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Involvement of a cyclic-AMP pathway in group I metabotropic glutamate receptor responses in neonatal rat cortex

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Abstract

3,5-dihydroxyphenylglycine (DHPG), (*S*)-3-hydroxyphenylglycine and (*S*)-4-carboxy-3-hydroxyphenylglycine (*S*-4C3HPG) stimulated phosphoinositide hydrolysis in neonatal rat cortical slices, but with lower maximal effect, in comparison with 2*S*,1′*S*,2′*S*-2-(2′-carboxycyclopropyl)glycine (L-CCG I) or (1*S*,3*R*)-1-aminocyclo-pentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD). DHPG, 1*S*,3*R*-ACPD, and *S*-4C3HPG also evoked a rapidly desensitizing increase in $[Ca^{2+}]_i$ in cortical layers of neonatal brain slices. (*R*,*S*)-α-methyl-4-tetrazolylphenylglycine (MTPG), and (*R*,*S*)-α-methyl-4-phosphono-phenylglycine (MPPG) inhibited the increase of phosphoinositide hydrolysis elicited by 1*S*,3*R*-ACPD but not that by *R*,*S*-DHPG. In contrast, the selective group II receptor agonist (1*S*,2*S*,5*R*,6*S*)-2-amino-bicyclo-[3.1.0]-hexane-2,6-dicarboxylate (LY 354740) potentiated the response of *R*,*S*-DHPG. Finally, 8-(4-chlorophenylthio)-cAMP, a membrane permeant analogue of cAMP, reversed the stimulatory effect of 1*S*,3*R*-ACPD and *S*-4C3HPG on phosphoinositide hydrolysis and $[Ca^{2+}]_i$ mobilization, without affecting the response induced by *R*,*S*-DHPG. These data suggest that, in neonatal rat cortex, the activation of group II metabotropic glutamate receptors potentiates the phosphoinositide hydrolysis and $[Ca^{2+}]_i$ responses mediated by group I metabotropic glutamate receptors. © 1997 Elsevier Science B.V.

Keywords: Ca^{2+} imaging; Cross talk; cAMP; Phenylglycine

1. Introduction

Eight metabotropic glutamate receptors (mGlu receptors) have been cloned from rat brain and they fall into three groups based on sequence homology, second messenger coupling and pharmacological characterization. $mGlu_1$ and $mGlu_5$ receptors, and their splice forms, are coupled to phosphoinositide (PI) hydrolysis and can be activated by (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD), quisqualate and ibotenate; they are classified as 'group I mGlu receptors'. Group II receptors include $mGlu_2$ and $mGlu_3$ receptors, which modulate cyclic AMP (cAMP) formation when expressed in mammalian cells, and can be activated by 1*S*,3*R*-ACPD, 2*S*,1′*S*,2′*S*-2-(2′-carboxycyclopropyl)glycine (L-CCG-I) and (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV), but are insensitive to L-2-amino-4-phosphonobutyrate (L-AP4). Group III is composed of $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ receptors, which also modulate adenylyl cyclase but are insensitive to 1*S*,3*R*-ACPD and activated by L-AP4 (Nakanishi, 1992; Pin and Duvoisin, 1995).

A number of phenylglycines have been reported to act on mGlu receptors in several in vitro preparations (Bedingfield et al., 1995; Birse et al., 1993; Eaton et al., 1993, Jane et al., 1993; Kemp et al., 1994; Sekiyama et al., 1996). These compounds seem to be promising for the functional characterization of mGlu receptor subtypes. (*R*,*S*)-3,5-Dihydroxyphenylglycine (*R*,*S*-DHPG), (*S*)-3,5-dihydroxyphenylglycine (*S*-DHPG) and (*S*)-3-hydroxyphenylglycine (*S*-3HPG) have been described as selective and potent agonists on the group I mGlu receptors (Baker et al., 1995; Hayashi et al., 1994; Ito et al., 1992; Schoepp et al., 1994; Thomsen et al., 1994) while (*S*)-4-carboxyphenylglycine (*S*-4CPG) and (*S*)-4-carboxy-3-hydroxyphenylglycine (*S*-4C3HPG) are considered to be antagonists of group I and agonists on group II mGlu receptors (Hayashi et al., 1994; Thomsen et al., 1994). More recently, however, an agonist effect of *S*-4C3HPG at high

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concentrations ($EC_{50} > 300$ μM) was shown on transfected $mGlu_5$ but not $mGlu_{1a}$ (Brabet et al., 1995). (1*S*,2*S*,5*R*,6*S*)-2-Amino-bicyclo-[3.1.0]-hexane-2,6-dicarboxylate (LY 354740) was recently described to be the most potent and selective group II agonist with IC_{50} values of 5 and 24 nM on transfected human $mGlu_2$ and $mGlu_3$ receptors, respectively. This compound was inactive on cloned human $mGlu_{1a}$, $mGlu_4$, $mGlu_{5a}$ and $mGlu_7$ receptors, up to a concentration of 100 μM (Schoepp et al., 1997). (+)-α-Methylcarboxyphenylglycine ((+)-MCPG), has been reported to be an antagonist of group I and II mGlu receptors (Hayashi et al., 1994) while (*R*,*S*)-α-methyl-4-tetrazolyl phenylglycine (MTPG) was found to be an antagonist of group II/III mGlu receptors, of $mGlu_{1a}$ and, with much less potency, of $mGlu_{5a}$ (Jane et al., 1995; Thomsen et al., 1996). (*R*,*S*)-α-methyl-4-phosphono-phenylglycine (MPPG) was found to be the most potent antagonist on transfected $mGlu_2$ and $mGlu_4$ (Gomeza et al., 1996). Finally, L-CCG I has been described to be a non-selective agonist with preferential activity on group II mGlu receptors (Hayashi et al., 1992).

