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The rat $mGlu_{1d}$ receptor splice variant shares functional properties with the other short isoforms of $mGlu_1$ receptor

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Abstract

Three splice variants of the rat metabotropic glutamate receptor 1 ($mGlu_{1a}$, $_{1b}$ and $_{1c}$ receptors) have been characterized so far. All have the same sequence up to the 46th residue following the 7th transmembrane domain, followed by different carboxyl-terminal tails. Whereas $mGlu_{1b}$ and $mGlu_{1c}$ receptors possess a short intracellular carboxyl-terminal tail, the $mGlu_{1a}$ receptor has a very long one. Compared to cells expressing $mGlu_{1b}$ or $mGlu_{1c}$ receptors, a higher agonist potency and basal phospholipase C activity were detected in cells expressing $mGlu_{1a}$ receptors. Another variant with a short carboxyl-terminal tail, the $HmGlu_{1d}$ receptor, has been recently isolated from human brain. Here we show that the $mGlu_{1d}$ receptor variant also exists in the rat. Like all rat $mGlu_1$ receptor variants, the $mGlu_{1d}$ receptor activates phospholipase C upon stimulation with $mGlu_1$ receptor agonists. Although the rank order of agonist potency is the same on $mGlu_{1a}$ and $mGlu_{1d}$ receptors, agonists are less potent in stimulating phospholipase C in $mGlu_{1d}$ receptor-expressing cells than in cells expressing $mGlu_{1a}$ receptors. Moreover, like the other short variants it has no significant constitutive activity. These results indicate that the $mGlu_{1d}$ receptor shares similar functional properties with the other short $mGlu_1$ receptor splice variants, and further suggests that the long carboxyl-terminal tail of the $mGlu_{1a}$ receptor increases phospholipase C coupling efficacy. © 1997 Elsevier Science B.V.

Keywords: Metabotropic glutamate receptor; Alternative splicing; Constitutive activity; G-protein coupled receptor; Phospholipase C coupling

1. Introduction

The metabotropic glutamate (mGlu), Ca^{2+}-sensing and $GABA_B$ receptors share primary sequence homology and constitute a new family of G-protein coupled receptors (Houamed et al., 1991; Masu et al., 1991; Brown et al., 1993; Nakanishi, 1994; Pin and Duvoisin, 1995; Conn and Pin, 1997; Kaupmann et al., 1997). Whereas the agonist binding site is located within a hydrophobic cleft formed by the 7 transmembrane domains in most G-protein coupled receptors, it is located within the large extracellular domain of mGlu receptors, a domain homologous to bacterial periplasmic binding proteins (O'Hara et al., 1993; Takahashi et al., 1993; Tones et al., 1995). Moreover, the second intracellular loop of mGlu receptors likely plays a role equivalent to that of the third intracellular loop of the other G-protein coupled receptors in G-protein coupling and activation (Pin et al., 1994, 1996; Gomeza et al., 1996a).

Among the 8 mGlu receptor subtypes cloned so far, the phospholipase C-coupled $mGlu_{1a}$ and $mGlu_5$ receptors, possess a surprisingly long carboxyl-terminal intracellular domain (350 residues), the role of which is not yet fully characterized. Two other splice variants of the $mGlu_1$ receptor differ in the length of their intracellular tail (Pin et al., 1992; Tanabe et al., 1992). In $mGlu_{1b}$ and $mGlu_{1c}$ receptors, the 313 carboxyl-terminal residues of the $mGlu_{1a}$ receptor are replaced by 20 and 11 residues, respectively. Some functional differences have been noticed between $mGlu_{1a}$ and the short receptors. Compared to $mGlu_{1b}$ and $mGlu_{1c}$ receptors, the $mGlu_{1a}$ receptor induces faster Ca^{2+} responses (Pin et al., 1992; Simoncini et al., 1993) and is activated by lower concentrations of agonists (Pickering et al., 1993; Flor et al., 1996). Moreover, in contrast to

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$mGlu_{1b}$ and $mGlu_{1c}$ receptors, the $mGlu_{1a}$ receptor stimulates cAMP formation (Gabellini et al., 1993; Pickering et al., 1993; Joly et al., 1995; Thomsen, 1996), and possesses a detectable constitutive activity (Gabellini et al., 1994; Prézeau et al., 1996).

