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Differentiation of group 2 and group 3 metabotropic glutamate receptor cAMP responses in the rat hippocampus

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Abstract

The effects of group 2- versus group 3-selective metabotropic glutamate (mGlu) receptor agonists were examined against forskolin (10 μM)-, vasoactive intestinal peptide (VIP; 1 μM)- and 5′-*N*-ethylcarboxamidoadenosine (NECA; 10 μM)-stimulated cAMP accumulations in adult rat hippocampal slices (in the presence of adenosine deaminase). Group 2 mGlu receptor-selective ((1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) and (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)-glycine (L-CCG I)) and group 3 mGlu receptor-selective (L-2-amino-4-phosphonobutyric acid (L-AP4) and L-serine-*O*-phosphate) agonists greatly inhibited forskolin-stimulated cAMP formation (> 80% at maximally effective concentrations). In contrast, stimulation of cAMP by VIP or NECA was inhibited by group 3, but not by group 2, mGlu receptor agonists. In fact, group 2 mGlu receptor agonists greatly potentiated cAMP accumulation evoked by NECA. Both the inhibitory effects of 1*S*,3*R*-ACPD on forskolin-stimulated cAMP and the potentiating effects on NECA-stimulated cAMP accumulation were reversed by the competitive group 1/2 mGlu receptor antagonist (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG). However, (+)-MCPG had no effects on L-AP4 inhibition of cAMP. Thus, the effects of group 2 versus group 3 mGlu receptor agonists on cAMP coupling can be pharmacologically as well as functionally differentiated in the rat hippocampus.

Keywords: Glutamate receptor, metabotropic; cAMP; Adenosine deaminase; Forskolin; VIP (vasoactive intestinal peptide); NECA (5′-*N*-ethylcarboxamidoadenosine); Hippocampus

1. Introduction

Metabotropic glutamate (mGlu) receptors are a heterogeneous class of G-protein-coupled receptors which differ structurally, functionally and pharmacologically from the ionotropic (ligand-gated ion channel) glutamate receptors (Schoepp and Conn, 1993; Pin and Duvoisin, 1995). At least eight mGlu receptor subtypes ($mGlu_1$–$mGlu_8$) have been cloned and characterized. These mGlu receptors can be classified into three groups, with members of each group having in common relatively high-sequence homology (about 70%) and similar agonist pharmacology (see Nakanishi, 1992, 1994; Schoepp, 1994). Group 1 mGlu receptors include $mGlu_1$ (Masu et al., 1991; Houamed et al., 1991) and $mGlu_5$ (Abe et al., 1992) which are coupled to phosphoinositide hydrolysis and are activated most potently by quisqualate, but are also sensitive to (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD). Group 2 mGlu receptors, including $mGlu_2$ (Tanabe et al., 1992) and $mGlu_3$ (Tanabe et al., 1992), are negatively coupled to cAMP formation and potently activated by 1*S*,3*R*-ACPD and (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)-glycine (L-CCG I) (Schoepp, 1994). Group 3 mGlu receptors include $mGlu_4$ (Tanabe et al., 1993), $mGlu_6$ (Nakajima et al., 1993), $mGlu_7$ (Okamoto et al., 1994; Saugstad et al., 1994) and $mGlu_8$ (Duvoisin et al., 1995), and they are also negatively coupled to cAMP formation, but are selectively activated by L-2-amino-4-phosphonobutyric acid (L-AP4) (Schoepp, 1994).

The expression of mGlu receptor subtypes in non-neuronal cells has been useful to characterize mGlu receptor subtype pharmacology, since these preparations represent homogeneous populations of receptors. However, in situ mGlu receptors likely couple to multiple second messenger systems in addition to those which have been demonstrated using recombinant receptors. Furthermore, the effects of mGlu receptor agonists on cAMP responses in situ can be

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greatly affected by the presence of endogenous substances which also modulate adenylate cyclase (Schoepp and Conn, 1993; Pin and Duvoisin, 1995). In the rat hippocampus, the effects of the mGlu receptor agonist 1*S*,3*R*-ACPD on forskolin-stimulated cAMP accumulation has been studied extensively. In the adult rat hippocampus, 1*S*,3*R*-ACPD potently inhibits forskolin-stimulated cAMP formation, producing about 50–60% inhibition at maximally effective concentrations (Schoepp et al., 1992). However, 1*S*,3*R*-ACPD can also enhance basal cAMP formation in both adult and neonatal rat hippocampus by a mechanism dependent on the presence of endogenous adenosine, since in the presence of adenosine deaminase (which metabolizes adenosine) this response to 1*S*,3*R*-ACPD is abolished (Casabona et al., 1992; Winder and Conn, 1992, 1993; Schoepp and Johnson, 1993a). 1*S*,3*R*-ACPD can also potentiate cAMP responses to other receptor agonists that activate adenylate cyclase through their effects on G_s, including vasoactive intestinal peptide (VIP), isoproterenol and prostaglandin E_2 (Winder et al., 1993; Winder and Conn, 1993). The cAMP potentiating effects of 1*S*,3*R*-ACPD in this tissue are likely mediated by activation of group 2 mGlu receptors, since this effect is not mimicked by the group 1 mGlu receptor-selective agonist 3,5-dihydroxyphenylglycine (Schoepp et al., 1994; Winder and Conn, 1995). Furthermore, group 2-selective agonists such as L-CCG-I (Schoepp et al., 1994) and (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) (Winder and Conn, 1995) potently potentiate cAMP responses in the rat hippocampus. The group 3-selective mGlu receptor agonists also inhibit forskolin-stimulated cAMP formation in the rat hippocampus (Schoepp and Johnson, 1993b), but an interaction between these receptors and other cAMP-coupled receptors has not been observed.

