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Fetal and Early Postnatal Development of Adenylate Cyclase and Cyclic AMP Phosphodiesterase Activities in Rat Brain

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Abstract. Brain cyclic AMP metabolism was studied in rat offspring at 17, 19 and 21 days of intrauterine life and 0, 1 and 4 postnatal days. Wet weight and protein and DNA and RNA concentrations in brain increased in parallel with body weight until the 4th postnatal day although during the perinatal phase some of these parameters suffered a manifest temporal retardation. In the brain, similar qualitative changes were observed in both adenylate cyclase and high K_m cyclic AMP phosphodiesterase activity while the low K_m enzyme showed a rise from 17-day fetuses to those of 19 days with no further changes. The increase in cyclic AMP concentration was very mild and the values found in the 21-day fetuses were already similar to those in adults. In the mother's brain a significant reduction in adenylate cyclase activity appeared at 19 and 21 days of gestation. The retardation in the ontogenic development of cyclic AMP metabolism during the perinatal phase in offspring is probably a consequence of their specific metabolic situation while in the mother it may be the result of its endocrine preparation for parturition.

Introduction

Adenosine 3′,5′-monophosphate (cyclic AMP) is known to function as an intracellular mediator for the actions of certain neurotransmitters in nerve cells [3, 4, 13]. It has recently been proposed that actions of cyclic AMP in the nervous system should not be considered in isolation but as part of an integrating system [14] which includes effects on metabolic processes besides the specific mechanisms on synaptic transmission. The concentration of cyclic AMP in the brain is controlled largely by the relative activities of adenylate cyclase (EC 4.6.1.1, ATP pyrophosphatelyase [cyclizing]) responsible for its synthesis, and cyclic AMP phosphodiesterase (EC 3.1.4.17, 5′-nucleotidohydrolase) responsible for the degradation of the nucleotide to 5′-AMP. The concentration of cyclic

AMP and the activities of these two enzymes have been extensively studied during postnatal development in the rat brain [20, 28, 29] but very few data are available concerning the fetal period.

The evaluation of these parameters during transition from the fetal to the postnatal phase in the rat deserves attention as it is known that in this species, most of the development of brain structures occurs after birth [32]. Thus it is of interest to determine whether these structural changes are followed by parallel changes in cyclic AMP metabolism in the brain. In the present investigation these determinations were obtained in the perinatal phase in the rat brain and the study was extended by estimating the DNA, RNA and protein concentrations as a gross index of cellularity.

Materials and Methods

Adenosine, guanosine, EDTA, 5′-nucleotidase, cyclic AMP, cyclic GMP, phosphocreatine, creatine phosphokinase and neutral alumine type VN-3 were purchased from Sigma Chemical Co., St. Louis, Mo. Isobutylmethylxanthine was from Aldrich Chemical Co., deoxyribonucleic acid from Calbiochem and *p*-nitrophenylhydrazine from Eastman Kodak Co. Anion exchange resin, AG 1X2, 50–100 mesh, was from BioRad Laboratories. Ready Solv-HP scintillation cocktail was from Beckman. (8-^{3}H)-cyclic AMP (30 Ci/mmol) and (α^{32}P)-ATP (17.8 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England. All other reagents were of the highest quality commercially available.

Animals

Female Sprague-Dawley rats, fed Purina chow diet, were mated at 2 months of age and placed in individual cages. Both mother and offspring were studied at 17, 19 or 21 days of pregnancy or at 0, 1 or 4 days postpartum. Female virgin rats of 2 months of age, maintained under the same conditions, were also studied. All animals were killed by decapitation and fetuses of the rats sacrificed before parturition were rapidly removed and decapitated. When used for enzyme activity, protein, RNA and DNA evaluations, the forebrain (defined as the portion of the brain located above the inferior colliculi and below the olfactory lobes) and cerebellum were immediately dissected and kept at −70 °C until processing. For cyclic AMP evaluation, offspring were introduced in toto into liquid N_2 and the complete frozen forebrain was removed and weighed for processing.

