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EFFECT OF L-TRYPTOPHAN ON DIURESIS AND 5-HYDROXYINDOLEACETIC ACID EXCRETION IN THE RAT

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Oral administration of L-tryptophan to rats produced two main biochemical and pharmacological effects: a marked increase in urinary 5-hydroxyindoleacetic acid excretion, and a significant reduction in the urine flow after a water load. Urinary 5-hydroxyindoleacetic acid excretion reached its maximum 2 to 6 hr after the administration of tryptophan, and it increased with the dose of the amino acid. Antidiuresis was seen after the administration of L-tryptophan, 200 mg/kg, or more. The effect appeared promptly and it was roughly proportional to the dose of the amino acid administered. Both antidiuretic effect and increase in urinary 5-hydroxyindoleacetic acid excretion were more intense after oral than after parenteral administration of L-tryptophan. D-Tryptophan, in oral doses up to 1,000 mg/kg, produced neither an increase in urinary 5-hydroxyindoleacetic acid nor a reduction of diuresis. Available evidence suggests that reduction of urine flow is a consequence of biosynthesis and release of 5-hydroxytryptamine by the gastrointestinal mucosa. Tryptamine produced by direct decarboxylation of L-tryptophan does not seem to play any important role.

In the course of experiments on precursors of 5-hydroxytryptamine, it was observed that oral administration of L-tryptophan, the initial precursor of the amine, produced two main effects: a conspicuous increase in the urinary excretion of 5-hydroxyindoleacetic acid, and a significant reduction in the urine flow following a water load. The last effect seemed particularly worth studying, as it was caused in the normal animal by a common dietary amino acid.

The present experiments were undertaken to investigate which compound was responsible for the observed antidiuresis, in order to provide additional information on the physiological role of 5-hydroxytryptamine in the control of diuresis, as well as on the biosynthesis and turnover rate of the amine in the enterochromaffin cell system, which is generally considered the main site of production of 5-hydroxytryptamine.

METHODS

Experimental animals. Adult albino rats of both sexes weighing 170 to 230 g, all of the same breed, were used. They were kept on a standard laboratory diet. During the afternoon preceding each experiment they received a diet rich in water (soaked bread and vegetables); in the night the animals were deprived of food but allowed free access to water. On the next morning the animals were given tryptophan either orally (amino acid dissolved in 5 ml. tepid tap water/100 g of rat) or parenterally (amino acid dissolved in 3 ml. 0.9% sodium chloride solution/100 g of rat). Doses of the administered L-tryptophan varied from 50 to 1,000 mg/kg; those of D-tryptophan from 200 to 1,000 mg/kg. In some experiments the

parenteral administration of L-tryptophan was repeated 3 to 4 times in 24 hr, at an interval of 4 to 5 hr between two administrations. The same amount of tepid tap water or of 0.9% sodium chloride solution was given to the controls.

Urine collection and estimation of urinary 5-hydroxyindoleacetic acid. The rats were placed in diuresis cages in groups of 4 animals each. Urine was collected in graduated cylinders containing 0.3 ml. chloroform and 0.3 ml. acetic acid in order to preserve the excreted 5-hydroxyindoleacetic acid. The urine output was measured for 5 hr after giving water and tryptophan, at intervals of 30 to 60 min. The 5-hydroxyindoleacetic acid was estimated by the colorimetric method of Macfarlane, Dalgliesh, Dutton, Lennox, Nyhus & Smith (1956), on samples of urine taken 2, 6 and 24 hr after loads. Already from the first experiments it became clear that this method could not be applied to the urine of rats treated with either 1,000 mg/kg L-tryptophan or D-tryptophan, owing to the presence of large amounts of interfering substances. In this case 5-hydroxyindoleacetic acid was estimated semi-quantitatively by paper chromatography. Urine was extracted with peroxide-free ethyl ether at *p*H 3 to 4 and the concentrated ether phase was chromatographed using the ascending method on Whatman no. 1 paper. The solvent was the *n*-butanol-acetic acid-water mixture (4:1:5); the developing reagents were: (*a*) a 2% alcoholic solution of *p*-dimethylaminobenzaldehyde, followed by exposure in a large glass chamber to hydrogen chloride vapours; (*b*) Gibbs' reagent (a 0.05% alcoholic solution of 2-6-dichlorochinonchlorimide); and (*c*) Heinrich and Schuler's NNCD reagent (4-nitro-2-chloro-1-diazobenzene-α-naphthalene sulphuric acid).