In this study, we have further investigated mGlu receptor agonist-induced cAMP responses in the adult rat hippocampus. Adenosine deaminase was included in the incubation buffer in order to eliminate any influence of endogenous adenosine on receptor coupling. The effects of both group 2- and group 3-selective mGlu receptor agonists on forskolin-, VIP- and 5′-*N*-ethylcarboxamidoadenosine (NECA)-stimulated cAMP responses were characterized. The effects of (+)-MCPG, which is a novel competitive antagonist for group 2 but not group 3 mGlu receptors (Thomsen et al., 1994; Watkins and Collingridge, 1994), were also determined in an attempt to differentiate these cAMP-coupled mGlu receptor groups.

2. Materials and methods

2.1. Materials

(1*S*,3*R*)-1-Aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD), L-2-amino-4-phosphonobutyric acid (L-AP4), (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)-glycine (L-CCG I) and (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG) were from Tocris Cookson (Bristol, UK). L-Serine-*O*-phosphate was from Sigma Chemical Corporation (St. Louis, MO, USA). Stock solutions of these compounds were prepared in water and adjusted to about pH 7 using 5 N sodium hydroxide solution. Forskolin (from Calbiochem Corp., La Jolla, CA, USA) and NECA (from Sigma Chemical Corporation, St. Louis, MO, USA) were dissolved in 50% water-ethanol. Adenosine deaminase (in glycerol solution) was from Boehringer Mannheim Corporation (Indianapolis, IN, USA). Vasoactive intestinal peptide (VIP) was from Sigma Chemical Corporation (St. Louis, MO, USA), and stock solutions were prepared in water.

2.2. Methods

Hippocampal slices were assayed for cAMP accumulation using the method previously described (Schoepp and Johnson, 1993a), with minor adaptations. Hippocampi from adult male (50–60 days old) Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were cross-chopped (300 μm) using a McIlwain tissue chopper, then suspended in Krebs-Bicarbonate buffer (approximately 50 volumes, usually 10 ml per determination which typically consisted of pooled tissue from 2 rats). Slices were allowed to settle by gravity and the excess buffer was aspirated. Slices were resuspended in approximately 30 volumes of buffer, centrifuged at $800 \times g$ (4°C), then resuspended in approximately 15 volumes of buffer to which adenosine deaminase had been added (1 U/ml). Slices were then preincubated under 5% CO_2-95% O_2 for 30 min at 37°C. In experiments where the stimulation of agonists was determined in the presence and absence of adenosine deaminase, the pooled slices were divided into equal parts prior to initial centrifugation and resuspended in buffer containing adenosine deaminase or an equivalent volume of glycerol (1 μl in 1 ml or 0.1% final assay volume). Following incubation, the tissue slices were centrifuged at $800 \times g$ and resuspended in the same volume of fresh buffer containing adenosine deaminase (or glycerol), and aliquots of tissue were added to 12×75 mm polypropylene tubes on ice. In most experiments adenosine deaminase (1 U/ml) was present in the buffer during the 30 min pre-incubation and all subsequent procedures including incubations with compounds which altered cAMP. Forskolin, VIP, NECA (5 μl per tube, final concentration 10, 1 and 10 μM, respectively) or the appropriate vehicle (50% ethanol in water for forskolin or NECA resulting in a final concentration of ethanol/water of 2%, or final concentration of 2% water for VIP) was added to the tubes prior to the tissue. Additionally, 5 μl of 1*S*,3*R*-ACPD, L-CCG I, L-AP4, L-serine-*O*-phosphate, (+)-MCPG or water (2% of final assay volume) was added to each tube. Final volume of each assay tube was 250 μl. Tubes were gassed and capped and incubated at 37°C for 15 min. The assay was terminated by adding 0.75 ml of ice-cold 6 mM

