STUDIES ON 5-HYDROXYTRYPTAMINE AND 5-HYDROXYTRYPTOPHAN

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The 5-HT content and the 5-HTP decarboxylase activity of several tissues of seven mammalian species has been estimated. Whereas 5-HT is concentrated in the spleen and gastrointestinal tract, the highest enzyme activity is found in the kidney, liver, gut and brain. In rats, treatment with reserpine or chlorpromazine lowers the 5-HT content of many tissues and reduces the 5-HTP decarboxylase activity of the kidney, but prolonged treatment with cortisone only lowers the 5-HT content of the skin.

During recent years, the discovery of new facts about 5-hydroxytryptamine (5-HT) has proceeded with astonishing speed. Its function in the body however remains uncertain, though several possibilities exist—(*a*) it may play a role in controlling the activity of the central nervous system, (*b*) it may act as the stimulus to peristalsis in the gut, (*c*) it may be the factor controlling capillary permeability in the tissues, or (*d*) it may be an important haemostatic agent. Knowledge of its distribution in the tissues[1,2] may give an indication of the part it is likely to play in the normal functioning of the body, but knowledge of its formation from 5-hydroxytryptophan (5-HTP) and its destruction is equally important. Preliminary studies on the biosynthesis of 5-HT have already been reported by Gaddum and Giarman[3] and the present work extends these observations and includes the effects of two ataractic drugs as well as cortisone on the distribution and formation of 5-HT. It was hoped that such studies might shed further light on the physiological function of this amine.

Methods

Preparation of Tissues for Determining their 5-HT Content

All tissues were freshly excised, cleaned and weighed in the wet state. The desired amount of tissue was cut into small pieces and extracted with acetone (5 ml./g. tissue) for 24 hours. After decanting the acetone, the tissues were re-extracted with a similar volume of 80 per cent (v/v) acetone. The acetone from the combined filtrates was removed by evaporation in air below 30°. The residue was brought to the desired volume (1–10 ml./g. of original material) with 0·9 per cent (w/v) NaCl solution and its activity measured. Each value in Table I represents the mean of four results.

Preparation of Tissues for Determining their Content of 5-HTP Decarboxylase

Similar portions of tissues were weighed, cut into small pieces, and ground in a mortar with a little sand and M/15 phosphate buffer (2 ml./g. tissue) at pH 8·0. Such treatment extracts the enzyme but not the 5-HT.

Aliquots of the homogenates containing the desired quantity of tissue (800 mg.) were measured into specimen tubes containing the co-enzyme, pyridoxal phosphate (100 μg.), and an inhibitor of mono-amine oxidase, iproniazid (100 μg.), to prevent the destruction of the 5-HT formed from 5-HTP. Phosphate buffer was then added to bring the volume to 4·6 ml., and the substrate, 5-HTP, was added last (400 μg. contained in 0·4 ml. water). Immediately after its addition, the mixture was shaken and incubated at 37° for 1 hour (as suggested by Gaddum and Giarman[3]). The reaction was then stopped by reducing the pH of the solution to 5·0 with N HCl, and the 5-HT content of the solution assayed. The amount of 5-HT formed per gram of tissue may be used as an indication of the 5-HTP decarboxylase content of that tissue. Each value in Table II represents the mean of four results.

Bioassay Procedure

Bioassays were carried out on the isolated uterus of the oestrous rat. An aerated 15-ml. bath of de Jalon's fluid containing atropine (10^{-7}) at 28° was used. On occasion, the extracts were also assayed on the rat colon suspended in a similar bath at 20°. Usually, both preparations were sensitive to 0·01–0·02 μg. 5-HT (i.e., approximately 10^{-9} g.). The specificity of the reaction was checked by using the 5-HT antagonist, 2-bromlysergic acid diethylamide. The standard 5-HT was used as its creatinine sulphate, but values given in the text refer to the base.

Depletion of 5-HT Stores in the Rat

Two groups of 10 female albino rats (100–150 g. in weight) received an intraperitoneal injection of either reserpine (1 mg./kg.) or chlorpromazine (25 mg./kg.) on each of 3 consecutive days. A third group of rats were given daily an intramuscular dose of cortisone (50 mg./kg.) for 14 days, whilst a fourth control group received a daily intraperitoneal dose of normal saline (0·5 ml.) for 14 days. All animals were killed 24 hours after the last injection and several tissues taken for 5-HT assay. The 5-HTP decarboxylase activity of the kidneys of rats from each group was also measured.

