Effects of selective phosphodiesterase inhibition on cyclic AMP hydrolysis in rat cerebral cortical slices

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- 1 The effects of selective inhibition of phosphodiesterase activities on the concentration and rate of hydrolysis of adenosine 3':5' cyclic-monophosphate (cyclic AMP) in rat cerebral cortical slices has been studied.
- 2 Isoprenaline caused a rapid, concentration-dependent increase in cyclic AMP concentration to new steady-state levels (basal: 7.1 ± 0.7 ; $10 \mu\text{M}$ isoprenaline: $14.3 \pm 1.4 \text{ pmol mg}^{-1} \text{ protein}$). Addition of a β -adrenoceptor antagonist to isoprenaline-stimulated cerebral cortical slices caused a rapid decrease in cyclic AMP concentration to basal levels ($t_{1/2}$: $58 \pm 18 \text{ s}$).
- 3 Preincubation of slices for 30 min with the phosphodiesterase inhibitors 1-methyl-3-isobutylxanthine, denbufylline, rolipram or Ro20 1724 caused concentration-dependent increases in basal and isoprenaline-stimulated cyclic AMP concentrations and decreased the rate of cyclic AMP hydrolysis measured after addition of a β -adrenoceptor antagonist. However, SKF 94120 and zaprinast had none of these effects.
- 4 The results are discussed with respect to previous studies of phosphodiesterase isozymic activities isolated from cerebrum and it is suggested that the Ca^{2+} /calmodulin-independent, low K_m cyclic AMP phosphodiesterase isozyme, which is selectively inhibited by denbufylline, rolipram and Ro20 1724, and is present in cerebrum is of critical importance to the regulation of cyclic AMP concentration in this tissue.

Introduction

Synthesis of the intracellular second messenger adenosine 3':5' cyclic-monophosphate (cyclic AMP) occurs exclusively by the activity of adenylate cyclase in all cell-types examined. The concentration of cyclic AMP achieved by agonist-mediated stimulation of the receptor-cyclase complex (Rodbell, 1980) is influenced by the rate of cyclic AMP hydrolysis (Barber & Butcher, 1980) by cellular cyclic nucleotide phosphodiesterase (PDE) activities (Strada *et al.*, 1984). Therefore, PDE is intimately involved in determining the intensity and duration of the responsiveness of cells to hormones and neurotransmitters acting via the adenylate cyclase system.

Our understanding of the mechanisms and roles for cellular regulation of PDE activities has lagged far behind that for receptor-mediated cyclic AMP production by adenylate cyclase and is heavily reliant upon *in vitro* studies of enzymes isolated from specific tissues. This is due to the multiplicity of PDE activities found in most cell-types which can be classified according to Ca^{2+} -dependence, relative substrate specificity for cyclic AMP and cyclic GMP and sensitivity to allosteric modulation by cyclic GMP (Thompson & Appleman, 1971; Strada *et al.*, 1984; Weishaar *et al.*, 1985; Beavo, 1988). Some inhibitors of PDE activities, such as the methylxanthines (e.g. isobutylmethylxanthine, IBMX) show little isoenzymic selectivity (Nicholson *et al.*, 1989), however, potent selective PDE inhibitors have been developed. Thus, Ca^{2+} /calmodulin-dependent PDEs are inhibited by zaprinast (Prigent *et al.*, 1988), the Ca^{2+} /calmodulin-independent, low K_m PDE sensitive to cyclic GMP inhibition is inhibited by agents such as amrinone, milrinone and SKF 94120 (Harrison *et al.*, 1986; Reeves *et al.*, 1987) and the Ca^{2+} /calmodulin-independent, low K_m PDE insensitive to cyclic GMP inhibition is inhibited by Ro 20 1724, rolipram and denbufylline (Reeves *et al.*, 1987; Nicholson *et al.*, 1989; Wilke *et al.*, 1989).