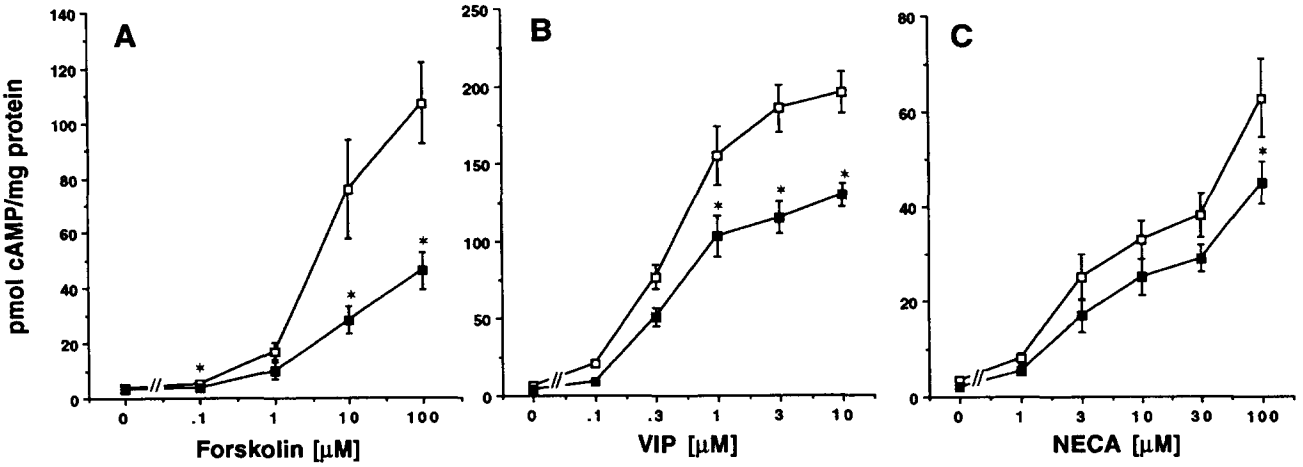

Fig. 1. Effects of adenosine deaminase on cAMP accumulation stimulated by forskolin (A), VIP (B) and NECA (C). Adult rat hippocampal slices were incubated with (■) or without (□) adenosine deaminase (1 U/ml) prior to and after addition of each stimulatory agent. Data are expressed as means ± S.E.M. ($n = 6$). * Indicates significance as compared to control, Student's t-test, $P < 0.05$.

disodium EDTA to each tube, immediately homogenizing the sample using a Tissuemizer from Tekmar (Cincinnati, OH, USA) and placing the tube into a boiling water bath for at least 10 min. Samples were centrifuged for 10 min at 10000 × g and supernatants were lyophilized and stored at 4°C until assayed for cAMP content. Levels of cAMP were determined on samples reconstituted in purified water using a commercially available radiobinding assay kit (Amersham TRK.432). Proteins were determined on the tissue pellets using the Biuret method (Layne, 1957).

Data were calculated as pmol cAMP per mg protein or normalized to a percentage of basal- or agonist-stimulated cAMP values in each experiment. In some experiments EC_{50} values were determined for the mGlu receptor agonists using a non-linear regression program (GraphPad Inplot, San Diego, CA, USA). Statistical significance was determined by Student's t-test or by a one-way analysis of variance (ANOVA) in conjunction with Duncan's Multiple Range procedure. Data were considered significant when $P < 0.05$.

