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mGluR-evoked augmentation of receptor-mediated cyclic AMP formation in neonatal and adult rat striatum

[1]Jayne Cartmell, Hervé Schaffhauser, Jürgen Wichmann & Vincent Mutel

Pharma Division Pre-clinical Research, F. Hoffmann-La Roche Ltd., CH-4002 Basel, Switzerland

1 The effects of selective agonists at group I, II and III metabotropic glutamate receptors (mGluRs) on adenosine A_2 receptor-mediated cyclic AMP formation were compared in cross-chopped slices of adult and neonatal (8 days old) rat striatum, in the presence of 1 u ml^{-1} adenosine deaminase.

2 The group II selective agonist, (2**S**,1**R**,2**R**,3**R**)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), elicited a potentiation of 5′-N-ethylcarboxamidoadenosine (NECA)-stimulated cyclic AMP production with similar potencies in adult (EC_{50} value 122 ± 35 nM) and neonatal (EC_{50} value 285 ± 6 nM) brain. In contrast, the group I selective agonist (**S**)-dihydroxyphenylglycine ((**S**)-DHPG) augmented the NECA cyclic AMP response in neonatal striatum (EC_{50} value 9 ± 1 μM), but at a concentration of 100 μM, (**S**)-DHPG failed to affect the NECA response in adult striatal slices.

3 The potentiation evoked by (**S**)-DHPG was specific for group I mGluRs as (2**S**,3**S**,4**S**,)-2-methyl-2-(carboxycyclopropyl)glycine (MCCG), a group II antagonist, was ineffective on the (**S**)-DHPG (100 μM) response at a concentration (500 μM) which reversed a similar augmentation elicited by DCG-IV (300 nM). Furthermore, a protein kinase C inhibitor (Ro 31-8220, 10 μM) markedly reversed the effect of (**S**)-DHPG without affecting the response to DCG-IV.

4 The mGluR agonist (2**S**,3**S**,4**S**,)-α-(carboxycyclopropyl)glycine (L-CCG-I), elicited a greater potentiation of NECA-stimulated cyclic AMP production in neonatal striatum in comparison with that observed in adult rat brain. Moreover, EC_{50} values obtained from adult and neonatal striatum were 2 ± 1 μM and 9 ± 1 μM, respectively. These differences in potency might reflect co-activation of both group I and group II mGluRs by L-CCG-I in neonatal striatum.

5 Distinct patterns of mGluR expression in various brain areas might account for previous conflicting data on the nature of the mGluR able to evoke such potentiated responses.

Keywords: Metabotropic receptors; cyclic AMP; (**S**)-DHPG; DCG-IV; striatum

Introduction

Metabotropic glutamate receptors are linked to a variety of second messenger systems via the activation of G-proteins. The primary transduction mechanism of group I receptors (mGluR1 and mGluR5) is the stimulation of phosphoinositide (PI) hydrolysis, whereas Group II (mGluR2 and mGluR3) and III (mGluR4, mGluR6, mGluR7 and mGluR8) evoke an inhibition of forskolin-stimulated adenosine 3′:5′-cyclic monophosphate (cyclic AMP) accumulation (for review see Pin & Duvoisin, 1995). In addition to these transduction mechanisms, mGluRs are able to interact with a diversity of other receptor systems.

It has been known for some time that mGluRs are able to augment cyclic AMP responses mediated via receptors directly linked to the production of cyclic AMP such as β-adrenoceptors, vasoactive intestinal peptide (VIP) and adenosine A_2 receptors (Alexander *et al*., 1992; Winder *et al*., 1993; Wright & Schoepp, 1996). The characterization of such cross-talk between receptors is necessary as these interactions can produce erroneous results with respect to mechanisms of receptor action. For example, mGluR agonists have been observed to elicit increases in basal levels of cyclic AMP in brain slices, suggesting the existence of an mGluR positively coupled to adenylyl cyclase (Winder & Conn, 1992). However, this increase in basal is, in fact, an indirect effect resulting from mGluR-evoked potentiation of the cyclic AMP responses of A_2 adenosine receptors stimulated by endogenous adenosine (Winder & Conn, 1993; Schoepp & Johnson, 1993; Schoepp *et al*., 1996).

