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Cerebral cortex
fetus
phosphodiesterase

Cyclic Nucleotide Phosphodiesterase Activities of the Fetal and Mature Human Cerebral Cortex

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Summary

Phosphodiesterase activities were examined in the supernatant and pellet fractions of a 30,000 × *g* preparation of brain tissues from human fetuses and young adults. Differences in total activity and distribution of the high and low K_m activity enzymes for adenosine and guanosine 3′,5′-monophosphate (cyclic AMP and cyclic GMP, respectively) were found. The mature cortex had 10 times more activity than the fetal brain for cyclic AMP hydrolysis and 15–20 times more activity for cyclic GMP hydrolysis. In the fetus, more activity for both nucleotides at both high and low concentrations is associated with the supernatant fraction. With maturity, a shift in localization of high K_m activity for cyclic GMP and low K_m activities for both nucleotides to the particulate fraction is observed.

Michaelis constants for both mature and immature brains are similar. The K_m values for cyclic AMP are 10^{-4} and 10^{-5} M and 10^{-4} and 10^{-6} M for cyclic GMP. The V_{max} values differed by a factor of 10 between the high K_m and the low K_m forms for each substrate.

Differences were observed between the fetus and the adult when the hydrolysis of one nucleotide was measured in the presence of varying amounts of the other nucleotide. Low concentrations of either nucleotide stimulated the hydrolysis of low concentrations of the other in the adult whereas, in the fetus, low concentrations of cyclic AMP inhibited cyclic GMP hydrolysis. At higher concentrations of either nucleotide, the addition of the other over a wide range of concentrations resulted in inhibition which was exaggerated in the fetus.

Speculation

Differences in responsiveness to modulators observed between fetal and adult enzymes suggest the possibility that these enzymes occur as isozymes. Substantiation of this possibility will require the separation of these enzymes in pure form.

Considerable evidence exists which suggests that the two naturally occurring cyclic nucleotides, cyclic AMP and cyclic GMP, are extensively involved in the regulation of the development and function of the nervous system. For instance, in model systems, (*1*) the induction of enzymes involved in neurotransmission has been demonstrated for the adenosine nucleotide (9, 23, 24, 26, 30), (*2*) control of growth and differentiation of neuronal tissue appear to be highly responsive to changes of the intracellular concentrations of cyclic AMP (29, 30, 45), and (*3*) the putative neurotransmitters elicit changes in the concentrations of the cyclic nucleotides during neurotransmission as determined by iontophoretic studies in both central and peripheral preparations (15, 20). In addition, evidence is also accumulating that cyclic AMP and cyclic GMP facilitate opposing effects in a number of tissues including nervous tissue (13, 38).

The concentration of these cyclic nucleotides in tissues are determined in part by the rate of their hydrolysis by specific phosphodiesterases. Under a common stimulus, the intracellular metabolism of responsive cells could vary widely depending on the functional status of the several forms of phosphodiesterase (PDE) which have been described in mammalian tissues.

In animals, developmental studies by Schmidt *et al.* (35) and Weiss (46) showed the presence of PDE in the brain of the newborn rat which increased to maturity levels by 15–23 days. Approximately 50% of the total PDE activity was associated with the particulate fraction according to the data of Weiss and Costa (47), whereas Schmidt and his associates found that the majority of the activity was located in the supernatant at a ratio of 4:1 for the particulate fractions at all ages studied. These studies were performed under experimental conditions which fail to account for the multiple forms of PDE since substrate was limited to cyclic AMP at concentrations of 10^{-5} and 10^{-4} M. Strada *et al.* (39) reinvestigated the postnatal development of this enzyme in the rat using physical separation methods in conjunction with kinetic analyses. The large total postnatal increase seen in the rat cerebrum was found to be due primarily to a 6-fold increase in the activity of the high K_m enzyme. The low K_m enzyme increased about 2.5-fold in activity during the same period.

It should be emphasized that age-function studies of the brain have been limited to the newborn and 60–100-day-old rat. Since maximum alterations in adaptive responsiveness to a variety of stimuli are observed between fetal and mature organs, the activities of the kinetically definable forms of PDE were examined in the cerebral cortices of the human fetus and adult. In addition, the effect of a variety of modulators of PDE activity was examined in the mature cortex and, in some instances, in the immature cortex.

MATERIALS AND METHODS

CLINICAL MATERIALS

Tissues for enzyme studies were collected at autopsy and kept frozen at −20° or −80° until analysis. Pertinent clinical features and the time elapsing between death or the expulsion of the fetus and the beginning of the postmortem examination for each subject are listed in Table 1. All autopsies were conducted routinely with the removal of the abdominal viscera preceding the removal of the brain. PDE assays were done on representative sections of frontal cortex containing both white and gray matter. All tissues were obtained with the informed consent of the responsible next of kin.

EXPERIMENTAL METHODS

Phosphodiesterase activity was assayed in the supernatant and particulate fractions of tissues which were homogenized in 3 volumes of glass-distilled water, freeze-thawed rapidly three times, spun at 30,000 × *g* (0–4°) for 30 min, and dialyzed overnight in 20 mM Tris buffer, pH 7.5, at 4°. PDE activity observed in the undialyzed fraction of a 30,000 × *g* homogenate of cortex from a 56-year-old male subject was 86.6 nmol/mg/min whereas the dialyzed fraction was 93.8. This increase in activity is probably due to the removal of endogenous substrates. Amounts of protein used for linearity over a 10-min period were 50–100 μg in 100 μl total volume for cyclic AMP PDE and 25–50 μg protein for cyclic GMP PDE. Incubation was for 10 min for specific activities and 1 min for kinetic studies at 34–36°. The divalent cation requirement was met by Mn^{++} at a final concentration of 0.1 mM and the reaction was conducted in 40 mM Tris buffer, pH 7.4. The two-step isotopic method of Thompson and Appleman (44) was used. Substrate concentrations varied over a range of 2×10^{-3} to 10^{-8} M. Theophylline was used at final concentrations of 0.9 to 7.5 mM. Final concentrations of imidazole were 10, 20, and 40 mM. Activator effects were studied with and without the addition of 10 μg partially purified protein from bovine brain, which was a gift from Dr. L. Liu. The percentage of conversion of labeled substrate to the 5′-derivative was determined in an aliquot of the supernatant after resin precipitation in Bray's solution in a spectrophotometer.