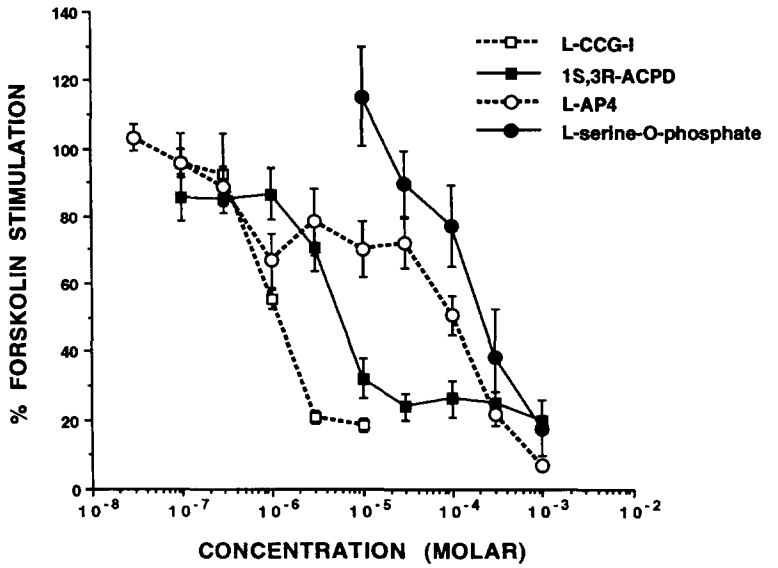

Fig. 2. Effects of various mGlu receptor agonists on forskolin-stimulated cAMP accumulation in adult rat hippocampal slices. After pretreatment with adenosine deaminase, tissue was incubated with 10 μM forskolin in the presence of adenosine deaminase (1 U/ml) and several concentrations of mGlu receptor agonists. (□) L-CCG I ($EC_{50} = 0.97 \pm 0.09$ μM), (■) 1*S*,3*R*-ACPD ($EC_{50} = 5.2 \pm 0.76$ μM), (○) L-AP4 ($EC_{50} = 246 \pm 87$ μM), (●) L-serine-*O*-phosphate ($EC_{50} = 285 \pm 125$ μM). Basal- and forskolin-stimulated levels of cAMP were 2.7 ± 0.02 and 59.9 ± 6.4 pmol/mg protein, respectively. Data are expressed as means ± S.E.M. ($n = 5$–9).

3. Results

3.1. The effect of adenosine deaminase on forskolin-, VIP- and NECA-stimulated cAMP formation

In preliminary experiments, we examined the effects of adding adenosine deaminase on concentration-effect increases in forskolin-, VIP- and NECA-stimulated cAMP accumulations. In the absence of adenosine deaminase, forskolin greatly stimulated the accumulation of cAMP over the concentration range of 1–100 μM. Addition of adenosine deaminase significantly reduced, but did not abolish, forskolin stimulations (Fig. 1A). In the presence of adenosine deaminase, forskolin (100 μM) significantly increased cAMP formation by about 14-fold over the basal values, and this concentration of forskolin was chosen for later experiments. Similar results were obtained with VIP,

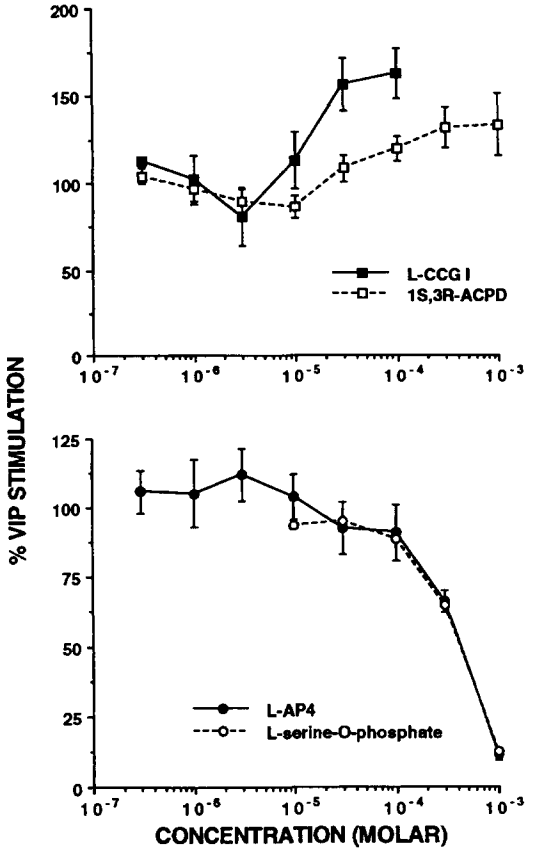

Fig. 3. Effects of various mGlu receptor agonists on VIP-stimulated cAMP accumulation in adult rat hippocampal slices. Adenosine deaminase-pretreated tissue was incubated with 1.0 μM VIP in the presence of adenosine deaminase (1 U/ml) and several concentrations of mGlu receptor agonists. (■) L-CCG I (EC_{50} = 12.5 μM), (□) 1*S*,3*R*-ACPD (EC_{50} = 61.9 μM), (●) L-AP4 (EC_{50} = 359 ± 56 μM), (○) L-serine-*O*-phosphate (EC_{50} = 405 ± 89 μM). EC_{50} values for L-CCG I and 1*S*,3*R*-ACPD were calculated using curves composed of averaged data. Basal- and VIP-stimulated levels of cAMP were 2.0 ± 0.1 and 192.5 ± 13.6 pmol/mg protein, respectively. Data are expressed as means ± S.E.M. (n = 3–6).