Recently, a new $mGlu_1$ receptor splice variant called the $HmGlu_{1d}$ receptor has been identified in human brain (Laurie et al., 1996). The 308 carboxyl-terminal residues of the $HmGlu_{1a}$ receptor are replaced by 22 residues in the $HmGlu_{1d}$ receptor making it similar in length to $mGlu_{1b}$ and $mGlu_{1c}$ receptors. Although the $HmGlu_{1d}$ receptor has been shown to induce Ca^{2+}-release from internal stores, its functional and pharmacological properties have not been fully analyzed yet (Laurie et al., 1996). The aim of the present study was to determine whether such an additional $mGlu_1$ receptor splice variant was also expressed in the rat brain and to compare its functional properties in heterologous expression systems to those of the other $mGlu_1$ receptor splice variants.

2. Materials and methods

2.1. Polymerase chain reaction (PCR) amplification of the $mGlu_{1d}$ receptor cDNA

Total RNA was prepared from adult rat brain as described (Sambrook et al., 1989). One microgram of RNA was used per reaction to synthesize cDNA with the Perkin Elmer Cetus GeneAMP RNA PCR Kit. PCR amplification was then performed using three sets of primers. Three cDNA fragments were thereafter amplified: an N terminal fragment (N, 1770 bp), a linker fragment (L, 872 bp) and a carboxyl-terminal fragment (C, 1860 bp). PCR primers were designed based on the rat $mGlu_{1a}$ receptor sequence (Masu et al., 1991): for the N fragment: 5′-TTC GCC ACA ATG GTC CGG CTC-3′ and 5′-GTC ACT CAC TCA AGA TAA CGG A-3′, for the L fragment: 5′-TGT CAT CAA TGC CAT CTA TGC CAT G-3′ and 5′-CTC ATG AAT CTG GGC TTC CGG GT-3′; and for the C fragment: 5′-ACA CTA CAG GGT GGA AGA GCT T-3′ and 5′-TCC GTT ATC TTG AGT GGA GTC AC-3′. The PCR reaction was run for 40 cycles of 1 min, at 95°C, 1 min at 55°C and 2 min at 72°C. The 10 × buffer II supplied by the manufacturer was substituted by a 10 × buffer III (300 mM tricine pH 8.4, 20 mM $MgCl_2$, 50 μM β-mercaptoethanol, 0.1% gelatin, 1% thesit (Ponce and Micol, 1992)). PCR products were separated by electrophoresis on a 1% agarose gel, excised and cloned into pBluescript-KS (Stratagene). The full length cDNA was then constructed using the unique restriction sites Nco-I and Bgl-II. The cDNA was sequenced using the Applied Biosystems Model 373A DNA sequencing system with Taq DyeDeoxy™ terminator kit (Perkin Elmer). Sequence analysis was performed using the Wisconsin Sequence Analysis Package (Genetics Computer Group, Madison, WI, USA).

2.2. PCR amplification of the $mGlu_1$ receptor mRNA splice site region

Total RNA was prepared from rat cerebellum, hippocampus and forebrain using the RNA NOW™ reagent (Biogentex). 0.5 μg of total RNA was reverse transcribed into cDNA using Moloney Murine Leukemia Virus Reverse Transcriptase and random hexamers from a Perkin Elmer Cetus GeneAMP RNA PCR Kit and fragments spanning the region of the splice site of $mGlu_1$ receptors were amplified. The 5′ and 3′ primers for PCR were 5′-TCA CGA CCT CTG ATG TTG TC-3′ and 5′-CTG CCA CAC GTG CTG TCC CT-3′. A schematic representation of the position of these oligonucleotides on $mGlu_1$ receptor mRNA is given in Fig. 1a. The PCR amplification reaction was performed for 30 cycles of 1 min at 95°C, 1 min at 65°C and 2 min at 72°C. The PCR products were analyzed on a 4% agarose gel (Metaphor agarose, Tebu) and cloned into the pCR-II™ vector (InVitrogen). DH5α bacteria (BRL/GIBCO) were transformed with pCR-II™ DNA containing splice variant inserts. The splice variants amplified were analyzed by size after digestion of plasmid DNA with *Bst*XI or double digestion with *Bam*HI and *Not*I followed by electrophoresis on a 4% agarose gel. Sequencing of the different fragments was done in both directions. Splice variant frequency was analyzed by selecting individual colonies of transformed bacteria and analyzing their insert as described above.

2.3. Construction of $mGlu_{1d}$ receptor expression plasmid

The Sph-I–Kas-I fragment of pRKG1a (rat $mGlu_{1a}$ receptor cDNA into the mammalian expression vector pRK5 (Joly et al., 1995)) was replaced by the Sph-I–Kas-I fragment of $mGlu_{1d}$ receptor cDNA assembled from PCR products.

