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DECREASE OF PHENYLALANINE HYDROXYLASE DURING HEPATOCYTE PROLIFERATION

R. MANJUNATH, VIDYA SHIVASWAMY and T. RAMASARMA*
Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India

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Abstract—In partially hepatectomized rats, the activity of phenylalanine hydroxylase decreased in the regenerating liver but not in the kidney. The concentration of corticosterone in the plasma of hepatectomized rats increased, and phenylalanine hydroxylase, despite being cortisol inducible, decreased in these as well as simultaneously adrenalectomized rats, showing lack of correlation between the changes of the steroid and the enzyme during the regeneration process. The decrease in the enzyme activity could be prevented by administering, during hepatic regeneration, only noradrenaline and adrenergic blocking agents, among the many hormones and phenyl compounds tested. A decrease in hepatic phenylalanine hydroxylase was also observed during two other conditions of hepatocyte cell proliferation obtained after giving chlorophenoxyisobutyrate and α-hexachlorocyclohexane.

The activity of phenylalanine hydroxylase in the liver increases in rats treated with cortisol [1–4]. This enzyme shows a delayed response compared to tryptophan pyrrolase and tyrosine aminotransferase, maximum increase being obtained 24 hr after the administration of the steroid [4]. Exposure of rats to low atmospheric pressure (0.5 atm) for a period of 24 hr also increases the activity of this enzyme, which is traceable to the concomitant increase in corticosteroids [5]. Giving a diet low in phenylalanine [6] and administering *p*-chlorophenylalanine [7] are the two conditions known to decrease phenylalanine hydroxylase and, interestingly, simultaneous administration of phenylalanine reverses the effect of *p*-chlorophenylalanine [8].

Liver, regenerating after partial hepatectomy, has been found to have low phenylalanine hydroxylase activity, the first case of such a decrease under conditions of hepatocyte proliferation [9]. It was of interest to investigate whether this decrease is due to changes in the concentration of adrenal and other hormones or to endogenous phenylalanine derivatives and also its occurrence in other conditions of cell proliferation. The results obtained suggest that decreased phenylalanine hydroxylase accompanies hepatocyte proliferation, that this decrease is not due to a change in cortisol, and that it is prevented specifically by noradrenaline and by adrenergic blocking agents but not by many phenyl compounds and hormones tested, including growth hormone.

MATERIALS AND METHODS

Chemicals. The compounds used in the experiments were obtained from the following sources: noradrenaline, adrenaline and cinnamate from Koch-Light Laboratories, Colnbrook, Bucks, U.K.; practalol and chlorophenoxyisobutyrate from Imperial Chemical Industries, Macclesfield, Cheshire, U.K.; phenoxybenzamine from Smith Kline & French (India), Bangalore; α-hexachlorocyclohexane from Dr. D. S. R. Sarma, University of Toronto, Toronto, Canada; and all other chemicals from the Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals and treatment. Male albino rats of the Wistar strain, weighing 140–160 g, from the Central Animal Facility of the Institute were used. The animals were fed *ad lib.* on a pellet diet obtained from Hindustan Lever, Bombay, India, containing 24% protein, 4% fat, 50% carbohydrate, and other nutrients. Water was given *ad lib.* Partial hepatectomy was done under ether anaesthesia normally between 10:00 a.m. and noon, and the rats were killed also during the same period by cervical dislocation, 48 hr after the operation unless otherwise specified. Adrenalectomy was done by the dorsal approach under Nembutal anaesthesia and the animals were given 0.9% NaCl to drink. The experiments on these animals were conducted after 3 days. Four or more rats were used in each group and the means ± S.D. were employed to calculate P values by Student's *t*-test. Because control values varied on different days, appropriate control of rats were killed simultaneously in each experiment, and the results were compared with the experimental group processed the same day. All compounds were administered intraperitoneally in 0.9% NaCl either as solution or suspension, unless otherwise specified; the control group received equivalent amounts of 0.9% NaCl.