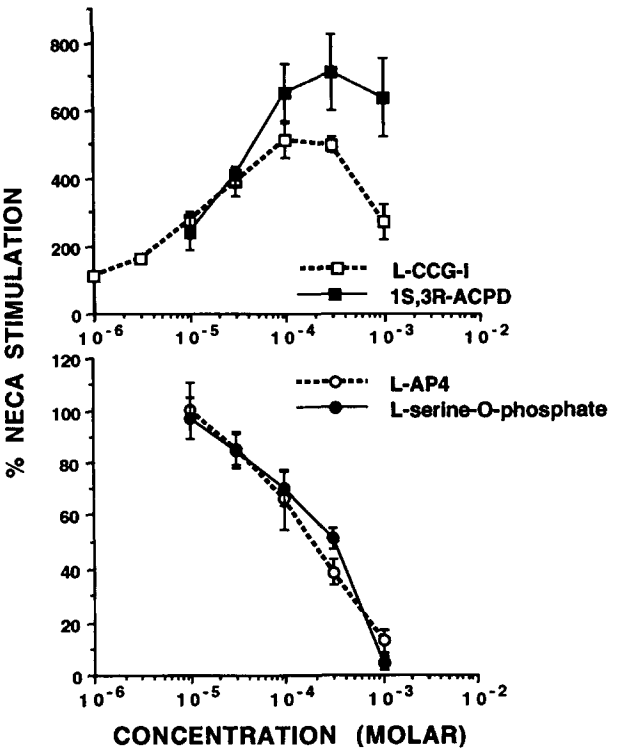

Fig. 4. Effects of various mGlu receptor agonists on NECA-stimulated cAMP accumulation in adult rat hippocampal slices. Tissue pretreated with adenosine deaminase was incubated with 10 μM NECA in the presence of adenosine deaminase (1 U/ml) and several concentrations of mGlu receptor agonists. (□) L-CCG I (EC_{50} = 13.5 μM), (■) 1*S*,3*R*-ACPD (EC_{50} = 34.3 μM), (○) L-AP4 (EC_{50} = 263 ± 70 μM), (●) L-serine-*O*-phosphate (EC_{50} = 223 ± 31 μM). EC_{50} values for L-CCG I and 1*S*,3*R*-ACPD were calculated using curves composed of averaged data. Basal and NECA stimulated levels of cAMP were 1.8 ± 0.1 and 27.8 ± 1.6 pmol/mg protein, respectively. Data are expressed as means ± S.E.M. (n = 3–6).

where in the absence of adenosine deaminase this agonist increased cAMP over the concentration range of 0.1–10 μM. Adenosine deaminase significantly reduced to about the same extent VIP stimulations at each concentration (Fig. 1B). 1 μM VIP was chosen for further experiments in the presence of adenosine deaminase, since cAMP formation at this agonist concentration was significantly increased by about 90-fold when compared to basal levels. In contrast to forskolin and VIP, cAMP stimulations by NECA were not greatly affected by adenosine deaminase. Significant inhibition by adenosine deaminase was only observed at 100 μM NECA. At 10 μM NECA (the concentration chosen for further experiments) basal cAMP levels were increased by about 12-fold (Fig. 1C).

3.2. mGlu receptor agonist effects on forskolin-, VIP- and NECA-stimulated cAMP production

The mGlu receptor agonists 1*S*,3*R*-ACPD, L-CCG I, L-AP4 and L-serine-*O*-phosphate almost completely inhibited (> 80%) forskolin-stimulated cAMP production (Fig.

2) in the presence of adenosine deaminase. L-CCG I was the most potent, followed by 1*S*,3*R*-ACPD, L-AP4 and L-serine-*O*-phosphate (see Fig. 2 legend for EC_{50} values). L-AP4 produced an inhibition curve which appeared biphasic. When the averaged L-AP4 data were analyzed for multiple sites (GraphPad Inplot), a two-component model with EC_{50} values of 0.12 μM (30%) and 241 μM (70%), respectively, fit the data with a high correlation ($r^2 = 0.995$, compared with 0.983 for a one-component model).

On the other hand, when VIP was used to stimulate cAMP production, only the group 3-selective mGlu receptor agonists, L-AP4 and L-serine-*O*-phosphate, were inhibitory (Fig. 3). 1*S*,3*R*-ACPD and L-CCG-I, which activate group 2 but not group 3 mGlu receptors, exhibited a slight potentiation of the cAMP response, with L-CCG I being more potent than 1*S*,3*R*-ACPD. The potencies of these effects were slightly lower for the group 2 agonists (about 10-fold) when compared to the effect on forskolin-stimulated cAMP production.

When NECA (a stable adenosine analog) was used to stimulate cAMP, the group 3-selective mGlu receptor agonists, L-AP4 and L-serine-*O*-phosphate, were again inhibitory, with similar potencies to those with forskolin and VIP, but group 2-selective agonists produced a robust potentiation of cAMP accumulation (Fig. 4). In this case,

