THE EFFECT OF THE ADMINISTRATION OF WATER OR ISOTONIC NaCl SOLUTION ON THE URINARY EXCRETION OF 5-HYDROXYINDOLEACETIC ACID IN THE RAT

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Administration of tap water by stomach tube or of isotonic NaCl solution orally or subcutaneously produces a conspicuous increase in the urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) in the rat. The same increase can be observed after administration of water plus posterior-pituitary antidiuretic hormone (ADH). Tap water is less effective than physiological saline; the latter, in its turn, is more effective by the subcutaneous route than by mouth. Increase of 5-HIAA may be, for short periods, as high as 3 times normal. The minimum oral dose of tap water causing a significant increase in urinary 5-HIAA is 2 ml./100 g., that of physiological saline 1 ml./100 g. Repeated doses of both tap water and physiological saline produce a more intense and long-lasting increase of 5-HIAA output than single doses. Generally, excess 5-HIAA coincides with excess urine elimination, but there is no obligatory correlation of the intensity of the two phenomena. Recovery, as urinary 5-HIAA, of exogenous 5-HIAA, 5-hydroxytryptamine (5-HT) and 5-hydroxytryptophan (5-HTP) is the same in control, non-hydrated rats and in rats given single or repeated doses of tap water, tap water plus ADH, or isotonic saline solution. The mechanism by which water or saline administration produces increased urinary excretion of 5-HIAA is discussed.

IN the course of other investigations it was observed that rats given a water load by stomach tube excreted more 5-hydroxyindoleacetic acid (5-HIAA) during the period of water diuresis than did control rats given no water.

This paper describes the results of a more complete investigation on the influence of administration of water and of physiological NaCl solution on urinary 5-HIAA excretion in rats.

EXPERIMENTAL

Experimental animals. Approximately 300 Wistar rats, of both sexes, weighing 180–240 g., were used. The same rats were given water or saline loads at intervals of 10–15 days. The diet was the standard laboratory diet.

Urine collection. Urine was collected in graduated cylinders from groups of four rats kept in diuresis cages.

Estimation of urinary 5-HIAA. 5-HIAA was estimated by the method of Macfarlane, Dalgliesh, Dutton, Lennox, Nyhus and Smith (1956) usually immediately after urine collection; if this was not possible the urine was treated with a few drops of acetic acid and the same volume of acetone and then stored in the refrigerator. When large volumes of urine were excreted they were concentrated at 40 to 50° under reduced

pressure. In recovery experiments 80 to 100 per cent of added 5-HIAA was found in urine after its concentration.

Estimation of 5-HT *in tissues.* Tissues and serum were extracted twice with 4 parts (4 ml./g. or 4 ml./ml.) of acetone. Bioassay of 5-HT was by the rat uterus preparation, as described previously (Erspamer, 1956).

Compounds. 5-Hydroxyindoleacetic acid, 5-hydroxytryptamine creatinine sulphate and DL-5-hydroxytryptophan were kindly supplied by the Farmitalia S.p.A., Milan. The posterior pituitary preparation used was Pitressin, Parke, Davis & Co.

RESULTS

The Effect of Single Oral Doses of Tap Water

Administration of 5 ml./100 g. of tepid tap water by stomach tube was followed by diuresis. This diluted urine contained about twice the 5-HIAA content of the urine of control rats. The increase in 5-HIAA output lasted only 2 hr. and ceased at the same time as did the water diuresis (Table I).

TABLE I

URINE VOLUME AND URINARY 5-HIAA EXCRETION (IN ML./KG. AND μG./KG. ± S.E.) AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF TAP WATER. IN PARENTHESES THE NUMBER OF GROUPS* OF RATS

Dose of administered water	(A) Unit volume (ml./kg.) (B) 5-HIAA(μg./kg.)	Urine collection period hr.			
		0–2	2–6	6–24	0–24
No water (24)	A	2·6 ± 0·32	3·7 ± 0·22	16·8 ± 0·77	22·4
	B	9·8 ± 0·59	17·6 ± 0·41	85·9 ± 0·71	113·3
5 ml./100 g. (24)	A	37·5 ± 1·73	11·9 ± 0·57	15·5 ± 0·61	64·9
	B	22·4 ± 0·86	19·7 ± 1·02	86·7 ± 2·0	128·8
3 ml./100 g. (6)	A	18·0 ± 0·29	9·0 ± 0·58	12·0 ± 0·58	39·0
	B	15·3 ± 1·16	22·6 ± 1·16	91·0 ± 2·90	129·0
No water (8)	A	6·4 ± 0·7	6·0 ± 0·18	—	—
	B	7·5 ± 0·49	21·2 ± 0·42	—	—
2 ml./100 g. (8)	A	18·0 ± 1·1	6·7 ± 0·6	—	—
	B	10·5 ± 0·57	21·2 ± 1·0	—	—
1 ml./100 g. (8)	A	12·1 ± 1·0	7·2 ± 0·7	—	—
	B	8·1 ± 0·67	25·0 ± 1·87	—	—

* 4 in each group.