2.4. Culture and transfection of human embryonic kidney (HEK) 293 cells

HEK 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal calf serum and transfected by electroporation as previously described (Gomeza et al., 1996b). Electroporations of 10 million cells were carried out in a total volume of 300 μl with 10 μg carrier DNA and 1 μg of plasmid DNA containing $mGlu_{1a}$, $_{1b}$, $_{1c}$ or $_{1d}$ receptor sequences. Cells were then spliced in 12 wells clusters in DMEM supplemented with 10% fetal calf serum. Two hours later the cells were washed and the medium was replaced by DMEM containing Glutamax-I instead of glutamine (Gibco BRL) and no serum.

2.5. Determination of inositol phosphates accumulation

Two hours after electroporation, the culture medium was changed for medium containing [^{3}H]myo-inositol (23.4

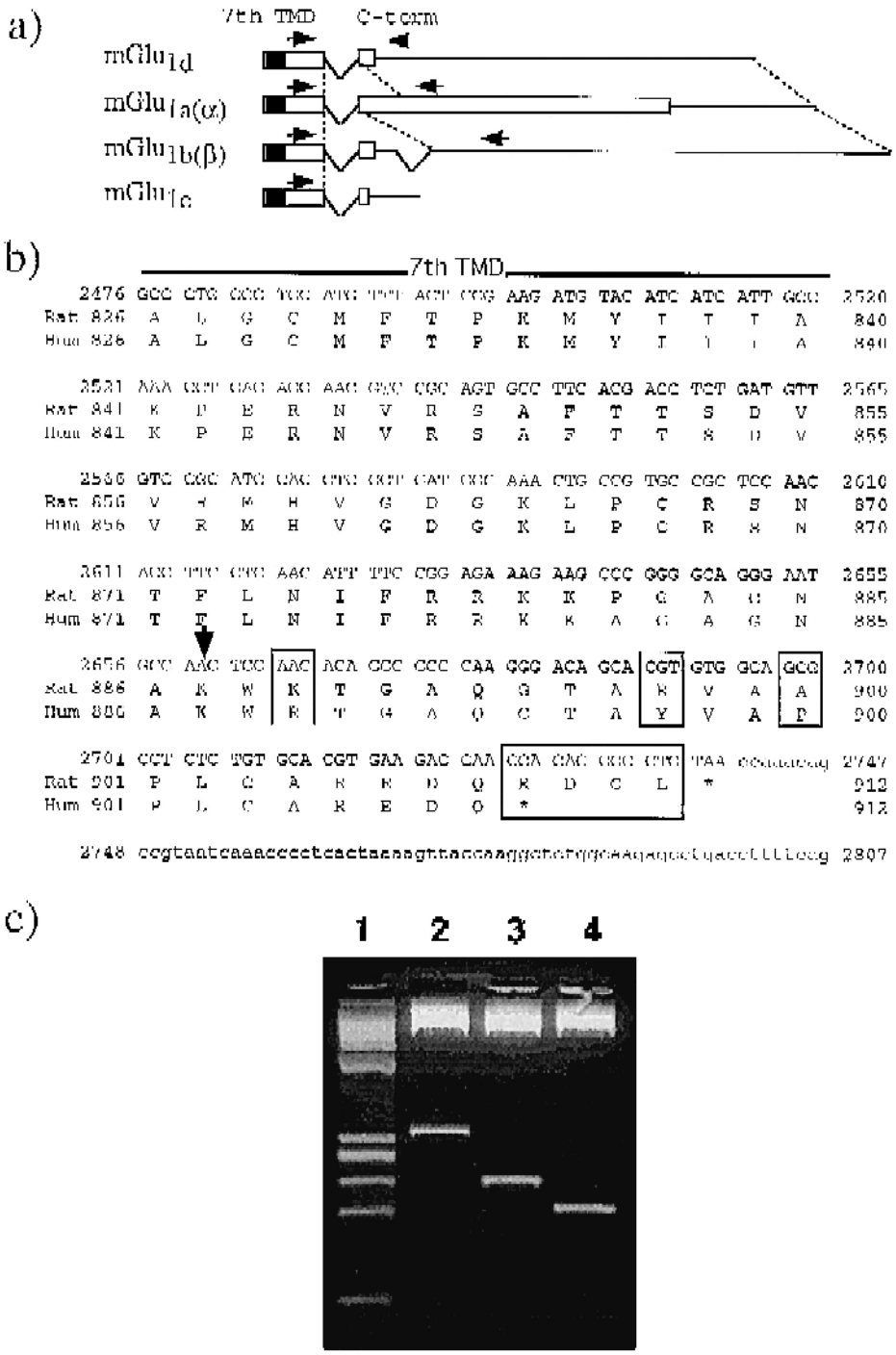

Fig. 1. Schematic representation, sequence and expression of the rat $mGlu_{1d}$ receptor splice variant. (a) Schematic representation of the C-terminal sequence of the $mGlu_1$ receptor splice variants. The C-terminal end of the coding sequence is represented as white boxes, the 7th transmembrane domain corresponds to the black square. The untranslated regions are indicated by horizontal lines. Identical sequences found in the different variants are joined by dashed lines. Introns involved in the generation of these splice variants are presented (V). The position of the oligonucleotides used to characterize the $mGlu_{1d}$ receptor mRNA are schematically represented by arrows. (b) Nucleotide and deduced amino acid sequence of the rat $mGlu_{1d}$ receptor and its comparison with the amino acid sequence of the $HmGlu_{1d}$ receptor, downstream of the 7th transmembrane domain. The position of the 35 bp deletion in the $mGlu_{1a}$ receptor sequence is indicated with an arrow. Residues that are different in the rat and human specific $mGlu_{1d}$ receptor sequences are boxed. (c) Analysis of splice variants generated by PCR from rat cerebellar RNA. PCR products spanning the splice site for $mGlu_{1a}$, $_{1b}$, and $_{1d}$ receptors were cloned into the pCR-II™ vector and analyzed by size after digestion with *Bst*XI. Lane 1 contains DNA size markers (a mixture of λDNA cut with *Hin*dIII and ϕX174 DNA cut with *Hae*III), lanes 2, 3 and 4 contain the digestion products of plasmids containing the $mGlu_{1b}$ (312 bp), $mGlu_{1a}$ (227 bp), and $mGlu_{1d}$ (192 bp) sequences, respectively. All inserts were confirmed by sequencing in both directions.