To determine the effect of differences in PDE activity due to variation in time between death and collection of tissues, cerebral cortices from single rats were frozen immediately and at 3, 5, and 24 hr after death, during which interval the intact carcass of each animal was refrigerated at 4°. All tissues were processed simultaneously for PDE measurements.

Adenosine 3′,5′-cyclic [8-^{3}H]phosphate was purchased from Schwarz-Mann and guanosine 3′,5′-cyclic [^{3}H-*G*]phosphate was obtained from New England Nuclear. Isotopes were further purified by chromatography on thin layer plates of cellulose in 2-propanol, ammonia, water (7:1:2) before use. All other chemicals were of reagent grade. Protein was determined by the method of Lowry with bovine serum albumin as a standard (25). DNA was measured by the diphenylamine method of Burton (7).

RESULTS

Since our samples were obtained at autopsy and the specific diseases, agonal conditions, and ages of our subjects varied considerably, the stability of the enzyme to storage effects and a common basis for comparison of enzyme activity had to be determined.

The specific activities observed for cyclic AMP hydrolysis of individual rat brains collected after varying periods of storage at 4° are presented in Table 2. No change in the specific activity of PDE was noted in tissues obtained from intact animals refrigerated for up to 24 hr. This observation is in agreement with the published literature which indicates that the crude enzyme is remarkably stable during storage (2).

Activity was based on DNA for the following reasons. (*1*) Increase in cellular protein, RNA, lipid, and water content of the cortex continues throughout the process of maturation which extends through adolescence (19). (*2*) Neuroblastic proliferation is completed by 20 weeks of gestational age in man and glial proliferation by the end of the first postnatal year (11, 49).

Table 1. *Clinical information of subjects*

Subject	Information
1	14-week male fetus, spontaneously aborted, twin of *subject 2*, 2 hr[1]
2	14-week female fetus, spontaneously aborted, twin of *subject 1*, 2 hr[1]
3	18-week male fetus, spontaneously aborted, 2 hr[1]
4	18-week male fetus, spontaneously aborted, 2–3 hr[1]
5	19–20-week fetus, spontaneously aborted, several hours[1]
6	15-year-old male, mental retardation, polycystic kidneys, uremia, 12 hr[1]
7	18-year-old male, treated acute lymphocytic leukemia, sepsis, 12–24 hr[1]
8	19-year-old male, acute hemorrhage from traumatic chest wound, 12 hr[1]

[1] Hours elapsing between death and autopsy. For fetuses, elapsed time from expulsion of fetus to collection of tissues.

Table 2. *Effect of delay in freezing of cerebral cortical tissues on phosphodiesterase activity*

Interval between death[1] and freezing of tissues, hr (n = 1)	Specific activity, nmol/mg protein/min[2]	
	50 μM cyclic AMP	1 mM cyclic AMP
0	3.25	69.97
3	3.48	74.92
5	3.40	70.34
24	3.58	75.91

[1] Sprague-Dawley rats of approximately same weight killed by ether anesthesia before removal of brain and freezing of tissue. Carcasses kept intact at 4° for the indicated duration.

[2] Averages of duplicate determinations.

Thus, the DNA content of the human cerebral cortex is essentially constant after approximately the first year of life. Activity per unit DNA may, therefore, be considered most representative of changes in cellular activity in the human cortex from subjects of widely different ages, reflecting largely neuroblastic activity in the fetus and combined neuroblastic and glial activity after the first year of life.

COMPARISON OF TOTAL ACTIVITY AND DISTRIBUTION OF PHOSPHODIESTERASE ACTIVITIES

CYCLIC AMP PDE ACTIVITY

Differences in distribution and total activity are found between the fetal and mature cortex. Maturation is accompanied by a 10-fold increase in activity for both the high and low K_m enzymes. Most of the high K_m activity is in the supernatant fraction in both fetal and mature brains. However, more low K_m activity is present in the pellet in the mature cortex, in contrast to the fetal brain, where more is located in the supernatant fraction (see Fig. 1). Activities based on brain weight are tabulated in Tables 3 and 4.

CYCLIC GMP PDE ACTIVITY

Differences in total activity and distribution are found again. Maturation results in a 15–20-fold increase in activity for both high and low K_m enzymes. In the mature brain, most of the high and low K_m activity is located in the pellet fraction, whereas these activities are located predominantly in the supernatant in the fetal brain (see Fig. 2).

MICHAELIS CONSTANTS

Nonlinear figures were obtained when the double reciprocals of substrate concentration and activity or velocity were plotted