The effect of a water load of 3 ml./100 g. was similar to that produced by 5 ml./100 g.; 2 ml./100 g. still produced a significant increase in 5-HIAA excretion ($P < 0{\cdot}01$); 1 ml./100 g. caused no appreciable effect ($P > 0{\cdot}4$). 1 ml. tap water plus 4 ml./100 g. paraffin oil provoked the same urinary excretion of 5-HIAA as 1 ml. tap water alone (Table II).

The Effect of Single Oral or Subcutaneous Doses of Isotonic and Hypertonic Solution of NaCl

Table III shows that both isotonic (0·9 per cent) and hypertonic (1·8 per cent) solution of NaCl given by mouth in amounts of 5 ml./100 g. produced an increase in the urinary output of 5-HIAA similar to that produced by tap water. Isotonic saline was more effective than, and hypertonic saline

TABLE II

URINE VOLUME AND URINARY 5-HIAA EXCRETION (IN ML./KG. AND μG./KG. ± S.E.) AFTER THE ORAL ADMINISTRATION OF PARAFFIN OIL. IN PARENTHESES THE NUMBER OF GROUPS* OF RATS

Treatment	(A) Urine volume (ml./kg.) (B) 5-HIAA(μg./kg.)	Urine collection period hr.			
		0–2	2–6	6–24	0–24
Tap water 1 ml./100 g. (12)	A	8·5 ± 0·57	6·3 ± 0·86	13·2 ± 0·29	27·7
	B	10·9 ± 0·73	22·8 ± 1·32	108·5 ± 2·35	141·4
Tap water 1 ml./100 g. + paraffin oil 4 ml./100 g. (12)	A	9·3 ± 1·17	5·1 ± 0·57	13·9 ± 1·17	28·3
	B	11·0 ± 0·29	23·3 ± 1·47	107·0 ± 2·64	141·0

*4 in each group.

approximately as effective as, tap water. With isotonic saline and hypertonic saline the excess urinary excretion of 5-HIAA lasted at least for 6 hr. The threshold dose of oral physiological saline causing a significant increase in urinary 5-HIAA during the first 2-hr. period was as low as 1 ml./100 g. ($P < 0·01$).

Subcutaneous isotonic saline caused a higher urinary output of 5-HIAA than oral isotonic saline. Maximum excretion of 5-HIAA occurred in the 6–11 hr. period, concomitantly with maximum diuresis.

TABLE III

URINE VOLUME AND URINARY 5-HIAA EXCRETION (IN ML./KG. AND μG./KG. ± S.E.) AFTER ORAL OR SUBCUTANEOUS ADMINISTRATION OF SINGLE DOSES OF TAP WATER, ISOTONIC NaCl SOLUTION (0·9 PER CENT), AND HYPERTONIC NaCl SOLUTION (1·8 PER CENT). IN PARENTHESES THE NUMBER OF GROUPS* OF RATS

Treatment	(A) Urine volume (ml./kg.) (B) 5-HIAA(μg./kg.)	Urine collection period hr.			
		0–2	2–6	6–11	11–24
Controls (12)	A	4·9 ± 0·69	5·5 ± 0·52	5·0 ± 0·59	7·0 ± 0·59
	B	7·6 ± 1·1	16·7 ± 0·87	20·0 ± 1·15	65·5 ± 1·73
Tap water by mouth 5 ml./100 g. (14)	A	33·0 ± 1·6	12·4 ± 0·56	6·1 ± 0·56	9·2 ± 0·67
	B	20·5 ± 1·1	16·5 ± 1·26	21·7 ± 2·68	69·0 ± 2·46
Isotonic NaCl by mouth 5 ml./100 g. (16)	A	9·2 ± 0·62	14·1 ± 0·82	11·5 ± 0·75	15·8 ± 0·60
	B	20·2 ± 0·87	21·5 ± 0·69	29·4 ± 0·81	78·0 ± 1·12
Isotonic NaCl by mouth 1 ml./100 g. (6)	A	11·5 ± 0·90	19·6 ± 1·22	—	—
	B	11·5 ± 0·65	23·1 ± 2·45	—	—
Isotonic NaCl by s.c. route 5 ml./100 g. (6)	A	5·8 ± 0·81	11·6 ± 0·81	23·0 ± 1·44	13·8 ± 1·46
	B	12·0 ± 1·44	25·0 ± 1·22	58·0 ± 3·26	71·5 ± 4·07
Hypertonic NaCl by mouth 5 ml./100 g. (6)	A	21·6 ± 2·04	7·2 ± 1·02	5·5 ± 0·94	10·0 ± 1·02
	B	17·0 ± 1·28	23·6 ± 0·91	20·0 ± 1·83	68·0 ± 2·73

*4 in each group.

