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THE RELEASE OF 5-HYDROXYTRYPTAMINE FROM THE RAT STOMACH *IN VITRO*

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SUMMARY

1. 5-HT was released into the lumen of the intact isolated rat stomach and into the bath fluid surrounding a preparation of the body and antrum stretched mechanically.

2. Release of 5-HT increased when the pressure inside the intact stomach was raised or when the body/antrum preparation was stretched.

3. This increased release was not prevented by hexamethonium, atropine, hyoscine or procaine, and was probably due to distortion of cells containing 5-HT.

4. During periods of peristalsis induced by transmural stimulation the pharmacological activity of the fluid in the stomach was usually increased owing to a greater release of 5-HT and also to the release of an unidentified substance.

5. In reserpine-treated rats, 5-HT was released into the stomach but transmural stimulation did not produce true peristalsis and only rhythmic contractions occurred.

6. Peristalsis was seldom reduced by methysergide, and it is concluded that 5-HT is not essential for gastric peristalsis in the rat.

INTRODUCTION

Although 5-hydroxytryptamine is present in the wall of the gastro-intestinal tract a physiological reason for its presence has not been established. It has been shown that the amount of 5-HT released into the lumen of guinea-pig ileum rises when the intraluminal pressure is increased (Bülbring & Lin, 1958; Bülbring & Crema, 1959*a*), and that 5-HT lowers the pressure threshold at which the peristaltic reflex occurs. Boullin (1964) found that normal intestinal peristalsis occurred in rats depleted of 5-HT although intraluminal or serosal application of 5-HT did stimulate peristalsis. Paton & Vane (1963) have described the release

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of 5-HT into the bath fluid surrounding the rat and guinea-pig stomach, and they suggest that its release is dependent on an increase in muscle tone and not on nervous activity. Armitage & Dean (1966), using an isolated transmurally stimulated rat stomach, showed that 5-HT added to the bath had no effect on vigorous peristalsis, but temporarily improved peristalsis in a failing preparation.

In order to examine the role of 5-HT in gastric peristalsis we have used the technique of Armitage & Dean (1962), and have estimated the release of 5-HT during peristalsis or when the intraluminal pressure was raised. In addition, we have examined the release of 5-HT from isolated preparations of stomach wall stretched mechanically.

METHODS

Isolated rat stomach preparation. The stomach was set up as described in the preceding paper by Armitage & Dean (1966) except that the transducer recording flow was not used, and a fine polythene tube with terminal perforations was passed through the duodenal cannula into the body of the stomach so that the stomach could be emptied by suction with a syringe. The preparation was rested at zero intragastric pressure for 20 min before the start of the experiment. 30 sec before the first collection period the stomach was washed out by drawing through 2 ml. of Krebs's solution. An 8 min cycle of collection was used throughout the experiment. The intragastric pressure was increased to a desired level for 3 min by raising the Marriotte bottle. When there was little or no flow through the duodenum, 1·5 ml. of fluid were aspirated half way through the 3 min period. At the end of this period the Marriotte bottle was lowered to the zero position, the stomach was emptied and a further 1 ml. of Krebs's solution was drawn through. The fluid removed from the stomach was added to any that flowed through the duodenum and kept on ice until assayed for 5-HT within 1–5 hr. After a 5 min rest at zero pressure the cycle was repeated. Two to four collections were made at the initial pressure, and the next collection was made either at the same pressure in the presence of drugs, or at the same pressure with simultaneous transmural stimulation (2 shocks/sec, 1 msec pulse width, and 50 V), or at a different pressure. Subsequently one or two collections were made at the initial pressure.