Assay of the enzymes. Phenylalanine hydroxylase was assayed as described by Bublitz [10] with modifications adopted by Namboodiri and Ramasarma [11]. The activity is expressed as nmoles/min per mg protein. Tyrosine hydroxylase was measured by the method of Nagatsu *et al.* [12]. Tryptophan hydroxylase was measured according to Inamdar *et al.* [13]. Protein was estimated by the biuret method [14].

* Author to whom all correspondence should be addressed.

RESULTS

Effect of partial hepatectomy on some hydroxylases. In the previous report, we showed that phenylalanine hydroxylase in the liver decreased maximally 48 hr after partial hepatectomy [9], at which time little change was found in tryptophan pyrrolase or tyrosine aminotransferase activity. To study the specificity of this effect, the activities of phenylalanine hydroxylase in the regenerating liver and the kidney, tryptophan hydroxylase in the regenerating liver, and tyrosine hydroxylase in the adrenals were measured in the same group of animals 48 hr after partial hepatectomy. The results in Table 1 confirm the decrease in phenylalanine hydroxylase in the regenerating liver tissue, while other hydroxylase activities tested showed small but not statistically significant changes.

The mean specific activity of phenylalanine hydroxylase in the liver lobes removed by partial hepatectomy ranged between 6.2 and 10.6 nmoles/min per mg protein in about twenty different groups of animals. With these serving as the controls, the regenerating liver tissue from the same groups of animals showed enzyme activities between 45 and 74% (average 54%) of the control values, and the changes were statistically significant in each experiment. Among the enzymes and tissues tested only phenylalanine hydroxylase activity in the regenerating liver tissue was found to decrease.

Lack of correlation with changes in corticosterone. During partial hepatectomy, the animal experiences surgical stress and a change in enzyme concentration might indirectly result through a change in the "stress hormone", corticosterone. This was verified by measuring the concentration of corticosterone in the plasma of rats operated on for partial hepatectomy. The data in Table 2 show that plasma corticosterone had increased 2-fold by 24 hr after partial hepatectomy, at which time the enzyme concentration showed no significant change. At 48 hr after partial hepatectomy, the corticosterone concentration was not higher than the control (statistically not significant) but the enzyme had decreased considerably. It had been found previously that cortisol retained the ability to induce the enzyme when injected (2.5 mg/rat, i.p.) before partial hepatectomy or during regeneration [9]. There must, therefore, have been some other factor in the regenerating tissue to prevent the enzyme from increasing. The mitotic activity was recorded in the livers of these animals and was found to be high at 24 hr and to have decreased thereafter (Table 2) in agreement with the previous report [15]. The decrease in phenylalanine hydroxylase was preceded by mitotic activity and coincided with the period of maximum weight increase of the tissue.

Effect of adrenalectomy. A simultaneous increase in corticosterone, an inducer of phenylalanine hydroxylase, might have counteracted the effect of a decrease in this enzyme during the early period of the regeneration process. Therefore, the effect was studied in adrenalectomized animals. The results in Table 3 show that the decrease in the enzyme in the regenerating liver appeared to be more pronounced in adrenalectomized animals. It is interesting to note that adrenalectomy by itself has no effect on the basal activity of the enzyme [5]. Thus, while the basal activity was independent of the concentration of corticosterone, absence of this hormone in partially hepatectomized and adrenalectomized animals may have potentiated the decrease obtained during regeneration.

Two other cortisol-induced hepatic enzymes, tyrosine aminotransferase and tryptophan pyrrolase, were also measured in the same set of animals to serve as markers. These enzymes showed no significant changes at 48 hr either after partial hepatectomy or after adrenalectomy, confirming earlier observations [9, 16, 17]. In the regenerating liver in adrenalectomized animals, the three enzymes showed differential responses (Table 3)—59%

Table 1. Effect of partial hepatectomy on the enzymes phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase*

Tissue	Enzyme	Enzyme activity (nmoles/min per mg protein)		
		Control animals	Animals with regenerating liver	% Control
Liver	phenylalanine hydroxylase	10.60 ± 0.90	7.90 ± 0.40	74†
Kidney	phenylalanine hydroxylase	0.76 ± 0.04	0.66 ± 0.08	87
Liver	tryptophan hydroxylase	0.23 ± 0.01	0.19 ± 0.02	84
Adrenal	tyrosine hydroxylase	0.12 ± 0.03	0.13 ± 0.02	107

* The animals were partially hepatectomized and their livers were allowed to regenerate for 48 hr. The tissues indicated were removed from these and from control animals, and the enzymes were assayed as given in Materials and Methods. The values are means ± S.D. of independent analysis of four rats in each group.