As has been emphasised by other workers, the biochemical properties of PDE isoenzymes isolated from tissues may be influenced by the procedure employed (Wells & Hardman,

1977), therefore the determination of the relative importance of the PDE isoforms responsible for cyclic AMP hydrolysis within intact tissue is crucial. In this study, the role of PDE activities in rat cerebral cortex has been investigated in cerebral cortical slices *in situ* using selective and non-selective inhibitors of the various PDE isoenzymes. Cyclic AMP concentration has been elevated to new steady-state levels by addition of a β -adrenoceptor agonist and the rate of decrease in cyclic AMP concentration to pre-stimulation levels, upon addition of a β -adrenoceptor antagonist, has been followed in the presence or absence of PDE inhibitors. A similar approach was used previously (Donaldson *et al.*, 1988) to investigate the effect of selective PDE inhibition by rolipram on rates of cyclic AMP hydrolysis in guinea-pig cerebral cortex slices following stimulation by histamine and adenosine. Using this novel assay system the effects of PDE inhibitors on cyclic AMP hydrolysis within the tissue can be assessed.

Methods

Incubation technique

Cross-chopped cerebral cortical slices ($0.3 \times 0.3 \text{ mm}$) were prepared from male Wistar rats (200–300 g). Slices were washed extensively with modified Krebs–Ringer buffer (KRB: mmol l^{-1} : NaCl 118, KCl 4.7, CaCl_2 1.3, KH_2PO_4 1.2, MgSO_4 1.2, NaHCO_3 25, HEPES 5, glucose 10, pH 7.4) saturated with 95% O_2 :5% CO_2 and incubated in 25 ml KRB at 37°C for 60 min in a oscillating waterbath. Every 15–20 min the slices were allowed to sediment and the buffer replaced. The medium was then replaced by 25 ml KRB containing various concentrations of PDE inhibitors. Incubation was continued for 30 min after which slices were sedimented, the supernatant removed, and aliquots of packed slices ($30\text{--}50 \mu\text{l}$) added to identical medium ($300 \mu\text{l}$). Additions of the β -adrenoceptor agonist isoprenaline were made at this point. Incubations were gassed with 95% O_2 :5% CO_2 , capped and incubated with gentle vortex shaking in a heated block at 37°C for appropriate time-periods. Where the rate of cyclic AMP hydrolysis was studied, the β -adrenoceptor antagonist

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Table 1 Basal and isoprenaline-stimulated cyclic AMP concentration and EC_{50} values for isoprenaline-stimulated cyclic AMP accumulation in cerebral cortical slices in the presence of various phosphodiesterase (PDE) inhibitors

PDE inhibitor concentration (μM)	Cyclic AMP concentration (pmol mg^{-1} protein)		EC_{50} isoprenaline (nM)
	Basal	+ isoprenaline $10 \mu\text{M}$	
Control	7.1 ± 0.7	14.3 ± 1.4 (7)	210 ± 41 (4)
Rolipram	1	17.9 ± 1.0	ND
	10	27.8 ± 2.5	265 ± 53 (4)
	100	39.0 ± 2.7	ND
Ro 20 1724	1	12.0 ± 2.3	186 ± 35 (4)
	10	23.9 ± 2.8	79 ± 20 (3)*
	100	25.2 ± 4.3	70 ± 21 (3)*
Denbutylline	1	15.6 ± 1.5	179 ± 51 (4)
	10	25.8 ± 2.0	149 ± 50 (4)
	100	29.3 ± 2.4	164 ± 58 (3)
IBMX	10	9.0 ± 1.5	ND
	100	17.3 ± 1.9	ND
	1000	27.6 ± 2.2	217 ± 15 (3)
8- ϕ -Theo	100	12.8 ± 1.7	ND
Zaprinast	100	7.3 ± 0.3	255 ± 74 (3)
SKF 94120	100	8.6 ± 1.8	284 ± 83 (3)

Values are presented as means $\pm$ s.e. for the number of separate experiments performed in triplicate indicated above. EC_{50} values were determined as described in the Methods section. Statistical significance (Student's *t* test) is indicated as * $P < 0.05$. IBMX is isobutylmethylxanthine; 8- ϕ -Theo is 8-phenyltheophylline. ND is not determined.

timolol was added to a final concentration of $20 \mu\text{M}$ at a time when an agonist-stimulated steady-state concentration of cyclic AMP had been achieved, the incubation rapidly gassed and incubation at 37°C continued for the appropriate length of time.

Incubations were terminated by addition of $20 \mu\text{l}$ concentrated HCl and immediately placed on ice. After 20 min, samples were neutralized by addition of 1 M NaOH and centrifuged at $2000 g$ for 15 min at 4°C . Cyclic AMP concentration was measured in $10\text{--}50 \mu\text{l}$ aliquots of supernatant using the competitive protein binding assay described by Brown *et al.* (1971). NaOH was added to the slice residues to a concentration of 1 M and after complete tissue disruption protein concentration was measured in each sample by the method of Lowry *et al.* (1951).