In the present study we have investigated the action of several phenylglycine derivatives on phosphoinositidase-coupled receptors in neonatal (post natal day 6 = PND 6) rat cortical slices with a particular interest for possible cross-talk between the signaling cascades respectively activated by group I and group II mGlu receptors.

2. Materials and methods

2.1. Materials

The experimental animals were either laboratory inbred Wistar rats or SPF rats obtained from Biological Research Laboratories (Füllinsdorf). Myo[2-^{3}H]inositol (S.A. 20 Ci/mmol, TRK 807) was purchased from Amersham (Zürich). 1-[2-(5-Carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*,*N*-tetraacetic acid pentaacetoxymethyl ester (FURA2-AM) was obtained from Molecular Probes (Eugene, OR) and tetrodotoxin from Sigma (Buchs). 1*S*,3*R*-ACPD, L-CCG I, *S*-3HPG, *S*-4CPG, *S*-4C3HPG, (+)-MCPG, *R*,*S*-DHPG, MPPG and MTPG were purchased from Tocris Cookson (Bristol). 8-(4-Chlorophenylthio)-cAMP [8(4ClPh*S*)cAMP] was obtained from RBI (Zürich). *S*-DHPG (Baker et al. (1995)) and LY 354740 were synthesized at Hoffmann La Roche by Dr. M.P. Heitz, Dr. B. Wirz and Dr. R. Jakob-Røtne. All others reagents used were of the highest purity available and purchased from Sigma or RBI.

2.2. Methods

2.2.1. Phosphoinositide hydrolysis

Six day old rats were decapitated and the cortex removed, bathed in an oxygenated cold Krebs buffer (NaCl, 118 mM; KCl, 3.2 mM; $CaCl_2$, 1.3 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 0.96 mM; glucose, 10 mM) and cross-sectioned (350 μm × 350 μm) using a McIllwain tissue chopper. After washing for 60 min in Krebs buffer at 37°C, the slices were incubated with 7.7 μCi myo [2-^{3}H]Inositol for 45 min. LiCl (10 mM final) was added followed, 15 min later, by the agonist for 30 min. For the inhibition experiments, phenylglycine derivatives or the cAMP analogue were incubated alone or in combination with 1*S*,3*R*-ACPD or *R*,*S*-DHPG for 30 min. The reaction was stopped with $HClO_4$ (final concentrations of 0.264 N). After neutralization the [^{3}H]IP1 fraction was separated using Dowex-AG1-X8 anion exchange columns and quantified by liquid scintillation counting. Data were normalized to the response obtained with 1 mM 1*S*,3*R*-ACPD. Statistical significance was determined using a unpaired Student's *t* test (RS1, BBN). The agonist curves were fitted to a four parameter logistic equation giving EC_{50} value, Hill coefficient and maximal effect using Deltagraph (Deltapoint, Monterey, CA).

2.2.2. Ca^{2+} imaging

Fresh cortical slices (300 μm thick) were obtained from ether-anesthetized 6 day old rats using a rotatory slicer. They were incubated in oxygenated Krebs buffer for 60 min at 34°C and then for 30 min in oxygenated Krebs buffer containing 2 μM FURA2-AM. After 30 min washing, the slices were superfused with Krebs buffer containing tetrodotoxin (0.5 μM). The tested drugs were applied for 30 s. The slices were alternatively illuminated at 350 and 380 nm and fluorescent images (510 nm), captured by an intensified CCD camera (Extended Isis, Photonic Science, Milham) were recorded every 10 s. The fluorescence ratio (F350/F380) image was calculated on an Imstar Computer Imaging system (Paris) according to Kudo et al. (1991). The viability of the preparation was tested in each experiment by a brief application of 30 mM KCl at the end of the recording period. The response was then semi-quantified by integrating the ratio value in different regions of interest in the recorded fields. This integrated value was then normalized to the value obtained with a full agonist and allowed an estimation of the drug effect. Statistical analysis was performed using the Mann–Whitney non-parametric test or a paired Student's *t* test.

3. Results

3.1. Phosphoinositide hydrolysis

In neonatal cortical slices phosphoinositide hydrolysis was stimulated in a concentration dependent manner by L-CCG I, 1*S*,3*R*-ACPD, *R*,*S*-DHPG, *S*-DHPG, *S*-3HPG and *S*-4C3HPG. These agonists exhibited different potencies (Fig. 1 and Table 1). At 1 mM concentration, the

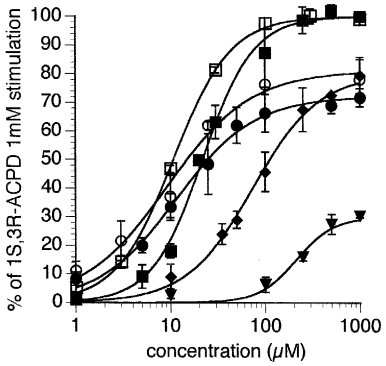

Fig. 1. Dose effect curves of agonists on PI hydrolysis in cortical slices from 6 days old rat brain. Effects have been normalized to the stimulation induced by 1*S*,3*R*-ACPD 1 mM ($n = 3$). (■) 1*S*,3*R*-ACPD; (□) L-CCG I, (▼) *S*-4C3HPG, (♦) *S*-3HPG, (●) *R*,*S*-DHPG, (○) *S*-DHPG.

responses to *S*-DHPG, *R*,*S*-DHPG and *S*-3HPG were similar, but all were statistically (Student's *t* test performed on dpm values obtained at 1 mM, $n = 3$) significantly of smaller magnitude than the maximal effect induced by 1*S*,3*R*-ACPD (i.e. $82 \pm 5\%$, $P = 0.03$; $73 \pm 3\%$, $P = 0.013$; and $78 \pm 2.5\%$, $P = 0.026$; respectively).