Stretched stomach preparation. A rat was stunned and bled and its stomach was removed. The duodenum and fundus were cut off; then the body and antrum were cut open along the lesser curvature and rinsed in Krebs's solution. One side of the stomach was sewn on to a glass holder and the opposite side was sewn to a fine glass rod attached by a thread to a lever. The tissue was mounted in a bath of 4·5 ml. capacity containing Krebs's solution at 32° C bubbled with 95 % oxygen and 5 % carbon dioxide. After resting the tissue for 15 min at zero tension, the bath fluid was drained off, discarded and replaced with fresh solution. An 8 min collection cycle was used, a 3 min period of collection being followed by a 5 min rest at zero tension. At the end of each collection period the bath fluid was removed and replaced. The removed fluid was cooled on ice until just before assay for 5-HT. At least two collections were made at zero tension; then one collection was made while tension was applied to the tissue for 3 min by adding a weight to the lever followed by two collections at zero tension. When the effect of a drug on 5-HT release was studied, it was added to the bath fluid during the 5 min rest period and to the fresh bath fluid at the start of the 3 min period.

Assay of 5-HT. The 5-HT content (expressed as base) of a solution was determined by assay against 5-hydroxytryptamine creatinine sulphate on the rat fundus strip preparation

(Vane, 1957) in the presence of hyoscine (10^{-7} g/ml.). Samples containing a drug antagonist were assayed against standards containing an equal concentration of the drug. Bromolysergic acid diethylamide (10^{-7} g/ml.) or methysergide (10^{-7} g/ml.) was used at the end of the assays to abolish the effect of 5-HT.

Treatment with reserpine. Rats were injected with 5 mg/kg reserpine I.P. on each of the two days previous to the experiment.

Drugs used. Acetylcholine perchlorate, 5-hydroxytryptamine creatinine sulphate, atropine sulphate, 2-bromo-D-lysergic acid diethylamide (BOL 148), hexamethonium bromide, hyoscine hydrobromide, methysergide (UML 491), procaine hydrochloride, reserpine (Serpasil, Ciba). The concentrations of all drugs except BOL and methysergide are expressed in terms of base.

RESULTS

Effects of intragastric pressure and of transmural stimulation on the release of 5-HT from the rat stomach

5-HT was released into the lumen of the isolated unstimulated rat stomach in all experiments. The amount released per minute remained fairly constant at a given intragastric pressure in any one experiment except during the first collection period when it was usually higher. In different preparations at the same intragastric pressure the release per minute varied greatly; at a pressure of 4 cm of water the range was from 0·05 to 1·6 ng/min and at 8 cm of water from 0·35 to 11·0 ng/min.

5-HT release was measured at intragastric pressures varying from 2 to 12·5 cm of water. As shown in Table 1, 5-HT release increased by 0·08 to 2·48 ng/min/cm of water rise in intragastric pressure and the percentage increase varied from 28% to more than 360%. In the first four experiments given in Table 1, 5-HT release was measured at three different intragastric pressures and a graded release was obtained. The results of one of these experiments (No. 4) are given in Fig. 1.

Table 2 gives the results of 5-HT release during peristalsis produced by transmural stimulation. During peristalsis low pressures are produced in the body of the stomach but when the peristaltic wave reaches the antrum pressures of up to 60 cm of water are produced (Armitage & Dean, 1963). Peristalsis was assessed as very good if the antral pressure exceeded 30 cm of water at least once per min, and good or moderate if the performance was correspondingly less. Very good peristalsis always increased the 5-HT release whereas good or moderate peristalsis was not always effective. In twenty-three of the thirty-one observations summarized in Table 2 the pharmacological activity of the fluid in the stomach increased by 29–1580% during peristalsis but between 20 and 80% of this increased activity was due to the release of an unidentified substance not blocked on the assay preparation by BOL or methysergide. During transmural stimulation there was often increased secretion of mucus.