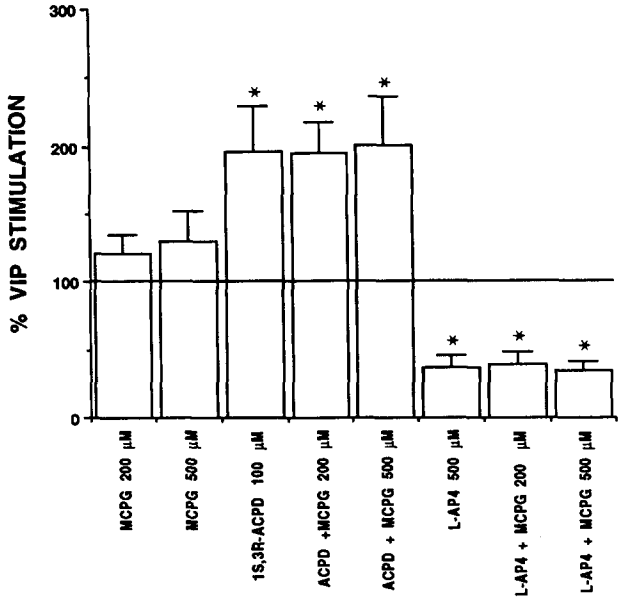

Fig. 6. Effect of group 2 mGlu receptor antagonist, (+)-MCPG, on 1*S*,3*R*-ACPD stimulation and L-AP4 inhibition of VIP-stimulated cAMP accumulation. Adenosine deaminase pretreated tissue was incubated with 1.0 μM VIP in the presence of adenosine deaminase (1 U/ml), 1*S*,3*R*-ACPD (100 μM), L-AP4 (500 μM) and/or (+)-MCPG (200 or 500 μM). * $P < 0.05$, compared to control (100% VIP stimulation, 145 ± 13 pmol/mg protein, $n = 6$). Statistical analysis by one-way ANOVA, with Duncan's Multiple Range procedure.

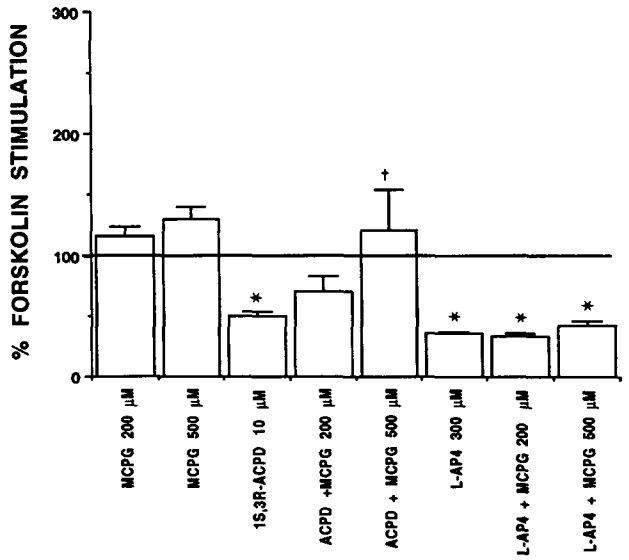

Fig. 5. Effect of group 2 mGlu receptor antagonist, (+)-MCPG, on 1*S*,3*R*-ACPD- and L-AP4 inhibition of forskolin-stimulated cAMP accumulation. After pretreatment with adenosine deaminase, tissue was incubated with 10 μM forskolin in the presence of adenosine deaminase (1 U/ml), 1*S*,3*R*-ACPD (10 μM), L-AP4 (300 μM) and/or (+)-MCPG (200 or 500 μM). * $P < 0.05$, compared to control (100% forskolin stimulation, 72 ± 8 pmol/mg protein, $n = 3$). † $P < 0.05$, compared to 1*S*,3*R*-ACPD in the absence of (+)-MCPG. Statistical analysis by one-way ANOVA, with Duncan's Multiple Range procedure.

