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Coupling of metabotropic glutamate receptors to phosphoinositide mobilisation and inhibition of cyclic AMP generation in the guinea-pig cerebellum

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1 The effects of metabotropic glutamate receptor (mGluR) agonists on cyclic nucleotide and phosphoinositide turnover were investigated in adult guinea-pig cerebellar slices by use of radioactive precursors.

2 L-Glutamate, 1-aminocyclopentane-1**S**,3**R**-dicarboxylate (1**S**,3**R**-ACPD) and **RS**-3,5-dihydroxyphenylglycine (DHPG) evoked concentration-dependent increases in the accumulation of [^{3}H]-inositol phosphates with pEC_{50} values of 2.98 ± 0.02, 4.45 ± 0.06 and 4.47 ± 0.07, respectively. Maximal responses to these agents were 43 ± 8, 52 ± 12 and $84 \pm 11\%$ of the response to 1 mM histamine, respectively.

3 The phosphoinositide response to 1**S**,3**R**-ACPD was antagonized in the presence of (+)-α-methyl-4-carboxyphenylglycine, with a calculated pK_i value of 3.55 ± 0.03.

4 Forskolin-stimulated accumulation of [^{3}H]-cyclic AMP was not significantly altered in the presence of 10 μM DCG-IV or 300 μM 1**S**,3**R**-ACPD. Similarly, 300 μM 1**S**,3**R**-ACPD failed to alter isoprenaline- (1 μM) or 2-chloroadenosine (2-CA, 30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP.

5 Forskolin-stimulated accumulation of [^{3}H]-cyclic AMP was concentration-dependently inhibited in the presence of L-glutamate and L-serine-O-phosphate (L-SOP) with pIC_{50} values of 2.91 ± 0.17 and 2.86 ± 0.04 with maximal inhibitions of 47 ± 2 and $92 \pm 3\%$, respectively. L-2-Amino-4-phosphonobutyrate (L-AP4) inhibited the forskolin response without saturating, evoking an inhibition of $71 \pm 7\%$ at 3 mM.

6 2-CA-evoked accumulation of [^{3}H]-cyclic AMP was also inhibited by L-glutamate and L-SOP with pIC_{50} values of 2.71 ± 0.03 and 2.72 ± 0.08 and maximal inhibitions of 51 ± 5 and $99 \pm 0\%$, respectively. L-AP4 inhibited the 2-CA response without saturating, evoking an inhibition of $68 \pm 1\%$ at 3 mM.

7 Isoprenaline-evoked accumulation of [^{3}H]-cyclic AMP was inhibited by L-glutamate and L-SOP with pIC_{50} values of 3.21 ± 0.01 and 2.96 ± 0.08 and maximal inhibitions of 88 ± 2 and $93 \pm 3\%$, respectively.

8 These results suggest that the guinea-pig cerebellum expresses Group I and Group III mGluRs coupled to phosphoinositide turnover and inhibition of cyclic AMP generation, respectively.

Keywords: Metabotropic glutamate receptors; cyclic AMP; phosphoinositide turnover; guinea-pig cerebellum

Introduction

Our knowledge of the mechanisms of signal transduction at receptors for the major excitatory transmitter, L-glutamate, has increased greatly in recent years through the application of molecular biological techniques (Nakanishi, 1992). Initially, glutamate receptors were thought to consist solely of ligand-gated ion channels; however, it is now clear that glutamate can activate specific G-protein-coupled receptors. At present, seven major subtypes of G-protein-coupled or metabotropic glutamate receptors (mGluRs) have been established (Nakanishi, 1992), with splice variants providing further multiplicity (e.g. Pin *et al.*, 1992; Minakami *et al.*, 1993). These receptors have been divided into three main groups on the basis of similarities in primary sequence, pharmacology and signal transduction properties (Nakanishi, 1992). Thus, Group I mGluRs comprise mGluRs 1 and 5, which couple to phosphoinositide mobilisation when transfected into naive cells (Pin *et al.*, 1992; Abe *et al.*, 1992). Group II is made up of mGluR2 and mGluR3 and couple to inhibition of cyclic AMP generation when expressed in naive cells (Prézeau *et al.*, 1992; Tanabe *et al.*, 1992, 1993). Group III receptors also couple to inhibition of adenosine 3':5'-cyclic monophosphate (cyclic AMP) generation in model cells, and comprise mGluRs 4, 6 and 7 (Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Tanabe *et al.*, 1993).