† $P < 0.05$.

Table 2. Effect of partial hepatectomy on the concentrations of hepatic phenylalanine hydroxylase and plasma corticosterone and on hepatic mitotic activity*

Time after partial hepatectomy	Hepatic phenylalanine hydroxylase (nmoles/min per mg protein)	Plasma corticosterone (μg/100 ml)	Hepatic mitotic activity (% dividing cells)
Control	7.4 ± 1.1	7.5 ± 2.6	0.04 ± 0.01
24	6.6 ± 1.0	14.5 ± 4.3†	17.60 ± 1.7†
48	3.3 ± 0.6†	9.6 ± 2.0	8.0 ± 0.7†

* Groups of rats were partially hepatectomized and the animals were killed at 24 or 48 hr; the regenerated livers were removed and blood was collected from heart. The plasma corticosterone was assayed as given in Materials and Methods. Mitotic activity is represented as the number of cells undergoing division per 100 cells. A total of 3000 cells at least were counted in each liver sample. The values are the means ± S.D. of independent analysis of four rats in each group.

† $P < 0.01$.

Table 3. Effect of adrenalectomy on the changes in the activities of phenylalanine hydroxylase, tryptophan pyrrolase, and tyrosine aminotransferase during liver regeneration*

Treatment	Enzyme activity (nmoles/min per mg of protein)		
	Normal liver	Regenerating liver	% of Normal liver
Phenylalanine hydroxylase			
Control	6.2 ± 0.6	3.7 ± 0.5	60†
Adrenalectomized	6.1 ± 0.4	2.5 ± 0.6	41‡
Tyrosine aminotransferase			
Control	8.6 ± 1.8	9.5 ± 1.0	110
Adrenalectomized	10.2 ± 0.8‖	14.9 ± 3.3‖	146§
Tryptophan pyrrolase			
Control	0.37 ± 0.03	0.32 ± 0.02	86
Adrenalectomized	0.35 ± 0.05	0.21 ± 0.05	61§

* Adrenalectomy was performed by the dorsal approach under ether anaesthesia. Animals were fed *ad lib.* and given 0.9% NaCl to drink. After 3 days, partial hepatectomy was performed, and the livers were allowed to regenerate for 48 hr. The values are the specific activities, means ± S.D.

† $P < 0.01$, compared to normal control.

‡ $P < 0.01$, compared to adrenalectomized-normal liver.

§ $P < 0.05$, compared to adrenalectomized-normal liver.

‖ $P < 0.05$, compared to control without adrenalectomy.

decrease in phenylalanine hydroxylase, 46% increase in tyrosine aminotransferase, and 39% decrease in tryptophan pyrrolase.

Effect of noradrenaline and other hormones. Noradrenaline, another hormone known to increase under conditions of stress, acts as a competitive inhibitor of phenylalanine hydroxylase [18]. It was found previously that noradrenaline, given along with pargyline before partial hepatectomy in four doses at 6-hr intervals, had no effect on phenylalanine hydroxylase in the livers of control animals or in the regenerating liver [9]. Rat growth hormone (0.2 mg/rat, two doses), serotonin (1.5 mg/rat), adrenaline (0.5 mg/rat), thyroxine + insulin (0.25 mg + 1 I.U./rat), estradiol (2 mg/rat, two doses), and glucagon (0.4 mg/rat) (injected intraperitoneally) given to normal animals had no effect on phenylalanine hydroxylase (data not shown). In another type of experiment in which noradrenaline was given in four doses at 6-hr intervals *during the 24–48 hr period of regeneration*, the enzyme activity in the treated livers increased significantly compared to the untreated, regenerating livers (Table 4). This result indicates that a regeneration-effected decrease in hepatic phenylalanine hydroxylase was prevented by continued treatment with noradrenaline during the process of liver regeneration. In the next set of experiments, the effects of adrenergic blocking agents were tested. Given in two doses during the first 24 hr of the regeneration process, both phenoxybenzamine (α-agent) and practalol (β-agent) were able to prevent the decrease in phenylalanine hydroxylase activity at 48 hr after hepatectomy (Table 4). These results prompt the interpretation that covering the adrenergic receptors either by noradrenaline or a blocking agent prevents the decrease in the enzyme activity obtained during the process of regeneration. However, this explanation may not