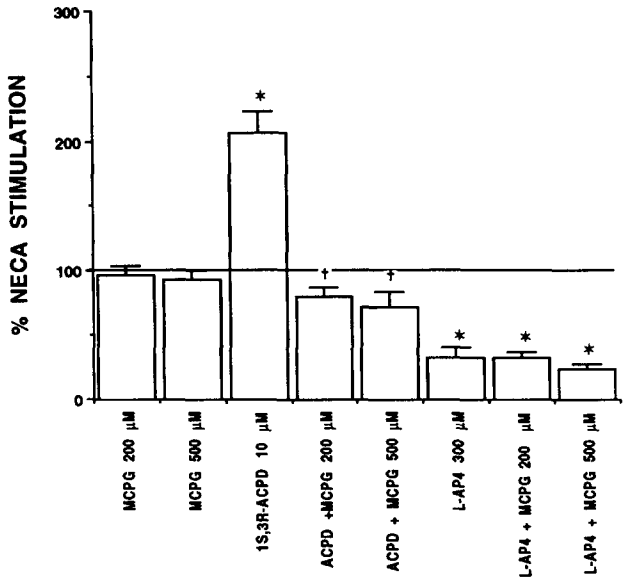

Fig. 7. Effect of group 2 mGlu receptor antagonist, (+)-MCPG, on 1*S*,3*R*-ACPD-stimulation and L-AP4 inhibition of NECA-stimulated cAMP accumulation. Tissue pretreated with adenosine deaminase was incubated with 10 μM NECA in the presence of adenosine deaminase (1 U/ml), 1*S*,3*R*-ACPD (10 μM), L-AP4 (300 μM) and/or (+)-MCPG (200 or 500 μM). * $P < 0.05$, compared to control (100% NECA stimulation, 25 ± 3 pmol/mg protein, $n = 3$). † $P < 0.05$, compared to 1*S*,3*R*-ACPD in the absence of (+)-MCPG. Statistical analysis by one-way ANOVA, with Duncan's Multiple Range procedure.

1*S*,3*R*-ACPD appeared to be slightly more efficacious than L-CCG I, but both of these agents had similar potency in this system to that with VIP stimulation.

3.3. Antagonist effects of (+)-MCPG versus 1S,3R-ACPD and L-AP4

(+)-MCPG alone did not significantly alter the cAMP accumulation stimulated by forskolin, nor did it have any effect on L-AP4 inhibition of forskolin-stimulated cAMP production (Fig. 5). On the other hand, the 1*S*,3*R*-ACPD inhibition of forskolin-stimulated cAMP accumulation was reversed by (+)-MCPG in a concentration-dependent manner.

When VIP was used to stimulate cAMP production, (+)-MCPG had no significant effects on VIP alone. In this case (+)-MCPG also had no significant effect on either L-AP4 inhibition or the more modest potentiation of cAMP production by 1*S*,3*R*-ACPD (Fig. 6).

(+)-MCPG reversed the 1*S*,3*R*-ACPD potentiation of NECA-stimulated cAMP production, but had no effect on L-AP4 inhibition (Fig. 7). In this case, (+)-MCPG completely reversed the potentiation by 10 μM 1*S*,3*R*-ACPD at both concentrations tested (200 and 500 μM).

4. Discussion

Cloned mGlu receptors in group 2 ($mGlu_2$ and $mGlu_3$) and group 3 ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) decrease forskolin-stimulated cAMP when expressed in non-neuronal mammalian cell lines (Schoepp, 1994; Nakanishi, 1994). Our laboratory and others have studied the effect of 1*S*,3*R*-ACPD and other mGlu receptor agonists on forskolin-stimulated cAMP in rat hippocampal slices and have demonstrated the existence of negatively coupled mGlu receptors, but have not distinguished which mGlu receptors are responsible for the effect, or what other receptors or transmitters may be involved in modulation of cAMP at the same sites at which mGlu receptors act. Thus, the aim of this study was to examine the actions of mGlu receptor agonists in systems where the influence of endogenous adenosine has been minimized by the addition of adenosine deaminase, and possibly differentiate by pharmacological means groups or subtypes of cAMP-coupled mGlu receptors.

Agents initially tested for stimulation of cAMP in the presence of adenosine deaminase included a stable adenosine analog (NECA), a neuropeptide agonist (VIP) and forskolin (a direct activator of adenylate cyclase). All of these agents produced significant increases in cAMP, either in the presence or absence of adenosine deaminase. NECA was the only one of the three that was minimally affected by adenosine deaminase. The effect of adenosine deaminase on forskolin and VIP was much more profound, but even in its presence cAMP stimulation by these agents was still highly significant when compared to basal levels. In the rat hippocampus, agents such as forskolin have been shown to stimulate the release of adenosine into the media (Casabona et al., 1994), and this could account for the adenosine deaminase-sensitive portions of the forskolin (and possibly VIP) stimulations shown here. Because of the complexity of the system being studied, we used submaximal concentrations of cAMP stimulatory agents under conditions which should minimize any influence of secreted endogenous adenosine, which itself can activate cAMP-coupled receptors in brain slices (Bruns et al., 1980).

In earlier studies of mGlu receptor inhibition of forskolin-stimulated cAMP in rat hippocampal slices, 1*S*,3*R*-ACPD and L-CCG I appeared to be only partially effective, with maximal inhibitory effects of approximately 50% at 100 μM (Schoepp et al., 1992). The data presented here support previous work in the rat cerebral cortex (Cartmell et al., 1993) suggesting that the partial efficacy of 1*S*,3*R*-ACPD inhibition may be due to an opposing adenosine-dependent increase in cAMP which is potentiated by mGlu receptor agonists. In the presence of adenosine deaminase, 1*S*,3*R*-ACPD and L-CCG I produced nearly full inhibition of forskolin-stimulated cAMP ($\approx$ 80% at 100 μM), but with approximately the same potencies as in previous studies (Schoepp et al., 1994).