trans-ACPD is a mixture of 1-aminocyclopentane-1**S**,3**R**-dicarboxylate (1**S**,3**R**-ACPD) and 1**R**, 3**S**-ACPD, and is a rigid, cyclic analogue of L-glutamate. The potency of either 1**S**,3**R**- or *trans*-ACPD has been assessed using examples of all three groups of these receptors. Thus, at Group II receptors, *trans*-ACPD has highest potency (EC_{50} value 5-8 μM, Tanabe *et al.*, 1992; 1993), and shows lower potency at Group I receptors (mGluR1 EC_{50} value ca. 50 μM, Abe *et al.*, 1992). 1**S**,3**R**-ACPD also appears to exhibit similar potency at Group III receptors (mGluR4 EC_{50} value 39 μM, Kristensen *et al.*, 1993). (2**S**, 1'**R**, 2'**R**, 3'**R**)-2,2',3'-dicarboxycyclopropylglycine (DCG-IV) has been suggested as a Group II-selective agonist (Pin & Duvoisin, 1995). Its potency at Group II mGluRs is in the submicromolar range (EC_{50} values ca. 250 nM) without significant activity at Group I or III mGluRs at concentrations up to millimolar (Hayashi *et al.*, 1993). Group III mGluRs, comprising mGluRs 4, 6 (which appears to be exclusively expressed in the retina, Nakajima *et al.*, 1993) and 7 appear to be selectively activated by the open chain glutamate analogues L-serine-O-phosphate (L-SOP) and L-2-amino-4-phosphonobutyrate (L-AP4). These two agents also appear to allow discrimination of subtypes mGluR 4 and 7 since mGluR4 shows increased potency for L-AP4 compared to L-SOP (EC_{50} values 0.5 and 4 μM, respectively, Tanabe *et al.*, 1993), while mGluR7 shows similar potency for the two agents (EC_{50} value 160 μM, Okamoto *et al.*, 1994).

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Due to the recent appearance of these agents, there are relatively few examples in the literature examining the use of putative antagonists against individual mGluRs expressed in naive cells. In one example, Thomsen *et al.* (1994) observed an mGluR-selective action of (±)-α-methyl-4-carboxyphenylglycine (compared to potential activity of ionotropic glutamate receptors), with an estimated K_i value at the mGluR1α of 172 μM (and an IC_{50} value of 340 μM at the mGluR2) without significant activity at mGluR4.

In the guinea-pig cerebral cortex, we have previously been able to show the presence of all three groups of mGluR. That is, phosphoinositide turnover is evoked by 1S,3**R**-ACPD and L-glutamate (Alexander *et al.*, 1990; Cartmell *et al.*, 1993). The response to 1S,3**R**-ACPD is concentration-dependent with an EC_{50} value of 35 μM (Cartmell *et al.*, 1993) while the response to L-glutamate shows a threshold concentration of ca. 1 mM and fails to saturate at concentrations up to 10 mM (Alexander *et al.*, 1990). Forskolin-induced cyclic AMP generation in the guinea-pig cerebral cortex is inhibited by a range of glutamate analogues with the rank order of potency (EC_{50} value) of DCG-IV (30 nM, Alexander *et al.*, unpublished observations) < L-CCG-1 (200 nM, Cartmell *et al.*, 1994) < 1S,3S-ACPD (500 nM, Cartmell *et al.*, 1993) < 1S,3**R**-ACPD (2 μM, Cartmell *et al.*, 1993) < L-AP4 (100 μM, Cartmell *et al.*, 1994) < L-SOP (1 mM, Alexander *et al.*, unpublished observations) = L-glutamate (1 mM, Alexander *et al.*, unpublished observations). The cyclic AMP response to the adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) was also inhibited by L-AP4 (Cartmell *et al.*, 1994), but enhanced in the presence of other, less selective glutamate analogues (e.g. 1S,3**R**-ACPD, Cartmell *et al.*, 1993) presumably through cross-talk mediated by products of phosphoinositide turnover (Cartmell *et al.*, 1994).