Estimation of tryptamine and indole-3-acetic acid. Semi-quantitative estimation of tryptamine and indole-3-acetic acid was carried out by paper chromatography after purification of the urine on alumina column. A volume of 50 ml. of urine, both normal and from rats treated with 400 mg/kg L-tryptophan, was evaporated to dryness under reduced pressure at 40° C. The residues were dissolved in 100 ml. 96% ethanol and then passed through an alumina column 3.3 cm in diameter. The column of alumina (aluminium oxide Merck, according to Brockmann) weighed 100 g and reached a height of 15 cm. Elution was performed with progressively decreasing concentrations of ethanol. Each fraction of eluate (100 ml.) was concentrated and examined by paper chromatography with the technique previously described. Tryptamine was found to be present in the 80% alcoholic eluate, indole-3-acetic acid in the 70% and 60% alcoholic eluates. The semi-quantitative estimation was performed by visual comparison of the spots obtained with a series of spots containing known concentrations of the corresponding pure substances.

Indole compounds. The L- and D- forms of tryptophan were purchased from Hoffman-La Roche (Basle) and indole-3-acetic acid from Fisher Scientific Company (U.S.A.); 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were kindly supplied by the Farmitalia Research Laboratories (Milan).

RESULTS

Effects of L-tryptophan and D-tryptophan on the diuresis of hydrated rats. The only pharmacological action produced by L-tryptophan was a reduction of the urine flow. This reduction was generally observed 1.5 hr after the water load and it was satisfactorily proportional to the dose of the amino acid administered. Results are shown in Table 1.

In rats treated with 50 and 100 mg/kg L-tryptophan, the reduction of diuresis was slight and not statistically significant, but in the groups of animals treated with 200 mg/kg L-tryptophan, the reduction was more conspicuous (14 to 30% less than the controls). It should be noted that, of the four groups of experiments carried out with a dose of 200 mg/kg, only two gave reductions that were highly statistically significant; in one the reduction was at the limits of significance; and in one it was

not significant. However, the average of all the values obtained from the groups treated with 200 mg/kg L-tryptophan was significantly lower ($P<0.05$) than that of the single control values.

As the dose of the amino acid was increased, the antidiuretic effect of L-tryptophan became more and more evident in both intensity and duration: a reduction of 38% was obtained with a dose of 400 mg/kg and one of 67% with 1,000 mg/kg of L-tryptophan. In this last instance, the reduction of the urine output was highly significant up to the 5th hr after the water load.

These results were all the more remarkable inasmuch as no action on diuresis was observed after oral administration of D-tryptophan, even at a dose of 1,000 mg/kg.

TABLE 1

ACTION OF L-TRYPTOPHAN AND OF D-TRYPTOPHAN ON RAT DIURESIS FOLLOWING WATER LOAD

The amounts of urine in ml./kg of body weight are shown, ±s.d. In parentheses the number of groups of rats is given; each group contained 4 animals. L-TP=L-tryptophan. D-TP=D-tryptophan