Although this cross-talk between mGluRs and other G-protein-coupled receptors is now widely accepted, a number of studies have yielded conflicting data with respect to the nature of the mGluR(s) responsible for the potentiating action. In guinea-pig cortex the mGluR agonist (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I) elicited a potentiation of the adenosine A_2 receptor cyclic AMP response that was markedly reduced in the absence of Ca^{2+} or in the presence of a selective protein kinase C inhibitor (Cartmell *et al*., 1994), suggesting a role for group I mGluRs. However, Winder & Conn (1995) demonstrated that, in adult rat hippocampus, selective group I agonists failed to evoke an augmentation of β-adrenoceptor-mediated cyclic AMP accumulation, but a potentiation was produced by those mGluR agonists active at group II mGluRs. Furthermore, this group II effect was resistant to protein kinase C inhibition, indicating that the mGluR-evoked augmentation in adult rat hippocampus was not mediated via PI hydrolysis.

Due to such discrepancies between the nature of mGluRs able to evoke potentiations of cyclic AMP responses, the aim of this study was to characterize further the augmentation of adenosine A_2 receptor cyclic AMP formation in rat striatum with the non-hydrolyzable adenosine derivative 5′-N-ethylcarboxamidoadenosine (NECA). Furthermore, mGluR-evoked potentiations of NECA-stimulated cyclic AMP accumulation in adult and neonatal (8 days old) striatum have been compared, so that potential developmental differences in the augmentation evoked by mGluRs could be determined.

Methods

Following decapitation of adult (male, 180–200 g) or neonatal (male and female, 8 day old) Sprague-Dawley rats, their striata were removed and placed in pre-gassed (95% O_2/5% CO_2), ice-cold Krebs buffer. The striata were then cross-chopped (350 μm × 350 μm) with a McIlwain tissue chopper and dispersed in pre-warmed (37°C), pre-gassed Krebs buffer. Sub-

[1] Author for correspondence.

sequent to three washes in buffer, the slices were incubated in a shaking water bath at 37°C; the surface of the buffer was continually gassed with 95% O_2/5% CO_2. Following 60 min pre-incubation with four intermediate changes of buffer, slices were transferred (in 30 μl gravity-packed aliquots) to flat bottomed vials containing Krebs buffer, adenosine deaminase and, where appropriate, receptor antagonists or Ro 31-8220. The final volume in each vial was 350 μl and experiments were performed in triplicate. Slices were incubated at 37°C for a further 10 min and then mGluR agonists or baclofen were added, in 20 μl aliquots. After 12 min, 20 μl aliquots of NECA were also added. The reaction was terminated after a further 12 min by boiling in a waterbath for 20 min. Tubes containing the slices were sonicated and centrifuged (20 min at 40 000 g) and then 200 μl supernatant were removed from each vial for cyclic AMP measurement by use of the acetylated enzymeimmunoassay system from Amersham (RPA 542).

5′-N-ethylcarboxamidoadenosine (NECA), **R**(+)-baclofen and phaclofen were purchased from RBI (Rahn AG, Zurich, Switzerland). (**S**)-3-Hydroxyphenylglycine ((**S**)-3HPG); (**R**,**S**)-dihydroxyphenylglycine ((**R**,**S**)-DHPG); (**S**)-dihydroxyphenylglycine ((**S**)-DHPG); (2**S**,3**S**,4**S**)-α-(carboxycyclopropyl)glycine (L-CCG-I), (2**S**,3**S**,4**S**)-2-methyl-2-(carboxycyclopropyl) glycine (MCCG), L(+)-2-amino-4-phosphonobutyric acid (L-AP4) and saclofen were purchased from Tocris Cookson (Bristol, U.K.). Adenosine deaminase was obtained from Boehringer Mannheim (Rotkreuz, Switzerland). (2**S**,1**R**,2**R**,3**R**)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) was synthesized at Hoffmann-La Roche (Basel, Switzerland) and Ro 31-8220 at Roche Products Limited (Welwyn Garden City, U.K.). All other laboratory reagents were purchased from Merck (Dietikon, Switzerland).

Results

Pharmacological characterization in adult striatal slices

The effects of various mGluR agonists on NECA (10 μM)-stimulated cyclic AMP formation in rat striatal slices were examined in the presence of 1 u ml^{-1}adenosine deaminase. None of the mGluR agonists tested evoked any significant effects on basal cyclic AMP levels in adult striatal slices ($P>0.05$, Student's t test). As shown in Figure 1a, the selective group I agonists (**S**)-3-hydroxyphenylglycine ((**S**)-3HPG), (**R**,**S**)-dihydroxyphenylglycine ((**R**,**S**)-DHPG) and (**S**)-dihydroxyphenylglycine ((**S**)-DHPG), all at concentrations of 100 μM, also failed to elicit any effects on the NECA cyclic AMP response in adult striatum. However, L-CCG-I (10 μM), an agonist with activity at all three mGluR groups, evoked a 344±18% increase of the NECA response. This increase was mimicked by the selective group II agonist (2**S**,1**R**,2**R**,3**R**)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) which augmented the cyclic AMP response to NECA by 372±36% at a concentration of 300 nM. In contrast, the group III selective agonist L(+)-2-amino-4-phosphonobutyric acid (L-AP4, 100 μM) inhibited the NECA cyclic AMP response by 54±8%.