Investigations of the regional distribution of mGluR mRNAs in rat brain have shown the cerebellum to be an area which expresses distinct levels of the subtypes of mGluR (e.g. Abe *et al.*, 1992; Fotuhi *et al.*, 1993; Pin *et al.*, 1993; Tanabe *et al.*, 1993). mGluRs are of especial interest in the cerebellum, since they have been implicated in cerebellar synaptic plasticity, in particular in the phenomenon of long term depression (Batchelor & Garthwaite, 1993), which has been suggested to be a cellular correlate of motor learning. Accordingly, we have investigated whether second messenger responses in the guinea-pig cerebellum reflect the pattern predicted from rat *in situ* hybridization studies.

Methods

Tissue preparation and second messenger accumulation

Preparation and incubation of slices were essentially the same as described previously (Hernández *et al.*, 1993). Cross-chopped cerebellar or cerebral cortex slices (350 × 350 μm) were prepared from guinea-pigs (Dunkin-Hartley, either sex, weighing 200–600 g) with a McIlwain tissue chopper. They were then incubated in a shaking water bath for 60 min at 37°C in several changes of Krebs-bicarbonate buffer which contained (mM): NaCl 118, KCl 4.7, $MgSO_4$1.2, $KH_2PO_4$1.2, glucose 11.7, $NaHCO_3$25, $CaCl_2$1.2, equilibrated to pH 7.4 with 95% O_2:5% CO_2. The slices were then used for estimation of cyclic AMP generation, phosphoinositide hydrolysis or L-glutamate uptake.

For estimation of [^{3}H]-cyclic AMP generation, slices were suspended in fresh Krebs buffer and [^{3}H]-adenine was added to a final concentration of 74 kBq ml^{-1} (ca. 80 nM). After an additional 60 min of incubation, the slices were washed and aliquots (25 μl approximately equivalent to 1 mg protein) transferred into flat-bottomed plastic vials containing Krebs buffer (to a final volume of 300 μl). Slices were allowed to equilibrate for 15 min prior to addition of agents. The tubes were resealed under 95% O_2:5% CO_2 after each addition. Incubations were terminated by the addition of 200 μl of HCl (1 M) containing [^{14}C]-cyclic AMP (30–35 Bq per tube), followed by 750 μl of ice-cold water. [^{3}H]-cyclic AMP was subsequently resolved by the double-column method of Salomon *et al.* (1974) using [^{14}C]-cyclic AMP as a recovery marker. Generation of cyclic AMP was estimated as the percentage conversion from total [^{3}H]-adenine nucleotides. Typical basal levels of [^{3}H]-cyclic AMP were 2452 ± 108 d.p.m.

Slices used for determination of phosphoinositide turnover were pre-equilibrated with Krebs-bicarbonate buffer for 60 min and distributed as 25 μl aliquots into flat-bottomed vials in the presence of [^{3}H]-inositol (ca. 40 kBq ml^{-1}) and LiCl (5 mM) to give a final volume of 300 μl, as previously described (Alexander *et al.*, 1989). After 40 min, agonist was added, and following an incubation period of 45 min, the reaction was halted by the addition of 7.5% perchloric acid. After neutralisation, [^{3}H]-inositol phosphates were then resolved by chromatography on Dowex-1 (chloride form) resin. In order to compare responses to excitatory amino acids between experiments, the phosphoinositide response to 1 mM histamine was estimated in each case.

Glutamate uptake was examined by slices of guinea-pig cerebral cortex and cerebellum in Krebs-Henseleit medium in a total volume of 300 μl. After a 30–40 min incubation in the presence of 10 mM L-glutamate containing [^{3}H]-L-glutamate (20 kBq ml^{-1}), parallel incubations maintained at 37°C or 4°C were rapidly filtered over Whatman GF/B filters, which were then washed with 3 × 3 ml ice-cold Krebs-Henseleit medium. Filters were allowed to extract overnight in liquid scintillation medium before counting. Maximal uptake of L-glutamate was less than 15% of the added [^{3}H]-L-glutamate.

