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THE RELEASE OF 5-HYDROXYTRYPTAMINE IN RELATION TO PRESSURE EXERTED ON THE INTESTINAL MUCOSA

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In a recent investigation (Bülbring & Lin, 1958) it was found that small amounts of 5-hydroxytryptamine (5-HT) were continuously released into the lumen of an isolated loop of intestine. The quantity released was directly related to the height of intraluminal pressure. Experiments carried out to ascertain whether 5-HT was liberated as a result of pressure on the mucosa, or as a result of peristaltic activity which was evoked by raising the pressure, were not entirely conclusive. When peristaltic activity was abolished either by blocking mucosal sensory receptors with procaine, or by blocking intramural ganglia with hexamethonium, the correlation between pressure and 5-HT release was still observed. However, the extent to which 5-HT release was augmented on raising the intraluminal pressure was less. It was therefore concluded that muscular activity contributed to the mechanism releasing 5-HT, probably by causing, during a peristaltic wave, a considerable additional rise in pressure on the mucosa.

The present work was carried out in order to investigate further the mechanism of 5-HT release.

METHODS

Experiments in vivo

Guinea-pigs were used throughout. The animal was anaesthetized by an intravenous injection of a mixture of 0·6% chloralose + 10% urethane (5 ml./kg). One hour after the injection the abdomen was opened and a piece of ileum close to the caecum, 5–7 cm long, was cannulated at both ends. Tyrode solution was passed through the lumen as in the method described by Bülbring & Lin (1958) for isolated loops of intestine. The fluid flowed from a Marriotte bottle, the height of which could be changed, through a narrow tube at a slow rate into the oral end of the intestine. This was connected to a vertical glass tube acting as a water manometer, the pressure being recorded, by air transmission, with a float recorder. The caudal end of the intestine was connected to a T-piece, one arm of which led to a valve. As a rubber valve constituted a certain resistance we used a float valve made of Perspex, as shown in Fig. 1. Fluid entered at *A* and lifted the conical Perspex float, which was, however, held in a vertical position by the side arm projecting across the tube. The fluid left the tube from the side arm *B*. The height of the water column in the tube was maintained by the outlet of the syphon tube.

When the side arm of the T-piece was closed fluid flowed only as a result of active peristaltic propulsion. In the absence of peristalsis fluid was collected from the side arm of the T-piece. The animal lay on its back and the cut edges of the body wall were lifted up to make a pool. The whole abdominal cavity with the perfused loop of intestine covered by a piece of muslin was bathed by a continuous drip of Tyrode solution at 37° C. Though the two cannulae were held rigidly, the intestine was not stretched between them and could move freely.

Experiments in vitro

A few experiments were carried out on isolated loops of intestine using the method described by Bülbring & Lin (1958). In order to study the effect of pressure exerted on the mucosa, it was necessary to exclude the contribution due to the contractions of the muscles in the gut wall. A piece of intestine was therefore turned inside out and slipped over a Perspex rod, 10 cm long

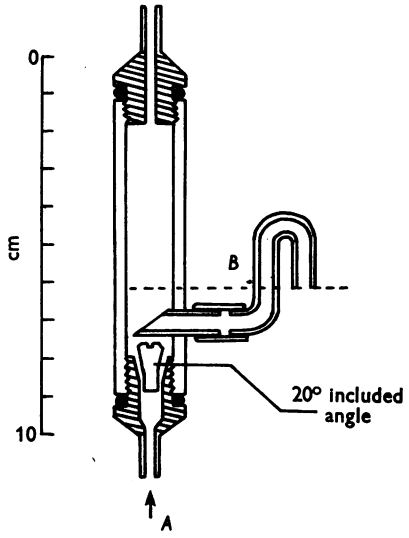

Fig. 1. Float valve to record fluid propulsion against constant water pressure (at broken line). Inflow at *A*, outflow at *B*. For further description see text.

and of 5 mm diameter, with grooves at both ends where the cut ends of the intestine were tied to the rod. In this way the muscle could only contract around the rod and the mucosa was on the surface. The rod with the intestine around it was placed in a Perspex tube of 11 mm internal diameter and 12 cm long; it was fixed along the axis of the tube, one end being held by the stopper. Tyrode solution entered through a hole in the stopper and left the tube through a hole at the other end. The whole perfusion tube with the inverted intestine inside was immersed in a constant temperature bath. The pressure exerted upon the mucosa could be altered by changing the height of the reservoir; the rate of flow could be regulated by adjusting the height of the outflow tubing. In this way it was possible to obtain different pressures while maintaining a constant flow, which was about 0·5 ml./min.