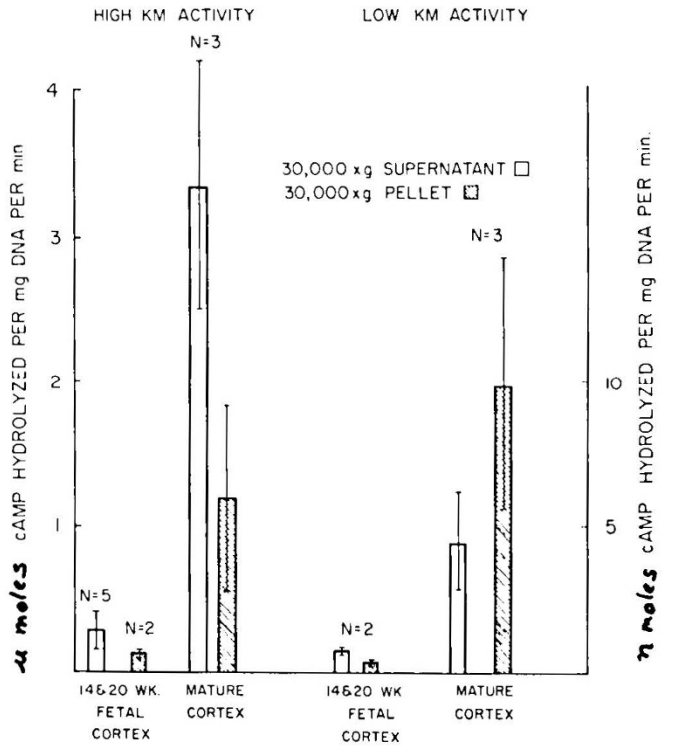

Fig. 1. Distribution of cyclic AMP (*cAMP*) phosphodiesterase activity in the fetal and mature human cerebral cortex. Frontal cortex containing both white and gray matter was homogenized in 3 volumes of glass-distilled water, freeze-thawed three times, spun at 30,000 × *g* (0–4°) for 30 min, and dialyzed overnight in 20 mM Tris buffer, ph 7.5, at 4°. Fifty to 100 μg protein were used; total volume 100 μl; Mn^{++} 0.1 mM in 40 mM Tris buffer, pH 7.4, incubated 34–36° for 10 min. Reaction terminated by boiling for 3 min before product, 5′-AMP, was hydrolyzed with snake venom (*Crotalus atrox*). Substrate concentrations, 2 mM and 2 μM.

Table 3. *Distribution of cyclic AMP activities in supernatant and particulate fractions of human cerebral cortex*

Fraction from *subject* no.	Cyclic AMP phosphosphodiesterase			
	High K_m activity (2 mM)[1]		Low K_m activity (2 μM)[1]	
	μmol/g tissue/min	μmol/mg DNA/min	nmol/g tissue/min	nmol/mg DNA/min
Supernatant				
1	1.00	0.29		
2	1.02	0.42		
3	0.84	0.35	1.70	0.70
4	0.41	0.24		
5	0.49	0.15	2.20	0.88
6	0.52	1.73	0.96	3.20
7	1.00	2.76	1.43	3.98
8	1.26	5.61	1.42	6.29
Particulate				
3	0.05	0.13	0.82	0.34
5	0.08	0.14	0.82	0.33
6	0.18	0.59	3.07	10.23
7	0.41	1.13	1.92	5.32
8	0.41	1.84	3.16	14.04

[1] Substrate concentration.

for determination of the Michaelis constants. Two kinetic forms were assumed, one exhibiting a low K_m or higher affinity and the other a high K_m or lower affinity for each substrate. (See Figure 3 for representative Lineweaver-Burk plot.) Values obtained for half-maximum saturation and maximal velocities are presented

Table 4. *Distribution of cyclic GMP activities in supernatant and particulate fractions of human cerebral cortex*

Fraction from *subject* no.	Cyclic GMP phosphodiesterase			
	High K_m activity (200 μM)[1]		Low K_m activity (2 μM)[1]	
	μmol/g tissue/min	μmol/mg DNA/min	nmol/g tissue/min	nmol/mg DNA/min
Supernatant				
3	0.074	0.031	4.1	1.7
5	0.083	0.025	4.2	1.2
6	0.054	0.178	2.7	8.9
7	0.113	0.312	2.7	7.4
8	0.150	0.665	3.9	17.1
Particulate				
3	0.020	0.047	0.6	0.2
5	0.042	0.075	1.5	0.6
6	0.245	0.817	8.7	29.1
7	0.118	0.327	4.0	11.2
8	0.122	0.544	5.6	25.0

[1] Substrate concentration.

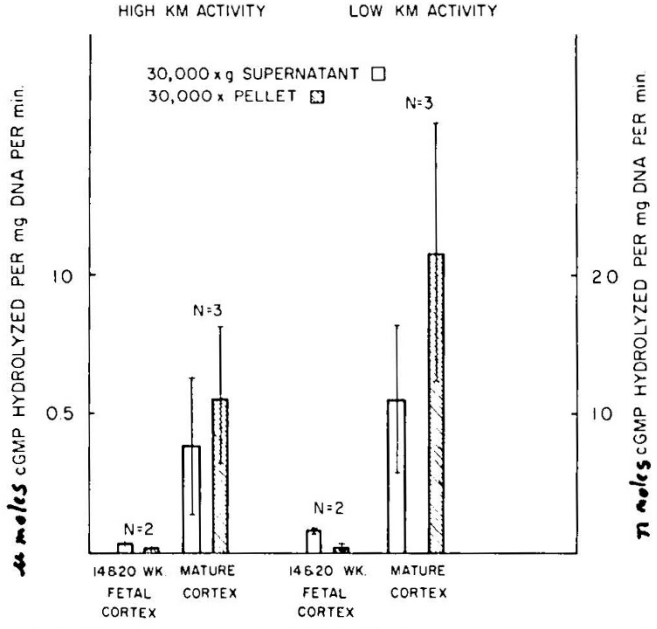

Fig. 2. Distribution of cyclic GMP (*cGMP*) phosphodiesterase activity in the fetal and mature human cerebral cortex. Incubation and assay conditions as for cyclic AMP phosphodiesterase using 25–50 μg protein. Substrate concentrations, 200 μM and 2 μM.

in Table 5. Lower K_m values were obtained for the high affinity cyclic GMP enzyme, a pattern seen in several tissues and species as reported by others (3). For the low affinity enzymes, the half-saturation values were higher for cyclic GMP than for cyclic AMP by a factor of 2–3. In particulate fractions at higher substrate concentrations, these enzymes exhibit slightly greater affinity for cyclic AMP but with maximum hydrolysis of cyclic AMP at a rate substantially lower than for cyclic GMP. No significant differences were noted between fetal and mature cortices.