Ci/mol, NEN, France) (Prézeau et al., 1996). The day after, cells were washed several times in Krebs buffer, and then incubated for 10 min in 10 mM LiCl prior addition of the agonist at the indicated concentration. The stimulation was stopped 30 min later by removing the medium and adding 0.5 ml perchloric acid (5%). To remove any contaminating glutamate from the culture medium, the washing steps were performed in the presence of the Glu degrading enzyme, glutamate pyruvate transaminase (1 U/ml) (Boehringer Mannaheim, Meylan) and 2 mM pyruvate. Glutamate pyruvate transaminase and pyruvate were also added in the medium during the stimulation period when the basal phospholipase C activity was determined. This was previously reported to be sufficient to prevent the action of any glutamate released from the cells (Prézeau et al., 1996). Results are expressed as the amount of inositol phosphate produced over the radioactivity present in the membranes. The dose–response curves were fitted using the equation $y = ((y_{max} - y_{min})/1 + (x/EC_{50})^n) + y_{min}$ and the kaleidograph program, where n is the Hill coefficient, x is the concentration of agonist, y_{max} and y_{min} are the maximal and minimal responses, respectively.

2.6. Estimation of $mGlu_1$ receptor levels by immunoblotting

HEK 293 cell membranes were prepared as previously described (Prézeau et al., 1996). Samples (20 μg of crude membrane protein) were solubilized with Laemmli sample buffer (2.5% w/v sodium dodecyl sulfate, 25 mM Tris–HCl pH 6.8, 5% v/v β-mercaptoethanol, and 6.25% glycerol), resolved by sodium dodecyl sulfate containing polyacrylamide gel electrophoresis (SDS-PAGE – 7.5% acrylamide) and transferred by electroblotting onto an Hybond C extra membrane (Amersham, France). Immunodetection of $mGlu_1$ receptor splice variants was performed as previously described (Prézeau et al., 1996) using a polyclonal antibody directed against the N-terminus of the $mGlu_1$ receptor (Conquet et al., 1994). For normalization of the results we used detection of α-actin with monoclonal antibody (Boehringer Mannaheim, Meylan). Proteins were visualized using secondary antibodies labeled with horseradish peroxidase for a chemiluminescence reaction (NEN RENAISSANCE reagents kit). Quantification was then performed on the GS-525 Molecular Imager (Biorad) using the volume analysis of the bands, as described by the manufacturer.