Finally, the Hill number for the agonist concentration-response curves was approximately one for *S*-DHPG, *R*,*S*-DHPG and *S*-3HPG but was higher than one for L-CCG I, 1*S*,3*R*-ACPD and *S*-4C3HPG.

Phosphoinositide hydrolysis was not directly affected by *S*-4CPG and (+)-MCPG but at concentrations of 1 mM, these drugs inhibited the effect of 1*S*,3*R*-ACPD (Fig. 2).

S-4C3HPG, which stimulated phosphoinositide hydrolysis in a concentration dependent manner (Fig. 1), did not exhibit antagonist activity on the IP3 effect elicited by 1*S*,3*R*-ACPD (Fig. 2). With regard to its agonist effect, the EC_{50} was much higher than that of the other agonists (Table 1) and the magnitude of the response was only $32 \pm 0.6\%$ of the maximal effect elicited by 1*S*,3*R*-ACPD (Fig. 1).

Table 1
Agonist effect of 1*S*,3*R*-ACPD and phenylglycine derivates on phosphoninositide hydrolysis, in 6 day old rat cortical slices

Compound	EC_{50}	Hill	E_{max}
S-DHPG	10 ± 2	0.88 ± 0.1	82 ± 5
L-CCG I	11 ± 0.24	1.4 ± 0.07	99 ± 1.9
R,*S*-DHPG	12 ± 3	0.97 ± 0.1	73 ± 3
1*S*,3*R*-ACPD	22 ± 9	2.1 ± 0.7	—
S-3HPG	88 ± 12	1.2 ± 0.3	78 ± 2.5
S-4C3HPG	270 ± 15	1.8 ± 0.2	32 ± 0.6
S-4CPG	N.E.	—	—

The mean EC_{50} (μM), Hill values and maximum effect (E_{max} in % of 1*S*,3*R*-ACPD maximum effect) ± standard deviation were derived from the individual dose curves performed at least 3 times (see Fig. 1).

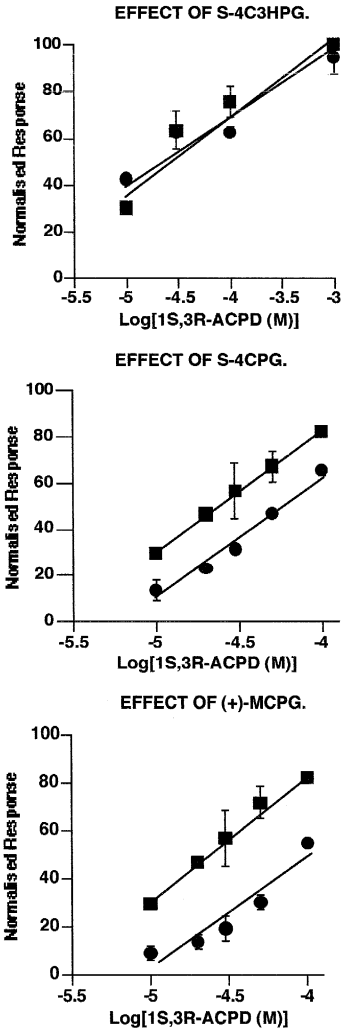

Fig. 2. Antagonist effect of *S*-4C3HPG, *S*-4CPG and (+)-MCPG at 1 mM on 1*S*,3*R*-ACPD induced PI hydrolysis in cortical slices from 6 days old rat brain. 1*S*,3*R*-ACPD alone, 1*S*,3*R*-ACPD in presence of the antagonist (1 mM). Responses were normalized to the effect of 1*S*,3*R*-ACPD at 1 mM ($n = 3$).

3.2. Ca^{2+} imaging

As 1*S*,3*R*-ACPD (not shown), *S*-DHPG, *R*,*S*-DHPG, and *S*-4C3HPG evoked an increase of $[Ca^{2+}]_i$ (Fig. 3A, B, and C) starting from a 1, 10 and 100 μM concentrations, respectively. At 500 μM, *S*-4CPG was devoid of agonist activity.

Repeated applications of these agonists showed a partial desensitization in the successive applications (Fig. 4); thus, for each antagonist tested on 1*S*,3*R*-ACPD, the observed responses were corrected for the desensitization induced by the agonist, measured in parallel experiments.

All the antagonists, when used at a concentration of 500 μM, inhibited the $[Ca^{2+}]_i$ response elicited by 1*S*,3*R*-ACPD to varying extents, as follows: *S*-4C3HPG less than 10%, *S*-4CPG by 15% and (+)-MCPG by 23% (Fig. 5).