The Effect of Repeated Doses of Tap Water and Isotonic Saline

Fig. 1 shows the effect on urinary 5-HIAA excretion and urine volume of tap water given by mouth, and of isotonic saline, given by mouth or subcutaneously on three occasions at 2-hr. intervals.

In another experiment, isotonic saline was given 6 times by mouth at 2-hr. intervals. During the 0–6, 6–12, 12–24 and 24–36 hr. periods the four groups of rats excreted 12·5, 13·6, 6·5 and 4 μg. 5-HIAA per kg./hr., respectively, and eliminated 15, 26, 4 and 1·7 ml./kg./hr. of urine. Total

excretion of 5-HIAA in the first 24-hr. period was 235 μg. compared with 136 μg. of the controls.

Repeated doses of tap water are seen to produce urinary excretion of 5-HIAA throughout the whole period of water diuresis (6 hr.); the amount of 5-HIAA was up to 3–4 times the control value. Isotonic saline given by mouth had a similar effect. Repeated subcutaneous doses of isotonic saline caused a more delayed diuresis and a conspicuous increase in urinary

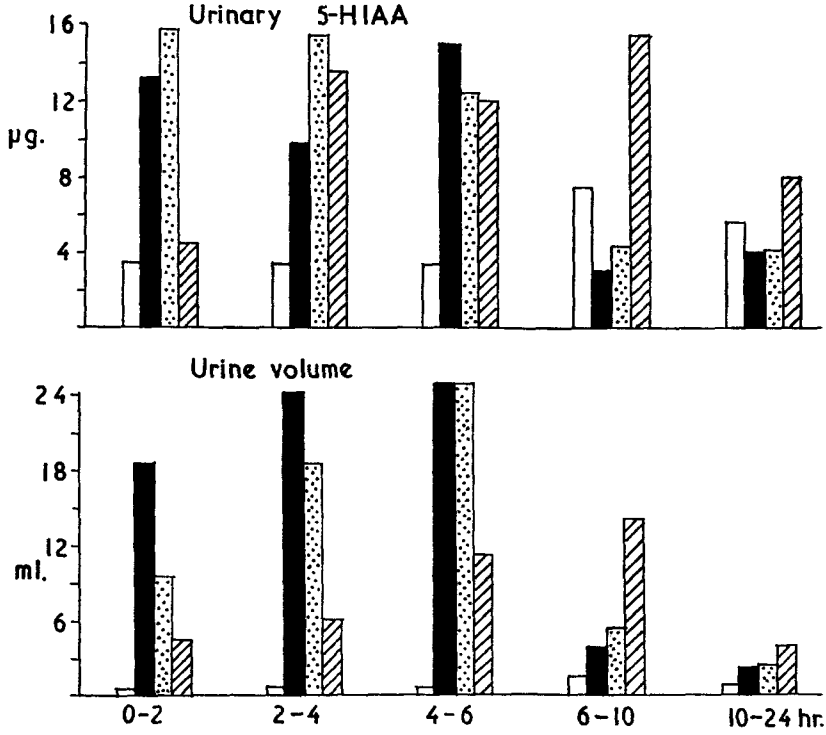

FIG. 1. Urinary 5-HIAA excretion (in μg./kg./hr.) and urine volume (in ml./kg./hr.) in control rats (unshaded columns), in rats given three doses of 5 ml./100 g. tap water by mouth (black columns), in rats given three doses of 5 ml./100 g. isotonic NaCl solution by mouth (dotted columns), and in rats given the same amount of isotonic saline by subcutaneous injection (hatched columns). Water and saline were given at 2-hour intervals, beginning at 0 time. Each value refers to the pool of urine obtained from 16 rats.

excretion of 5-HIAA lasting over 12 hr. During the whole period the injected rats excreted twice as much 5-HIAA as did the control rats.

Repeated doses of subcutaneous saline caused more 5-HIAA excretion than did repeated doses of oral saline.

