

STUDIES WITH THE SEROTONIN PRECURSOR, 5-HYDROXY-TRYPTOPHAN, IN EXPERIMENTAL ANIMALS AND MAN

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There is a widespread interest in the pharmacology of serotonin and its possible roles in normal physiology and pathologic states. Diverse actions of this substance in man are suggested by the syndrome of vasomotor disturbances, bronchoconstriction, intestinal hypermotility and valvular heart disease associated with hyperserotonemia due to secreting carcinoid tumors (1, 2). The potent stimulant action of serotonin on the estrous rat uterus *in vitro* (3) suggests that this substance might also affect uterine function *in vivo*. Suspicions concerning a role of serotonin in brain function have been supported by the demonstration that reserpine releases serotonin from the brain, as well as from other body depots (4). Although the importance of this agent is apparent, studies of its effects in the intact animal are limited by its rapid transformation to inactive metabolites after parenteral administration.

Recently, Udenfriend, Weissbach, and Bogdanski (5) demonstrated prolonged elevations of serotonin in various tissues following the administration of its precursor, 5-hydroxytryptophan (5HTP). It was found that 5HTP in a dosage of 30 to 60 mg. per Kg. in dogs produced marked effects which lasted several hours. These included tremors, ataxia, loss of contact plantar reflex, pupillary dilation, loss of light reflex, lacrimation, apparent blindness, salivation, hyperpnea, and tachycardia. These effects were considered to be similar to those produced by the hallucinogenic agent, lysergic acid diethylamide (LSD). It seemed worth while to ascertain whether chronic administration of 5HTP to animals would produce a sustained state of serotonin excess and whether alterations similar to the carcinoid syndrome, particularly endocardial fibrosis, would develop in animals treated in this manner. Also, the similarity in the actions of 5HTP and LSD in animals

made it desirable to determine whether the former agent would induce central disturbances in man.

This paper consists of two parts. The first deals with observations on rats during chronic administration of 5HTP and includes studies on the effects of 5HTP on pregnant animals and their fetuses. The second part is devoted to the metabolism and pharmacological effects of 5HTP in man.

MATERIALS AND METHODS

The experimental animals were fed a standard commercial rat food¹ and allowed water *ad libitum*. Sprague-Dawley males weighing approximately 100 Gm. were used for the chronic studies. Pregnant rats were obtained from the Holtzman Rat Company; the duration of pregnancy was calculated from the time of observed mating.

The human subjects used in these studies were patients without evident renal or hepatic disease.

Platelet counts were done using direct phase contrast microscopy (6). For histologic studies tissues were fixed in 10 per cent buffered formalin. Sections were cut at a thickness of 6 microns and stained as follows: hematoxylin and eosin-y, azure and eosin, elastica (orcein) and Hale's iron stain for "mucopolysaccharides" (Longley's variant). Serotonin and 5-hydroxyindoleacetic acid (5HIAA) were measured by methods developed in this laboratory (7, 8).

Biochemical, pharmacologic and pathologic effects of 5HTP in rats. From previous observations made in this laboratory, it was considered likely that the administration of 5HTP at an appropriate dosage every 12 hours would maintain a state of chronic serotonin excess in animals. Accordingly, 5-hydroxy-DL-tryptophan was administered to rats in a dosage of 200 mg. per Kg. intraperitoneally. Twelve rats were injected every twelve hours for periods up to four months, and twelve others served as untreated controls. They were observed for changes in behavior, skin color, character of the feces and weight. Animals were randomly selected from each group and sacrificed at monthly intervals using pentobarbital intraperitoneally. The serotonin content of

¹ Purina rat pellets, Purina Mills, St. Louis, Missouri.

various tissues was determined and autopsy examinations were done. The following organs were examined microscopically: heart, liver, kidney, lung, skin, stomach, and cecum.