Analysis of data

The concentration of isoprenaline producing half-maximal stimulations of cyclic AMP accumulation (EC_{50}) was obtained by subjecting the concentration-response curves to a four-parameter logistic model using computer-assisted iterative curve-fitting (ALLFIT; Delean *et al.*, 1978). Statistical significance was determined by Student's *t* test.

Chemicals

[2,8- ^3H]-cyclic 3':5' adenosine monophosphate was purchased from NEN Products, Du Pont (U.K.) Ltd. Isoprenaline bitartrate, cyclic AMP, 8-phenyltheophylline and 1-methyl-3-isobutylxanthine (IBMX) were purchased from Sigma. The following compounds were obtained as gifts: Denbutylline (BRL 30892—1,3-di-*n*-butyl-7-(2'-oxopropyl)xanthine; Beecham-Wulff, Gronau (Leine), F.R.G.), Rolipram (ZK 62711—4-(3'-cyclopentyl-4'-methoxy-phenyl)-2-pyrrolidone; Schering Health Care Ltd., Burgess Hill, W. Sussex), Ro 20 1724 (d-4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; Roche Products Ltd., Welwyn, Herts), SKF 94120 (5-(4-acetamidophenyl) pyrazin-2(1H)-one; Smith Kline and French Research Ltd., Welwyn, Herts), zaprinast (MB22948—2-o-propoxyphenyl-8-azapurin-6-one; May and Baker Ltd., Dagenham, Essex) and timolol maleate (Leo Laboratories, Hayes, Middlesex).

Results

Isoprenaline-stimulated cyclic AMP accumulation

Incubation of cerebral cortical slices with isoprenaline ($10 \mu\text{M}$) resulted in a 2–3 fold increase in cyclic AMP concentration which reached a maintained new steady-state level within 5 min of agonist addition. Pre-incubation with rolipram, Ro 20 1724, denbutylline or IBMX for 30 min had a concentration-dependent effect on basal cyclic AMP levels (Table 1) and addition of isoprenaline ($10 \mu\text{M}$) resulted in a 2–4 fold increase in cyclic AMP concentration (Table 1) which was maximal within 15 min of isoprenaline addition. In all cases the effect of PDE inhibition was maximal within the 30 min pre-incubation period (illustrated for denbutylline in Figure 2). 8-Phenyltheophylline exerted a small, but significant effect in elevating basal and isoprenaline-stimulated cyclic AMP levels, whereas SKF94120 and zaprinast were ineffective at concentrations ($100 \mu\text{M}$) that have been shown to be at least 5 times greater than the IC_{50} values for these inhibitors in isolated enzyme preparations (Reeves *et al.*, 1987; Prigent *et al.*, 1988).

Incubation of slices with various concentrations of isoprenaline, in the presence and absence of PDE inhibition, produced concentration-dependent increases in cyclic AMP levels. In the absence of PDE inhibition the EC_{50} value for isoprenaline-stimulated cyclic AMP accumulation was $210 \pm 41 \text{ nM}$ (Table 1). Increasing concentrations of denbutylline ($1\text{--}100 \mu\text{M}$) increased basal and isoprenaline-stimulated cyclic AMP accumulation without affecting the EC_{50} (Table 1). Rolipram, Ro 20 1724 and IBMX had qualitatively similar effects (Table 1), however, Ro 20 1724 was unique in that, at higher concentrations, it also modified the EC_{50} value for isoprenaline stimulation of cyclic AMP accumulation (Table 1).

The effects of denbutylline and IBMX concentration on basal and isoprenaline-stimulated cyclic AMP accumulation are illustrated in Figure 1. It can be seen that denbutylline was approximately 100 fold more potent than IBMX in elevating basal and isoprenaline-stimulated cyclic AMP accumulation.