Tryptophan dose (L-TP or D-TP)	Urine (ml./kg) excreted after:		
	1½ hr	3 hr	5 hr
L-TP 50 mg/kg	35·2±3·2 (6)	46·8± 6·3 (6)	52·6±11·0 (6)
Controls	38·2±8·3 (6)	46·8±12·6 (6)	52·8±11·7 (6)
	$P>0.4$		
L-TP 100 mg/kg	33·8±4·2 (6)	47·3± 9·7 (6)	54·5± 3·4 (6)
Controls	38·2±8·3 (6)	46·8±12·6 (6)	52·8±11·7 (6)
	$P>0.2$		
L-TP 200 mg/kg	31·0±2·3 (5)	47·6±2·5 (5)	62·0± 4·2 (5)
Controls	44·0±6·3 (5)	56·0±3·4 (5)	62·6± 3·7 (5)
	$P<0.01$	$P<0.01$	
L-TP 200 mg/kg	27·5±4·1 (6)	38·5± 1·9 (6)	55·6± 3·2 (6)
Controls	35·5±2·3 (6)	41·2± 3·2 (6)	54·8± 4·1 (6)
	$P<0.01$	$0.2>P>0.1$	
L-TP 200 mg/kg	27·8±4·0 (6)		
Controls	32·5±3·1 (6)		
	$P>0.4$		
L-TP 200 mg/kg	25·7±4·3 (6)		
Controls	31·3±6·1 (6)		
	$0.1>P>0.05$		
D-TP 200 mg/kg	34·0±3·2 (7)	42·0±3·0 (6)	54·5±3·4 (6)
Controls	35·8±9·9 (14)	43·0±1·0 (6)	54·0±4·2 (6)
	$P>0.8$		
L-TP 400 mg/kg	18·3±4·1 (6)	34·7±4·3 (6)	46·1±2·5 (6)
D-TP 400 mg/kg	29·2±4·9 (6)	42·3±2·3 (6)	47·1±1·8 (6)
	$P<0.01$	$P<0.01$	
L-TP 1,000 mg/kg	10·0±3·2 (4)	20·5±5·4 (4)	40·7±8·0 (4)
Controls	30·5±5·5 (4)	46·5±4·2 (4)	55·0±7·0 (4)
	$P<0.01$	$P<0.01$	$P<0.01$
L-TP 1,000 mg/kg	8·4±2·2 (5)	23·2±4·9 (5)	36·0±2·9 (5)
D-TP 1,000 mg/kg	27·2±3·6 (5)	40·0±5·1 (5)	45·0±5·9 (5)
Controls	24·4±2·7 (5)	39·2±2·6 (5)	46·4±3·8 (5)

Action of L-*tryptophan on the urinary excretion of 5-hydroxyindoleacetic acid.* Observations on the urinary excretion of 5-hydroxyindoleacetic acid in the 24-hr period after an administration of L-tryptophan are shown in Table 2.

TABLE 2

URINARY EXCRETION OF 5-HYDROXYINDOLEACETIC ACID IN THE 24-HR PERIOD FOLLOWING ADMINISTRATION OF L-TRYPTOPHAN

The means±s.d. of 5-hydroxyindoleacetic acid in μg/kg are shown. or.=orally; s.c.=subcutaneously; i.p.=intraperitoneally.

Dose of L-tryptophan	5-Hydroxyindoleacetic acid μg/kg	% increase	*P*
50 mg/kg, or.	138·2±14·9 (5)	7	>0·2
Controls	129·0± 7·5 (5)		
100 mg/kg, or.	171·5±17·2 (6)	35	<0·01
Controls	126·7±11·7 (7)		
200 mg/kg, or.	221·2±44·2 (19)	67	<0·01
Controls	133·5±26·7 (22)		
400 mg/kg, or.	261·6±64·0 (5)	106	<0·01
Controls	125·8± 7·3 (5)		
1,000 mg/kg, or.	400 to 500 (4)		
Controls	131·7±12·0 (4)		
200 mg/kg, s.c.	163·6±35·1 (10)	23	<0·05
Controls	132·8±25·4 (10)		
200 mg/kg, i.p.	155·0±11·6 (4)	37	<0·01
Controls	113·0± 5·8 (4)		

It clearly appears from Table 2 that all groups of rats treated with L-tryptophan showed an increase of urinary 5-hydroxyindoleacetic acid in comparison with the controls. When L-tryptophan was administered orally, this increase was barely evident and not significant with the dose of 50 mg/kg ; it became more conspicuous with higher doses ; with the dose of 400 mg/kg the amounts of 5-hydroxyindoleacetic acid excreted were twice those excreted by the control groups. Chromatographic estimation of 5-hydroxyindoleacetic acid after administration of 1,000 mg/kg L-tryptophan revealed values 3 to 4 times higher than the control values.

An increased excretion of 5-hydroxyindoleacetic acid, even if not so striking, was observed also in the groups of animals given L-tryptophan by the subcutaneous or intraperitoneal route.

In further experiments it was demonstrated that the effect on 5-hydroxyindoleacetic acid excretion of 3 to 4 successive doses of 100 mg/kg each, given subcutaneously at 4- to 6-hr intervals, did not significantly differ from that of a single dose of L-tryptophan 400 mg/kg.