EFFECT OF CYCLIC GMP ON CYCLIC AMP PHOSPHODIESTERASE ACTIVITY

Since phosphodiesterase activity is reported to be variably affected by the additional presence of the other cyclic nucleotide

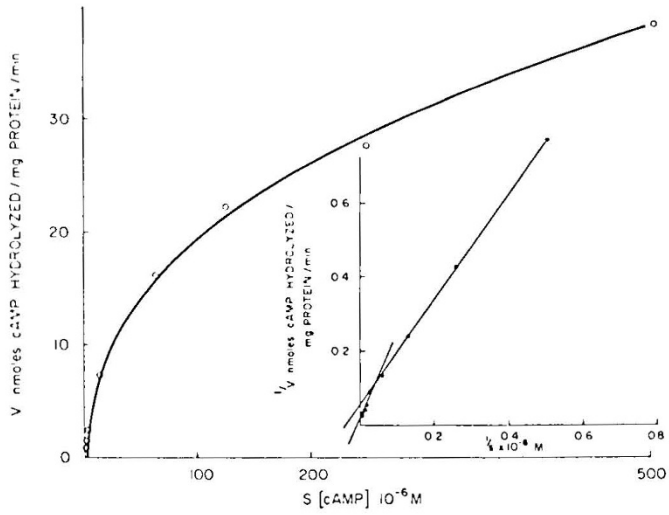

Fig. 3. Lineweaver-Burk plot of cyclic AMP (*cAMP*) hydrolysis by the 30,000 × *g* supernatant fraction of a homogenized and freeze-thawed specimen of human cerebral cortex.

(4), the effect of one nucleotide on the hydrolysis of the other was examined.

In the mature cortex, the hydrolysis of 10^{-7} M cyclic AMP was stimulated by the addition of cyclic GMP over the concentration range of 10^{-7}–10^{-5} M as depicted in Figure 4. Increasing the concentration of the added nucleotide resulted in a progressive reduction of stimulation. When cyclic GMP concentrations were increased to 10^{-4} M, hydrolysis of cyclic AMP was inhibited 85–90%.

The hydrolysis of higher concentrations of cyclic AMP (10^{-5}–10^{-4} M) was progressively inhibited competitively with increasing amounts of cyclic GMP.

EFFECT OF CYCLIC AMP ON CYCLIC GMP PHOSPHODIESTERASE ACTIVITY

The addition of 10^{-9}–10^{-7} M cyclic AMP inhibited the hydrolysis of 2×10^{-4} M cyclic GMP in the fetus and adult. The fetus exhibits greater sensitivity to inhibition. When the added nucleotide is increased from 10^{-6} to 10^{-4} M, relief of inhibition was seen in the adult cortex (Fig. 5). Even at lower substrate concentrations, the addition of 10^{-9}–10^{-7} M cyclic AMP also inhibited hydrolysis of cyclic GMP in the fetus. The mature brain, in contrast, was slightly stimulated under the same conditions. When the inhibitor concentrations were raised to 10^{-6} to 10^{-4} M, the brain of the fetus exhibited progressive release from inhibition (Fig. 6).

Since N^6,2′-*O*-dibutyryl (db)-cyclic AMP has been observed to mimic some of the effects of cyclic AMP and is believed not to be hydrolyzed by PDE (36), the effect of varying concentrations of this analog on cyclic GMP hydrolysis was examined in the mature cortex.

A stimulatory effect was observed on cyclic GMP hydrolysis at high concentrations by 10^{-9} M db-cyclic AMP which was sustained over increasing concentrations of the dibutyryl compound to 10^{-4} M. At low concentrations of cyclic GMP, 10^{-9} M db-cyclic AMP inhibited the hydrolysis of the guanosine nucleotide; this inhibition was also sustained over increasing amounts of the added db-cyclic AMP (Fig. 7). These findings are suggestive that db-cyclic AMP may be an allosteric effector of the cyclic GMP phosphodiesterases which is permissive and stimulatory for the low affinity enzyme and inhibitory for the high affinity enzyme.

These results indicate that the hydrolysis of each cyclic nucleotide is variously affected by the presence of different concentrations of the other nucleotide and significant differences occur as a result of maturation. These findings may represent differences in the apoproteins, or they may represent the sum effect of a

Table 5. *Michaelis constants of cerebral cortical phosphodiesterases (PDE's)*

Fraction from *subject* no.	Cyclic AMP PDE				Cyclic GMP PDE			
	High K_m enzyme		Low K_m enzyme		High K_m enzyme		Low K_m enzyme	
	K_m[1] ($\times 10^{-4}$)	V_{max}[2]	K_m[1] ($\times 10^{-5}$)	V_{max}[2]	K_m[1] ($\times 10^{-4}$)	V_{max}[2]	K_m[1] ($\times 10^{-6}$)	V_{max}[2]
Supernatant								
1	0.77	52.6	0.77	6.7	2.22	54.1	5.0	6.67
5	0.59	34.5	1.0	20.0	3.85	68.9	4.0	3.33
6	1.06	66.7	2.0	6.7	2.54	71.4	10.0	10.00
7	1.11	76.9	2.0	10.0	1.37	62.5	5.0	7.14
8	1.43	125	2.0	10.0	1.67	76.9	5.0	10.00
Particulate								
1	2.86	1.70	1.25	0.10			5.0	0.67
5	1.25	1.25	0.67	0.20	6.67	10.0	1.0	0.50
6	1.10	1.67	1.0	0.10	1.67	62.5		
7	0.77	1.43	1.67	0.20	6.67	100	3.57	1.67
8	1.43	0.50	1.67	0.25			2.63	1.11

[1] Molar concentration.
[2] Nanomoles per mg per min.