Although during the first 6 hr. rats given tap water excreted almost 4 times more 5-HIAA than control rats (76 μg. compared with 20·5 μg./kg.), the 24-hr. output of 5-HIAA was the same in the two groups of rats (140 μg. compared with 136 μg./kg.). This is due to the less intense

excretion of 5-HIAA shown by hydrated rats in the 10–24 hr. and, still more so, in the 6–10 hr. periods. Yet, during these periods hydrated rats excreted a half to twice as much more urine than control rats. This affords a striking example of the lack of a clear-cut and constant relation between diuresis and 5-HIAA excretion.

The Effect of Single or Repeated Oral Doses of Tap Water Plus Antidiuretic Hormone of the Posterior Pituitary (ADH)

The effect on 5-HIAA excretion of single oral doses of 5 ml./100 g. of tap water given at the same time with a subcutaneous injection of 0·1 or 1 unit/kg. of ADH (in 0·5 ml. distilled water/100 g.) is shown in Fig. 2.

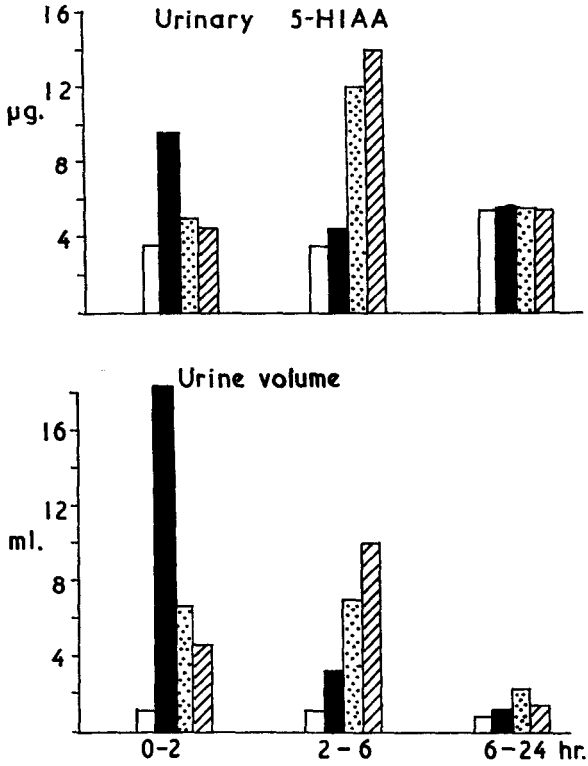

FIG. 2. Urinary 5-HIAA excretion (in μg./kg./hr.) and urine volume (in ml./kg./hr.) in control rats (unshaded columns), in rats given 5ml./100 g. tap water by mouth (black columns), and in rats given the same amount of tap water plus 0.1 unit/kg. (dotted columns) or 1 unit/kg. (hatched columns) of posterior-pituitary antidiuretic hormone, subcutaneously. Each value refers to the pool of urine obtained from 12 rats.

In another experiment, two water loads were given at 2-hr. intervals, simultaneously with two subcutaneous injections of 1 unit/kg. ADH. After 4, 6 and 8 hr., three other injections of ADH were given without any further water load. During the 0–6, 6–12, 12–24 and 24–36 hr.

periods the four groups of rats given water plus ADH excreted 5·1, 6·3, 10·8 and 4·2 μg. 5-HIAA per kg./hr., respectively, and eliminated 3, 2·5, 9 and 1·6 ml./kg./hr. of urine; four groups of untreated control rats excreted 3·4, 7·8, 5·7 and 4 μg./kg./hr. of 5-HIAA, and eliminated 0·8, 1·5, 1 and 1 ml./kg./hr. of urine, respectively.

It seems that after the administration of ADH both water diuresis and maximum excretion of 5-HIAA were much delayed. After a single dose of ADH, maximum diuresis and 5-HIAA excretion occurred in the 2–6 hr. period; after repeated doses of ADH, in the 12–24 hr. period, or more than 10 hr. after the water loads. In the first 12-hr. period the amount of 5-HIAA excreted by rats given six injections of ADH did not exceed that of the control rats, in spite of excreting more than twice the volume of urine.

Single or repeated injections of ADH did not show an appreciable effect on the urinary excretion of 5-HIAA in non-hydrated, control rats.

The Recovery, as Urinary 5-HIAA, *of Exogenous* 5-HTP, 5-HT *and* 5-HIAA *in Control Rats and in Rats Given Isotonic Saline or Tap Water plus* ADH

Numerous experiments were made to check the influence of repeated oral doses of physiological saline or of tap water plus ADH on the urinary excretion of strictly exogenous 5-HIAA and also of the 5-HIAA originating in the tissues from exogenous 5-HTP or 5-HT.