Estimation of 5-HT

The isolated rat stomach preparation (Vane, 1957) was used for estimating 5-HT. Samples were tested at 5 min intervals and allowed to act for 90 sec, after which the fluid in the bath was changed every 30 sec. A vibrator was used to prevent friction of the lever (Bülbring, Crema & Saxby, 1958). The preparation was sensitive to $1–2 \times 10^{-11}$ 5-HT. Even so, the amounts of 5-HT

in the effluent collected in the absence of peristalsis were occasionally so small that none could be detected. The rat stomach preparation was suspended in Locke's solution containing hyoscine and mepyramine, each in a concentration of 1×10^{-7}/ml., to prevent contractions due to acetylcholine or histamine which might have been present. The contractions produced by the samples and by the 5-HT standard solutions were completely abolished by BOL (2-brom-D-lysergic acid diethylamide) 10^{-5}.

RESULTS

The effect of atropine on the correlation between intraluminal pressure and 5-HT release

Raising the intraluminal pressure in normal loops of intestine produced, in the experiments of Bülbring & Lin (1958), on the average a fourfold increase in 5-HT release (maximum 50 times). In the presence of hexamethonium or procaine the 5-HT release at comparable pressures was only doubled. When the peristaltic reflex was blocked, co-ordinated peristaltic waves were, of course, abolished. However, during the persistence of a high intraluminal pressure and the prolonged distension of the intestinal wall, incoordinated muscle contractions still occurred; they were often strong and caused irregular pressure changes. We observed similar contractions in the presence of atropine. For example, in normal conditions the threshold of pressure required to elicit the peristaltic reflex was 18 mm H_2O. The 5-HT release at subthreshold pressure of 8 mm was 1·34 ng/min, and at 25 mm water pressure it was 3·33 ng/min. In the presence of atropine 5×10^{-6} in the bathing solution no peristalsis occurred at 25 mm. Instead there were spastic contractions; these persisted although the atropine concentration was raised to 10^{-5} and, perhaps because of the spasm, the 5-HT release was 5·67 ng/min. When the pressure was lowered it was not possible to pass fluid through the lumen at 8 mm pressure as before, and in order to overcome the spasm the pressure had to be 16 mm, at which the 5-HT release was 1·67. Thus atropine like hexamethonium and procaine abolished the peristaltic reflex, but muscle contractions still occurred and the release of 5-HT at high intraluminal pressure was three times greater than at low pressure.

Experiments on inverted loops of intestine concerning the correlation between intraluminal pressure and 5-HT release

In order to exclude the changes in pressure produced by muscle contractions, experiments were carried out on loops of guinea-pig ileum which were turned inside out and slipped over a Perspex rod. The loop could not shorten, and circular contractions could exert pressure on the rod inside but not on the mucosa. The preparation was placed inside a tube through which Tyrode solution was passed. The perfusion pressure could thus be changed and the 5-HT content in the effluent be estimated. In each experiment the pressure was raised twice and the results are shown in Table 1. Each pressure was

tested for a period of 30 min, thus every experiment lasted for 2 hr. The 5-HT release rose gradually during this period, except in Expt. 3. The second change in pressure caused a smaller change in 5-HT release than the first, except in Expt. 2. The degree of change produced by raising the pressure varied. On the average the 5-HT release was increased about three times.