L-AP4 inhibited forskolin-stimulated cAMP completely in the presence of adenosine deaminase, but the concentration effect was biphasic with high- and low-affinity components. Studies on the pharmacology of cloned mGlu receptors indicate that this L-AP4 effect may reflect activation of group 3 mGlu receptors. Of the cloned group 3 (L-AP4-sensitive) mGlu receptors, $mGlu_4$ and $mGlu_7$ have been shown via in situ hybridization studies to be present in the rat hippocampus (Saugstad et al., 1994). When expressed in non-neuronal cells the $mGlu_7$ receptor has somewhat lower affinity for L-AP4, and it is expressed at much higher levels throughout the rat hippocampus when compared to the $mGlu_4$ receptor. Interestingly, we found that the low-affinity component in the rat hippocampus comprised the majority of the L-AP4 inhibitory effect, suggesting that the $mGlu_7$ receptor may be responsible for the predominant hippocampal response. L-AP4 and L-serine-*O*-phosphate (both agonists at the cloned group 3 mGlu receptors) also inhibited both VIP- and NECA-stimulated cAMP with similar potencies to the low affinity L-AP4 effect on forskolin, but the group 2 agonists (1*S*,3*R*-ACPD and L-CCG I) potentiated the responses to VIP and NECA, thus clearly demonstrating functional differences between cAMP coupling of group 2 versus group 3 mGlu receptors in the rat hippocampus.

To further characterize the pharmacology of mGlu receptor agonist effects, experiments using (+)-MCPG, a phenylglycine analog which has been shown to inhibit a group 1 mGlu receptor ($mGlu_1$) and a group 2 mGlu receptor ($mGlu_2$), but not a group 3 mGlu receptor ($mGlu_4$)

(Thomsen et al., 1994; Watkins and Collingridge, 1994) was performed. We showed that (+)-MCPG reversed both 1*S*,3*R*-ACPD potentiation of NECA stimulation and 1*S*,3*R*-ACPD inhibition of forskolin-stimulated cAMP. At concentrations of (+)-MCPG which reversed both the inhibition of forskolin-stimulated cAMP and the potentiation of NECA-stimulated cAMP by 1*S*,3*R*-ACPD, there was no effect on inhibition of cAMP formation by L-AP4. Recently, Winder and Conn (1995) reported that 1*S*,3*R*-ACPD and the $mGlu_{2,3}$ receptor-selective agonist DCG-IV potentiate cAMP responses (in this case to isoproterenol) in the rat hippocampus. Unlike DCG-IV, the group 1-selective agonist 3,5-dihydroxyphenyl glycine did not potentiate cAMP responses. These data provide strong evidence that both the inhibitory effects of 1*S*,3*R*-ACPD on forskolin-stimulated cAMP and the cAMP-potentiating effects of 1*S*,3*R*-ACPD on agonists such as NECA or isoproterenol are mediated by activation of group 2 mGlu receptors. However, Cartmell et al. (1994) showed that the cAMP potentiating effects of the non-selective agonists 1*S*,3*R*-ACPD and L-CCG I are reduced by a protein kinase C inhibitor and in the absence of Ca^{2+}. This suggests a role for group 1 mGlu receptors in increasing cAMP through activation of PKC and mobilization of Ca^{2+}. However, their work was done in another species (guinea pig) and brain region (cerebral cortex) and thus may not be relevant to the rat hippocampus. No antagonists which block group 2, but not group 1 mGlu receptors (and vice versa) are currently available to more clearly address this question.

It is more difficult to interpret data on potentiation of VIP-stimulated cAMP by 1*S*,3*R*-ACPD, since this effect was not blocked by (+)-MCPG. Possibly this effect of 1*S*,3*R*-ACPD is not receptor-mediated and at least not due to activation of a group 2 mGlu receptor such as $mGlu_2$. It should also be noted that the relative antagonist potencies of (+)-MCPG across all cloned mGlu receptors have not yet been reported.

In summary, this study demonstrates that the rat hippocampus can be used to characterize the receptor subtype or 'group' selectivity of mGlu receptor-selective compounds under conditions where the receptor is in its natural environment. Specifically, group 3 mGlu receptor agonist effects can be clearly distinguished pharmacologically from group 2 mGlu receptor agonists. In the case of NECA-stimulated cAMP, activation of group 2 mGlu receptors results in increases in cAMP, while group 3 mGlu receptor agonists produce decreases in cAMP accumulation. Although both group 2 and group 3 mGlu receptor agonists decrease forskolin-stimulated cAMP formation, the effects of these agonists also appear mediated by distinct ((+)-MCPG-sensitive and -insensitive, respectively) receptor populations. In the future, selective compounds may be discovered that further distinguish the individual receptors within the mGlu receptor groups. At that time it may be possible to sort out the relative contributions of specific mGlu receptor subtypes within each group that mediate different cAMP responses in the rat hippocampus.

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