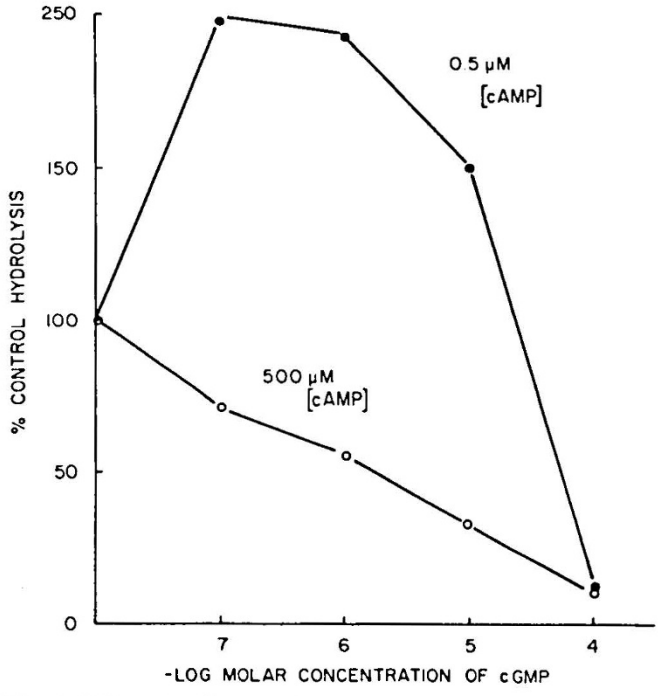

Fig. 4. Effect of cyclic GMP (*cGMP*) on cyclic AMP (*cAMP*) phosphodiesterase. Plotted as percentage of control or baseline hydrolysis. ●——●: cyclic AMP concentration 0.5 μM. ○——○: cyclic AMP concentration 500 μM. Supernatant fractions used. (*Subject 8:* initial activity with 500 μM cyclic AMP was 38.9 nmol/mg protein/min and with 0.5 μM cyclic AMP activity was 181 pmol/mg protein/min).

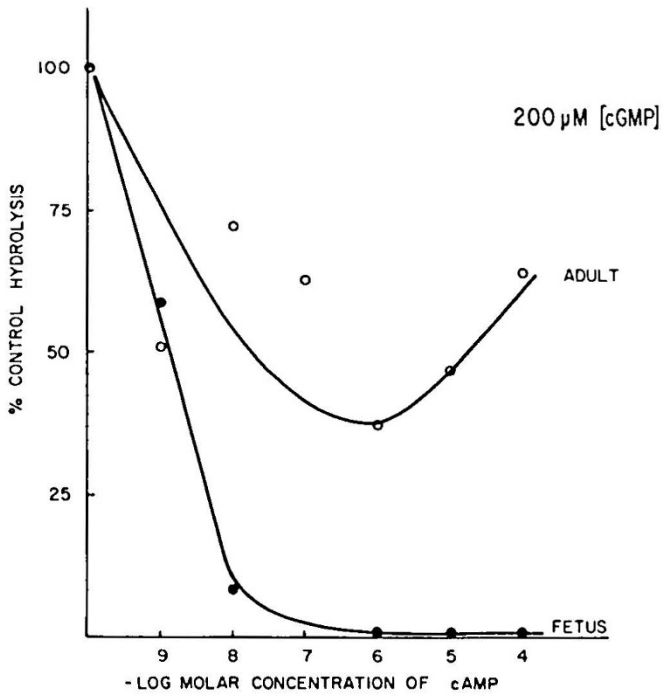

Fig. 5. Effect of cyclic AMP (*cAMP*) on cyclic GMP (*cGMP*) phosphodiesterase. Values were plotted as percentage of control or baseline hydrolysis which were as follows: *subject 2*, 37.03 nmol/mg protein/min and *subject 8*, 34.53 nmol/mg protein/min. ●——●: supernatant fractions of the fetus (*subject 2*) when cyclic GMP concentration was 200 μM; ○——○: supernatant fractions of the mature cortex (*subject 8*) with 200 μM cyclic GMP.

variety of factors as, for instance, in the binding proteins and protein kinases which are also undergoing developmental change. In the absence of differences of the Michaelis constants or further purification of the enzymes, the basis of the observed findings is not apparent. However, variability of the effect of one nucleotide on the hydrolysis of the other does not reflect concordance, suggesting that simple competitive inhibition may be but one of several mechanisms by which one cyclic nucleotide affects the hydrolysis of the other.

EFFECTS OF A NONIONIC DETERGENT, TRITON X-100

In the rat, homogenates of brain reportedly exhibit a latent phosphodiesterase activity which could be unmasked by the use of nonionic detergents (10). The effect of 0.2% Triton X-100 (v/v) on the two kinetic forms of the cyclic AMP and cyclic GMP PDE of the human cerebral cortex was, therefore, examined. In the mature cortex, over a range of 5×10^{-8}–10^{-4} M cyclic AMP concentration, Triton X-100 had a slightly inhibitory effect on cyclic AMP hydrolysis, which decreased the V_{max} by 55% at high substrate concentrations and the affinity (increased in K_m by 2-fold) over low substrate concentrations.

Over comparable concentration ranges of cyclic GMP, inhibition due to Triton X-100 was again seen and was due to reduction of the maximal velocities in both the mature and immature brains and in the several fractions examined.

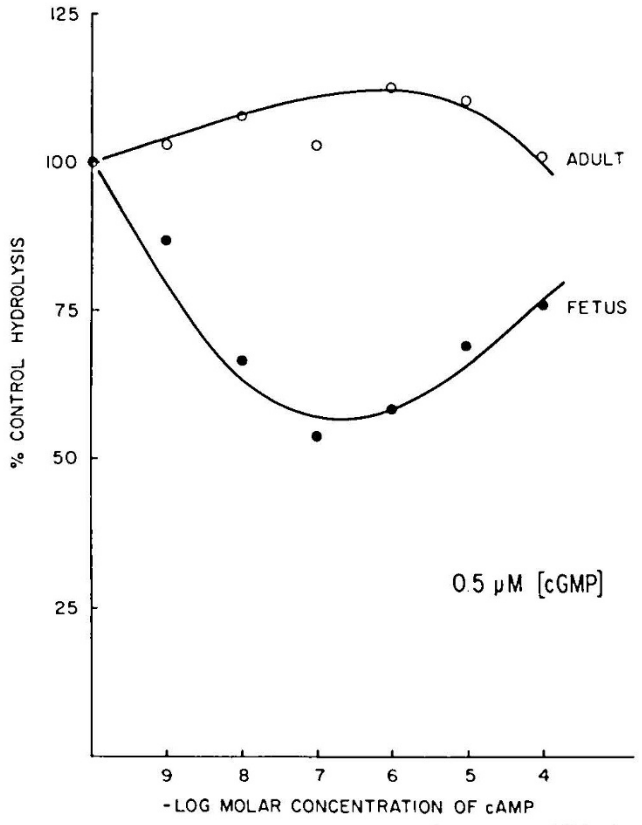

Fig. 6. Effect of cyclic AMP (*cAMP*) on cyclic GMP (*cGMP*) phosphodiesterase. Values were plotted as percentage of control or baseline hydrolysis and were 495 pmol/mg protein/min for the mature cortex (*subject 8*) and 336 pmol/mg protein/min for the fetal cortex (*subject 2*). Supernatant fractions of the fetus (●——●) and of the mature cortex (○——○). Cyclic GMP, 0.5 μM.