Effects of phosphodiesterase inhibition on rates of cyclic AMP hydrolysis

The effect of addition of the β -adrenoceptor antagonist timolol (at a concentration of $20 \mu\text{M}$, a value 2000 times greater than its K_i for β -adrenoceptor antagonism in rat cere-

Figure 1 Effect of denbufylline and isobutylmethylxanthine (IBMX) on cyclic AMP accumulation in cerebral cortex slices. Each data point represents mean of at least 3 separate experiments performed in triplicate; s.e.mean shown by vertical bars. Denbufylline (○), denbufylline, isoprenaline $10 \mu\text{M}$ (●); IBMX (□), IBMX plus isoprenaline $10 \mu\text{M}$ (■).

bral cortex; Dickinson & Nahorski, 1981) on isoprenaline-stimulated cyclic AMP concentration was studied. The experimental protocol is illustrated in Figure 2 for a single experiment performed in the presence of denbufylline ($10 \mu\text{M}$).

Data for experiments performed in the absence of PDE

Figure 2 Effect of denbufylline on the rate of cyclic AMP hydrolysis. Cerebral cortex slices were pre-incubated for 30 min with denbufylline $10 \mu\text{M}$ (○). At zero-time, isoprenaline $10 \mu\text{M}$ was added to incubations of slices and cyclic AMP accumulation followed (●). After 20 min timolol $20 \mu\text{M}$ was added and cyclic AMP hydrolysis followed (□). Data are shown for a single experiment performed in triplicate.

inhibition are shown in Figure 3d. Upon addition of timolol to slices (which had been incubated with isoprenaline ($10 \mu\text{M}$) for 10 min to achieve a new elevated steady-state cyclic AMP concentration), the cyclic AMP concentration decreased rapidly towards pre-stimulation levels. Inclusion of zaprinast or SKF 94120 at a concentration of $100 \mu\text{M}$ had no detectable effect on the rate of decrease in cyclic AMP concentration

Figure 3 Rate of cyclic AMP hydrolysis in cerebral cortex slices in the presence of PDE inhibitors. Each decay-curve represents means for 3 to 5 separate experiments performed in triplicate; s.e.mean shown by vertical bars (a) Denbufylline: 1 (○), 10 (□) or 100 (■) μM ; (b) rolipram: 1 (○), 10 (●) or 100 (■) μM ; (c) Ro 20 1724: 1 (△), 10 (▲) or 100 (▲) μM ; (d) isobutylmethylxanthine: 100 (◇) or 1000 (◆) μM . Control data for no PDE inhibitor addition are shown in (d) (▼).

after antagonist addition. The effects of PDE inhibition by rolipram, denbufylline, Ro 20 1724 or IBMX on the rate of cyclic AMP hydrolysis upon timolol addition to cerebral cortical slice incubations are shown in Figures 3a–d. Increasing concentrations of PDE inhibitors prolonged the time required for cyclic AMP to return to pre-stimulation values. The half-recovery times, derived from the data presented in Figure 3, are shown in Figure 4. Both denbufylline and rolipram exerted significant effects on the rate of cyclic AMP hydrolysis at concentrations as low as $1 \mu\text{M}$ and the effects of these agents on cyclic AMP hydrolysis appeared to reach maxima at $100 \mu\text{M}$. At this concentration, a 6–7 fold decrease in the rate of cyclic AMP hydrolysis was observed. Ro 20 1724 inhibition of PDE activity appeared to reach the same maximal effect, however, this agent was approximately 10 fold less potent in exerting this action (Figure 4). At the maximum tested concentration (1 mM), IBMX caused a 5 fold decrease in the rate of cyclic AMP hydrolysis. IBMX was at least 100 times less effective compared to denbufylline or rolipram, and 10 times less effective compared to Ro 20 1724 in exerting this effect on cyclic AMP metabolism.

Discussion

Selective inhibition of PDE activities may lead to a variety of discrete pharmacological responses, and considerable interest has focussed on the development of potent, selective inhibitors of PDE isoforms which might allow discrete control of various biological processes (Weishaar *et al.*, 1985). As in many other mammalian tissues, multiple forms of PDE are present in the central nervous system (Thompson & Appleman, 1971; Strada *et al.*, 1984; Kincaid *et al.*, 1984), which in rat cerebral cortical extracts can be resolved into three fractions (Nicholson *et al.*, 1989). Fractions I and II are Ca^{2+} /calmodulin-dependent and have a low affinity for cyclic AMP; hydrolysis of cyclic AMP by fraction II is stimulated by cyclic GMP. Fraction III is Ca^{2+} /calmodulin-independent and has a high affinity for cyclic AMP. Cerebral PDE activities thus have somewhat different regulatory characteristics to the isoenzymes present in other tissues such as heart (Reeves *et al.*, 1987; Weishaar *et al.*, 1987; Silver *et al.*, 1988; Nicholson *et al.*, 1989).