3. Results

3.1. Characterization of the $mGlu_{1d}$ receptor sequence in the rat brain

A PCR product was obtained from rat brain RNA using specific $mGlu_1$ receptor oligonucleotides and corresponding to the 3′ half of the open reading frame of the $mGlu_{1a}$ receptor cDNA. Its sequence was found to be identical to the $mGlu_{1a}$ receptor cDNA, except for the deletion of 35 bases downstream of position 2660 of the coding sequence. This position corresponds to the end of an exon in the rat $mGlu_1$ receptor gene (Houamed et al., 1991), suggesting that this PCR product results from the amplification of an isoform of the $mGlu_1$ receptor mRNA generated by alternative splicing (Fig. 1a). Accordingly, a full length $mGlu_1$ receptor cDNA also lacking these 35 bases has been identified in the human brain, and has been named the $HmGlu_{1d}$ receptor (Laurie et al., 1996). In the deduced amino acid sequence of the rat $mGlu_{1d}$ receptor isoform, the 313 carboxyl-terminal residues of the rat $mGlu_{1a}$ receptor are replaced by 26 residues. Between the rat and human specific $mGlu_{1d}$ receptor sequences, 19 residues are conserved, 3 are different and the rat sequence is 4 residues longer (Fig. 1b). Further DNA amplification of retrotranscribed total RNA (RT-PCR) experiments performed using specific primers designed to amplify the region surrounding the splice site in $mGlu_{1a}$, $mGlu_{1b}$ and $mGlu_{1d}$ receptors mRNA confirm the presence of $mGlu_{1d}$ receptor mRNA in the rat brain (Fig. 1a, c).

3.2. Effects of $mGlu_1$ receptor agonists on $mGlu_{1a}$ versus $mGlu_{1d}$ receptors

The functional properties of the $mGlu_{1d}$ receptor isoform were examined in transiently transfected HEK 293 cells and compared in parallel experiments to those of the other $mGlu_1$ receptor splice variants. Like the other $mGlu_1$ receptor isoforms, the $mGlu_{1d}$ receptor stimulates inositol

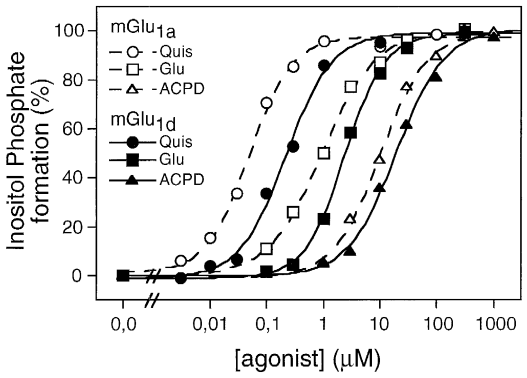

Fig. 2. Effect of quisqualate (circles), Glu (squares) and 1*S*,3*R*-ACPD (triangles) on inositol phosphate formation in cells expressing $mGlu_{1a}$ (empty symbols) or $mGlu_{1d}$ (filled symbols) receptors. One day after transfection, cells labelled with [^{3}H] myo-inositol were washed several times, incubated for 10 min in 10 mM LiCl Krebs buffer, and then stimulated for 30 min with the indicated concentration of agonists. Total inositol phosphate production was then determined as described in the text. Results are means of 3–4 independent experiments performed in triplicates, and are expressed as the percent of the maximum of the agonist-stimulated inositol phosphate production. Experiments with $mGlu_{1a}$ and $mGlu_{1d}$ receptors-expressing cells were done in parallel. Error bars were not included for clarity.

Table 1
Potency and Hill coefficient of various $mGlu_1$ receptor agonists on $mGlu_{1a}$ and $mGlu_{1d}$ receptors

	Quisqualate		Glutamate		1*S*,3*R*-ACPD	
	EC_{50} (μM)	n_H	EC_{50} (μM)	n_H	EC_{50} (μM)	n_H
$mGlu_{1a}$	0.05 ± 0.01	1.1 ± 0.4	0.86 ± 0.10	0.9 ± 0.2	7.3 ± 0.9	1.0 ± 0.3
$mGlu_{1d}$	0.21 ± 0.02	1.3 ± 0.1	2.35 ± 0.10	1.3 ± 0.1	18.1 ± 1.2	1.1 ± 0.2

EC_{50} values (μM) and Hill coefficients (n_H) were determined as described in the text. Values are means ± SEM of at least three independent experiments performed in triplicates. EC_{50} values determined on $mGlu_{1a}$ receptor-expressing cells are statistically different (*t*-test, $P < 0.05$) from those measured in parallel in $mGlu_{1d}$ receptor-expressing cells.

phosphate formation upon treatment with various $mGlu_1$ receptor agonists — quisqualate, glutamate (Glu) and 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) — in a concentration-dependent manner (Fig. 2). The rank order of potency of these agonists is quisqualate > Glu > 1*S*,3*R*-ACPD, in agreement with that obtained with the other $mGlu_1$ receptor isoforms (Fig. 2 and Table 1). However, the EC_{50} values of these agonists determined in cells expressing $mGlu_{1d}$ receptors were larger than those determined in $mGlu_{1a}$ receptor-expressing cells (Fig. 2 and Table 1), but similar to those obtained with the other $mGlu_1$ receptor short isoforms (data not shown).