These results suggest that latency of PDE activity is not encountered in the preparations used in our study. Differences between our results and the animal study may represent species differences, although Hardman (16) observed an inhibitory effect of 0.01% Triton X-100 on the activity of the cyclic AMP PDE in the rat liver which was as much as 50% inhibited at high substrate concentrations.

EFFECT OF THEOPHYLLINE

Variability of drug effects has been demonstrated for the PDE's of the two cyclic nucleotides and is exemplified by the greater selectivity of theophylline for cyclic AMP rather than cyclic GMP PDE in the lung of the guinea pig (2). In the human cerebral cortex, a similar selectivity for the cyclic AMP PDE is seen. At 1 mM substrate concentration, cyclic AMP hydrolysis is markedly inhibited, whereas cyclic GMP hydrolysis is relatively unaffected by millimolar amounts of theophylline (see Fig. 8) in either the mature or immature cortex. At micromolar concentrations of substrate, the hydrolysis of both nucleotides was inhibited to a degree comparable to that observed for cyclic AMP at 1 mM. At all substrate concentrations studied, the fetal cortex was slightly more sensitive to the inhibitory effects of theophylline than the mature cortex.

Although the *in vivo* effects of theophylline are not limited to PDE inhibition alone and theophylline concentrations of 1 to 8 mM are rarely achieved clinically, the difference exhibited by the low affinity enzymes indicate that tissue concentrations of the two cyclic nucleotides would vary considerably in the presence of theophylline.

EFFECT OF IMIDAZOLE

Imidazole has been demonstrated to stimulate the high K_m PDE of the brain preparations of a number of species (12), although no effect is observed on the low K_m PDE for a number of them (1).

In both fetal and mature brains, concentrations of imidazole of 20 and 40 mM had a stimulatory effect of 25–50% on cyclic AMP and cyclic GMP hydrolysis at high substrate concentrations, whereas an inhibitory effect of 25% was seen at micromolar concentrations of cyclic GMP. Although a mild inhibitory effect was observed at micromolar concentrations of cyclic AMP in mature brains, a 20% stimulation of activity was observed in fetal brains in the presence of 40 mM imidazole.

EFFECT OF BOVINE ACTIVATOR PROTEIN

Activation of brain PDE activity by a heat-stable, nondialyzable factor present in brain extracts has been ascribed to a calcium binding protein by a number of workers (14, 21, 22, 42). The possible regulatory role played by this factor is not clear since it is ubiquitous (37), is usually present in excess even in tissues where PDE activity is barely detectable (37), appears to activate only one of the several forms of PDE obtained by physical separation methods (48), and appears to stimulate adenylate cyclase activity as well (6).

The addition of 10 μg bovine activator protein, which has been shown to stimulate the PDE's of other species, had no significant effect on the tissues examined. This could indicate that saturating amounts of the endogenous activator were present in the samples or that the preparation of bovine activator used did not activate the human enzyme (see Table 6).

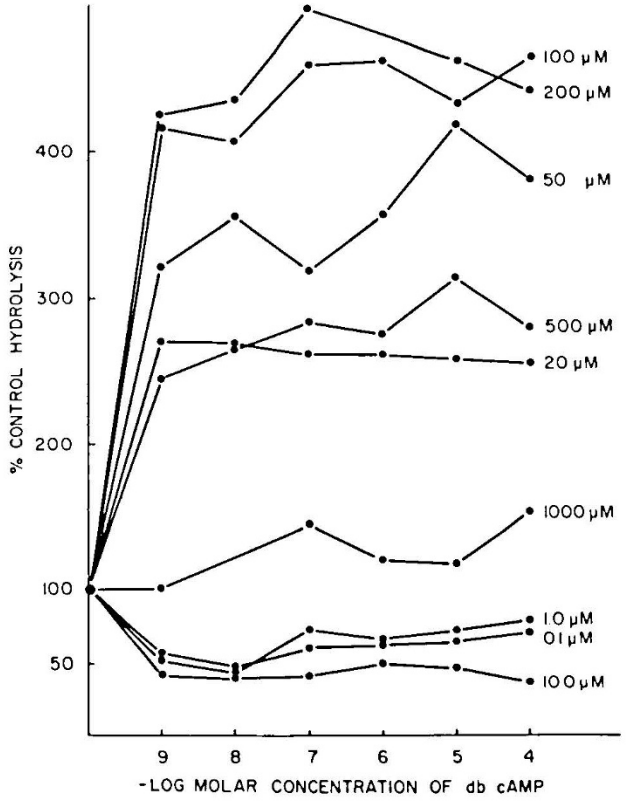

Fig. 7. Effect of dibutyryl-cyclic AMP (*db cAMP*) on cyclic GMP hydrolysis. Plotted as percentage of control or baseline hydrolysis at varying substrate concentrations of cyclic GMP, 0.1 to 1,000 μM. Supernatant of mature cortex used.