Calculations and statistical analysis

The computer programme Prism (GraphPad, California, U.S.A.) was used to generate parameters from concentration-response curves. In the text, values represent mean ± s.e.mean (except where indicated) of n independent experiments (in all cases, $n \geq 3$) conducted in triplicate or quadruplicate. Statistical analysis was performed with Student's unpaired t test.

Chemicals

[8-^{3}H]-adenine (962 GBq mmol^{-1}) was from Amersham International, Bucks., UK. [Adenine-U-^{14}C]-adenosine 3',5'-cyclic phosphate (11.4 GBq mmol^{-1}) and [^{3}H]-inositol (455.1 GBq mmol^{-1}) were purchased from DuPont NEN, Herts, UK. Adenosine deaminase was obtained from Boehringer Mannheim. DCG-IV was a generous gift from Professor H. Shinozaki, Japan, while 1S, 3**R**-ACPD, DHPG and MCPG were purchased from Tocris-Cookson (Bristol, UK). All other reagents were obtained from either Sigma (Dorset, UK) or Fisons (Leics, UK).

Results

Phosphoinositide turnover

The effect of 1S,3**R**-ACPD, DHPG and L-glutamate on phosphoinositide turnover in cerebellar slices was investigated (Figure 1a). In [^{3}H]-inositol-prelabelled guinea-pig cerebellar slices, 1S,3**R**-ACPD elicited a concentration-dependent phosphoinositide response with a pEC_{50} value of 4.45 ± 0.06, and with a maximal response of 52 ± 12% (n = 4) of the 1 mM histamine response (ca. 5 fold basal [^{3}H]-inositol phosphates accumulation). L-Glutamate stimulated phosphoinositide turnover with a similar maximal response (43 ± 8% of the response to 1 mM histamine) but with a calculated pEC_{50} value of 2.98 ± 0.02 (n = 3). The response to DHPG was equipotent with the 1S,3**R**-ACPD response (pEC_{50} value 4.47 ± 0.07) with a slightly increased maximal response (84 ± 11% histamine response, n = 3).

The effect of the 'selective' mGluR antagonist, (+)-α-me-

thyl-4-carboxyphenylglycine ((+)-MCPG, Pin & Duvoisin, 1995) was examined on the phosphoinositide response to 1S,3R-ACPD (Figure 1b). In this series of experiments, 1S,3R-ACPD evoked a concentration-dependent generation of [^{3}H]-inositol phosphates with a calculated pEC_{50} value of 4.69 ± 0.14 ($n=3$). In the presence of 1 mM (+)-MCPG, the concentration-response curve to 1S,3R-ACPD was shifted rightward with a mean increase of 4.6 fold, allowing the calculation of an apparent pK_i value for (+)-MCPG of 3.55 ± 0.03 ($n=3$).

L-Glutamate uptake

Since we previously observed that phosphoinositide responses to L-glutamate failed to saturate at concentrations up to 10 mM in the guinea-pig cerebral cortex (Alexander *et al.*, 1990), we investigated whether the saturating response to L-glutamate in the cerebellum was due to differences in the rate of uptake of L-glutamate in the two brain regions. Conditions for uptake were chosen which were similar to those for phosphoinositide turnover, that is, 25 μl tissue slices in a total volume of 300 μl, with a high glutamate concentration (10 mM) and for an extended period (30–40 min). Uptake at 4°C was identical in both brain regions (1.9 ± 0.2 nmol min^{-1}). However, uptake at 37°C was greater in the cerebral cortex (11.9 ± 1.3 nmol min^{-1}) than in the cerebellum (6.3 ± 0.4 nmol min^{-1}). Temperature-dependent uptake of L-glutamate was calculated to be 10.1 ± 1.2 and 4.4 ± 0.3 nmol min^{-1} per 25 μl slices for cerebral cortex and cerebellum ($n=3$ throughout).