TABLE IV

RECOVERY, AS URINARY 5-HIAA, OF 5-HT AND 5-HIAA ADMINISTERED TO CONTROL RATS, TO RATS GIVEN TAP WATER AND TO RATS GIVEN TAP WATER PLUS ADH. WATER, 5-HT AND 5-HIAA WERE ADMINISTERED BY MOUTH, ADH BY SUBCUTANEOUS ROUTE IN SINGLE OR REPEATED DOSES, AT 2-HR. INTERVALS. IN PARENTHESES THE NUMBER OF GROUPS* OF RATS

Administered water	Dose/kg. of 5-HT or 5-HIAA	5-HIAA excretion (μg./kg. ± s.e.)		
		0–6 hr.	6–12 hr.	12–24 hr.
Nil (4)	5-HT, 1 mg. × 2	92·2 ± 3·92	48·2 ± 3·12	57·5 ± 5·03
5 ml./100 g. × 2 (4)	5-HT, 1 mg. × 2	108·5 ± 2·82	39·0 ± 2·61	61·0 ± 2·43
5 ml./100 g. × 2 + ADH, 1 unit/kg. × 3 (4)	5-HT, 1 mg. × 2	87·7 ± 4·17	69·7 ± 7·01	57·0 ± 1·8
Nil (4)	5-HIAA, 1 mg. × 2	365·5 ± 16·0	164·7 ± 15·0	65·0 ± 8·7
5 ml./100 g. × 2 (4)	5-HIAA, 1 mg. × 2	307·5 ± 10·1	95·0 ± 2·1	76·5 ± 5·2
5 ml./100 g. × 2 + ADH, 1 unit/kg. × 3 (4)	5-HIAA, 1 mg. × 2	229·5 ± 11·3	137·5 ± 9·7	91·2 ± 8·6
Nil (3)	Nil	18·0 ± 1·15	35·3 ± 2·6	—
Nil (3)	5-HIAA, 1 mg.	223·2 ± 9·38	69·3 ± 12·7	—
5 ml./100 g. (3)	Nil	26·3 ± 2·18	36·0 ± 2·88	—
5 ml./100 g. (3)	5-HIAA, 1 mg.	246·7 ± 3·34	61·0 ± 2·33	—
Nil (3)	5-HIAA, 1 mg.	146·6 ± 26·1	—	—
5 ml./100 g. (3)	5-HIAA, 1 mg.	132·6 ± 9·1	—	—

* 4 in each group.

To avoid the pharmacological actions of 5-HTP the dose was restricted to 1 mg./kg. since Erspamer and Bertaccini (1962) showed the minimum antidiuretic dose of subcutaneous DL-5-HTP to be 10–20 mg./kg. The dose was chosen so that the amount of 5-HT produced would be similar to that produced by endogenous 5-HTP. For the same reasons 5-HT was

administered only by mouth at a dose which proved to be completely ineffective on diuresis (Erspamer and Ottolenghi, 1953).

The most important results obtained in these experiments are shown in Tables IV and V.

TABLE V

RECOVERY, AS URINARY 5-HIAA, OF DL-5-HTP ADMINISTERED BY SINGLE OR REPEATED SUBCUTANEOUS OR INTRAPERITONEAL INJECTIONS TO CONTROL RATS AND TO RATS GIVEN SINGLE OR REPEATED ORAL LOADS OF WATER (WITH OR WITHOUT ADH) OR ISOTONIC SALINE. ADH WAS GIVEN BY SUBCUTANEOUS ROUTE. IN PARENTHESES THE NUMBER OF GROUPS* OF RATS