TABLE 1. The release of 5-HT from the rat stomach *in vitro* at different intragastric pressures

Expt.	Initial pressure (cm water)	5-HT release (ng/min)	Raised pressure (cm water)	5-HT release (ng/min)	Increase in 5-HT rel/cm rise in pressure (ng/min)	% increase/ cm rise in pressure
1	3	1·1	7·5	6·0	1·1	100
	3	1·1	12·5	9·2	0·85	77
2	4	< 0·05	8	0·35	> 0·08	> 160
	4	0·25	12	0·93	0·09	36
	4	< 0·05	8	0·77	> 0·18	> 360
	4	0·08	12	0·93	0·11	137
3	4	0·6	8	2·0	0·35	58
	4	0·8	12	6·9	0·76	95
4	3·5	1·1	10·5	5·4	0·61	55
	3·5	1·1	7·0	2·5	0·40	36
	3·5	1·0	10·5	4·8	0·54	54
5	4	1·1	8	11·0	2·48	226
6	4	1·6	8	9·3	1·93	120
7	7·5	1·1	10	2·1	0·40	36
8	2	1·8	6	6·0	1·05	58
9	3	1·0	7	2·8	0·45	45
10	3	1·1	7	8·2	1·78	162
	3	0·6	7	7·6	1·75	290
11	3	2·3	7	11·8	2·38	103
12	4	0·8	8	1·7	0·23	28

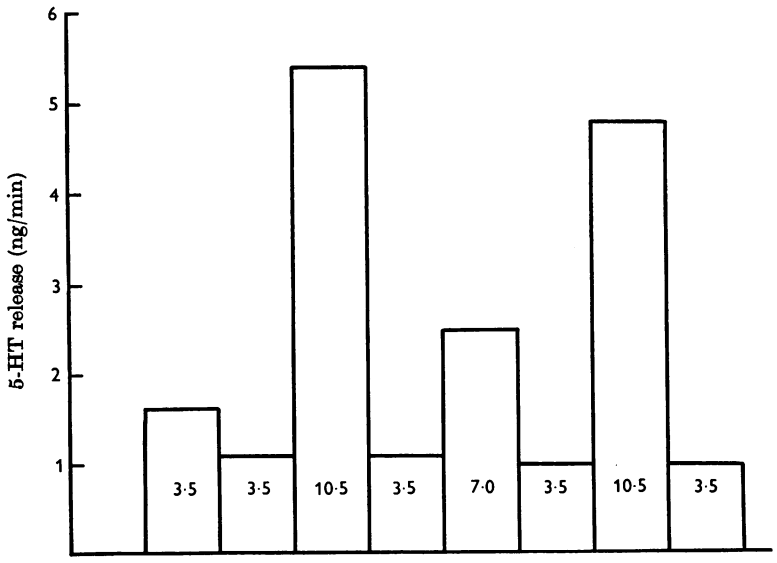

Fig. 1. The release of 5-HT at different intragastric pressures. The number in each column represents the intragastric pressure in cm of water.

The effect of stretch on the release of 5-HT

Applying weight (5–20 g) to the longitudinal or circular muscle fibres of the body and antrum of the rat stomach in the absence of drugs increased the 5-HT release by 0·04–0·32 ng/min/g increase in weight (Table 3); the percentage increase/g/min varied from 4 to 420 %.

TABLE 2. Changes in the pharmacological activity of the fluid from the rat stomach during transmural stimulation. Peristalsis is assessed as moderate, good or very good on an arbitrary scale (+ to + + +) from the pressure recordings obtained

Expt.	Intra-gastric pressure (cm water)	% increase in activity of stomach fluid after stimulation	Peristalsis
1	5	−9	+
	5	−11	+
2	4	375	+
	4	0	+
3	4	300	+
	4	375	+ +
	8	−19	+ +
	8	−7	+
4	5	0	+
	5	0	+ +
5	5	106	+ +
	5	350	+ + +
6	5	175	+
	5	113	+ +
7	4	100	+
	6	52	+
	6	100	+
8	4	33	+
	4	−19	+
	4	50	+ + +
	4	108	+ + +
9	4	29	+ + +
	4	43	+ + +
10	4	167	+ + +
	4	1580	+ + +
11	5	115	+ +
	5	79	+ + +
	5	400	+ +
	5	196	+ + +
12	4	55	+
13	5	188	+ + +

The effect of drugs on 5-HT release

Hexamethonium. The presence of hexamethonium in the bath fluid did not abolish the increase in the release of 5-HT produced by raising the intraluminal pressure (Table 4), or by applying tension to the stretched stomach preparation (Table 3).