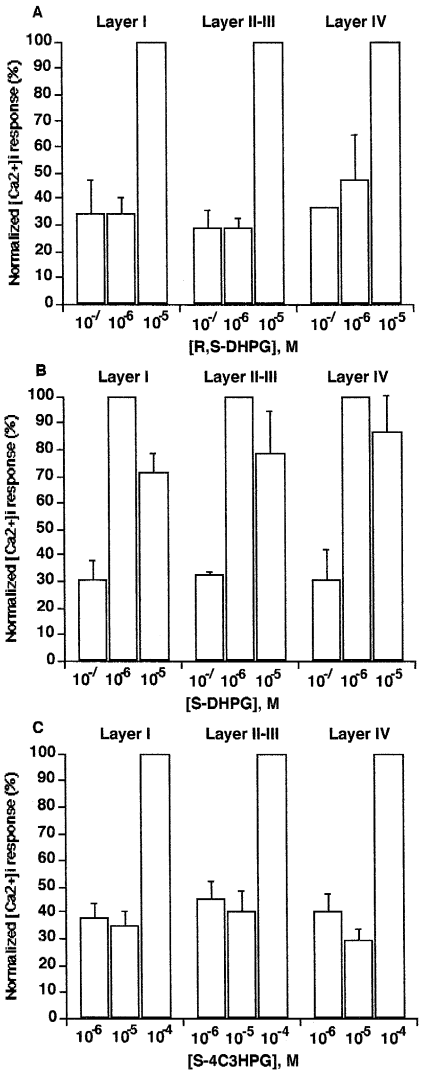

Fig. 3. Dose dependent effect of (A) *R,S*-DHPG (B) *S*-DHPG and (C) *S*-4C3HPG on $[Ca^{2+}]_i$ in layers I, II–III and IV in 6 days old rat cortical slices ($n = 3$). The responses are normalized to the maximal effect obtained with each drug. Note the higher potency and the desensitization observed with *S*-DHPG.

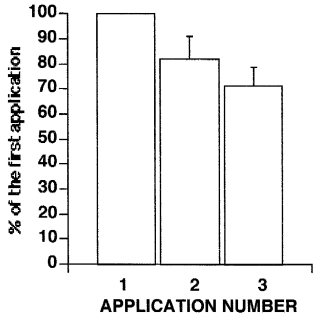

Fig. 4. Desensitization of 1*S*,3*R*-ACPD-induced $[Ca^{2+}]_i$ response in 6 days old rat cortical slices after repeated agonist applications. Results are expressed as percent of the first agonist response ± SD ($n = 3$). One hundred micromolar 1*S*,3*R*-ACPD was applied for 30 s and the slice was washed, with Krebs buffer, for 3 min between applications.

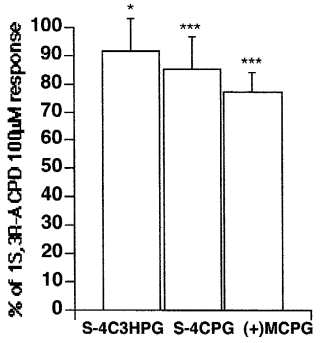

Fig. 5. Antagonist effect of 500 μM *S*-4C3HPG, *S*-4CPG and (+)-MCPG on the 100 μM 1*S*,3*R*-ACPD-induced $[Ca^{2+}]_i$ in 6 days old rat cortical slices. These effects were corrected for desensitization ($n = 3$ to 11). $^{*}P < 0.05$, $^{***}P < 0.01$ versus 1*S*,3*R*-ACPD alone; Mann–Whitney test.

3.3. Cross-talk

The maximal phosphoinositide response induced by the specific group I agonists, *R,S*-DHPG, *S*-DHPG and *S*-3HPG was lower than that induced by 1*S*,3*R*-ACPD which is agonist on both group I and group II mGlu receptors.

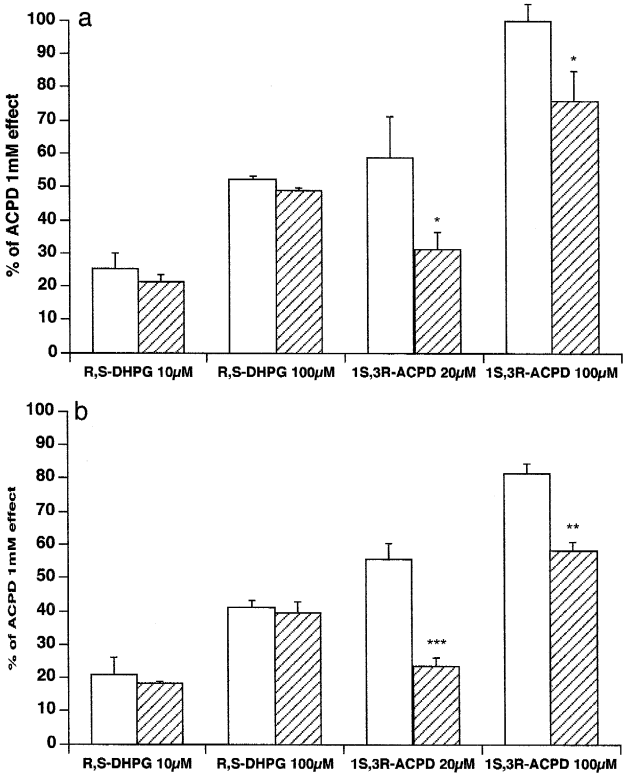

Fig. 6. Effect of 500 μM MTPG (a) or MPPG (b) on PI hydrolysis induced by *R,S*-DHPG and 1*S*,3*R*-ACPD in 6 days old rat cerebral cortex slices ($n = 3$). Empty bars: without antagonist; striped bars: with antagonist. Response were normalized to the effect of 1*S*,3*R*-ACPD at 1 mM. $^{*}P < 0.01$, $^{**}P < 0.005$, $^{***}P < 0.001$ versus agonist alone; unpaired Student *t* test.