To obtain evidence that prolonged, elevated serotonin levels occurred in the chronic experimental animals, they were sacrificed at varying intervals of time after a given dose of 5HTP, and brain, ileum, kidney and liver were assayed. As shown in Table I the serotonin content of kidney and liver was markedly increased even 12 hours after 5HTP. In the brain, the serotonin returned to normal values within a few hours. The gut normally contains large amounts of serotonin, usually in the range of 7 to 15 μ g. per Gm. The largest amount found in our chemically treated animals was 22 μ g. per Gm. A value of this magnitude is occasionally encountered in control animals. Since the hearts were used entirely for histologic studies, separate experiments were carried out to demonstrate elevation of heart serotonin. In two rats, administration of 50 mg. per Kg. of 5HTP increased the levels of serotonin in the heart from less than 0.1 to over 2.5 μ g. per Gm. in two hours. It is apparent that the administration of 200 mg. per Kg. of 5HTP every 12 hours is a satisfactory procedure for obtaining prolonged elevations of tissue serotonin levels *in vivo*.

Considering the large amounts of serotonin in their tissues, the animals which were on this regimen of 5HTP for periods of one to four months tolerated the agent remarkably well. The gain in weight of the experimental group was considerably less than that of the control group. The mean weights in grams at the beginning

TABLE I
Tissue levels of serotonin after chronic administration of 5HTP

Hours after last injection	Weeks of treatment	Serotonin (microgm./Gm.)*		
		Brain	Kidney	Liver
0†	0	0.40	<0.10	<0.10
2	12	1.00	18.70	5.75
4-6	16	0.54	3.35	1.95
12	8	0.35	1.37	1.00

* Each value represents results obtained on homogenate of two separate rats.

† Controls.

and end of the experiment in the treated and control groups were: 104 ± 10.2 ; 109 ± 9.6 ; 236 ± 17.4 ; and 375 ± 23.4 , respectively. In spite of this, only one animal died during the study and this death was due to pneumonia. No distinct central effects were observed although within a few weeks the experimental animals appeared lethargic and relatively unresponsive to stimuli such as handling and injections. No significant gross or microscopic pathologic changes were found, even after four months of continuous treatment. Lesions of myocarditis, pyelonephritis and pneumonitis were observed but these were found to a comparable degree in both the control and experimental animals. Extensive study of the sections from the heart and skin showed no significant anatomic alterations. Platelet counts on three animals during the twelfth week of study ranged from

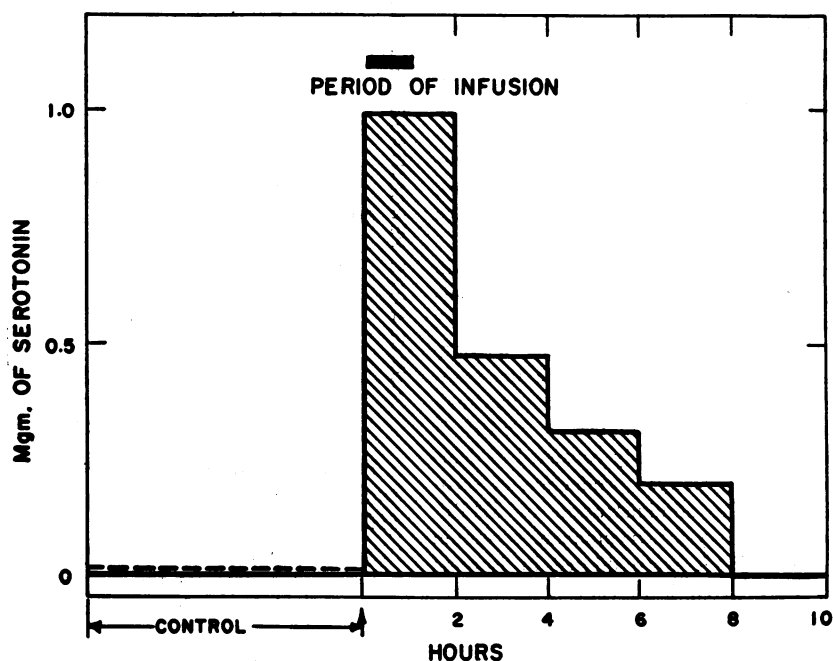


FIG. 1. URINARY EXCRETION OF SEROTONIN (MG. PER TWO HOURS) IN MAN AFTER INTRAVENOUS ADMINISTRATION OF 50 MG. OF 5HTP

Values are the average of two studies.