TABLE 3. The release of 5-HT from the stretched rat stomach preparation at different tensions

Expt.	Muscle fibres stretched	Drug (g/ml.)	Initial weight (g)	5-HT release (ng/min)	Increased weight (g)	5-HT release (ng/min)	Increase in 5-HT rel/g incr. in weight (ng/min)	% increase in 5-HT rel/g rise in weight
1	Longitudinal	—	0	1·4	6·5	2·8	0·22	16
		—	0	1·1	13	2·7	0·12	11
2	Longitudinal	—	0	0·3	5	0·9	0·12	40
		—	0	0·2	10	1·7	0·15	75
3	Longitudinal	—	0	0·2	10	0·7	0·05	25
		—	0	0·2	10	0·8	0·06	30
		C_6 5×10^{-6}	0	< 0·1	10	5·1	0·5	> 500
		C_6 5×10^{-6}	0	< 0·1	10	1·7	0·16	> 160
		C_6 5×10^{-5}	0	0·3	10	0·9	0·06	20
		C_6 5×10^{-5}	0	< 0·1	10	0·8	0·07	> 70
4	Longitudinal	—	0	0·57	15	1·2	0·04	7
		Hyoscine 10^{-7}	0	0·45	15	1·4	0·06	13
		C_6 10^{-5}	0	0·88	15	2·5	0·11	13
		Procaine 10^{-5}	0	0·72	15	2·7	0·13	18
5	Circular	—	0	8·6	20	15·0	0·32	4
6	Circular	—	0	0·05	10	2·2	0·21	420
		—	0	0·06	20	1·4	0·07	117
7	Circular	—	0	0·55	15	1·1	0·04	7
		Hyoscine 10^{-7}	0	0·44	15	0·82	0·025	6
		C_6 10^{-5}	0	1·2	15	3·1	0·13	11
		Procaine 10^{-5}	0	1·4	15	4·1	0·18	13

TABLE 4. The effect of drugs on the increased release of 5-HT produced by raising intragastric pressure. Drugs were added to the bath fluid except in Expt. 3 in which the procaine was administered intraluminally

Expt.	Drug	Initial pressure (cm water)	5-HT release (ng/min)	Raised pressure (cm water)	5-HT release (ng/min)	Incr. in 5-HT rel/cm rise in pressure (ng/min)	% incr./cm rise in pressure
1	—	3	2·3	7	11·8	2·38	103
	C_6 10^{-5}	3	< 1·0	7	4·2	> 0·8	> 80
2	—	3	0·6	7	7·6	1·75	290
	C_6 2×10^{-5}	3	1·2	7	10·7	2·38	198
3	—	2	1·8	6	6·0	1·05	58
	Procaine 2×10^{-6}	2	1·3	6	7·8	1·63	125
	Procaine 10^{-5}	2	3·3	6	11·9	2·15	65
4	—	3	1·0	7	2·8	0·45	45
	Procaine 5×10^{-5}	3	1·1	7	8·0	1·73	157
5	Atropine 5×10^{-7}	3	2·5	7	13·0	2·62	106
6	Atropine 10^{-6} reserpinized rat (5 mg/kg)	3	1·7	7	3·6	0·48	28
7	Reserpinized rat (5 mg/kg)	3	7·0	7	15·6	2·15	31
		3	6·5	7	13·5	1·75	27

Atropine. In concentrations which abolished peristalsis in the transmurally stimulated stomach, atropine in the bath fluid did not affect the increased release of 5-HT produced on raising the intragastric pressure (Table 4).

Hyoscine. When tension was applied to the stretched stomach preparation in the presence of hyoscine there was an increased release of 5-HT (Table 3).

Procaine. The presence of procaine hydrochloride in the lumen or in the bath fluid inhibited peristalsis in the transmurally stimulated stomach but did not affect the increased release of 5-HT on raising the intragastric pressure (Table 4). Procaine also did not prevent the increased release of 5-HT which occurred when tension was applied to the stretched stomach preparation.