Determinations

Adenylate cyclase activity was measured in tissue homogenates made in 40 m*M* Tris-HCl containing 5 m*M* $MgCl_2$, pH 7.6 with a final protein concentration of 2 mg/ml. A slightly modified version of the method of *Farber and Lolley* [7] was used. Briefly, 50–100 µg of sample protein were incubated for 10 min at 30 °C in the presence of 40 m*M* Tris-HCl buffer (pH 7.6), 5 m*M* $MgCl_2$, 12 m*M* phosphocreatine, 7 units of creatine kinase, 1 m*M* cyclic AMP containing 10,000 cpm of (8-^{3}H)-cyclic AMP, 0.08 m*M* isobutylmethylxanthine and 1 m*M* ATP containing 1 × 10^6 CPM of (α^{32}P)-ATP, in a final volume of 200 µl. The reaction was stopped by adding 50 µl of 200 m*M* EDTA-disodium salt. The cyclic ^{32}P-AMP formed in the reaction was isolated in neutral alumina columns, eluated with 3.5 ml of 40 m*M* Tris-HCl buffer (pH 7.6) and its radioactivity was counted.

For cyclic AMP determination, samples were extracted with 0.1 *N* HCl [8], using a high specific activity ^{3}H-cyclic AMP as internal marker. The nucleotide was measured by the binding protein method of *Gilman* [11].

Cyclic AMP phosphodiesterase activity was assayed [16] as a coupled reaction with 5′-nucleotidase (0.6 units) by using substrate concentrations of 500 or 5 µ*M* when assaying the high or low K_m enzyme, respectively. The product (adenosine) was isolated by using AG 1X2 slurry resin and the supernatant was counted for radioactivity.

RNA was measured by using the method of *Schmidt and Thannhauser* [22] as modified by *Munro and Fleck* [18]. DNA was extracted from alkaline digests with hot acid and assayed by the method of *Webb and Levy* [28]. Proteins were measured [17] by using bovine serum albumin as standard.

Statistical evaluation of the data was done by the Student's t test.

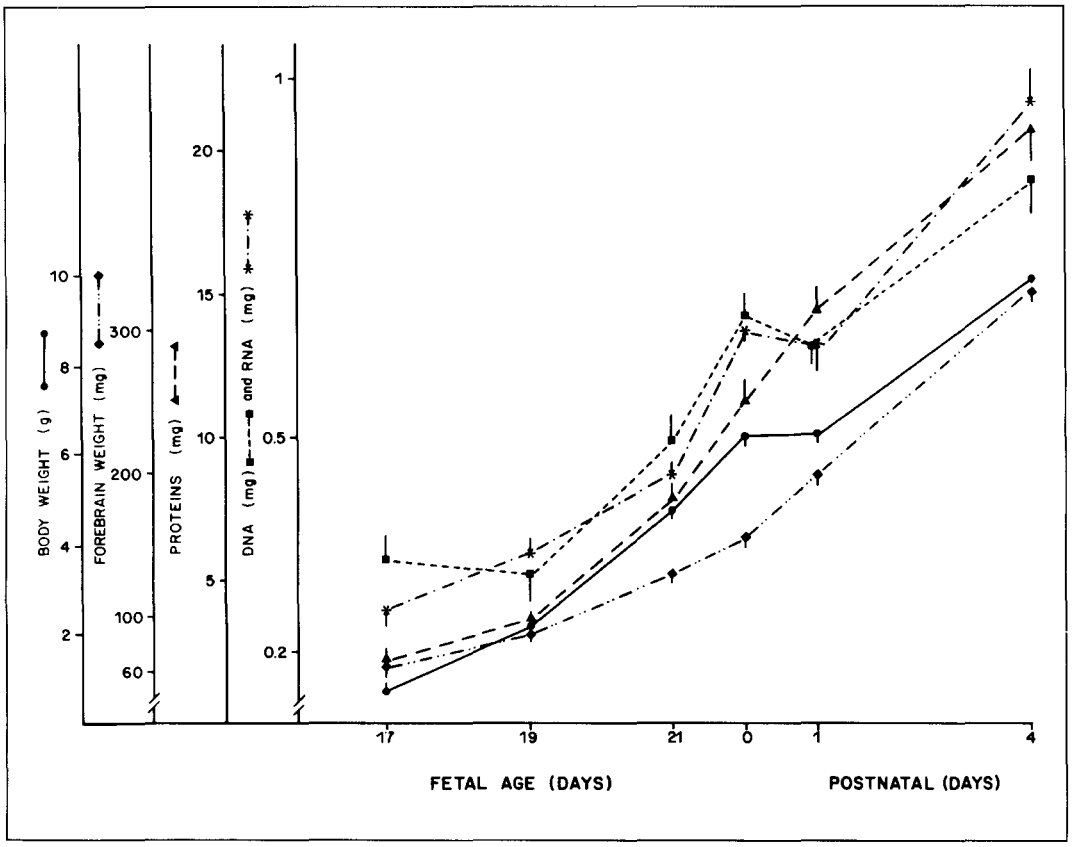

Fig. 1. Body and forebrain weights and forebrain proteins, DNA and RNA content in the rat during the perinatal phase.