Concentration-response curves for the effects of DCG-IV and L-CCG-I on NECA-stimulated cyclic AMP accumulation in adult striatum are shown in Figure 2a. DCG-IV evoked a concentration-dependent potentiation (EC_{50} value 122±35 nM) with a maximal augmentation of 409±7% at 1 μM. L-CCG-I produced a similar potentiation of the NECA response (maximal increase 406±51% at 30 μM) with an EC_{50} value of 2±1 μM.

The augmentation of the NECA cyclic AMP response in adult striatum was not specific for mGluR agonists. The selective γ-aminobutyric $acid_B$ ($GABA_B$) receptor agonist, baclofen, also elicited a concentration-dependent potentiation of NECA-stimulated cyclic AMP formation in adult striatal slices with an EC_{50} value of 2±1 μM. However, the maximal response of baclofen was somewhat lower than that elicited by the mGluR agonists; 249±53% augmentation of the NECA cyclic AMP response at a concentration of 30 μM. The effect of baclofen was markedly reduced in the presence of the $GABA_B$ receptor antagonists, phaclofen (500 μM) or saclofen (500 μM). Both compounds elicited approximately 40% inhibition of the potentiation evoked by 10 μM baclofen (Figure 3), but were without significant effect on basal cyclic AMP levels ($P>0.05$, Student's t test).

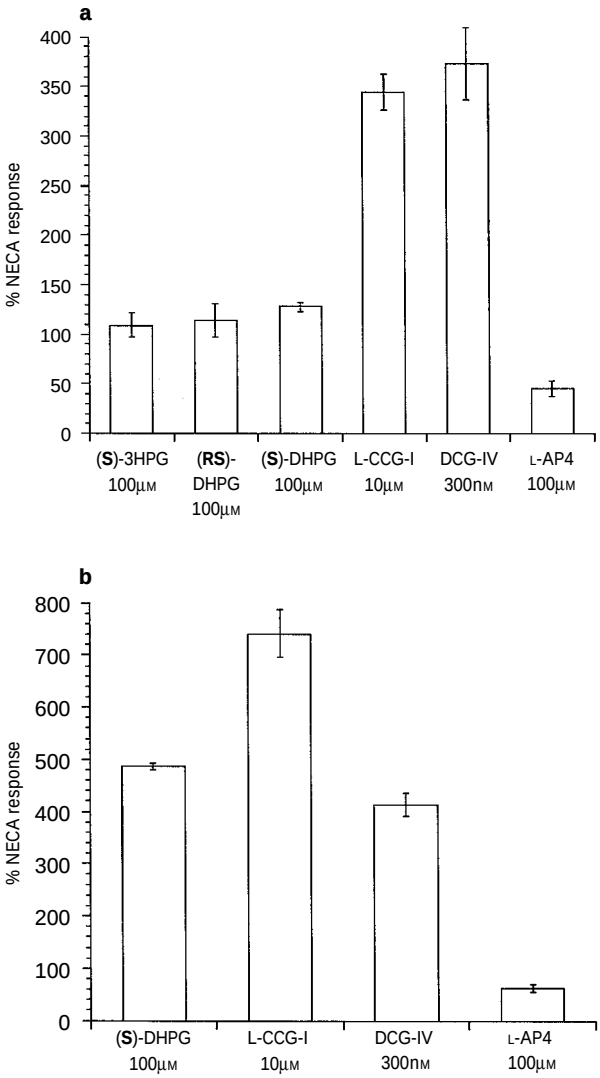

Figure 1 Effect of various mGluR agonists on NECA (10 μM)-stimulated cyclic AMP production in (a) adult or (b) neonatal rat striatal slices. Basal cyclic AMP levels were 227±48 and 3657±553 fmol mg^{-1} protein and stimulation by 10 μM NECA was 376±65 and 245±41% basal, in adult and neonatal striatal slices, respectively. The results are expressed as the mean±s.e.mean of at least three independent experiments in triplicate.