3.3. Determination of basal phospholipase C activity in $mGlu_1$ receptor-expressing cells

In cells expressing $mGlu_{1d}$ receptors, Glu stimulated inositol phosphate formation to a similar extend as cells expressing other forms of the $mGlu_1$ receptor (Fig. 3a). However, as in cells expressing the other short isoforms $mGlu_{1b}$ and $mGlu_{1c}$ receptors, no significant increase in the basal phospholipase C activity was observed in $mGlu_{1d}$ receptor-expressing cells. On the contrary, a higher basal phospholipase C activity was measured in $mGlu_{1a}$ receptor-expressing cells (Fig. 3b), in agreement with our previous results (Prézeau et al., 1996). Besides, we reported that high basal phospholipase C activity observed in $mGlu_{1a}$ receptor-expressing cells does not result from activation by endogenous agonists, but is due to constitutive activity of this receptor (Prézeau et al., 1996). Basal and Glu-induced inositol phosphate formation were measured in cells transfected with increasing amounts of $mGlu_{1d}$ receptor plasmid DNA. The total amount of DNA was however maintained constant by varying the amount of carrier DNA so that the number of transfected cells remained constant (data not shown). While Glu-stimulated phospholipase C activity increased with increasing amount of $mGlu_{1d}$ recep-

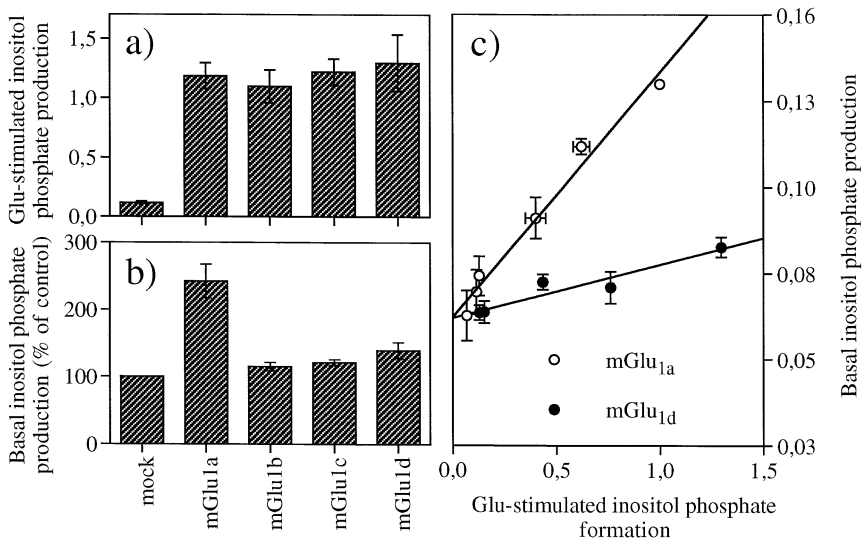

Fig. 3. Comparison of the phospholipase C-activation by $mGlu_{1d}$ and the other $mGlu_1$ receptor splice variants. Glu (1 mM)-stimulated (a) and basal (b) inositol phosphate formation were determined in cells transfected in parallel with carrier DNA alone (mock) or with plasmid DNA encoding $mGlu_{1a}$, $mGlu_{1b}$, $mGlu_{1c}$ or $mGlu_{1d}$ receptors (500 ng per 5 million cells). Values in (a) correspond to the [^{3}H]inositol phosphate produced divided by the amount of radioactivity in the membranes. Values in (b) are expressed as the percentage of the basal inositol phosphate formation determined in mock transfected cells. Values in (a) and (b) are means ± SEM of 8 to 21 independent experiments performed in triplicates. (c) Glu-stimulated (*X* axis) and basal (*Y* axis) inositol phosphate formation were determined in cells transfected with 0, 10, 30, 100, 300 or 1000 ng of plasmid DNA containing the $mGlu_{1a}$ (open circles) or $mGlu_{1d}$ (filled circles) receptor cDNA. Values are means ± SEM of triplicate determinations from a typical experiment.

tor plasmid DNA, the basal phospholipase C activity remained very low in contrast to that measured for $mGlu_{1a}$ receptor-expressing cells (Fig. 3c). Interestingly, the basal activity was found to be directly proportional to the Glu-stimulated phospholipase C activity indicating that the ratio basal over Glu-stimulated phospholipase C activity is independent of receptor expression levels (Fig. 3c). This parameter which corresponds to the slope of the curve presented in Fig. 3c, is much higher for the $mGlu_{1a}$ receptor than for the $mGlu_{1d}$ receptors, indicating that this functional difference between these two receptor isoforms cannot be explained by different levels of receptor expression.