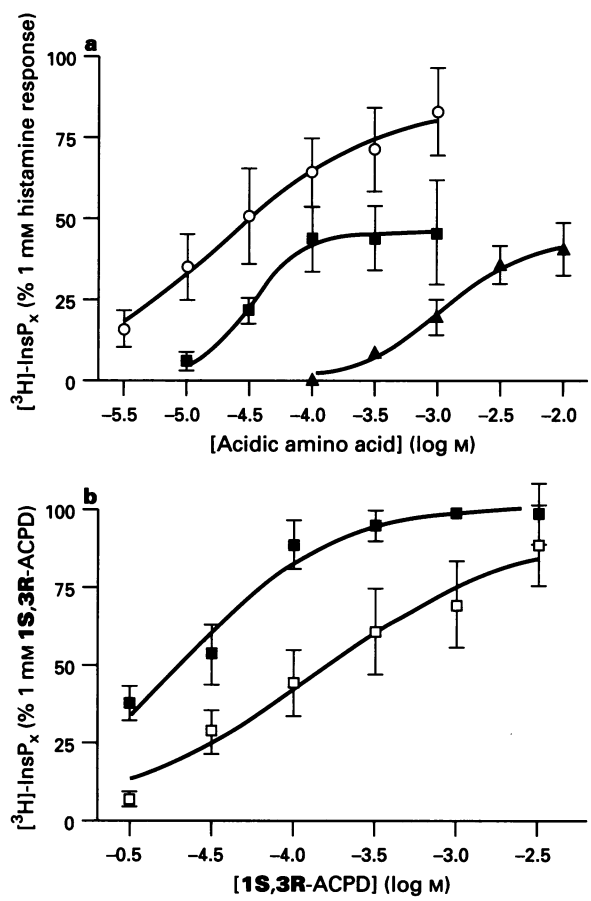

Figure 1 Phosphoinositide turnover in the guinea-pig cerebellum in response to increasing concentrations of acidic amino acids. (a) Accumulation of [^{3}H]-inositol phosphates evoked in the presence of DHPG (○), 1S,3R-ACPD (■) or L-glutamate (▲). Data are the means ± s.e.mean from 3 experiments expressed as a percentage of the response to 1 mM histamine (basal and histamaine-evoked responses were 1308 ± 579 and 6285 ± 1592 d.p.m., respectively). (b) Accumulation of [^{3}H]-inositol phosphates evoked by increasing concentrations of 1S,3R-ACPD in the absence (■) or presence (□) of 1 mM (+)-MCPG. Data are the means ± s.e.mean from 3 experiments expressed as a percentage of the response to 1 mM 1S,3R-ACPD (basal accumulation of [^{3}H]-inositol phosphates was 2908 ± 130 d.p.m.).

Modulation of cyclic AMP generation

In order to examine Group II and III mGluR-mediated inhibition of cyclic AMP generation, forskolin (30 μM), isoprenaline (1 μM) and 2-chloroadenosine (30 μM) were used to stimulate cyclic AMP levels with responses of ca. 60, 20 and 9 fold basal, respectively.

Forskolin-stimulated cyclic AMP generation was unaffected in the presence of DCG-IV (10 μM, 100 ± 15% control) or 1S,3R-ACPD (300 μM, 89 ± 4% control). In addition, cyclic AMP responses to isoprenaline and 2-chloroadenosine were unaffected in the presence of 300 μM 1S,3R-ACPD (87 ± 23 and 89 ± 6% control, respectively, $n=3/4$).

Examination of concentration-response curves for inhibition of forskolin-stimulated cyclic AMP accumulation (Figure 2a) revealed pIC_{50} values for L-glutamate and L-SOP of 2.91 ± 0.17 and 2.86 ± 0.04, respectively ($n=3$), with maximal inhibitions of 47 ± 2 and 92 ± 3%, respectively. L-AP4 evoked a concentration-dependent inhibition of forskolin-stimulated cyclic AMP generation without attaining a maximal response, evoking an inhibition of 71 ± 7% at 3 mM ($n=3$).

