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Ca^{2+}/calmodulin-dependent cyclic GMP phosphodiesterase activity in granule neurons and astrocytes from rat cerebellum

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Abstract

Cyclic GMP (cGMP) formation induced by agonist stimulation of Ca^{2+}/calmodulin-dependent nitric oxide (NO) synthase type I is known to occur in both granule cell and astrocyte cultures from rat cerebellum. Here we show that in these same cells cGMP is predominantly hydrolyzed by a Ca^{2+}/calmodulin-dependent phosphodiesterase. At 10 μM cGMP, Ca^{2+} (25 μM) stimulated basal (Ca^{2+}-independent) phosphodiesterase activity about 6 times in granular neurons and 15 times in astrocytes. The calmodulin antagonist calmidazolium blocked the Ca^{2+}-dependent phosphodiesterase activity and exogenous calmodulin increased 3–4-fold the stimulatory potency of Ca^{2+} in both cell types (EC_{50} values 1.26 ± 0.20 and 1.50 ± 0.42 μM in the absence and 0.38 ± 0.11 and 0.39 ± 0.14 μM in the presence of 1 μM calmodulin, for neurons and astrocytes, respectively). In both cell types K_m values for cGMP at 25 μM Ca^{2+} were similar (1.72 ± 0.20 and 1.92 ± 0.09 μM) and phosphodiesterase activities were inhibited by isozyme-selective phosphodiesterase inhibitors with potencies analogous to those described for Ca^{2+}/calmodulin-phosphodiesterases or phosphodiesterase type 1 isoforms in other preparations. The nonselective phosphodiesterase inhibitor 3-isobutyl-1-methylxantine (IBMX) effectively blocked the Ca^{2+}/calmodulin-phosphodiesterase activity in granule cell and astrocyte extracts (IC_{50} values at 1 μM cGMP: 31 ± 10 μM and 46 ± 6 μM, respectively), in contrast to the apparent inability of this compound to inhibit the Ca^{2+}-dependent activity reported in whole brain extracts. These results demonstrate that comparable phosphodiesterase type 1 activities are found in the cytosols of cerebellar granule cells and astrocytes and suggest that these activities may play an important role in controlling cGMP levels in cells where the Ca^{2+}-dependent NO synthase type I is stimulated.

Keywords: cGMP; Phosphodiesterase; Ca^{2+}; Granule cell; Astrocyte

1. Introduction

Cyclic GMP (cGMP) is now recognized as an important second messenger in the central nervous system although its functions are still unclear. It is known to activate protein kinase G, to stimulate or inhibit cyclic nucleotide phosphodiesterases and to have direct and indirect effects on channel gating (Garthwaite and Boulton, 1995). cGMP is synthesized by two types of guanylyl cyclases, one is membrane associated and activated by natriuretic peptides and the other is cytosolic and its major activator is nitric oxide (NO). In the normal brain, NO is largely generated by NO synthase type I, a Ca^{2+}/calmodulin-dependent enzyme. NO synthase type I has been immunohistochemically localized in all brain areas, but is particularly enriched in cerebellum (Bredt et al., 1990). In this region, it is present in granule and basket cells (Dawson et al., 1991) and also in astrocytes and Bergmann glia (Schmidt et al., 1992; Kugler and Drenckhahn, 1996; Arbonés et al., 1996). Production of NO in cerebellar granule neurons occurs mainly as a result of stimulation of excitatory amino-acid receptors of the *N*-methyl-D-aspartate (NMDA) type (Kiedrowski et al., 1992) whereas in cerebellar astroglial cells the effect is elicited by noradrenaline acting through α_1-adrenoceptors (Agulló et al., 1995). In both cell types, agonist effects require extracellular Ca^{2+} (Kiedrowski et al., 1992; Agulló et al., 1995).