Table 4. Effect of noradrenaline and adrenergic blocking agents on phenylalanine hydroxylase in normal and regenerating liver*

Treatment	mg/rat	Phenylalanine hydroxylase (nmoles/min per mg protein)		
		Normal liver	Regenerating liver	% of Normal liver
None		8.4 ± 0.6	6.1 ± 0.5	73†
Noradrenaline	0.1 (4 doses)		8.9 ± 0.3	106
None		8.5 ± 0.6	5.0 ± 2.0	58†
Isoproterenol	4 (2 doses)		6.8 ± 1.4	80
Practalol	5 (2 doses)		9.0 ± 1.4	106
None		8.7 ± 0.8	6.0 ± 1.5	69†
Phenoxybenzamine	10 (2 doses)	11.0 ± 1.0	11.1 ± 3.2‡	101

* Groups of rats were partially hepatectomized and killed 48 hr after operation in all the cases. All the drugs were administered in 0.9% saline, in two or more doses at specified intervals after partial hepatectomy: noradrenaline—24, 30, 36 and 42 hr; isoproterenol, practalol and phenoxybenzamine—4 and 24 hr. The values are specific activity, means ± S.D.

† $P < 0.05$, compared to normal liver.

‡ $P < 0.05$, compared to untreated control.

be valid in view of the lack of a similar effect with isoproterenol, an analogue of noradrenaline, with agonistic action and the ability to activate the adrenergic receptors with greater efficiency (Table 4). Phenoxybenzamine, given similarly to normal rats, also increased phenylalanine hydroxylase (Table 4) as well as tryptophan pyrrolase in the liver. Absence of this effect in adrenalectomized rats (data not shown) suggests that corticosteroids may have a role in this action of phenoxybenzamine.

Effect of drugs causing hypertrophy. Regeneration of the liver is a case of hepatocyte proliferation, and the decrease in phenylalanine hydroxylase, a catabolic enzyme, may be related to the cell proliferation process. In this context it is important to note that phenoxybenzamine, which counteracted the enzyme decrease during regeneration, also has the ability to delay the initiation of DNA synthesis [19]. It was therefore of interest to study other conditions of hepatocyte proliferation on phenylalanine hydroxylase. Two drugs causing this effect were selected—chlorophenoxyisobutyrate [20] and α-hexachlorocyclohexane [21]; their administration led to a significant decrease in phenylalanine hydroxylase (Table 5).

Effect of phenylalanine and related compounds. The endogenous effector responsible for the decrease in phenylalanine hydroxylase in the regernating liver may bear a relationship to catecholamines in view of the counteracting effect of noradrenaline. A similar situation had been noted previously in the case of phenylalanine counteracting the decrease of this enzyme on treatment with *p*-chlorophenylalanine [8]. In the present experiments on regenerating liver, treatment with phenylalanine and the related compounds, cinnamic acid, *p*-coumaric acid and norfenfluramine, failed to reverse the decrease in the enzyme (data not shown). Phenylalanine, *p*-chlorophenylalanine and *O*-methyl tyrosine, on the other hand, potentiated the effect obtained during regeneration. Therefore, the mechanism of decrease in the enzyme on *p*-chlorophenylalanine treatment appears to be different from that in regeneration, and phenylalanine may not be the source for the active compounds.