Pharmacological characterization in neonatal striatal slices

In common with adult striatal slices, DCG-IV and L-CCG-I were without effect on basal cyclic AMP levels, but elicited concentration-dependent augmentations of NECA-stimulated cyclic AMP production in neonatal striatal slices; EC_{50} values were 286±6 nM and 9±1 μM, respectively (Figure 2b). When statistical analysis was performed on the log of the relevant EC_{50} values, the difference between potencies obtained for L-CCG-I in the adult and neonatal preparations was found to be significant ($P<0.01$, Student's t test). The EC_{50} value obtained for the DCG-IV-elicited potentiation in neonatal slices, although significantly different from that obtained in adult slices

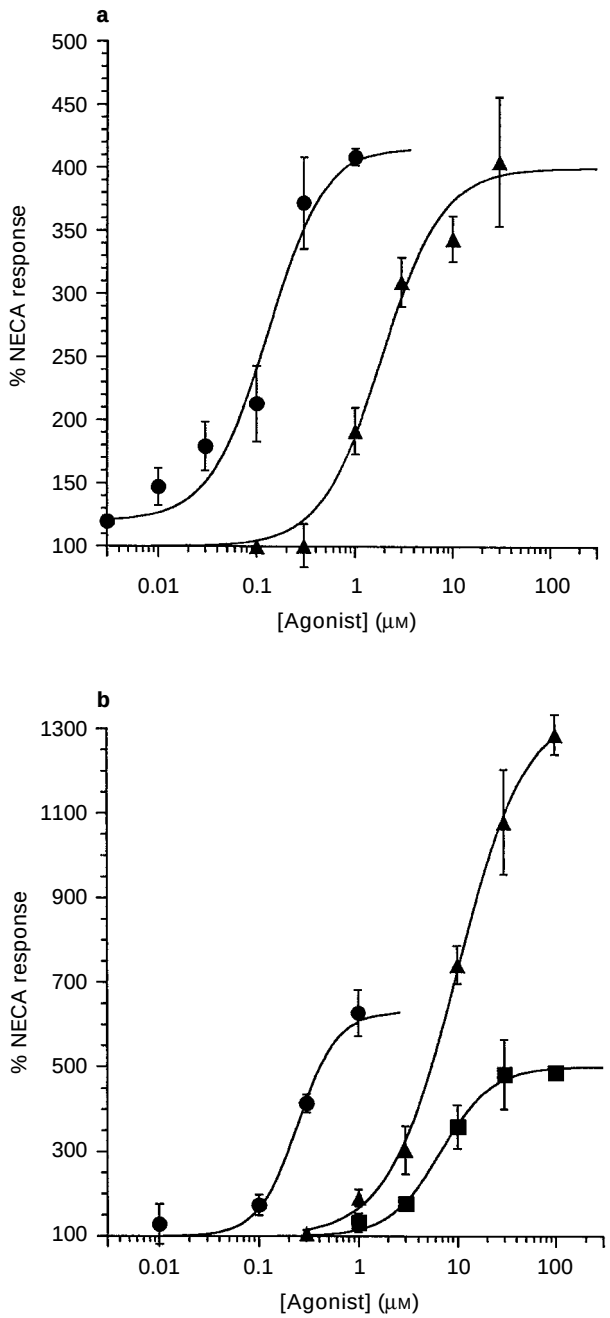

Figure 2 Effect of DCG-IV (●), L-CCG-I (▲) and (S)-DHPG (■) on NECA (10 μM)-stimulated cyclic AMP production in (a) adult and (b) neonatal rat striatal slices. Basal cyclicAMP levels were 219 ± 33 and 3545 ± 619 fmol mg^{-1} protein and stimulation by 10 μM NECA was 366 ± 39 and $280 \pm 52\%$ basal, in adult and neonatal striatal slices, respectively. The results are expressed as the mean of three independent experiments in triplicate; vertical lines show s.e.mean.

($P < 0.05$, Student's t test), could only be estimated as the response did not reach a definite maximum. However, concentrations of DCG-IV greater than 1 μM were not tested as the compound has some NMDA receptor activity at higher concentrations (Ishida *et al*., 1993). In comparison with responses in the adult brain, DCG-IV and, in particular, L-CCG-I evoked much larger increases in neonatal striatum; maximal increases were $628 \pm 54\%$ with 1 μM DCG-IV and $1286 \pm 48\%$ with 100 μM L-CCG-I.

L-AP4, at a concentration of 100 μM also evoked an inhibitory effect on NECA-stimulated cyclic AMP accumulation in neonatal brain, resulting in a response $63 \pm 7\%$ of control values (Figure 1b). At this concentration, L-AP4 also evoked a slight, but significant ($P < 0.05$, Student's t test), inhibition