Stimulation of NO formation is often determined indirectly by measuring the cGMP accumulation sensitive to NO synthase inhibition. However, it has been reported that in brain extracts formation of cGMP by NO donor stimulation of soluble guanylyl cyclase is inhibited by Ca^{2+} concentrations in the same order as those that stimulate

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NO synthase type I (Knowles et al., 1989). Since the effect was observed in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), a Ca^{2+}-inhibited soluble guanylyl cyclase was postulated to be present in the extracts. A later report, however, demonstrated that brain soluble guanylyl cyclase is not sensitive to Ca^{2+}, but that cGMP is degraded by a highly active Ca^{2+}/calmodulin-dependent phosphodiesterase relatively insensitive to IBMX (Mayer et al., 1992).

There are more than 30 phosphodiesterase isozymes that are expressed in different degrees in particular cell types and that are grouped into seven broad categories (Beavo, 1995). The phosphodiesterase type 1 family or Ca^{2+}/calmodulin-phosphodiesterases are the predominant forms in mammalian brain where at least the 61, 63 and 75 kDa isoforms of this group are known to be present (Sharma et al., 1984; Shenolikar et al., 1985). In the cerebellum, the 63 and the 75 kDa forms have been detected in Purkinje cells (Polli and Kincaid, 1994; Yan et al., 1994; Yan and Beavo, 1994) and only the 75 kDa isoform in granule cells (Yan and Beavo, 1994). There have been so far no reports on the presence of phosphodiesterase type 1 in astroglial cells.

Recent results from our laboratory show that NO donor-stimulated cGMP formation is inhibited by agents that increase intracellular Ca^{2+}, both in granule cells and in astrocyte primary cultures from rat cerebellum (Baltrons et al., submitted), suggesting that Ca^{2+}-dependent phosphodiesterases may be operating in both cell types. In this work we have characterized the phosphodiesterase activity present in cytosolic fractions of granule cell and astrocyte cultures and show that in both cell types cGMP is largely hydrolyzed by a Ca^{2+}/calmodulin-phosphodiesterase or phosphodiesterase type 1 activity.

2. Materials and methods

2.1. Cell cultures

Primary cultures enriched in astrocytes or in granule neurons were prepared from cerebella of 7-day-old Sprague-Dawley rats after mechanical dissociation of the tissue as previously described (Agulló et al., 1995). To obtain astrocyte-enriched cultures, cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and seeded in 35-mm-diameter plastic Petri dishes (1.25×10^5 cells/cm^2). Medium was changed after 1 week, and cells used after 14–19 days. As described elsewhere (Agulló et al., 1995), these cultures contain small numbers of neurons, fibroblasts and oligodendrocytes (about 5% of total cells) and variable amounts of microglia, which is the major contaminant. For granule neuron cultures, cells were resuspended in DMEM supplemented with 25 mM KCl and 10% fetal calf serum and seeded in polyornithine-precoated 35-mm-diameter dishes (2.5×10^5 cells/cm^2). Neuronal cultures were treated with 10 μM cytosine arabinoside after 24 h in culture and used at day 8. Around 95% of the cells in these cultures are positive for neuron-specific enolase (Agulló et al., 1995).

2.2. Preparation of cytosolic fractions

Culture dishes were washed twice with cold Hepes-buffered saline (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 and 20 mM Hepes, pH 7.4) and then harvested and homogenized in cold 50 mM Tris/HCl buffer, pH 7.4 at 37°C, containing 0.5 mM dithiothreitol, 0.1 mm EDTA, 0.1 mM EGTA, 0.2 mM benzamidine, 10 mg/l trypsin inhibitor and 100 mg/l phenylmethylsulfonyl fluoride. Homogenization was performed by 20 hand strokes in a glass-Teflon Potter-Elvehjeim type homogenizer and homogenates centrifuged at $100\,000 \times g$, for 60 min, at 4°C. Supernatants were stored at −80°C until use.

