Biochemical Pharmacology, Vol. 19, pp. 1951–1954. Pergamon Press. 1970. Printed in Great Britain

NEONATAL DIFFERENCES IN THE INDUCTION OF HEPATIC AMINOLEVULINIC ACID SYNTHETASE*

JAMES S. WOODS and ROBERT L. DIXON

Department of Pharmacology, School of Medicine, University of Washington, Seattle, Wash. 98105, U.S.A.

(*Received* 21 *June* 1969; *accepted* 3 *October* 1969)

Abstract—The induction of hepatic aminolevulinic acid (ALA) synthetase, an enzyme postulated to be normally regulated by genetic repression, was studied in neonatal and adult rabbits. The basal activity *in vitro* of ALA synthetase in newborn rabbits is six to eight times that seen in adult animals. Subcutaneous treatment of each age group with the inducing agent, 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC), demonstrated that the neonatal animals were relatively much less responsive to the inducing agent than the adult rabbits. The basal enzyme activity in newborn animals was only doubled while, in contrast, the synthetase activity in adult animals was increased 19-fold. These findings suggest that the regulation of the synthesis of hepatic heme in neonatal animals may be less strictly controlled than in adults. It is suggested that synthesis of ALA synthetase during the neonatal period is semiconstitutive.

THE SYNTHESIS of hepatic delta-aminolevulinic acid (ALA) synthetase, the first and rate-limiting enzyme in the biosynthesis of heme, has been shown in micro-organisms to be controlled by means of both end product inhibition[1] and repression.[2] Evidence has also been presented that in chick embryo liver cells[3] and mammalian liver cells[4] the major mechanism of regulation of ALA synthetase synthesis is through repression by the end product heme. Recently, the induction of hepatic ALA synthetase by a variety of chemical substances has been demonstrated.[5–7] Compounds such as 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC), when given to experimental animals, cause changes in porphyrin levels similar to those seen in persons suffering from acute intermittent porphyria. This disease is characterized by a marked increase in the level of hepatic ALA synthetase, which has been shown to be responsible for the increased synthesis and excretion of porphyrin precursors.[8]

Studies of the sources of bile pigments in the newborn[9] have indicated that bilirubin derived from nonerythropoietic sources constitutes up to 28 per cent of the total bilirubin load and 30 per cent in premature infants. This is nearly twice the amount seen in adults. The majority of these nonerythropoietic heme compounds are produced predominantly by hepatic ALA synthetase. It is therefore interesting to consider the possibility that some of the problems associated with heme metabolism in the neonate, such as physiological jaundice and perhaps the inability to metabolize various drugs, may be related to differences in the ability to regulate the activity of this enzyme. Previous experiments in this laboratory have shown that the normal activity of hepatic ALA synthetase during the perinatal period of several species is four to eight times

* This research was supported in part by USPHS Grants T01-Gm 01458 and HD 02350.

greater than that of the adult. This study was undertaken to determine if the age of the animal has an effect on the degree to which ALA synthetase can be induced.

MATERIALS AND METHODS

Preparation of the animals. Adult and newborn Dutch rabbits were used in these experiments. Pregnant animals were received several days prior to delivery and were housed in individual cages equipped with removable kindling boxes. Breeding dates were obtained from the supplier.

Induction of ALA synthetase. DDC was purchased from Eastman and used after recrystallization from ethanol–water. Adult and newborn animals were originally divided into four groups of two animals each. Treatments were performed according to the following schedule: (1) daily for 3 days; (2) daily for the last 2 days; (3) once on the third day; (4) ethanol daily. DDC was injected subcutaneously only once in the day(s) indicated at a dose of 500 mg/kg. DDC was dissolved in ethanol. Twenty-four hours after the last treatment, all animals were sacrificed by cervical dislocation and the livers were removed for analysis of ALA synthetase activity.

Enzyme assay. Liver homogenates were prepared according to Marver *et al.*[10] Livers were washed and homogenized with 3 vol. of 0·9% NaCl solution containing 0·5 m-moles EDTA and 10 m-moles Tris buffer adjusted to pH 7·4. Each incubation mixture was 2 ml and contained: 0·5 ml homogenate, 200 μmoles glycine, 20 μmoles EDTA, 0·4 μmole pyridoxal phosphate, 150 μmoles Tris-HCl buffer adjusted to pH 7·2. The 2-ml incubation mixture was placed in 25-ml flasks and incubated in air at 37° for 1 hr. Six of the 2-ml incubation flasks were combined for each ALA determination. Duplicate mixtures were prepared and used without incubation for a determination of background readings. All samples (12 ml total) were precipitated using 4·5 ml of a mixture (8:1) of 1 M acetate buffer (pH 4·6) and 1 N HCl, and heating for 3 min in boiling water. The solutions were cleared by centrifugation. The quantity of ALA in 10 ml of deproteinized solution was determined as follows: Two columns were arranged so that the solution flowed from one to the other according to Davis and Andelman.[11] The top column contained an anion-exchange resin (Bio-Rad AG 1-X8) which retained the porphobilinogen. The ALA was retained by the second column (lower) which contained a cation-exchange resin (Bio-Rad AG 50W-X4). The upper column was discarded and the ALA eluted from the lower column with 1 M sodium acetate. ALA was then determined colorimetrically as described by Mauzerall and Granick.[12] The synthesis of aminoacetone is inhibited 90 per cent by these conditions of incubation.