Using 2-chloroadenosine as a stimulant of cyclic AMP generation, L-glutamate and L-SOP elicited concentration-dependent inhibitions (Figure 2b) with calculated pIC_{50} values of 2.71 ± 0.03 and 2.72 ± 0.08, respectively, and maximal inhibitions of 51 ± 5 and 99 ± 0% respectively ($n=3/4$). L-AP4 evoked an inhibition of 2-chloroadenosine-elicited cyclic AMP generation of 68 ± 1% at 3 mM ($n=3$).

When isoprenaline was used to stimulate cyclic AMP levels (Figure 2c), L-glutamate and L-SOP inhibited cyclic AMP production with calculated pIC_{50} values of 3.21 ± 0.01 and 2.96 ± 0.08, respectively, with maximal inhibitions of 88 ± 2 and 93 ± 3%, respectively ($n=3$).

Discussion

The object of this investigation was to investigate the second messenger responses to mGluR agonists and an antagonist in the guinea-pig cerebellum. The data indicate the presence of Group I and III mGluRs, but fail to indicate the presence of Group II mGluRs.

Group I mGluRs

Stimulation of phosphoinositide hydrolysis by the non-selective mGluR agonist, 1S,3R-ACPD exhibited a potency in the guinea-pig cerebellum (32 μM) almost identical to that which we have previously observed in the cerebral cortex (35 μM, Cartmell *et al.*, 1993). However, in contrast to our previous observations in the cerebral cortex the phosphoinositide response to L-glutamate reached a maximal response (at 3–10 mM), allowing the calculation of an EC_{50} value for L-glutamate of approximately 1 mM. This discrepancy between cerebral cortex and cerebellum is apparently due to the increased uptake of L-glutamate in the former region compared to the latter; temperature-dependent uptake of 10 mM L-glutamate in the cerebellum is only ca. 40% of the uptake in the cerebral cortex. However, there is still substantial uptake of L-glutamate in the cerebellum so the current figures for the potency of L-glutamate at mGluRs in both tissues are likely to be underestimates (cf. Garthwaite, 1985). The comparable potencies of 1S,3R-ACPD in the cortex and cerebellum imply that this compound is not subject to uptake via L-glutamate uptake systems. Further evidence for the mediation of the phosphoinositide response to 1S,3R-ACPD by Group I