3.4. Immunodetection of $mGlu_{1d}$ receptor protein in HEK 293 cells

Levels of expression of $mGlu_1$ receptor splice variants in HEK 293 cells were compared using western blot detection since binding experiments cannot be performed accurately due to the low affinity of [^{3}H]Glu, the only radioactive ligand for the $mGlu_1$ receptor. Samples of similar protein amounts were resolved on SDS-PAGE, blotted onto nitrocellulose, and $mGlu_1$ receptor splice variants were immunodetected using a polyclonal antibody directed against a portion of their N-terminus (Conquet et al., 1994). These blots revealed the presence of a protein of approximately 165 kDa in $mGlu_{1a}$ receptor-expressing cells, and 110 kDa in $mGlu_{1b}$, $mGlu_{1c}$ and $mGlu_{1d}$ receptor expressing cells (Fig. 4a). Such a pattern of migration on SDS-PAGE gels was previously described for the glycosylated forms of $mGlu_{1a}$ and $mGlu_{1b}$ receptor proteins (Pickering et al., 1993). Quantitation using phosphor imager technology revealed that bands with the lowest intensity always corresponded to the $mGlu_{1a}$ receptor, whereas bands with highest intensity corresponded to the $mGlu_{1d}$ receptor (Fig. 4b). Additional bands which might correspond to receptor dimers (Romano et al., 1996) were also detected with much lower intensity (Fig. 4a).

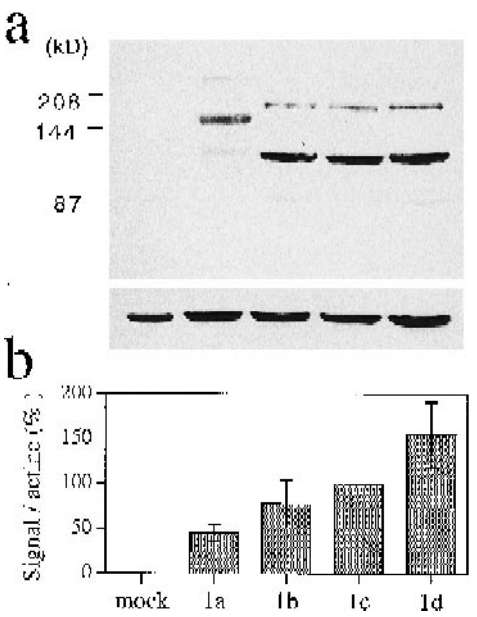

Fig. 4. Immunoblots of $mGlu_1$ receptor splice variants expressed in HEK 293 cells. Cells transfected in parallel with carrier DNA alone (mock) or with 500 ng plasmid containing the $mGlu_{1a}$, $mGlu_{1b}$, $mGlu_{1c}$ or $mGlu_{1d}$ receptor cDNAs. In each individual experiment a subset of cells was used to measure basal and Glu-induced inositol phosphate formation. High basal activity was always detected in $mGlu_{1a}$ receptor-expressing cells, and Glu stimulated inositol phosphate formation to similar extend in cells expressing any of the $mGlu_1$ receptor variants. Membranes were prepared from the remaining cells, solubilized and subjected to SDS-PAGE. After transfer on nitrocellulose membrane, $mGlu_1$ receptors and actin were detected using selective antibodies ((a), upper and lower panels, respectively). The upper band observed with the $mGlu_1$ receptor antibody likely corresponds to the mGlu receptor dimer, as described (Hampson et al., 1994; Prézeau et al., 1996; Romano et al., 1996). (b) Histogram of the ratio of the metabotropic to the actin signals measured as described in Section 2. Values in (b) are expressed as percentage of those determined with $mGlu_{1c}$ receptor-expressing cells, and are means ± SEM of 4–8 independent experiments.