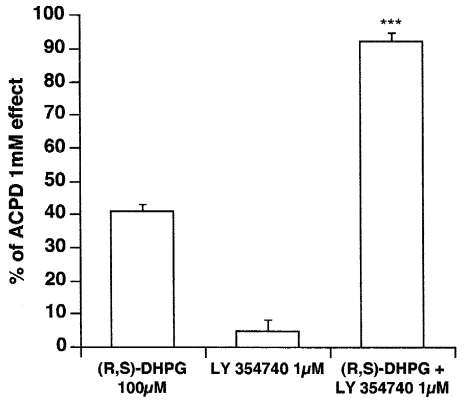

Fig. 7. Effect of 1 μM LY 354740 on PI hydrolysis stimulation induced by 100 μM *R,S*-DHPG in 6 days old rat cerebral cortex slices ($n = 3$). Response were normalized to the effect of 1*S*,3*R*-ACPD at 1 mM. $^{***}P < 0.001$ versus *R,S*-DHPG alone; unpaired Student *t* test.

Also, surprisingly, *S*-4C3HPG, which is considered as a $mGlu_1$ antagonist, moderately stimulated the IP3/Ca^{2+} signaling cascade. This compound, however, did not antagonize the effect of 1*S*,3*R*-ACPD as would be expected for a partial agonist. We thus hypothesized that the simultaneous activation of group I and group II mGlu receptors by non-selective agonists such as 1*S*,3*R*-ACPD resulted in a stimulation of the IP3/Ca^{2+} signaling cascade greater than that generated by the activation of group I mGlu receptors alone.

In order to test this hypothesis, we attempted to block the stimulation of group II mGlu receptors by using MTPG and MPPG. Five hundred micromolar MTPG or MPPG were without effect on phosphoinositide hydrolysis evoked by *R,S*-DHPG, but inhibited the responses induced by 20 μM and 100 μM 1*S*,3*R*-ACPD, or by 250 μM and 500 μM *S*-4C3HPG (Fig. 6a and b). A reciprocal experiment was performed using LY 354740, a selective and potent group II agonist. While 1 μM LY 354740 did not affect significantly phosphoinositide hydrolysis, the compound potentiated the response evoked by a saturating concentration of (*R,S*)-DHPG, up to the same magnitude of response obtained with the non-selective agonists L-CCG I and 1*S*,3*R*-ACPD (Fig. 7).

A membrane-permeant cAMP analogue, 8(4ClPh*S*)cAMP, which by itself did not display any significant effect on phosphoinositide hydrolysis (Fig. 8), inhibited the stimulatory effects of 1*S*,3*R*-ACPD and of *S*-4C3HPG (both used at the concentrations above) on phosphoinositide hydrolysis and was without effect on the *R,S*-DHPG-induced response. Three mM 8(4ClPh*S*)cAMP reversed the stimulatory effect of 100 μM *S*-4C3HPG on $[Ca^{2+}]_i$ increase in cortical layer I and more effectively in layer IV

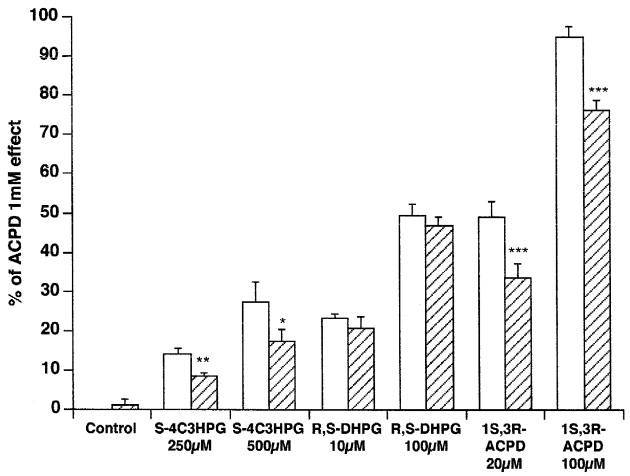

Fig. 8. Effect of 500 μM 8(4ClPh*S*)cAMP on PI hydrolysis induced by *S*-4C3HPG, *R,S*-DHPG and 1*S*,3*R*-ACPD in 6 days old rat cerebral cortex slices ($n = 3$). Empty bars: no 8(4ClPh*S*)cAMP added; striped bars: 8(4ClPh*S*)cAMP added. Responses were normalized to the effect of 1*S*,3*R*-ACPD at 1 mM. $^{*}P < 0.01$, $^{**}P < 0.005$, $^{***}P < 0.001$ versus agonist alone; unpaired Student *t* test.