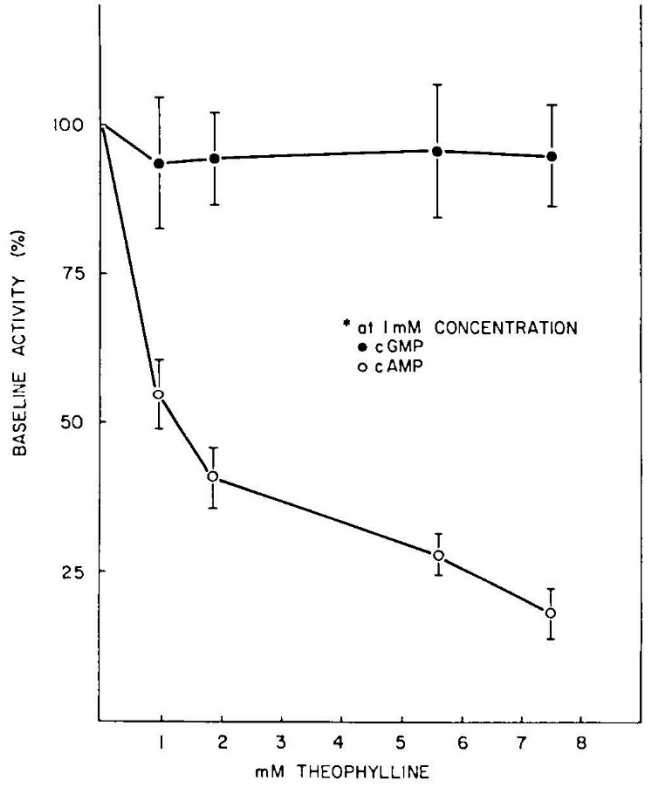

Fig. 8. Effect of theophylline on cyclic nucleotide hydrolysis in the human cerebral cortex. Plotted as percentage of control or baseline hydrolysis at 1 mM cyclic AMP (*cAMP*) concentrations (○——○) and 1 mM cyclic GMP (*cGMP*) concentrations (●——●) using the supernatant fractions.

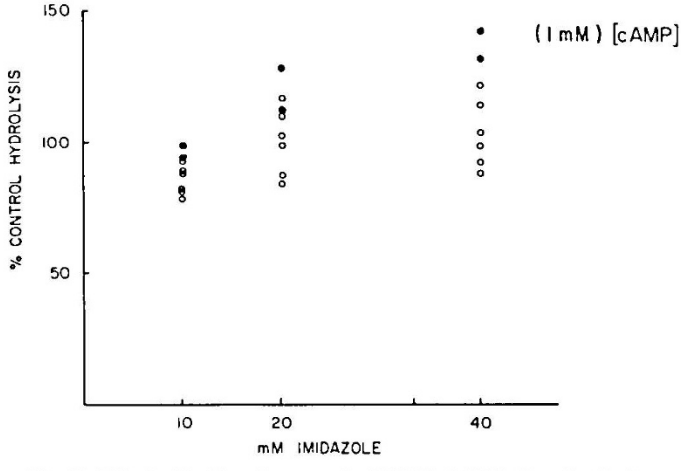

Fig. 9. Effect of imidazole on cyclic AMP (*cAMP*) phosphodiesterase activity. Plotted as percentage of control or baseline hydrolysis of the supernatant fractions of eight separate subjects (○: mature subjects, ●: fetuses).

DISCUSSION

When total phosphodiesterase activity is compared on a DNA basis, measurable but low activities are found in the fetus whereas high activities are found in the cortex from young adults. At high substrate concentrations, both mature and immature brains hydrolyze cyclic AMP more readily than cyclic GMP and at low substrate concentrations this preference is reversed.

In the fetus, more activity for both nucleotides at high and low concentrations is associated with the supernatant fraction. With maturity, there is a shift in localization of high K_m activity for cyclic GMP and low K_m activities for both nucleotides to the particulate fraction.

Table 6. *Effect of bovine activator protein on phosphodiesterase activities of human cerebral cortex*

	% of control hydrolysis at various substrate concentrations			
	Cyclic AMP		Cyclic GMP	
Parameter	1 mM	2 μM	500 μM	2 μM
Mean (%)	79.3	104	113	103
Range (%)	61.3-92.5	82.9-124.6	63.5-216	90.7-116
SD (%)	±9.4	±13.6	±49.1	±8.0
No. of subjects	8	8	8	7

These findings differ in several respects from reports of animal studies. Beavo *et al.* (4) found that whereas the hydrolysis of cyclic GMP and cyclic AMP by homogenate and subcellular fractions from bovine heart and several rat tissues was nearly the same at millimolar levels of substrate, at μM concentrations, cyclic GMP was hydrolyzed more efficiently than cyclic AMP in most fractions studied. This activity was attributed by them to a low K_m PDE exhibiting a higher affinity for the guanosine than the adenosine nucleotide. Kakiuchi *et al.* (22) found the supernatant fractions of a variety of rat tissues to contain much more activity for the hydrolysis of cyclic GMP than for cyclic AMP, which led these authors to suggest that the function of the Ca^{++}- and Mg^{++}-dependent cyclic nucleotide PDE was probably to hydrolyze cyclic GMP preferentially.

Localization of the majority of the low K_m activity with the particulate fraction of preparations has been reported for many mammalian tissues (43). The low K_m PDE is believed to play a more significant role in regulating the basal, intracellular concentration of the cyclic nucleotides since the half-maximal concentration of substrate for maximum velocity is at physiologic tissue levels of the nucleotides.

In the mature cortex, localization of the bulk of the low K_m activity for cyclic AMP hydrolysis in the particulate fraction might offer an advantage for regulating the intracellular concentration of cyclic AMP since it has been established that the enzyme of synthesis, adenylate cyclase, is membrane bound (41). Such an advantage may not exist for the low K_m cyclic GMP PDE since guanylate cyclase appears to be soluble in most of the tissues studied (18). It has been suggested, however, that the solubility of guanylate cyclase may be a dissociative artifact of the processing of tissues (17) and that guanylate cyclase is also membrane bound. In the latter case, the proximity of the synthetic and degradative enzymes for cyclic GMP would allow for the close monitoring of the intracellular level of the cyclic nucleotide expected of a highly regulated system.

The influence of one cyclic nucleotide on the hydrolysis of the other was variable in the fetal and mature cortex. A diminution of inhibition at high concentrations of added nucleotide was seen in the adult cortex for cyclic GMP PDE. The addition of millimolar amounts of unlabeled cyclic AMP or GMP to the reactants of our PDE assays after boiling and before the addition of the snake venom followed by the addition of resin according to the method of Thompson and Appleman (44) did not increase the amount of recoverable label in the supernatant. Thus, a technical artifact could not be found to explain this observation.