TABLE II
Effect of 5HTP* on fetal and uterine serotonin

Tissue	Serotonin (microgm./Gm.)†	
	Controls	2 hours after 5HTP
Fetus	<0.10	5.0
Uterus	<0.10	2.5

* Dosage, 200 mg. per Kg.

† Each value represents results obtained on homogenate of two separate rats.

600,000 to 990,000 per cubic mm. These were comparable to values obtained on control animals.

The most obvious effects of 5HTP were on the gastrointestinal tract and on cutaneous vasculature. The first consisted of marked diarrhea which became apparent within one week and continued for about two weeks, after which it persisted in a milder form. Secondly, within a few weeks a bluish discoloration was observed on the skin of the nose, ears, feet, and tail. This "cyanotic" appearance, indicative of a marked vasomotor disturbance, was most evident four to six hours after each injection, but was constantly present on the tails.

As shown in Table II, administration of 5HTP to pregnant rats resulted in a marked elevation in the levels of serotonin in the uterus and fetus. The following experiments were designed to investigate the effects of serotonin on uterine function during pregnancy and on cardiogenesis *in utero*. Twelve pregnant rats were given 200 mg. per Kg. of 5HTP twice a day on the seventh, eighth, ninth, and tenth days of gestation,² the period of most active cardiogenesis. In no instance did abortion occur. The fetuses, all of which were viable, were removed surgically on the twenty-first day, and fixed in 40 per cent formaldehyde. The hearts of 17 fetuses were examined under a dissecting microscope and compared with fetal hearts obtained from control rats. No abnormalities were seen in the great vessels, heart valves, chordae tendinae or septa. Histologic study of twelve fetal hearts revealed no alterations.

In an attempt to detect uterine effects of serotonin in the later stages of gestation, 200 mg. of 5HTP was administered in a single dose on the twentieth day to 12 rats. None of these animals delivered within a four-hour period of observation, during which time the serotonin levels in the uterus were presumed to be maximal.

Biochemical and pharmacologic studies of 5HTP in man. The first part of the human study consisted of demonstrating an increase and prolonged production of serotonin in man following the administration of 5HTP. Various amounts of 5-hydroxy-DL-tryptophan, serotonin creatinine sulfate and 5HIAA were diluted in isotonic NaCl solution and were given intravenously at a constant rate over a period of one hour, in the fasting state. Fifty mg. of 5HTP administered to two subjects resulted in a marked increase in the urinary excretion of serotonin for several hours (Figure 1). The percentages of

² Period of gestation in the rat is 21 days.

the administered dose of 5HTP represented by urinary serotonin in the two subjects were 4 and 7 per cent, respectively. There was no detectable increase in urinary serotonin following the administration of 10 mg. of serotonin itself. Thus, in man as in experimental animals, it would appear that 5HTP produces a prolonged increase of serotonin in the body. No attempt was made to measure the effect of 5HTP on circulating serotonin since previous studies with animals indicated that at the dosage used in these experiments, the increase in blood serotonin would be barely perceptible. Further information concerning the fate of serotonin, its precursor (5HTP), and its metabolite (5HIAA) in man, was obtained by measurement of the urinary excretion of 5HIAA following the administration of each of the three substances. As shown in Figure 2, the average cumulative percentage of each substance excreted as 5HIAA was found to be: serotonin, 77 per cent (range, 65 to 92); 5HIAA, 55 per cent (range, 40 to 70); and 5HTP, 28 per cent (range, 14 to 35).