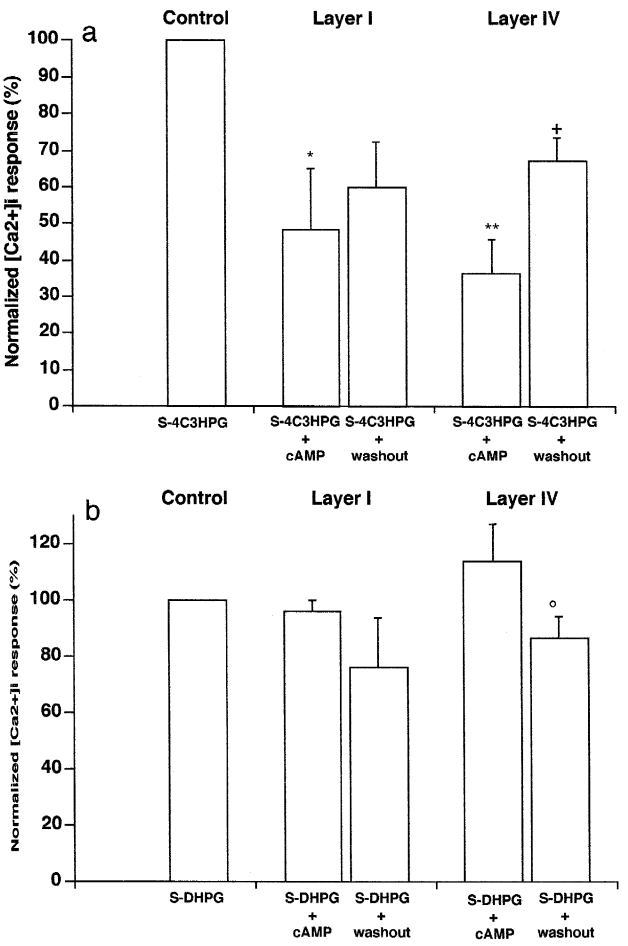

Fig. 9. Effect of 8(4ClPh*S*)cAMP on $[Ca^{2+}]_i$ increase induced by (a) 100 μM *S*-4C3HPG, or (b) 10 μM *S*-DHPG in layer I and IV of 6 days old rat cortical slices ($n = 3$). Responses were normalized on the effect induced by the agonist alone. Each drug application lasted for 30 s and the drugs were washed out for 3 min between each application. $^{*}P < 0.01$, $^{**}P < 0.001$ versus *S*-4C3HPG alone; $^{+}P < 0.02$ versus *S*-4C3HPG + cAMP analogue, $^{\circ}P < 0.03$ versus *R,S*-DHPG + cAMP analogue; paired Student *t* test.

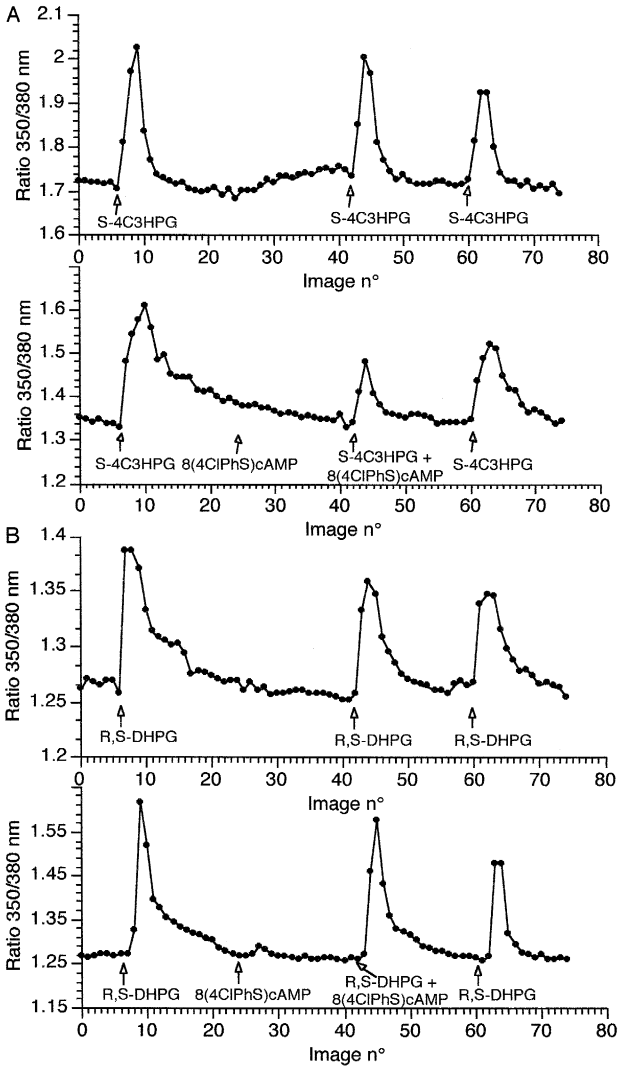

Fig. 10. Effect of 100 μM *S*-4C3HPG (A) or 10 μM *R,S*-DHPG (B) on the $[Ca^{2+}]_i$ measured in layer I of 6 days old rat cortical slices. The images were taken every 10 s according to the methods described above and the ratio F350/380 nm was measured. No significant effect was elicited by 3 mM 8(4ClPh*S*)cAMP alone. Three mM 8(4ClPh*S*)cAMP decreased the effect of *S*-4C3HPG without affecting the response induced by *R,S*-DHPG. The traces represent the results obtained in a typical experiment. The arrows indicated the application of compounds.

of the cortex (Fig. 9a, Fig. 10A and Fig. 11). The effect elicited by *S*-4C3HPG was partially restored upon the washing out of 8(4ClPh*S*)cAMP. In contrast, the cAMP analogue did not affect the increase of $[Ca^{2+}]_i$ elicited by 10 μM *S*-DHPG (Fig. 9b) or *R,S*-DHPG (Fig. 10b).