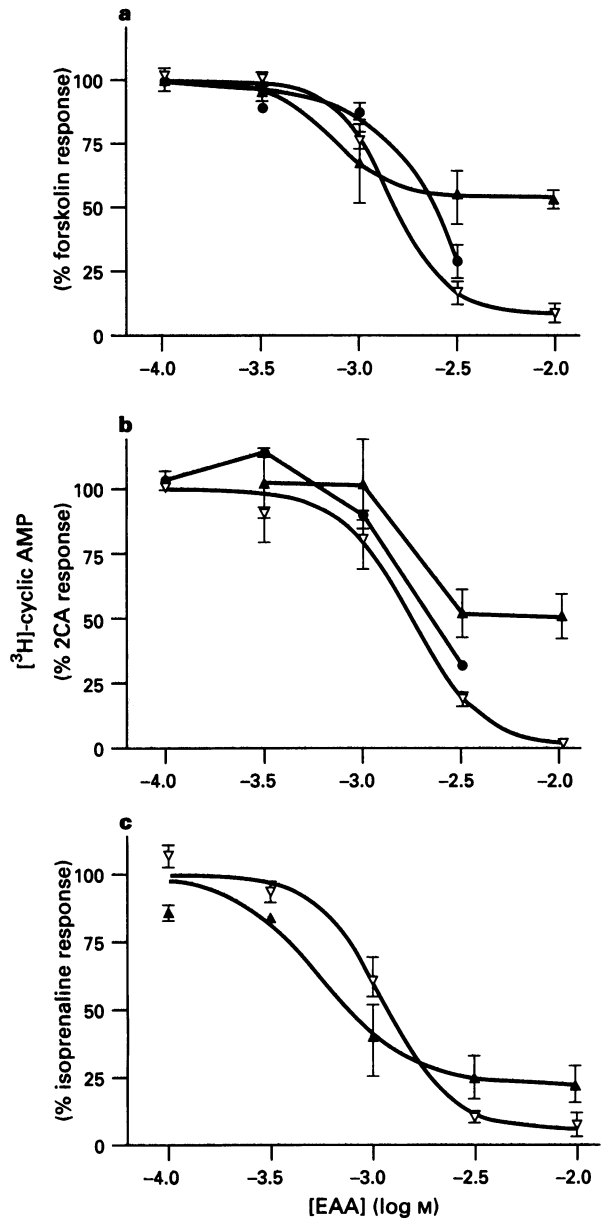

Figure 2 Inhibition of cyclic AMP generation in the guinea-pig cerebellum in the presence of increasing concentrations of acidic amino acids. (a) Accumulation of [^{3}H]-cyclicAMP evoked in the presence of 30 μM forskolin, (b) 30 μM 2-chloroadenosine and (c) 1 μM isoprenaline in the presence of increasing concentrations of L-SOP (▽), L-AP4 (●) or L-glutamate (▲). Data are means ± s.e.mean from 3/4 experiments conducted in quadruplicate, expressed as a percentage of the response to forskolin, 2-chloroadenosine or isoprenaline alone (basal, forskolin, 2-chloroadenosine and isoprenaline-evoked responses were 0.17 ± 0.04, 9.89 ± 0.81, 2.95 ± 0.34 and 1.48 ± 0.17% conversion, respectively).

mGluRs is obtained from the experiments using the more selective agonist DHPG (Ito *et al.*, 1992; Schoepp *et al.*, 1994) and the putative mGluR antagonist MCPG, which lacks activity at ionotropic glutamate receptors (Thomsen *et al.*, 1994). The agonist DHPG, has an EC_{50} value at Group I mGluRs in the range 20–60 μM (Ito *et al.*, 1992; Schoepp *et al.*, 1994), a potency which is also found for phosphoinositide turnover in the present study. The racemic mixture of MCPG has a reported K_i value at the mGluR1α transfected into baby hamster kidney cells of 172 μM (Thomsen *et al.*, 1994), similar to the K_i value estimated in the current study for the (+)-stereoisomer (281 μM).

In the guinea-pig cerebral cortex, 1S,3R-ACPD evokes a concentration-dependent enhancement of the A_{2b} adenosine receptor cyclic AMP response through a Group I mGluR (Cartmell *et al.*, 1993; 1994). However, in the cerebellum, a high concentration of 1S,3R-ACPD does not modify the A_{2b} adenosine receptor response, implying that Group I mGluRs and A_{2b} adenosine receptors are differentially located in this brain region.

The Group I mGluRs comprise the two subtypes mGluR1 and mGluR5, both of which exhibit further splice variants (Pin *et al.*, 1992; Tanabe *et al.*, 1992; Minakami *et al.*, 1993). *In situ* hybridization studies in the rat cerebellum suggest the presence of mGluR1a and mGluR1b (Fotuhi *et al.*, 1993) and lower expression of mGluR1c (Pin *et al.*, 1992). The expression of mGluR5 is also relatively low in the rat cerebellum (Abe *et al.*, 1992). Immunocytochemical studies have also shown the presence of mGluR1α immunoreactivity in the rat cerebellum (Görcs *et al.*, 1993). The current understanding of the pharmacology of these individual isoforms does not, however, allow their discrimination.

The phenomenon of long term depression at parallel fibre-Purkinje cell synapses, elicited by simultaneous stimulation of cerebellar climbing and parallel fibres may be mimicked by depolarization in the presence of the mGluR agonist, ACPD in a calcium-dependent manner (Batchelor & Garthwaite, 1993). This suggests the presence of Group I mGluRs in the Purkinje cell layer, a theory supported by the findings of Fotuhi *et al.* (1993) and Görcs *et al.* (1993). The former report also identified co-localization of *trans*-ACPD-evoked phosphoinositide turnover together with mGluR1, 1,4,5-$InsP_3$ receptor and protein kinase C immunoreactivities in the molecular layer of the cerebellum, implying close association of the mGluR1 with the Ca^{2+} and protein kinase C signalling systems (Fotuhi *et al.*, 1993).