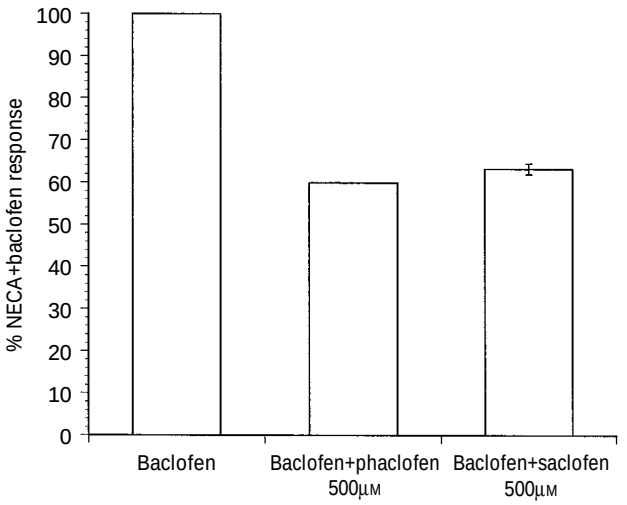

Figure 3 Effect of phaclofen (500 μM) and saclofen (500 μM) on the potentiation of NECA (10 μM)-stimulated cyclic AMP production by baclofen (10 μM) in adult rat striatal slices. Basal cyclic AMP levels were 268 ± 87 fmol mg^{-1} protein and stimulation by 10 μM NECA was $366 \pm 16\%$ basal. Baclofen (10 μM) elicited a $219 \pm 8\%$ potentiation of the NECA cyclic AMP response, this augmentation was not affected by 500 μM MCCG. The results are expressed as the mean $\pm$ s.e.mean of three independent experiments in triplicate.

(approximately 20%) of basal cyclic AMP levels in neonatal striatal slices.

Although the group I selective agonist (S)-DHPG failed to elicit an effect on the NECA cyclic AMP response in adult striatal slices, in neonatal striatum (S)-DHPG evoked a similar increase to that of the group II selective compound DCG-IV, but without significantly affecting basal cyclic AMP levels ($P > 0.05$, Student's t test). Figure 2b shows the concentration-response curve of (S)-DHPG on the NECA response in neonatal striatal slices, with an EC_{50} value of 9 ± 1 μM and a maximal potentiation of $487 \pm 6\%$.

Effect of MCCG on mGluR-evoked potentiation

The antagonist, (2S,3S,4S)-2-methyl-2-(carboxycyclopropyl)-glycine (MCCG), has recently been shown to be selective for group II mGluRs (Jane *et al*., 1994). Therefore, MCCG was tested on the augmentation of NECA cyclic AMP responses in both adult and neonatal striatal slices, in order to confirm that the potentiations elicited by DCG-IV were mediated via group II receptors. Although MCCG has previously been shown to have some agonist activity (Bushell *et al*., 1996), we failed to observe any effect of the compound, at a concentration of 1 mM, on basal or NECA (10 μM)-stimulated cyclic AMP levels in adult or neonatal tissue. MCCG (500 μM) failed to reduce the potentiation evoked by 10 μM baclofen (data not shown), but inhibited the DCG-IV (300 nM)-elicited increase of NECA cyclic AMP responses in adult brain by $46 \pm 2\%$, and by $74 \pm 5\%$ at 1 mM MCCG (Figure 4a). MCCG also reduced the augmentation evoked by 10 μM L-CCG-I in adult tissue by $39 \pm 5\%$ and $67 \pm 1\%$ at 500 μM and 1 mM MCCG, respectively (Figure 4a).

In neonatal striatum MCCG, at concentrations of 500 μM and 1 mM, reduced the DCG-IV (300 nM)-elicited potentiation by $67 \pm 1\%$ and $78 \pm 6\%$, respectively (Figure 4b). The potentiation elicited by 10 μM L-CCG-I in neonatal striatal slices was reduced by $21 \pm 3\%$ in the presence of 500 μM MCCG, and by $56 \pm 8\%$ with 1 mM MCCG (Figure 4c). However, MCCG, at concentrations of 500 μM and 1 mM failed to affect significantly the potentiation evoked by 100 μM L-CCG-I (Figure 4c).

In addition, 500 μM MCCG failed to have any effect on the augmentation of the NECA response by (S)-DHPG (100 μM). However, when the concentration of MCCG was increased to