The effect of ACh on the release of 5-HT

Though, in the inverted intestinal loop, muscle contractions could not contribute to the pressure exerted on the mucosa, ACh might still be liberated and might cause a release of 5-HT. Bülbring & Lin (1958) found that ACh added to the fluid passing through the intestinal lumen, in concentrations from 10^{-7} to 10^{-5}, stimulated peristalsis. There was the possibility that it did so by releasing 5-HT. This was tested in three experiments on the

TABLE 1. Release of 5-HT from inverted pieces of intestine at difference pressures (ng/min)

Pressure (mm H_2O) ...	6–10	45–50	Factor $\frac{\text{High}}{\text{Low}}$
Expt. 1	0·033	0·33	10·0
	0·18	1·00	5·5
2	0·048	0·28	5·8
	0·06	0·57	9·5
3	0·30	0·44	1·5
	0·10	0·13	1·3
4	0·06	0·17	2·8
	0·42	0·83	2·0
5	0·22	1·20	5·5
	1·10	1·20	1·1
6	1·00	2·30	2·3
	1·15	2·50	2·2
7	0·80	6·30	7·9
	1·10	3·20	2·9
Mean	0·47	1·46	3·2

inverted intestine at pressures from 8 to 25 mm H_2O. It was found that the addition of ACh to the perfusion fluid in concentrations from 1 to 3×10^{-6} had no effect on the 5-HT release.

The effect of BOL and of atropine on the action of ACh and 5-HT on peristalsis

In another series of experiments the actions of 5-HT and of ACh were tested on peristaltic activity of isolated loops of guinea-pig ileum, using the method described by Bülbring & Lin (1958). At a constant pressure head of about 20 mm H_2O regular peristaltic waves occurred at a rate of about 1–2/min, propelling about 1 ml. of fluid each time. In order to produce transient effects, drugs were not added to the perfusion fluid in the reservoir but doses were injected into the fluid at a short distance before it entered the lumen of the intestine. ACh 50–200 μg stimulated peristalsis, as did 5-HT in the same or half the dose, lowering the threshold of intraluminal pressure at which the

reflex occurred. The effect of such a short application of the drug was very transient, affecting only one or two contractions, but equivalent doses of ACh and 5-HT could be found and they could be given repeatedly.

When BOL was injected into the lumen in a single large dose (300 μg) the action of 5-HT was abolished but that of ACh was not. Figure 2*a* shows the effect of 100 μg 5-HT before injecting BOL (*a*), and the ineffectiveness of 300 μg 5-HT after this (*b*), when ACh 200 μg was still effective (*c*). The addition of BOL 10^{-6} to the perfusion fluid diminished the action of 5-HT leaving that of ACh unchanged, whereas BOL 10^{-5} at first produced the differential effect like that of a single large dose, but after prolonged exposure to BOL 10^{-5} the action of ACh was gradually diminished and finally also abolished.

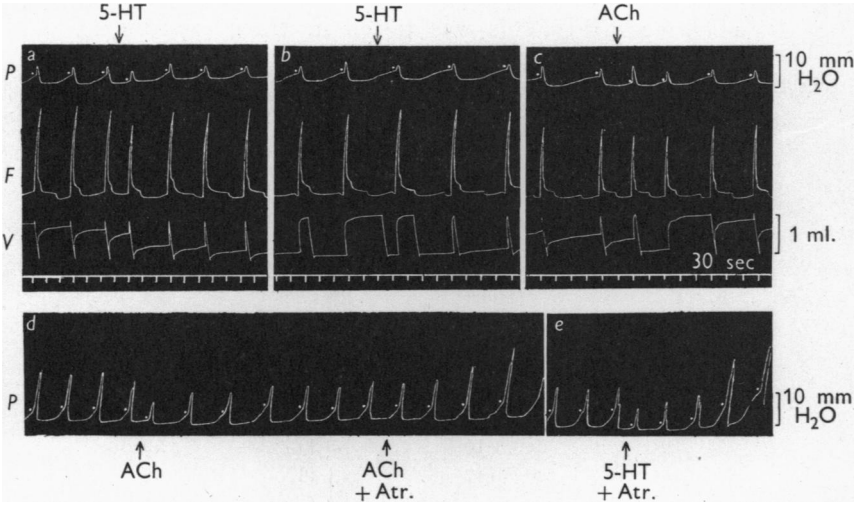

Fig. 2. Isolated guinea-pig ileum. Records in upper tracings: P = intraluminal pressure (threshold marked by dots); F = fluid propulsion through the valve; V = volume of fluid transported. (*a*) intraluminal injection of 100 μg 5-HT; (*b*) 300 μg 5-HT; (*c*) 200 μg ACh. Between (*a*) and (*b*) 300 μg BOL was injected into the lumen. Records in lower tracings from another experiment; intraluminal pressure (P) only recorded. (*d*) 100 μg ACh and 100 μg ACh + 40 μg atropine; (*e*) 100 μg 5-HT + 80 μg atropine.