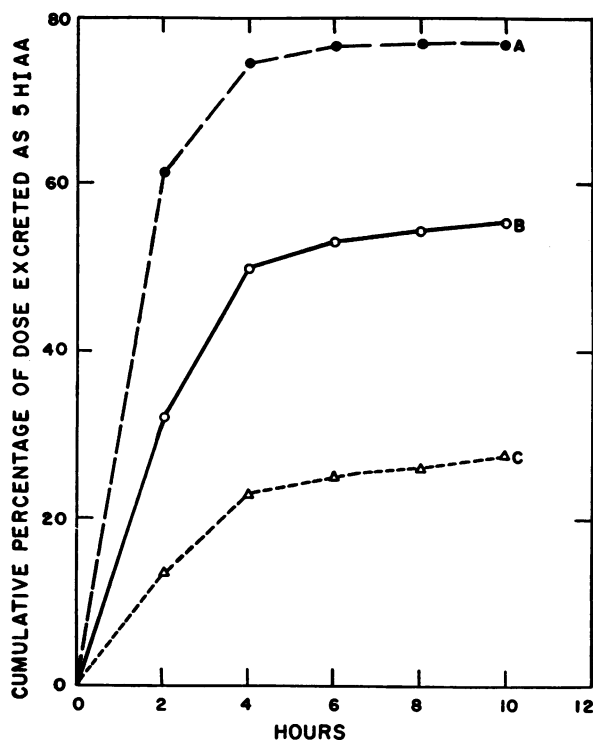


FIG. 2. URINARY EXCRETION OF 5HIAA FOLLOWING THE ADMINISTRATION OF SEROTONIN, 5HTP, AND 5HIAA

All compounds were infused over a one-hour period.

Curve A represents the average of three infusions of 22.8 mg. of serotonin creatinine sulfate (10 mg. free base).

Curve B represents the average of two infusions of 10.8 mg. of 5HIAA.

Curve C represents the average of five infusions in which the dose of 5HTP varied from 12.5 to 100 mg.

TABLE III
Effects of 5HTP in human subjects

Patient No.	Total dose (mg.)	Period of infusion	Effects
1	10.4	10 sec.	Moderate nausea beginning in 20 min., lasting an hour
2	25.0	30 min.	Mild intermittent nausea and abdominal cramping
3	25.0	10 sec.	Mild nausea for 10 minutes
	25.0	60 min.	Mild nausea for two hours after end of infusion
	34.0	51 min.	Severe nausea and frequent retching for two hours after end of infusion
4	20.0	10 sec.	Mild nausea for 5 min.
5	22.5	10 sec.	Moderate nausea for 10 min.
	25.0	5 min.	Moderate nausea, abdominal cramps and urge to defecate lasting 10 min.
	29.0	10 min.	Moderate nausea, abdominal cramps and urge to defecate lasting 1½ hours
	41.0	60 min.	Severe nausea, abdominal cramps, urge to defecate, and borborygmi lasting 2 hours
	50.0	60 min.	Severe nausea, retching, cramps, flatus, urge to defecate, and borborygmi lasting 2 hours
6	25.0	60 min.	Mild nausea for 5 min.
7	25.0	60 min.	Mild nausea for 5 min.
8	27.0	40 min.	Moderate nausea, occasional retching, and increased bowel sounds lasting 5 hours
9	30.0	10 sec.	Mild nausea for 10 min.
10	50.0	60 min.	Mild nausea, cramping, increased bowel sounds, and projectile vomiting for 10 min.
11	120.0	60 min.	Mild nausea for 30 min.

The pharmacologic effects observed following 5HTP administration in 11 human subjects are summarized in Table III. Initially, infusions were given over a period of 10 seconds and in each case nausea developed after a latent period of 15 to 20 minutes with a dosage of 10 to 30 mg. (Patients Nos. 1, 3, 4, 5, 9). Subsequent infusions were given over longer periods of time (5 to 60 minutes) in the hope of obviating the nausea and thereby administering a dose sufficiently large to produce mental disturbances. Although prolonging the period of infusion enabled administration of up to 120 mg. (Patient No. 11), nausea, with or without vomiting, was produced consistently. In one patient (No. 5), the most pronounced symptoms were severe abdominal cramps with borborygmi and an urge to defecate. The apparent increase in intestinal motility following 5HTP was confirmed by recordings of intraluminal pressure changes in the small bowel. The results of further studies on the gastrointestinal effects of 5HTP in man will be reported elsewhere (9). An untoward effect in one patient (No. 3) was the occurrence of a transient period of hypotension following each of three separate infusions. No changes in blood pressure were observed in the other subjects, however, and no alterations in pupil size, respiratory rate and electrocardiograms were noted. No obvious changes in mental function were observed following infusion of 5HTP in these patients (including Patient No. 10, who was schizophrenic). However, the gastrointestinal symptoms were often of such a severity as to invalidate psychologic testing and precluded the achievement of dosages which might have produced definite central effects.