2.3. Measurement of phosphodiesterase activity

Phosphodiesterase activity was determined by a modification of the method of Hidaka and Asano (1976). The reaction mixture (final volume 100 μl) contained 50 mM Tris/HCl, pH 7.4, 1 mM $MgCl_2$, [^{3}H]cGMP (0.075 μCi, from 0.3 μM to 1 mM), 0.5 mM EGTA and different concentrations of $CaCl_2$ to obtain the desired free Ca^{2+} concentrations that were calculated by the program RECIPC (S. Roberston, University of Cincinnati, 1981). When no $CaCl_2$ was added, the free Ca^{2+} concentration in the assay medium was less than 1 nM. Reactions were started by adding 50 μl of the cytosolic fractions and were incubated at 37°C. Enzyme concentration (15–40 μg/tube) and incubation time (5–30 min) were adjusted so that no more than 20% of the substrate was hydrolyzed in order to be in the linear range. Incubations were terminated by boiling for 5 min. Then 50 μg of snake venom in 1 ml of H_2O were added and mixtures incubated for another 15 min at 37°C. Denatured protein was removed by centrifugation and supernatants were applied to 0.75 ml cation exchange resin columns (Dowex 50W-X4, 200–400 mesh). The product, [^{3}H]guanosine, was eluted with 1.5 ml of 3 N ammonium hydroxide after washing the columns with 15 ml of water. Over 95% of [^{3}H]guanosine was recovered from the columns. Inhibitors were usually dissolved in dimethyl sulfoxide (final concentration in reaction mixtures 1%, v/v). At this concentration dimethyl sulfoxide inhibited enzyme activity by approximately 10%, as previously described by Weishaar et al. (1986). In these conditions vinpocetine was not soluble at concentrations higher than 100 μM.

2.4. Materials

DMEM and fetal calf serum were obtained from Flow Laboratories; [^{3}H]cGMP (33 Ci/mmol) from New England Nuclear; Dowex 50W-X4 (200–400, H^+ form) from Bio-Rad. Vinpocetine was from Calbiochem-Novabiochem

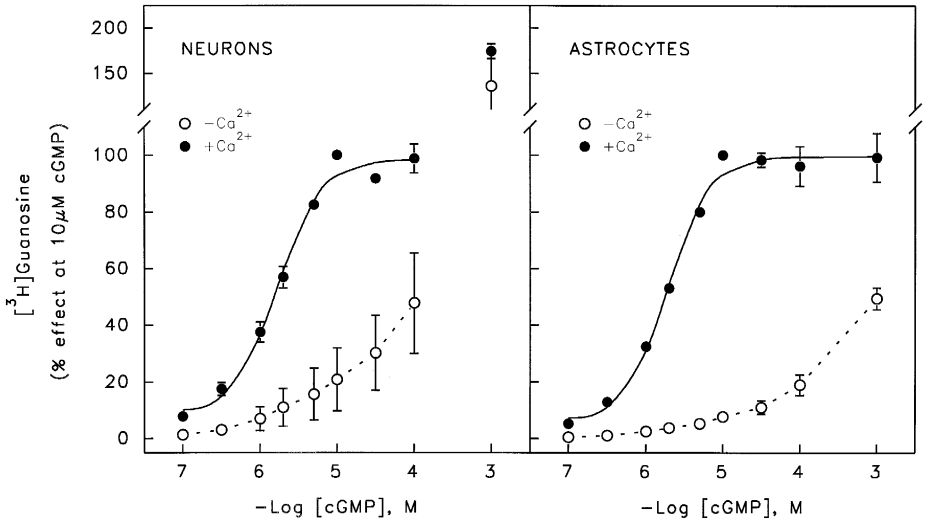

Fig. 1. Effect of increasing cGMP concentrations on phosphodiesterase activity in granule cell and astroglial cytosolic fractions. Phosphodiesterase activity in cytosols was determined by measuring [^{3}H]guanosine formation in the absence of Ca^{2+} (○) or in the presence of 25 μM free Ca^{2+} (●). Results are expressed as percentage of the activity at 10 μM cGMP in the presence of Ca^{2+} and are means ± range of two experiments performed in different culture preparations.

and IBMX, nicardipine, zaprinast and snake venom (*Crotalus atrox*) were from Sigma. Ro 20-1724 (4-[(3-butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone) was a gift from Hoffmann-La Roche.