In tissue homogenates, the non-selective PDE inhibitor IBMX produces a concentration-dependent inhibition of cyclic AMP hydrolysis by total cyclic nucleotide PDE activity (Nicholson & Wilke, 1987; Wilke *et al.*, 1989), with the IC_{50} of IBMX for inhibition of total tissue cyclic nucleotide PDE activity being very similar to its IC_{50} for inhibition of the separate PDE isoenzymes (Davis, 1984; Wilke *et al.*, 1989). On

the other hand, denbufylline, which selectively inhibits cerebral Ca^{2+} -independent, low K_m cyclic AMP PDE (Nicholson *et al.*, 1989) is a relatively ineffective inhibitor of cyclic AMP hydrolysis by total cyclic nucleotide PDE activity in cerebral homogenates (Nicholson & Wilke, 1987). Concentrations 100 fold in excess of the K_i for denbufylline inhibition of the low K_m cyclic AMP PDE ($0.9 \mu\text{M}$; Wilke *et al.*, 1989) produce less than a 50% inhibition of total cyclic nucleotide PDE activity in cerebral homogenates (Nicholson *et al.*, 1989). The relative inability of denbufylline to inhibit PDE activity in cerebral homogenates can be most plausibly explained by the known predominance of Ca^{2+} /calmodulin-dependent PDE in this tissue (Kincaid *et al.*, 1984; Strada *et al.*, 1984).

In the present study, we have investigated the effects of PDE inhibitors on agonist-stimulated cyclic AMP concentrations and the kinetics of cyclic AMP hydrolysis in cerebral cortical slices and in this way gain information about the contributions of PDE isoenzymes to cyclic AMP turnover in a cellular system. Using this approach we have shown that inhibition of the Ca^{2+} /calmodulin-independent isoenzyme, using the selective inhibitors denbufylline, rolipram or Ro 20 1724, can elevate basal cyclic AMP levels and potentiate the effects of β -adrenoceptor stimulation, whereas the potent Ca^{2+} /calmodulin-dependent PDE inhibitor, zaprinast, had no effect. Furthermore, the assay used in the present study showed that the action of denbufylline and rolipram was indeed due to PDE inhibition as both inhibitors decreased the rate of cyclic AMP hydrolysis in cortical slices, an effect only attributable to PDE inhibition. Therefore, selective inhibition of the low K_m cyclic AMP PDE in cerebral cortex is sufficient to elevate cellular cyclic AMP concentration. Ro 20 1724 which selectively inhibits the same isoenzyme as rolipram (Davis, 1984; Weishaar *et al.*, 1987), although somewhat less potently, showed similar activity, but diminished potency, in comparison to rolipram, in the assay used in the present study.

IBMX is approximately 20–25 fold less effective than rolipram and denbufylline as an inhibitor of the Ca^{2+} /calmodulin independent, low K_m cyclic AMP PDE (Davis, 1984; Nicholson *et al.*, 1989). This is less than the 100 fold potency difference between IBMX and denbufylline and rolipram in this study. The reason for this discrepancy is unclear at present but is unlikely to be due to differences in the relative abilities of these compounds to access their sites of action in the tissue slice preparation, as for all agents investigated the maximal effect on elevation of basal cyclic AMP was observed within 15 min of PDE inhibitor addition (see for example Figure 2). The relative inability of IBMX and zaprinast to change cortical cyclic AMP levels and to reduce the rate of breakdown of cyclic AMP in the cerebrum suggests that the Ca^{2+} /calmodulin-dependent, high K_m cyclic AMP PDEs play little role in controlling cellular cyclic AMP levels in the rat cortex, at least under the conditions used in the present study. This is surprising as although these PDEs have a low affinity for cyclic AMP they are of high maximal activity. The Ca^{2+} /calmodulin-dependent PDE activity in cerebrum has been shown to be activated by Ca^{2+} and inhibited by K^+ (Davis, 1984), therefore it is possible that under conditions where *in vivo* cellular Ca^{2+} and K^+ concentrations are maintained, the activity of this PDE isoform is low.