Similar experiments were carried out using atropine. In order to prevent blockage of the peristaltic reflex atropine was not added to the perfusion fluid in the reservoir but was always injected simultaneously with the dose of the drug. Doses of atropine which were less than 1/10 of the dose of ACh had no effect but doses of atropine which were more than 1/10 of that of ACh abolished its action. When the same or twice the dose of atropine was injected with 5-HT its action remained unchanged. This is illustrated in Fig. 2*d* and *e*.

Thus BOL abolished the action of 5-HT but not that of ACh, whereas atropine abolished the action of ACh but not that of 5-HT. These results suggest that the action of ACh was not due to a liberation of 5-HT.

5-HT release from the intestinal mucosa in relation to intraluminal pressure in anaesthetized guinea-pigs

We confirmed in the whole animal the results obtained on isolated loops of intestine. Guinea-pigs were anaesthetized with chloralose-urethane. A piece of ileum of length similar to those used *in vitro* was cannulated at both ends and Tyrode solution was passed through at different pressures. The 5-HT content of the effluent was estimated and the 5-HT release was calculated in ng/min. The threshold at which the peristaltic reflex occurred was higher *in vivo* than *in vitro* (Straub & Viaud, 1933) and it varied from one experiment to another, probably because of variations in the depth of anaesthesia. Moreover, the pressure had to be maintained at a higher level and peristaltic activity had

TABLE 2. Release of 5-HT from intestinal mucosa in relation to intraluminal pressure in anaesthetized guinea-pigs

Expt.	Pressure (mm H_2O)	5-HT (ng/min)	Pressure (mm H_2O)	5-HT (ng/min)	Factor of change
1	25	0·8	70	2·0	2·5
	25	2·6	60	10·0	3·9
	20	0·8	40	3·0	3·7
2	15	8·0	80	40·0	5·0
	15	1·4	45	10·0	7·2
	15	2·8	45	95·0	34·0
3	25	7·0	60	18·0	2·6
4	15	1·3	40	7·5	5·8
5	15	3·0	60	28·0	9·0
6	15	2·1	45	14·0	6·7
7	20	0·6	60	15·0	25·0
8	25	1·2	50	5·5	4·6
Mean	15–25	2·6	40–80	20·5	7·9

a different pattern. It was not as regular *in vivo* as *in vitro*. Sometimes it occurred in bursts lasting about 2–3 min which were separated by intervals of 2–10 min; sometimes it was quite irregular.

The changes in 5-HT release produced by raising the intraluminal pressure (40–80 mm) are given in Table 2. The amount of 5-HT released in different animals varied over as wide a range as that found *in vitro* by Bülbring & Lin (1958). In some animals the resting 5-HT release was as high as that of other animals during peristalsis. Nevertheless, in each individual experiment there occurred an increase in 5-HT release when the intraluminal pressure was raised. The increases varied over a very wide range, from 2 to 34 times; the average increase was 8 times. Bülbring & Lin (1958) applying lower pressures (25–30 mm) *in vitro* found a range from 2 to 50 times, but in the inverted intestine the increase was never more than 10 times. *In vivo* there was no decline in the extent to which 5-HT release was increased when the pressure was raised repeatedly. Nor was there a clear relation between 5-HT release and the actual pressure head in the reservoir. But there was a relation to intestinal motility and to secretion of mucus. Samples which contained a large amount of mucus also contained a large quantity of 5-HT.

The effect of hexamethonium

The influence of motility was further studied by giving hexamethonium (1–2 mg/kg) and determining the 5-HT release upon raising the intraluminal pressure. The results are given in Table 3. Though the peristaltic reflex was abolished, frequent muscle contractions still occurred. Hexamethonium depressed 5-HT release. It reduced, but did not abolish, the increase in 5-HT release upon raising the intraluminal pressure. These results supported the view that the contribution to the pressure exerted upon the mucosa by peristaltic contractions was considerable.