3. Results

Phosphodiesterase activity was measured in 100 000 × *g* supernatants obtained from homogenates of primary cultures enriched in granule cells or in astrocytes from rat cerebellum, at 10 μM [^{3}H]cGMP and in the presence (25 μM) or absence (less than 1 nM) of free Ca^{2+}. As shown in Table 1, in the absence of Ca^{2+} activity was three times higher in neurons than in astrocytes. Addition of 25 μM Ca^{2+} increased phosphodiesterase activity about 6-fold in neurons and 15-fold in astrocytes. Thus, under this condition activities were of similar magnitude in both cell types. The non-selective phosphodiesterase inhibitor IBMX at 1

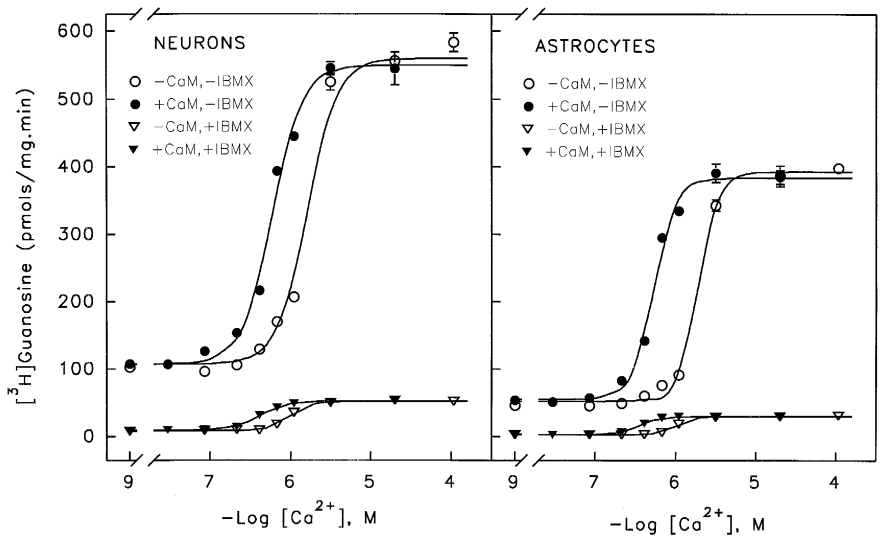

Fig. 2. Effect of increasing Ca^{2+} concentration on cGMP-phosphodiesterase activity in granule cell and astroglial cytosolic fractions. Cytosols were incubated with [^{3}H]cGMP (10 μM) in the presence of increasing concentrations of free Ca^{2+} with no added calmodulin (○, ▽) or with 1 μM calmodulin (●, ▼), in the presence (▽, ▼) or absence (○, ●) of 1 mM IBMX. Results are means ± S.D. of triplicate determinations in a representative experiment that was replicated 3 times in neurons and 2 times in astrocytes.

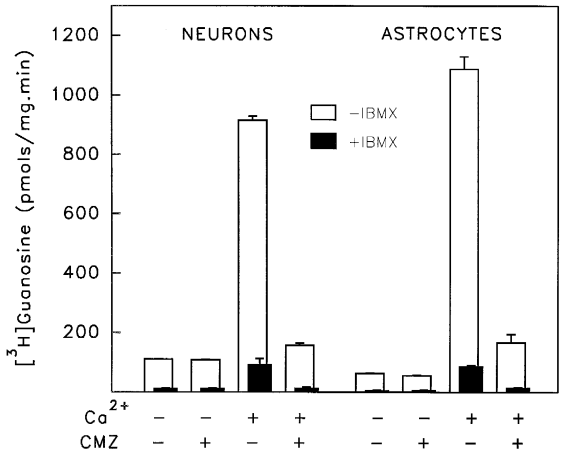

Fig. 3. Effect of calmidazolium on phosphodiesterase activity. Cytosols were incubated with [^{3}H]cGMP (10 μM) at 25 μM or <1 nM free Ca^{2+}, with or without 30 μM calmidazolium (CMZ) and in the presence (solid bars) or absence (open bars) of 1 mM IBMX. Results are means ± S.D. of triplicate determinations in a representative experiment.