4. Discussion

Our results reveal the existence of an additional rat $mGlu_1$ receptor splice variant with a short carboxyl-terminal intracellular domain homologous to the recently identified $HmGlu_{1d}$ receptor (Laurie et al., 1996). PCR analysis of the relative abundance of rat $mGlu_{1a}$, $mGlu_{1b}$ and $mGlu_{1d}$ receptor mRNAs revealed that $mGlu_{1d}$ receptor mRNA was found at a significant lower frequency than $mGlu_{1a}$ and $mGlu_{1b}$ receptor transcripts (data not shown) which may explain why this variant had not been identified earlier. Although this analysis did not allow us to estimate the relative abundance of the $mGlu_{1c}$ receptor mRNA, our previous study already suggested that this isoform was expressed at much lower levels than the other isoforms (Pin et al., 1992).

Functional analysis of the $mGlu_{1d}$ receptor expressed in HEK 293 cells revealed that like the other forms of the $mGlu_1$ receptor, it couples to phospholipase C. The pharmacological profile of the $mGlu_{1d}$ receptor was found to be similar to that of the other $mGlu_1$ receptor isoforms, in agreement with the large extracellular domain of the receptor being responsible for the ligand recognition in this receptor family (O'Hara et al., 1993; Takahashi et al., 1993; Tones et al., 1995). However, the different agonists tested are less potent in stimulating inositol phosphate formation in cells expressing $mGlu_{1d}$ receptors than in cells expressing $mGlu_{1a}$ receptors. A similar difference in the agonist potency has already been reported between the other $mGlu_1$ receptor short isoforms and the $mGlu_{1a}$ receptor (Pickering et al., 1993; Flor et al., 1996). Morevover, like the other short isoforms $mGlu_{1b}$ and $mGlu_{1c}$ receptors (Prézeau et al., 1996), the $mGlu_{1d}$ receptor does not induce an increase in basal phospholipase C activity in transfected cells in the absence of agonist, whereas such an agonist-in-

dependent activity is observed with the $mGlu_{1a}$ receptor. This further suggests that the long carboxyl-terminal intracellular domain of the $mGlu_{1a}$ receptor confers high basal constitutive activity to the receptor. This difference in relative basal activity and agonist potency between $mGlu_{1a}$ and $mGlu_{1d}$ receptors is not due to a lower level of expression of short isoform. When western blots were performed to compare the relative expression level of the different $mGlu_1$ receptor splice variants, the intensity of the band corresponding to the $mGlu_{1d}$ receptor protein was always higher than that of the $mGlu_{1a}$ receptor protein. Moreover, by varying the amount of plasmid DNA used for transfection, we were able to show that the Glu-stimulated to basal activity ratio is independent of the receptor density, and that the basal activity of the $mGlu_{1d}$ receptor is much lower than that of the $mGlu_{1a}$ receptor.

Other functional differences have been described between the $mGlu_{1a}$ receptor and the short splice variants $mGlu_{1b}$ and $mGlu_{1c}$. These include fast activation of the Cl^--current signaling when the $mGlu_{1a}$ receptor was expressed in *Xenopus* oocytes, in contrast to small and slowly developing currents induced by $mGlu_{1c}$ receptor activation (Pin et al., 1992). Moreover, in contrast to $mGlu_{1b}$ and $mGlu_{1c}$ receptors, the $mGlu_{1a}$ receptor stimulates adenylyl cyclase (Aramori and Nakanishi, 1992; Pickering et al., 1993; Joly et al., 1995; Thomsen, 1996). We were unable to obtain any activation of the Cl^--current in *Xenopus* oocytes expressing $mGlu_{1d}$ receptors (data not shown), and our data indicate that like $mGlu_{1b}$ and $mGlu_{1c}$ receptors, the $mGlu_{1d}$ receptor does not stimulate cAMP formation in transfected cells. Taken together, these results confirm that the long carboxyl-terminal tail of the $mGlu_{1a}$ receptor enables it (i) to have a higher coupling efficacy for the G protein (Lechleiter et al., 1990, 1991; Fong et al., 1992; Pin et al., 1992; Kunkel and Peralta, 1993; Spengler et al., 1993; Gomeza et al., 1996a), (ii) to activate various subtypes of G-proteins allowing it to stimulate cAMP formation (Aramori and Nakanishi, 1992; Pickering et al., 1993; Joly et al., 1995; Thomsen, 1996), and finally (iii) to activate phospholipase C even in the absence of agonist (Prézeau et al., 1996). These functional properties observed in heterologous expression systems may not necessarily be relevant in cells naturally expressing these receptors. However, they clearly indicate that the long carboxyl-terminal intracellular domain of $mGlu_{1a}$ receptor confers specific G-protein coupling properties not shared by the short variants, including the $mGlu_{1d}$ receptor.

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