TABLE 3. The effect of hexamethonium (1–2 mg/kg) on release of 5-HT in relation to pressure, in anaesthetized guinea-pigs

	5-HT (ng/min)			
	Controls		Hexamethonium	
Pressure ...	Low	High	Low	High
Expt. 1	7·0	18·0	0·8	3·0
2	1·3	7·5	1·0	2·4
3	3·0	28·0	2·3	7·7

The effect of peristaltic activity

Several experiments were therefore carried out in which, at a constant pressure head, samples of fluid were collected during periods of peristaltic activity and during the intervening periods of rest. Fig. 3 illustrates an experiment of this kind. While the pressure was subthreshold the 5-HT release was very low (0·7 ng/min). When the pressure was raised, the release during the first 10 min increased 10 times (shaded column). In this period there were bursts of peristalsis and also periods of rest. The next sample was collected during a period of inactivity (white column) and the next during peristalsis (black column). This was repeated three times, collecting either during movement, or during rest, or collecting a mixed sample over a period of 10 min. The 5-HT release rose above 30 ng/min when there was a burst of peristaltic waves. During the intervals it fell as low as 3·6 ng/min (average 8·7), and the mixed samples varied between 5 and 15 ng/min. Though in this experiment the 5-HT release declined somewhat, there was no progressive decline of 5-HT release in other experiments. In one experiment at a pressure of 45 mm it remained at 15 ng/min for $1\frac{1}{2}$ hr; in another experiment the 5-HT release varied widely, but at a pressure of 50 mm it was as great at the end of the fourth hour as it was at the end of the first hour.

5-HT release as a result of pressure exerted on the mucosa

In order to establish whether the release of 5-HT was the result of pressure exerted on the mucosa or whether it depended on the muscle contractions during peristalsis, all the figures available have been summarized in the graph

shown in Fig. 4. The logarithm of the amount of 5-HT released at low pressure has been plotted in relation to the logarithm of the amount of 5-HT released in the same experiments when the pressure was raised. Each point therefore represents a pair of observations. The initial amounts at low pressure varied between 0·03 and 14·0 ng/min, and the amounts released upon raising the pressure varied from 0·13 to 95·0 ng/min. In general, if the initial 5-HT release was small, the amount appearing at high pressure was also relatively small; and in those preparations in which the initial release was large, relatively large amounts of 5-HT appeared when the pressure was raised. Thus the relative effect of raising the pressure was similar in all

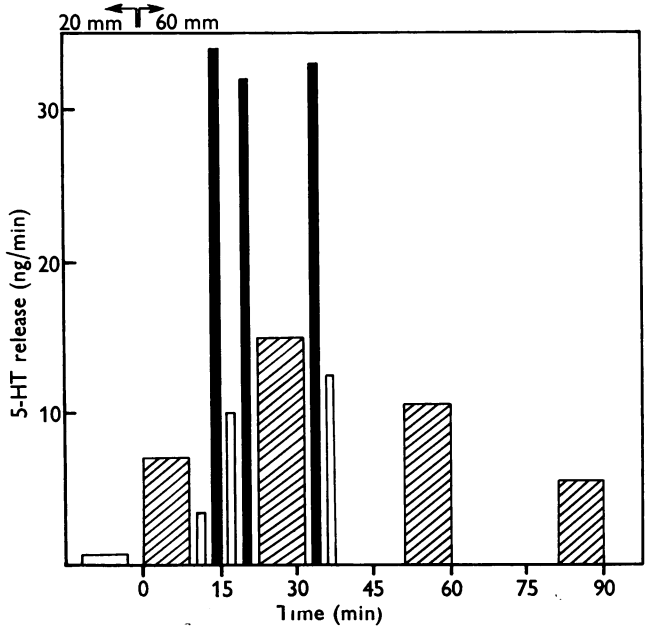

Fig. 3. Ordinates = 5-HT release (ng/min) from guinea-pig ileum *in vivo*; abscissa = time. White columns = samples collected during inactivity; black columns = samples collected during peristalsis; shaded columns = mixed samples collected over 10 min period. For further details see text.

experimental conditions tested, as is shown by the points which are grouped around the calculated regression line which was highly significant ($P < 0{\cdot}001$).