Highly variable effects on the hydrolysis of the nucleotides have been reported for a number of different tissues by the addition of the other nucleotide. Beavo *et al.* (4) reported that in bovine heart and rat liver the presence of 10^{-6}–10^{-5} M concentrations of either nucleotide was inhibitory to the hydrolysis of

10^{-6} M concentrations of the other nucleotide. At low concentrations of cyclic GMP (10^{-7} M), the hydrolysis of cyclic AMP was stimulated 2-fold in rat liver but not in bovine heart (4). In a follow-up study on other tissues of the rat, a stimulatory effect of 10^{-8} M concentration of cyclic GMP was seen on particulate preparations of the liver, brain, kidney, heart, and thymus and on soluble preparations of the liver and thymus. Increasing the concentration of cyclic GMP to 10^{-5} M, on the other hand, was inhibitory (5).

In a rat brain preparation, O'Dea *et al.* (28) observed that although 10^{-7} to 10^{-6} M cyclic GMP was competitively inhibited by 10^{-6} to 10^{-5} M cyclic AMP, the hydrolyses of cyclic AMP was unaffected by comparable amounts of cyclic GMP. Similar findings were reported by Rosen (31) for the frog erythrocyte at millimolar concentrations of substrate and inhibitor.

At more physiologic concentrations of substrate and inhibitor, Sakai *et al.* (34) observed in the fat cells of the Wistar rat that the hydrolysis of low concentrations of either cyclic nucleotide was stimulated by the presence of 10^{-8}–10^{-7} M concentrations of the other. Higher concentrations of the added nucleotide, 10^{-5} M, were inhibitory by a noncompetitive mechanism for cyclic AMP hydrolysis and competitively for cyclic GMP hydrolysis (34).

The physiologic significance of our findings is not readily apparent, particularly with respect to the high concentrations of substrate and "inhibitor" interrelationships. If a simplistic view is taken that two levels for each cyclic nucleotide may be critical in tissues, the following interpretation may be made. At the concentration of each nucleotide in tissues, a stimulatory influence is exerted on each PDE to ensure the maintenance of low, basal concentrations of each nucleotide. In the mature cortex, the stimulatory effect of cyclic GMP on cyclic AMP hydrolysis is greater than the influence of cyclic AMP on cyclic GMP degradation. Since cortical cells tend to degrade more cyclic GMP than AMP at low substrate concentrations, this is suggestive that the net effect of rather comparable tissue levels may result for both nucleotides, assuming that rates of synthesis of each are similar. However, the apparent greater affinity of these enzymes for cyclic GMP at low concentrations (see Table 5) indicates that tissue cyclic GMP levels would be lower than cyclic AMP if rates of synthesis of the two cyclic nucleotides are indeed similar. When adenylate or guanylate cyclase is maximally stimulated, the presence of basal amounts of the other nucleotide serves to raise the tissue concentration of the nucleotide being synthesized even further, allowing a critical level to be reached more quickly for the activation of specific kinases. Under such maximal stimulation, the presence of the other nucleotide in high concentrations results in a difference of effect on the specific PDE responsible for the hydrolysis of the newly synthesized nucleotide. When adenylate cyclase is stimulated, high levels of cyclic AMP are reached in the presence of high levels of cyclic GMP. If guanylate cyclase is stimulated, cyclic GMP levels reached are comparable to that observed in the absence of added cyclic AMP despite the presence of 10^{-5} and 10^{-4} M cyclic AMP. If the two cyclases can be comparably stimulated in the adult cerebral cortex, these findings indicate that higher levels of cyclic GMP may not be favored by at least one of the factors regulating the concentrations of the cyclic nucleotides in the brain. In the fetus, on the other hand, these interrelationships appear to favor the sparing of cyclic GMP from degradation.

Since db-cyclic AMP, which is probably not actively metabolized by the phosphodiesterases, inhibits the hydrolysis of cyclic GMP catalyzed by enzyme with low K_m and stimulates the high K_m enzyme, it is possible that one nucleotide exerts an allosteric effect on the hydrolysis of the other at low and high substrate concentrations.

Evidence of the separateness of the several forms of PDE has been reported by Russell *et al.* (33), Kakiuchi *et al.* (22), and others on the basis of kinetic analysis of physically separated proteins; the fact that these enzymes are under separate control was reported by Pastan's group (32). The effects of theophylline on cyclic nucleotide hydrolysis are attributed to inhibition of PDE (40), and correlations between theophylline concentration and physiologic responsiveness have been shown (8). Our observation of differences in sensitivity of the cyclic GMP PDE's to theophylline suggests that the active sites for each of the GMP enzymes may be separate. Further, the variability of the effect of theophylline on the guanosine and adenosine PDE's at high substrate concentrations also tends to support the concept that these sites may be different.

CONCLUSION

Since cyclic AMP has been shown to play a significant role in the differentiation of developing tissues and hormone production, and responsiveness may be present in a number of tissues from early stages in man (27), characterization of the enzymes which regulate the levels of the cyclic nucleotides in the cerebral cortex of man at various ages may be of importance because the putative neurotransmitters utilize these nucleotides to effect their neuroendocrine functions. The data reported here, although limited to the immature and mature cortex, indicate that total enzyme activity and distribution of PDE in the cerebral cortex of man are age dependent. In addition, differences in response to stimulators and inhibitors can be found at both extremes of maturation. This was most notable with respect to the influence of one nucleotide on the hydrolysis of the other. In the absence of differences of the kinetic constants, these findings may be the result of other factors which are undergoing changes simultaneously but which are not directly part of the apoprotein of the PDE's. Further purification of the enzymes will be necessary to explore the possibility of isozymes. Whether these differences are due to maturation of neuroblasts, glial proliferation, or to both processes cannot be determined from our studies. Finally, differences noted at low and high substrate concentrations in the presence of the other nucleotide at both extremes of maturity together with differences in responsiveness to theophylline confirm the notion that the active sites for the hydrolysis of the guanosine and adenosine nucleotides may be separate.

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