In order better to establish the time of maximum output of urinary 5-hydroxyindoleacetic acid, this metabolite was estimated in some groups of rats also in the urine collected 2 and 6 hr after the tryptophan load. Results are shown in Table 3.

It can be seen that the time of highest excretion of 5-hydroxyindoleacetic acid after L-tryptophan load was that ranging between the 3rd and 6th hr. In this period 5-hydroxyindoleacetic acid excretion was 3 to 4 times higher than that of controls. A remarkable increase, though less conspicuous, was observed also in the following 18-hr period.

TABLE 3

URINARY EXCRETION OF 5-HYDROXYINDOLEACETIC ACID

The figures represent mean excretion of 5-hydroxyindoleacetic acid in μg/kg$\pm$s.d. In parentheses number of groups of rats; 4 animals in each group

Time in hr	Controls	Treated with L-tryptophan (200 mg/kg)
0–2	28·4±6·8 (5)	28·0± 4·0 (5)
2–6	16·0±2·6 (5)	51·4± 7·6 (5)
6–24	84·7±18·2 (8)	144·9±49·3 (8)

Chromatographic estimation of tryptamine and indole-3-acetic acid. Chromatographic estimation of tryptamine and indole-3-acetic acid gave the following values: (*a*) Normal urine: tryptamine undetectable, indole-3-acetic acid 300 μg/kg/24 hr. (*b*) Urine of rats given 400 mg/kg L-tryptophan by oral route: tryptamine 250 μg/kg/24 hr (recovery approximately 0.06%), indole-3-acetic acid approximately 4 mg/kg/24 hr (recovery roughly 1%).

DISCUSSION

Administration of L-tryptophan to rats produced two effects: a remarkable increase in the urinary excretion of 5-hydroxyindoleacetic acid, and a significant reduction of urine flow.

The observed increase in urinary 5-hydroxyindoleacetic acid is in accordance with the results obtained by other investigators in man (Lauer, Inskip, Bernsohn & Zeller, 1958 ; Kopin, 1959).

It is highly probable that the 5-hydroxyindoleacetic acid found in urine originates from L-tryptophan via 5-hydroxytryptophan and 5 -hydroxytryptamine. Alternative pathways, although possible from a theoretical point of view (L-tryptophan→ tryptamine → 5-hydroxytryptamine → 5-hydroxyindoleacetic acid ; L-tryptophan → indolepyruvic acid→indole-3-acetic acid→5-hydroxyindoleacetic acid ; L-tryptophan →5-hydroxytryptophan→5-hydroxyindolepyruvic acid→5-hydroxyindoleacetic acid), seem to be of no practical importance. The main site of 5-hydroxylation of L-tryptophan is in all probability the gastrointestinal mucosa. In fact, the increase in urinary 5-hydroxyindoleacetic acid obtained with oral L-tryptophan was greater than that obtained with similar subcutaneous or intraperitoneal doses of L-tryptophan. Moreover, it has been shown in previous experiments that in rats from which the whole gastrointestinal tract had been removed by operation, L-tryptophan given by subcutaneous route did not prevent the disappearance of urinary 5-hydroxyindoleacetic acid (Bertaccini, 1960).

Apparently the urinary output in 5-hydroxyindoleacetic acid increases with the dose of the administered L-tryptophan, and maximum excretion rate of 5-hydroxyindoleacetic acid occurs between the 2nd and the 12th hr following the administration of the amino acid.

A dose of 1,000 mg/kg L-tryptophan given by mouth produced a 300% increase in the 24-hr excretion of 5-hydroxyindoleacetic acid. It may therefore be inferred that 5-hydroxylation of L-tryptophan and biosynthesis of 5-hydroxytryptamine are

at least trebled following a tryptophan load. If under normal conditions the half-life of gastrointestinal 5-hydroxytryptamine in the rat is approximately 6 to 7 hr (Erspamer, 1955), after a tryptophan load it may be reduced to 2 hr. This means that under normal conditions the biosynthetic capacity of the enterochromaffin cells of the gastrointestinal mucosa are far from being saturated.