Table 1
cGMP phosphodiesterase activity in cytosolic fractions of granule neurons or astroglial cells from rat cerebellum

	[^{3}H]Guanosine (pmol/mg protein·min)	
	$-Ca^{2+}$	$+Ca^{2+}$
Neurons		
−IBMX	221 ± 42 (7)	1317 ± 230 (7)
+IBMX	22 ± 4 (6)	115 ± 23 (6)
Astrocytes		
−IBMX	70 ± 13 (6)	1087 ± 271 (6)
+IBMX	7.3 ± 1.4 (6)	75 ± 16 (6)

Phosphodiesterase activity was determined incubating cytosolic fractions of neuronal or astroglial primary cultures (15–40 μg) with [^{3}H]cGMP (10 μM), in the absence of Ca^{2+} (less than 1 nM) or in the presence of 25 μM Ca^{2+}, with or without 1 mM IBMX. The product of the reaction, [^{3}H]GMP, was transformed into [^{3}H]guanosine that was separated by cation exchange chromatography and quantified as indicated in Section 2. Results are means ± S.E.M. of the number of experiments indicated in parentheses that were performed in triplicate in different culture preparations.

mM inhibited around 90% of the activity of both cell extracts whether Ca^{2+} was present or not (Table 1).

The dependence on cGMP concentration was different for the Ca^{2+}-dependent and independent activities (Fig. 1). In the presence of Ca^{2+}, phosphodiesterase activity in the range of 0.1–100 μM cGMP was saturable in both cell types (without subtracting the activity in the absence of Ca^{2+}) showing similar K_m values (1.72 ± 0.20 and 1.92

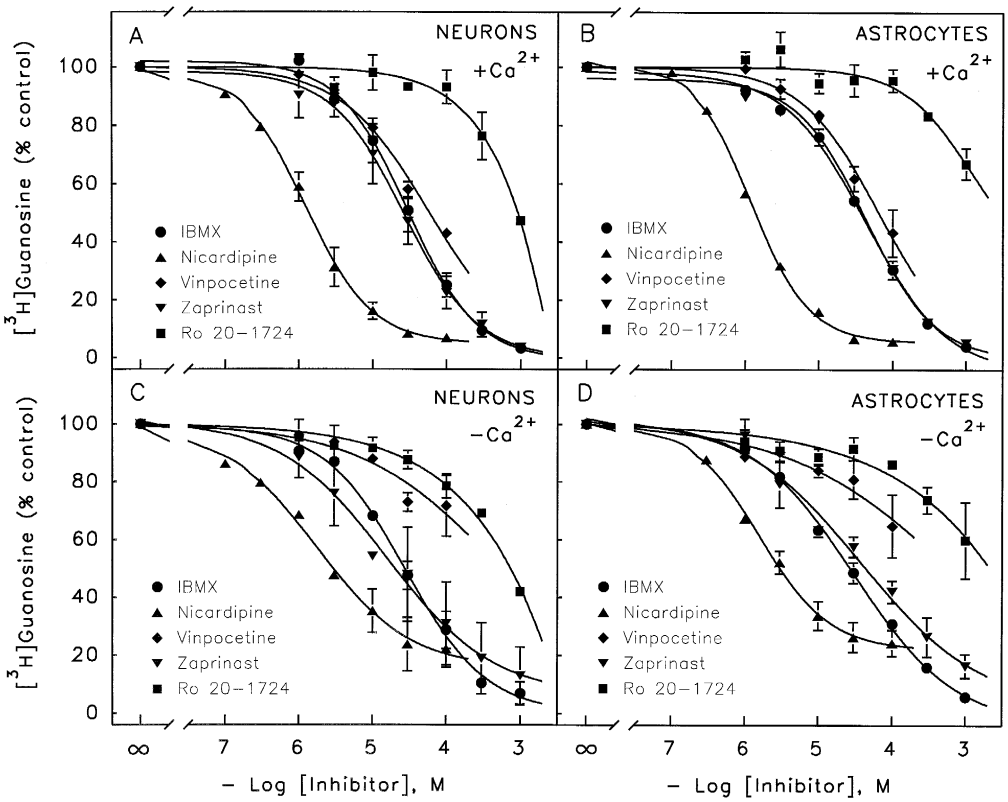

Fig. 4. Effect of phosphodiesterase inhibitors on cGMP-phosphodiesterase activity in granule cell (A,C) and astroglial (B,D) cytosolic fractions. Cytosols were incubated in the absence of Ca^{2+} (C,D) or in the presence of 25 μM Ca^{2+} (A,B) with increasing concentrations of IBMX (●), Ro 20-1724 (■), nicardipine (▲), vinpocetine (◆) or zaprinast (▼). Results, expressed as percentage of the activity in the absence of inhibitors, are means ± S.E.M. of three experiments for nicardipine or means ± range of two experiments for the other inhibitors.

