HYDROXYLATION OF PHENYLALANINE AND TRYPTOPHAN BY LIVER ENZYME OF ADRENALECTOMIZED RATS

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The primary step in the biosynthesis of serotonin is the hydroxylation of tryptophan to 5-hydroxytryptophan which is followed by decarboxylation of the latter compound (Udenfriend et al., 1956). It has been demonstrated by Freedland et al. (1961a) that cell free liver enzyme hydroxylates tryptophan in vitro. Renson et al. (1962a) have shown that this hydroxylase is identical with phenylalanine hydroxylase which converts phenylalanine to tyrosine.

Hicks and West (1958) have found an increased serotonin level in the body tissues of adrenalectomized rats maintained on water instead of saline; on the other hand, Davis (1963) has reported a decreased 5-hydroxytryptophan decarboxylation in such animals. In order to clarify these apparently contradictory results, a study of the phenylalanine and tryptophan hydroxylase system in the liver of adrenalectomized animals was undertaken.

Male rats of local stock weighing 100-120 grams were used. Adrenalectomy was performed under ether anesthesia and the animals were kept on saline unless stated otherwise. The rats were sacrificed and examined 51 hours after the operation. Both nor-

mal and sham-operated rats were employed as controls.

The liver soluble fraction was obtained by centrifugation of the homogenate at 100,000xg for half an hour. The tryptophan hydroxylating assay was performed according to Freedland et al. (1961a). The phenylalanine hydroxylating assay was carried out according to Kenney et al. (1958). The formation of 5-hydroxyindoles was ascertained with the aid of the 1-nitroso 2-naphthol reaction of Udenfriend et al. (1955). The tyrosin was estimated according to Kaufman (1957).

The results obtained for the hydroxylation of phenylalanine and tryptophan by the liver enzyme of normal, sham-operated, and bilaterally adrenalectomized rats are presented in Table I; results obtained with bilaterally adrenalectomized animals maintained on water instead of saline are also included.

TABLE I

Effect of Adrenalectomy on Phenylalanine and Tryptophan Hydroxylation.

Animals	Enzyme Activity m μ moles per 100 mg liver protein	
	Tyrosine	5-Hydroxyindole
Normal	5000 ±195 (12)	220± 6 (12)
Sham-operated	5220 ±275 (6)	218±12 (7)
Adrenx.: saline	5620 ±285 (9)	167± 7 (12)
Adrenx.: water	6150 ±625 (4)	172±10 (4)

In all cases incubation was carried out at 37°C for one hour. Numbers in parentheses indicate number of animals employed. Values given are averages plus minus standard errors of the mean.

The values of Table I do not show any significant change in hydroxylation of phenylalanine in the adrenalectomized rats as compared to the sham-operated ones. On the other hand, tryptophan hydroxylation is markedly suppressed follo-

wing adrenalectomy. This latter result is of interest since Garattini et al. (1961) have demonstrated that the serotonin level in tissues is not changed by removal of the adrenals. Furthermore, according to Hicks and West (1958), animals maintained on water show a higher tissue serotonin level than those kept on saline. This finding is to be compared with the evidence (Table I) that the suppression of the tryptophan hydroxylating activity of liver enzyme is independent of whether the animals are given water or saline.

The lack of correlation between the serotonin level in tissues and the hydroxylating activity of liver enzyme, support the assumption (Renson et al. 1962b) that the phenylalanine (tryptophan) hydroxylase does not play an important physiological role in the overall biosynthesis of serotonin.

Since it may be supposed that the aforementioned decreased activity of tryptophan hydroxylase is associated with a reduced activity of glucocorticoids due to adrenalectomy, the influence of cortisone on the phenylalanine and tryptophan hydroxylating systems has been investigated. For this purpose, 3 intramuscular injections of cortisone acetate were administered to rats - immediately after operation, 24 hours later, and 3 hours before sacrifice.

The results are summarized in Table II. The table shows that, in sham-operated animals, the hydroxylation of both phenylalanine and tryptophan is enhanced in a highly significant manner by the administration of cortisone. In adrenalectomized rats, phenylalanine hydroxylation - which is not affected by removal of the adrenals (Table I) - remains unaffected by injection of the hormone. On the other hand, tryptophan hydroxylating activity, which is markedly suppressed

TABLE II

Effect of Cortisone on Phenyalanine (Tryptophan) Hydroxylase Activity in Sham-Operated and adrenalectomized rats

Treatment	Enzyme Activity mμ moles per 100 mg liver protein		
	Tyrosine		
	Sham	Adrenx.water	Adrenx.saline
Control	6410± 60 (5)	6650±375 (9)	6050±255 (12)
¥Cortisone	7900±130 (4)	7080±345 (4)	6660±225 (7)
	5 - Hydroxyindole		
Control	219± 8 (8)	176± 6 (8)	166± 10 (11)
¥Cortisone	256± 14 (6)	239± 20 (4)	254+ 8 (6)

¥ Cortisone (50 mg per kg) was administered three times at 24 hours intervals.

Numbers in parentheses indicate number of animals employed.

Values given are averages plus minus standard errors of the mean.

by adrenalectomy (Table I), is completely restored by cortisone.

It is to be pointed out that only bilateral adrenalectomy and triple injections of cortisone influence the tryptophan hydroxylation system in the manner reported; unilateral adrenalectomy as well as single injections of cortisone to bilaterally adrenalectomized animals have been found to leave the system unaffected.

The results of this study conform with the hypothesis (Freedland et al. 1961b) that, although phenylalanine hydroxylase is essentially identical with tryptophan hydroxylase, additional factors may be involved which cause dissimilar behavior in the two systems under various conditions. Further investigations are in progress which aim to throw more light on the relationship existing between adrenal

secretions, liver hydroxylase activity, and the serotonin level in tissues.

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