Table 5. Effect on phenylalanine hydroxylase of drugs causing hypertrophy

Treatment		Enzyme activity (nmoles/min per mg protein)		
Compound	Days	Control	Treated	% Control
Chlorophenoxyisobutyrate	3	8.2 ± 0.5	7.6 ± 0.6	93
	7	7.2 ± 1.6	5.1 ± 0.3	71†
	14	7.1 ± 0.3	5.2 ± 0.3	73†
Hexachlorocyclohexane	2	7.2 ± 0.8	5.5 ± 0.3	76†
	4	9.1 ± 1.5	5.1 ± 1.4	56†

* Groups of animals were fed with the standard diet containing chlorophenoxyisobutyrate (0.5%, w/w) for various periods of time as specified, and the control animals received a standard diet. α-Hexachlorocyclohexane was sonicated in groundnut oil (30 mg/ml) and was administered intraperitoneally (0.5 ml/rat each day) while the control animals received only the oil. The values are specific activity, means ± S.D.

† $P < 0.01$.

DISCUSSION

Decrease in the catabolic enzyme, phenylalanine hydroxylase, during hepatic proliferation seems appropriate for the economy of the cells. The decrease in the hepatic enzyme activity during liver regeneration and in animals treated with chlorophenoxyisobutyrate and α-hexachlorocyclohexane amply supports this view. It must be pointed out that the decrease in the present experiments probably represents the enzyme concentrations and not the changes in the reversible phosphorylated forms discovered by Donlon and Kaufman [22] since the synthetic cofactor used in the present experiments will estimate the total activity of the enzyme.

The two mitogenic compounds tested are both chloro-substituted and unnatural. While they gave supporting evidence, along with partial hepatectomy, they must have been acting indirectly through modification of some cellular process. It seems likely that the effector modulating phenylalanine hydroxylase is a metabolite that is altered during cell proliferation. The large number of compounds—hormones, drugs and metabolites—tested in the present set of experiments, both in intact and partially hepatectomized animals, gave us few clues on the nature of this effector.

Phenylalanine hydroxylase in the liver was induced on treatment of rats with cortisol but the maximum response was observed only after 24 hr. This property of inducibility was retained during regeneration, and any changes in the enzyme in a given condition must take into account the corresponding changes in endogenous corticosteroids. During the early phase of hepatic regeneration, corticosterone, in fact, increased, and this should have resulted in an increase in the enzyme. The absence of a corresponding increase in the enzyme suggests that the endogenous effector may have been counteracting the cortisol induction. This effect was marked at a 24-hr time interval (Table 2) wherein a small decrease of enzyme activity was observed despite a large increase in the circulating corticosterone. This is also supported by the potentiation of the effect obtained in the adrenalectomized animals. It seems reasonable to conclude that the decrease in the enzyme during regeneration was not due to a change in the concentration of corticosteroids.

A related situation, encountered with tryptophan pyrrolase, deserves mention here. The well-known induction of tryptophan pyrrolase by cortisol was inhibited by noradrenaline [23]. Both being "stress-hormones", a possibility is thus provided for fine control of metabolic processes in animals exposed to stressful conditions. Noradrenaline, however, was not the active effector in the case of phenylalanine hydroxylase as its administration prevented a decrease during regeneration, instead of the expected potentiation.

The interesting features of the effect of noradrenaline are its specificity and the requirement of its presence during regeneration. The same effect being obtained with both types of adrenergic blockers but not by an agonist raised a doubt whether membrane receptor mediation was involved in the case of noradrenaline. Another related enzyme, tyrosine aminotransferase, was found to increase on treatment with an adrenergic blocking agent, phentolamine, but this was found to be due to an increase in plasma corticosterone rather than to adrenergic blockade [24]. The possibility of increased corticosterone in animals treated with phenoxybenzamine, and even noradrenaline, accounting for the observed effects during regeneration need not be considered in view of the strong evidence on the lack of correlation between the decreased phenylalanine hydroxylase in regenerating liver and plasma corticosterone.

The evidence obtained in this investigation suggests the need for a natural effector, elaborated during hepatocyte proliferation, whose action of decreasing phenylalanine hydroxylase is counteracted by noradrenaline and adrenergic blocking agents but not by a variety of hormones including growth hormone.

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