RESULTS

Tissue distribution of 5-HT

The results are shown in Table I. Briefly, 5-HT is concentrated in the spleen and gut of all the species studied. Another important site in the rat

TABLE I

5-HT CONTENT (μG./G.) OF ANIMAL TISSUES

Tissue	Rat	Mouse	G'pig	Hamster	Rabbit	Dog	Cat
Kidney	0·1	0·1	0·1	0·1	0·1	0·1	0·1
Liver	0·1	0·7	0·1	0·2	0·6	0·5	0·6
Spleen	2·5	2·7	1·1	20·5	24·3	4·6	8·5
Skin	1·3	0·4	0·1	0·1	0·1	0·1	0·1
Stomach	1·4	1·0	1·4	1·2	4·9	5·2	0·5
Duodenum ..	1·2	1·2	5·0	0·9	3·3	3·7	0·9
Ileum	1·2	1·0	3·4	1·3	3·7	4·3	0·5
Brain	0·2	0·3	0·3	0·2	0·3	0·2	0·3

is the skin where over half of the 5-HT content of the body is located[2]. Only traces of 5-HT are found in the kidney and brain.

Tissue Distribution of 5-HTP Decarboxylase

The enzyme activities of the tissue preparations are recorded in Table II. By far the most striking result is that the highest concentrations of enzyme are found in the kidney and liver, two tissues where the 5-HT content is low. The 5-HT-forming capactity of the spleen, however, is

TABLE II

THE RELATIVE 5-HTP DECARBOXYLASE ACTIVITY OF VARIOUS ANIMAL TISSUES, RECORDED AS THE 5-HT FORMED (μG./G. TISSUE) FROM ADDED 5-HTP

Tissue	Rat	Mouse	G'pig	Hamster	Rabbit	Dog	Cat
Kidney	188	187	171	62	56	47	24
Liver	125	22	156	62	94	12	55
Spleen	1	6	2	93	19	2	2
Skin	1	1	1	1	1	1	1
Stomach ..	1	2	64	45	34	3	25
Duodenum ..	2	2	391	9	13	2	23
Ileum	1	1	190	1	6	1	8
Brain	32	9	13	20	5	2	2

small, except in the hamster and rabbit, and the skin likewise is deficient in this enzyme. Only traces of enzyme activity are found in the gut of the rat, mouse and dog, but exceptionally high values exist in this region in the guinea pig. The brain of several species is also capable of forming much 5-HT.

Action of Reserpine in the Rat

After reserpine treatment, the 5-HT content of several tissues was reduced to less than 10 per cent of the control values of untreated animals (Table III). The gut on the other hand lost only about 50 per cent of its content. The 5-HTP decarboxylase activity of the kidneys of these rats was reduced to 48 per cent of that of saline-injected rats. This reduction in enzyme activity was due in part to the residual reserpine, since reserpine (10^{-5}) added to homogenates of normal rat kidney always slightly reduced the conversion rate of 5-HTP to 5-HT.

Action of Chlorpromazine in the Rat

Chlorpromazine lowered the 5-HT content of the tissue studied though it was less active than reserpine (Table III). Similarly the 5-HTP

TABLE III

THE 5-HT CONTENT OF TISSUES OF THE RAT AFTER DRUG TREATMENT. ALL VALUES ARE EXPRESSED AS PERCENTAGES OF THOSE OF UNTREATED ANIMALS

Tissue	Reserpine	Chlorpromazine	Cortisone
Spleen ..	5	25	100
Skin	7	17	35
Stomach ..	50	70	110
Duodenum ..	45	50	100
Ileum	70	90	109
Brain	9	40	90

decarboxylase activity of the kidneys of the treated rats was reduced, this time to 64 per cent of that of the saline-treated animals.

Action of Cortisone in the Rat

The action of cortisone on the 5-HT levels in the tissues differed from that of reserpine or chlorpromazine. Only the skin showed a major change, being reduced to 35 per cent of that of the control level (Table III) There was no alteration in the 5-HTP decarboxylase activity of the kidney.

Discussion

The finding that 5-HT is present in extracts of brain aroused considerable speculation a few years ago concerning its function in the central nervous system. Hallucinogens were thought to exert their effect by antagonising the brain 5-HT content and the hypothesis was advanced that the 5-HT content of the brain is one of the factors controlling the activity of nerve cells in the brain. Recent work however has indicated that this is not the full explanation and the role of 5-HT in the brain is still open. In the present experiments, the 5-HT-forming enzyme has been detected in the brain of all species studied, and it is particularly active in the rat, hamster and guinea pig.