RESULTS AND DISCUSSION

Table 1 presents the effect of ethanol treatment and age on the production of ALA by fortified liver homogenate. It can be seen that ethanol pretreatment had no effect on enzyme activity. Ethanol was used to solubilize DDC for treatment of animals in the induction studies. In contrast, the effect of age was very dramatic. Newborn rabbits had enzyme activity seven to eight times that of adult animals. The perinatal development of ALA synthetase activity has been reported recently.[13]

Table 2 demonstrates that newborn rabbits are much less responsive to the inducing effect of DDC than adult animals, while the maximal induced ALA synthetase levels in both newborn and adult rabbits are similar. Adult and newborn rabbits were

treated with either ethanol or DDC, once, twice or three times before enzyme assay. The last treatment was always approximately 24 hr before determinations *in vitro*. It can be seen that with adult animals the activity of the enzyme after a single DDC treatment increased 220 per cent more than control activity. After two treatments this increase was 1598 per cent, and after three treatments the increase was 1783 per cent. In contrast, single DDC treatment of newborn rabbits results in only a 22 per cent increase. A second treatment caused a 100 per cent increase, but the subsequent DDC

TABLE 1. EFFECT OF AGE ON ALA SYNTHETASE ACTIVITY

	Age groups†	
Treatments*	Adult	Newborn
None	4·1 ± 1·4 (5)‡	33·2 ± 5·4 (4)
Ethanol	4·0 ± 1·1 (5)	30·9 ± 2·7 (4)

* There is no significant difference between treatments in either group.
† Newborn activity is significantly greater ($P < 0.05$) than adult activity with both treatments.
‡ Values are means ± standard deviations and express metabolism as mμmoles ALA/g liver/hr. The number of individual animals is shown in parentheses.

TABLE 2. DDC INDUCTION OF ALA SYNTHETASE IN NEWBORN AND ADULT RABBITS

Treatment* (days)	Adult	% Increase	Newborn	% Increase
No DDC	4·0 ± 1·1 (5)		30·9 ± 2·7 (4)	
DDC, 1	12·8† (11·2, 14·4)	220	37·6 (27·2, 48·0)	22
DDC, 2	67·9 (65·3, 70·4)	1598	61·9 (67·6, 56·1)	100
DDC, 3	75·3 (83·7, 66·8)	1783	65·6 (74·1, 57·2)	112

* Animals were treated subcutaneously daily with either ethanol or DDC for 1, 2 or 3 days. The last treatment was always approximately 24 hr before determination of enzyme activity *in vitro*.
† Values are means of two experiments with the actual values presented in parentheses. Values express metabolism as mμmoles ALA/g liver/hr. Per cent increase is the increase in activity greater than control expressed as a per cent of control.

treatment had very little additional effect. The fact that the maximum activity after DDC treatment of newborn and adult rabbits was nearly identical might also indicate that the inherent capacity of the enzyme systems to be induced had been achieved.

A recent abstract by Song *et al.*[14] reported that newborn rats are refractory to ALA synthetase induction by DDC and allylisopropylacetamide, another potent porphyria-inducing agent.

Newborn animals have higher basal levels of ALA synthetase than do adult animals, but respond relatively much less to the inducing agent DDC. This fact indicates that the regulation of hepatic ALA synthetase in the newborn rabbit is not as well developed as in the adult animal, and suggests an interesting analogy with the proposed etiology of hepatic porphyria.[3] It has been suggested that the mechanism underlying acute

intermittent porphyria is a mutation which has produced a defective, poorly repressed, operator gene. The affected individual appears normal at most times, but is subjected to episodes characterized by the overproduction of porphyrin precursors after treatment with porphyria-inducing drugs. This overproduction of porphyrin precursors is associated with an elevated ALA synthetase activity. Based on the findings of these experiments, one can visualize a condition in the neonatal rabbit characterized by an underdeveloped system for the regulation of the synthesis of this enzyme which is compatible with the data presented here. The enhanced basal level of ALA synthetase and the decreased effect of DDC as an inducer suggest an operator gene with decreased responsiveness to both repression and induction. This poorly responding enzyme system might be described as semiconstitutive. Further studies are planned of the regulation of this enzyme system in perinatal animals.

REFERENCES

1. B. F. BURNHAM and J. LASCELLES, *Biochem. J.* **87**, 462 (1963).
2. J. LASCELLES, *J. gen. Microbiol.* **23**, 487 (1960).
3. S. GRANICK, *J. biol. Chem.* **241**, 1359 (1966).
4. N. HAYASHI, B. YODA and G. KIKUCHI, *J. Biochem.* **63**, 446 (1968).
5. S. GRANICK and G. URATA, *J. biol. Chem.* **238**, 821 (1963).
6. D. P. TSCHUDY, F. H. WELLAND, A. COLLINS and G. HUNTER, JR., *Metabolism* **13**, 396 (1964).
7. H. S. MARVER, D. P. TSCHUDY, M. G. PERLROTH and A. COLLINS, *Fedn Proc.* **24**, 721 (1965).
8. D. P. TSCHUDY, M. G. PERLROTH, H. S. MARVER, A. COLLINS, G. HUNTER, JR. and M. RECHCIGL, JR., *Proc. natn. Acad. Sci. U.S.A.* **53**, 841 (1965).
9. S. SCHWARTZ, G. IBRAHIM and C. J. WATSON, *J. Lab. clin. Med.* **64**, 1003 (1964).
10. H. S. MARVER, D. P. TSCHUDY, M. G. PERLROTH and A. COLLINS, *J. biol. Chem.* **241**, 2803 (1966).
11. J. R. DAVIS and S. L. ANDELMAN, *Archs environ. Hlth* **15**, 53 (1967).
12. D. MAUZERALL and S. GRANICK, *J. biol. Chem.* **219**, 435 (1956).
13. J. S. WOODS and R. L. DIXON, *Life Sci.* (in press)
14. C. S. SONG, J. W. SINGER, R. D. LEVERE, D. F. HARRIS and A. KAPPAS, *J. Lab. clin. Med.* **72**, 1019 (1968).