Reserpine. 5-HT was released from the stomach of reserpine-treated rats and the release increased when the intraluminal pressure was raised (Table 4).

The effect of drugs on gastric peristalsis

Reserpine. Transmural stimulation of the stomachs from reserpine-treated rats produced rhythmic contractions of the stomach wall. These waves did not appear to close the antrum from the body so that high antral pressures were not produced and flow through the pylorus was poor.

Methysergide. The presence of methysergide (10^{-8} to 10^{-6} g/ml.) in the bath fluid, or in both the bath fluid and the gastric lumen did not affect peristalsis or only slightly reduced it.

DISCUSSION

The finding that 5-HT was released into the lumen of the rat stomach and that the release increased when the intraluminal pressure was raised agrees with the results of Bülbring & Lin (1958) and Bülbring & Crema (1959*a*) on the guinea-pig ileum, and of Paton & Vane (1963) on the guinea-pig and rat stomach. A possible relation between 5-HT release and muscle tone is indicated by the work of Lee (1960) who found that the intraluminal release of 5-HT was greater in proximal guinea-pig colon where the tone of the circular muscle was high, than in the caudal part where tone was low. In our experiments differences in muscle tone may account in part for the wide range of 5-HT release observed in different preparations at the same intragastric pressure.

The increased release of 5-HT from the rat stomach when the intraluminal pressure was raised may be due to stimulation of pressure or tension receptors, or to mechanical distortion of cells containing 5-HT when the wall is stretched. As the increased release from the intact stomach

and from the stretched stomach preparation was not inhibited by hexamethonium, procaine or atropine, in doses which prevent peristalsis in response to transmural stimulation, a cholinergic nervous reflex is apparently not involved. It is likely that the release is due to mechanical distortion of cells containing 5-HT. This agrees with the conclusions of Bülbring & Crema (1959*a*) from their experiments on guinea-pig ileum.

5-HT may play a role in peristalsis in the guinea-pig ileum, since its intraluminal application stimulates peristalsis and lowers the pressure threshold, and serosal application of LSD or BOL abolishes peristalsis. On the other hand, intraluminal administration of BOL slows but does not stop peristalsis, and in guinea-pig ileum, depleted of 5-HT by reserpine, peristalsis is abnormally active (Ginzel, 1957; Bülbring & Lin, 1958; Bülbring & Crema, 1959*b*). In the rat intestine depletion of 5-HT did not affect peristalsis and intraluminal or serosal application first stimulated and then inhibited peristalsis (Boullin, 1964). Armitage & Dean (1966) have shown that 5-HT did not stimulate peristalsis in the transmurally stimulated rat stomach unless activity had decreased owing to prolonged stimulation.

In some of our experiments there was no increased release of 5-HT during transmural stimulation, indicating that 5-HT is not primarily associated with peristalsis in the rat stomach. Further, peristalsis was either unaffected or only slightly reduced by the presence of the 5-HT antagonist methysergide inside and outside the stomach. Transmural stimulation of stomachs of rats treated with reserpine in an attempt to deplete 5-HT produced contractions but true peristalsis did not occur. 5-HT was still released into the lumen, however, and these results only confirm the finding of Shore (1962) that it is difficult to deplete the 5-HT in rat stomachs with reserpine.

In the experiments where the output of 5-HT increased during transmural stimulation, this may have been due to the increase in intraluminal pressure produced by waves of peristalsis. Between 20 and 80% of the increased activity in the fluid from the stimulated stomach was due to an unidentified substance which contracted the rat fundus in the presence of BOL or methysergide. This unidentified substance has properties (Bennett & Vane, unpublished observations) similar to those of the acid lipid which diffuses into the fluid bathing isolated frog intestine (Vogt, 1949).

From the findings that 5-HT release was not always increased during peristalsis, that release was unaffected by drugs which abolished peristalsis, and that methysergide had little or no effect on peristalsis, we conclude that 5-HT is not essential for gastric peristalsis in the rat.

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