$\pm$ 0.09 μM for neurons and astrocytes, respectively, $n = 2$). However, in the absence of Ca^{2+} phosphodiesterase activities increased with increasing concentrations of cGMP but did not reach saturation even at 1 mM (Fig. 1). At all cGMP concentrations used the Ca^{2+}-independent activity was 2–3-fold higher in neurons than in astrocytes but the difference became much larger at 1 mM. At this cGMP concentration there was a dramatic increase in the Ca^{2+}-independent activity of neurons which may also be responsible for the increase above saturation levels of the activity in the presence of Ca^{2+}.

As shown in Fig. 2, dependence on Ca^{2+} concentration was also similar for the phosphodiesterase activities of both cell types (EC_{50} values 1.26 $\pm$ 0.20, $n = 3$, and 1.50 $\pm$ 0.42 μM, $n = 2$, for neurons and astrocytes, respectively). Maximal stimulation was reached with an increase in Ca^{2+} concentration of less than an order of magnitude, indicating a highly co-operative effect, typical of Ca^{2+}/calmodulin-activated systems. Addition of 1 μM calmodulin had no effect at saturating Ca^{2+} concentrations, but increased the Ca^{2+} potency about 3–4-fold (EC_{50} values 0.38 $\pm$ 0.11, $n = 3$, and 0.39 $\pm$ 0.14 μM, $n = 2$, for granule cells and astrocytes, respectively) (Fig. 2). The calmodulin antagonist calmidazolium (30 μM) inhibited around 90% the phosphodiesterase activity in the presence of Ca^{2+} but not in its absence (Fig. 3). The Ca^{2+}/calmodulin dependency of the small response remaining in the presence of 1 mM IBMX was the same as in the absence of inhibitor (Figs. 2 and 3).

To further characterize the phosphodiesterase activities of neurons and astrocytes, the inhibitory potencies of a series of phosphodiesterase inhibitors were examined at 1 μM cGMP, in the presence and absence of Ca^{2+}. Very similar results were obtained in both cell types (Fig. 4, Table 2). The most potent inhibitor was the selective Ca^{2+}/calmodulin-phosphodiesterase inhibitor nicardipine (Matsushima et al., 1987). This compound almost completely eliminated the activity in the presence of Ca^{2+} but left 10–15% of the activity in the absence of the cation. Vinpocetine, another phosphodiesterase type 1-selective inhibitor, at 100 μM reduced the activity in the presence of Ca^{2+} around 60%, but only 30% in the absence (Fig. 4). Ro 20-1724, a selective phosphodiesterase type 4 (cAMP-specific phosphodiesterase) inhibitor, and zaprinast, selective for phosphodiesterase type 5 (cGMP-specific phosphodiesterase), showed inhibitory potencies compatible with phosphodiesterase type 1 inhibition. At 1 μM cGMP, zaprinast as well as the non-selective inhibitor IBMX at 1 mM totally inhibited the activity with and without Ca^{2+} (Fig. 4). The inhibitory potency of IBMX was the same in the presence and absence of Ca^{2+} in neurons and astrocytes (Table 2) and also in rat cerebellar cytosols (not shown).

4. Discussion

Results shown here demonstrate the existence of a Ca^{2+}/calmodulin-phosphodiesterase or phosphodiesterase type 1 of similar characteristics and levels of activity in cultured granule neurons and astrocytes from rat cerebellum. This Ca^{2+}-dependent activity appears to account for most of the cGMP hydrolytic activity in the cytosol of these cells, since at μM concentrations of cGMP the Ca^{2+}-independent activity is much lower. Ca^{2+}/calmodulin-phosphodiesterases are known to be the predominant physiological regulators of cyclic nucleotide degradation in brain (Kincaid et al., 1981a). A phosphodiesterase type 1 isoform of 75 kDa was recently localized by in situ hybridization in cerebellar granule cells (Yan and Beavo, 1994) but none of the phosphodiesterase type 1 isoforms has been reported to be present in astroglial cells. This is to our knowledge the first report on the presence of a phosphodiesterase type 1 activity in astrocytes.