Water or saline load	Dose of DL-5-HTP	5-HIAA excretion (in μg./kg. ± s.e.)		
		0–6 hr.	6–12 hr.	12–24 hr.
1. Nil (4)	1 mg./kg. s.c. × 2	124·5 ± 3·8	68·2 ± 8·16	48·75 ± 2·13
Water 5 ml./100 g. × 2 (4)	1 mg./kg. s.c. × 2	160·0 ± 4·3	46·2 ± 2·4	41·75 ± 1·5
Water 5 ml./100 g. × 2 + ADH, 1 unit/kg. × 3 (4)	1 mg./kg. s.c. × 2	131·5 ± 3·8	79·5 ± 7·0	48·5 ± 3·4
2. Nil (3)	Nil	19·3 ± 1·3	41·0 ± 2·08	—
Nil (3)	1 mg./kg. s.c. × 2	103·3 ± 8·83	71·0 ± 0·58	—
Saline 5 ml./100 g. × 2 (3)	Nil	61·6 ± 4·48	37·0 ± 4·93	—
Saline 5 ml./100 g. × 2 (3)	1 mg./kg. s.c. × 2	184·3 ± 2·26	30·0 ± 1·73	—
3. Nil (3)	1 mg./kg. i.p.	106·7 ± 5·95	60·0 ± 9·75	77·3 ± 3·6
Water 5 ml./100 g. (3)	1 mg./kg. i.p.	147·0 ± 7·23	43·3 ± 6·20	72·0 ± 8·2
ADH, 1 unit/kg. × 3, no water (3)	1 mg./kg. i.p.	108·3 ± 4·4	78·3 ± 9·3	73·7 ± 6·87
Water 5 ml./100 g. + ADH, 1 unit/kg. × 3 (3)	1 mg./kg. i.p.	120·0 ± 11·6	43·0 ± 3·6	57·0 ± 4·04
4. Nil (6)	Nil	19·8 ± 1·39	40·8 ± 1·26	—
Nil (6)	1 mg./kg. s.c. × 2	72·2 ± 4·86	54·5 ± 1·55	—
Saline 5 ml./100 g. × 2 (3)	Nil	40·0 ± 3·5	31·7 ± 1·55	—
Saline 5 ml./100 g. × 2 (3)	1 mg./kg. s.c. × 2	94·3 ± 3·02	27·0 ± 1·8	—
ADH, 1 unit/kg. × 3, no water (3)	1 mg./kg. s.c. × 2	73·7 ± 5·06	54·7 ± 1·22	—
Water 5 ml./100 g. × 2 + ADH, 1 unit/kg. × 3 (3)	1 mg./kg. s.c. × 2	90·0 ± 0·81	34·3 ± 1·63	—
5. Nil (3)	Nil	47·0 ± 0·41		61·7 ± 1·63
Nil (6)	1 mg./kg. s.c. × 3	208·0 ± 14·7		84·3 ± 2·92
Saline 5 ml./100 g. × 2 (6)	Nil	74·5 ± 4·74		58·5 ± 5·6
Saline 5 ml./100 g. × 2 (6)	1 mg./kg. s.c. × 3	198·0 ± 8·17		59·5 ± 4·9
Water 5 ml./100 g. × 2 + ADH, 1 unit/kg. × 3 (3)	1 mg./kg. s.c. × 3	203·3 ± 17·3		44·7 ± 4·49

*4 in each group.

The following conclusions are valid. Total recovery of 5-HTP, as urinary 5-HIAA, in rats given repeated doses of physiological saline or of tap water plus ADH was the same as, or lower than that observed in control rats. ADH administration to non-hydrated rats given 5-HTP did not produce any change in the urinary excretion of 5-HIAA.

Similar results were obtained after administration of exogenous 5-HT and 5-HIAA. In no instance was recovery of 5-HIAA greater after water loads or water plus ADH.

In experiments 1 and 5 of Table V excess 5-HIAA produced by the precursor amino-acid was completely eliminated within the first 6-hr. period by hydrated rats, whereas in non-hydrated rats it appeared also in the urine of the second 6-hr. period. This fact may be interpreted to be due to a more thorough washing out of the urinary bladder in hydrated rats. In control rats some of the small amount of urine collected in the bladder during the first 6-hr. period was probably evacuated during the second period, because of a lack of stimulus to urinate.

The Effect of Repeated Oral Administration of Physiological Saline or of Tap Water plus ADH *on the* 5-HT *Content of the Gastrointestinal Mucosa and other Tissues*

Four groups of two rats each of 180–250 g. were given 6 doses of physiological saline by mouth (5 ml./kg.) at 2-hr. intervals, and then killed by bleeding 2 hr. after the last dose. Four other groups were given 2 doses of tap water at 2-hr. intervals and at the same time two subcutaneous injections of 1 unit/kg. of ADH. ADH was given on four further occasions at 2-hr. intervals. Finally, four groups of rats served as controls. The results of 5-HT estimation are summarised in Table VI.

