slices. After L-glutamate, the potency order exhibited was L-quisqualic acid, followed by *trans*-ACPD and ibotenic acid. L-CCG-I exhibited poor displacement. This rank order of affinity is very similar to that described by Pang et al. (1994) for inhibition of L-[^{3}H]glutamate binding to the olfactory plasma membrane-rich fraction, and to that reported by Catania et al. (1994) in different rat brain regions. However, these results do not agree with those reported by Thomsen et al. (1993) for a cloned baby hamster kidney (BHK) cell line whose affinity order was L-quisqualic acid > L-glutamate > ibotenic acid > *trans*-ACPD and with those of Cha et al. (1990), who found a

rank order of L-quisqualic acid > ibotenic acid > *trans*-ACPD in cerebellar slices. These discrepancies argue in favour of the hypothesis of different metabotropic glutamate receptor responses in different brain regions, as it has been reviewed by Schoepp et al. (1990). The high potency exhibited by L-glutamate and L-quisqualic acid and the lower displacement of L-[^{3}H]glutamate bound produced by L-CCG-I in our case suggest that the L-glutamate binding site found in C6 plasma membranes could be the $mGlu_1$ receptor subtype.

mGlu receptors are activated by L-glutamate, L-quisqualic acid, ibotenic acid and *trans*-ACPD but not by NMDA, kainate or AMPA. Among these agonists *trans*-ACPD has been found to be the only specific compound for the mGlu receptors (Palmer et al., 1989; Desai and Conn, 1990). Recently, the radiolabeled mGlu receptor-specific agonist *trans*-ACPD has become available, which we have used in binding assays to further characterize the mGlu receptor present in our preparation. When assays were performed with this radioligand, [^{3}H](±)-*trans*-ACPD bound to plasma membranes in a saturable manner (Fig. 3) with very similar kinetic parameters to those obtained when L-[^{3}H]glutamate was used (Fig. 1). On the other hand, when competition experiments were performed with different mGlu receptor-specific agonists, a comparable pharmacological profile was obtained with a rank order of potency L-glutamate > L-quisqualic acid > *trans*-ACPD (Fig. 4). The different IC_{50} values found between the same ligand in L-[^{3}H]glutamate and [^{3}H](±)-*trans*-ACPD binding conditions could be explained by the different specificity of these radioligands.

It is known that metabotropic glutamate receptors are coupled to G-proteins and stimulate phosphoinositide hydrolysis (Sladeczek et al., 1985; Nicoletti et al., 1988; Sugiyama et al., 1989; Manzoni et al., 1992; Milligan, 1993). To see whether or not the mGlu receptor present in C6 plasma membranes is coupled to a G-protein, we have studied the kinetic and pharmacological characteristics in the absence and in the presence of 100 μM Gpp(NH)p, a nonhydrolyzable GTP analogue.

As shown in Fig. 5, when competition binding experiments were performed in the presence of 100 μM Gpp(NH)p, the dose-response curve was shifted to the right, increasing the IC_{50} value, demonstrating the coupling of these receptors to G-proteins. The fact that Gpp(NH)p brings about an increase in the kinetic parameters (K_d and B_{max}) when saturation assays were performed in the presence of 100 μM of the nonhydrolyzable analogue, also demonstrates the coupling of these receptors to G-proteins (Fig. 6). Although the kinetic binding experiments performed with L-[^{3}H]glutamate and [^{3}H](±)-*trans*-ACPD only detect the binding sites corresponding to the low-affinity state, the increase in the B_{max} and K_d values observed in the presence of Gpp(NH)p suggest that a transition from the high- (undetectable) to the low-affinity state could take place in the presence of guanine nucleotides (Green, 1984). All these results confirm the coupling of the mGlu receptors found in C6 plasma membranes to a G-protein.

In this report we present evidence of the presence of mGlu receptors in rat C6 glioma cells and its coupling to G-proteins. Also, the data presented herein suggest that this receptor could be the $mGlu_1$ receptor subtype. The fact that these receptors are present in this type of cells (tumoral cell line) and the participation of this receptor in neurotoxicity phenomena could open new perspectives in the study of tumoral diseases.

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