4. Discussion

The availability of highly selective pharmacological tools is a prerequisite for investigating the role of each of the mGlu receptors. Until recently, very few compounds exhibited the desired selectivity, however, reports on the activities of various phenylglycines derivatives have suggested that these compounds might be of help for further characterization of mGlu receptors (Baker et al., 1995; Bedingfield et al., 1995; Eaton et al., 1993; Hayashi et al., 1994; Ito et al., 1992; Jane et al., 1993; Kemp et al., 1994; Pin and Duvoisin, 1995; Schoepp et al., 1994; Sekiyama et al., 1996; Thomsen et al., 1994). In the present study we have used neonatal cortical slices to investigate the action of phenylglycine derivatives on mGlu receptors coupled to phosphoinositidase C.

R,S-DHPG and *S*-DHPG have been described as selective agonists on metabotropic glutamate receptors coupled to phospholipase C (Hayashi et al., 1994; Thomsen et al., 1994). Our results confirm that, in six day old rat cortical slices, *S*-3HPG, *R,S*-DHPG and *S*-DHPG stimulated phosphoinositide hydrolysis with various potencies, but with responses of similar magnitudes.

The maximal responses elicited by the two hydroxyphenylglycines were, however, significantly smaller than those elicited by the non-selective group I and group II mGlu receptor agonists, 1*S*,3*R*-ACPD or L-CCG I (Pin and Duvoisin, 1995). Furthermore, the Hill coefficients of the hydroxyphenylglycine concentration-response curves (i.e. approximately one) were different from those of 1*S*,3*R*-ACPD and L-CCG I (i.e. > 1) (Table 1). To explain the differences in the effects of 1*S*,3*R*-ACPD or L-CCG I and the hydroxyphenylglycine derivatives, we can hypothesize that the various compounds exhibited selective preferences for the different group I mGlu receptors subtypes present in the cortex, each expressed at different densities (hypothesis 1). Romano et al. (1996), using specific antibodies in Western blots, showed a high density of $mGlu_{5a}$ in PND 7 rat cortex membranes. Northern blot analysis showed that the mRNA encoding $mGlu_{5a}$ was three times more abundant than that encoding for $mGlu_{5b}$ (Romano et al., 1996). In contrast $mGlu_{1a}$ has been shown to be expressed at a low level in the cortex at all ages (Catania et al., 1994; Shigemoto et al., 1992), although information is lacking concerning its splice forms at PND 6. At present, the absence of analogs enabling us to discriminate between the different mGlu receptors belonging to group I does not allow further verifications of this hypothesis.

It is also possible that part of the stimulatory properties of the two non-selective agonists results from the co-activation of group II receptors, and, from the subsequent cross-talk, a greater response is obtained than with group I stimulation alone (hypothesis 2). The almost exclusive agonist effect of *S*-4C3HPG observed on phosphoinositide hydrolysis and $[Ca^{2+}]_i$ changes can be explained by the two possible mechanisms described. In fact, Brabet et al. (1995) have shown that, in transfected cells, *S*-4C3HPG, generally considered as a group I antagonist, exhibits some agonist activity on $mGlu_{5a}$, with an EC_{50} value exceeding 300 μM. Our results, i.e. the agonist effect of *S*-4C3HPG, agree with the data of these authors and with the abundance $mGlu_5$ described by Romano et al. (1996). Due to the apparent partial agonism of this compound in our