The Ca^{2+} /calmodulin independent, low K_m cyclic AMP PDE which is selectively inhibited by cyclic GMP and by inotropic agents such as SKF 94120 (Gristwood *et al.*, 1986; Reeves *et al.*, 1987), milrinone and imazodan (Harrison *et al.*, 1986; Weishaar *et al.*, 1987) appears to be absent (or present at a very low activity) in rat cerebrum (Kariya & Dage, 1988; Nicholson *et al.*, 1989). The inability of SKF 94120 to elevate basal or isoprenaline-stimulated cyclic AMP levels in the present study confirms the lack of the SKF 94120 sensitive isoenzyme in rat cerebral cortex.

The results of the present study demonstrate that although PDE IV forms only a minor part of the total cyclic nucleotide PDE pool (Kincaid *et al.*, 1984; Nicholson & Wilke, 1987), it may play a predominant role in controlling the rate of cyclic

Figure 4 Concentration-response curves for the effect of various phosphodiesterase (PDE) inhibitors on the rate of cyclic AMP hydrolysis. Each data point represents mean for half-recovery times estimated in at least 3 experiments performed in triplicate (see Figure 3); s.e.mean shown by vertical bars. Half-recovery time in the absence of PDE inhibition (∇). Denbufylline ($\blacksquare$), rolipram ($\bullet$), Ro 20 1724 ($\blacktriangle$), isobutylmethylxanthine ($\blacklozenge$).

AMP hydrolysis in rat cerebral cortex. Our data extend the work of Hill and colleagues (Donaldson *et al.*, 1988; Stanley *et al.*, 1989), who have shown that inhibition of PDE IV potentiates the effect of histamine on cyclic AMP accumulation in guinea-pig cortical and hippocampal slices. The significance of these results is illustrated by the fact that, when assayed in rat cerebral cortex homogenates, inhibitors of PDE IV are relatively ineffective as inhibitors of cyclic AMP hydrolysis (Nicholson & Wilke, 1987). These results obtained for cerebral cortex homogenates would appear to indicate that inhibitors of PDE IV should neither elevate cerebral cyclic AMP levels, nor significantly influence the rate of cyclic AMP hydrolysis in response to agonist stimulation. In direct contrast, this study has shown that inhibitors of PDE IV are very effective modulators of cyclic AMP turnover, indicating that data obtained in assays measuring PDE enzyme activity can only be extrapolated with caution when estimating the functional importance of PDE isoenzymes in modulating tissue levels of cyclic nucleotides.

8-Phenyltheophylline is normally regarded as a selective adenosine receptor antagonist with little ability to inhibit PDE (Smellie *et al.*, 1979; Bruns, 1981). However, the compound has more recently been shown to be effective as a PDE inhibitor in tissues in which denbufylline is particularly active (Nicholson & Wilke, 1989), suggesting that 8-phenyltheophylline may selectively inhibit the same isoenzyme as denbufylline. Thus, PDE inhibition rather than adenosine receptor antagonism may explain why this compound elevates cyclic AMP levels in the rat cerebral cortex slice preparation. If adenosine antagonism were to elevate cyclic AMP levels,

then basal adenosine release must be reducing cyclic AMP levels via adenosine A₁ receptors and inhibition of adenylate cyclase in rat cortical slices. Two pieces of evidence argue against this however; first, adenosine deaminase has no effect on basal cyclic AMP levels, and second, IBMX, which is both a non-selective PDE inhibitor and an adenosine receptor antagonist (Daly, 1982; Weishaar *et al.*, 1985; Prigent *et al.*, 1988), was not a very effective elevator of cyclic AMP levels and was effective in the same concentration-range in increasing basal and β -adrenoceptor-stimulated cyclic AMP levels and in reducing the rate of cyclic AMP breakdown.

Denbufylline has utility in the treatment of dementia (O'Connolly *et al.*, 1986) whilst rolipram is an antidepressant (Eckmann *et al.*, 1988). The elevation of cerebral cortical cyclic AMP levels may contribute to the clinical usefulness of these compounds. Interestingly, the central effects of denbufylline and rolipram are not accompanied by marked cardiovascular effects (Angersbach & Ochlich, 1984; Weishaar *et al.*, 1987).

In conclusion, the present study has characterized a model for the evaluation of cyclic nucleotide PDE inhibitors in a cellular system. It has been demonstrated that the Ca²⁺/calmodulin-independent, low K_m cyclic AMP PDE appears to have a predominant functional role in cyclic AMP turnover in rat cerebral cortex under the conditions used in these experiments.

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