Group II mGluRs

In the guinea-pig cerebral cortex, DCG-IV or 1S,3R-ACPD at the concentrations used here elicit at least 95% inhibition of the forskolin-stimulated cyclic AMP response (Cartmell *et al.*, 1992; Alexander *et al.*, unpublished observations). *In situ* hybridization studies in the rat suggest that both mGluR2 and mGluR3 are present in the cerebellum but expression is moderate compared with other regions (Genazzani *et al.*, 1993; Tanabe *et al.*, 1993). It remains to be established whether species differences may explain the lack of inhibition of cyclic AMP generation by these Group II mGluR agonists in the guinea-pig cerebellum compared to the pattern of mGluRs expected from *in situ* hybridization studies using rat brain. The lack of inhibition of forskolin- or agonist-stimulated cyclic AMP generation with either of these agonists may possibly indicate coupling of these receptors to other transduction mechanisms. Previous investigations in our laboratories have established the presence of other receptors coupled to inhibition of adenylyl cyclase in the guinea-pig cerebral cortex but not in the cerebellum, although both tissues display abundant radioligand binding (i.e. A_1 adenosine receptors, Hernández *et al.*, 1993; Alexander *et al.*, 1994). In the absence of a radioligand binding assay for the Group II mGluRs, it is not possible to establish the presence of these receptors in the guinea-pig cerebellum from the data obtained in the current study.

Group III mgluRs

Of the Group III mGluRs, both subtypes mGluR4 and mGluR7 are expressed in the rat cerebellum (Tanabe *et al.*, 1993; Okamoto *et al.*, 1994), while mGluR6 appears to be confined to the retina (Nakajima *et al.*, 1993). It appears unlikely, therefore, that mGluR6 mediates responses to mGluR agonists in the guinea-pig cerebellum. The Group III-selective mGluR agonists L-SOP and L-AP4 both evoked concentration-dependent inhibitions of forskolin- and receptor-stimulated cyclic AMP formation in the guinea-pig cerebellum. The

potencies of these agents were similar with EC_{50} values of ca. 1 mM (although the L-AP4 inhibition failed to reach saturation at concentrations up to 3 mM). This might be taken as evidence against the presence of mGluR4 in the guinea-pig cerebellum, since the rat mGluR4 receptor expressed in Chinese hamster ovary cells shows an almost 10 fold higher potency for L-AP4 compared to L-SOP (Tanabe *et al.*, 1993). The rat mGluR7 receptor, in contrast, shows similar potency for these agents (Okamoto *et al.*, 1994). Further evidence for the absence of the mGluR4 receptor in the guinea-pig cerebellum is the lack of effect of 300 μM 1S,3R-ACPD (at the cloned rat mGluR4 it displays a potency of ca. 40 μM, Kristensen *et al.*, 1993).

L-Glutamate evoked a concentration-dependent inhibition of the cyclic AMP responses to forskolin, 2-chloroadenosine and isoprenaline with IC_{50} values ranging from 0.6 to 2 mM. Given that L-glutamate is subject to uptake in the guinea-pig cerebellum (see above) it is likely that these potencies are underestimates. The higher maximal inhibition observed for L-glutamate using isoprenaline as a cyclic AMP stimulus might be taken as evidence for the greater degree of co-localization of Group III mGluRs and β-adrenoceptors compared to mGluRs and A_{2b} adenosine receptors in the guinea-pig cerebellum. Alternatively, it is possible that the difference in the maximal inhibitions arises from the smaller response to isoprenaline compared to 2-chloroadenosine.

Concluding remarks

In conclusion, therefore, it is apparent that mGluRs coupled to either phosphoinositide mobilisation or inhibition of cyclic AMP generation are expressed in the guinea-pig cerebellum. It is possible that Group II mGluRs are present in this tissue, but coupled to a signal transduction system other than inhibition of cyclic AMP generation. The data do, however, indicate the presence of Group I and Group III (possibly mGluR7) mGluRs coupled to phosphoinositide and cyclic AMP metabolism, respectively. These latter subtypes of mGluR may prove to be important in the function of particular cellular populations in the cerebellum, for example, in the phenomenon of long term depression.

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