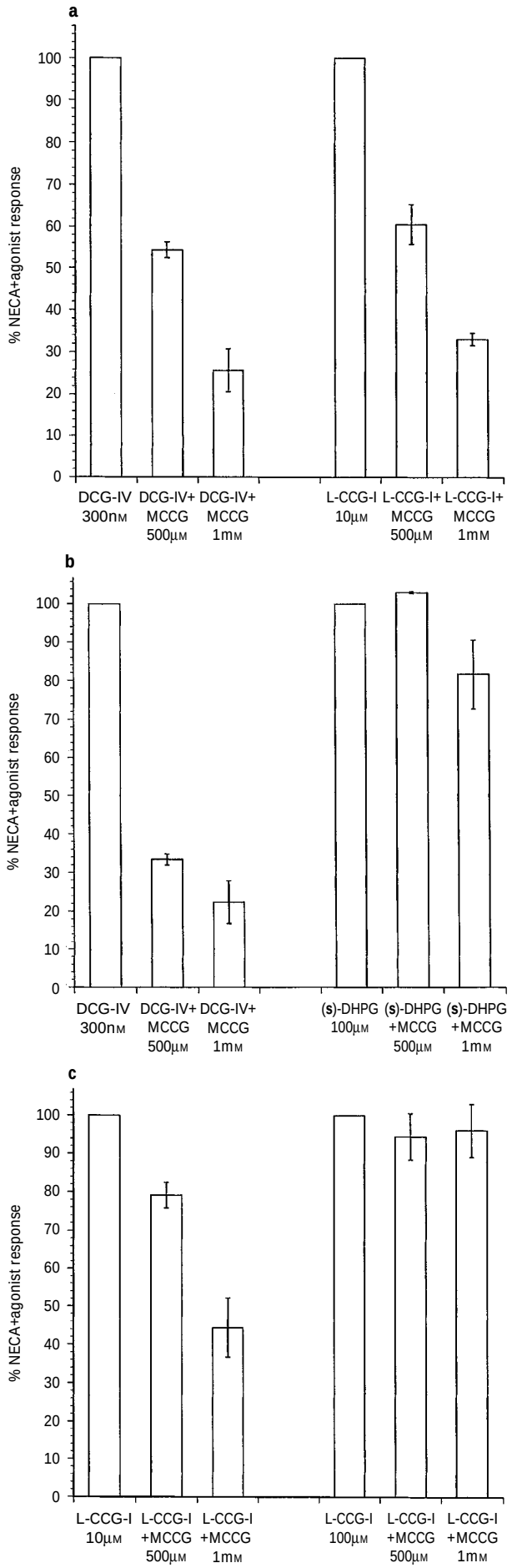

Figure 4 Effect of MCCG on the potentiation of NECA (10 μM)-stimulated cyclic AMP production by DCG-IV (300 nM), L-CCG-I (10 μM or 100 μM) and (**S**)-DHPG (100 μM) in (a) adult or (b) and (c) neonatal rat striatal slices. Basal cyclic AMP levels were 208 ± 46 and 4318 ± 218 fmol mg^{-1} protein, and stimulation by 10 μM NECA was 399 ± 81 and 211 ± 33% basal, in adult and neonatal striatal slices, respectively. The results are expressed as the mean $\pm$ s.e.mean of at least three independent experiments in triplicate.

1 mM, the potentiation evoked by (**S**)-DHPG was reduced by approximately 20%, indicating an apparent non-selectivity of MCCG at this concentration (Figure 4b).

Mechanism of mGluR-evoked potentiation

In order to investigate a possible involvement of PI hydrolysis in the augmentation produced by group I mGluR agonists, the effects of the selective protein kinase C inhibitor Ro 31-8220 (Davis *et al*., 1989) were examined. In neonatal striatum, 10 μM Ro 31-8220, a concentration that completely abolished potentiation of the NECA cyclic AMP response by phorbol-12,13-dibutyrate in guinea-pig cortex (Cartmell *et al*., 1994), had no significant effect on basal cyclic AMP levels or the potentiation of the NECA cyclic AMP response by 300 nM DCG-IV (P values > 0.05, Student's t test). However, the effect of (**S**)-DHPG (100 μM) was markedly reduced (47 ± 2% control values), but not abolished, when incubated with the same concentration of Ro 31-8220 (Figure 5).

Discussion

The effects of subtype-selective mGluR agonists on receptor-mediated cyclic AMP formation have been compared in cross-chopped slices of adult and neonatal rat striatum.

In contrast to the inhibition of forskolin-stimulated cyclic AMP accumulation by DCG-IV previously described (Genazzani *et al*., 1993), the group II mGluR agonist increased cyclic AMP levels stimulated by the adenosine receptor agonist, NECA (10 μM). This result supports the data of Wright & Schoepp (1996) who showed a potentiation of cyclic AMP response to VIP and NECA, in adult rat hippocampal slices, by the non-selective mGluR agonist 1**S**,3**R**-ACPD and L-CCG-I. In adult rat cortex, Winder & Conn (1995) also showed a potentiation of β-adrenoceptor-mediated cyclic AMP production by DCG-IV with a similar potency (EC_{50} value approximately 240 nM) to that obtained for the augmentation evoked by DCG-IV in adult striatum (120 nM) in the present study (see Table 1). Both of these values correlate well with potencies obtained for the action of DCG-IV at group II mGluRs expressed in CHO cells (IC_{50} values of approximately 300 and 200 nM at mGluR2 and mGluR3, respectively; Hayashi *et al*., 1993). Furthermore, the potency observed for L-CCG-I in adult striatum (EC_{50} value approximately 2 μM) is also in agreement with that of L-CCG-I at mGluR2-or mGluR3-transfected Chinese hamster ovary (CHO) cells (300 nM and 1 μM, respectively; Hayashi *et al*., 1992; Pin & Duvoisin, 1995).