The K_M values for cGMP obtained in both cell types were close to those reported for other Ca^{2+}/calmodulin-phosphodiesterases in different tissues and species (Shenolikar et al., 1985; Sharma and Kalra, 1994), but given the wide range of kinetic constants reported (Beavo, 1995) it is not possible to ascribe the activity in our cultures to a particular isoform within the phosphodi-

Table 2
Inhibitory potency of different phosphodiesterase inhibitors

	IC_{50} (μM)				
	IBMX	Nicardipine	Vinpocetine	Zaprinast	Ro 20-1724
Neurons					
$-Ca^{2+}$	28 $\pm$ 6	1.7 $\pm$ 0.4	200 $\pm$ 78 [a]	29 $\pm$ 17	866 $\pm$ 13 [a]
$+Ca^{2+}$	31 $\pm$ 10	1.3 $\pm$ 0.3	60 $\pm$ 7 [a]	28 $\pm$ 9	987 $\pm$ 72 [a]
Astrocytes					
$-Ca^{2+}$	31 $\pm$ 6	1.7 $\pm$ 0.2	150 $\pm$ 38 [a]	45 $\pm$ 4	> 1500 [a]
$+Ca^{2+}$	46 $\pm$ 6	1.2 $\pm$ 0.1	70 $\pm$ 24 [a]	44 $\pm$ 5	> 1500 [a]

Results shown are means $\pm$ range ($n = 2$) or means $\pm$ S.E.M. ($n = 3$) of the IC_{50} values estimated from individual inhibition curves of the experiments represented in Fig. 4. The inhibition curves were fitted to a Hill equation using a non-linear regression program. [a] IC_{50} values obtained setting maximal inhibition to 100% and Hill coefficient to 1.

esterase type 1 family. Ca^{2+} concentrations eliciting half-maximal activity (EC_{50}) at 1 μM calmodulin were lower than those described for 61 or 63 kDa phosphodiesterase type 1 isoforms purified from bovine brain (Sharma and Kalra, 1994). However, the Ca^{2+} potency obtained in this study is probably overestimated due to the presence of an unknown concentration of endogenous calmodulin in the cell extracts. The effect of calmodulin, increasing the Ca^{2+} potency but not the activity at saturating concentrations of Ca^{2+}, is analogous to that described for purified Ca^{2+}/calmodulin-phosphodiesterases (Sharma and Kalra, 1994). The EC_{50} values for Ca^{2+} found here in the presence of 1 μM calmodulin for phosphodiesterase activities in granule neurons and astrocytes are the same as those we reported for NO synthase type I activities in the same cells (Arbonés et al., 1996).

The inhibitory potencies of the different phosphodiesterase inhibitors assayed are also in agreement with data on other Ca^{2+}/calmodulin-phosphodiesterases described. Nicardipine and vinpocetine, selective phosphodiesterase type 1 inhibitors, show IC_{50} values in the presence of Ca^{2+} similar to those reported for purified Ca^{2+}/calmodulin-phosphodiesterases (Matsushima et al., 1987; Saeki and Saito, 1993). In the absence of Ca^{2+}, the inhibitory potency of vinpocetine is decreased. Different sensitivity to selective inhibitors in the presence or absence of Ca^{2+} has been described for some isoforms of phosphodiesterase type 1 (Matsushima et al., 1987). Zaprinast and Ro 20-1724, selective phosphodiesterase type 5 and phosphodiesterase type 4 inhibitors, respectively, inhibit the activities of neurons and astrocytes at much higher concentrations than those required to inhibit their specific isoforms (Lugnier et al., 1986; Saeki and Saito, 1993). These two compounds are more potent in neurones, cells that show more Ca^{2+}-independent phosphodiesterase activity than astrocytes.

