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PRECOCIOUS INDUCTION OF HEPATIC ANILINE HYDROXYLASE AND AMINOPYRINE *N*-DEMETHYLASE WITH HYDROCORTISONE IN NEONATAL RAT*

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Abstract—The pre- and postnatal development of hepatic aniline hydroxylase and aminopyrine *N*-demethylase activities was studied in rats in order to identify the natural trophic factors, if any, responsible for early neonatal formation of the enzymes. The postnatal development of these two enzymes up to 3 weeks of age was comparable with that of cytoplasmic tyrosine aminotransferase. They could be precociously induced in 7-day-old rats by hydrocortisone. Cycloheximide inhibited their induction.

HUMAN neonates appear to have only a limited capacity to metabolize drugs which require microsomal biotransformation before being excreted.[1] Similarly, new-born rabbits are not able to metabolize hexobarbital, aminopyrine, amphetamine, acetanilide, chlorpromazine or 4-nitrobenzoic acid, although the oxidative activity of the enzymes mediating the metabolism of these drugs rises to near adult levels at 4 weeks of age.[2] The relative immaturity of drug metabolizing enzymes in young animals has been the subject of many investigations.[3–13] However, no attempt has been made to explore the reasons for the low activity or to identify the naturally occurring trophic factors responsible for development of the relevant enzymes. The present report on developmental formation of aniline hydroxylase and aminopyrine *N*-demethylase in rats shows that the appearance of these enzymes is dependent on the activation of the adrenal pituitary axis. This is substantiated by the fact that precocious induction of these enzymes can be achieved by hydrocortisone in 7-day-old rats.

MATERIALS AND METHODS

Rats of the Charles Foster strain (Central Drug Research Institute) were separated from the colony at parturition and housed with their mothers. The young were weaned 21 days after birth. The sexes were separated about 5 days after birth and the male rats were used for further study. The foetuses were delivered surgically. The livers excized, minced and homogenized in 4 vol. of chilled 250 mM sucrose, using a Potter–Elvehjem homogenizer fitted with a motor-driven Teflon pestle. The homogenates were centrifuged at 9000 *g* at 0 for 20 min. The supernatants were used as the enzyme source.

Enzyme assays. Tyrosine aminotransferase was assayed according to the procedure of Diamondstone.[14] The activity of aminopyrine *N*-demethylase was estimated by measuring the amount of formaldehyde formed (Nash[15]) according to the method

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of Cochlin and Axelrod.[16] Aromatic hydroxylation of aniline was determined by measuring the 4-aminophenol formed (Kato and Gillette).[17] Protein was estimated according to the procedure of Lowry *et al.*,[18] using bovine plasma albumin as a standard.

NADP was purchased from C. F. Boehringer & Soehne GmbH, Mannheim, glucose-6-phosphate from Sigma, and isocitrate from Biochemical Unit, V.P. Chest Institute, Delhi. Aminopyrine was a gift from Dr. Gautum of Indian Drugs and Pharmaceuticals Ltd., Hyderabad, India. All other reagents used were analytically pure.

RESULTS AND DISCUSSION

Developmental formation of the enzymes. Representative values of the activities of hepatic tyrosine aminotransferase, aniline hydroxylase and aminopyrine *N*-demethylase in the developing rat are given in Table 1. Tyrosine aminotransferase starts to

TABLE. 1. DEVELOPMENTAL FORMATION OF HEPATIC TYROSINE AMINOTRANSFERASE, ANILINE HYDROXYLASE AND AMINOPYRINE *N*-DEMETHYLASE IN THE RAT

Age (days)	Enzyme activity (units*/mg protein)		
	Tyrosine aminotransferase	Aniline hydroxylase	Aminopyrine *N*-demethylase
−4	0·8 ± 0·1 (3)	N.D.	N.D.
−2	0·9 ± 0·2 (3)	N.D.	N.D.
0	16·3 ± 1·4 (4)	8·0 ± 0·6 (3)	5·3 ± 0·9 (4)
4	6·8 ± 0·4 (4)	38·0 ± 2·0 (4)	6·6 ± 1·7 (4)
10	8·3 ± 0·2 (4)	228·0 ± 20·0 (4)	7·2 ± 1·9 (4)
21	22·3 ± 3·4 (4)	264·0 ± 31·0 (4)	13·0 ± 1·9 (4)
56	15·6 ± 2·2 (6)	173·0 ± 15·0 (6)	17·9 ± 3·6 (4)
84	10·7 ± 1·6 (4)	66·0 ± 5·0 (3)	20·3 ± 2·1 (4)
180	16·4 ± 1·2 (4)	29·0 ± 6·0 (3)	17·7 ± 1·4 (4)

* *n*-Moles product formed/min at 37 in the case of tyrosine aminotransferase and aminopyrine *N*-demethylase and *p*-moles product formed per min in the case of aniline hydroxylase. Values are expressed as the mean ± S.D. for the number of experiments in parentheses.

N.D.—Not detectable.