Although the potency observed for DCG-IV-elicited augmentation of the NECA cyclic AMP response in neonatal tissue also correlates well with values obtained from group II mGluR-transfected cells, the EC_{50} value for L-CCG-I in this preparation (approximately 10 μM) does not correlate with the potency of L-CCG-I at any currently characterized mGluR. The potency observed for L-CCG-I might reflect co-activation of group I and group II mGluRs in neonatal brain.

Although agonists selective at group I mGluRs failed to have any effect on NECA-stimulated cyclic AMP production in adult rat striatal slices, (**S**)-DHPG produced a potentiation in the neonatal brain similar in magnitude to that observed with DCG-IV. A potentiation of NECA-stimulated cyclic AMP formation by (**RS**)-DHPG has recently been observed by Alexander *et al*. (1996); they obtained an EC_{50} value of approximately 5 μM in guinea-pig cortical slices. The EC_{50} value for the augmentation evoked by (S)-DHPG (9 ± 1 μM) in the

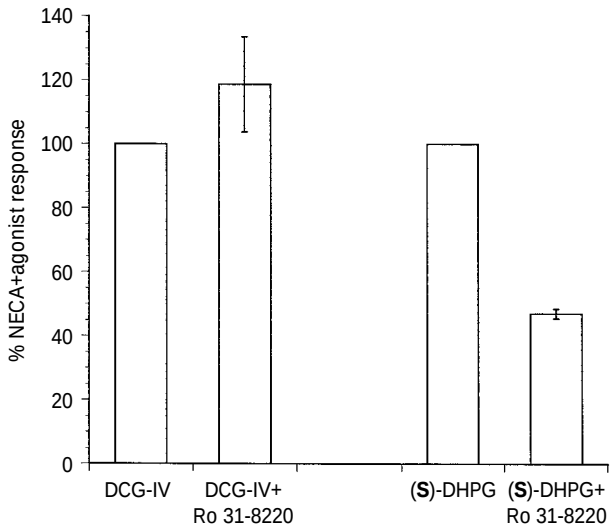

Figure 5 Effect of the protein kinase C inhibitor Ro 31-8220 (10 μM) on the potentiation of NECA (10 μM)-stimulated cyclic AMP production by DCG-IV (300 nM) and (**S**)-DHPG (100 μM) in neonatal rat striatal slices. Basal cyclic AMP levels were 3400 ± 455 fmol mg^{-1} protein, and stimulation by 10 μM NECA was 258 ± 66% basal. The results are expressed as the mean $\pm$ s.e.mean of at least three independent experiments in triplicate.

neonatal striatum is almost identical to that obtained for (S)-DHPG-stimulated PI hydrolysis in neonatal rat hippocampal slices (8 μM; Schoepp *et al*., 1994), suggesting the involvement of group I mGluRs in this response. Indeed, a role for protein kinase C in the (**S**)-DHPG-evoked potentiation in neonatal striatum was confirmed by the observation that the selective protein kinase C inhibitor Ro 31-8220 (10 μM) markedly reduced the effect of (**S**)-DHPG (100 μM), but not that of 300 nM DCG-IV. This finding supports previous data showing a similar inhibitory effect of Ro 31-8220 on L-CCG-I-produced augmentation of the NECA cyclic AMP response in guinea-pig cortex (Cartmell *et al*., 1994).

Evidence presented in this study implicates a role for phospholipase C-coupled mGluRs in the potentiation of NECA cyclic AMP responses in the neonatal brain, even though group I selective agonists failed to have an effect on the NECA response in adult striatum. Interestingly, Schoepp *et al*., (1991) have shown that the magnitude of the PI hydrolysis response stimulated by 1**S**,3**R**-ACPD in adult rat hippocampus is approximately half that of the response observed in neonate. These differences between adult and neonatal brain may well reflect a differential expression of group I mGluRs. Indeed, a developmental regulation of group I mGluR expression has been demonstrated by Catania *et al*. (1994). These authors found that mGluR1 transcripts were expressed at a low level at birth and increased with development whilst, in contrast, mGluR5 mRNA was highest at birth and expression decreased with age. These findings have been confirmed recently by Romano *et al*. (1996) who showed that in the striatum, hippocampus and cortex (as well as other areas) more mGluR5 protein is present in developing brain in comparison with adult. Therefore, the lack of group I effect in adult rat cortex (Winder & Conn, 1995) as well as adult rat striatum might be due to the low expression of mGluR5.