The phosphodiesterase activity observed in the absence of Ca^{2+} could be due to the presence in the cell extracts of other phosphodiesterase isoforms different from phosphodiesterase type 1 specially at cGMP concentrations higher than 10 μM. However, at 1 μM cGMP, the activity in the absence of Ca^{2+} is strongly inhibited in both cell types by the highly selective phosphodiesterase type 1 inhibitor nicardipine. Moreover, the inhibitors of other phosphodiesterases, zaprinast and Ro 20-1724, inhibit the Ca^{2+}-independent activity with IC_{50} values compatible with phosphodiesterase type 1 inhibition and not with inhibition of their respective phosphodiesterases. Thus it seems that the major part of the Ca^{2+}-independent activity measured has pharmacological characteristics of a phosphodiesterase type 1 activity. Basal or Ca^{2+}-independent activity has been described extensively for different purified phosphodiesterase type 1 isoforms (Kincaid et al., 1981a, 1984; Sharma et al., 1984; Shenolikar et al., 1985). Tucker et al. (1981) suggested that this was a result of enzyme degradation, the product becoming totally active even in the absence of Ca^{2+}. This does not seem to be our case since it was not necessary to subtract Ca^{2+}-independent activities in order to reach saturation in the presence of Ca^{2+}. Other authors have reported the existence of an equilibrium between monomers that show activity in the absence of Ca^{2+}/calmodulin and dimers that need association with Ca^{2+}/calmodulin to reach a substantial activity (Kincaid et al., 1981a,b). Our results could agree with the presence of an equilibrium between monomers and dimers where monomers would have an increased affinity for cGMP when Ca^{2+} is present.

Increases in Ca^{2+} were reported to decrease cGMP levels produced by NO donor stimulation of soluble guanylyl cyclase in brain extracts in the presence of the non-selective phosphodiesterase inhibitor IBMX (Knowles et al., 1989), suggesting that Ca^{2+} was inhibiting soluble guanylyl cyclase. However, Mayer et al. (1992) demonstrated that purified synaptosomal guanylyl cyclase was not affected by Ca^{2+}, but that cGMP hydrolysis was highly stimulated by the ion in cytosolic fractions. IBMX at 1 mM was apparently ineffective in blocking cGMP breakdown in the presence of Ca^{2+} leading the authors to suggest that the phosphodiesterase involved was insensitive to IBMX. Our results are in contrast with this suggestion. We show here that 1 mM IBMX can totally inhibit the Ca^{2+}/calmodulin-phosphodiesterase activity of both granule cell and astroglial cytosols assayed at 1 μM cGMP and that the inhibitory potency of IBMX is close to that reported for rat cerebrum phosphodiesterase type 1 (Nicholson et al., 1989). However, as shown in Table 1, at 10 μM cGMP, concentration used by Mayer et al. (1992), 1 mM IBMX leaves uninhibited about 10% of the activity both in the presence and absence of Ca^{2+}, thus relative stimulation by Ca^{2+} is similar whether IBMX is present or not. Since the Ca^{2+}/calmodulin-phosphodiesterase activity in brain extracts is very high it is possible that this remaining activity may account for the apparently IBMX-insensitive phosphodiesterase type 1 activity suggested by Mayer et al. (1992).

Recent results from our laboratory show that agents that increase intracellular Ca^{2+} and stimulate NO formation in cerebellar granule cells and astrocytes in culture are able to inhibit cGMP accumulation induced by NO donors in the same cells (Baltrons et al., submitted). These data are explained by the present results showing the presence of a similar Ca^{2+}/calmodulin-phosphodiesterase activity in both cell types. Thus, in a single cell increases in Ca^{2+} concentration induced by agonists would stimulate cGMP formation by activation of NO synthase type I and cGMP degradation by activation of phosphodiesterase type 1. This can be an important mechanism to control cGMP levels in the cells where NO is generated and would be in agreement with previous data showing that cGMP increments due to agonist-induced NO formation occur in cells different from those where NO synthase is activated (Garthwaite and Garthwaite, 1987; De Vente et al., 1990).

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