DISCUSSION

It is apparent from these studies that a prolonged increase in the production of endogenous

serotonin can be achieved in both man and experimental animals by the administration of 5HTP. Furthermore, biochemical adaptation does not develop since, in the chronic studies, a state of continuous serotonin excess was found in rat tissues. The chronic studies in rats were also of importance in providing information concerning the toxicity of 5HTP prior to studies in man.

Serotonin has been implicated in a variety of pathologic states. It has been considered that the endocardial fibrosis and concomitant valvular heart disease in patients with malignant carcinoid tumors might be due to serotonin secreted by these tumors (2). Also, the possibility occurred to Waldenström and Ljungberg (10) and ourselves that serotonin in the fetal circulation might be involved in the pathogenesis of endocardial fibroelastosis of infancy. Renal cortical necrosis (11, 12) and thrombocytosis (13) have been reported following injections of serotonin in rats. The renal lesion is known to occur in such clinical conditions as *abruptio placentae*, peritonitis and acute pancreatitis (14). However, rats subjected to huge amounts of serotonin administered through its precursor over a period of their life span comparable to about 10 to 15 years of human life exhibited no significant pathologic alterations. Considering the potent pharmacologic actions of serotonin, this was a most surprising result but was reassuring in terms of administering the compound to human subjects. Although limited supplies of

5HTP prevented giving the compound throughout the entire period of gestation in the rat, fetal hearts subjected to excess serotonin *in utero* during the period of cardiogenesis showed no abnormalities.

Since the rat uterus in estrous is sensitive to serotonin *in vitro*, it was also suspected that 5HTP might be a powerful abortifacient. In spite of the fact that high levels of serotonin were attained in the uterine muscle of pregnant rats, abortion did not occur. It would appear that elevation of uterine serotonin *in vivo* causes no marked alteration in uterine activity. Although it has been pointed out that much of the serotonin found in tissues a few hours after administration of 5HTP exists in a bound, inactive form, it was also shown that within the first two hours after 5HTP administration high levels of free, pharmacologically active serotonin are found in the plasma (5).

The cutaneous vasomotor disturbances and diarrhea in 5HTP-treated rats are consistent with known actions of serotonin and are similar to symptoms which are seen in patients with malignant carcinoid. In patients given 5HTP, nausea and increased intestinal motility were the only consistent effects, further demonstrating the sensitivity of the human gut to serotonin. Since the gastrointestinal tract contains most of the serotonin in the body, these findings may signify a role of serotonin in gastrointestinal motility.

Although no mental alterations were noted in the human subjects, the dosage of 5HTP was limited by the intestinal effects. Since 20 to 60 mg. per Kg. of 5HTP were required to produce central disturbances in dogs and cats (15) it is not surprising that the amounts used in our studies (0.1 to 2.0 mg. per Kg.) had no central effect. Further attempts should be made to infuse larger amounts of this substance in man. In this regard, it may be possible to block the peripheral effects of 5HTP pharmacologically and thereby achieve dosages sufficiently high to produce central effects in man. Such studies may help clarify recent speculation concerning the effects of serotonin in the brain, which has been gathered exclusively from studies with drugs structurally related to serotonin.

SUMMARY

1. The chronic administration of the serotonin precursor, 5-hydroxytryptophan (5HTP), has been shown to produce sustained elevations of

serotonin levels in rats. Rats so treated for periods up to 120 days exhibited diarrhea and cutaneous vasomotor disturbances but showed no discernible anatomic alteration on histologic examination.

2. Prolonged increases in tissue serotonin in man also were achieved with 5HTP. The predominant pharmacologic effect of 5HTP in man was stimulation of the gastrointestinal tract which precluded the attainment of a dosage sufficiently high to produce central effects comparable to those seen in experimental animals.

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