The absolute amounts of 5-HT released from normal intestine *in vivo* and *in vitro* were of the same order. The slightly greater effect of pressure *in vivo* is shown by the fact that the points obtained *in vivo* are found in the upper region of the group. On the other hand, the points obtained after treatment with hexamethonium, atropine and procaine are found in the lower region of the group, showing that the effect of raising the pressure was reduced when peristaltic contractions were abolished. Neither of the two differences was

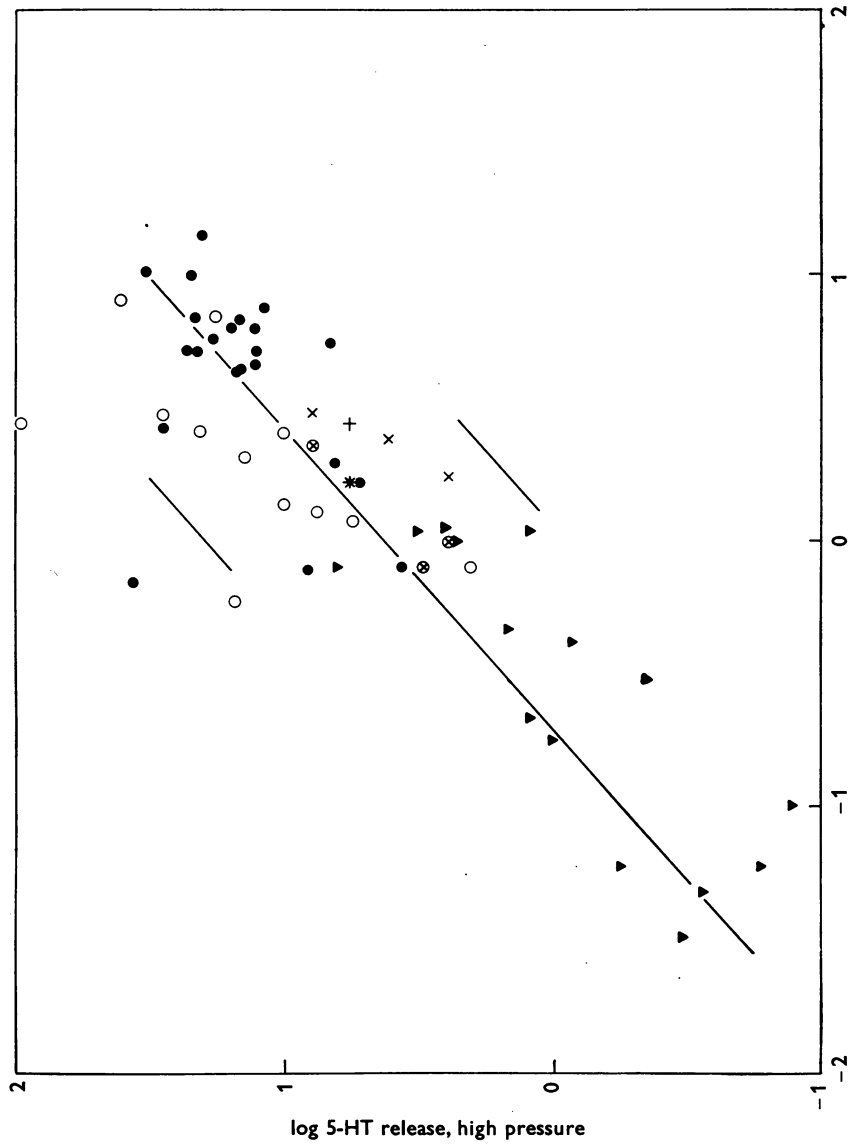

Fig. 4. Logarithm of 5-HT release observed at low pressure (abscissae) plotted in relation to logarithm of 5-HT release at high pressure observed in the same experiment (ordinates). ● = *in vitro*, normal conditions (Bülbring & Lin, 1958); ▼ = *in vitro*, inverted; × = *in vitro*, peristalsis blocked by serosal hexamethonium (Bülbring & Lin, 1958); + = *in vitro*, peristalsis blocked by serosal procaine (Bülbring & Lin, 1958); ✱ = *in vitro*, peristalsis blocked by serosal atropine; ○ = *in vivo*, normal conditions; ⊗ = *in vivo*, peristalsis blocked by hexamethonium I.V. The central line is the calculated regression ($b = 0{\cdot}872$), the outer lines are the 95% confidence limits.