TABLE VI

THE 5-HT CONTENT IN DIFFERENT TISSUES OF CONTROL RATS, RATS GIVEN REPEATED ORAL DOSES OF ISOTONIC SALINE AND RATS GIVEN TAP WATER PLUS ADH. IN PARENTHESIS THE NUMBER OF GROUPS* OF RATS. FIGURES WITHOUT S.E. REFER TO A POOL OBTAINED FROM 8 RATS

Tissue	5-HT content (in μg./kg. or μg./ml. ± s.e.)		
	Control rats	Saline rats	ADH rats
Stomach (4)	1·86 ± 0·15	1·70 ± 0·24	1·41 ± 0·16
Small intestine, first quarter (4)	2·30 ± 0·13	1·86 ± 0·06	1·59 ± 0·14
Small intestine, last three quarters (4)	2·57 ± 0·16	1·71 ± 0·15	1·70 ± 0·16
Large intestine (4)	6·32 ± 0·5	5·65 ± 0·5	5·40 ± 0·3
Lung	2·0	1·5	1·55
Spleen	2·15	2·0	2·15
Serum	0·65	0·60	0·62
Brain	0·48	0·30	0·26
Gastrointestinal tract	1·87	1·31	1·22
Lung	0·84	0·66	0·64
Spleen	1·65	1·65	1·60
Serum	0·52	0·54	0·60

*2 in each group.

In a second experiment three additional groups of 8 rats, each of 130–160 g. were treated exactly as above, with the results shown in the lower part of the Table.

The urine volume and the total 5-HIAA excretion during the 12-hr. period elapsing from the first water or saline load until death were in the first experiment: Control rats 20 ml./kg.; 71 μg./kg.; isotonic saline rats 262 ml./kg.; 210 μg./kg., and ADH rats 38 ml./kg.; 78 μg./kg.

And in the second experiment: Control rats 19 ml./kg.; 64 μg./kg., isotonic saline rats 266 ml./kg.; 221 μg./kg., and ADH rats 33 ml./kg.; 57 μg./kg.

It may be seen that at the time of death the rats treated with physiological saline had eliminated most of the administered liquid and as much as 3 to 3·5 times more 5-HIAA than the controls. Rats treated with ADH, on the contrary, still retained enormous amounts of water (in some instances there was haemoglobin in urine, indicating an incipient haemolysis) and the excreted amount of 5-HIAA did not exceed that of controls.

In the first experiment the weight of the intestines was 50·7 g./kg. in control rats, 53·2 g. in isotonic saline rats, and 50·3 g./kg. in ADH rats; in the second experiment 52·2, 55·5 and 51·8 g./kg., respectively. Thus the decrease in the 5-HT content of the intestines cannot be ascribed,

except to an insignificant extent and this only in isotonic saline rats, to an increase in the weight of the tissue produced by water or saline loads.

In a third experiment, 100 rats served as controls and 20 were treated, without any water load, with 5 subcutaneous doses of 1 unit/kg. ADH in 0·25 ml./100 g. physiological saline. At the end of the 12-hr. observation period 5-HIAA excretion was 49 μg./kg. in control rats and 47 μg./kg. in ADH rats. The 5-HT content of the gastrointestinal tract was 2·8 and 2·45 μg./g., respectively; that of the brain 0·41 and 0·38 μg./g., respectively. It is evident that ADH alone does not interfere either in the storage or metabolism of 5-HT.

The above estimations of 5-HT in tissues must be considered as preliminary to a more thorough investigation, and more evidence is needed before it may be concluded that excess urinary excretion of 5-HIAA produced by isotonic saline or water plus ADH is accompanied by a moderate reduction in the 5-HT content of the gastrointestinal tract, lung and brain. The only thing which seems certain is that the 5-HT content of serum and spleen remains unchanged.

Discussion

Administration of water or isotonic saline produced in rats a variable but always conspicuous increase in the urinary excretion of 5-HIAA. This fact seems to be firmly established, but the interpretation of the phenomenon appears to be extremely difficult, owing to the existence of at least three possible explanations.

The first which may be put forward is that oral water or saline increases 5-HIAA output by stimulating the release of 5-HT from the gastrointestinal mucosa through increase of the intraluminal pressure, particularly in the stomach and upper part of the small intestine. Attention on this possibility has been called especially by Bülbring and Lin (1958) and Bülbring and Crema (1959), who showed that, in the guinea-pig, increase of intraluminal pressure in an isolated intestinal loop produced the release into the lumen of measurable amounts of 5-HT.

In the present experiments the participation of intestinal distension in producing excess urinary excretion of 5-HIAA can be excluded by the observation that subcutaneous saline was more effective than oral saline, and that paraffin oil (40 ml./kg.), provoking a distension of the entire gastrointestinal tract, failed to cause any increase in urinary 5-HIAA. The latter observation does not favour the hypothesis that the physiological stimulus for release of 5-HT by the intestinal mucosa, at least for the release into the blood, is represented by increased intraluminal pressure.