For each estimation: livers from all the foetuses of a litter were pooled; at parturition livers from six neonates were pooled; at 4 days of age livers from four rats were pooled; two livers were pooled from 10-day-old animals and single livers were used for the animals of subsequent ages.

appear in late foetal life and attains a significant level around the 15th day of gestation. The activity increases up to the 20th day, shows a peak at parturition, falls for the following 4 days and then increases during the 2nd week of life. At the onset of puberty and thereafter the activity decreases. There appears to be a correlation between the endocrine status of the animal at different ages and tyrosine aminotransferase activity. Hypoglycemia associated with parturition is known to lead to secretion of glucagon from the alpha cells of the pancreas and, therefore, glucagon may be the stimulant at birth.[19,20] Activation of the adrenal pituitary axis during the second week of postnatal life of the rat[21] coincides with the stimulation of hepatic tyrosine aminotransferase actvity and hydrocortisone is known to be the natural stimulant at this age.[19]

In contrast to tyrosine aminotransferase, aniline hydroxylase activity is not detectable during foetal life and is barely detectable in neonatal rat. Activity appears on the 4th day after parturition and rises to a maximum the 2nd week after birth. This is comparable with the development of tyrosine aminotransferase. Activation of the adrenal pituitary axis may also be required for the synthesis of this enzyme. Like tyrosine aminotransferase, aniline hydroxylase also shows a gradual decline in activity with the onset of puberty and senescence.

The developmental pattern of aminopyrine *N*-demethylase is similar to that of aniline hydroxylase until the 21st day of life. Activation of the adrenal pituitary axis and elaboration of hydrocortisone could be responsible for the first burst in activity of this microsomal enzyme. However, unlike tyrosine aminotransferase and aniline hydroxylase, the demethylase activity increases gradually until puberty, levels off as the animal attains adulthood and decreases with senscence. Besides the adrenal pituitary axis, which triggers the demethylase activity in the neonates, male sex hormones may also be the natural trophic factors at puberty.

TABLE 2. PRECOCIOUS INDUCTION OF HEPATIC ANILINE HYDROXYLASE AND AMINOPYRINE *N*-DEMETHYLASE WITH HYDROCORTISONE IN THE 7-DAY-OLD RAT

Treatment	Units/mg protein	
	Aminopyrine *N*-demethylase	Aniline hydroxylase
Single dose of:		
Normal saline	4·0 ± 0·4	180 ± 19
Hydrocortisone	5·0 ± 0·7	225 ± 16
Two consecutive doses of:		
Normal saline	4·5 ± 0·4	230 ± 30
Hydrocortisone	8·1 ± 0·6	360 ± 32

Each value is the mean of four experiments ± S.D. (livers of two rats were pooled for each estimation). For single dose experiments the rats were killed 24 hr after the administration of saline or hydrocortisone (5 mg/100 g body wt) and for two consecutive dose experiments a repeat dose of saline or hydrocortisone was administered to the animals 24 hr after the first. The animals were killed 24 hr after the final injections.

Precocious induction of the microsomal enzymes. From the pattern of development of aniline hydroxylase and aminopyrine *N*-demethylase, it would appear that hydrocortisone is a primary natural stimulus for increasing the activities of these enzymes. To confirm this, we examined the possibility that these two enzymes could be induced precociously in the 7-day-old rat. The results of these experiments are presented in Table 2. Aniline hydroxylase and aminopyrine *N*-demethylase were both induced about twofold in the 7-day-old rat by two consecutive injections of hydrocortisone. These results suggest that hydrocortisone is indeed the triggering agent for the development of these drug metabolizing enzymes.

Effect of cycloheximide on precocious induction of the enzymes. Hydrocortisone may cause the above effect either by stimulating the conversion of the enzyme precursor into an active enzyme or by stimulating *de novo* synthesis of the enzymes. Our results

(Table 3) suggest that hydrocortisone stimulates *de novo* synthesis of the enzymes. Seven-day-old rats were divided into four groups: the first group served as a control and was injected with normal saline twice with 24 hr interval in between; the second group received two injections of hydrocortisone in normal saline (the second being administered 24 hr after the first); the third group was injected with cycloheximide 30 min before each injection of normal saline, while the fourth group received hydrocortisone 30 min after cycloheximide. From the results it can be seen that cycloheximide completely inhibits the induction of aniline hydroxylase and aminopyrine *N*-demethylase.

TABLE 3. INHIBITION OF THE PRECOCIOUS INDUCTION OF ANILINE HYDROXYLASE AND AMINOPYRINE DEMETHYLASE BY CYCLOHEXIMIDE IN THE 7-DAY-OLD RAT

Treatment	Units/mg protein	
	Aminopyrine *N*-demethylase	Aniline hydroxylase
Normal saline	5·0 ± 0·6	225 ± 27
Hydrocortisone	8·2 ± 0·9	340 ± 33
Cycloheximide	4·0 ± 0·3	175 ± 18
Cycloheximide + hydrocortisone	4·2 ± 0·4	188 ± 20

Each value is the mean of four experiments ± S.D. (livers of two rats pooled for each estimation). Two consecutive doses of saline, hydrocortisone, cycloheximide or cycloheximide plus hydrocortisone were given as described under Table 2. Cycloheximide (1 mg/100 g body wt) was given 30 min prior to the administration of hydrocortisone or normal saline. The animals were killed 24 hr after the last injection.

Hydrocortisone thus appears to be an important trophic factor in the differentiation of the hepatocyte with regard to the development of these two drug metabolizing enzymes. Similar studies with other drugs, particularly those used in pediatric practice, may help to find the cause of certain untoward reactions associated with them, such as chloroamphenicol, and may also help in finding remedies by combination therapy or otherwise.

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