sufficiently large to be statistically significant. Muscle contractions, often of high frequency or of spastic nature, causing irregular pressure changes, were present in all preparations in which the peristaltic reflex was abolished by pharmacological means. The only experiments in which muscle contractions could no longer contribute to the pressure exerted on the mucosa were those carried out with the inverted intestine. The absolute amounts of 5-HT released in these conditions were of a lower order of magnitude. But the proportionality between the low and high pressure release of 5-HT was also in this group not significantly different from that in normal conditions.

The observations summarized in Fig. 4 suggested that 5-HT release was the result of pressure exerted on the intestinal mucosa. Muscular contractions contributed, particularly when they were peristaltic waves which caused a considerable further increase of pressure.

DISCUSSION

The view that pressure on the intestinal mucosa causes the release of 5-HT is supported by the present work. The results obtained *in vivo* are similar to those obtained by Bülbring & Lin (1958) *in vitro*. Indeed, *in vivo* the increase in 5-HT release produced by raising the pressure was on the average greater than *in vitro*. This may be due to the fact that *in vivo* a higher pressure is required to elicit the peristaltic reflex. Moreover, the initial filling pressure is usually more than doubled during a peristaltic wave. As in the living animal bursts of peristaltic waves occur at more or less regular intervals it was possible to make two comparisons: first, the comparison of 5-HT release at a constant filling pressure during periods of peristalsis with the 5-HT release during the intervals of rest; and secondly, the comparison of the 5-HT release during the intervals of rest at high filling pressure with the resting 5-HT release at subthreshold pressure (see Fig. 3). The results leave no doubt that in the absence of peristaltic activity more 5-HT is released at high pressure than at low pressure, and that at high filling pressure the peristaltic contractions greatly augment the release of 5-HT into the lumen.

When the peristaltic reflex was abolished by hexamethonium or atropine, a rise in intraluminal pressure still increased the release of 5-HT. These drugs did not, however, abolish all muscular activity and the resulting irregular pressure changes. The effect of muscular activity was excluded in experiments on inverted intestinal loops, in which the muscle contracted around a rod and could not exert any pressure on the mucosa. Also in these conditions the 5-HT release was increased when the mucosa was exposed to a higher pressure.

The experiments exclude neither a nervous nor a humoral mechanism as a cause for the liberation of 5-HT. There is no evidence for a cholinergic mechanism. Acetylcholine applied to the mucosa had no effect on the amount of

5-HT released. Moreover, in the presence of atropine, hexamethonium and procaine, the 5-HT release was still increased by raising the pressure.

The enterochromaffine cells frequently extend along the base of the epithelium. In this position they may be particularly exposed to mechanical effects resulting from the distension of the gut wall normally associated with increased filling pressure. The possibility suggests itself that they might be neurosecretory cells in which deformation leads to the release of 5-HT.

SUMMARY

1. Experiments were carried out *in vivo*, on guinea-pigs anaesthetized with chloralose-urethane, in which fluid was passed through a loop of intestine and the release of 5-HT was measured in relation to intraluminal pressure.

2. A rise of pressure from 15–20 mm H_2O, which was subthreshold, to 40–80 mm H_2O, when peristalsis occurred, increased the release of 5-HT on the average eight times.

3. At a constant filling pressure the release of 5-HT was very much greater during bursts of peristalsis than during the intervening periods of rest.

4. Hexamethonium or atropine, which abolished the peristaltic reflex, did not abolish the augmentation of the release of 5-HT upon raising the intraluminal pressure.

5. In inverted loops of intestine, in which muscle contractions could not exert pressure upon the mucosa, the release of 5-HT was reduced. However, exposing the mucosa to higher pressure in the perfusion system still increased the release of 5-HT.

6. No evidence was found indicating that acetylcholine might be a mediator liberating 5-HT.

7. It is concluded that the release of 5-HT is a result of pressure upon the mucosa.

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