Results

Figure 1 shows the body and forebrain weights and forebrain proteins, DNA and RNA concentrations in rat fetuses of 17, 19 and 21 days of gestation and neonates of 0, 1 and 4 days of age. Although certain differences appeared in these parameters during development, their increases remained very parallel and seemed slower during late intrauterine life than soon after birth. The main difference among these parameters appeared at the perinatal phase when the rate of increase in forebrain weight was slower from day 21 of fetal age to day 0 of birth than that of the other parameters and the increase in body weight and in forebrain DNA and RNA concentrations was stopped from day 0 to day 1 of postnatal life. The activities of both adenylate cyclase and cyclic AMP phosphodiesterase and the concentration of cyclic AMP in the forebrain of the fetuses and neonatal rats is shown in table I. All these parameters increased during ontogenesis in the rat forebrain. Adenylate cyclase in the 17-day fetus was only 13% of that found in adults

Table I. Developmental changes of adenylate cyclase, cyclic AMP phosphodiesterase activities and cyclic AMP concentration in rat forebrain (mean ± SEM of 8–12 animals)

Age, days	Adenylate cyclase pmol/min/mg protein	Cyclic AMP phosphodiesterase nmol/min/mg protein		Cyclic AMP concentration pmol/mg tissue
		high K_m	low K_m	
Prenatal 17	76 ± 9	3.5 ± 0.5	0.29 ± 0.02	ND
19	164 ± 10***	4.1 ± 0.6 NS	0.43 ± 0.01**	0.77 ± 0.06 NS
21	170 ± 12 NS	10.4 ± 0.3***	0.42 ± 0.02 NS	1.10 ± 0.15 NS
Neonate	277 ± 18***	10.8 ± 0.6 NS	0.44 ± 0.08 NS	1.48 ± 0.18 NS
Postnatal 1	287 ± 14 NS	11.6 ± 0.7 NS	0.50 ± 0.03 NS	1.06 ± 0.21 NS
4	355 ± 21*	12.6 ± 0.9 NS	0.48 ± 0.03 NS	1.21 ± 0.10 NS
Virgins (adults)	590 ± 23***	52.3 ± 3.3***	1.38 ± 0.10***	1.15[a]

Enzyme activities and nucleotide levels were determined as described in Methods. The statistical significance between every two values during development: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS = not significant. ND = Not determined.

[a] From *Schmidt* et al. [21].

Table II. Adenylate cyclase and cyclic AMP phosphodiesterase activities in mothers' brains during pregnancy (mean ± SEM)

	Adenylate cyclase pmol/min/mg protein	Cyclic AMP phosphodiesterase nmol/min/mg protein	
		high K_m	low K_m
Mothers			
17-day pregnancy	537 ± 40 (7)	40.9 ± 2.8 (6)	1.49 ± 0.14 (4)
19-day pregnancy	504 ± 30 (6)*	45.7 ± 2.9 (7)	1.32 ± 0.05 (4)
21-day pregnancy	470 ± 22 (9)**	45.7 ± 2.2 (10)	1.56 ± 0.18 (7)
1 day postpartum	567 ± 44 (6)	50.8 ± 5.4 (5)	1.50 ± 0.16 (5)
4 days postpartum	637 ± 24 (7)	44.7 ± 1.5 (6)	1.54 ± 0.15 (6)
Virgins	590 ± 23 (9)	52.3 ± 3.3 (6)	1.38 ± 0.10 (6)

Enzyme activities were assayed as described in Methods. The number of animals is given in parentheses. Statistical significance between mothers and virgins: ** $p < 0.01$; * $p < 0.05$.

but it increased rapidly until the time of birth, reaching values at day 0 that were 47% of the amount in adults. During the first 24 h of postnatal life, there was no change in forebrain adenylate cyclase activity, while from 1 to 4 days of age there was another important increment. The cyclic AMP phosphodiesterase activity of high K_m in the forebrain in 17-day fetuses was only 7% of the adult amount and it reached values of 20% in 21-day fetuses. From this time up to 4 days of age there was only a slight increase in this

enzyme activity. The low K_m enzyme showed an activity that was much lower than that of the high K_m at all ages studied, but its values in the 17-day fetuses were 21% that of adults. There was a significant increase in this activity from the 17th to 19th fetal days with no further change until the 4th postpartum day when values were 35% of those in adults. The concentration of cyclic AMP in the forebrain (table I) observed in 19-day fetuses was 67% of that reported by others [21] for adults. The change in this parameter during the perinatal phase was very mild, with a progressive increase until the time of birth and no change thereafter.