It has been found that the gastrointestinal tract of the rats commonly used in this laboratory weighs 40 to 50 g/kg body weight. The overall 5-hydroxytryptamine content of the gastrointestinal tract is approximately 2 to 4 μg/g and the daily urinary excretion of 5-hydroxyindoleacetic acid is 90 to 140 μg/kg. Assuming that the 5-hydroxytryptamine synthesized by the enterochromaffin cells is three times more than the 5-hydroxyindoleacetic acid excreted (Erspamer & Testini, 1959), it may be calculated from the data given above that under basal conditions the gastrointestinal tract synthesizes 5-hydroxytryptamine at a rate of 0.35 μg/g/hr, and that after a tryptophan load the rate of 5-hydroxytryptamine biosynthesis may be at least trebled for some hours, probably reaching or even surpassing values of 1 μg/g/hr.

Oral doses of 200 mg/kg L-tryptophan and, more evidently, larger doses produce a significant antidiuretic effect. D-Tryptophan is completely ineffective even with doses of 1,000 mg/kg. It is therefore certain that antidiuresis is produced by some metabolite which can originate solely from L-tryptophan. Practically only two metabolites may come into play: tryptamine and 5-hydroxytryptamine.

It is highly probable that 5-hydroxytryptamine is the main, if not the sole, agent responsible for antidiuresis. This claim is based on the following evidence. The amount of 5-hydroxytryptamine synthesized and released by the gastrointestinal mucosa following an oral tryptophan load is largely sufficient to cause antidiuresis. In fact, it has been repeatedly demonstrated that subcutaneous doses of as little as 4 to 10 μg/kg of 5-hydroxytryptamine produce a significant reduction of urine flow in hydrated rats, and that 5-hydroxytryptamine is 100 to 300 times more potent than tryptamine in reducing diuresis (Erspamer, 1954). Although the amount of tryptamine produced in the organism by decarboxylation of L-tryptophan cannot be calculated directly, strong indirect evidence supports the view that this amount is not sufficient to cause antidiuresis.

In this respect one has to consider that urine of rats given 400 mg/kg of L-tryptophan orally contains only 250 μg/kg tryptamine, whereas urine of rats given 400 mg/kg of DL-5-hydroxytryptophan (that is, 200 mg/kg L-5-hydroxytryptophan) contains as much as 33 mg 5-hydroxytryptamine (13 mg as free base and 20 mg as the glucuronide) (Erspamer & Bertaccini, 1962). This signifies that decarboxylation of L-tryptophan in the rat organism is at least 200 times less rapid than that of 5-hydroxytryptophan. Now, in spite of the easy attack by decarboxylase, the minimum oral dose of L-5-hydroxytryptophan causing a significant reduction of urine flow is as high as 100 mg/kg (Erspamer & Bertaccini, 1962), whereas the minimum oral dose of L-tryptophan active on diuresis is only twice as great. These results strongly suggest that tryptamine, which is produced from the precursor amino acid at a rate at least 200 times less than 5-hydroxytryptamine and which is 100 to 300 times less potent than 5-hydroxytryptamine, cannot be responsible for the antidiuresis provoked by L-tryptophan.

It may be observed that maximum antidiuretic effect appears during the first 1 or 2 hr following tryptophan load, whereas maximum excretion of 5-hydroxyindoleacetic acid is more delayed. This demonstrates that 5-hydroxytryptamine synthesis and liberation by the gastrointestinal tract follows very promptly the absorption of the precursor amino acid and provides additional proof that the amount of 5-hydroxytryptamine required to reduce urine flow is extremely small, as it is not detectable in urine in the form of excess 5-hydroxyindoleacetic acid. It may be that, as biosynthesis of 5-hydroxytryptamine continues, the released amine acts on the renal effectors less intensely, owing to the appearance of tachyphylaxis.

The production of an evident, easily repeatable pharmacological effect with a common dietary amino acid given by mouth is not an unusual phenomenon. It should be added that the antidiuretic effect of L-tryptophan was obtained in completely normal, non-pretreated animals.

It is difficult to decide whether this observation can be taken as evidence in favour of the hypothesis that 5-hydroxytryptamine may interfere in rats in the physiological control of diuresis. Certainly the circumstance that antidiuresis has been provoked by an excess of 5-hydroxytryptamine of endogenous origin (the remote precursor of endogenous 5-hydroxytryptamine is in fact L-tryptophan) is in agreement rather than in disagreement with such a hypothesis.

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