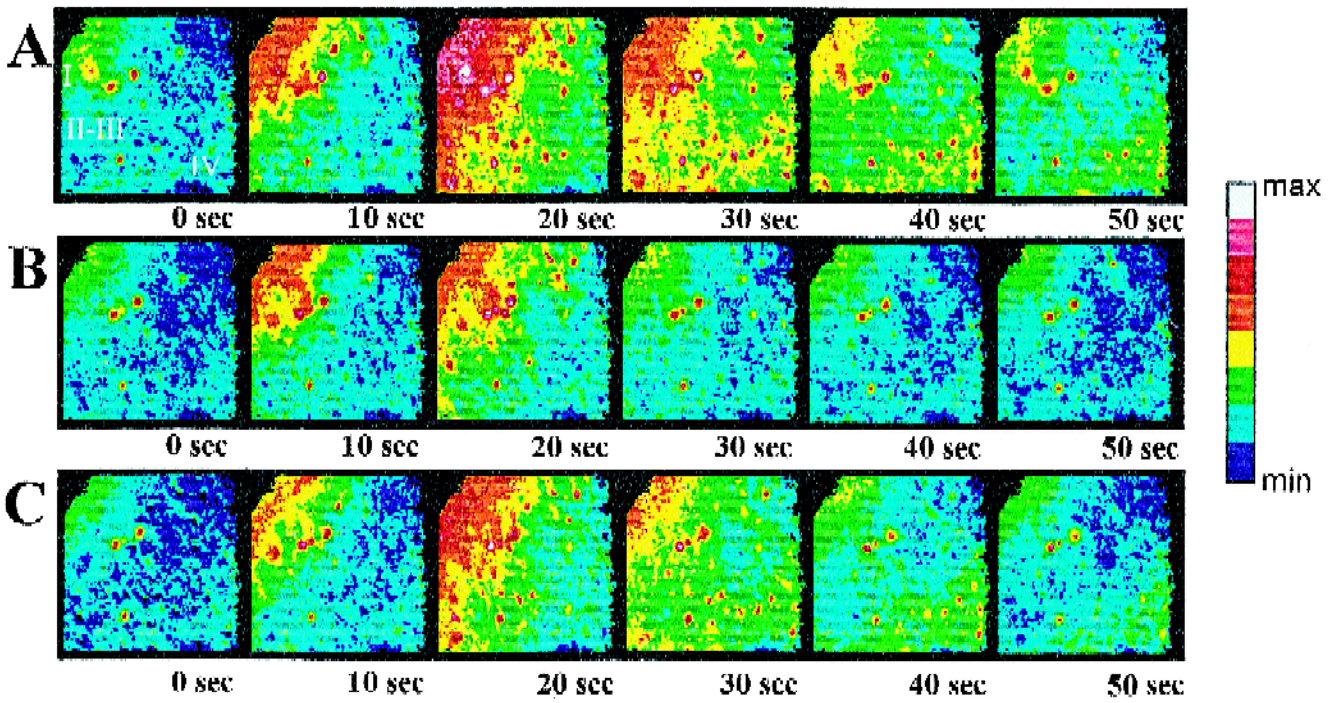

Fig. 11. Pseudo colored ratio image (F350/F380) of a Fura-2 loaded occipital cortex slice from a 6 day old rat. The slice was sequentially superfused with different drugs for 30 seconds and washed for 3 min between each drug application. 100 μM *S*-4C3HPG was first applied (panel A), then *S*-4C3HPG and 3 mM 8(4ClPh*S*)cAMP (panel B), and finally *S*-4C3HPG (panel C). Note the maximal inhibitory effect of cAMP in layer IV and the partial restoration of the response after washing.

preparation, an antagonism of *S*-4C3HPG on the 1*S*,3*R*-ACPD induced response was expected. The absence of this effect is not easy to understand. However, the complexity of the *S*-4C3HPG action (e.g. simultaneous activation of $mGlu_5$ and $mGlu_2/mGlu_3$ and blockade of $mGlu_1$) may explain the lack of antagonist effect in our experimental conditions.

The inhibitory effect of two group II/II antagonists, MTPG and MPPG, on phosphoinositide hydrolysis evoked by 1*S*,3*R*-ACPD or *S*-4C3HPG, but not on that induced by *R*,*S*-DHPG, in cerebral cortex supported the hypothesis of a cross talk between pathways activated by group I and II receptors. Recently it has been described that 1 mM MPPG blocked only 50% of $mGlu_{1a}$ response and 20% of the $mGlu_{5a}$ response in transfected cells (Thomsen et al., 1996). Thus, in our experimental conditions, 500 μM MPPG should have only a small inhibitory effect on $mGlu_1$ and $mGlu_5$, which would therefore explain its lack of effect on the DHPG-induced response. Moreover, as 1 mM MTPG blocked 80% of the $mGlu_{1a}$ in the same cells, but only 30% of $mGlu_{5a}$, it supported the predominant involvement of $mGlu_5$ in the DHPG stimulation. The inhibitory effect of MPPG and MTPG on the 1*S*,3*R*-ACPD response should thus be mediated by group II receptors. Indeed both compounds were reported to antagonize group II receptors in hippocampus (Bushell et al., 1996) and spinal cord (Jane et al., 1995), and more recent studies, in tranfected cells, showed the activity of MPPG and MTPG on $mGlu_2$ (Gomeza et al., 1996) and suggested a preferential effect of MTPG on $mGlu_3$ (Thomsen et al., 1996).

Finally, the potentiating effects of LY 354740, which was itself inactive on the phosphoinositide hydrolysis, on the DHPG stimulation is also in agreement with the cross-talk hypothesis, and the inhibitory effects of 8(4ClPh*S*)cAMP on phosphoinositide hydrolysis and on $[Ca^{2+}]_i$ may suggest a role of cAMP levels in the control of the $IP3/[Ca^{2+}]_i$ cascade. In agreement with the data obtained in this study, Schoepp et al. (1996) have recently reported that the selective group II agonist, 2*R*,4*R*-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC), also potentiates the stimulation of phosphoinositide hydrolysis by DHPG. However these authors observed that 2*R*,4*R*-APDC induced an increase of the maximum effect of DHPG only in adult rat hippocampal slices, whereas in 7 days old animals, 2*R*,4*R*-APDC induced an increase of DHPG potency without change of maximum effect. This is in contrast with our study in 6 days old rat brain cortex where the group II agonists induced an increase of the maximum effect of DHPG and we provided some evidences that cAMP may directly modulate the PI response.

The involvement of protein kinase A and the point of regulation in the cascade (receptor, G-protein or phospholipase C) are currently under evaluation. DCG-IV which is a selective and potent agonist of the group II mGlu receptors was described to potentiate the effect of quisqualate on phosphoinositide hydrolysis in adult rat hippocampal slices

(Nicoletti et al., 1993). Although this effect was similar to what we observed in 6 days old rat cortex, it was not sensitive to intracellular cAMP level changes but was sensitive to protein kinase C activation (Genazzani et al., 1994). This discrepancy may reflect the existence of several control mechanisms of mGlu receptors and/or may point to a common regulatory system which may be the target for both kinases. It is interesting to note that the potentiating effect of DCG-IV was found exclusively in hippocampal but not in cortical slices.

In conclusion, the most relevant finding of this study is that in neonatal rat cerebral cortex, the activation of group II mGlu receptors potentiated the phosphoinositide response induced by a concomitant activation of group I mGlu receptors and this cross talk may involve a cAMP dependent mechanism.

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