Both adenylate cyclase and cyclic AMP phosphodiesterase activities were also measured in the forebrain of mothers and compared with those of female adult virgins. As shown in table II, there was a significant reduction in the activity of adenylate cyclase in the mother's brain at 19 and 21 days of gestation with no other change in this enzyme or in either high or low K_m cyclic AMP phosphodiesterase activity.

Discussion

The slow increase in forebrain weight and DNA and RNA concentrations, observed in fetuses at late gestation, and the fast increase, seen during early postnatal life, are in agreement with the findings of *Brasel* et al. [5], suggesting that, in the rat, there is an initial rapid brain cell proliferation in intrauterine life that lasts until the 16th day of pregnancy and then decreases until birth, after which it again increases. This postnatal cell proliferation is manifested by the marked increase in both DNA and RNA concentrations in the brain from the 1st to the 4th day and corresponds to the intense DNA and RNA synthesis observed during this phase by *Sung* [24]. The retention of DNA and RNA concentrations in the rat brain during the first 24 h of postnatal life deserve some consideration. They appeared together with a parallel body weight increase and could be interpreted as a consequence of the specific metabolic situation occurring at this time. During the first hours of life, blood glucose levels are reduced [10, 12] and blood ketone bodies are not yet augmented [9]. Thus metabolic fuels for the brain become limited and could cause a temporary halt in cell proliferation until those metabolites become available again due to the development of sufficient gluconeogenesis and ketogenesis which occurs after the first few hours of extrauterine life [1, 2].

In the study of ontogenic changes in enzyme activities, the question arises of how to express the data. Here they are expressed per unit of tissue protein as this gives the specific change of the enzyme activity compared with that of other proteins present. Adenylate cyclase is known to be composed of at least three membrane proteins [19] and it plays an important role in synaptic transmission [14]. Here it was found that the activity of that enzyme increased progressively from the 17th day of fetal age until the 4th postnatal day. As far as we know this is the first time these findings during intrauterine life have been reported, with the exception of *Von Hungen* et al. [27] who reported the values of adenylate cyclase activity in the 1-day prenatal rat. The progressive increase in activity found during the postnatal phase was also observed by others [20, 27, 30] who reported that the increase lasted until about the 20th day of age. In some of these experiments, the observed values were lower than those found here, but in one study EGTA was used in the

medium [27] and it is known that this agent reduces adenylate cyclase activity by trapping Ca^{++} ions [26] while in another study [30] no corrections were made for the degradation of cyclic AMP by the phosphodiesterase activity always present in the homogenates. *Schmidt* et al. [20] found that adenylate cyclase activity in the rat brain was not stimulated by norepinephrine until the 3rd to 6th postnatal days. Thus it appears that the enzyme activity in the rat brain is developed earlier than their adrenergic receptors. The decrease in adenylate cyclase activity in the mother's brain at late gestation may be interpreted as the result of increased levels of prostaglandins and oxytocin present at this time in the mother. These substances have been reported to decrease such activity in other preparations [15] and it has been proposed that a similar inhibitory action on adenylate cyclase activity may be functioning on the myometrial receptors' side [6] as a hormonal control of parturition.

Although the ontogenic changes of adenylate cyclase activity in the brain must influence the actual levels of cyclic AMP, these are also influenced by the activity of cyclic AMP phosphodiesterase which controls its degradation. The latter enzyme is known to be composed of multiple forms [23, 31]. It has been proposed that the low K_m enzyme controls the basal cyclic AMP levels [25, 30], while the high K_m would only operate when concentrations of the nucleotide are greatly enhanced. We do not know which of these forms of the enzyme actually influences the changes in cyclic AMP levels in the brain during the perinatal phase in the rat, but the fact that they are very mild in spite of the marked increases in adenylate cyclase activity would suggest that in addition to the basal role of the low K_m enzyme, the high activity and the progressive rise in high K_m play an important role in preventing an unnecessary accumulation of the nucleotide.

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