Interestingly, in neonatal striatal slices, L-CCG-I elicited a much larger potentiation (1000% NECA response at 30 μM) in comparison with that observed in adult tissue (400% NECA response at 30 μM). This difference, coupled with the low potency of L-CCG-I in neonatal striatum, might reflect the lack of subtype-specificity of L-CCG-I, as co-activation of both group I and group II mGluRs would be expected to elicit at least additive responses. Such large augmentations in neonatal brain have also been published by Winder *et al*. (1993).

Table 1 EC_{50} values (expressed in μM) of different agonists tested on NECA (10 μM)-stimulated cyclic cAMP production in adult and neonatal rat striatal slices

	Adult	*Neonate*
(S)-DHPG	No effect	8.76 ± 0.55
L-CCG-I	1.61 ± 0.65	9.17 ± 0.80
DCG-IV	0.12 ± 0.04	0.29 ± 0.01

Values are the mean $\pm$ s.e.mean of at least three independent experiments in triplicate.

They showed that 1**S**,3**R**-ACPD, an agonist at both group I and II mGluRs, elicited potentiations of the 2-chloroadenosine and VIP cyclic AMP responses which were four and nine-fold larger, respectively, than those seen in adult rat. Although the non-selectivity of L-CCG-I might account for the low potency of the compound in neonatal brain, a similar discrepancy in the potency of L-CCG-I has been obtained in adult rat cortex (Winder & Conn, 1995). These authors observed a potency of 35 μM for the augmentation of the isoprenaline cyclic AMP response by L-CCG-I, even though selective group I mGluR agonists failed to evoke a potentiation in this preparation.

To determine which mGluR subtypes contribute to the actions of non-selective agonists such as L-CCG-I, it is necessary to use a potent, selective mGluR subtype antagonist. Although the putative group II antagonist, MCCG, at a concentration of 1 mM, reduced the augmentation evoked by 10 μM L-CCG-I by approximately 60% in neonatal tissue, this concentration of MCCG failed to affect the response elicited by 100 μM L-CCG-I. The lack of effect of MCCG on the potentiation evoked by 100 μM L-CCG-I might be indicative of the recruitment of group I mGluRs in the potentiation, together with an abolition of the MCCG antagonist effect by an increase in the concentration of agonist acting at group II receptors. Despite failing to block completely the effects of 10 μM L-CCG-I or 300 nM DCG-IV, 1 mM MCCG reduced the potentiation evoked by 100 μM (**S**)-DHPG in neonatal brain, demonstrating that the selectivity of the compound was compromised at such high concentrations. Furthermore, although not observed in this study, Bushell *et al*. (1996) have shown some agonist activity of MCCG, thereby bringing into question the suitability of MCCG as a selective group II antagonist. More potent and selective antagonists at mGluR subtypes must be developed in order to characterize the effects of non-selective mGluR agonists.

In contrast to the potentiations evoked by group II agonists, the group III selective agonist, L-AP4, inhibited NECA-stimulated cyclic AMP formation in adult and neonatal striatal slices. These results support previous findings of an inhibition of NECA cyclic AMP responses in guinea-pig cerebral cortical (Cartmell *et al*., 1994) and rat hippocampal (Wright & Schoepp, 1996) slices, and may be indicative of distinct coupling mechanisms between group II and group III mGluRs.

In summary, the potentiation of NECA-stimulated cyclic AMP formation in adult and neonatal striatal slices can be augmented by group II mGluR agonists. In neonatal striatum, but not adult, group I mGluRs can mediate a similar potentiation, which is partly reduced by a protein kinase C inhibitor and therefore implicates products of PI hydrolysis in the group I effect. The non-selective agonist L-CCG-I evoked a greater increase of NECA-stimulated cyclic AMP production in neonate, compared with adult striatum, which may be due to co-activation of both group I and II mGluRs. The mechanism underlying the potentiation evoked by group II agonists is at present unknown, but there is much speculation that the effect is mediated via G-protein $\beta\gamma$ subunits (Schoepp *et al*., 1996; Winder & Conn, 1995). If this is the case, in order for the augmentation to occur, the expression of an adenylyl cyclase that is sensitive to positive modulation by $\beta\gamma$ (eg. types II, IV,

VII) is also necessary. Therefore, receptor cross-talk is dependent not only on the expression of particular receptors but also the expression of suitable effector mechanisms.

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