The present results also show that there is generally little or no relation between the 5-hydroxytryptophan decarboxylase activity of a tissue and the amount of 5-HT that can be extracted from that tissue. For example, the decarboxylase activity of the kidney and liver is high yet their 5-HT content is low. In these two tissues, this can probably be explained by the high activity of mono-amine oxidase, an enzyme which rapidly inactivates the 5-HT. In contrast, the spleen of most species contains much 5-HT but little enzyme. Both the decarboxylating enzyme and 5-HT have been found in high concentration in the stomach, duodenum and ileum of most species, and this is in accord with Erspamer's view that the gut is one of the most important sites of 5-HT production.

The hypothesis has been advanced that reserpine owes its pharmacological properties to its ability to interfere with the binding sites of 5-HT in the body[4]. Reserpine mobilizes the 5-HT from the brain, blood platelets and gastrointestinal tract and it is now apparent that the 5-hydroxytryptophan decarboxylase activity of the kidney of the rat can be reduced by treatment with reserpine. This result is in sharp contrast to that of Brodie and his colleagues[4] who found that this drug did not modify the decarboxylase activity of the brain of the rabbit. Chlorpromazine also reduces the 5-HT content of the brain, spleen and skin and lowers the decarboxylase activity of the kidney. Cortisone however reduces the 5-HT content of only the skin without modifying the kidney decarboxylase activity. Thus it is clear that much work still remains to be done on the action of drugs on the biosynthesis and metabolism of 5-HT.

The 5-hydroxytryptophan decarboxylase activity of a particular tissue has been determined as the amount of 5-HT formed in 60 minutes by 1 g. of tissue. It is possible that different conclusions might have been reached if the decarboxylation had been allowed to proceed for longer

periods of time than 1 hour or if the amount of substance in the system was altered.

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DISCUSSION

The papers were presented by MISS SHIRLEY A. P. PRICE and DR. G. B. WEST.

The CHAIRMAN. Could more details be given of the technique of filling the ampoules in an atmosphere of nitrogen, and had the oxygen content been determined in any filled ampoules?

DR. G. F. SOMERS (Liverpool). How did one associate the presence of a substance like 5-hydroxytryptamine in a particular tissue with its function?

DR. F. HARTLEY (London). Was Dr. West satisfied with the specificity of the decarboxylases he had determined, and would the extraction process used for 5-HT deal with bound as well as free 5-HT?

MR. J. J. LEWIS (Glasgow). The action of reserpine appears to be non-specific. For example, in the brain it reduces not only the 5-HT content of a tissue but also its noradrenaline content.

MR. S. G. E. STEVENS (London). What were the decomposition products in the solutions? Had the authors looked for 3:5-dihydroxyindole, which could arise from 5-HTP?

MR. C. A. JOHNSON (Nottingham). The ultra-violet figures in Table I (p. *88 T*) indicated a full content of indole even in coloured solutions; this throws doubt on the chromatographic system used.

MR. H. B. HEATH (Sudbury). Was the loss of 50 per cent in the biological activity sudden and were solutions examined at periods between 3 and 6 weeks?

DR. WEST replied. The presence of 5-HT in a tissue is not indicative of its site of action since its concentration depends upon the activities of the enzymes responsible for its formation and its destruction. For example, the 5-HT content of the brain can easily be increased by an inhibitor of the inactivating enzyme. The homogenates of liver had been made in the usual way and contained all the enzymes originally present, yet incubation of these homogenates with several amino acids other than

5-hydroxytryptophan failed to yield detectable amounts of the corresponding amines. This indicated the specificity of the reaction when carried out with the reported method. The extraction process used dealt with free 5-HT. Bound 5-HT is extracted only with difficulty.

MISS PRICE also replied. Some solutions 10 months old filled under nitrogen with 0·1 per cent sodium metabisulphite were water-white. She was uncertain about the nature of the brown compound, though it was probably a melanin derivative. The solutions had been boiled for 20 minutes and then cooled whilst nitrogen was bubbled through and the ampoules were then filled under nitrogen. The solutions were not examined at periods between 3 and 6 weeks. The coloured solution had never shown any indole derivative other than 5-HTP by two-dimensional chromatography in several solvent systems.