The second possibility is that water or saline administration causes increased 5-HT release from the gastrointestinal tract and possibly from other tissues, and hence increased 5-HIAA excretion in urine, as a consequence of the expansion of blood volume or hydraemia it produces or both. Finally, a third possibility is that increased urinary excretion of 5-HIAA is of merely renal origin, being due to a facilitated excretion by

the tubules of the circulating 5-HIAA or to a defect in the tubular reabsorption of the filtered or excreted 5-HIAA.

On the whole, the third hypothesis seems to be most plausible, as it gives a satifactory explanation of the fact that increased 5-HIAA excretion generally paralleled increased urine elimination and that following administration of oral water plus ADH, maximum 5-HIAA excretion was delayed until cessation of block of diuresis produced by ADH.

However, the following experimental observations are difficult to explain and to reconcile with the hypothesis that water or saline loads simply act through renal mechanisms. Although, as stated above, increased 5-HIAA excretion generally coincided with increased urine elimination, there was no constant and obligatory correlation of the two phenomena. For example, repeated subcutaneous doses of isotonic saline produced during the second 2-hr. observation period the excretion of 6 ml./kg./hr. urine and 13·6 μg./kg./hr. 5-HIAA, whereas repeated oral doses of saline caused the excretion of 18·2 ml./kg./hr. urine and 15·6 μg./kg./hr. 5-HIAA (Fig. 1); single oral doses of water caused, during the first 2-hr. period, the excretion of three times more urine than single doses of isotonic saline, yet in both instances urine contained the same absolute amount of 5-HIAA (Table III).

In some experiments, hyperexcretion of 5-HIAA by hydrated rats was followed by a period of reduced excretion, in spite of the urine volume being greater than that of the controls (Fig. 1).

Urinary recovery, as 5-HIAA, of exogenous 5-HTP was satisfactorily the same in hydrated rats (with and without ADH) and in control rats and, at any rate, never larger. The same was true of urinary recovery, as 5-HIAA, of exogenous 5-HT and 5-HIAA. This signifies that saline or water loads (with or without ADH) do not interfere either with the formation of 5-HIAA, via 5-HT, or with the renal excretion of 5-HIAA. Moreover, whereas in rats given water plus ADH, the excretion of 5-HIAA originating from exogenous 5-HTP, 5-HT or 5-HIAA was maximum in the first 6-hr. period, i.e. during ADH antidiuresis, maximum excretion of strictly endogenous 5-HIAA occurred only in the second 6-hr. period, in which as much as 70 to 80 per cent of administered water was eliminated.

Our preliminary studies on the 5-HT content of rat tissues after repeated doses of isotonic saline or of tap water plus ADH have not given conclusive results. At any rate the release of as little as 20 to 30 per cent of the 5-HT contained in the gut, lung and other tissues would certainly not be sufficient to explain the observed huge increase in the urinary 5-HIAA excretion. However, it should be emphasised that static measurements of the 5-HT content of tissues do not reflect the rate of 5-HT formation and metabolism. The 5-HT found at a given moment in tissues corresponds merely to the algebraic sum of the 5-HT formed and that released, and it may well be that administration of water or physiological saline produced not only a moderate reduction of the storing capacity of the tissues, but also an accelerated biosynthesis of 5-HT.

The problem will be further investigated by the administration of L-tryptophan, the remote precursor of 5-HT, α-methyldopa, an inhibitor of the biosynthesis of 5-HT, and reserpine, a powerful 5-HT liberator.

It has been recently claimed that the renal excretion rate of weak organic acids and bases, such as indoleacetic acid, 5-HIAA and 5-HT may depend on the pH of urine. The interference of the urine reaction in the results of present experiments can be excluded, since the pH of urine was practically the same in control rats, in rats given water and in rats given saline, and varied in different experiments only within a small range (6·2–6·9). Moreover, Milne, Crawford, Girão and Loughridge (1960) were unable to find any significant alteration in urinary excretion of exogenous 5-HIAA in the rat by changing the pH, and any alteration in excretion of endogenous 5-HIAA in man; and similarly Sandler and Spector (1961) observed no change in the urinary elimination of 5-HIAA after administration of 5-HT in "acid" rats (pH of urine 5·5–6) and in "alkaline" rats (pH of urine 8–8·5).

Summing up, it is clear that the problem of the origin of excess urinary 5-HIAA after water or isotonic NaCl administration cannot be considered to be solved. Further extensive investigation must be made not only in rats, but also in other animal species. Preliminary experiments in man seem to confirm the data obtained in rats.

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